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FOREWORD

A Word from the Editor-in-Chief

Dear colleagues,

In your hands is the Book of Proceedings of the X International Scientific Agricultural Symposium “AGROSYM 2019”, which I hope you will find useful in your work. As many as 900 contributions, from 82 countries, have been accepted for oral or poster presentations. Symposium themes cover all branches of agriculture and are divided into 7 sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, 7) Forestry and agroforestry. Papers dealing with agricultural engineering and technology were included into one of the seven sessions depending on their focus.

In the plenary lectures were addressed interesting topics; one keynote was on biotechnology and two others dealt with organic farming in Australia and Europe. This confirms the role of AGROSYM as a forum for open discussions and exchanges on agriculture, food, the environment and rural development in the Balkans and beyond. Many of the papers identify a number of approaches and market-based incentives to encourage producers to achieve higher levels of performance (from both economic and environmental points of view) and as a result to meet the expectations of governments and consumers.

The successful management of agricultural resources to satisfy changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, indicate a long-term agricultural development imperative. Advances in productivity, profitability and stability of modern cropping, animal and forestry systems will have to be achieved globally on an ecologically sustainable basis. Today, it is obvious that conventional methods of agricultural production, while providing sufficient food and various products to humanity, have led to a number of negative impacts, including the transgression of many planetary boundaries. These negative impacts raise serious questions about the long-term sustainability of high-input agriculture and call for a genuine transition towards sustainable agro-food systems, which achieve food and nutrition security for present and future generations within the safe operating space for humanity.

Full texts of the submitted communications will be available on the website of AGROSYM (http://agrosym.ues.rs.ba). Each paper included in the present Book of Proceedings was positively reviewed.

Much appreciation is due to the authors of all papers submitted and presented at the symposium as well as to all symposium participants whose ideas and contributions allowed rich and lively discussions during the various sessions. Many thanks to all reviewers, session moderators and colleagues for their help in editing the Book of Proceedings. Special thanks go to all co-organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief

Dušan Kovačević

Dusan Kovacevic, PhD

East Sarajevo, 12 October 2019

CONTENT

1. PLANT PRODUCTION

NUTRIENTS STATUS IN MAIZE GRAIN FROM SUSTAINABLE AGRICULTURE

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Abstract

Maize cultivation with application of proper organic and bio-fertilizers could increase nutritional value of crop grain and maintain soil fertility. The aim of the study was to examine variations in concentrations of phytate, soluble phenols, total glutathione (GSH), yellow pigment (YP), DPPH radical scavenging capacity (DPPH), Ca, Mg, Fe, Zn and Mn in maize hybrids with white, yellow and red colour grain, under the influence of bio-, organic fertilizer and urea in regard to control (without fertilization). Results indicated that phytate, DPPH, Ca, Mg and, Mn varied slightly (< 10%). Red grain maize was characterized with the highest concentrations of phenols, Ca, Mg, Fe, Zn, and DPPH. White grain maize, particularly in urea and bio-fertilizer treatment, accumulated higher GSH values, while red and especially yellow grain hybrid accumulated higher YP amount in urea treatment. Irrespective to lower variations in Mn concentration, higher values were determined in yellow hybrid. Organic fertilizer mainly induced increase in Mg bio-fertilizer which positively affected Fe accumulation, while urea caused higher Zn and Mn accumulation in maize grain. It could be concluded that yellow and particularly red grain hybrid enabled increased accumulation of mineral elements, together with higher DPPH values, mainly in treatments with organic fertilizer and urea giving it advantage in production of highly nutritious food.

Keywords: *Colored maize grain, Sustainable production, Mineral nutrients, Antioxidants.*

Introduction

All nutrients that humanity consumes came mainly from the agricultural production. When ability of agricultural systems to produce enough of diverse foods fail, people will suffer, mortality and morbidity rates will increase, working productivity will be reduced, societies will deteriorate (Welch 2002). That is one of the main reasons why nutritional deficiencies affect over three billion people globally, irrespective that they live in developed or developing countries, causing chronic diseases, such cancer, cardio-vascular diseases, stroke, diabetes, osteoporosis etc. (Graham et al., 2007). At the same time, soils are deteriorated worldwide Maintenance and even increase of soil is of particular importance for agricultural production. This means that proper mineral nutrition is significant measure when crops are grown on infertile soils. When pea and wheat were grown on soil with adequate Zn and Se supply, they accumulated about three-fold more Zn and five-fold more Se in their grains, when compared to plants grown on Zn- and Se-deficient medium (Miller and Welch, 2013).

Different fertilizer types, such as organic and bio-fertilizers contribute to the maintenance and/or increase of soil organic matter and status of mineral nutrients, thus contributing to the soil fertility, together with the improved availability of nutrients to plants and increased yielding potential. Mineral nutrition is complex practice, since some nutrients are able to promote or reduce absorption of the other ones, like application of higher N rates, which decreases Mg and Zn absorption and promotes Mn absorption and accumulation, thus altering nutritional quality of maize grain (Feil et al., 2005). There are many records about contribution of bio-fertilizers to improved crop production. Vessey (2003) find that bio-fertilizers improve availability of nutrients from the rhizosphere, promoting crop growth and their nutritional quality. El-Sirafy et al. (2006) underlined that bio-fertilizer significantly affected Fe, Mn and Zn concentration in wheat tissues. Nevertheless, there are some negative aspects of bio-fertilizer application, such as decreased Fe and β-carotene concentrations in maize grain with elevated phytate concentration (Dragicevic at al., 2015).

Irrespective to the status of mineral nutrients in grains, their further bio-availability from digestive organs depends on chemical composition of grain (Nuss and Tanumihardjo, 2010; Welch and Graham, 2000), so it is important to evaluate relation between essential elements and factors that contribute or restrain their bio-availability, like phytic acid, phenols, carotenoids, glutathione, fiber, etc.

The relation between phytate and mineral elements is important trait for evaluation of their potential bio-availability (Dragičević et al., 2014; Dragicevic et al., 2016; Šimić et al., 2012; Wiesler, 2012), indicating that genotypes and cropping practices that reduce phytate content and increase concentrations of essential mineral elements are desirable, from the nutritional standpoint.

The aim of the study was to examine variations in concentrations of phytate, soluble phenols, total glutathione (GSH), yellow pigment (YP), DPPH radical scavenging capacity (DPPH), Ca, Mg, Fe, Zn and Mn in maize hybrids with white, yellow and red colour grain, under the influence of bio-, organic fertilizer and urea in regard to control (without fertilization).

Material and Methods

The research was conducted in Zemun Polje (44°52'N 20°20'E), in the vicinity of Belgrade, on a slightly calcareous chernozem soil in 2017, within an on-going experiment in dry-land conditions. The field experiment was arranged in a split-plot design with 4 replications. Three maize hybrids were used in experiment: ZP 737, ZP 5048*c* and ZP 522*b* with yellow, red and white grain, respectively. Also, three fertilizers were tested: bio-fertilizer (BF) Team Mycoriza Plus (containing *Glomus spp*. 300 spore^{-1} g and rizoshere bacteria 10 UFC^{-1} g, in amount of 0.5 kg 100^{-1} l water; organic fertilizer (OF) – Fertor (containing 65% of organic matter, 65% N, 4.1% organic N, 2.7% K, 2.3% K, 1.1% MgO, 9.3% CaO in amount of 2.5 t ha^{-1}; urea (U), as a standard mineral fertilizer, in amount of 200 kg ha^{-1}; and control (C) – without fertilization. Fertilizers were incorporated into soil at the end of April, prior to sowing.

After harvesting (second half of October) maize grain was milled and chemical composition was determined. Phytate (Pphy) was determined by the method of Dragičević et al. (2011), total glutathione (GSH) by the method of Sari Gorla et al. (1993), yellow pigment (YP) by the method described by Vancetovic et al. (2014), soluble phenols by method of Simić et al. (2004), DPPH radical scavenging capacity (DPPH) by the method suggested by Abe et al. (1998). Mineral elements were determined by Inductively Coupled Plasma - Optical Emission Spectrometry.

Results were analysed with ANOVA and the significance of the treatments effect were determined by the Fisher's least significant difference (LSD) test at p = 0.05 and by correlation (Pearson's coefficients).

Results and Discussion

The main source of variability in content of examined parameters was hybrid (for phytic P, phenols, glutathione, yellow pigment DDPH radical scavenging capacity, Mg and Zn), as well as interaction between hybrid and fertilizer (for all examined factors) (Table 1). Feil et al. (1990) and Mladenović Drnić et al. (2009) also acquired high genotypic variability in seed phosphorus, i.e. phytate content. Fertilizer expressed significant influence on the variation of Mg, Fe, Zn and Mn content in maize grain. Besides, red grain maize was characterised with the highest content of phenols, yellow pigment DPPH scavenging capacity, and all examined minerals, as well as the lowest content of phytic P, signifying it mainly as the favourable source of antioxidants, and so factors that contribute to the potentially increased bio-availability of mineral nutrients. Nevertheless, white grain maize had the highest GSH content. When impact of applied fertilizers was taken into consideration, it could be assumed that bio-fertilizer expressed the highest impact on phytate decrease, as well as increase in content of phenols, GSH, Ca and Fe, while urea was mainly important for increased accumulation of yellow pigment, Cu and Zn and organic fertilizer was reflected positive on increase of Mg and Mn. These results were mainly supported by results of Dragicevic at al. (2015) who pointed rather negative effect of bio-fertilizer, which led to decreased Fe and β-carotene concentrations in maize grown as intercrop with soybean, as well as in maize monocrop. Nevertheless, El-Sirafy et al. (2006) proved that bio-fertilizer increased absorption and accumulation of Fe, Mn and Zn in wheat tissues. Also, Singh and Reddy (2011) and Kaur and Reddy (2015) showed that particular bio-fertilizers could be used to improve availability of some target mineral elements, like P is, for maize and wheat crops, even from insoluble forms.

Table 1. Variations in the content of phytic P (Pphy), phenols (Phen.), total glutathione (GSH), yellow pigment (YP), DPPH radical scavenging capacity, Mg, Ca, Fe, Cu, Zn and Mn in grain of yellow, red and white maize

	Pphy mg g^{-1}	Phen. ug g^{-1}	GSH nmol g^{-1}	YP ug g^{-1}	DPPH %	Mg	Ca	Fe μg g^{-1}	Cu	Zn	Mn
					Yellow grain						
BF	2.73	302.81	995.5	12.43	79.78	1918.3	213.39	54.46	26.97	26.93	9.96
U	2.98	245.85	937.2	17.39	84.14	2234.0	169.70	37.17	36.51	36.50	10.77
OF	2.93	230.86	748.3	16.41	90.30	2305.6	262.07	42.74	31.67	31.73	11.05
C	2.97	203.88	1058.2	15.51	94.66	2394.1	209.17	47.51	38.24	38.31	12.09
Mean	2.90	245.85	934.8	15.43	87.22	2213.0	213.58	45.47	33.35	33.37	10.97
					Red grain						
BF	2.62	776.52	1011.0	14.95	96.51	2129.1	263.09	37.59	29.72	29.80	9.68
U	2.47	668.59	856.1	14.97	98.15	2422.5	329.51	51.35	55.07	54.99	11.00
OF	2.54	623.62	987.1	15.25	91.44	2507.4	177.40	75.33	44.52	44.73	12.03
C	2.60	713.56	897.6	16.72	95.64	1985.5	192.44	88.51	32.29	32.31	10.51
Mean	2.56	695.57	938.0	15.47	95.44	2261.1	240.61	63.19	40.40	40.46	10.80
					White grain						
BF	2.57	326.80	1195.5	1.98	97.11	1584.5	192.48	161.16	37.41	37.09	9.35
U	2.96	317.81	1281.8	1.93	97.17	2123.0	145.36	32.71	31.29	31.36	12.65
OF	2.76	260.84	1172.0	1.87	92.86	1972.5	226.91	25.60	33.16	33.20	9.47
C	2.80	284.83	1021.4	1.39	93.90	1921.6	249.76	32.00	30.58	30.53	9.93
Mean	2.77	297.57	1167.7	1.79	95.26	1900.4	203.63	62.87	33.11	33.05	10.35
					Mean						
BF	2.64	468.71	1067.3	9.79	91.14	1877.3	222.99	84.40	31.37	31.27	9.66
U	2.80	410.75	1025.0	11.43	93.15	2259.9	214.86	40.41	40.96	40.95	11.47
OF	2.74	371.77	969.1	11.18	91.53	2261.8	222.12	47.89	36.45	36.56	10.85
C	2.79	400.75	992.4	11.21	94.73	2100.4	217.12	56.01	33.70	33.72	10.85
					LSD 0.05						
Hyb.	0.14	53.79	115.8	1.91	4.10	204.1	47.39	36.31	15.68	6.75	1.05
Fert.	0.20	223.60	163.5	7.28	5.73	208.6	50.50	33.26	15.87	6.73	0.86

H x F	0.10	39.98	77.4	1.92	1.13	23.2	2.33	1.76	17.27	0.10	0.05

Due to the fact that accumulation of essential elements in maize grain depend on agro-ecological conditions and applied cropping measures (Bender et al., 2013), and that further bio-availability from digestive organs depends on chemical composition of grain (Nuss and Tanumihardjo, 2010; Welch and Graham, 2000), it is important to evaluate relation between essential elements and factors that contribute or restrain their bio-availability. The correlation between these factors and examined minerals from maize grain shown significant and negative dependence between phytate and majority of minerals (Ca, Fe, Cu and Zn) (Table 2), indicating that any factor which reduce phytate content, such as particular usage of bio-fertilizer in yellow and white grain maize, positively impacts accumulation of mineral elements, with exception of Mn, which correlates positive with phytate in maize grain. Feil et al. (2005) also pointed that mineral nutrition, such as application of higher N and P rates affects absorption and accumulation of other mineral elements, thus changing nutritional quality of maize grain. Increased accumulation of mineral elements, particularly micro-nutrients with parallel phytate decrease is positive trend, since many data support finding that parallel increase in concentration of mineral elements and reduction of phytate concentration is desirable for their improved bio-availability (Dragičević et al., 2014; Dragicevic et al., 2016; Šimić et al., 2012; Wiesler, 2012). Potentially increased Mg bio-availability is also supported by increased content of yellow pigment, emphasizing yellow grain maize as potentially better source of Mg in nutrition. Nevertheless, increased phenols content, as it was found in red grain maize, correlates significantly and positive with Cu and Zn, reducing their potential bio-availability to some extent. This is essential, since results demonstrate that red grain maize accumulates higher concentration of mineral elements, when compared to yellow and particularly white grain maize. What is more, increased DPPH radical scavenging capacity, as it was find in red and white grain maize, also positively correlates with Cu and Zn, indicating the importance of antioxidants for potential bio-availability of mineral elements, mainly Cu and Zn.

Table 2. Correlation between phytic P (Pphy), phenols (Phen.), total glutathione (GSH), yellow pigment (YP), DPPH radical scavenging capacity and Mg, Ca, Fe, Cu and Zn

	Pphy	Phen.	GSH	YP	DPPH
Mg	0.119	0.204	-0.471*	0.646*	-0.005
Ca	-0.375*	0.291	-0.532*	0.181	0.227
Fe	-0.510*	0.162	0.145	-0.100	0.216
Cu	-0.463*	0.327*	-0.228	0.259	0.344*
Zn	-0.460*	0.331*	-0.232	0.266	0.342*
Mn	0.364*	-0.054	-0.003	0.272	0.118

*Pearson correlation coefficients at the level of significance of 0.05

Conclusions

Based on obtained preliminary results, it could be concluded that accumulation, and potential bio-availability of mineral elements from maize grain is complex trait, depending mainly on genotype characteristics and its interaction with production conditions. It is shown that red grain maize is better accumulator of mineral elements, having lower phytate and higher content of phenols, yellow pigment and DPPH radical scavenging capacity, making it as desirable source of antioxidants and mineral in nutrition. But, when increased level of phenols is taken into consideration, potential bio-availability from digestive system could be compromised to some extent. Owing to the higher yellow pigment content and little bit lower values of mineral elements, yellow grain maize could be also a great source of carotenoids and minerals. Irrespective that white grain maize is lower among all three genotypes in mineral elements it is also a good source of antioxidants, such as GSH. Among tested fertilizers, bio-fertilizer expressed full potential in increase of antioxidants content and phytate reduction, together with increase of Ca and Fe content in maize grain, while urea is much more important for accumulation of yellow pigment, Cu, Zn and Mn.

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INCREASE IN GROWTH AND YIELD OF SOYBEAN IN FIELD TRIAL BY IAA-PRODUCING MUTANT *BACILLUS* SP. CO-INOCULATED WITH *B. JAPONICUM*

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Abstract

Application of bioinoculants using plant growth promoting rhizobacteria (PGPR) as root-colonizing non-pathogenic bacteria and their metabolites can increase plant resistance to biotic and abiotic stress factors. Co-inoculation using bacteria belonging to *Bacillus* and *Bradyrhizobium* genera, as PGPR members, can lead to synergistic activity and improvement of growth, nodulation and yield of leguminous plants, as well as to better tolerance against drought as abiotic stress. Assessment of soybean (*Glycine max* L.) growth and yield enhancement by co-inoculation with *Bacillus* sp. and *Bradyrhizobium japonicum* was the aim of this study. The field trial was conducted in Vajska locality (Vojvodina, Serbia) during 2017 on 0.5 ha plot per treatment under low rainfall. Commercial fertilizer containing *B. japonicum* strain (Kf) was used as positive control, while negative control (K) was without any inoculation. *Bacillus* sp. strain Q10b (B), mutant for the production of growth stimulating IAA (indole acetic acid), and its extracellular metabolites (Em) in addition to *B. japonicum* strain 526 were used in field trial. *Bacillus* sp. strain Q10b, as induced mutant, produced eightfold higher amount of IAA than wild type strain Q10. The B, Em and Kf treatments significantly improved morphological parameters of plant growth at flowering and maturity stage comparing to K. The values of plant height, trifoliate leaf number, pod number per plant and SDW were higher for treatments B and Em than in Kf at the flowering stage, as well as grain mass per plant at the maturity stage. The seed yields of 1600 (K), 1800 (Kf), 2220 (B) and 2480 kg ha^{-1} (Em) were obtained, suggesting soybean exerted high response to IAA-producing mutant *Bacillus* sp. Q10b and its metabolites in the addition to *B. japonicum* strain.

Keywords: *Bacillus sp., B. japonicum, IAA, soybean, coinoculation.*

Introduction

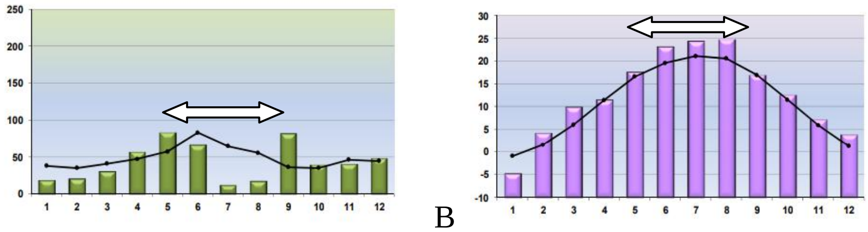
Soybean [*Glycine max* (L.) Merr.], a member of the Leguminosae family, is one of the most important oil seed crops worldwide; it is the best source of proteins, oil, minerals (calcium, zinc and iron) and vitamins (folic acid, riboflavin, thiamin and niacin) (Ali, 2010). Soybean is a source of food for livestock and human, and the food industry source for many products (flour, oil, milk, vegetable cheese, lecithin). Improvement in production of this legume depends on many factors, including edaphic factors and biological processes related to the host plant and microorganisms.

Among the large number of microbes living in the endosphere, rhizosphere and the root surrounding soil are some beneficial bacteria. PGPR are distributed around the plant root system and have impact on plant growth and productivity due to effect of its metabolic activities and plant interactions (Berg, 2009; Lugtenberg and Kamilova, 2009). The important mechanisms of growth promotion include biofertilization through production of growth stimulating phytohormones, solubilization and mobilization of phosphate, siderophore production, N_2 Fixation, as well as biological control of pathogens through antibiosis, secretion of lytic enzymes, production of antibiotics and induction of plant systemic resistance to pathogens. Plant growth promotion by beneficial bacteria is usually a result of combined action of two or more of these mechanisms. PGPR may synthesize phytohormones such as

auxins, cytokinins and gibberellins able to enhance or regulate various stages of plant growth. Indole-3-acetic acid (IAA), as auxin, is able to stimulate root growth, to enhance lateral root and root hair development and to increase surface area (Idris et al. 2007; Richardson et al. 2009; Verma et al. 2010). Phytohormones also help the plants to escape abiotic stress or survive stressful conditions (Skirycz and Inze, 2010; Fahad et al. 2015).
Rhizobacteria belonging to the genus *Bacillus* (*Bc*.) and *Bradyrhizobium* (*B*.) may improve plants growth by producing extracellular metabolites, including IAA. *Bacillus,* rod-shaped Gram-positive bacteria, are the most commonly commercialized member of PGPR because of their ability to produce endospores tolerant to heat and desiccation (Akinrinlola et al. 2018). *B. japonicum* is one of the symbionts of soybean that possess the ability to reduce molecular nitrogen into ammonia during biological nitrogen fixation (BNF) (Bottomley et al. 2007; Franche et al. 2009). Although *Bradyrhizobium* inoculation is very effective at enhancing BFN and crop yields in soybean, co-inoculation of *Bradyrhizobium* and other PGPR significantly improved soybean growth and yield as compared with the sole application of *Bradyrhizobium* (Wasule et al. 2007). Co-inoculation is effective practice considering demands of agricultural, economic and environmental sustainability (Chaparro et al. 2012).
The aim of this study was the estimation of the production of growth stimulating phytohormone IAA by mutant strain *Bacillus* sp. Q10b and its evaluation as co-inoculant with *B. japonicum* on growth promotion and yield improvement in soybean under drought conditions.

Material and methods

Bacteria were grown aerobically in nutrient broth (NB) (*Bacillus* sp.) or yeast mannitol broth (YMB) (*B. japonicum*) for 24 h or 48 h at 28ºC on a rotating shaker (150 rpm). The density of culture was measured spectrophotometrically (Shimadzu Spectrophotometer UV-160) at 600 nm, diluted in sterile medium to a final concentration of 5 x 10^8 CFU $ml^{-1,}$ and used for further analyses. Cell free supernatant containing extracellular metabolites (Em) of *Bacillus* sp. mutant strain Q10b was obtained by centrifugation at 12000 rpm for 15 min and filtration through 0.22 μm filters (Merck Millipore Ltd.).
Quantitative analysis of IAA production was tested according to standard procedure (Glickman and Dessaux, 1995). Bacterial suspension (10 μl standardized to OD_{600} of 0.625) was inoculated in NB or YMB medium supplemented with of 2.5 and 5 mM L-tryptophan. After 24h and 48h of incubation at standard temperature, the density of the culture was measured spectrophotometrically at 600 nm for optimization to value 1 and the bacterial cells were removed from the culture medium by centrifugation. The supernatant was mixed (1:2 v/v) with Salkowski reagent (2% 0.5M ferric chloride in 35% perchloric acid) and pink color intensity was measured at 530 nm for IAA production or IAA standard curve (1-100 μg ml^{-1}).
The field experiment was conducted during 2017 from May 12th to September 8th in Vajska locality (Bačka District, Vojvodina, Serbia) using soybean cultivar Tambor. Field trial was performed on 0.5 ha plot per treatment under low rainfall and high temperature (fig. 1). Four treatments were used: K - negative control without any inoculation; Kf - commercial fertilizer containing *B. japonicum* strain used as positive control; B - *Bacillus* sp. mutant strain Q10b and *B. japonicum* strain 526; Em - extracellular metabolites of *Bacillus* sp. mutant strain Q10b in addition to *B. japonicum* strain 526.
At flowering and maturity period, 40 plants per treatment were sampled and main growth parameters such as plant height, trifoliate leaf number, root weight, root length, nodule and pod number per plant, grain mass per plant and mass 1000 grain were measured. The yield was compared to both control treatments.



A B

Figure 1. Average values of monthly precipitation (mm) (A) and air temperatures (^{o}C) (B) during the 2017 and (•) average values in period 1961 – 1990 (RHMZ RS).

Statistical analysis. Data were analyzed using Statistica 8 software. One-way general linear model ANOVA was used to determine the effect of each treatment. The Duncan test was used for post hoc analysis to determine differences between means. Differences were considered significant at $p < 0.05$.

Results and discussion

The results of IAA measurements reflected the ability of *Bacillus* sp. wild type (wt) Q10 and mutant strain Q10b to produce indole compounds. The tested strains exhibited a pink to red color with a variation in intensity. Mutant strain *Bacillus* sp. Q10d produced significantly higher amount of IAA in NB medium than wt Q10, without or in addition of tryptophan. IAA produced naturally by Q10 ranged from 1.63 to 1.87 μg ml^{-1} after 24 h and 48 h of cultivation, while mutant strain Q10b produced about eightfold higher amount. The highest value of auxin production (60 μg ml^{-1}) was obtained by mutant strain Q10b after 48h of cultivation in NB supplemented with 5 mM L-tryptophan. Influence of two tryptophan concentrations on wt and mutant strain is shown in table 1.

Table 1. *Bacillus* sp.: IAA production (μg ml^{-1}) by wild type Q10 and mutant strain Q10b

Strain	L-tryptophan (mM)					
	0		2.5		5	
	24 h	48 h	24 h	48 h	24 h	48 h
Q10	1.63 ±0.11	1.87±0.10	4.91±0.28	6.02±0.12	6.28±0.89	7.81±0.15
Q10b	13.27±0.42	15.63±0.42	27.99±0.13	37.70±3.03	48.92±0.29	60.06±2.28

Tryptophan has been identified as a main precursor molecule for biosynthesis of IAA in bacteria. Tryptophan-dependent production of IAA affects level of plant growth promotion by some *Bacillus* strain (Idris et al. 2007). In our earlier investigation, IAA production from 3.76 μg ml^{-1} in medium without the addition of tryptophan, to 10.62 μg ml^{-1} in medium supplemented with 5 mM L-tryptophan, was observed for *Bacillus* sp. Q3 strain (Starovic et al. 2013). Similar values were observed in this investigation for wild type Q10 strain supplemented with 5 mM L-tryptophan, as well as for several PGP *Bacillus* and *Paenibacillus* strains that showed IAA production of 5-7 μg ml^{-1} in non-supplemented medium (Erturk et al. 2010). The same authors quantified higher values of IAA in the presence of 25 μg ml^{-1} of tryptophan for *Bacillus* RC23 strain that produced 20.4 μg ml^{-1} and *Bc. simplex* RC19 that produced 33.6 μg ml^{-1}. The two fold higher values were quantified for *Bacillus* mutant strain Q10b, although tenfold lowest tryptophan concentration was added.

The effect of co-inoculation treatments on soybean growth and yield components is shown in tables 2 and 3. Data illustrated that *B. japonicum* 526 inoculation amended with extracellular metabolites of *Bacillus* sp. mutant strain Q10b, containing high amount of IAA, or its co-inoculation with the *Bacillus* sp. Q10b strain, caused significant increases in all growth components, as compared with the commercial fertilizer applied or uninoculated treatments. The yield and yield increment compared to two controls are shown in table 4.

Table 2. Growth promotion of soybean cv. Tambor as affected by *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526 at flowering period

Treatment	Plant height (cm)	Root length (cm)	Trifoliate leaf No plant^{-1}	Pod No plant^{-1}	Nodule No plant^{-1}	Shoot fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
K*	68.29^{b**}	16.88^{b}	10.55^{d}	8.13^{c}	6.79^{c}	46.68^{b}	4.06^{b}	31.54^{c}	4.92^{b}
Em	79.54^{a}	24.39^{a}	26.08^{b}	32.87^{a}	19.32^{a}	183.98^{a}	14.24^{a}	88.97^{a}	12.98^{a}
B	79.76^{a}	22.47^{a}	34.92^{a}	36.00^{a}	15.92^{b}	183.31^{a}	15.80^{a}	90.93^{a}	13.84^{a}
Kf	77.50^{a}	22.08^{a}	22.50^{c}	19.50^{b}	18.47^{a}	117.51^{a}	14.49^{a}	57.05^{b}	12.37^{a}

*K - negative control without any inoculation; Kf - commercial fertilizer containing *B. japonicum* strain used as positive control; B - *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526; Em - extracellular metabolites of *Bacillus* sp. mutant strain Q10b in addition to *B. japonicum* strain 526. **According Duncan test, different letters above data indicate significant differences among treatments ($p < 0.05$).

Table 3. Growth promotion of soybean cv. Tambor as affected by *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526 at maturity period

Treatment	Plant height (cm)	Root length (cm)	Pod No plant^{-1}	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Grain mass (g plant^{-1})	Mass 1000 grain (g)
K*	47.97^{c**}	16.13^{b}	19.87^{c}	19.02^{b}	10.11^{c}	13.20^{c}	121.5^{d}
Em	82.63^{a}	21.2^{a}	39.14^{a}	24.86^{a}	13.02^{ab}	23.18^{a}	175.3^{a}
B	84.26^{a}	20.33^{a}	36.15^{ab}	25.73^{a}	13.95^{a}	21.75^{a}	168.8^{b}
Kf	63.82^{b}	21.23^{a}	34.20^{b}	24.33^{a}	12.50^{b}	17.29^{b}	137.8^{c}

*K - negative control without any inoculation; Kf - commercial fertilizer containing *B. japonicum* strain used as positive control; B - *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526; Em - extracellular metabolites of *Bacillus* sp. mutant strain Q10b in addition to *B. japonicum* strain 526. **According Duncan test, different letters above data indicate significant differences among treatments ($p < 0.05$).

The appearance of 0-13 (6.79 in average) nodules per plant in uninoculated control on the plot of soil without earlier soybean cultivation could be explained due to the presence of indigenous population of *B. japonicum* different from introduced strains (data not shown). The effectiveness of indigenous *B. japonicum* was lower than applied strain and commercial fertilizer comparing to growth parameter and yield of soybean.

According to Masciarelli et al (2014), *Bc. amyloliquefaciens* strain LL2012 produces high levels of gibberellins and salicylic acid in addition of IAA (18.8 μg ml^{-1}). In co-inoculation of soybean plants with *B. japonicum*, strain LL2012 improves nodulation and other plant growth parameters. IAA production of *Bacillus* sp. strain Q10b was higher than LL2012 and showed PGP effect. Several *Bacillus* strains impact root colonization and growth promotion not only soybean, but also other plants, as *Bc. cereus* YL6 of wheat and Chinese cabbage (Ku et al. 2018).

Table 4. Yield of soybean cv. Tambor and its increase as affected by *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526

Treatment	Yield (kg ha^{-1})	Yield increase (%) compared to	
		negative control	positive control
K*	1600	-	-11.11
Em	2480	55	37.78
B	2220	38.75	23.33
Kf	1800	12.5	-

*K - negative control without any inoculation; Kf - commercial fertilizer containing *B. japonicum* strain used as positive control; B - *Bacillus* sp. mutant strain Q10b co-inoculated with *B. japonicum* strain 526; Em - extracellular metabolites of *Bacillus* sp. mutant strain Q10b in addition to *B. japonicum* strain 526.

Drought affects plant–water potential and change physiological and morphological traits in plants (Rahdari and Hoseini, 2012). It influences the availability and transport of soil nutrients, affects at various sub cellular compartment, cell organs and whole plant level (Rahdari et al. 2012). Improvement in soybean growth and yield in the field trial under drought stress using *Bacillus* sp. wt strain Q10 in co-inoculation with *B. japonicum* 526 and *Pseudomonas chlororaphis* Q16 are reported (Iličić et al. 2017). Growth and yield reduction under drought stress were also observed during vegetation of soybean in our field trial. Comparing to results obtained earlier on the neighbor locality, yield increment of 7.15% impacted by wt Q10 strain in co-inoculation with *B. japonicum* 526 on soybean cv. Angela is lower than yield increment of soybean cv. Tambor under *Bacillus* sp. mutant strain Q10b co-inoculated with the same *B. japonicum* strain (23.33%). Soybean inoculated with mutant strain Q10b showed good response to drought stress. *Bc. thuringiensis* AZP2 application on wheat seedlings under drought stress increased plant biomass and survival under drought by reducing emissions of volatile compounds and increasing photosynthesis, suggesting that bacterial inoculation improved plant stress tolerance (Timmusk et al. 2014).

Conclusion

Co-inoculation of *Bacillus* sp. mutant strain Q10b, as producer of IAA and other PGP substances, and *B. japonicum* 526, or application of *B. japonicum* with addition of extracellular metabolites of Q10b, may be an efficient strategy for enhancing the productivity of soybean in drought conditions.

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EVALUATION OF RAPE SEED GENETIC DIVERSITY AND CREATION OF DROUGHT-RESISTANT GENOTYPES USING *IN VITRO* CELL CULTURE

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Abstract

Rape seed (*Brassica napus* L.) is one of the major industrial high-yielding oilseed and fodder crops. Water deficit in the limits plants genetic potential in all agro-climatic zones of growing. One of the main ways to increase rape seed productivity is to create new hybrids with the optimal response to environmental changes. The efficiency of breeding is largely determined by the genetic diversity of plant breeding genotypes, which is accepted to be evaluated by morphological features in the process of state variety testing and by using DNA markers. The purpose of this study was to evaluate the genetic polymorphism of spring and winter rape seed varieties using morphological and DNA markers, followed by their use for obtaining drought-resistant lines with the aid of *in vitro* cell selection. The study involved varieties of winter and spring rape seed. Resulted from cluster analysis were two clusters formed by morphological features. According to the varietal distribution by SSR markers (Ra3-H09, Na12-A02, FITO-063, Na10-B07), three clusters were obtained. No correlation between the matrices of genetic distances by morphological features and the DNA markers under study was found; however, the distribution obtained by genetic distances allowed to detect the difference between the studied rape seed varieties. As a result of the mutagenic action (γ-irradiation in a dose of 40 Gy) on micro calluses of rape seed varieties, which significantly differed by morphological and DNA markers, and subsequent gradual cell selection *in vitro*, about 4% of the drought-resistant rape seed clones were selected. Thus, the obtained distribution of varieties by morphological traits and SSR markers ensures a reliable basis for identification of varietal differences in selection for drought-resistant rape seed.

Keywords: *SSR markers; morphological traits; cluster analysis; cell selection*.

Introduction

Rapeseed (*Brassica napus* L.) is one of the leading oilseed and forage crops in Ukraine and in the world. The breeding programs dedicated to winter and spring rapeseed are aimed at the obtaining of high-yielding varieties and hybrids of various types based on the content and composition of the oil, with high plasticity to meteorological and agro-ecological factors (Sytnik and Kliachenko, 2010; Klyachenko, 2016; Clapp *et al*., 2018). The efficiency of breeding is largely determined by the heterogeneity of the plant parent material, which requires new approaches to increase the genetic heterogeneity of the breeding genotypes (Ana *et al*., 2009; Jamali *et al*., 2017). The description of marker morphological traits is known to be one of the effective methods for determining the differences between varieties in the process of their registration which used in Ukraine and in European countries (Ana *et al*., 2009). However, today one of the fastest and most effective methods for assessing the polymorphism of crops is the use of SSR markers. In our previous studies, an assessment of the genetic diversity of rapeseed varieties of different origin was made and the efficiency of the marker system for determining the variance of varieties was demonstrated (Klyachenko *et al*., 2018). Scientists have described the use of cell selections *in vitro* to obtain crop genotypes resistant to biotic and abiotic stress factors (Liu *et al*., 2003). Drought stress significantly limits plant growth and yield. However, in some crops possessing high adaptive capacity, such as rapeseed, morphological and metabolic changes occur in response to drought, contributing to adaptation to such environmental constraints (Rad and Abbasian, 2011). The action of ionizing (X-rays and γ-rays) and ultraviolet radiation induces mutagenesis with the widest spectrum of mutations and significantly increases the efficiency of cell selections (MacDonald *et al.*, 1991). The purpose of our study was to evaluate the genetic polymorphism of spring and winter rapeseed varieties using morphological and DNA markers, which will be used to obtain drought-resistant lines with the aid of cell selection *in vitro*.

Material and Methods

In the study, we used rapeseed varieties of Ukrainian and foreign origin: five winter varieties (Senator Liuks, Aliot, NK Technik, NK Petrol and Nelson) and two spring ones (Kliff and Geros). The varieties differed in terms of ripeness: mid-early and early-ripening, as well as different levels of drought resistance (Klyachenko *et al.*, 2018). The research was carried out during the 2016−2018 period on the basis of the Biotechnology Laboratory of the National University of Life and Environmental Sciences of Ukraine and the Laboratory of Molecular Genetic Analysis of the Ukrainian Institute for Plant Variety Examination.

Determination of the genetic diversity of rapeseed varieties

The description of the morphological traits of rapeseed varieties was made using 22 markers in the process of qualification examination of plant varieties for distinctness, uniformity, and stability (DUS). According to the DUS Test Guidelines, the degree of detection of the traits is indicated by numeric values from 1 to 9.

The molecular genetic polymorphism of rapeseed varieties was determined using four SSR markers: Ra3-H09, Na12-A02, FITO-063 and Na10-B07. In accordance with the obtained alleles, the number of polymorphic alleles and the level of polymorphism was determined (Klyachenko *et al.*, 2018).

The evaluation of genetic distances between rapeseed varieties was made by the cluster analysis using unweighted pair-group average method for SSR markers and single-link relationships based on morphological traits with a calculation of Euclidian distances (Fortin *et al.*, 2002; Everitt *et al.*, 2011). The correlation between the investigated SSR markers and the degree of manifestation of morphological traits was determined by genetic distances by the Mantel test using the computer program XLSTAT 2018 (Trial version) (Tommasini *et al.*, 2003; Legendre *et al.*, 2010; Diniz- Filho *et al.*, 2013; Klyachenko and Prysiazhniuk, 2018).

Obtaining of drought-resistant rapeseed lines in vitro

Rape seeds (100 seeds of each variety) were sterilized using 0.9% NaCl at the 15-min exposure and then three times rinsed with sterile distilled water. The callus tissue was obtained from the stems of aseptic seedlings through cultivating them in a thermostat at a regulated temperature from 25 to 26°C, relative humidity from 70 to 80%, without illumination, followed by transfer of the formed primary callus to the medium of the same composition every 21 days. To achieve the mutagenic effect of ionizing irradiation, callus tissues were treated with γ-rays at a dose of 40 Gy (Sydorov, 1990). A suspension culture for determining the sublethal concentration of selective agents was obtained according to Sidorov (1990).

The incremental *in vitro* cell selection of rapeseed for drought resistance was carried out according to the following scheme: seed germination in solutions with a selective agent → 3 passages in a selective environment → 3 passages without a selective factor → 3 passages in a selective environment → plant regeneration (Klyachenko, 2016).

As stress factors 15−20% mannitol and 5−25% high molecular weight PEG 6000 on the callusogenic medium MS (MS+0.5 mg/L BAP, 0.5 mg/L NOC, 0.05 mg/L of GA_3) were used (Sydorov, 1990). To study the influence of the concentration of selective agents in the selection process, the growth rate of the callus tissue was determined (Kucherenko *et al.*, 1991; Butenko, 1999). To obtain the regenerated plants, the callus was planted on an MS medium supplemented with 6-BAP at a concentration of 0.05 mg/L and cultivated in a light culture chamber at 25°C, illumination of 3000−4000 lux and a 16-hour photoperiod. Statistical data processing was carried out using the computer program STATISTICA 12.0 (Trial version).

Results and Discussion

Estimation of the genetic diversity of rapeseed varieties

To study the polymorphism of the studied rapeseed varieties, a cluster analysis was carried out with the purpose to evaluate the genetic distances between the objects. Showed in the Fig. 1 is a phylogenetic tree of the hierarchical rapeseed clustering by morphological markers.

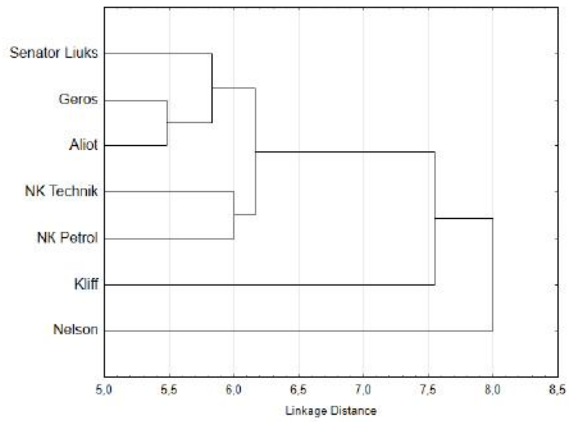

Fig. 1. Distribution of rapeseed varieties by the degree of proximity on the basis of morphological traits

It can be seen that two clusters are formed by Geros and Aliot, NK Technik and NK Petrol varieties, while other varieties are placed in adjacent clusters. The least values of genetic distances were found between varieties Geros and Aliot, NK Technik and NK Petrol (5.48 and 6.00, respectively). Noticeably, that the most distant varieties by morphological traits appeared Kliff and Nelson with the value of the genetic distance of 12.1. Thus, the obtained results reflect the degree of proximity in the manifestation of the specified characteristics and are not related to the type of development of a variety, unless indicated by the characteristics on which these varieties differ, namely the tendency of formation of inflorescences in the year of spring and late summer sowing (Official bulletin, 2010).

Analysis of rapeseed varieties by SSR markers yielded 36 detected alleles, with an average of 9 alleles per marker. The highest level of polymorphism was determined by the marker Na12-A02 (83%) while FITO-063 was the least polymorphous (40%). The markers Ra3-H09 and Na10-B07 have a polymorphism of 60 and 44%, respectively. These results are in the line with our previous studies of a large number of rapeseed varieties and are consistent with the results obtained by other researchers for these markers (Tommasini *et al.*, 2003; Hasan *et al.*, 2006; Li *et al.*, 2011; Klyachenko *et al.*, 2018).

The results of the cluster analysis of rapeseed varieties by the four SSR markers proved the presence of three genetically remote groups of genotypes (Fig. 2).

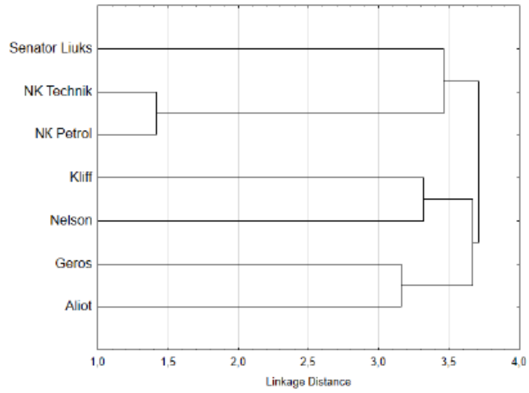

Fig. 2. Distribution of rapeseed varieties by the degree of affinity based on SSR markers

Varieties NK Technik and NK Petrol, Kliff and Nelson, Geros and Aliot have formed separate clusters. The most related by markers Ra3-H09, Na12-A02, FITO-063 and Na10-B07 appeared varieties NK Technik and NK Petrol (genetic distance of 1.41), and the most distant ones were Kliff and Nelson (4.00) included in one cluster. Senator Liuks variety did not fit into any cluster by both marker systems. Thus, for an objective assessment of the genetic diversity of rapeseed varieties as breeding genotypes for the production of drought-resistant lines, it is advisable to determine the correlation between marker systems.
The evaluation of the correlation between genetic distances obtained by SSR markers and morphological features was performed using the Mantel test (linear correlation by Pearson). As a result of the analysis, the calculated significance level p-value and the coefficient of correlation r (AB) for the theoretical significance level α=0.05 were obtained. According to the interpretation of the results of the comparison test, these indicators allow accepting one of the hypotheses about the presence (Ha) or absence of correlation (H0) (Fig. 3).

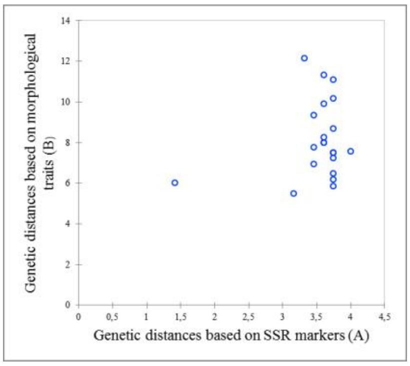

Fig. 3. Relationship between genetic distances of rapeseed varieties by morphological characteristics and SSR markers

The calculated p-value 0.345 was higher than the significance level α=0.05. Consequently, we have to accept the hypothesis H0 of the absence of a correlation which condition is $p > \alpha$ (Diniz-Filho *et al.*, 2013). Some scientists reported the absence and presence of correlation between genetic distances (Karuri *et al.*, 2010; Darvishzadeh, 2012). Tommasini *et al.* (2003) and investigated the relationship between genetic distances by morphological traits and using 46 SSR markers in ten winter and spring rapeseed varieties. According to the Mantel test, there was a lack of correlation between the studied markers and the morphological traits. It should be noted that, due to a sufficiently large number of SSR markers, the authors described only five morphological features. Ana *et al.* (2009) examined the polymorphism of rapeseed varieties with 9 RAPD markers and 10 quantitative morphological features. The authors obtained the results which indicated a lack of correlation between the studied DNA markers and morphological characteristics. The accuracy of determining the genetic proximity or distance based on the DNA markers may be affected by many factors, such as the number of markers used, their distribution by the genome, and the degree of accuracy of the analysis. In addition, it should be noted that molecular markers cannot be used in making conclusions about intergalactic interactions that result in the expression of individual genes (Ana *et al.*, 2009). Consequently, the distribution by genetic distances obtained by us made it possible to detect the difference between the studied rapeseed varieties using SSR markers and morphological traits. We found that the distribution of the studied varieties by clusters along with the absence of correlation between two marker systems does not have significant differences and proves the genetic proximity of the varieties included in one cluster by both morphological and SSR markers.

Rape selection in vitro for drought tolerance

To obtain drought-resistant lines on the basis of the studied varieties we used winter rapeseed varieties Aliot and Nelson. It is known that winter crops, under other equal conditions, are more productive due to the full use of autumn-winter moisture reserves. As a result of the gradual change in climate and the increase in the average annual temperature in the regions of rapeseed growing, insufficient rainfall is increasingly observed in winter which in turn affects the stock of moisture in the soil (Dyirenko, 2012; Clapp *at al.*, 2018). The lack of water in the soil inhibits growth processes in plants, changes the intensity and direction of the processes of photosynthesis, respiration, the course of carbohydrate, nitrogen and nucleic exchanges, the activity of enzymes, etc. (Bray, 2002; Rabbani *et al*., 2003).

According to the description provided by the breeders, the varieties under study possess medium to high capacity to withstand drought conditions and belong to different groups of ripening: Aliot is early ripening and Nelson is middle ripening (Official bulletin, 2010). It is known that the selection of varieties and lines attributed to different geographical groups and their involvement in crossbreeding will allow the use of all genetic breeding potential of rapeseed (Ashadullin and Ashadullin, 2007). It should be noted that varieties Aliot and Nelson are quite different both on the basis of morphological traits and for DNA markers. The values of genetic distances between them were 8.00 and 3.61, respectively.

According to the available literature, agar nutrient media supplemented by osmotically active substances that reduce the external water potential of cells are usually used to induce drought stress *in vitro* (Sydorov, 1990; Rai *et al.*, 2011; Masoabi *et al.*, 2018). To determine the concentrations of PEG 6000 and mannitol, which can be used as selective agents in the process of *in vitro* incremental selection, the growth of callus at different concentrations of osmotically active substances was determined (Table 1).

Table 1. Callus increment in winter rapeseed at different concentrations of PEG 6000 and mannitol

Variety	Control	Callus increment (%)					
		PEG 6000 (%)			Mannitol (%)		
		5	12	20	15	17	20
Aliot	92.2	90.3	55.3	5.7	61.8	48.6	4.0
Nelson	91.8	79.0	43.9	4.0	62.3	39.8	3.8
$LSD_{0.5}$	4.5	3.4	1.4	0.2	2.6	1.8	0.6

When studying the effects of different doses, it was found that action of 12% PEG 6000 and 17% mannitol caused significant differences between the studied varieties of winter rapeseed and about 50% decrease in the growth of callus tissue. The studies conducted by Masoabi *et al.* (2018) showed that 20% PEG 6000 was effective in *in vitro* selection of sugar cane. However, in the same studies, the ability of the callus tissue to grow at the 10% concentration of PEG 6000 was noted. In the works by Rahayu and Sudarsono (2015), 15% PEG 6000 concentration appeared sublethal during *in vitro* selection of peanut varieties. Similar studies were carried out by Galovic *et al.* (2005) for winter wheat establishing the efficiency of PEG 6000 at a concentration of 5% for drought-resistant lines. Research on the selection of drought-resistant rapeseed varieties was carried out by Ashraf and Mehmood (1990). However, genotypes were selected based on field studies and evaluation of the osmotic potential of leaves.

Analysis of the obtained data shows, that PEG 6000 (12%) and mannitol (17%) can be used as selective agents in the work with the studied winter rapeseed genotypes.

In our studies, in the subsequent cell selection scheme, PEG 6000 was used, since it impedes water stress without penetration into a cell and acts as an osmotic agent (Chandler and Thorpe, 1987; Priyanka *et al.*, 2011; Jan *et al.*, 2018). The microcalluses treated with γ-irradiation were planted in a medium with a sublethal concentration of PEG 6000. The sublethal concentrations of PEG 6000 were determined by seeding a suspension culture in nutrient media of various concentrations (10%, 20%, 30%, and 40%). As a result, the sublethal concentration of PEG 6000 (20%) was determined by a significant decrease in the growth of the mass of the callus tissue. This concentration was subsequently used for *in vitro* incremental cell selection. To do this, at the end of the first passage only light portions of the callus tissues were isolated and transferred to a freshly prepared medium with a selective agent. A count of the colonies that grew under selective and non-selective conditions was carried out. It was found that up to the seventh passage the number of the first selected colonies substantially decreased (Table 2).

Table 2. Dynamics of elimination of adaptive variants in winter rapeseed genotypes

Variety	The number of treated with γ-rays microcolonies	The number of live colonies in passages (% of the colonies treated with γ-rays)						
		1	2	3	4	5	6	7
Nelson	275	45.7±2.2	34.8±1.6	28.3±1.4	28.3±1.4	28.3±1.4	12.8±0.5	4.7±0.2
Aliot	310	54.8±2.6	32.1±1.5	20.4±1.0	20.4±1.0	20.4±1.0	15.4±0.7	4.8±0.2

Note: $p < 0.05$

The data presented in Table 2, show that the number of viable colonies in the first passage decreased to 35−50% as a result of treatment with γ-irradiation, with the elimination of colonies by passage being almost the same in quantitative terms for the studied rapeseed varieties. After three selective passages, the number of live colonies ranged between 15 and 25%. Beversdorf and Kott (1987) used γ-rays for an embryonic rapeseed culture. The authors determined that the survival rate of callus in the first passage after irradiation was 50%. To obtain forms tolerant to oxalic acid, Liu *et al.* (2003) also applied the mutagenesis of rapeseed with γ-rays. MacDonald *et al.* (1991) obtained rapeseed clones resistant to herbicides by applying ultraviolet rays to microcolonies. In this case, the proportion of surviving embryos was 4%.

In our studies, after carrying out two passages in a medium without a selective agent and checking the growth of the microcolonies under selective conditions, it was possible to isolate about 4% of the resistant rapeseed clones that stably manifested the sign of drought-resistance.

The calluses of all clones were similar in morphotype, forming a dense, globular structure of greenish coloration, and grew slowly. The resulting drought-tolerant rapeseed lines were used for plant regeneration by adding exogenous hormones to various nutrients in different concentrations.

Thus, in order to induce somatic embryogenesis in the callus tissue of winter rapeseed, the concentration of growth regulators in the medium was changed in accordance with the procedure and record of sprout formation was performed after 3−5 weeks of cultivation (Table 3).

Table 3. Morphogenesis and regeneration of shoots in the callus culture of winter rapeseed

Variety	Callus count	Morphogenetic calluses (%)	Regeneration (%)
Nelson	30	57	38
Aliot	30	59	40
$LSD_{0.5}$	-	2.8	1.8

According to the presented data, the percentage of regeneration of the studied rapeseed varieties was 38 and 40%. The obtained regenerated plants of drought-resistant lines of winter rapeseed after rooting and adaptation were transferred for use in breeding programs.

Conclusions

In summary, in this study, the polymorphism of winter and spring rapeseed varieties on the basis of morphological traits and using SSR markers was determined, and the rapeseed line tolerant to drought were obtained. Geros and Aliot varieties were found the most related on the basis of morphological traits with the genetic distance value of 5.48. The studied rapeseed varieties have differences by at least seven traits. The distribution of varieties by SSR markers is similar to the obtained distribution by morphological marker traits. Cluster analysis shows that varieties NK Technik and NK Petrol are the most related varieties with the genetic distance value of 1.41. The results of the Mantel test showed a lack of correlation between the marker systems under study on the ground of the genetic distances between the varieties. However, it should be noted that the application of an integrated assessment of the genetic variation of varieties is promising for use in the selection and examination of new varieties.

It was found that in the process of cell selection *in vitro* aimed at the production of drought-resistant rapeseed lines, it is expedient to use PEG 6000 at a concentration of 12%. The study proved the effectiveness of incremental selection *in vitro* with the use of γ-irradiation as a mutagenic agent. According to the proposed scheme, we obtained about 4% of drought-tolerant winter rapeseed clones that stably retained the trait.

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CULTIVATION PRACTICES EFFECT *CANNABIS SATIVA* YIELD

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Abstract
An industrial crop which can produce high yield of a quality fiber is *Cannabis sativa* subsp. *sativa*. For the purposes of the study, a field experiment was established at the Experimental Farm of the Technological Educational Institute of Thessaly in Greece (TEI; Larissa plain) in 2017, in order to determine height, dry biomass and fiber yield of *Cannabis sativa* subsp. *sativa* (cv. Fibranova). The effect of two different plant populations (P_1 = 160 plants/m^2 and P_2 = 80 plant/m^2), two irrigation levels (I_1 = 100 % ETo, I_2 = 60 % ETo) and two N-fertilization levels (F_1: 244, F_2: 184 kg ha^{-1}) were investigated. It was found that at harvest period the average plant height was 3.67 m, while the final plant height was significantly affected by irrigation input. Moreover, the average biomass dry yield and fiber yield may overcome 13t ha^{-1}and 4 t ha^{-1}, respectively. Dry biomass partitioning showed that 17% of total biomass was the dry yield of the leaves and the 83% the dry yield of the stems, while the 36-37% of the dry stem yield was the fiber dry yield. Therefore, it could be concluded that *Cannabis sativa* subsp. *sativa* (cv. Fibranova) is a very promising annual crop for fiber production in Greece and other areas of the Mediterranean region with similar environmental conditions, but further investigation is needed.

Keywords: *yield, plant density, fertigation, fiber.*

Introduction
Hemp (*Cannabis sativa* subsp. *sativa*) is an annual herbaceous crop considered to be one of the oldest crops known to man (Yang, 1991) and it is grown in many regions for its fiber production (Amaducci et al., 2015). Moreover, hemp is a remarkable plant containing many valuable natural components. It has been cultivated throughout the world for use as a food, fuel source, nutritional supplement, body care product, source of paper, building material, medicine, and in textiles (Small and Marcus, 2002; Bertoli et al., 2010; Mihoc et al., 2012).
Although industrial hemp can be used in a wide range of industrial applications, the growing popularity of synthetic fibers and the increase in labor costs are probably responsible for the continual drop in hemp cultivation worldwide since the 19th century (Allavena, 1962).
Furthermore, hemp production has been forbidden due to its Δ^9-tetrahydrocannabinol (THC) content, which is a phyto-chemical drug component.
Unlike medical cannabis abundant in the psychoactive ingredient Δ^9-THC, modern hemp has been selectively bred to produce low levels of Δ^9-THC and high levels of fiber, seed, and, more recently, cannabidiol (CBD). Most of the European Union and Canada have recognized the value of hemp and have defined a legal limit of 0.3% Δ^9-THC in the dry plant material (Small and Marcus, 2003), with the exception of Italy (0.2% Δ^9-THC) (Cappelletto et al., 2001). To date, 51 hemp cultivars have been approved for commercial use by the European Union (Directive, 2013).
Industrial hemp is a high-yielding crop (Struik et al., 2000) characterized of little technical inputs (Amaducci et al., 2015) and of a positive environmental impact (Bouloc and Werf, 2013). Its stem contains high-quality cellulose (De Meijer, 1994), the seed contains high-

quality oil (Callaway, 2004) and the inflorescence contains valuable resins (Bertoli et al., 2010).
Moreover, industrial hemp is an excellent break crop that can improve soil structure due to its extensive root system (Amaducci et al., 2008), reduce weed pressure and increase the yield of the sub-sequent crop (Bósca and Karus, 1997). Due to its numerous crop characteristics, hemp has great potential as an alternative rotation crop and could improve the agronomic and economic sustainability of farmers (Finnan and Styles, 2013).
Nowadays, environmental concerns and multi-purpose production have brought renewed interest in industrial hemp; however there is great lack of agronomical data to support hemp cultivation (Tang et al., 2016). It is really important to evaluate hemp phenology and crop adaptability in order to provide farmers with decision support information concerning cultivation practices.
The aim of this study is the assessment of the impact of plant density, irrigation and N fertilization on the yield of leaves, stems, and fiber, under the Mediterranean agro-climatic conditions of Thessaly plain, central Greece. Such data will be useful for the evaluation of hemp production and its future include in the cropping systems of Greece and the Mediterranean region in general.

Material and Methods

Experimental site

For the purpose of the study, a field experiment was established in a typical soil-climatic environment of Thessaly plain, in central Greece. The experimental site is located at the Experimental Farm of the Technological Educational Institute of Thessaly (TEI; Larissa plain, coordinates: latitude 39°62′69″ N, longitude 22°38′14″ E).

Field management and experimental design

A modern cereal seeding machine was used (on April 2017), applying 22.5 and 45 kg ha^{-1} of seed cv. Fibranova, in a row-distance of 25 and 12.5 cm, respectively.
A split-split-plot design 2 × 2 × 4 with four replications was used and the plot size was 14 m^2 (2 m width × 7 m length). Plant density was the main factor at two different populations [P_1 = 160 plants/m^2 and P_2 = 80 plant/m^2], irrigation the sub-factor at two treatments [I_1 = 100 % ETo, I_2 = 60 % ETo, because the precipitation during summer months in central Greece ranges from 90 to 200 mm, and the evapotranspiration is 500 mm, depending on the year and the microclimate of different sites], and N-fertilization the sub -sub-factor at 2 levels (F_1: 244, F_2: 184 kg ha^{-1}). Irrigation took place in 8 applications during the dry summer period (May–July) using a drip irrigation system. N-fertilization occurred at 4 applications through the irrigation system and the used type was 34,5-0-0. Weed management took place only during the establishment year (mechanically) while during the different plant growing stages, cultivation could compete the weeds. Complete weather data were recorded hourly in an automatic meteorological station which was installed next to the experimental field.

Laboratory measurements and data analyses

Soil analysis

Soil samples were analyzed using the following methods which are referred by Page et al. (1982).
Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.
Inorganic forms of nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) were extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy.

Exchangeable forms of potassium were extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK). Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylenetriaminepentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. In the case of the determination of total metals Mn, Cu and Zn, 1 g of wet material, was digested at 350 ^oC + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by (Allen et al., 1974 and Varian, 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture.

Plant analysis

Fresh and dry biomass partitioning was monitored by means of samplings. To avoid any border effect sampling took place in the inner plot, where 1 m^2 was harvested in each sampling on 10 cm above ground. Plant height was determined by the average of all harvested plants per plot. The samples were weighed fresh and then a subplot of 10 plants was taken for further laboratory measurements. The plants of each subplot divided into the various plant components: leaves and stems in the laboratory. Then stems were placed into specific water basins for a week as to be able to separate the fibers from the stem. Leaves, stems and fiber were dried until constant weight and weighed again to determine the final dry weights.
The dry leaves were chopped and grounded. Nitrogen content in leaves was measured using the standard Kjeldhal method (Nelson and Sommers, 1973), while the total phosphorus and potassium contents were determined with the of dry combustion method, using a spectrophotometer (P) and flame photometry (K) (Jones and Case, 1990).
Then, the dry weight of the leaves was multiplied by the respective N-P-K concentration to calculate the final nutrient removal (uptake) of the sampled biomass through the canopy.

Statistical Analysis

The statistical package GenStat (7th Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data in order to evaluate the main effects and interactions of all factors. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982).
Moreover, Tukey's procedures were used to detect and separate the mean treatment differences of soil samples at P = 0.05, where were four replications. Statistical analyses for the soil measure were performed by the statistical program MINITAB (Ryan et al., 2005).

Results and Discussion

Weather Conditions

The study area is characterized by a typical Mediterranean climate with cold humid winters and hot-dry summers. In particular, the average air temperature ranged to 22.9 ^oC while the noticed precipitation was 290 mm during the growing period of hemp in 2017 (Figure 1). The 33 % of the precipitation was noticed in May (113.4 mm) during the first growing stages and the 33% during July. In the same period the average temperature was 21 and 28 ^oC, respectively.

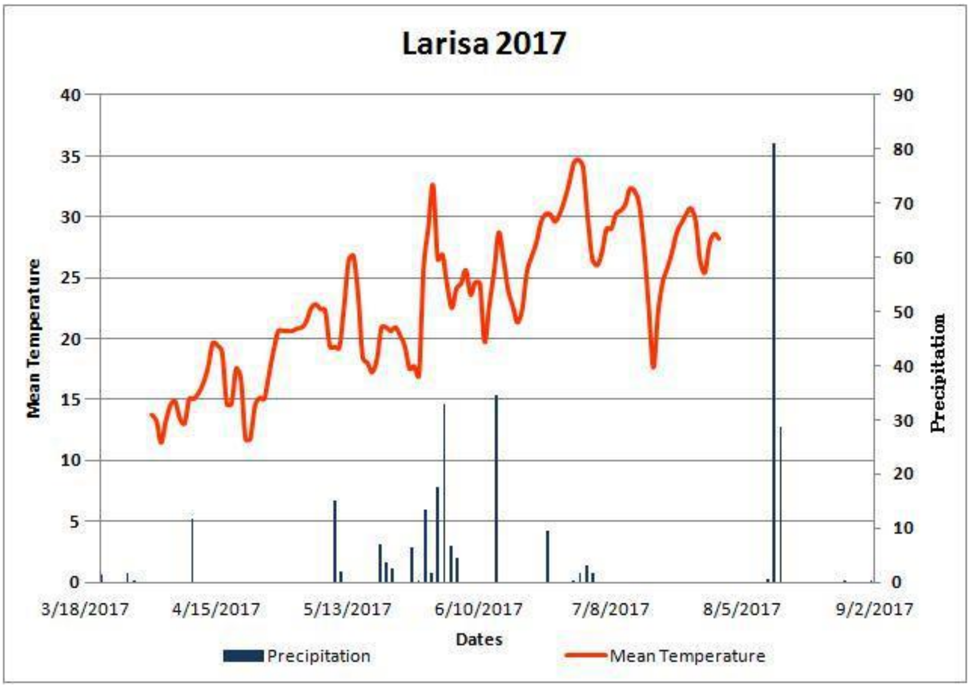

Figure 1. Mean air temperature and precipitation noticed to the study site in 2017.

Soil characteristics

The soil of the experimental site is characterized as a, calcareous (pH = 7.26), sandy clay loam (sand 46.5-48,5%, loam 17-19%, clay 34.5%), with soil organic matter content of 1.44% at a depth of 30 cm. An underground pumping irrigation network is used in this territory (east Thessaly plain), which indicates a deep groundwater table.
Moreover, the soil properties and the contained elements of the experimental field are shown in Table 1, 2.

Table 1.Soil properties/elements.

Properties / Elements	Soildepth
	0-30 cm
Texture	Sandy Clay Loam
pH, extract (1:5)	7.26 ± 0.05
EC, extract (1:5), dS m^{-1}	0.20 ± 0.01
*CEC (cmol kg^{-1})	19.3 ± 0.80
$CaCO_3$ (%)	2.42 ± 0.14
Organic matter(%)	1.44 ± 0.07
Cu- DTPA (mg kg^{-1})	0.93 ± 0.11
Zn- DTPA (mg kg^{-1})	0.70 ± 0.04
Fe- DTPA (mg kg^{-1})	0.37 ± 0.17
Mn- DTPA (mg kg^{-1})	4.92 ± 0.80
Cd- total (mg kg^{-1})	< 0.01
Pb- total (mg kg^{-1})	< 0.1

Data represent average means and SE deviation. n= (4).

Table 2. Soil elements at soil depth 0-30 cm.

Treatments	Cu	Zn	Mn	Na	P	K	Mg	Fe
	(mg/kg soil)					(g/kg soil)		
	25.6	514.3	1391	447	347.4	9.09	14.71	44.17

Plant height

At the harvest period the average plant height was 3.67 m. The final plant height was significantly affected by irrigation input (Table 2). This height was higher than the reported 2.4 m (Cosentino et al., 2013; Campiglia et al., 2017) and the reported height measured in Italy (Tang et al., 2016) and in agreement with the measured height in Czech Republic (Tang et al., 2016).

Yield and Fiber yield

Hemp management depends primarily on soil and climatic conditions of the region, which determine the genotype and variety choice (Tang et al., 2016).
Average dry biomass was higher for the irrigated treatment without significant differences (Table 3). The average harvested yield was 13.3 t ha^{-1}. The above dry yield is separated to 2.23 t ha^{-1} dry leaves and 11.07 t ha^{-1} dry stems. Table 3 shows a significant difference for the interactions of the three factors (plant density, irrigation and fertilization) for dry yield of leaves and stems. Furthermore, the produced stem yield is higher with reported stem yield in Italy and France (Tang et al., 2016) and in agreement with the results in Czech Republic and Latvia (Tang et al., 2016). According to the Table 3 the average dry fiber yield was 4.15 t ha^{-1}. A significant difference was noticed for the interactions of plant density, irrigation and fertilization.
Finally, as it has already been mentioned, the total average dry yield was 13.3 t ha^{-1} where the 17% is the dry yield of the leaves and the 83% the dry yield of the stems. Moreover, the 36-37% of the dry stem yield was the fiber dry yield.

Nutrient concentration in leaves

The data on N-P-K uptake from the leaves per hectare for all treatments are summarized in Table 3.
The average N-concentration in leaves is 3.26%, which is the triple than the N-content in switchgrass leaves [1.2-1.4%; (Giannoulis et al., 2017)] or six times the N-content in rice leaves [0.5%; (Abbeddou et al., 2011)]. Therefore, protein content of hemp leaves is 20.3%, while the average nitrogen removal from the leaves is 66.05 kg ha^{-1} and there was found a significant difference for the interactions of the three factors (plant density, irrigation and fertilization; Table 3).
The average P and K concentration in leaves is 0.35% and 1.89%, respectively. The above values lead to an average phosphorus and potassium removal up to 6.83 and 38.1 kg ha^{-1}, respectively. In case of P and K there was found a significant difference for the interactions of the three factors (plant density, irrigation and fertilization; Table 3).

Table 3. Hemp yield and leaves N-P-K content (%) as affected by 2 plant densities (P1, P2), 2 irrigation (I1, I2) and 2 N-fertilization levels (N1, N2).

	Height (m)	Yield (t/ha)	Dry Yield (t/ha)	Stems (t/ha)	Leaves (t/ha)	Fiber (t/ha)	N-uptake (kg/ha)	P-uptake (kg/ha)	K-uptake (kg/ha)
P1	3.636	46.65	13.31	11.08	2.232	4.045	72.8	7.56	42.3
P2	3.677	45.91	13.23	11.43	1.800	4.247	59.3	6.09	33.9
$LSD_{0.05}$	ns	ns	Ns	ns	ns	ns	ns	ns	ns
I1	3.748	48.67	13.97	11.89	2.077	4.173	67.8	7.03	39.5
I2	3.565	43.89	12.57	10.62	1.954	4.119	64.2	6.62	36.7
$LSD_{0.05}$	0.1035	4.587	Ns	1.209	ns	ns	ns	ns	ns
F1	3.659	46.06	13.24	11.33	1.905	4.129	58.9	6.46	41.1
F2	3.654	46.49	13.30	11.17	2.127	4.163	73.1	7.20	35.2
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	11.12	ns	ns
P1I1	3.660	48.55	13.85	11.50	2.349	4.155	76.6	7.99	44.6
P1I2	3.611	44.74	12.78	10.67	2.114	3.935	68.9	7.14	39.9
P2I1	3.836	48.78	14.09	12.28	1.805	4.191	59.0	6.08	34.4
P2I2	3.519	43.04	12.37	10.57	1.795	4.303	59.5	6.11	33.5
$LSD_{0.05}$	0.1436	ns	ns	1.622	0.4654	ns	ns	ns	7.02
P1F1	3.630	49.15	14.07	11.86	2.214	4.284	68.4	7.51	47.5
P1F2	3.642	44.14	12.56	10.31	2.250	3.806	77.1	7.62	37.0
P2F1	3.688	42.98	12.41	10.81	1.596	3.974	49.4	5.40	34.6
P2F2	3.667	48.84	14.04	12.04	2.004	4.520	69.1	6.78	33.3
$LSD_{0.05}$	ns	6.360	ns	1.502	0.4838	0.4212	16.63	1.676	9.10
I1F1	3.667	50.95	14.66	12.60	2.061	4.382	63.9	7.02	44.4
I1F2	3.829	46.38	13.28	11.18	2.093	3.964	71.7	7.05	34.6
I2F1	3.650	41.18	11.82	10.07	1.749	3.877	53.9	5.90	37.7
I2F2	3.480	46.61	13.32	11.16	2.160	4.361	74.5	7.35	35.7
$LSD_{0.05}$	ns	5.216	1.510	1.374	0.3580	ns	12.45	1.189	8.37
P1I1F1	3.518	53.82	15.41	13.01	2.407	4.790	74.5	8.20	51.4
P1I1F2	3.803	43.27	12.28	9.99	2.291	3.520	78.8	7.78	37.8
P1I2F1	3.742	44.47	12.73	10.71	2.020	3.779	62.4	6.82	43.6
P1I2F2	3.481	45.02	12.83	10.62	2.208	4.092	75.5	7.46	36.3
P2I1F1	3.817	48.07	13.90	12.19	1.715	3.973	53.4	5.84	37.4
P2I1F2	3.854	49.49	14.27	12.38	1.895	4.408	64.6	6.32	31.4
P2I2F1	3.558	37.88	10.91	9.44	1.477	3.975	45.4	4.97	31.7
P2I2F2	3.479	48.20	13.82	11.70	2.113	4.631	73.6	7.25	35.2
$LSD_{0.05}$	0.1829	7.649	2.188	1.912	0.5778	0.9536	20.05	1.987	12.22
CV (%)	3.4	9.4	9.4	10.2	19.9	11.9	21.9	19.6	26.4

Conclusions

Hemp dry yield may reach the 13.3 t ha^{-1} under Mediterranean environmental conditions where the 17% is the dry yield of the leaves and the 83% the dry yield of the stems. Moreover, the 36-37% of the dry stem yield was the fiber dry yield. The fiber yield can overcome the 4 t ha^{-1}, which is an acceptable yield.

The N-P-K content in leaves is variable among interactions of the three factors (plant density, irrigation and fertilization). The average N-concentration in leaves is 3.26%, while P-content is lower 0.35% and the K-content 1.89%.

Hemp removes from soil (from its leaves) 66.05, 6.83 and 38.1 kg ha^{-1} nitrogen, phosphorus and potassium, respectively.

As an overall conclusion, *Cannabis sativa* subsp. *sativa* (cv. Fibranova) is a very promising annual crop for fiber production in Greece and similar Mediterranean environments, characterized by high yields, and its introduction in land use systems should be seriously taken into consideration.

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TEMPERATURE EFFECT ON SEED GERMINATION RATESOFDIFFERENT WINTER LEGUMES AND SPRING CEREALS

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Abstract

The most crucial stage in the life cycle of cultivation is the seed germination as it significantly determines the evolution of the crop. Temperature is one of the most important factors affecting the vegetation. Therefore, a laboratory experiment was conducted to determine the vegetation rate of different varieties of three winter legumes (*Lupinus albus* L. vs Multitalia and Ultra, *Pisum arvense* vs. Arvica and Olympus, *Vicia faba* vs. Tanagra, Favino, Solon and Scuro di Torre Lama), 3 varieties of spring cereals (*Sorghum bicolor* vs EJ 7282, Skyscraper, Grain sorghum – Pacific Graze), and 2 varieties of a perennial cereal (Switchgrass – *Panicumvirgatum* vs. EC 1101 and EC 1102) at 9 different temperatures (24, 20, 16, 12, 10, 8, 6, 4, and 2^{o}C) in a constant parameter chamber (temperature). For each variety, 100 seeds were placed in 2,4 or 5 separate petri dishes depending on the size of the seed. Observations were taken each day for high temperatures and every second day for low temperatures. The hierarchy of the vegetation rate of the studied species was as follows: *Sorghum bicolor* EJ 7282 >*Sorghum skyscraper* >*Grain Sorghum*, *Panicum virgatum*: EC 1101 > EC 1102, *Lupinus albus*: Ultra > Multitalia, *Pisum arvense*: Arvica > Olympus and *Vicia faba*: Scuro di Torre Lama > Solon > Favino > Tanagra. The higher temperatures for the studied seed caused the germination in a shorter period, and as the temperature dropped, it took more days to reach the total vegetation rate. Finally, the speed of day-to-day germination between varieties and species was different.

Keywords: *germination rate, winter legumes, spring cereals, temperature.*

Introduction

Cereals are of enormous economic importance, while *Sorghum* and switchgrass occupy the 5thand 6th position, respectively, in the global production (FAOSTAT Database). *Sorghum* in Europe is mainly grown for its grain and as animal feed in Italy and France (FAOSTAT 2006). In some regions of Africa, it has been found that switchgrass can cover 90% of energy in nutrition. Switchgrass, as a feed, has similar properties to oats and barley (Giannoulis *et a*l., 2013).

On the other hand, legumes are in the second place, in terms of their importance. They are crops of low input requirements and are suitable to sustainable agriculture schemes. Legumes are grown for human and animal nutrition, and as green fertilization. Lupine contains the 2nd highest protein content (33-46%) in the seeds compared to the rest of the legumes, while beans (22-35%) and pea (16-32%) are following.

Successful crop establishment depends on seed quality, environmental factors and genotypes. Literature confirmed that there are differences in the response of the seeds and seedlings to different temperatures in association with their geographic origin.

The interest of Europe and the need to produce animal feed of high protein content and lower cost has increased and farmers are growing such crops over larger areas. Seedling establishment is a crucial stage in crop production and influences yield variations. Literature states that there is a major change in the past 20 years, where legume production has been to

adapt to earlier sowing dates in case to shift successful crop establishment and have plants of higher frost tolerance during winter (Bourion et al., 2003; Vocanson and Jeuffroy, 2008).
Ecological factors and seed characteristics are effective in crop growing in order to obtain a desirable yield and quality. Temperature is one of the most crucial factors that affect plant growth rate, development and the most critical stage in crop life cycle which is seed germination (Walck et al., 2011). Furthermore, germination rate is really important in crop life cycle and can differ according to species, soil structures, sowing methods, soil moisture ratios etc.
Different temperature ranges are needed for the different types of seeds to germinate. Maximum germination can be achieved in minimum time when the ideal temperature prevails. The effect of temperature can be modelled by thermal time and predict seed germination progress and to provide "a measure of physiological time" and yield coefficients (Bradford 2002). The purpose of this study was to quantify the germination temperatures of 8 varieties of winter legumes and 5 varieties of spring cereals.

Material &Methods

This research was carried out in the laboratory of Agronomy and Applied Crop physiology in the University of Thessaly, Greece. The study was performed in a growth chamber adjusted to 9 different temperatures (24, 20, 16, 12, 10, 8, 6, 4, 2) for 8 varieties of winter legumes (*Lupinus albus* L.–vs Multitalia and Ultra, *Pisum arvense*–vs. Arvica and Olympus, *Vicia faba*–vs Tanagra, Favino, Solon and Scuro di Torre Lama), 3 varieties of spring cereals (*Sorghum bicolor* vs EJ 7282, *Skyscraper*, *Grain sorghum – Pacific Graze*), and 2 varieties of a perennial cereal (Switchgrass – *Panicumvirgatum* vs. EC 1101 and EC 1102). Each treatment was repeated four timesfor each temperature value. For each temperature treatment, 100 seeds were placed indifferent separate petri dishes with sheets of Watman No. 1 filter paper. The trial was commenced as soon as the seed was exposed to the moist filter paper. The measurements were taken every day for the high temperatures and every second day for the low temperatures. When a 1mm of radical was visible (Gimeno-Gilles et al., 2009), the seed was thought to have germinated. The number of germinants was measured at frequent and regular intervals by the rate of germination. The maximum germination percentage for each temperature treatment was calculated as the average of the replicates.

Results and Discussion

As it is illustrated in Figure 1, comparing the two varieties of *Lupinus albus*, Ultra had higher growth rates than Multitalia. Regarding Ultra variety, in almost every tested temperature, germination was completed on the third or fourth day. As it was expected, germination rates were higher at the increased temperatures (24, 20, and 16^{o}C), while by decreasing the temperature, also the germination rate was reduced. On the other hand, Multitalia needed seven to eight days to reach the rates of Ultra variety (except the 24 ^{o}C. At low temperatures, germination could not exceed even the 50%.

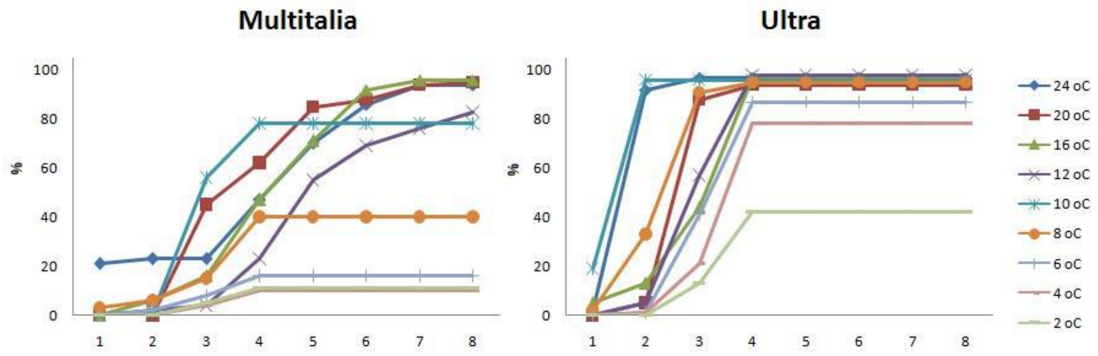

Figure 1. Seed germination percentage of *Lupinus albus* (Multitalia and Ultra) for the different tested temperature.

In case of the two tested varieties of pea (Arvica and Olympus; Figure 2), the germination rates were similar and the same number of days to reach the final germination were needed. At 24 ^{o}C, the germination rate reached 50% for both varieties from the first day.At lower temperatures, this rate was succeeded on the second or third day, while at the very low temperatures the number of days increased. Only in case of 2 ^{o}C only the 50% of the seeds germinated, classifyingthe crop more resistant to low temperatures compared to lupine.

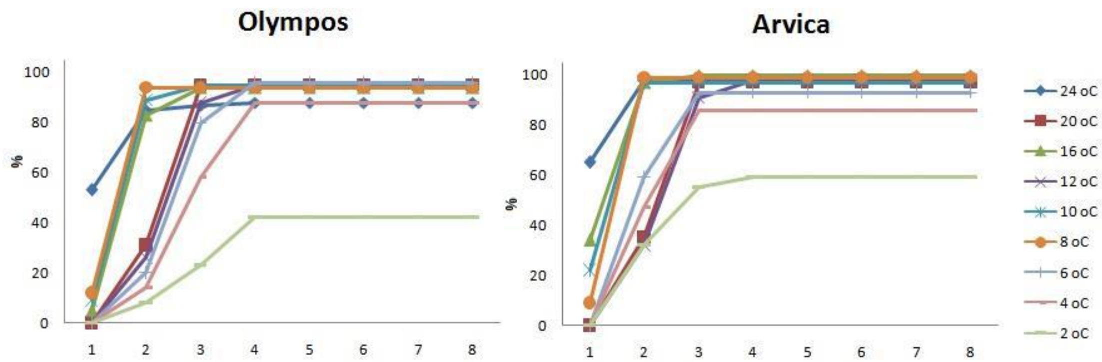

Figure 2. Seed germination percentage of *Pisum arvense* (Olympos and Arvica) for the different tested temperatures.

Figure 3 shows the germination of the four *Vicia faba* varieties. Scuro di Torre Lamahas has the greatest germination capacity, second comes Solon, third Favino and Tanagra is the last one. Scuro di Torre Lama was the only one that managed to reach almost the 100% at almost all temperatures. At the higher temperatures (24 and 20 ^{o}C) seeds germinated from the first day while as the temperature decreased during the first few days there were not germinated seeds.

Solon germination rates for the higher temperatures reached the 85%, while at lower temperatures it was less than 55%. In case of Favino, the percentage of germination was less than that recorded in the previous variety, with the only exception of 16 ° C where all seeds germinated. Finally, Tanagravariety was the variety with the worst germination rates.

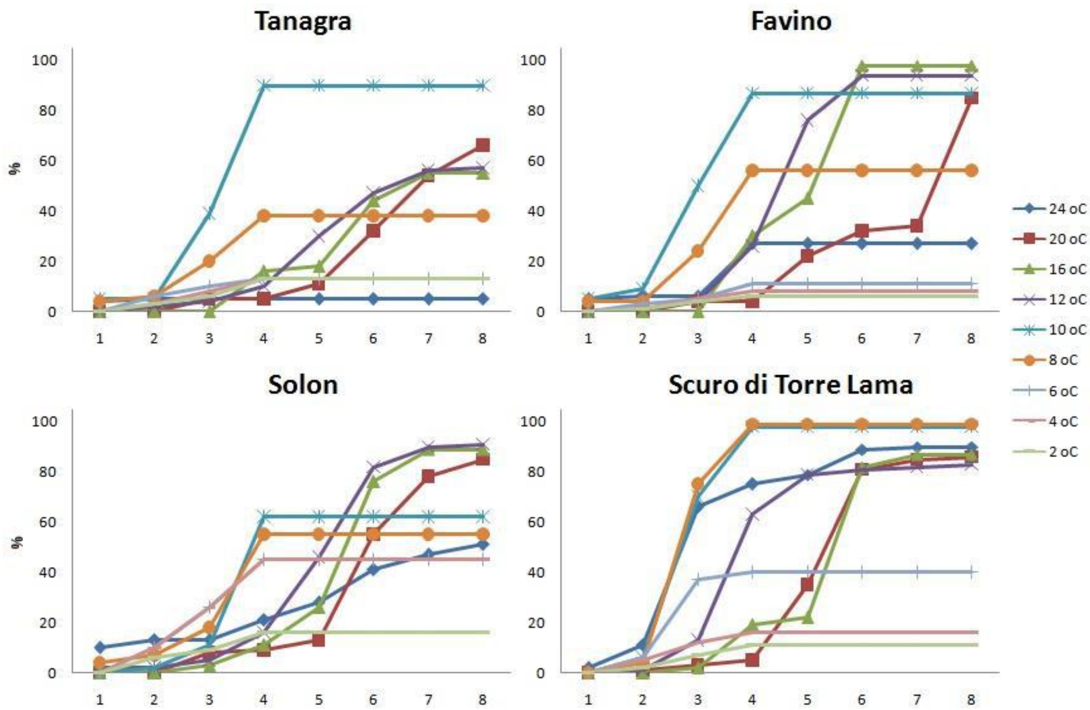

Figure 3. Seed germination percentage of *Vicia faba* (Tanagra, Favino, Solon and Scuro di Torre Lama) for the different tested temperature.

In case of the five studied varieties of spring cereals, three were *Sorghum* varieties (*Sorghum bicolor* EJ 7282, *Sorghum* skyscraper, Grain sorghum) and the other two were *Panicum virgatum* varieties (EC 1101 and EC 1102), which is a perennial crop.
In the case of *Sorghum*, Grain sorghum showed the lowest germination rate compared to the other two varieties which had similar rates. This varietyhad lower germinated seeds but the most important was the delay in reaching the final germination rate regardless of temperature. Sorghum bicolor EJ 7282 variety had the highest rate of vegetation than the other two varieties. At 24 °C and 20 °C, for both varieties (Sorghum bicolor EJ 7282, Sorghum skyscraper) took seven to eight days to reach the final rate.

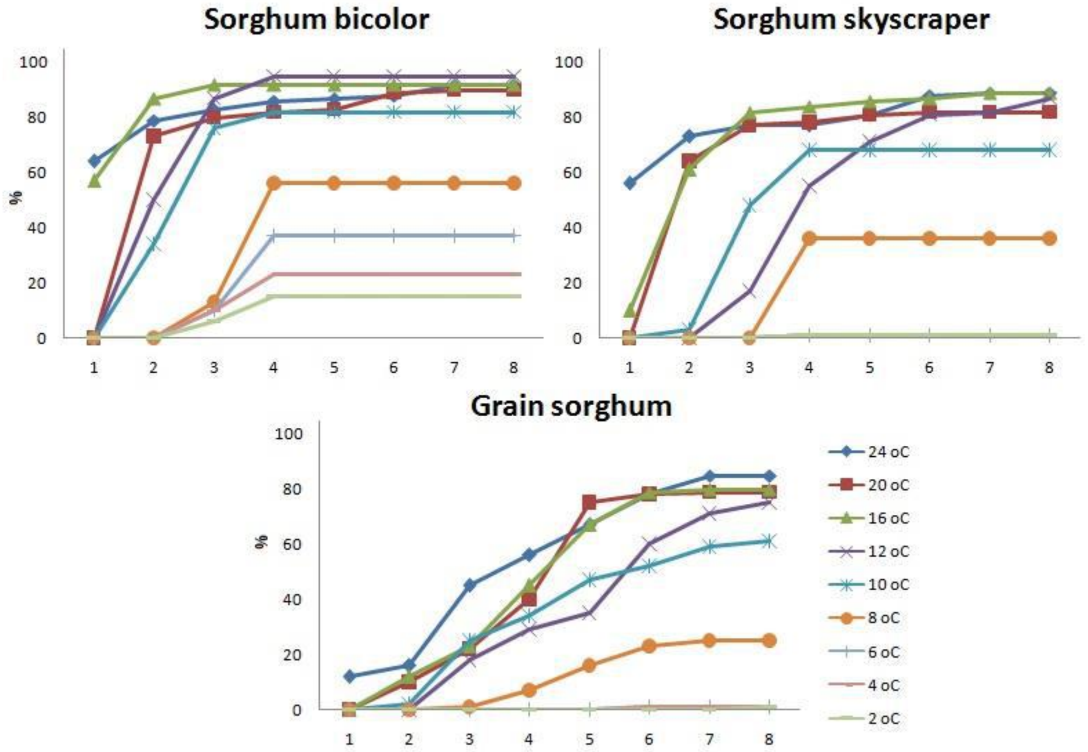

Figure 4.Seed germination percentage of *Sorghum bicolor* (*Sorghum bicolor* EJ 7282, *Sorghum skyscraper*, *Grain sorghum*) for the different tested temperature.

Switchgrass (*Panicum virgatum* L.) for both varieties shown that temperature below 10 ^{o}C is a limiting germination factor. At 12 ^{o}C the germination rate reached the 20% for both tested varieties. Temperatures higher than 16 ^{o}C seem to be the best for in case to reach up to 80-90% germination rates. Of course, between these two varieties a higher rate of vegetation from day to day had Switchgrass EC 1101 (Figure 5).

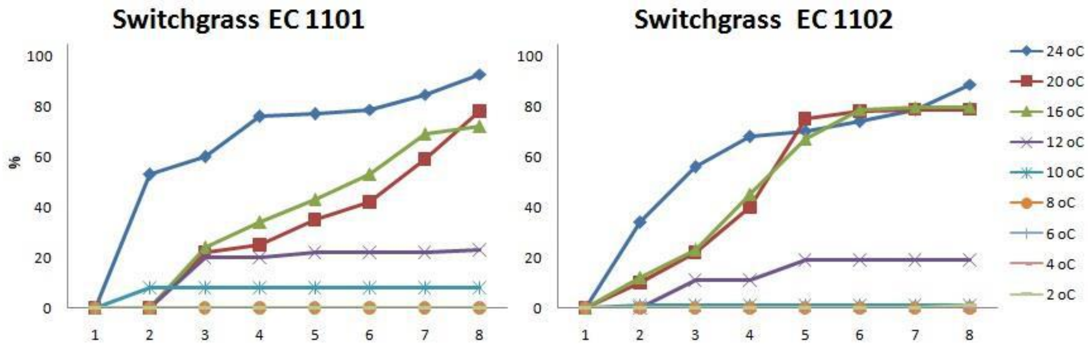

Figure 5. Seed germination percentage of switchgrass (EC 1101 and EC 1102) for the different tested temperature.

Conclusions

Almost in all varieties of the different species that were studied, it was observed that at high temperatures the vegetation rate was higher and as the temperature decreased the seeds needed more days to germinate. In each species there were variations in the rate of germination, some varieties displayed a faster rate than others.

Firstly, from the spring cereals, the germination capacity of sorghum varieties was Sorghum bicolor EJ 7282 > Sorghum skyscraper> Grain Sorghum while *Panicum virgatum* varieties were EC 1101> EC 1102.

In terms of winter legumes, the germination rate of lupine, pea and field beans varieties was as follows: Ultra > Multitalia, Arvica > Olympus and Scuro di Torre Lama > Solon > Favino > Tanagra. In the above mentioned varieties, the speed of day-to-day germination between varieties and species was different.
In few species the low temperature was restricted for their germination and should be taken in consideration.
As a general conclusion is that *Vicia faba* may satisfactorily germinate in rather cool micro-environments, and their sowing in the fall may be postponed for some weeks without substantial germination risk comparing to the rest legume species, while switchgrass is forbidden to be sowed if temperature is lower than 12 ^{o}C.

Acknowledgment

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RESPONSE OF SUNFLOWER HYBRIDS TO STAND DENSITY

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Abstract

Stand density plays an important role in sunflower productivity. It is one of the basic yield components, which depends on the characteristics of hybrids and agroecological conditions of the growing region. This study investigated the influence of stand density on seed yield of six sunflower (*Helianthus annuus* L.) hybrids, created in the Institute of field and vegetable crops, Novi Sad, Serbia. In each of two years, six sunflower hybrids (NS Kruna, NS Horizont, NS Ronin, NS Romeo, NS Dukat, Sumo 1 PR) were sown in six stand densities (from 30000 to 80000 plants/ha with the increasing step of 10000). The trial was arranged as a randomized complete block design (RCBD) with three replications. Analysis of variance (ANOVA) showed that the main effects year (Y), hybrid (H), stand density (SD) and year × hybrid interaction were highly significant for seed yield. Seed yield was predominantly influenced by the hybrid (48.52%). Year contributed to this trait with 22.77% and stand density with 5.03%. Only interaction Y × H for seed yield was significant, indicating that six hybrids responded differently to varying production years. Seed yield was significantly higher in 2018 (3.86 t/ha) in relation to 2017 (3.46 t/ha).The significantly highest seed yield was stated in NS Ronin (4.01 t/ha) and NS Kruna (3.91) on the basis of average for all densities. Regarding stand density, seed yield ranged from 3.50 (SD1) to 3.76 t/ha (SD4). In average for two years the highest seed yield was achieved at 60000 plants/ha (SD4). This study showed that stand density had a significant effect on seed yield in sunflower hybrids.The results may be helpful in recommending optimal sunflower stand density in this region.

Keywords: *Main effects, Interaction, Seed yield, Stand density, Sunflower.*

Introduction

One of the most widely cultivated oil crops in the world is a sunflower, with a harvested area of about 26.5 million hectares in which 48 million tons of seeds are produced on average (FAOSTAT, 2017). In Serbia, sunflower is the most important crop for the production of edible oil. In our country, the surface under sunflower varies depending on the year and range between 150000 and 230000 hectares.

Stand density is one of the significant production factor contributing to the final yield of crops including also sunflower. It is very important to determine the optimum stand density in order to get a high seed yield. Stand density and genotype have significant effects on seed yield and oil yield, as stated by Alam et al. (2003), Ali et al. (2012), Eco et al. (2017). The extreme weather conditions could increase the risk of sunflower production. As Szabó (2011) concluded in order to reduce the unfavourable weather effects as much as possible, the agrotechnical factors need to be optimized. Selection of hybrids, the proper sowing technology (sowing date, stand density), optimized and rational crop protection are of special importance (Szabó, 2012).

The purpose of this study was to examine the response of sunflower hybrids to different levels of stand density on the seed yield during two vegetation seasons.

Material and methods

Field experiments were conducted in the 2017 and 2018 seasons to determine the response of sunflower (*Helianthus annuus* L.) hybrids to different stand densities on seed yield at the experimental field of the Institute of field and vegetable crops, Novi Sad, Serbia. In each of the 2 seasons six sunflower hybrids (NS Kruna, NS Horizont, NS Ronin, NS Romeo, NS Dukat, Sumo 1PR) were sown in six stand densities: 30000 plants/ha (SD1), 40000 plants/ha (SD2),50000 (SD3), 60000 (SD4), 70000 (SD5), 80000 (SD6). The experiments were arranged in a randomized complete block design (RCBD) with three replications. Seed yield (t/ha) was measured after the harvest. Data were analyzed using three-way analysis of variance (ANOVA), in the STATISTICA 12.0 package computer program (*StatSoft*).Least significance difference (LSD) test at $P \leq 0.05$ was used to compare the differences among treatments means.

Results and discussion

Seed yield is a quantitative trait, which expression is the result of the genotype, environment and the interaction between the genotype and the environment. On the basis of ANOVA, it can be seen that all main effects (year, hybrid, stand density) for seed yield in sunflower hybrids were highly significant. Seed yield was predominantly influenced by the hybrid (48.52%). This is in agreement with the results communicated by Mrđa et al. (2012), that on average the seed yield of the studied hybrids was affected most by the genotype. Year contributed to this trait with 22.77% and stand density with 5.03% in our investigation. Popa et al. (2017) also reported high significance for the main effects for seed yield. The year showed the highest influence on seed yield, then the hybrid.

Table 1. ANOVA for seed yield in sunflower hybrids (2017 and 2018)

Source of variation	df	SS (%)	MS	*P*
Year (Y)	1	22.77	8.37	0.000**
(H)	5	48.52	3.57	0.000**
Stand density (SD)	5	5.03	0.37	0.013**
Y × H	5	8.45	0.62	0.000**
Y × SD	5	0.61	0.05	0.866
H × SD	25	7.31	0.11	0.644
Y × H × SD	25	7.31	0.11	0.644
Error	142		0.12	

$^{*}P < 0.05$; $^{**}P < 0.01$

Concerning interactions only Y × H for seed yield was significant, indicating that six hybrids responded differently to varying production years. The non-significant H × SD for seed yield showed that hybrids reacted similarly to stand density (Table 1). On the contrary Ali et al. (2012), Balalic et al. (2016) reported significant interaction between hybrid × stand density.

Table 2.Mean values (t/ha) and variability for seed yield in sunflower hybrids (2017, 2018)

Year (Y)	Hybrid (H)	Stand density (SD)							
		SD1	SD2	SD3	SD4	SD5	SD6	Mean (Y×H)	Mean (Y)
2017	NS Kruna	3.57	3.77	3.96	4.04	3.80	3.92	3.84	3.46
	NS Horizont	3.14	3.72	3.23	3.24	3.34	3.38	3.34	
	NS Ronin	3.60	3.84	3.70	3.97	3.51	3.64	3.71	
	NS Romeo	3.40	3.59	3.86	3.42	3.76	3.63	3.61	
	NS Dukat	2.65	2.83	2.67	3.04	2.90	2.98	3.85	
	Sumo 1 PR	3.34	3.48	3.44	3.58	3.49	3.18	3.42	
	Mean	3.28	3.54	3.48	3.55	3.47	3.45		
2018	NS Kruna	3.82	3.92	3.58	3.96	4.45	4.18	3.99	3.86
	NS Horizont	3.43	3.92	3.71	4.04	3.37	3.34	3.63	
	NS Ronin	4.22	4.46	4.32	4.42	4.17	3.23	4.30	
	NS Romeo	3.72	4.31	4.17	4.44	4.24	3.89	4.13	
	NS Dukat	3.51	3.61	3.72	3.64	3.56	3.47	3.58	
	Sumo 1 PR	3.59	3.55	3.54	3.33	3.77	3.19	3.50	
	Mean	3.71	3.96	3.84	3.97	3.93	3.72		
Mean (2 years)	NS Kruna	3.69	3.84	3.77	4.00	4.13	4.05	Mean (H)	3.91
	NS Horizont	3.28	3.82	3.47	3.64	3.35	3.36		3.49
	NS Ronin	3.91	4.15	4.01	4.20	3.84	3.93		4.01
	NS Romeo	3.56	3.95	4.02	3.93	4.00	3.76		3.87
	NS Dukat	3.08	3.22	3.19	3.34	3.23	3.22		3.22
	Sumo 1 PR	3.47	3.52	3.49	3.46	3.63	3.18		3.46
	Mean	3.50	3.75	3.66	3.76	3.70	3.59		

V (%) = 10.41

LSD	Y	H	SD	Y×H	Y×SD	H×SD	Y×H×SD
0.05	0.10	0.16	0.16	0.22	0.22	0.40	0.55
0.01	0.13	0.21	0.21	0.29	0.29	0.53	0.74

LSD	2017			2018		
	H	SD	H×SD	H	SD	H×SD
0.05	0.22	0.22	0.53	0.23	0.23	0.58
0.01	0.29	0.29	0.71	0.31	0.31	0.77

Seed yield was significantly higher in 2018 (3.86 t/ha) in relation to 2017 (3.46 t/ha). Comparing the mean values for six investigated hybrids in the two years experiment it can be seen that between them highly significant differences were stated. Seed yield varied from 3.22 t/ha (NS Dukat) to 4.01 t/ha (NS Ronin). Significantly highest seed yield was stated in NS Ronin and NS Kruna. In the second year of the experiment hybrid NS Ronin had also the highest mean value for seed yield (4.30 t/ha). Hybrid NS Horizont gave the lowest seed yield on average for two years (Table 2).

Regarding stand density, seed yield ranged from 3.50 (SD1) to 3.76 t/ha (SD4). In average for two years the highest seed yield was achieved at 60000 plants/ha (SD4). The results were similar when analyzing the results for each year separately (Table 2). At the optimum rate of plant density crop plants can acquire the optimum rate of light for the process of photosynthesis, at a high plant density the plant species will be more competitive to receive

light and their efficiency for growth and yield production may decrease (Soleymani, 2017). Optimal stand density according to seed yield was nearly 60.000 plants per hectare reported also Crnobarac et al. (2007). Crnobarac et al. (2006) reported that in the average for all investigated hybrids in 2005, seed yield increased to its highest density 60000 and 70000 plants/ha, which was significantly higher than in the case of lower density, while in 2004 only the lowest and highest density gave significantly lowest seed yield. Seed yield (3.6 t/ha) was highest at 75000 plants/ha in the experiment carried out by Ibrahim (2012) with five sunflower hybrids (Malabar, Romson 32, Horizon, Recordand, Galla) and four stand densities (45000, 60000, 75000 and 90000 plants/ha, respectively). Rasool et al. (2015) commented that plant density had a significant influence on seed yield. The maximum value of seed yield (2889 kg/ha) was observed in plots where plant density was 8.33 plants/m^2 and the minimum value of seed yield (2526 kg/ha) of 5.56 plants/m^2. Salehi and Bahrani (2000) reported that increasing plant population of sunflower increased oil and seed yield and reached the maximum at a plant population of 6.67 plants/m^2 with 25 cm plant spacing. Seed yield was increased up to a plant population of 85000 plants/ha while higher stand density led to decrease in production, stated some authors (Mojiri and Arzani, 2003, Ravichandran and Srinivasan, 2017). Lower values of optimum stand density (40000 plants/ha) were reported by Szabó and Pepó (2005). The differences in the results of optimum stand density depended on the material used in the experiments, so as on environment and the interaction between them.
The variability of seed yield was 10.41%. Similar variability (V=9.60%) was stated by Balalić et al. (2018), analyzing 10 hybrids, grown in 15 locations in Serbia. Balalić et al. (2019) have gotten higher variability for seed yield (V=20.21%) in the experiment carried out with 3 hybrids and 8 sowing dates, during two vegetation seasons.

Conclusions

The present investigation revealed that all main effects (year, hybrid, stand density) had significant effects on seed yield in sunflower. Seed yield was predominantly influenced by the hybrid (48.52%). Year contributed to this trait with 22.77% and stand density with 5.03%. Only interaction Y × H for seed yield was significant, indicating that six hybrids responded differently to varying production years. The analysis of variance revealed the significant differences among the hybrids for seed yield indicating the presence of sufficient variability among the genotypes for this trait. In average for two years the highest seed yield was achieved at the stand density of 60000 plants/ha (SD4). The results were similar when analyzing the results for each year separately. The results of this investigation showed the significance of stand density on seed yield in sunflower hybrids.

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INFLUENCE OF AGROTECHNICAL MEASURES ON YIELD AND QUALITY OF GREEN ONION

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Abstract

Green onion has high nutritional values reflected in the high content of vitamin C and other bioactive substances, primarily essential oil with high antioxidant properties. This essential oil has a high phytochemical effect and is considered as one of powerful plant antibiotics. Due to its specific chemical composition, spring onion takes a special place in the diet of people, most specifically in the early spring when offer of fresh vegetables is limited. The aim of this research was to determine how production method, ie. adequately applied general and special agrotechnical measures influence the yield and the quality of green onion. The influence of various soil mulching materials (control variant, agro-textile, straw, combination of straw and agro-textile) and fertilizers (control plot without fertilization, NPK (N_{120},P_{100},K_{120}),NPK(N_{120},P_{100},K_{120}) + 30g phytofert 20:20:20/100m²/day, NPK (N_{120}, P_{100}, K_{120}) + 40g phytofert 4:10:40/100m²/day) were tested.

The results indicated that the joint effects of mulch and fertilization were exerted through a positive effect on the yield of green onion. The use of agro-textile showed a statistically higher yield in plants fertilized with NPK + 40g phytofert (1,45 kg/m^2) compared to the control variant (1,06 kg/m^2). In addition, the use of straw contributed to statistically much higher yield in fertilized plants with NPK + 30g phytofert (1,53 kg/m^2) compared to the control variant (1,17 kg/m^2). Applied variants of mulch material had a positive influence on the content of vitamin C. The highest content of vitamin C was found in variants with straw mulching (13,41mg/100 g).The increased quantities of fertilizers did not positively affect the qualitative characteristics of green onion.

Keywords: green onion, production method, yield, quality.

Introduction

Onion is produced on around 3,7 million hectares in the world with an average yield of about 17 t / ha. The three largest producers of this vegetable are China (22,3 million tons), India (19,3 million tons) and the United States (3,2 million tons) with a total production of about 45 million tons, or about half of the world's total production. In the European Union, about 190692 ha (40 t / ha) are cultivated, the highest in the Netherlands is 32723 ha (44,29 t / ha), Spain 23174 (54,14 t / ha) and Germany 11294 (46,27 t / ha). http://www.faostat.fao.org

In Bosnia and Herzegovina production is usually carried out on smaller areas, where very low yields of 9.13 t / ha are realized. In the structure of production, onions represent about 13% and onion is ranked as the fourth product by the volume and significance of vegetable production in B&H. The realized production of the onions in 2016 amounted to 45 thousand tons and it is 19% higher than in the previous year (Statistics Agency of B&H, 2016). A large number of products based on the onion basis has found its application in ready to eat food products. In addition to being used in nutrition, it is part of many medicines and cosmetics (Kumar et al., 2010).

For the domestic market, continuous production of onion with sowing or planting from September to the first days of April. From the adequate application of general (plowing,

fertilization, inter-row cultivation, irrigation ...) and special agro-technical measures (mulching, covering of plants with agro-textile) depends largely yield of green onions.
The aim of this research was to determine how production method, ie. from adequately applied general and special agrotechnical measures influence the yield and quality of green onion.

Matherial and methods

The experiment was carried out in the first decade of March 2014. Two-factorial trial (mulching, fertilization) was arranged on a random block system in a greenhouse without heating. The influence of various soil mulching materials (control variant, agro-textile, straw, combination of straw and agro-textile) and fertilizers (control plot without fertilization, NPK (N120; P100; K120); NPK (N120; P100; K120) + 30g phytofert 20:20:20/100m²/day, NPK (N120; P100; K120) + 40g phytofert 4:10:40/100m²/day) was tested. A variety StuttgarterRiesen was used for trial.The size of the experiment plot was $1,5m^2$.
The basic properties of the soil on which the experiment was placed were 7.13 pH (in KCl); 2.81% CaCO3; 3.66% humus; 3.27 mg 100g-1 Al-P2O5 and 15.17 mg 100g-1 Al-K2O.
The content of vitamin C was determined by the titration method.
The obtained results were analyzed by the method of analyzing the variance (ANOVA) using SPSS 4.5 software. The significance of differences in individual environments was tested with LSD test.

Results and Discussion

Young green onions are harvest in the phase of 6-8 leaves and 40 cm high of the stem.According to Lazić et al., (2001), the yield of green onions depends on the harvest time and ranges from 0.5 to 15 kg/m^2, depending on whether the green onion or bulb is harvested. The average yield of green onions in our research was 1,93 kg / experiment plot, or 1,28 kg / m^2 (Table 1).

Table 1. The yield of the green onion on the experiment plot($kg/1,5m^2$)

Mulching	Fertilization				Average C
	F_1	F_2	F_3	F_4	
C_0	2,11	1,65	1,87	1,93	1,89
C_1	1,60	1,72	1,92	2,17	1,90
C_2	1,76	1,91	2,29	2,09	2,01
C_3	1,87	2,32	1,96	1,87	2,01
Average F	1,83	1,90	2,01	2,01	1,93
LSD	A	B	AxB		
0,05	0,171	0,171	0,342		
0,01	0,231	0,231	0,462		

F-fertilization (F_1- control, F_2- $N_{120}P_{100}K_{120}$, F_3- $N_{120}P_{100}K_{120}$+30gphytofert 20:20:20/ 100m² /day, F_4- $N_{120}P_{100}K_{120}$+40g phytofert 4:10:40/ 100m² /day); C-covering land (C_0-control, C_1- agrotextile,C_2-straw,C_3 straw+ agrotextile)

The results of the variance analysis showed that the investigated factors (mulching, fertilization) did not have a statistically significant effect on the average yield of the onion. However, if we look at the joint effects of the two factors (mulching, fertilization), it is noted that on the second variant of mulching the difference in the average yield of the fourth variant of fertilization b_4 ($2.17kg/1,5m^2$) was statistically significant compared to the first b_1 ($1.6/1,5m^2$).
Also, the interaction effect of the variant of straw mulching showed a statistically significant difference in the third variant of fertilization ($2.29kg/1,5m^2$) compared to the first ($1.76kg/1,5m^2$).

Similar results show Lazić at all., (1968). They conclude that the onion is well responding to the increase in the total quantity of fertilizers, in particular the higher the presence of potassium and nitrogen, if produced from the onion set.

Content of vitamin C

The daily requirement of vitamin C per person is 30 to 75 mg which means that the daily needs of the organism for this vitamin can be easily satisfied with the daily use of fresh vegetables in the diet (Nikolić,1980). Vitamin C (L-ascorbic acid) has a strong antioxidant and antibacterial effect, affects the functioning of the immune system and enhances resistance to infectious diseases.

It works on stress and has an anticancer role in the body (Ilin, 1999).

The nutritional values of the leaves of the onion are even greater than those contained in the bulb. About 8,5 g of vitamin C is contained in a kilogram of onion, and in green leaves about 38 g.

Within the mulching factor (Table2), the highest content of vitamin C was obtained on a variant with straw (13,41 mg/100g), and the smallest in the variant of combination straw+ agrotextile (11,45mg/100g).

If we observe the fertilization factor, we will see that the highest content of vitamin C was recorded on the control variant (14,92mg/100g), and the smallest in the variant with NPK + 40g phytofert(10,44 mg/100g). The results of Šifrina (1955) also show that fertilization of onion affects the decrease in the content of vitamin C, unlike Lazić (1968), which in its studies indicates the increased content of vitamin C in bulbs, using the combination of fertilizers $N_{120}P_{50}K_{160}$ and $N_{120}P_{25}K_{60}$.According to the same author (Lazić,1968) combination of $N_{120}P_{50}K_{160}$ fertilization increase the yield by 47% and the combination of $N_{120}P_{25}K_{60}$ fertilization increase yield by 21% compared to the control.

Table 2.Content of vitamin C in green onion (mg/100g)

Mulching	Fertilization				Average C
	F_1	F_2	F_3	F_4	
C_0	13,69	15,31	12,56	8,13	12,42
C_1	16,22	15,76	16,28	7,75	14,00
C_2	16,17	10,86	10,98	15,63	13,41
C_3	13,61	13,64	8,32	10,25	11,45
Average F	14,92	13,89	12,04	10,44	12,82

F-fertilization (F_1- control, F_2- $N_{120}P_{100}K_{120}$, F_3- $N_{120}P_{100}K_{120}$+30g phytofert 20:20:20/ 100m² /day, F_4- $N_{120}P_{100}K_{120}$+40g phytofert 4:10:40/ 100m² /day); C-covering land (C_0-control, C_1- agrotextile,C_2-straw,C_3 straw+ agrotextile)

Vitamin C is directly dependent on fertilization and mulching of the soil.On average, the highest content showed agrotextile variance (14,00 mg/100g), while the variantstraw+agrotextile had significantly lower content of vitamin C (11,45 mg/ 100g). The results of the research are in parallel with Govedarica-Lučić (2012) which shown that the content of vitamin C in the salads depend of mulching.

According to the same author, the greatest content of vitamin C was shown by the combination of mulching + agrotextile (26,77 mg/100 g), while the control variant had significantly lower content of vitamin C(21,10mg/100g).

Conclusion

Based on the results of the study about the impact of agrotechnical measures on yield and vitamin C content in the green onion, the following can be concluded:

•The achieved average yield during the survey was at a satisfactory level. The lowest yield was determined on the agro-textile in the control variant of fertilization (1,06 kg/m^2), and the highest yield was recorded in the variant straw+agrotextile of fertilization with NPK (1,54 kg/m^2).
• The joint impact of mulch and fertilizers is manifested through a positive impact on the yield of the onion. The use of agro-textile showed a statistically higher yield in variant with fertilizer NPK + 40 g phitofert (1,45 kg/m^2) compared to the control variant (1,06 kg/m^2).
•By using straw and fertilization statistically higher yield was obtained in variant NPK + 30 g phytofert (1,53 kg/m^2) compared to the control variant (1,17 kg/m^2).
•The content of vitamin C depended on the covering material. The highest content of vitamin C was obtained on agrotextilevariant (14,00 mg/100g), and the smallest on variant with straw + agrotextile (11,45 mg/100g).
• The increased quantities of fertilizers did not positively affect the vitamin C content.

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RESPONSE OF SOME WHEAT GENOTYPES TO DROUGHT AT GERMINATION AND EARLY SEEDLING GROWTH

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Abstract

The aim of this study was to determine the most tolerant winter wheat varieties against osmotic stress at germination stage and early seedlings growth. The osmotic stress was simulated in controlled environmental conditions by adding different concentrations of mannitol solution to the growing media of five winter wheat variety. In all studied varieties the benchmark water potential in which they had germinated and had a good seedlings growth was of -0.3 MPa. Under the stronger stress, -0.6 MPa, all varieties showed reduction in all examined parameters. The variety Bosanka had the highest final germination and germination energy under mannitol. The parameters defining the development or percentage of strong seeds, coleoptile and root length, fresh and dry weight of root and coleoptile of a seed were more affected by water deficit stress than germination and germination energy. Biplot analysis showed that wheat cultivars grown under -0.6 MPa osmotic had higher values of root/coleoptile ratio in relation to control and -0.3 MPa treatment which was the most reliable for screening properties of the genotypes for drought resistance in seedling stage.

Key words: *Winter wheat (Triticum aestivum L.), tolerance, mannitol, biplot analysis.*

Introduction

Drought stress is also a globally widespread and ever growing environmental phenomenon encountered by wheat crop which cause severe reduction in overall crop production (Nezhadahmadi *et al.*, 2013). Under drought stress condition decreasing pattern was experienced in morphologically yield contributing characters like plant height, grains per spike, spikes per plant, thousand grain weight, and reduction in number of fertile tillers per plant, which ultimately caused noticeably low grain productivity in wheat (Kilic and Yagbasanlar, 2010; Joudi, 2017). Generally drought and other stress factors cause the formation of reactive oxygen compound essentially in plants (Reactive Oxygen Species, ROS), which results in biomembranes and macromolecules damage (Sharma *et al.*, 2012). The availability of water and its movement in the seed are important for the process of germination, early growth and elongation of coleoptile and root. Wheat (*Triticum aestivum* L.) is regarded as one of the most important cereal crop, grown in different regions that can be characterized with increased salt level in the soil (Rana *et al.*, 2013). The selection of tolerant wheat genotypes against drought stress at seedling stage is important, especially if the tolerance at the germination stage correlated with tolerance to drought in grain filling period.
The aim of this study was to examine the seed viability of selected winter wheat cultivars in osmotic stress conditions and determine the most tolerant cultivars to osmotic stress at germination stage and early seedlings growth.

Materials and Methods

The experiment

The tests included examination parameters of germination in five different varieties of winter wheat (Jelena, Kristina, Orion, Bosanka and Nova Bosanka) under controlled conditions in the laboratory of the Faculty of Agriculture in East Sarajevo, Bosnia and Herzegovina. Three replicates of 50 uniform, healthy seeds were sterilized in 96 % alcohol for 30 seconds, washed with distilled water several times, and transferred into sterile Petri dishes. A double layered filter paper saturated with solution of a determined concentration mannitol (Table 1) was placed on a dish. Mannitol was dissolved with water potential close to zero (control), -0.3 and -0.6MPa. In each petri dish 15 ml of solution was dispensed. Control petri dishes were filled with distilled water. Alcohol mannitol was used to induce water stress. Petri dishes were incubated for 7 days at 25°C. During this experiment, the following parameters were determined: germination energy, final germination, Vigour Test or percentage of strong seeds, coleoptile and root length, fresh and dry weight of root and coleoptile, and root/coleoptile dry weight ratio. To determine the dry weight of the root and coleoptile seedlings were dried for 24 hours at 80 °C.

Table 1. Amounts of Mannitol in different levels of water deficit

Ψ_O the level of MPa	NaCl (g/l distilled water)
0	0
-0.3	4.20
-0.6	8.40

Data analysis

Data was processed using two-way analysis of variance, with software Infostat 10. Means were compared using Duncan's multiple range test. Principal component analysis (PCA) was used to determine interdependence between the traits.

Results and Discussion

Results from our study showed that increase in osmotic potential had an adverse effect on wheat final germination and germination energy (Table 2). Generally, germination energy was lowest in the presence of the lowest osmotic potential (-0.6MPa). Further, a significant difference among diverse wheat varieties under different treatments was observed. The variety Orion was the most sensitive variety under the lowest osmotic potential (-0.6MPa) and its germination potential compared to control was reduced from 97% to 8%. Results from our study showed a significant effect of mannitol concentration on wheat final germination. By increasing mannitol concentration (decreasing osmotic potential), germination percent decreased almost linearly in the studied wheat cultivars. For example, in the variety Orion with a higher water deficit (-0.6MPa) the percentage of germination decreased by 95% in mannitol stress compared to the control. The cultivar with highest germination percentage at zero potential, -0.3MPa and -0.6 MPa was Bosanka. Therefore, this cultivar could be grown under decreased osmotic conditions. According to Al – Taisan (2010) results like this could be attributed to absence of energy to start a germination process, as energy was obtained by increments in the respiratory pathway after the imbibition and in low levels of water potential tax water absorption was processed slowly.

Table 2. Germination (%), Germination energy (%) of five winter wheat varieties exposed to w ater deficits induced by different concentration of mannitol during germination

Treatment	Germination				Germination energy			
	Mannitol							
	0 MPa	-0.3 MPa	-0.6 MPa	average	0 MPa	-0.3 MPa	-0.6 MPa	average
Bosanka	92.33ab	92.33ab	86.67b	90.44A	90.00a	89.00a	86.33a	88.44A
Jelena	90.00ab	66.33e	70.67de	75.67B	90.00a	64.00def	69.33bcde	74.44B
Kristina	76.67cd	74.67cd	73.00cde	74.78B	75.67b	73.33bc	72.00bcd	73.67B
Nova Bosanka	78.67c	77.00cd	69.67de	75.11B	60.00f	65.67cdef	62.67ef	62.78C
Orion	94.67a	72.67cde	8.00f	58.44C	92.00a	72.67bcd	5.00g	56.56D
Average	86.67A	76.6B	61.6C	74.89	81.53A	72.93B	59.07C	71.17

Different letters indicate significant difference at P < 0.05 level.

Table 3. Vigor (%) of five winter wheat varieties exposed to water deficits induced by different concentration of mannitol during germination

Treatment	Vigor			
	Mannitol			
	0 MPa	-0.3 MPa	-0.6 MPa	average
Bosanka	60.33b	25.00f	0h	28.44C
Jelena	80.33a	28.00ef	0h	36.11A
Kristina	49.67c	43.67d	2.33h	31.89B
Nova Bosanka	25.67f	9.33g	0h	11.67E
Orion	47.67c	30.33e	0h	26.00D
average	52.73A	27.27B	0.47C	26.82

Different letters indicate significant difference at P < 0.05 level.

There was a significant difference in the parameter vigor classification (Table 3) and by decreasing osmotic potential vigor percent declined gradually from 0MPa to -0.3MPa. Generally, most wheat varieties were sensitive to the influence of osmotic potential of -0.6 MPa where their values drastically falls close to zero.

Table 4. Coleoptile length (mm) and root length (mm) of five winter wheat varieties exposed to water deficits induced by different concentration of Mannitol during germination

Treatment	Coleoptile length				Root lenght			
	Mannitol							
	0MPa	0.3MPa	0.6MPa	average	0MPa	0.3MPa	0.6MPa	average
Bosanka	69.4c	56.77de	5.00j	43.72C	67.37c	79.23a	30.83g	59.14A
Jelena	59.37d	35.67g	13.27i	36.1D	71.10bc	38.56f	30.30g	46.65C
Kristina	88.97a	49.90f	31.70g	56.86A	72.47b	47.40e	45.10e	54.99B
Nova Bosanka	80.18b	23.97h	9.00ij	37.72D	52.5d	39.80f	21.00h	37.77E
Orion	53.95def	49.17f	52.07ef	51.73B	78.1a	20.93h	31.67g	43.5D
average	70.37A	43.09B	22.21C	45.22	68.31A	45.19B	31.78C	48.43

Different letters indicate significant difference at P < 0.05 level.

Coleoptile and root length (Table 4) in our experiment interfered differently. At zero potential, both coleoptiles and root lengths reached their highest values. The Nova Bosanka variety showed highest coleoptile length at zero potential. On the other hand Nova Bosanka variety was the most sensitive variety under decreased osmotic potential since its coleoptiles length significantly decreased at -0.6 MPa. Similar results were reported by Qayym *et al.* (2911) who investigated the effect of water deficiency on germination in different wheat genotypes, and concluded that the length of the coleoptiles was significantly decreased at a water potential of 0.8 bar.

Table 5. Coleoptile fresh weight (g) and root fresh weight (g) of five winter wheat varieties exposed to water deficits induced by different concentration of mannitol during germination

	Coleoptile fresh weight				Root fresh weight			
Treatment	Mannitol							
	0MPa	0.3MPa	0.6MPa	average	0MPa	-0.3 MPa	-0.6 MPa	average
Bosanka	0.545^{c}	0.280^{g}	0.096^{i}	0.307^{D}	0.031^{g}	0.035^{f}	0.016^{j}	0.027^{D}
Jelena	0.683^{b}	0.372^{e}	0.175^{h}	0.410^{B}	0.054^{cd}	0.056^{cd}	0.055^{d}	0.055^{A}
Kristina	0.737^{a}	0.490^{d}	0.160^{h}	0.463^{A}	0.062^{b}	0.047^{e}	0.057^{c}	0.055^{A}
Nova Bosanka	0.689^{b}	0.324^{f}	0.093^{i}	0.369^{C}	0.053^{d}	0.027^{h}	0.019^{i}	0.033^{C}
Orion	0.553^{c}	0.371^{e}	0.296^{fg}	0.407^{B}	0.036^{f}	0.075^{a}	0.017^{ij}	0.043^{B}
average	0.641^{A}	0.367^{B}	0.164^{C}	0.391	0.048^{A}	0.047^{A}	0.033^{B}	0.043

Different letters indicate significant difference at P < 0.05 level.

Fresh weight of coleoptiles and roots (Table 5) were differently affected by water deficits. Alcohol mannitol significantly reduced coleoptile fresh weight across treatments. Moreover, the varieties Bosanka and Nova Bosanka were the most sensitive against the lowest osmotic potential (-0.6 MPa). There was no significant difference in root fresh weight in all wheat varieties between control and -0.3 MPa mannitol treatments. Our results are in agreement with those of Demir *et al*. (2008) and Braga *et al*. (1999) who had examined the effect of polyethylene glycol on the germination of seeds of pepper and beans respectively. Wang et al. (2009) reported that the fresh weight, root length and the above-ground part in the two varieties of alfalfa were significantly inhibited by 35% PEG solution.

Table 6.Coleoptile dry weight (g) and root dry weight (g) of five winter wheat varieties exposed to water deficits induced by different concentration of mannitol during germination

	Coleoptile dry weight				Root dry weight			
Treatment	Mannitol							
	0 MPa	-0.3 MPa	-0.6 MPa	average	0 MPa	-0.3 MPa	-0.6 MPa	average
Bosanka	0.066^{d}	0.040^{h}	0.008^{j}	0.038^{C}	0.012^{g}	0.016^{cd}	0.007^{h}	0.011^{C}
Jelena	0.077^{c}	0.060^{e}	0.033^{i}	0.057^{A}	0.018^{b}	0.014^{ef}	0.017^{c}	0.016^{B}
Kristina	0.083^{b}	0.042^{gh}	0.042^{gh}	0.056^{A}	0.014^{f}	0.024^{a}	0.017^{bc}	0.018^{A}
Nova Bosanka	0.090^{a}	0.052^{f}	0.011^{j}	0.051^{B}	0.015^{e}	0.012^{g}	0.006^{i}	0.011^{D}
Orion	0.073^{c}	0.045^{g}	0.030^{i}	0.049^{B}	0.012^{g}	0.011^{g}	0.008^{h}	0.010^{E}
average	0.078^{A}	0.048^{B}	0.025^{C}	0.050	0.014^{B}	0.015^{A}	0.011^{C}	0.013

Different letters indicate significant difference at P < 0.05 level.

The results showed that different mannitol concentration effected significantly the wheat coleoptile and root dry weight (Table 6). Coleoptile dry weight gradually decreased with water deficit increase, while the highest root dry weight was recorded under conditions of -0.3 MPa. The highest average result on shoot dry weight under the conditions of the highest mannitol concentration was observed in the variety Kristina. The variety Bosanka was the more sensitive to water stress and had the lowest shoot dry weight at -0.6 MPa. Root dry weight did not suffer any decrease until -0.6 MPa, and the variety Orion presented lowest result. According to Marur *et al.* (1994), the lack of water slows physiological and biochemical processes of soybean seeds and low water deficit affected poor growth and reduced the accumulation of dry matter.

Table 7. Root / coleoptile ratio of five winter wheat varieties exposed to water deficits induced by different concentration of mannitol during germination

Root /coleoptile ratio				
Treatment	Mannitol			
	0 MPa	-0.3 MPa	-0.6 MPa	average
Bosanka	0.179^{fg}	0.401^{d}	0.898^{a}	0.493^{A}
Jelena	0.231ef	0.236^{e}	0.513^{c}	0.327^{C}
Kristina	0.165^{g}	0.578^{b}	0.403^{d}	0.382^{B}
Nova Bosanka	0.170^{g}	0.230^{ef}	0.587^{b}	0.329^{C}
Orion	0.163^{g}	0.254^{e}	0.269^{e}	0.229^{D}
average	0.182^{C}	0.340^{B}	0.534^{A}	0.352

Different letters indicate significant difference at P < 0.05 level.

Root/coleoptile (RCR) ratio is one of the several ratios which give estimates of the distribution of dry matter between the different plant organs (Hunt 1990). It is a measure of distribution of dry matter between the root and the shoot systems and it is a good indicator for effects on root and shoot dry weights. Under mannitol treatment average of RCR raised from 0.182 (control) to 0.340 (-0.3 MPa) and to 0.534 (-0.6MPa). In relation to the other varieties, the variety Bosanka had the highest RCR under -0.6MPa. High ratio RCR in the germination of winter wheat is a good indicator of resistance to drought (Baalabaki *et al*., 1999).

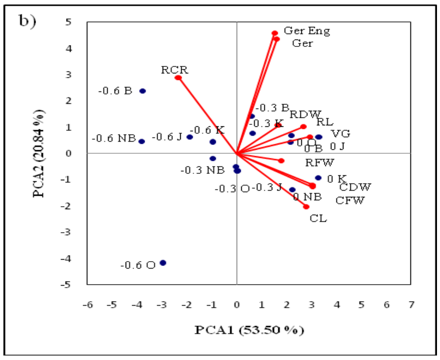

Figure 1. PCA analysis of trait association of winter wheat cultivars (Bosanka – B, Kristina – K, Jelena – J, Nova Bosanka – NB and Orion – O) grown under different levels (0 MPa – O, -0.3 MPa – -0.3 and -0.6 MPa – -0.6) of mannitol

The biplot of the principal component analysis (PCA) illustrates the relationships between the means of the studied traits in wheat cultivars grown under different levels of mannitol (Figure 1). The cultivar × treatment points and traits vector are placed on biplot according to their PCA scores. Biplot revealed similar interrelationship between the studied traits in wheat cultivars at different mannitol levels. Germination (Ger) and germination energy (GerEng) were highly and positive associated as indicated by acute angles. Moreover, root fresh (RFW) and dry weight (RDW) and coleoptile fresh weight (CFW) and dry weight (CDW) were highly associated under the influence of different mannitol levels. There was positive association between root and coleoptile weight (both dry and fresh). Root length was positively associated to GerEng, indicating that early germination improves root growth. An increase in root growth is an indicator of the ability of plants to withstand under water stress, It could be also used to screen plant cultivars for drought tolerance. Biplot showed that wheat cultivars grown under lowest osmotic potential (-0.6MPa) had higher values of root/coleoptiles ratio (RCR) compered to control at -0.3MPa treatment. This result indicates that coleoptile growth is more suppressed than root under condition of low osmotic potential.

Conclusion

Based on the results of this study it can be concluded that there are significant differences between the examined cultivars in their response to drought tolerance. Generally a gradual increase of mannitol treatments was followed by a reduction in all the analyzed parameters in selected cultivars. Highest decrease occurred at increased mannitol treatments (-0.6MPa) and it could be an indication of the tested varieties sensitivity to stressing conditions. In order to enhance plant tolerance to stressing environmental conditions, especially drought stress, studying the reaction of different cultivars in stressing environments is importance in improving the efficiency of breeding and selection. The limit value of water resources when a plant, under the influence of mannitol, can germinate quite well and have a good increase in seedling was at -0.3MPa, i.e. in low stress. Besides the standard techniques and commonly used data analysis, biplot offers additional options, preferably in the portion of a visual representation and understanding important interactions revealed in the present data sets from surveys of seed science research.

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SOWING QUALITIES AND YIELDING PROPERTIES OF SUDAN GRASS (*Sorghum sudanense*) DEPENDING ON FRACTIONATION OF SEEDS AND SEEDING RATE

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Abstract

Currently, fodder production is important in providing livestock feed and has a huge impact on agricultural production in the Russian Federation.
To create a sustainable fodder base for meeting the needs of animal husbandry, an important forage crop is Sudan grass (*Sorghum sudanense*) – which is an annual plant. It has a high drought resistance, good aftermath, it is versatile to it, produces a high yield of forage with good fodder merits. Green crop reaches 35-40 tons per hectare (Shitikova, A.V., 2019).
With proper cropping practices, it produces record yields among fodder annual grasses.
Data on the optimal sowing rate during cultivation for feed and seeds vary from 0.5 to 5 million units per hectare (Shapsovich, S.N., 2013). In this regard, it is necessary to determine the optimal seeding rate for the Central Black Earth region. In addition, the lack of scientifically based recommendations on the preparation of seeds for sowing indicates the relevance of research aimed at improving the elements of agricultural technology of this crop.
When conducting research on Sudan grass Voronezh 24, data were obtained on the dependence of the quantity of the green mass of Sudan grass, on the seeding rate and fractionation of seeds.
In the conditions of the Central Black Earth region, seeds of the Sudanese grass of Voronezh 24 variety must be separated on an aerodynamic separator. For sowing, seeds of large fractions are use. The highest yield of green mass can be obtained by using the seeding rate of 2.5 million pcs / ha.

Keywords: *Sudan grass, annual grasses, seed fractionation, feed production, feeding animals*

Introduction

In modern conditions, fodder production is of decisive importance not only in the provision of animal husbandry with feed, but it also has a huge impact on agricultural production in the Russian Federation as a whole.
In solving the problem of creating a sustainable fodder base for increasing the productivity of animal husbandry, Sudanese grass is the most promising crop. It has not only high drought resistance, but also good aftermath, versatility of use, high yield of forage with good fodder merits.
Sudan grass is a high-yielding agricultural crop, which with proper cropping practices of cultivation, produces a record harvest of forage annual grasses (Muslimov, M.G., 2013).
Sudan grass forms a well-developed root system, extending to a depth of more than 2 meters (Fundamentals of programming crop yields, 2014). The stem is a cylindrical made. Plants are up to three meters high. The lower stem node is the tillering node. Sudanese grass can form up to 25 shoots in a bush. The leaf is wide-linear, up to 60 cm long, smooth, slightly rough along the edge. The leaf of sorghum consists of a vagina and a leaf blade, the length of which sometimes reaches 1 m. The surface of the leaf can be smooth or corrugated. Inflorescence is panicle of various shapes, densities and colors, size 15-70 cm (Private selection of field crops, 2016). Inflorescence is a panicle, about 40 centimeters long. The fruit is a caryopsis, tightly enclosed in spikelet scales. 4-5 grams of seeds are obtained from each panicle (Plant growing, 2015). It is undemanding to soils. It grows poorly in wetlands.

Sudanese grass is cultivated for green fodder, grass meal, hay, silage and haylage. It is included in the rations of cattle, sheep and pigs. It suffers from trampling a little and quickly grows when it is etched. Therefore it is of great importance as a pasture plant, especially for late grazing (Solovjev B.F., 1960).
The feeding value of green mass and hay is higher than other cereal grasses due to its high content of protein, carbohydrates, carotene, sugars and fiber. There is also a large number of macro-and microelements. The beneficial properties of Sudanese grass are also due to the presence of vitamins. The green mass and hay of Sudanese grass contain a lot of sugar, and are well eaten by animals (Kokonov, S.I., 2013).
It is of particular value in the second half of the growing season, as a reliable source of green fodder, and a promising link in the raw materials conveyor.
The objective is to establish the influence of the seeding rate and seed fractionation of Sudanese grass seeds on sowing qualities and yield of green mass.

Material and Methods

The object of the study was variety of Sudanese grass Voronezh 24, zoned by the Central Black Earth Region. The variety was created at the department of breeding and seed production of Voronezh State Agrarian University.
The experiments were carried out on the fields of Voronezh State Agrarian University in 2016-2018. The option without separation of seeds with a seeding rate of 2.5 million units per hectare was taken as the standard. The seeds were divided into five fractions using an aerodynamic separator. The mass of 1000 grains and seed germination were determined by standard methods. Field experiments were carried on plots with an accounting area of 4 square meters in fourfold repetition. Sowing was carried out by the SSFC-1.6 breeding drill with a seeding rate of 2 million pieces per hectare and 2.5 million pieces per hectare. Row spacing was 15 centimeters. Green mass harvesting was performed manually, the yield was taken into account by weighing.
Seeds of Sudanese grass Voronezh 24 were calibrated using the SAD-4 machine for 5 fractions. Clods of soil and other heavy impurities fell into the first fraction, seeds with the highest specific gravity in the 2nd and 3rd, the seeds with the lowest specific gravity in the 4th, and lightweight impurities in the fifth.

Results and Discussion

Sowing qualities (germination energy, field and laboratory germination) were determined from the seeds of the isolated fractions (Table 1).

Table 1 - Sowing qualities of seeds of Sudan grass

Seed fractions	Option	Mass 1000, g	Energy germination,%	Laboratory germination,%	Field germination,%
Control St	St	13,6	75,0	85,7	78
II fraction	1	15,5	84,6	90,0	80
III fraction	2	14,1	82,5	92,2	83
IV fraction	3	11,9	71,3	80,1	68

In the experiment on the determination of germination energy and field germination, the 1st and 2nd variants exceeded the control by more than 9%, and in laboratory germination the best result was noted on variant 2 (laboratory germination is 92.2%).
In general, it can be concluded that the size of the seeds has an effect on germination, but the largest seeds do not have the highest germination rates, which may be due to the fact that

large seeds are more severely injured during harvesting (Kryukova T.I., Goleva G.G. and Borovkova A.N., 2015).
To study the yield properties of seeds of different fractions, field experiment was carried out.
The average seed embedment depth was 4-5 cm, whereas in the condition of late sowing and arid spring, seeds were embedded more deeply. Sowing was carried out at the optimum time when the soil warmed to the temperature required for sowing.
In the field experiment, phenological observations were performed during the entire growing season (Table 2).

Table 2 - The number of days of the onset of development phases in Sudan grass

Seed fractions	The number of days from sowing to the onset of the development phases			
	seedlings	tillering	heading stage	flowering phase
Control St	12	31	36	47
II fraction	10	28	34	43
III fraction	12	31	34	43
IV fraction	13	34	38	50

It was noted that the plants grown from the seeds of the second fraction entered the initial phases of development faster, and the plants grown from the seeds of the third fraction grew slowly at the beginning of the growing season, and entered the heading and flowering phases faster than in the control. This may be due to the fact that these seeds were formed in the middle part of the panicle and were characterized by the highest specific weight, and, consequently, the highest biological value. Smaller seeds entered the flowering phase of the control after 3 days. Caring for crops included pre-emergence harrowing, hand weeding, applying potassium humate and loosening the row spacing.
Harvesting Sudan grass for hay and green fodder starts a few days before the beginning of the cutting phase. Later, the stems grow coarser, the quality of the feed decreases, and the animals eat it worse (Bogatyrev, V. M., 1951).
Before mowing for the green mass, the plants were counted according to productivity elements (Table 3). For this, 30 plants were selected from each plot.

Table 3 - The structure of the yield of green mass of Sudanese grass at different seeding rates, 2016-2018

Seed fraction	Plant height, cm	Stem diameter, cm	Quantity, pcs.		Size of the largest sheet, cm	
			shoots	leaves	length	width
2.0 million pieces per hectare						
Control St	150,0	0,75	6,2	6,2	45,6	3,1
II fraction	161,9	0,90	6,5	6,7	47,6	2,7
III fraction	165,5	0,82	6,5	7,0	58,4	2,9
IV fraction	138,4	0,65	7,0	5,3	43,1	2,7
2.5 million pieces per hectare						
Control St	148,8	0,39	6,0	5,9	49,4	2,5
II fraction	169,2	0,50	5,9	6,1	54,6	2,3
III fraction	164,5	0,48	5,8	6,8	52,9	2,6
IV fraction	132,5	0,35	6,7	5,2	42,0	2,5

It was established that the highest height was observed in plants grown from the seeds of the third fraction of 165.5 cm with a seeding rate of 2.0 million units / ha^{-1}, and 164.5 cm with a seeding rate of 2.5 million units / ha^{-1}. The remaining biometric indicators did not have significant differences depending on the density of standing plants.

The quality of the feed of Sudanese grass largely depends on the diameter of the stem and its foliage. As a rule, the thicker the plant stem, is the rougher the obtained green fodder and hay is.

It was established that the stem diameter depended on both the seeding rate and the size of the seeds. Plants with a reduced seeding rate formed a thicker stem. A direct dependence of the stem diameter on the size of the seeds was observed: the larger the seeds were, the larger the stem diameter was in plants. The thinnest stems were in the control variant and in plants grown from seeds of 4th fraction, and in terms of the number, size of leaves and bushiness, plants grown from seeds of the third fraction were the best. The plants grown from the seeds of the fourth fraction and 2.5 million pieces per hectare, formed stems with a diameter of 0.35 cm. Such tender food is well eaten by animals.

The yield of green mass at a seeding rate of 2.5 million units / ha^{-1} was higher in all variants (Table 4).

Table 4 - Green mass yield of Sudanese grass, 2016-2018

Seed fraction	Option	Green mass yield, kg / ha		
		seeding rate		increase
		2.0 million pcs.	2, 5 million pcs.	
Control St	St	278	308	30
II fraction	1	295	329	34
III fraction	2	325	340	15
IV fraction	3	226	252	27

The highest level of yield of green mass was noted in the second variant, where in the total for two cuttings the yield was 325 centners per hectare with a seeding rate of 2.0 million pcs / ha, 340 with a seeding rate of 2.5 million pcs / ha, which is 47 and 32 centners per hectare higher than in the control, respectively.

Small seeds (option 3) showed low yield properties, where the yield of green mass was only 226 centners per hectare with a seeding rate of 2.0 million pcs / ha, and 252 centners per hectare with a seeding rate of 2.5 million pcs / ha.

The largest increase in yield, depending on the increase in the seeding rate, was noted in variant 1, and amounted to 34 centners per hectare. Such an increase in the yield of green mass may be related to the fact that the seeds of the second fraction have low germination rate, and an increase in the seeding rate has a significant impact on the yield of green mass.

Conclusion

1. The best indicators of sowing qualities of seeds: 82.5% - germination energy, 92.2% - laboratory germination, 83% - field germination were marked on the option 2 (average size is fractions 3).
2. According to the number of days from sowing until the onset of the development phases, in variants 1 and 2, where the seeds are larger, it was observed that the *period of seedlings-the beginning of flowering* was for 4 days shorter.
3. The dependence of the yield of green mass of Sudanese grass on the seed fraction and seeding rate was revealed. The highest yield of green mass was obtained with a standing density of 2.5 million pcs / ha on the variant with seeds of the third fraction (340 centners per hectare), which is 32 centners per hectare more than the control. According to the biometric

parameters of the plants, a significant difference was noted in the stem diameter (with an increased seeding rate in all variants, the stem diameter was 0.5 cm thinner on average).

Thus, according to the results of the research, it was established that in the conditions of the Central Black Earth region, seeds of Sudan grass of variety Voronezh 24 should be divided into an aerodynamic separator, and sown with the seeds of the second and third fraction. With a seeding rate of 2.5 million pcs / ha, 330-340 centners per hectare of green mass can be produced.

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IDENTIFICATION OF MOLECULAR MARKERS FOR FOREGROUND AND BACKGROUND SELECTION IN *Ga1-S* INCORPORATION INTO MAIZE LINES

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Abstract
Marker assisted selection (MAS) significantly increases efficiency of conventional breeding. Molecular markers are utilized as selection markers for target genes (foreground selection) and also for identification of the genotypes (progenies) with the highest proportion of recurrent parent's genome (background selection). Maize Research Institute "Zemun Polje" has a breeding program with the aim to create lines with incorporated incompatibility dominant gene *Gametophytic Factor 1-S (Ga1-S),* using the integrated conventional and molecular breeding approach. *Ga1-S* is the most described gene belonging to the group of genes specific to the pollen development, germination and pollen tube growth. The *Ga1-S* system is the most commonly used to prevent the pollination of sweetcorn, popcorn and white kerneld hybrids by standard maize. The objectives of this study were identification of gene-specific molecular marker for foreground selection, as well as the set of SSR markers polymorphic between parental lines to be used in background selection. Genetic variability between two donor and three recurrent parental inbred lines was analyzed with 42 SSRs distributed over the maize genom. Total number of alleles detected with 30 informative markers was 83, average being 2.77. The genetic similarity values calculated on Dice coefficient ranged from 0.47 to 0.71. Among 12 gene-specific markers tested on parental lines, two showed distinct polymorphism for *Ga1-S*. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* into our inbred lines which will be used for the creation of white kernel hybrids.

Keywords: *Gametophytic Factor 1-S, Maize, Molecular markers, Foreground selection, Background selection.*

Introduction
The inability of certain maize genotypes to successfully pollinate other genotypes is attributed to genes known as *gametophyte factors (ga)*. Gametophyte factors regulate the success of pollen-pistil interactions and affect the success of fertilization by causing the aberrant Mendellian genetic ratios in certain crosses (Nelson, 1994). These genes have two functions associated with them: a female function that produces a barrier to non-self-type pollen and a male function that enables self-type pollen to overcome that barrier (Lu *et al*., 2014). When present in pistil, they discriminate against or completely exclude pollen lacking the same allele (Nelson, 1994).
The *Ga1* was the first characterized gametophyte factor (Mangelsdorf and Jones, 1926) and has been intensively studied. It was mapped to the short arm of chromosome 4 in maize (Bloom and Holland, 2011; Lausser *et al.*, 2010; Liu *et al.*, 2014; Zhang *et al.*, 2012). *Ga1-s* is considered the "strong" variant of *Ga1*. In the *Ga1-s* barrier, pollen tubes do not grow straight and demonstrate heavy accumulation of clustered callose plug deposits (Lu *et al.*, 2014). The homozygous *Ga1-S/Ga1-S* genotype confers complete nonreciprocal cross-incompatibility (or unilateral cross-incompatibility) with *ga1* pollen (Kermicle and Evans, 2010; Zhang *et al.*, 2012).

Various strategies (e.g. physical barriers, temporal and spatial isolation) have been taken to reduce and avoid cross-fertilization among adjacent maize fields. Specialty types maize, such as sweet and waxy maize, popcorn, as well as the white kernel maize, are required to be free from foreign pollen due to the xenia effect. Seed production and non-GM maize fields require foreign- and GM-free pollen to maintain high hybrid purity and avoid contamination. The *Ga1-S* might be used as a biological reproductive barrier for the containment of gene flows between different types of maize (Liu *et al.*, 2014).
The incorporation of *Ga1-S* from popcorn to non-crossable maize elite lines has been performed using marker assisted selection (MAS) that greatly improve the efficiency and reliability of the backcrossing process by eliminating difficulty of phenotyping of the segregating population (Zhang *et al.*, 2012). Also, *Ga1-S* was introgressed by MAS into parental lines of an elite white waxy maize hybrid by six generations of backcrossing and one generation of selfing (Liu *et al.*, 2014). Maize Research Institute "Zemun Polje" (MRI) has a breeding program aimed at incorporation of the incompatibility gene *Ga1-S* into parental components of the hybrids with specific traits (white kernel). The main objectives of the research presented herein were to identify: 1) the SSR marker specific to the *Ga1-S* gene that will be used in foreground selection and 2) the set of SSR markers polymorphic between parental lines that will be used in background selection.

Material and Methods

Plant material

Two inbred lines (provided by the gene bank in Illinois, USA) were used as the donor parents (D_1 and D_2) of the favourable allele of the *Ga1-S* gene. Three MRI commercial inbred lines adapted to the local environmental conditions in Serbia were used as the recurrent parents (R_1, R_2 and R_3). These lines are components of the white kernel MRI hybrids.

DNA extraction

Total DNA was isolated from the kernel bulk according to Doyle and Doyle (1987). Bulks were prepared by pooling an equal amount of flour obtained by grounding 10 kernels per sample. The DNA was quantified using biospectrometer BioSpetrometer kinetic (Eppendorf, Germany) and diluted to a working concentration of 20 ng/µL.

Identification of molecular markers for foreground selection

Twelve gene-specific primers were chosen according to Liu *et al.*, (2014) and Zhang *et al.*, (2012). Polymerase chain reaction was carried out in 25 µL reaction volume containing: 1×DreamTaq Green Buffer (Thermo Scientific, USA), 100 µM dNTP (Thermo Scientific, USA), 0.5 µM of each primer, 1U DreamTaq DNA Polymerase (Thermo Scientific, USA) and 20 ng DNA template. Amplifications were performed in thermocycler Biometra TProfessional Standard 96 (Biometra, Germany) with the following program: an initial denaturation at 95ºC/5min, followed by 35 cycles each of denaturation at 95ºC/1min, annealing at 55ºC/30s and extension at 72ºC/1min with final elongation at 72°C for 10 min. The amplified fragments were separated by electrophoresis on 8% polyacrylamide gel in 1xTBE buffer. Gels were run on small format vertical gel system (Mini Protean Tetra-Cell, BioRad, USA), at 80 V for 1.5 h. After staining with ethidium bromide, they were visualized under UV transilluminator and documented in gel documentation system BioDocAnalyze (Biometra, Germany). Allele designations were made and approximate size range for the amplification products for each SSR locus was determined based on the positions of the bands comparing to the 100 bp molecular weight ladder (Thermo Scientific, USA).

Identification of molecular markers for background selection

Simple sequence repeat (SSR) analysis was done with 42 primer pairs spanning over the whole genome, selected from the maize database (www.maizegdb.org). Polymerase chain reaction (PCR) was carried out in 25 µL reaction volume containing: 1×DreamTaq Green Buffer (Thermo Scientific, USA), 200 µM dNTP (Thermo Scientific, USA), 0.5 µM of each primer, 1U DreamTaq DNA Polymerase (Thermo Scientific, USA) and 20 ng DNA template. The following touch-down program in the thermocycler Biometra TProfessional Standard 96 (Biometra, Germany) was performed: an initial denaturation at 95ºC/5min, followed by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5ºC/1min (-0.5ºC/cycle) and extension at 72ºC/1min; another 22 cycles of 95ºC/30 s, 56ºC/1min and 72ºC/1min with final elongation at 72°C for four min. The PCR products were separated by electrophoresis on 8% polyacrylamide gel, with 20 bp molecular weight ladder (Thermo Scientific, USA) as a marker. After staining with ethidium bromide, they were photographed under UV light using BioDocAnalyze gel documentation system (Biometra, Germany). SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. Genetic similarity (GS) was calculated in accordance with Dice (1945): GS_{ij} = 2a/2a+b+c; where: a is the number of fragments present in both variety *i* and *j* (1,1), b is the number of fragments present in *i* and absent in *j* (1,0), c is the number of fragments absent in *i* and present in *j* (0,1). Marker data analyses were performed using statistical NTSYSpc2 program package (Rohlf, 2000).

Results and Discussion

The main advantages of the marker assisted selection for simply inherited traits are direct selection of target gene with specific SSR markers (foreground selection) and fast recovery of recurrent parent's genome (background selection). Properties such as high polymorphism, co-dominant inheritance, random and frequent distribution throughout the genome, simple detection at any stage of plant development, independence of environmental conditions, high reproducibility, as well as cost-effectiveness, characterize SSR markers suitable for wide implementation into modern grain breeding programs (Semagn *et al*., 2006).

In our research, 12 gene-specific markers were tested on parental lines. Ten markers failed to give scorable bands due to absence of amplification product or poor amplification, while two markers showed distinct polymorphism for *Ga1-S* (Table1). The PR1 amplified ~410 bp fragment in donor parents (DPs) and ~390 bp fragment in recurrent parents (RPs). The PR2 amplified ~550 bp fragment in DPs and ~450 bp fragment in RPs. Amplification with these gene-specific SSR markers is given in Figure 1. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* into our inbred lines which will be used for the creation of white kernel hybrids.

Table 1. Primers identified for foreground selection for the *Ga-1* gene

Primer	Reference	Sequence
PR1 F	ID2 F in Liu *et al*., (2014)	5′-CAAATTGAGCCCATTACC-3′
PR1 R	ID2 R in Liu *et al*., (2014)	5′-TTCATTCTATTGCGGGTC-3′
PR2 F	SD9 F in Zhang *et al*., (2012)	5′-GAGAGCTACGCACGACTTAT-3′
PR2 R	SD9 R in Zhang *et al*., (2012)	5′-CAAGACTTGCACAATCGAGG-3′

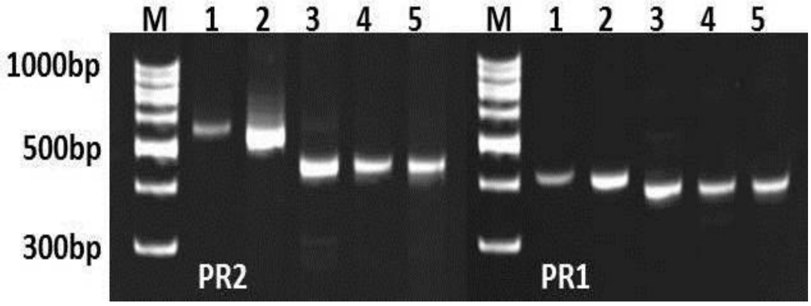

Figure 1. Parental polimorphism with the *Ga-1*-specific markers. M: 100 bp DNA ladder, 1-2: donor parents D_1 and D_2, 3-5: recurrent parents R_1, R_2 and R_3.

Genetic variability between two donor and three recurrent parental inbred lines was analyzed with 42 SSRs distributed over the maize genom. Total number of alleles detected with 30 informative markers was 83, average being 2.77, which is similar to those previously reported in maize inbreds (Bante and Prasanna, 2003; Legesse *et al.*, 2007; Kostadinovic *et al*., 2018). These SSR markers that showed polymorphism between parental lines will be employed for identification of the genotypes with the highest proportion of recurrent parent's genome in BC_2 generation. The genetic similarity values calculated on Dice coefficient ranged from 0.47 to 0.71 (Table 2). These results can be useful for identifying the most appropriate parental lines to be crossed, i.e. predicting the yields of crosses between lines (Ribaut and Hoisington, 1998).

Table 2. The pairwise genetic similarity values calculated on Dice coefficient between donor (D_1 and D_2) and recurrent parent lines (R_1, R_2 and R_3).

	D_1	**D_2**
R_1	0.52	0.47
R_2	0.56	0.49
R_3	0.71	0.56

As concluded by Zhang *et al*., (2012), the introduction of *Ga1-S* performed by backcrossing without markers is laborious, lengthy and inefficient because of the difficulty of phenotyping of the segregating population. Since 50% individuals of the backcrossing population do not contain *Ga1-S* allele, the tightly linked markers can greatly improve the efficiency and reliability of the backcrossing process by eliminating those without *Ga1-S* allele prior to pollination, reducing the size of the breeding population and saving both time and money. Also, background selection accelerates recovery of the recurrent parent's genome. According to Hospital *et al.* (1992), two generations can be saved by conducting marker assisted background selection.

Conclusions

Among 12 gene-specific SSR markers tested on parental lines, two markers showed distinct polymorphism for *Ga1-S*. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* gene. Also, the set of 30 SSR markers polymorphic between parental lines is chosen for the background selection. The identification of these markers represents the first step towards the incorporation of the incompatibility gene *Ga1-S* into parental components of the white kernel hybrids.

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EFFECT OF CULTIVAR ON THE YIELD AND ANTIOXIDANT ACTIVITY OF OKRA (*Abelmoschus esculentus* L. Moench) GROWN IN SLOVAK REPUBLIC

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Abstract

The okra (*Abelmoschus esculentus* L. Moench.) is less-known vegetable species in Slovak Republic. The goal of experiment was to evaluate the yield potential and antioxidant activity of okra grown in conditions of Slovak Republic. The field experiment was established at the Slovak University of Agriculture in Nitra in 2018. Within experiment, seven okra cultivars with different fruit color were tested (Cajun Delight F1, Clemson Spineless, Burgundy, Blondy, Baby Bubba, Jing Orange, Pure Luck F1). Okra seedlings (18 of each cultivar) were planting in spacing 0.60 x 0.40 m on 23th May 2018. The harvest of okra fruits was realized according to fruit production from 3rd July 2018 to 27th August 2018. The antioxidant activity was measured in fresh pulp and seeds of okra fruits by method of DPPH. The yield of okra fruits per plant were ranged from 293.2 (Burgundy) to 470.7 g (Clemson Spineless). The average weight of okra fruits was varied from 25.6 (Cajun Delight F1) to 36.1 g (Jing Orange). The okra yield per hectare, counted according to yield per plant, was ranged from 12.22 (Burgundy) to 19.61 $t.ha^{-1}$ (Clemson Spineless). The antioxidant activity of okra fruit pulp was ranged from 77.9 (Clemson Spineless) to 84.2 %DPPH (Baby Bubba). The antioxidant activity of okra seeds was only slightly varied, concretely from 98.3 (Cajun Delight F1) to 99.0 %DPPH (Pure Luck F1). Obtained results indicate that okra can be considered as a crop with good yield potential in the Slovak Republic. The important factor, influencing on the yield and quality of okra, is its cultivar.

Keywords: *Okra, Cultivar, Yield, Antioxidant activity*

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench., syn. *Hibiscus esculentus* L.) belongs to the *Malvaceae* family (George, 2011). The exact origin of okra is unknown, but it is thought to have come from Africa, where it has been grown as a crop for centuries. Some evidence suggests it was grown in Egypt as long ago as 2,000 BC (Chanchal *et al.*, 2018). Okra is known and marked by various names in the world (Singh et al., 2014), e. g. lady's fingers (English speaking countries), bhindi (India), krajiab kheaw (Thailand), ochro, okoro, quingumbo, kacang bendi (South East Asia), bamya (Middle East), quiabo (Angola), quimbombo (Cuba), gombo, gumbo (France), mbamia, mbinda (Sweden), okura (Japan) or qiu kui (Taiwan). According to the database of Faostat (2019), average world production of okra in period 2008-2017 was 8.682 mil. tonnes. The highest share of okra was produced in Asia (69.18%) and Afrika (30.06%); Europe production of okra was presented only by 0.1% share from total world production. The highest okra producers are India, Nigeria, Sudan, Pakistan or Ivory Coast.

Okra is an annual or perennial, dicotyledonous plant related to species such as cotton, cocoa and *Hibiscus* (Jain *et al.*, 2012). According to Singh *et al.* (2014), okra plays an important role in the human diet - it is a food source of protein, carbohydrates, vitamins, calcium, potassium and enzymes which are often lacking in the diet, mainly in developing countries. Its medicinal value was reported in curing of ulcers and relief from haemorrhoids. Okra has found medicinal application as a plasma replacement or blood volume expander and it is also useful in genito-urinary disorders, spermatorrhoea and chronic dysentery.

Okra is a multi-purpose crop due to its various use of fresh leaves, buds, flowers, pods, stems and seeds. Young, immature fruits can be used in salads or soups. Fruits offer a mucilaginous consistency after cooking (Gemede *et al.*, 2015). Okra mucilage has the potential for use as food, non-food products and medicine (Haruna *et al.*, 2016). It is used to modify the food quality in terms of food stability, texture, and appearance properties by acting as an emulsifier, thickener, gelling agent, or texture modifier (Noorlaila *et al.*, 2015). Okra mucilage also contributes to improved functionality, especially water-binding, emulsifying, and foaming properties of food products (Jideani and Bello, 2009). Okra seeds may be roasted and ground to form a caffeine-free substitute for coffee (Calisir *et al.*, 2005). Okra leaves can be harvested and used as a cooked green vegetable (George, 2011). The okra plant consists of stalk fibre and bast fibre and it can be an effective alternative for paper production (Jahan *et al.*, 2012).

The goal of experiment was to evaluate the yield potential and antioxidant activity of okra grown in conditions of Slovak Republic. This study was supported by the grant VEGA 1/0087/17.

Material and Methods

The field experiment was established at the Slovak University of Agriculture in Nitra in 2018. The climate of experimental area is characterized by warm and dry summer and slightly warm, dry or very dry winter. According to the climatic normal 1951 - 2000 for Nitra, annual mean temperature is 9.9 °C and mean rainfall total is 548 mm (Šlosár *et al.*, 2016). Within vegetation period (May - August), the average air temperature was 18.1 °C and the rainfall total was only 89 mm. Individual experimental month were evaluated as hot-very hot and dry-extremely dry.

Within experiment, seven okra cultivars with green (Baby Bubba; Cajun Delight F1, Clemson Spineless; Pure Luck F1), red (Burgundy; Jing Orange) and creamy (Blondy) fruit colour were tested. Okra seedlings were planting in spacing 0.60 x 0.40 m on 23th May 2018. The variant of each okra cultivar was divided to three plots with 6 plants, thus, 18 plants of all okra cultivars were planted. Within fertilization strategy, nitrogen was only applied because phosphorus and potassium content in soil before experiment establishment was evaluated as sufficient for okra production (Table 1).

Table 1 Agrochemical soil characteristics before experiment establishment in 2018

pH_{KCl}	Humus (%)	Nutrient content (mg.kg^{-1} of soil)					
		$N_{min.}$	P	K	Ca	Mg	S
7.04 N	32.9 G	6.4 L	140.5 H	368.0 H	6350.0 H	763.4 VH	26.3 M

Note: N_{min} - N mineral (N inorganic); N - neutral; G - good; L - low; M - medium; H - high; VH - very high.

The harvest of immature okra fruits was realised according to fruit production from 3rd July 2018 to 27th August 2018 (12 dates). The yield of okra fruits per hectare was (t.ha^{-1}) was calculated according to the yield of okra fruits per one plant. Within calculation, density of 41667 plants per hectare was used. The plant density was calculated according to the plant spacing (0.6 x 0.4 m) used in this experiment.

The antioxidant activity of okra was determined in fruits harvested on the 10th August 2018. After harvest, seeds were divided from okra fruits. The antioxidant activity was separately

measured in fresh pulp and seeds of okra fruits by method of DPPH (Hegedűsová *et al.*, 2018).
The statistical analysis of obtained results was performed by using of the Statgraphic Centurion XVII (StatPoint, USA). Results were evaluated by analysis of variance (ANOVA) and average values were tested by LSD test performed at the significance level of 95% ($p < 0.05$).

Results and Discussion

Yield per plant

Most of differences in the yield of fruit per plant among tested okra cultivars were evaluated as statistically significant (table 2). The yield of okra fruits per plant was ranged from 293.2 (Burgundy) to 470.7 g (Clemson Spineless). Similar values of okra yield per plant (320.0 - 433.3 g) were showed in the field experiment in Bangladesh (Saha *et al.*, 2016a). Saifullah and Rabbani (2009) realised the extensive study focused on the effect of genotype to the yield of okra fruit per plant in Bangladesh. Values of okra yield per plant in 121 genotypes were ranged from 92.16 to 641.42 g. Singla *et al.* (2018) also presented significant variability of okra yield per plant (299.1 - 496.7 g), dependent on cultivar, in the field experiment realised in India. Results of our experiment are in the range found by mentioned authors.

Table 2 Yield parameters and antioxidant activity of okra

Cultivar	Yield/plant (g ±SD)	Average weight (g ±SD)	Yield ($t.ha^{-1}$ ±SD)
Baby Bubba	396.2 ±8.8^{c}	29.6 ±0.7^{c}	16.51 ±0.37^{c}
Blondy	467.7 ±7.8^{d}	32.0 ±0.5^{d}	19.49 ±0.33^{d}
Burgundy	293.2 ±7.7^{a}	32.9 ±0.9^{de}	12.22 ±0.32^{a}
Cajun Delight F1	307.6 ±8.4^{b}	25.6 ±0.7^{a}	12.82 ±0.35^{b}
Clemson Spineless	470.7 ±9.2^{d}	33.4 ±0.7^{e}	19.61 ±0.38^{d}
Jing Orange	462.6 ±5.4^{d}	36.1 ±0.4^{f}	19.28 ±0.22^{d}
Pure Luck F1	398.3 ±8.2^{c}	27.3 ±0.6^{b}	16.60 ±0.34^{c}

Note: SD - standard deviation; Note: Values with different letters among rows are significantly different at $p < 0.05$ by LSD in ANOVA.

Average weight of fruits

The statistical analysis of obtained results showed statistically significant differences of average fruit weight among tested okra cultivars (table 2). The average weight of okra fruits was ranged from 25.6 (Cajun Delight F1) to 36.1 g (Jing Orange). Saha *et al.* (2016b) found weight of okra fruits in the range from 19.0 to 24.4 g in Bangladesh. Within another study in Bangladesh (Saifullah and Rabbani, 2009), the weight of fruits was also influenced by okra cultivar (15.3 - 26.2 g). Singh *et al.* (2018) examined the effect of cultivar on the okra fruit weigh in India. Authors found variability of its values from 11.68 to 14.09 g. In mentioned studies, values of fruit weight were lower in comparison with results found in our experiment. In US Virgin Islands, Nandwani (2012) found comparable weight of okra fruits in cultivar 'Clemson Spineless' (32.6 g) to our experiment.

Yield per hectare

The statistically significant differences of yield per hectare among tested okra cultivars were found. The yield per hectare was ranged from 12.22 (Burgundy) to 19.61 $t.ha^{-1}$ (Clemson Spineless). According to Chanchal *et al.* (2018), value of 10 $t.ha^{-1}$ can be considered as a good yield and yield over 40 $t.ha^{-1}$ can be reached only under optimal conditions. According to Faostat database (2019), average yield of okra in the world during 2008-2017 was 4.81 $t.ha^{-1}$. It is significantly lower value compared to results of our experiment with okra.

In Bangladesh experiment, Saifullah and Rabbani (2009) compared the yield of okra fruits in 121 cultivars and its values were ranged from 2.76 to 19.24 $t.ha^{-1}$. The yield of okra fruits, reached in our experiment, was varied in the range presented by authors. Similarly, the significant variability of okra yield, dependent on its cultivar, was also presented by Singh *et al.* (2018) in India (8.18 - 13.67 $t.ha^{-1}$). On the contrary, Saha *et al.* (2016a) found lower yield of okra fruits per hectare (9.28 - 12.56 $t.ha^{-1}$) in Bangladesh. Abubaker *et al.* (2012) tested the effect of plant density on the yield of okra fruits in Jordan. In 'Clemson Spineless' okra cultivar and comparable plant density (40000 per hectare), the significantly lower yield (2.57 $t.ha^{-1}$) was found compared to the same okra variety tested in our experiment.

Antioxidant activity (AOA)

The statistical analyses of AOA values in pulp or whole fruit were showed among tested okra cultivars. The AOA of okra pulp was ranged from 77.9 (Clemson Spineless) to 84.2 %DPPH (Baby Bubba). The average AOA value of whole fruit (pulp+seeds) was ranged from 88.0 (Cajun Delight F1) to 91.6 %DPPH (Baby Bubba). Differences of AOA in okra seeds among tested cultivars were evaluated as a statistically non-significant.

Ahiakpa *et al.* (2013) analysed AOA in whole fruits of 25 okra cultivars in Ghana and its values were ranged from 16.74 to 55.97 %. In okra cultivar 'Clemson Spineless', AOA of fruits was 45.27 %. All okra cultivars were showed by lower AOA compared to cultivars tested in our experiment. Petropoulos *et al.* (2018) determined the AOA of fruits in eight okra cultivars by method of DPPH and results were expressed in EC50 values (sample concentration providing 50% of AOA or 0.5 of absorbance in the reducing power assay). Authors found significant variability of AOA in dependency on the okra cultivar. The AOA was ranged in the interval 0.66 - 1.27 (small fruits) or 0.70 - 1.18 $mg.ml^{-1}$ (large fruits). Differences between the lowest and highest AOA were presented by 92.4 % or 68.6 %.

The antioxidant activity of okra seeds was only slightly varied, concretely from 98.3 (Cajun Delight F1) to 99.0 %DPPH (Pure Luck F1). Graham *et al.* (2017) found significantly lower AOA of seeds in six okra cultivars (36.9 - 60.4 %) in comparison with cultivars tested in our experiment.

Table 3 Antioxidant activity of okra

Cultivar	Antioxidant activity of okra (%DPPH ±SD)		
	pulp	seeds	fruit
Baby Bubba	84.2 ±0.7^{c}	98.9 ±0.4^{a}	91.6 ±0.4^{c}
Blondy	80.4 ±0.6^{b}	98.4 ±0.4^{a}	89.4 ±0.2^{b}
Burgundy	80.6 ±0.7^{b}	98.7 ±0.5^{a}	89.7 ±0.5^{b}
Cajun Delight F1	79.3 ±1.2^{ab}	98.3 ±0.4^{a}	88.0 ±0.7^{ab}
Clemson Spineless	77.9 ±1.2^{a}	98.9 ±0.5^{a}	88.4 ±0.7^{a}
Jing Orange	83.0 ±0.6^{c}	98.7 ±0.5^{a}	90.9 ±0.3^{c}
Pure Luck F1	83.2 ±0.7^{c}	99.0 ±0.4^{a}	91.1 ±0.6^{c}

Note: SD - standard deviation; Note: Values with different letters among rows are significantly different at $p < 0.05$ by LSD in ANOVA.

Conclusions

The okra is less-known vegetable species in Slovak Republic, or Middle Europe generally. The goal of experiment was to evaluate the yield potential and antioxidant activity of okra grown in conditions of Slovak Republic. Results of this study indicate that okra has a promising yield potential in Slovak Republic, despite of its tropic origin. The okra cultivar 'Clemson Spineless' was expressed by the highest yield of okra fruits per plant or hectare. Generally, all tested okra cultivars were showed by yield quantity comparable to studies realized in countries where its cultivation has a long-term tradition. The okra fruits and seeds were also showed by high antioxidant activity which was significantly influenced by tested cultivar.

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RESULTS OF TESTING THE SOWING AGGREGATES FOR PLANTING THE NARROW-ROW CROPS WITH CONVENTIONAL AND REDUCED TILLAGE

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Abstract

The main goal of sowing the narrow-row crops is to make the seed distribution even more uniform in the vegetation space. Specifies of sowing the narrow-row crops in interaction with the effects of sowing aggregate operations significantly influence the profitability of production, bearing in mind the fact that failures in the sowing process cannot later be corrected. The paper presents the results of testing the quality of work of various sowing aggregates on two tillage systems during sowing of narrow-row crops. The tests were carried out in the vicinity of Knjaževac, and the quality of sowing aggregates effects included the determination of the longitudinal, transverse and seed distribution along the depth of the seed bed in the sowing of winter wheat of the Sosthena variety on the reduced tillage of the Amazone D9 4000 Super sowing machine, and on the conventional tillage of the seedling machine OLT Gama 18. The obtained results showed that the planting aggregate Amazone D9 4000 Super performed better and achieved a more even seed arrangement. The highest content of grain in the transverse distribution of 67% was grouped at a group distance of 120.1 - 140 mm, while the longitudinal seed content of over 64% was in the group distance of 40.1-60 mm. The second tested aggregate obtained the uneven distribution of seeds, bearing in mind that in the group distribution 120.1 - 140 mm there were 45% of the grains, while in longitudinal 55% of the grains were within the group distance of 40.1 - 60 mm. Distribution of seed at depth with both sowing aggregates was moderately satisfactory.

Keywords: *Sowing aggregate, Quality, Seed, Distribution, Wheat.*

Introduction

Wheat production can be carried out by using conventional and conservational tillage, as a substitute for classical tillage due to numerous advantages, especially in sowing the winter crops (Kovačević *et al.*, 2005; Rusu *et al.*, 2011; 2013). The basic goal of wheat sowing is the balanced seeding to optimal depth and even distribution in vegetation, which is not satisfactorily obtained with the existing solutions of universal wheat sowing machines and tillage systems (Šumanovac *et al.*, 2005). Successful sowing in precise agriculture is difficult to achieve without the use of appropriate sowing aggregates, and knowing their performances (Auernhammer, 2004; Wiesenhoff and Koller, 2004). The performance of aggregates for sowing is significantly influenced by crop remains (Doan *et al.*, 2005). Conventional tillage does not provide a good soil structure, so the quality of sowing is inadequate. Distribution of wheat seeds at the soil surface in different tillage systems and direct sowing was not satisfactory, while the distribution results were satisfactory in depth (Šumanovac *et al.*, 2006). At the planned depth by wheat sowing machine "Vederstad Rapid 400S Super XL", 75% of the plants were analysed, and 15% at depths bigger or smaller than 1 cm from the set depth, while the seeders "Lemken Solitaire 9" and "Amazone RP AD 403" set depth of seeding achieved in 85% of analyzed plants (Mehandžić *et al.*, 2006). Choosing the wrong tillage system and seeding aggregate adversely affects moisture conservation, yield and production costs (Boydas *et al*., 2007; Farkas *et al*., 2009; Sarauskis, 2009). The assessment of the quality of the work of the seeders is being reflected in the fulfillment of the requirements related to the achievement of the desired sowing norm and the uniform distance of seeds in a row (Marković *et al*., 2009). Seed drills are equipped with sowing devices for every row or central sowing device that distributes seed to all drill's depositors. In comparison with individual metering, the central metering apparatus has higher values of non-uniformity coefficient over seed depositors and higher values of variation coefficient (i.e. more expressed non-uniformity) of longitudinal and transverse seed distribution over the plot (Malinović *et al*., 2001; 2003; Barać *et al*., 2015). The research was carried out in order to determine the effects of labor and qualitative indicators of the work of various sowing aggregates in the reduced tillage and winter wheat sowing under the test conditions.

Material and Methods

In the conditions of production in the vicinity of Knjaževac 43°35'30.2"N 22°12'56.0"E during 2017/18 we examined sowing aggregates for reduced tillage and seeding the winter wheat of the Sosthena variety. The tests included an assessment of the quality of the work of two types of seeding aggregates: Amazone D9 4000 S with RoTeC-Control applicators (Type A) in the variant of reduced tillage and sowing and seeding aggregate OLT Gama 18 (Type B) in the variant of conventional tillage and sowing. Seedlings were distinguished by the construction of the seeding device, the installed seed applicators as well as by the additional equipment for the soil preparation for winter wheat seeding. The Type A seeder was equipped with a seeding device for single seeding, combined with multiple working devices groups of 4 m the working width. The first working organ is a rotary harrow with the pins, the next working is a steel-toothed roller, and behind the bladed barriers for seeds covering, and its end with pressing wheels. The Type B seeder was a standard seed drill with a central seeding device for seeding and standard discs. The seeders were aggregated with tractors of an appropriate power of 147 kW, or 46.5 kW. In both tillage varieties, the pre-culture was corn. In the conventional tillage variant, plowing was done with ram plows, then harrowing and pre-sowing preparation, after which seedlings with OLT Gama 18 were done. In the variant of reduction tillage, the plate was made at an angle of 10^o in relation to the direction of the pre-culture lines, and then the preparation of the soil and sowing was done with the Amazone D9 4000 S. Seed quantity was 220 kg ha $^{-1}$, the planned distance in the row was 5 cm, and sowing depth 3-5 cm, and inter-row distance 12 cm. The analysis of the effects of the seedlings was done after the wheat crop, and it was introducing and including the assessment of the longitudinal, transversal as well as the seed distribution by depth. The longitudinal seeds arrangement was determined when the plants had 2-3 leaves, by counting the plants in every second row at 3 m in length, while the transversal distribution of wheat seeds was determined by measuring the spacing between the rows on the working width of the seeders. The depth of sowing was determined by picking up the plants and measuring the etiolated part of the plants up to the transition to dark green color. Taking into account the slipping of the soil, and all the values were read in 5 repetitions. The obtained results were processed using the Microsoft Office Excel 2007 package.

Results and Discussion

Figure 1, 2 and 3 show the results of the longitudinal and transversal distribution as well as the one by the depth of winter wheat seeding of the Sosthena variety according to the treated variants of reduced and conventional tillage (Type A and Type B).

The obtained results show that the Amazone D9 4000 S sowing aggregate has a higher operating quality and has achieved more even distribution of the seeds compared to the second sowing aggregate. Analysing the longitudinal distribution of wheat seeds by group

spacing, it is observed that in the first variant (Type A) the highest average grain content of wheat grains of 64% was in the group spacing of 40.1 - 60 mm, which was considered very satisfactory, while in the other variant type B), in the group distance of 40.1 - 60 mm, the share of grains was significantly lower and amounted to an average of 55%.
It is important to note that in both examined variants there was no higher presence of wheat grains in the group distance of 0-10 mm, which was 2% - Type A and 3% - Type B, while in group distance 10.1 - 20 mm it was recorded 5% of winter wheat seed in both tested variants. In the group spacing 30.1 - 40 mm, wheat grain content ranged from 14% (Type A) up to 23% (Type B). In the other group intervals, there were no major deviations between the examined variants (Figure 1).

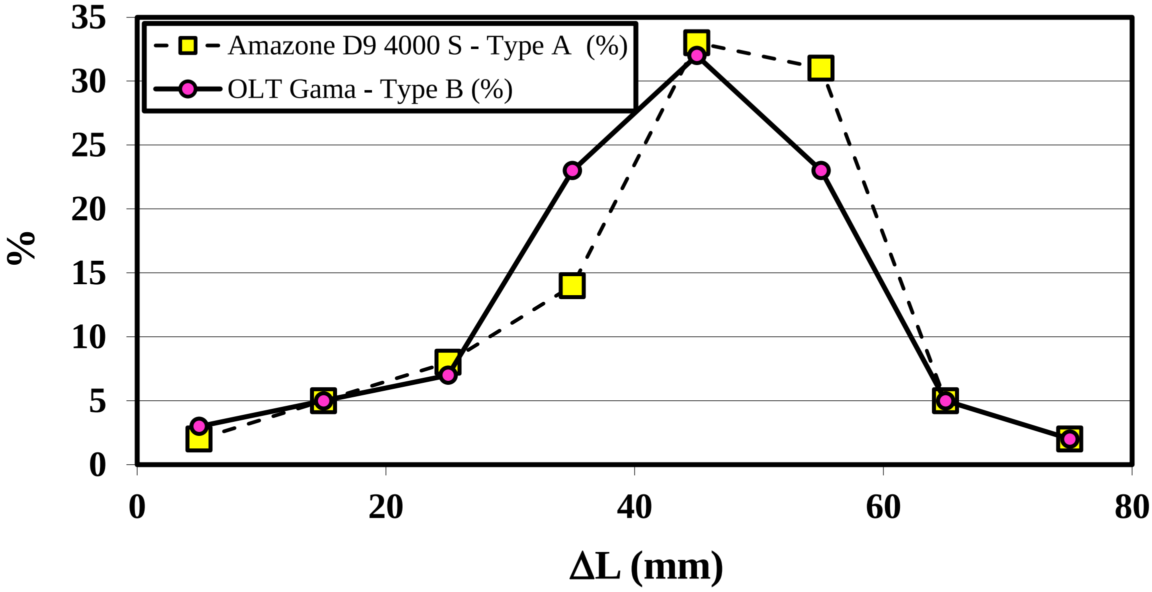

Figure 1. Relative frequency of longitudinal distribution of wheat seed by group spacing and examined variants

Figure 2 shows the results of cross-distribution of wheat seed according to treated variants. The results shown in Figure 2 indicate that the Amazone D9 4000 S seeder obtained more uniform cross-section density of wheat grain compared to the second variant. Thus, for variant A, in the group distance of 120.1 - 140 mm, there were 67% of wheat grains, which can be considered very satisfactory, while at the same group distance, for variant B, 45% of wheat grains were recorded, which is not within satisfactory limits. In the group distance of 0-100 mm, a negligible share of wheat grains was recorded. Namely 1% - Type A and 2% - Type B.
The wheat grain content in the group spacing of 110.1-120 mm ranged from 7% in the Type A seeder up to 9% in the Type B seeder. At a group spacing of 140.1-150 mm, 15% of wheat grains (Type A) or 21% (Type B), while in the group distance of more than 150 mm, the content ranged from 6 to 20%.

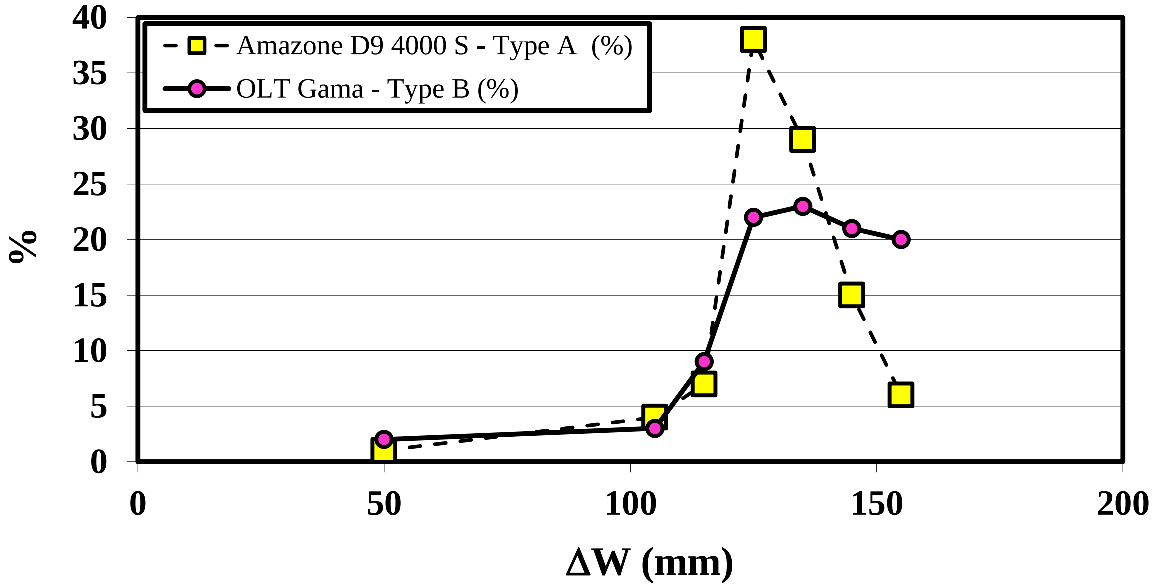

Figure 2. Relative frequency of cross-seed distribution by group intervals and examined variants

More evenly longitudinal and transversal arrangement of wheat seed with seeder Amazone D9 4000 S is primarily explained by the characteristics of the seeding device that performs better seeding and RoTeC-Control seeding depositors in comparison to the seeder OLT Gama 18 with a central sowing machine that achieves unsatisfactory seeding as it groups seeds into piles and standard disc depositors, as well as pre-seeding soil preparation..

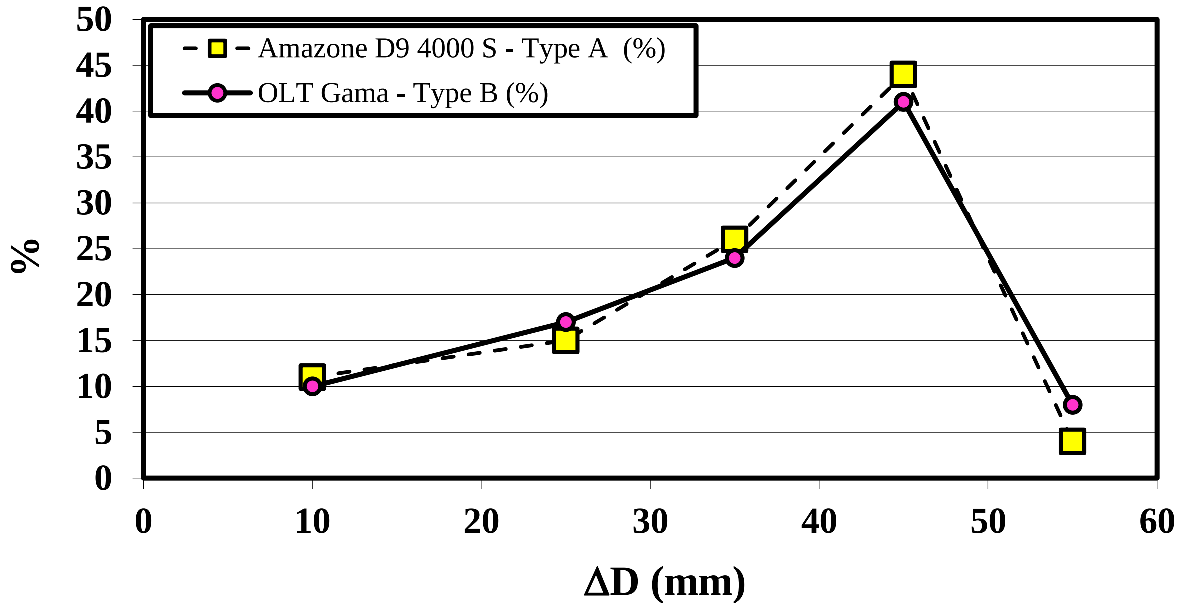

Figure 3. Relative frequency of seed distribution in group distances by the depth

Figure 3 shows the results of wheat seeding by depth, depending on the variants of the seeders aggregates examined. On the basis of the results shown, it can be noticed that in terms of the depth of winter wheat seed of the Sosthena variety, significant differences were not observed at the analyzed group intervals in both types of tested seed aggregates. Thus, the type A seeder, in the group spacing of 10.1-20 mm, sowed 11% of the seed, while in the group

distance 20.1-30 mm, 15% of wheat seed was seeded. In the group spacing of 30.1 - 50 mm, a total of 70% was seeded, while at a depth of more than 50 mm, only 4% of wheat was seeded.
During the examination of Type B seeders, similar values were obtained for observed group intervals. Within the group distance from seeding depth of 10.1 - 20 mm was recorded 10%, while 17% of wheat seeds were sown within a depth of 20.1-30 mm. 65% of wheat seeds were sown on the depth of sowing of 30.1 - 50 mm (Figure 3).
Other authors came up with similar results in their research (Malinović et al., 2003; Wiesenhoff *et al*., 2004; Šumanovac *et al*., 2006; Mehandžić *et al*., 2006; Marković *et al*., 2009; Rusu *et al*., 2013; Barać *et al*., 2015).

Conclusions

Based on the obtained results, it can be concluded that the examined seed aggregates obtained the different quality of work on two tillage systems during winter wheat sowing. The sowing aggregate of type A, operated better and achieved an evener distribution of wheat seed in relation to the second seeding aggregate, since in the longitudinal distribution at group intervals the highest average grain content of wheat grain from 64% was in the group distance of 40.1 - 60 mm which is considered to be very satisfactory, while in the other examined variants - type B in the group distance of 40.1 - 60 mm the grain share was significantly lower and amounted to an average of 55%. It is important to note that in both examined variants there was no higher presence of wheat grains in the group spacing of 0 - 10 mm, which was 2% - type A and 3% - type B. In the case of an aggregate type A, it was more uniform than the transversal distribution of wheat grains compared to the second variant, while for the total examined variant in the group distance of 120.1 - 140 mm, which is very satisfactory, while for the same group distance, for wheat variant B, 45% of wheat grains were recorded, which is not within the satisfactory values. In the group spacing of 0 - 100 mm, a negligible share of wheat grains was recorded, namely 1% - Type A and 2% - Type B. The more uniform longitudinal and transversal distribution of wheat seeds with the Amazone D9 4000 S seeder is explained by the characteristics of the planting apparatus that carries higher quality seed deposers, and RoTeC-Control seed depositors compared to the OLT Gama 18 seeder with a central sowing device that achieves unsatisfactory sowing because it drops seeds into piles, and standard disc deposers as well as pre-seeding soil preparation. In terms of the depth of seeding the winter wheat, the examined seed aggregates did not obtain significant differences in analyzed group distances so that seed distribution by depth can be considered relatively satisfactory. The seeding aggregated Type A, in a group spacing from 30.1 - 50 mm seeded 70%, while the Type B seeding aggregate at a depth of sowing of 30.1 - 50 mm sowed 65% of wheat seeds.

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THE SIGNIFICANCE OF CLIMATE VARIABILITY ON THE PRODUCTION OF WHEAT AND RAPESEED IN SERBIA

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Abstract

Yields of wheat and rapeseed in the Republic of Serbia in the period 2011-2018 were analyzed on the basis of data on sown areas and yield taken from the Statistical Yearbook of the Republic of Serbia. The trends of planted areas and yields in the Republic of Serbia and by region of analyzed meteorological stations (Novi Sad, Kraljevo, Negotin, Valjevo, Vranje and Kragujevac) have been determined, in order to analyze the impact of climate on yields of crops. The average sown area of wheat in the period 2011-2018 was 558,823 ha, and of rapeseed 12,679 ha. A slightly better eight-year average yield of wheat was achieved in Region I (4.76 t ha^{-1}); while in Region II the yields were 3.5 t ha^{-1}. The average yield of rapeseed from one hectare of sown area on the basis of the eight-year production in Serbia was 4.27 t ha^{-1}.

Keywords: *climate, wheat, areas and yield, rapeseed*

Introduction

Primary The climate is variable in its nature and this variability has been increasing in recent years. Climatic extremes are becoming more and more frequent, more intense and more costly. In addition to the significant impact on yield, they significantly affect both the sown area and the total quantities produced. However, science and the profession in agriculture are working to improve new technologies. Breading by creating stress-tolerant varieties also tries to reduce the impact of climate variability on yields. However, in the changing climatic conditions, a different response to the yield of certain species should be expected, which will also condition the cultivation perspective.

Wheat belongs to the family *Poaceae* genus *Triticum*. Over 70% of the population of the globe is fed with wheat bread making it world's most important bread plant. At the same time, Europe is the main production center where the highest yields per unit area are achieved. In the agroecological conditions of Serbia, the sensitive stages in the production are germination and grain-filling. The germination process requires a minimum temperature of 1^{o}C, but under such conditions germination runs slowly, while the optimal temperature for germination is 15-20^{o}C. During the grain-filling process, the grain passes through the milk and dough state. In the milk state, the grain reaches its full length, the color is green, and the plants at that time have their green color, with the lower leaves fading. In the dough state the grain is full and glossy; the plants at that stage become more yellow, although the green color is mostly preserved only by the peak leaves. In agroecological conditions of Serbia the grain filling lasts 16-22 days. The wax maturity phase lasts 6-8 days and sometimes more and grain moisture is 35-25%. Plants at the end of waxy maturity become yellow, leaves die off, and grain moisture is 21-24%. The duration of this phase differs from 3-4 days in arid conditions, while in humid conditions it can be extended to about 20 days, which can significantly affect the yield mainly through the hectolitic weight of the grain. Full maturity occurs at a moisture of 16-17%, which can be reduced to 14-15%. All these stages of wheat development and growth are followed by climatic conditions. From the phase of filling the grain to the maturation in the wheat vegetation, temperatures ranging from 25 to 30°C are considered high. Temperatures above 30°C are considered high and harmful to wheat. A very high temperature above 35°C in the presence of dry winds destroys photosynthesis in plants, slows the growth, and can cause death of plants, but in agroecological conditions this is characteristic of the ripening phase. High temperatures combined with low relative humidity lead to poor grain fulfillment, i.e. its leanness and premature maturation. This is then called a forced maturation that inevitably leads to low yields (Statistical Yearbook of the Republic of Serbia 2012-2018).

Rapeseed belongs to the family *Brassicaceae*, the genus *Brassica*. Rapeseed is grown for seeds that contain 40 to 49% of oil, and 18 to 25% of protein. The refined oil is used for food or technical purposes for the production of lubricants, biodiesels or as a raw material in pharmaceutical, textile and others industries. In Central and Western Europe, rapeseed is increasingly important for the production of bio-diesel. The largest producers of rapeseed in the world are the European Union, and China as an individual country. The optimal period of sowing of rapeseed is at the end of August-early September, with enough moisture in the soil, there are seedlings appearing in 4-6 days (*Crnobarac et al*., 1999). But in this period, there is often a lack of precipitation which in some years prevents successful germination where in addition to the temperature soil moisture is needed in order to initiate the metabolic processes necessary for germination. In the dry autumn conditions characteristic for rapeseed, is that even during the sowing the insufficiently humid soil germination is lagging till the rain falls, and the sprouted seedlings enter the period of autumn frosts in the phase of the cotyledon, which can cause the uneven germinated crops or complete destruction of newly-formed crops (*Gašparov et al*., 1988). The uniformity of germination of rapeseed may also be affected by the color of the seed (*Knežević et al.,* 2019). In the production of rapeseed, the collecting-harvesting process belongs to a more fragile phases since involves at this stage fruits of rapeseed are prone to cracking and seed dispersal, which is particularly pronounced in combination with weather conditions such as winds with periods of rainy and sunny weather.

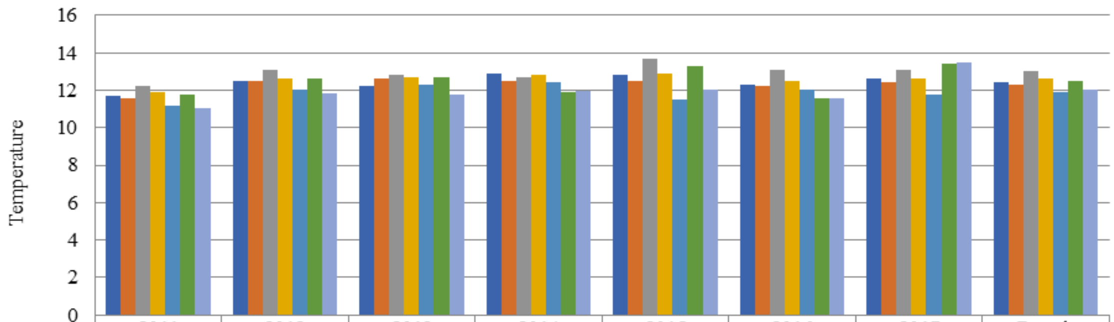
The aim of this paper was to analyze the impact of climatic conditions that accompany production by regions, areas under cultivation and yield per unit area of wheat and rapeseed in Serbia for the period from 2011 to 2018.

Material and Methods

Yields of wheat and rapeseed in the Republic of Serbia in the period 2011-2018 were analyzed on the basis of data on sown area and yield taken from the Statistical Yearbook of the Republic of Serbia (G2012, G2013, G2014, G2015, G2016, G2017 and G2018). Trends in planted areas and yields in the Republic of Serbia and by regions (Region I - North of Serbia and Region II - Šumadija, western, southern and eastern Serbia) were determined. In order to analyze the influence of observed climate change trends on yields of examined cultures, average annual temperatures and precipitation amounts in the Republic of Serbia and at regions in which the analyzed meteorological stations are located (Novi Sad, Kraljevo, Negotin, Valjevo, Vranje and Kragujevac) are calculated.

Results and Discussion

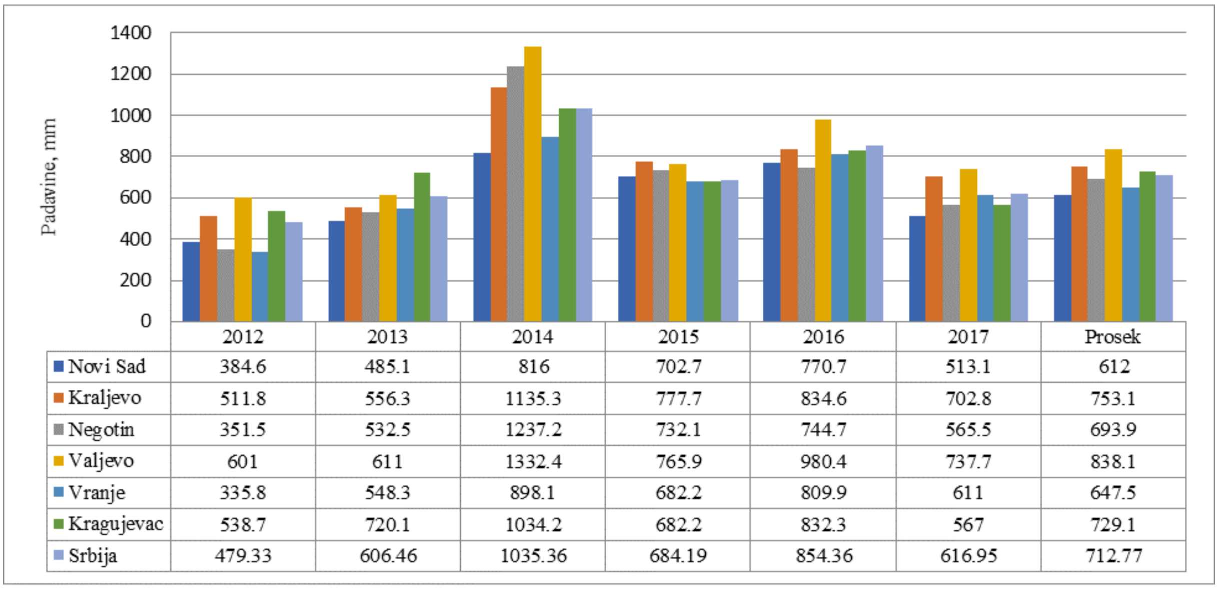
Graph 1 shows the average annual air temperature in Serbia in the period from 2011 to 2017. The average air temperature in Serbia during the seven-year period was 12°C and was higher by 0.95°C compared to 2011, by 0.18°C compared to 2012, by 0.21°C compared to 2013 and by 0.43°C compared to 2016 year, while it was lower by 1.5°C compared to 2017. The average air temperature in 2014 and 2015 was approximately the average for Serbia. The highest average seven-year temperature value was established in Negotin (13^{o}C) and the lowest in Vranje (11.9^{o}C). Based on the data from Graph 1, it can be concluded that two years ago, out of seven tested, had a slightly higher average temperature than the perennial average for Serbia. Various stress abiotic factors (high and low temperatures, drought, acid and saline soil) in various stages of wheat development limit the expression of maximum genetic potential. Drought has become the main limiting factor in global plant production reducing yields in developed agricultures in the world. The drought-stress is usually followed by high temperatures, which additionally increases the effect of stress (*Đekić et al*., 2017, *Terzić et al.,* 2018). Global climate changes condition increasingly warmer summers and milder winters, which in the future will shift the dates of sowing and spiking, as well as the growing areas of stubble grain cereals (*Đekić et al*., 2019).



	2011	2012	2013	2014	2015	2016	2017	Prosek
Novi Sad	11.7	12.5	12.2	12.9	12.8	12.3	12.6	12.4
Kraljevo	11.6	12.5	12.6	12.5	12.5	12.2	12.4	12.3
Negotin	12.2	13.1	12.8	12.7	13.7	13.1	13.1	13
Valjevo	11.9	12.6	12.7	12.8	12.9	12.5	12.6	12.6
Vranje	11.2	12	12.3	12.4	11.5	12	11.8	11.9
Kragujevac	11.8	12.6	12.7	11.9	13.3	11.6	13.4	12.5
Srbija	11.05	11.82	11.79	11.98	12.05	11.57	13.5	12

Graph 1. Average annual air temperature in Serbia (2011-2017)

The data shown in Graph 2 for the studied vegetation period clearly indicate that the average annual rainfall varied from the seven-year average for Serbia. The average annual rainfall was lower than the seven – year average of 233.44 mm, in 2012, 106.31 mm, in 2013, 28.58 mm in 2015 and 95.82 mm in 2017 while the distribution of rainfall was uneven. The average rainfall was greater by 322.59 mm in 2014 and by 141.59 mm in 2016. The average amount of precipitation compared to the studied cities was 40.33 mm higher in Kraljevo, 125.33 mm in Valjevo and 16.3 mm in Kragujevac. Slightly lower than seven-year average values for precipitation compared to Serbia were in Novi Sad, Negotin and Vranje. The average yield of wheat above 4 t ha^{-1} was achieved in 2013, 2015, 2016, 2017 and 2018 (Table 1), which shows that they were favorable both for wheat and rapeseed cultivation. *Đekić et al.,* (2017) and *Terzić et al*. (2018) pointed out that the total amount of precipitation is important at the annual level, but the arrangement of it, especially in critical phases of plant development, is significantly disturbed. The same authors point out that in addition to the necessary reserves for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil. In terms of wheat growing fields, wheat is the second crop in Serbia. Rapeseed is highly variable by the areas on which it is grown. Wheat and rapeseed are botanical and usable cultures, but they are very competitive in the areas they occupy.



Graph 2. Average annual precipitation in Serbia (2012-2017)

Table 1. Areas and realized yields of wheat and rapeseed in Serbia

Year	Wheat						Rapeseed	
	Region I north Serbia		Region II Šumadija, western, southern and eastern Serbia		Average in Serbia Region I + II		Average in Serbia Region I + II	
	Areas sown ha	Yield t ha^{-1}	Areas sown ha	Yield t ha^{-1}	Areas sown ha	Yield t ha^{-1}	Areas sown ha	Yield t ha^{-1}
2018	363570	5.42	284513	3.64	648083	4.74	-	-
2017	314480	4.62	245222	3.40	557702	4.08	19934	4.11
2016	329187	5.78	265931	3.69	595118	4.85	13476	4.83
2015	325766	4.79	363997	3.29	589763	4.12	12226	4.12
2014	308398	4.30	240334	3.49	548732	3.94	9815	3.96
2013	320923	4.64	236473	3.55	557396	4.18	9686	4.33
2012	268241	4.33	214372	3.52	482613	3.97	8258	4.01
2011	270514	4.17	220660	3.39	491174	3.82	15357	4.23
Average	312635	4.76	258938	3.50	558823	3.84	12679	4.27
(CV)	9.377	11.93	17.40	3.819	9.11	8.979	31.77	6.949

For the analyzed period and both regions, wheat was grown on the smallest areas in 2012 (482,613 ha) and also in 2011 it was grown on the areas of less than 500,000 ha. In the remaining of the analyzed years, it was cultivated on the areas greater than 500,000 ha. For the analyzed period for wheat in both regions, the area variability expressed in the coefficient of variation (CV%) was 9.11%, while for the rapeseed the area variability was much higher (CV=31.77%). (Table 1).

In the analyzed period, wheat produced the total yield per unit area of 3.82 t ha^{-1} (in 2011) to 4.85 t ha^{-1} (in 2016) (the region average I + II) with the variability expressed in the coefficient of variation CV=8.979%, In rapeseed the corresponding values ranged from 3.96 t ha^{-1} (in 2014) to 4.83 t ha^{-1} (in 2016) and with lower variation (CV=6.949%).

Observing the achieved yields of wheat by years and regions in all the years north of Serbia (Region 1 Belgrade and Vojvodina) compared to the south of Serbia (Šumadija, western

Serbia, southern Serbia and eastern Serbia) generated higher yields of 1.26 t ha^{-1}, the yield variability was higher in the region I (CV=11.93%) than the variability in the region II (CV=3.819%), (Table 1).

Đekić et al. (2019) and *Terzic et al.* (2018) point out that grain yield shows a tendency of growth in years with higher intensity and better precipitation patterns during critical stages of plant development. They also concluded that the sowing structure should be based on more than one variety in order to reduce the risk bearing unpredictability of each vegetation, regardless to the reliability of the selection criteria for the seed sorting in a particular year of research.

Conclusions

The paper presents an analysis of the production of wheat and rapeseed in the Republic of Serbia in the period 2011-2018. With regard to the yield of wheat and rapeseed, the record year for the period 2011-2018 was 2016, with an average wheat yield of 4.85 t ha^{-1} and a 4.84 t ha^{-1} of rapeseed. In 2016, the largest production of wheat and rapeseed in the last decade was recorded. When it comes to total production, it can be said that there is a tendency of growth with smaller fluctuations. Grain yield shows a growth tendency in years with higher levels and better rainfall pattern during critical stages of plant development.

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EFFECT OF CULTIVAR AND YEAR ON YIELD AND GRAIN QUALITY OF TWO-ROW SPRING BARLEY

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Abstract

Grain yield and quality components were evaluated in four ('Novosadski 448', 'Novosadski 456', 'Dinarac' and 'Dunavac') two-row spring barley cultivars. Comparative studies were conducted during the 2008-2010 year period on the experimental field of the Small Grains Research Centre, Kragujevac. The experiment was laid out in a randomized block design with three replications on a Vertisol soil. At tillering, 50 kg ha^{-1} nitrogen was applied. Calcium ammonium nitrate (CAN) with a nitrogen content of 27% was used for top dressing. The cultivars showed significant differences in grain yield, thousand kernel weight and germination energy, and no substantial differences in protein contents. Grain yield was highest in cv. Dinarac and lowest in cv. Dunavac. The analysis of the effect of the cultivar-year interaction reveals a specific response of each cultivar to germination energy. All cultivars were found to have germination energy of above 90%. Environmental conditions had no significant effect on grain protein content. This suggests that all of the tested cultivars can serve as equally valuable raw materials in the brewing industry.

Keywords: *Spring barley, grain yield, thousand kernel weight, germination energy, protein content*

Introduction

The most important traits that a barley genotype should possess is the yield and quality of the grain. Grain yield has changed during the last century, with yield increases mostly resulting from the development of plant selection and breeding techniques that lead to the genetic yield potential of newly created spring barley cultivars of above 11 t ha^{-1} (Pržulj *et al.* 2010).
Yield increase is associated with improvement of the genetic basis of cultivars and use of adequate production technology. Temperature and rainfall are not necessarily the most important factors that govern the level of production and grain quality in malting barley, since the harvested grain is often of poor quality when these factors are close to optimal (Pržulj *et al.* 2014).
Apart from high yield, thousand kernel weight and good biotic and abiotic stress resistance, malting barley cultivars should have a low content of chaff and proteins and a high starch content. Thousand kernel weight is not just a component of yield, but also a very important component of quality malting barley grains (Ullrich, 2002; Madić *et al.* 2016). Pržulj and Momčilović (2008) stated that the brewing grain quality should have a mass of thousand grains of 45 to 50 g. Grains of greater weight usually has more starch and less protein (Lagassé *et al*. 2006; Marquart *et al.* 2007). Thousand-kernel weight and test weight are among initial major indicators of grain quality (Fox *et al.* 2006, Fox *et al.* 2007). The malting barley varieties divided into groups on the basis of 1000-kernel weight: >45 g large grain, 41 to 44 g medium grain, 37 to 40 g small grain (Malcev, 1967). Protein content is the initial indicator of qualitative analysis of barley grain. Malting barley should have a low content of proteins (below 11.5 %), soluble ones in particular, since a high content of soluble proteins

gives a saturated taste to the beer produced and makes the colour and taste of beer difficult to control.

Apart from the genetic factors (choice of cultivar), an increase in protein content was substantially affected by environmental factors such as inadequate use of nitrogen fertilisers, water deficiency, high temperatures during the kernel filling stage, etc. (Maksimović *et al.* 2001). Furthermore, many studies suggest differences in grain yield stability and adaptability of cultivars to environmental and growing conditions, as well as different cultivar responses to biotic and abiotic stresses (Mirosavljević *et a*l. 2015).

Production technology should be strictly applied adhered to in order to fully implement the genetic potential for grain yield and quality in malting barley (Pržulj and Momčilović 2002; Madić *et al.* 2006; Paunović *et. al.* 2008).

Materials and Methods

Four cultivars of two-row spring barley, (Novosadski 456, Novosadski 448, Dinarac and Dunavac) were analysed for grain yield and quality components. The research was conducted during the 2008-2010 year period on the experimental field of the Small Grains Research Centre, Kragujevac, on a Vertisol soil. According to the analysis, this is a soil of medium acid reaction (pH $_{KCl}$ 4.9), poor in organic matter (2.68 %), very poor in readily available phosphorus (P_2O_5 < 1 mg*100^{-1} g soil), and medium provided with easily accessible potassium (K_2O 10.5 mg*100^{-1} g soil).

The experiment was laid out in a randomized block design with three replications. Each treatment was planted in plots of 5.0 m^2 area, consisting of ten 5.0 m long rows, spaced 0.1 m between rows, 0.5 m between plots and 1.0 m between blocks.

Seeds were sown by hand into the experimental plots in all three years on mid-March, at a seed rate of 450 germinating seeds m^{-2}. Maize was the preceding crop in each experimental year. During the growing season the standard measures of plant protection were used. At tillering 50 kg ha^{-1} nitrogen was applied. Calcium ammonium nitrate (CAN) with a nitrogen content of 27% was used for top dressing.

Grain yield was determined on each plot and expressed in t ha^{-1} at 14% moisture content. Thousand-kernel weight, germination energy (%), protein content was assessed four months upon harvest under laboratory conditions.

Grain protein content (% d.m.) was determined by Kjeldahl method. Results were subjected to a two-way ANOVA (year, cultivar) for the experimental period using the statistical package SAS/STAT, 2000. User's Guide, Version 9.1.3. The significance of difference between the means was assessed by LSD test at the 95% level.

Results and Discussion

Grain yield of barley is a complex trait of tremendous economic importance resulting from the effect of genotype and the environment during the entire plant life cycle. It is difficult to create a variety that has all the positive properties in different cultivation conditions and resistant to biotic and abiotic stress factors (Pržulj *et al.* 2010; Knežević *et al.* 2015; Madić *et al.* 2016). The analysis of variance for grain yield showed significant differences among the cultivars, with the highest and lowest yields being obtained with cvs. 'Dinarac' and 'Dunavac', respectively (Table 1).

The yield was significantly influenced by the conditions of growth (years), and there was also a significant interaction effect between the genotype and the year.

An analysis of the interactive effects between genotypes and year reveals a similar tendency of cvs. 'Novosadski 448', 'Dinarac' and 'Dunavac'. The variety 'Dunavac' had a significantly lower grain yield in the first year. In the third year the yield of cv. 'Dunavac' was at the level of the second year, so it can be concluded that the cv. 'Dunavac' does not react to improved environmental conditions (Table 1).

Table 1. Grain yield (t ha^{-1}), 1000-kernel weight (g), germination energy (%) and grain protein content (%) of barley cultivars over a three-year period

		Grain yield	1000-kernel weight	Germination energy	Grain protein content
Cultivars (A)	Novosadski 456	5.3ab‴	39.8bc	93.9b	10.84
	Novosadski 448	5.2ab	39.6c	95.6a	10.44
	Dinarac	5.5a	45.3a	93.6b	11.31
	Dunavac	4.8b	41.7b	92.8b	10.48
Years (B)	2008	4.1c	40.5b	93.7	-
	2009	5.5b	43.8a	93.3	10.92
	2010	6.3a	44.2a	93.7	11.22
Novosadski 456	2008	4.7g	37.7gh	91.0fg	-
	2009	5.6cde	41.9ef	94.3cde	10.90
	2010	6.7a	40.6f	96.6a	11.18
Novosadski 448	2008	4.2g	36.3h	95.7abc	-
	2009	5.4cde	41.5def	94.6abc	10.40
	2010	6.4ab	41.1ef	96.3ab	10.67
Dinarac	2008	4.2g	44.3bc	90.5g	-
	2009	5.7b-e	45.6a	94.3cde	11.39
	2010	6.8a	45.1a	95.9abc	10.83
Dunavac	2008	4.0fg	38.9fg	94.7abc	-
	2009	5.0ef	42.8ab	92.8ef	10.49
	2010	5.3cde	44.1a	90.6fg	10.23
ANOVA	Cultivar (A)	*	**	**	ns
	Year (B)	**	**	ns	ns
	AxB	**	**	**	ns

‴Means followed by different lowercase letters in columns for cultivars and years indicate significant differences according to LSD test ($P \leq 0.05$).
*NS - non-significant; ** F - test significant at the 99% level; * F - test significant at the 95%

Thousand kernel weight is not only a yield component, but also a very important component of grain quality in malting barley. Malting barley is of good quality if its 1000-kernel weight is above 38 g (Gaćeša *et al.* 1992). The analysis of variance for 1000-kernel weight suggested significant differences among cultivars (Table 1). The highest 1000-kernel weight had cv. 'Dinarac' and the lowest cvs. 'Novosadski 448' and 'Novosadski 456'. Environmental conditions over the years also produced statistically significant effect, with significant interaction effects between cultivar and year being observed. Thousand kernel weight was substantially lower in cvs. 'Novosadski 456', 'Novosadski 448' and 'Dunavac' during the first year of the study as compared to cv. 'Dinarac', but showed an increasing tendency in the second and third years. Cultivar 'Dinarac' had a significantly larger thausand kernel weight in all years in relation to other varieties (Table 1).

The strongest response to variable environmental conditions was exhibited by cv. 'Dunavac', whereas the least variations, irrespective of the conditions, were observed with cv. 'Dinarac'. Substantial effects of production conditions on technological quality parameters were reported by Lookhart *et al. (*2001), Knežević *et al.* (2004) and Pržulj *et al.* (2014). Malt is defined as the germinated barley, and the maltsters expect the bar to be 365 days regardless of the storage conditions (Pržulj *et al.* 2000).
The grain yield of barley can be improved by selecting plants of medium plant height with thousand kernel weight above 41.0 g and grain protein content of about 11.0 g 100^{-1} g dm (Mirosavljević *et al.,* 2015). Certain grain traits, such as dormancy and hydrosensibility, are desirable agronomic traits though undesirable in technological terms; therefore, breeders are required to satisfy production and storage requirements, on the one hand, and malting requirements, on the other (Pržulj *et al.* 2010). The highest germination energy had cv. 'Novosadski 448'. Between the varieties there was no difference in the energy of germination except in cv. 'Novosadski 448', also the impact of the year did not have a significant effect. All cultivars were found to possess the required germination energy of above 90%. The lowest variation across years was observed in cv. 'Novosadski 448'. Conversely, cvs. 'Dinarac' and 'Novosadski 456' exhibited significantly higher values in the second and third years. The analysis of the cultivar-year interaction effect indicates a specific response of each cultivar to germination energy.
Grain protein content of barley grains is one of the limiting parameters of malting barley. To be good raw material for beer industry, barley grains should be balanced uniform in size, with the absolute weight over 40 grams and with the total protein below 12% (Maleševic *et al.* 2010). The cultivars tested did not significantly differ in the grain protein content, nor did environmental conditions produce any significant effect (Table 1). This suggests that all of the tested cultivars can serve as equally valuable raw materials in the brewing industry. The choice of the most favourable cultivar can be determined by other production traits.

Conclusion

The grain yield of all of the cultivars tested was above 6 t ha^{-1}, being the highest in cv. 'Dinarac' and lowest in cv. 'Dunavac'. The environmental conditions (year), as well as the genotype-year interaction significantly influenced the grain yield and thousand kernel weight. All cultivars in the first year had significantly less thousand-kernel weight, compared to the second and third year. The strongest response to variable environmental conditions was exhibited by cv. 'Dunavac', whereas the least variations, irrespective of the conditions, were observed with cvs. 'Dinarac' and 'Novosadski 456'. All cultivars were found to have germination energy of above 90%. The analysis of the effect of the cultivar-year interaction reveals a specific response of each cultivar to germination energy. Environmental conditions had no significant effect on grain protein content. This suggests that all of the tested cultivars can serve as equally valuable raw materials in the brewing industry.

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CROP ROTATION PRODUCTIVITY WITH CEREALS AND LEGUMES: A SHORT REVIEW

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Abstract

Crop rotation is considered to be an instrument of sustainable cropping system assisting in varying the set of soil nutrients. Thereby reducing the likelihood of soil erosion, building soil organic matter, increasing carbon sequestration, improving soil quality, water holding capacity, nutrient availability, soil structure and minimizing greenhouse gas emissions. Additionally, reducing the outbreak of pests, weeds and other diseases on the farmland, reducing the reliance on chemical fertilizers, minimizing agricultural crop production dangers and heighten crop yield in comparison with monoculture practices. Crop rotation is an agricultural practice of growing different or non-similar crops on the same farmland in different seasons. Also, this cropping system has the potential to increase the diversity of cropping systems, to maximize resiliency of the cereals-based system under variable weather conditions. Higher cereal yields have been gained by including legumes in the rotations. Legume crops could play an important role by delivering multiple services in line with sustainability principles. Crop type can impact soil temperature and water content by affecting shade intensity and evapotranspiration. Crop residues returned to the soil are the main input in maintaining soil organic carbon (SOC), which generally seems to increase with the diversified crop rotation compared to the mono-cropping. Especially, crop rotation and grazing system can affect crop residue mineralization, root and microbial respiration, so as to play a major role in regulating soil surface GHG emissions.

Keywords: *rotation, legumes, cereals, productivity.*

Introduction

Rotations are an important part of any sustainable agricultural system. Yields of crops grown in rotations are typically 10% higher than those of crops grown in monoculture in normal growing seasons, and as much as 25% higher in droughty growing seasons. When you grow a grain or vegetable crop following a forage legume, the extra supply of nitrogen certainly helps (Chuan, 2013; Paul *et al*., 2014). However, yields of crops grown in rotation are often higher than those of crops grown in monoculture, even when both are supplied with plentiful amounts of nitrogen. Research in Iowa found that even using 240 pounds of N per acre when growing corn after corn, yields were not as good as corn grown following alfalfa with little or no N applied. In addition, following a non legume crop with another non legume produces higher yields than a monoculture using recommended fertilizer rates. For example, when you grow corn following grass hay, or cotton following corn, you get higher yields than when corn or cotton is grown year after year. This yield benefit from rotations is sometimes called a *rotation effect.* Another important benefit of rotations is that growing a variety of crops in a given year, spreads out labor needs and reduces risk caused by unexpected climate or market conditions. Other benefits may occur when perennial forages (hay-type crops) are included in the rotation, including decreased soil erosion and nutrient loss (Craig *et al*., 2006; Abdul Quddus *et al.*, 2017).

In Mediterranean-type ecosystems, given the low rainfall, the practices followed in the context of modern agriculture have often led to a deterioration in the quality of resources. Soil

quality must be maintained and resource exhaustion limited. Consequently, the adoption of multiple crop systems, such as the economy, continued productivity and environmental security, must be a primary objective of the corresponding agricultural policy. Among these systems, rotation and green fertilization of legumes, contributing to plant health, controlling the population of enemies, pathogens and weeds, the availability of nutrients, the improvement and rational use of soil, the protection of flora and fauna and, more generally, their impact on future crops. (Bao-Luo, 2016; Lampurlanes *et al.*, 2009).
It is known that 75% of the nitrogen contained in legume biomass comes from conventional nitrogen sequestration (Grath and Wohlrab 1992, Wendland *et al.*, 1993). Beans and peas are always associated with a significant nitrogen gain. Legumes fix the atmospheric nitrogen, release in the soil high-quality organic matter in the soil and facilitate soil nutrients' circulation and water retention (Neugschwandtner *et al.*, 2015; Ghanem *et al.*, 2011). Legumes could be competitive crops and, due to their environmental and socioeconomic benefits, could be introduced in modern cropping systems to increase crop diversity and reduce use of external inputs. Field pea and faba bean accumulate about 130 and 153 kg Nha^{-1} in their aboveground biomass, respectively and significant quantities (30–60% of the accumulated total N) may also be stored in belowground biomass. Faba beans can contribute large amounts of nitrogen to the soil (up to 100kg N/ha over an extended period), while helping to control crown rot and improving overall soil health. Field pea varieties developed provide a viable pulse alternative for grain growers. (Sallaku *et al.*, 2016; Denton, 2013).
The purpose of this article is to present a brief review about the advantages and applications of the crop rotation technique, so that restoring this 'old' method which has been forgotten despite the great prospects in both agronomic and environmental science.

Literature review

Literature review is a thorough study into the already existing knowledge of a topic selected by researchers. This literature search concerns the Crop rotation. The information was obtained from the main online scientific sites including Science Direct, Google Scholar and Scopus. Searches were also undertaken in the University of Thessaly library, dissertation and thesis search engines like ProQuest, Open-thesis and National Documentation Centre.

Experimental results

The switching of fallow crops is considered the oldest and most basic agricultural practice of payment for agriculture, basic procedures before the emergence of modern cultivation techniques. The final succession option is a management decision based on the desire to optimize economic, agricultural or environmental goals. The key to economic goals is to optimize profit, for agricultural purposes, maximize production from a variety of crops, and for the environment, minimize the use of chemical inputs.
The succession of a row of crops in crop rotation is likely to face problems with government regulations - or regulations agro - ecological conditions (such as climatic conditions, topographical and climatic conditions) soil), the presence of insects, weeds, diseases or their varieties cultivation and available mechanical equipment (Castellazi *et al*., 2008).
According to Tillman *et al*. (2004) winter crop rotation with spring crops helps to preserve biodiversity by offering shelter and food to a beneficial effect insects. Also, the use of legumes and soil cover plants in general crop rotation systems reduce the likelihood of crop failure by diseases and enemies, due to lack of insults or reduction of numbers of the pathogens during their development.
Crop rotation combined with reduced soil treatment (or non-cultivation) increases the population of worms and therefore improves the quality of the soil, reduces oxidation rates,

improves the percentage of organic matter so long-term availability and supply of macro- and micro-elements in crops (Francis, 2005).

A modern rotation system was first applied in England in the 18th century, in Rothamsted, England, for 100 years (since 1843). Some of experiments are still ongoing. In addition, 30-year experiments in America showed that the yield of wheat increased by three years crop rotation corn and oats by 140%, 86% and 64%, respectively on the above species (Karlen *et al*., 1994).

The results showed that wheat as monoculture is lagging behind in crop yield over two years crop rotation and especially those that included legumes. Of these, pea (*Pisum sativum*) helped to obtain higher yields of wheat without the addition of any chemical fertilizer while the use of chemical fertilizers gave higher yields on cereals. There are very few long-term studies which have quantified the effect of different crop systems in soil conditions. One of these studies in Cyprus showed that long-term crop rotation leads to impeccable, environmental cultivation system perspective, with higher productivity (Papastylianou, 1993), better income for farmers (Papastylianou & Panayiotou, 1993), as well as lower nitrogen fertilizer requirements (Papastylianou, 1993).

Unless chemical fertilizers are used, green lubrication, using in particular the pea incorporated in the soil, is considered to be the most productive crop rotation for wheat, because it achieves rapid stability of its yields at high levels. "Maintenance is used in almost 40% of cultivated area in the South US, while winter plant, cover crops play an important role in farming systems with regard to the protection against erosion as well as the improvement of the soil productivity (CTIC, 2004).

In an experiment in Alabama, US three-year crop rotation: cessation - corn and tropical legumes (sunny hemp) – corn found that N content in maize seed was much higher in the yields followed by the legumes after the set-aside. Or the contribution of nitrogen fertilization to the segments that received it and preceded the pulp, ranged around 58 kg N ha^{-1} based on biomass production and about 33 kg N ha^{-1} (16.3 kg N ha^{-1} higher than set-off) based on the seed content. Maize production after oil compared to set-aside was 85% higher (Balcom & Reeves, 2005).

A rotational experiment with legumes was conducted in the southwest England. The results showed that the organic matter of the soil was maintained, improving both its physical and chemical characteristics, providing the soil with additional N and reducing surface erosion due to intense winter rainfall (Boquet & Dabney, 1991). Also, Oyer and Touchton (1990) have shown experimentally that an increase in N after winter-leguminous cultivation has led to an increase in successive cotton and sorghum crops without fertilization in spring crops but a decrease in organic soil content.

Table 1. Comparison of Monoculture, Fixed-Sequence Rotations, and Dynamic Cropping Systems (Source: Modified from Hanson *et al.*, 2007).

	Monoculture	**Fixed-Sequence Rotations**	**Dynamic Cropping Systems**
Numbers and types of crops	Single crop	Multiple crops: number depends on regionally adapted species, economics, farmer knowledge, infrastructure.	Multiple crops: number depends on regionally adapted species, economics, farmer knowledge, infrastructure.
Crop diversity	N/A	Diversity depends on length of fixed sequence.	Diversity high due to annual variation in growing conditions and marketing opportunities, as well as change in producer goals.
Crop-sequencing flexibility	N/A	None, although fixed-sequence cropping systems that incorporate opportunity crops increase flexibility.	High. All crops, in essence, are opportunity crops.
Biological and ecological knowledge	Basic knowledge of agronomy	Some knowledge of crop interactions is necessary.	Extended knowledge of complex, multiyear crop and crop-environment interactions.
Management complexity	Generally low, though variable depending on crop type	Complexity variable depending on length of fixed sequence and diversity of crops grown.	Complexity inherently high due to annual variation in growing conditions, markets, and producer goals.

Advantages

The advantages of such a farming system are related to:

- Maintaining or increasing soil fertility, which is achieved through more efficient use of soil nutrients, increased organic matter and nitrogen (N), erosion protection and preservation or improvement of the physical composition of the soil.
- The fight against plant pests, which in combination with other factors combat weeds, pets and plant diseases.
- Increasing performance, qualitatively and quantitatively.
- Addressing economic and technical problems (Selim, 2019).

The rotation system that we will follow and maximize our profit over a period of many years depends on the following factors:

- The species and varieties included in the crop rotation, as well as the crop rotation cycle (number of years).
- The ratio of the total area to be occupied by each crop.
- The succession of crops in the crop rotation cycle (Chongtham *et al.*, 2016).

Conditions for successful crop rotation

In determining the above factors, account should be taken of the following:

- the adaptability of plants to the soil and climate conditions of each region, as well as the economic and technical conditions determining the competitiveness of crops (product prices, cost, demand for products, allocated production facilities and capital);
- the existence of water resources and their better management,
- the presence of pests (weeds, enemies and diseases) and the possibility of dealing with them,
- the existence and distribution of working hands and mechanical equipment,
- the effect of one crop on the next. (Bulldock 1992; Charles *et al*., 2009).

Examples of crop rotation

A simple rotation of a broadleaf and a narrow-leaf is a **two-year** crop rotation.
Potatoes - Winter rye
Sugar beet - Summer barley
Cotton - Winter wheat
Tobacco - Winter wheat
Under certain conditions of the regions we can make three-year, four-year and five-year crop rotation, consisting of a broad leaf and two consecutive narrow-leafs, etc.

Three-years
Corn - Winter wheat - Winter barley
Cotton - Winter wheat - Oats
Sugar beet - Summer wheat - Winter barley

Four Years
Potatoes - Winter wheat - Winter barley - Oats
Corn - Oats - Winter wheat - Summer Barley
Potatoes - Potatoes - Winter wheat - Winter barley - Oats - Winter rye

Five Years
Potatoes - Winter wheat - Winter barley - Summer rye - Oats
Beans - Winter wheat - Summer wheat - Summer barley - Oats
Dual plant rotation can be made from two broadleaves and two narrow leaves:
Potatoes - Sugar beet - Winter wheat - Summer wheat
They can be grown for two consecutive years of broadleaf leaves and followed by a narrow leaf:
Sugar beet - Potatoes - Oats
Corn - Corn - winter wheat

It is reported that diversified crop rotations have also greater energetic productivity from above-ground biomass (grain / seed yield and by-products) if compared with crops grown in repeated sowings or in monoculture. Crop rotation in combination with different tillage methods (conventional tillage, reduced or minimum tillage and no-tillage) is the way to improve soil quality, but it is not clear whether the soil treatment method has a significant impact on the overall crop rotational energy productivity.

The most nitrogen-demanding crop, corn, followed the pasture, and grain was harvested only two of every five to seven years. A less nitrogen-demanding crop, oats, was planted in the second year as a "nurse crop" when the grass-legume hay was seeded. The grain was harvested as animal feed, and oat straw was harvested to be used as cattle bedding; both eventually were returned to the soil as animal manure. This rotation maintained soil organic matter in many situations, or at least didn't cause it to decrease too much. On prairie soils, with their very high original contents of organic matter, levels still probably decreased with the below rotation.

Year 1. Corn Year 2. Oats (mixed legume–grass hay seeded) Years 3, 4, and 5. Mixed grass–legume hay Years 6 and 7. Pasture (Hanson *et al.*, 2007).

There are literally dozens of rotations that might work well on a particular farm. The specific selection depends on the climate and soils, the expertise of the farmer, whether there are livestock on the farm or nearby, equipment and labor availability, family quality-of-life considerations, and financial reality (potential price minus the cost of production). (However, vegetable farmers will sometimes include low-return crops in their rotations because customers expect to find them in the mix at a farm stand or farmers' market.)

Conclusions

The introduction of new cultivation techniques has enabled the same field to be cultivated continuously refined with the same crop culture. This practice leads to the loss of fertility in the fields, increases weed problems and diseases, thereby augmenting the cost of producing more fertilizers and pesticides. This concomitant waste of natural resources as well as the uncontrolled use of chemicals are leading ecosystems to environmental degradation. The concept of crop rotation is known to farmers from the earliest stages of agricultural development on the planet. As has been the case with many practices, industrialization and changing lifestyles have made producers withdraw from this environmentally beneficial of this cultivation technique. Crop rotation is a method that begins to be applied again after a year of negligence. The advantages of crop rotation systems can help solve some of the major problems of modern agriculture such as maintaining or increasing soil fertility to improve plant health, increase yields and income for farmers in combination with environmentally friendly practices. Agriculture is one of the most anthropogenic activities with the greatest negative environmental impacts, so scientific research on methods that reduce contamination is imperative in order for agriculture to become environmentally friendly sector.

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INTERCROPPING – MAY BE AN OLD PRACTICE BUT TIMELESS: A SHORT REVIEW

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Abstract

Intercropping is known as the achievement of a high and stable production that not only raises complementary products in the area but also reduces the harmful effects of diseases and pests, prevents pollution and results in effective use of resources. Intercropping is an agricultural practice of cultivating two or more crops in the same place of land at the same time which is commonly practiced in many parts of the world in order to increase the productivity per unit area of the land. The crops are not necessarily sown at the same time and their harvest time may be quite different, but they are simultaneously grown for significant growing periods. In this review study, we are informed about the use and importance of the intercropping system which is mainly based on very old ones and which is of great importance in recent years in agricultural production. There are several intercropping systems such as mixed, strip and row intercropping patterns. Supplemental effects in models of resource use should be taken into consideration so as to get better yield and quality in intercropping systems. Cereal/legume intercropping increased dry-matter production and grain yield more than their monocultures. The N transfer from legume to cereal increased the cropping system's yield and efficiency of N uses. The taller cereal reduces biological N fixation and yield of the associated legume. Also, intercropping can be a method of improving diversity in agricultural ecosystem. It provides farmers with the chance to simulate natures´ principle of diversity on their farms. Complementarities in models of resource use should be considered to get better productivity in intercropping systems.

Keywords: *intercropping, productivity, legumes, agricultural practice.*

Introduction

As a result of the rapid population growth, the demand for food production is increasing, but unfortunately the availability of cultivated land remains the same or decreases. Thus, the only way to increase agricultural output is to increase yields per year (Odedina *et al.*, 2014).
Intercropping has a very old history and it is define as the agricultural practice of cultivating two or more plant species in the same area at the same time. Sowing time and harvest time may not be the same but most of the times are time-matched (Ghanbari and Lee, 2003; FAO, 1983). In an intercropping system it is possible to combine annually with annual plants, annual with perennials and perennials with perennial plants without any problem (Eskandari *et al*., 2009; Mousavi and Eskandari, 2011).
The intersection provides territorial coverage throughout the year or at least for longer than monocultures in order to protect the soil from dehydration and erosion. By cultivating more than one crop at a time in the same field, farmers maximize the efficiency of water use, maintain soil fertility and minimize soil erosion, which are serious disadvantages of monoculture. It also reduces the peak of seasonal work as a result, of different planting and harvesting time between crops. In addition, it could serve to increase production per unit area, in particular with low levels of external input, since a mixture of species better uses the available nutrients and water in the soil(Waktola, 2014).

Several scientific studies have been carried out on cereals and legumes interconnection systems and prove their success compared to monocultures. Some studies have shown that intercropping culture was more productive than the monoculture due to the complementary effect of the intercrops of such studies included, cucumber-cowpea, amaranth-cowpea (Bhatti *et al*., 2013) cowpea-corn (Waktola, 2014) and cowpea mushrooms (Gomez and Gomez, 1983).
In this review study, we are informed about the use and importance of the intercropping system which is mainly based on very old one practices and which is of great importance in recent years in agricultural production.

Mechanisms

Complementarity is a general term describing the positive effects that can result from intercropping. There are two mechanisms that contribute to complementarity: resource allocation and facilitation. Resource allocation, also known as segregation and position differentiation, describes the fullest use of available resources from combining crops compared grown separately. The choice of plants for the method of mating depends on the depth of rooting, phenology and plant architecture between the cultivated species at a junction can minimize competition and increase resource allocation. A classic example is the combination of corn seeds (*Zea mays* L., *Phaseolus vulgaris* L. and *Cucurbita pepo* L. respectively) (Litrico and Violle, 2015). In this case, the allocation of resources depends on the differences in the characteristics between the beans that determine the nitrogen, the low growth of bean which covers the soil and suppresses the weeds and the tall corn that serves as a pergola for the beans (Pleasant and Burt, 2010).
The facility refers to processes whereby one type of crop provides a limiting resource or improves the environmental status of another crop. A classic example is the supply of nitrogen from legumes to the soil. Another example is when a deep-rooted species provides water to species difficult to absorb groundwater by hydraulic lifting. As an indirect facilitation, you also consider the beneficial change in the rhizosphere through the terrestrial microorganisms that can grow a plant species thus improving the availability and transport of nutrients from one plant to another (Brooker *et al.*, 2015).

Types of intercropping

Cultivations can be grown together in a variety of ways. **Mixed** intercropping is the practice in which two or more species simultaneously develop in a field without the use of any particular spatial configuration. In this type of intercropping, there are no distinct rows of plant species.
Opposite, the **strip** intercropping is the practice of cultivating two or more crops in separate but adjacent rows at the same time close enough to each other to interact. While these types of intercropping vary depending on spatial configuration, crops can also be inserted in different ways that change in time. The **relay** intercropping comprises the step-by-step planting of two or more cultivations together so as to overlap only parts of their life cycles (Altieri, 1995; Brooker et al., 2015).

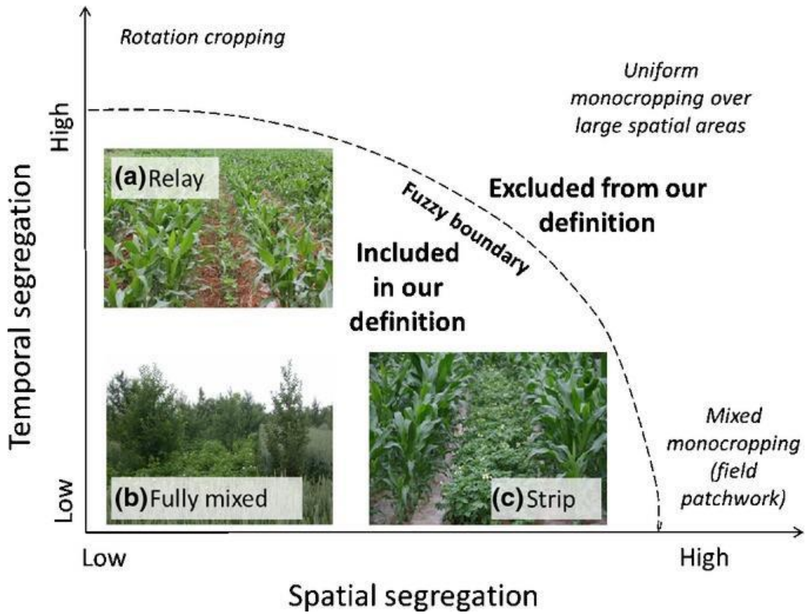

Figure 1. Presentation of the distinction according to degree of both spatial (x-axis) and temporal separation (y-axis) two (or more) cultivars using different intercropping types (Brooker *et al.*, 2015).

Advantages of intercropping

Intercropping is used to gain the most benefit. The advantage of intercropping is that increases productivity by reducing external inputs. It has a low cost that's why is very useful for low income farmers (Eskandari, 2012). There are many reports on the positive results and the superiority of intercropping than the monoculture. The most important advantages of intercropping are:

- **Increased crop production**

One of the main reasons for the use of intercropping is that increased crop production more than a pure cropping(Caballero and Goicoechea, 1995).Intercropping has been shown to reduce the risk of crop failure by increasing crop yield stability over time and in all locations (Bybee *et al*., 2016; Raseduzzaman and Jensen, 2017). Sustainability of crop yield can be increased by decreasing the variation of years at the same site or the increase of production coherence across the region year (Williamson, 2018).For example, experiments have determined that the wheat and bean combination increases production and stability as compared to pure culture (Ghanbari and Lee, 2002). We also, obtained the same results from the bean stew with barley (Martin and Snaydon, 1982).

- **Better resource management**

Increased crop productionoften seen at intercropping compared to unique crops was the result of the full exploitation of natural resources such as solar energy, soil nutrients and water (Szumigalski and Van-Acker, 2008).

- **Management of weeds**

It is known that weeds interact with crops, causing problems through competition (for light, water, nutrients and space) or allopathy. Intercropping is more effective than monoculture to suppressing weeds, but their efficacy varies greatly (Girjesh and Patil, 1991). The benefits of weed control over pure cultivation are two ways. First, there may be weed suppression or may provide yield advantages without suppressing weed growth below the levels observed in net harvesting of components, if the intersections use resources that are not exploitable by the weed (Matt and Dyck, 1993; Hauggaard *et al.* 2003).

- **Reduction of pests, diseases**

An important advantage of the interaction is that it mitigates harmful organisms and diseases (Willey, 1990). Generally strategies to reduce pest infestations can be divided into three groups:

1) By breaking down the ability of a pest to attack the host due to the many existing cultivations(Watiki *et al.*, 1993).
2) Residual trapping of parasitesor pathogens to prevent damage to the main species.
3) Remediation of parasites from natural enemies present in the species (Danso *et al*., 1987).

Though the junction does not happenalways reduce parasites or pathogen, most reports delay the reduction of parasite and disease populations in the region(Fujita *et al.,* 1992).

Example of successful intercropping (*Panicum miliaceum – Pisum sativum*)

Panicum miliaceum, also known as proso millet, belongs to the family of *Poaceae*, and it is considered one of the oldest domesticated plants. Nowadays, the *Panicum miliaceum* is mainly grown in India, China, Russia, USA (McDonald and Dowey, 2003). It has a superficial root system. The height of plants ranges from 30 to 100 cm depending on genotype and environmental conditions. Its stems are internally hollow and the stem diameter ranges from 20 to 50 mm. Oftenlaterally branches develop at αsufficient height from the ground surface. The leaves are lanceolate, about 30 cm long, with a small tongue. Strains and leaves are covered with light fluff (Chai, 1999). It does not have high nutrient requirements, withstanding drought and high temperatures with low water requirements compared to cereal crops (Lyon *et al*., 2008; Rajput *et al.,* 2016).

Pisum sativum is an annual herbaceous plant. It is known fresh as vegetable and dryas legume. It thrives in cold areas of the beloved areas up to 670 north width and height up to 2,000m. The root system consists of a strong pile root and a rich networklateral roots. The root can reach a depth of 1m or more. The stem is thin, tender, has an angular or round cross section and is hollow internally.

The length of the shoots ranges from 45 to 120cm, but the plants usually do notshow this height because they are sloping. The pea can be adapted to a variety of soils, however it prefers fertilizer clay with very good drainage pH 5.6 -7.5 (Pavek, 2012).

Generally, the intercropping of cereals and *Pisum sativum* L. leads to the reduction of external inputs. The nitrogen produced by the plants covers some of the grain needs, resulting in greater productivity and growth of plants by reducing the use of compound fertilizers. In addition, the combination of the two plant species improves plant health (Jensen *et al.,* 2006; Kadziuliene *et al.,* 2011).

Intercropping had increase in the protein content of cereals, increasing their nutritional value to a high level (Sarunaite *et al.,* 2012). This is also shown by experiments carried out by intercropping millet with *Fabaceae* family species like (cowpea) which have an increase in protein content compared to monocultures. The content of crude fiber are lower in intercropping experimentthe fiber content is low in intercropping system in relation to monocultures which areconsidered positive because it facilitates the digestion of lives. Percentof ash is higher in sole millet (Islam *et al.,* 2018). Finally,intercropping helps to control weeds because the combination of plant species gives very good results having a suppressive effect on weeds (Bellwood, 2005).

Conclusions

Intercropping is a useful tooland has been recognized through numerous scientific studies. Farmers can achieve an increase in production of the main crop combined with additional income from the second or third crop, making maximum use of agricultural land. The concentration does not only increase the farmer's revenue due to the increase in production but also reduces his costs in fertilizers, herbicides and water. This is a major asset and can improve the financial situation of small farmers in particular. Finally, it is a friendly method for environments that reduces the negative impacts of modern agriculture and can contribute to sustainable intensification of industrialized agricultural landscapes.

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A MISUNDERSTANDING BUT VERY PROMISING CROP: LUPINS

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Abstract

Grain legumes, also called pulses, are crops of the botanical family *Fabaceae*.Genus *Lupinus*spp. isalso included in this family.There are over 150 different species of lupines. Some of them are ideally suited to agricultural production due to their nature as nitrogen fixing grain legumes that develop seeds with high protein and high energy contents, which can be grown effectively in northern and southern climates. There are three lupine species of agricultural significance at present: narrow-leafed (*L.angustifolius*), white (*L. albus*) and yellow (*L. luteus*).The inclusion of lupine in crop rotation positively influences biodiversity and soil fertility as the bacteria at the root of lupine symbiotically absorb nitrogen (N) from the air. Lupine plant residues provide the next culture with 32–96 kg N/ha, while the accumulation of nitrogen in lupine biomass ranges from 199–372 kg N/ha, 86% of which is the result of symbiotic fixation.Lupine can be grown on less fertile, acidic and sandy soils where other crops produce lower yield. This is also very important for crop rotation, especially in organic agricultural production, due to its positive impact on the yield of subsequent crops, mainly cereals.Lupine seeds are of low price and non-genetic modified ingredients that constitute of good protein sources (ca. 40%), fiber (ca. 28%), healthy fatty acids, vitamins, minerals and other metabolites with recognized antioxidant properties (e.g., polyphenols). Despite the low European production of grain legumes, European countries exhibit suitable soil-climatic conditions in order for this crop to be cultivated.Measures towards increasing their local production have already been purposed by the European Commission as a way of decreasing the external dependence on soybeans.

Keywords:*lupine, cultivation, animal feed, nutritional value.*

Introduction

The value of lupine in recent centuries has grown considerably over the past few years. As a legume, Lupine provides nitrogen to the soil by setting a very good choice for crop rotational systems.Legumes are a very promising category of plants that grow without the need of special irrigation, which fits perfectly mainly in the dry-heat conditions of the less fertile areas (Naumkin *et al.*, 2015). Although humans have been consuming lupines for many years, beneficial properties in human and animal body have become known in recent years through scientific research. The lack of knowledge regarding the great benefits of lupines for dairy products has led to a global decline in world production (Naumkin *et al.*, 2015**;** Tan *et al.*, 2014).

Nowadays, lupine cultivation has begun to grow again by creating new varieties that eliminate the disadvantages of cultivation. Lupine plant residues contain about 32-96 kg N/ha, while in its biomass the nitrogen content is 199-372 kg/ha. (Unkovich et al., 1994).

Lupine is one of the best choices for animal feedingdueto the high protein and carbohydrate content and the low starch levels. Soluble and insoluble non-starch polysaccharides positively influencefood quality, allowing proper absorption of other nutrients. In terms of seed composition, lupine has a high level of protein (40%), fat (28%), antioxidants and vitamins that increase their nutritional value (Curti*et al.*, 2018; Melde *et al.*, 2016). However, some

varieties contain alkaloids which can cause stomach upsets in animals and humans (Magalhã es*et al.*, 2017).
In addition, production costs in relation to other feeds are particularly competitive, making it a good choice for a ruminant. Finally, the lupine is easy to store and manage so it can be easily used by livestock units.
Although the lupine is one of the best choices as a feed with nutritional value equals and sometimes can be better than other feeds.It is not used widely due to lack of knowledge and information. The purpose of the work is to inform the scientific community about the advantages of lupine and to try to solve some misunderstanding of previous years.

Climate, Soil, Yield and Cultivars

Despite the low European production of grain legumes,European countries present suitable edapho-climatic conditions to cultivate lupines. Guidelines toincrease local production have already been proposed by the European Commission as a way to reduce soybean external dependence.
Yellow lupine (*L. luteus*) breeding is restricted in Europe. Despite the fact that it is extremely resistant to drought and that it can withstands pH=4, due to the low resistance to diseases,it is not cultivated.In Poland, the yields of this variety were 1.5-2.5 t/ha on sandy soil(Gresta *et al*., 2017).
Narrow-leafed lupine (*L. angustifolius*) is grown in northern Europe and requires a wide range of pH values between 5 and 6.8(Gresta *et al*., 2017).The largest yields in Germany over the period 2009-2011 were 3.5-5 t/ha and related to the 'Boregine', 'Haagena' and 'Sonate' varieties. On the other hand, the cultivation of the same varieties on sandy soil produced 2-3.5 tn/ha (Guddat *et al*., 2011).
White lupine (*L. albus*) is well adapted to different soil types, with greater preference for loamy and light clay soils. Furthermore, lupine has excellent calcium resistance and a biological cycle of many days (140-200 days; Gresta *et al*., 2017).

Sowing time

In Mediterranean areas,lupines' sow is usually carried out in autumn as to be able to get higher yields compared with yields of different sowing time (Annicchiarico and Carroni, 2009). White lupine has a temperature resistanceup to -8 ^{0}C while the narrow-leafed lupine can withstand up to -10^{0}C. Therefore, narrow-leafed lupinehas a higher temperature resistance and is recommended for early sowing in spring. In North areas, sowing is not recommended in autumn, as the prolonged low temperaturesof winter have negative effects on growth (Prins, 2014).

Fertilization

Potassium sulphate fertilization in Dutch had a positive effect on the final quality of the lupine and reduced the levels of alkaloids, while there was not observed any differences on the harvested yield (Prins, 2014; Prins and Nuijtnet, 2015). Finally, nitrogen fertilization had not any effect on crops in northern Europe (Prins, 2014).

Weed Control

There is a high competition between weeds and lupines. Weed control or restriction can be achieved in two ways; either by applying high planting density (Herbert *et al*., 1978; Isaac *et al*., 2000) or by using chemical-mechanic weed control.The application of chemical control is limited to lupine cultivation (Prins, 2014) due to its sensitivity to herbicides.Weed population decreased significantly with grubbing 4-5 times in the first two months (Jensen *et al*., 2004a).

Irrigation
According to previous studies, irrigation (at the required levels) increased crop yield, while *Botrytis cinerea* Pers. infestation has led to a yield decrease (Jensen *et al*., 2004b).

Alkaloids in lupines
Lupines contain nine alkaloid compounds with quinolizidine: lupinine, sparteine, angustifoline, α-isopolinone, lupanine, 11,12-dehydrolupanine, 13α-hydroxylupanine, piperidine (smipine) and indole (gamine). Sparteine is the most dominant alkaloid of the mentioned above in Yellow Lupine, while in White and Narrow-leafed lupines lupanine is the major one (Magalhães *et al*., 2015).
Lupine is used extensively in livestock farming. According to its high protein content,lupine is used for the nutrition of the ruminants and the monogastric animals. The prolonged use of such foods for ruminants increases the resistance to the harmful effects of alkaloids. However, lupine varieties with alkaloid content greater than > 1000 mg/100 g dry matter must be avoided (Magalhães *et al*., 2015).
Consequently, sweet white and yellow lupine are suitable for animal feeding, while in case of other varieties a debittering process before using these is necessary (Magalhães *et al*., 2015).

Tolerance to biotic stress
White lupine is predominantly attacked by an insect called *Phorbiaplatura*. The infestation occurs in the roots and hypocotyls of the larvae have a higher incidence during autumn sowing. The control can be done in two ways, either by application of soil insecticides or by coating of systemic seeds insecticides. Regarding fungal diseases, white lupine is affected by 3 fungi (Huyghe, 1997). *Pleiochaetasetosa* is characterized mainly by the appearance of brown spots on the leaves of the plant and, secondly, by the destruction of the roots of *L. angustifolius.* The use of winter-hardy progenitors had positive results on increasing the plants resistance to fungi (Cowling, 1988).
Uromyceslupinicolus occurs in hot and dry periods such as in summer period. The reduction in biomass production and leaves premature fall can be treatedby the application of triazole group fungicides (Huyghe, 1997).
Colletotrichum gloeosporioides, known as Anthracnose, primarily affects the seed. Consequently, the appearance of the symptoms (cankers on the stems) occurs in the early stages of the plant's life cycle resulting in its destruction before flowering (Von Baer and Hashagen, 1996)

Tolerance to abiotic stress
Abiotic stress is mainly due to drought, high pH and frost (Huyghe, 1997). The varieties sown in autumn are highly resistant to frost (Huyghe and Papineau, 1990).Primarily, when sowing takes place in the early autumn, the plants have a better developed and larger root system and therefore have greater resistance to soil cooling.White lupine is cold-tolerant, but temperatures of -6^{0}C to -7^{0}C are harmful at germination. Cool temperate weather conditions are important during the vegetative stage. Temperatures lower than 10^{0}C and short days are required to induce flowering (Putnam, 1993). It appears that bitter cultivars tolerate cold and disease stress better than sweet ones.(Jansen, 2006). Finally, it has been found that the resistance is inherited (Huyghe, 1997).
A second abiotic factor is water logging, which can cause root damage resulting in *Fusarium* and *Botrytis cinerea* infection. The solution to this problem is the early sow (Huyghe, 1997).White lupine thrives on soils with pH ranging from 4.5-7.5. However, aluminium toxicity and iron chlorosis have been observed at valuesbelow 4.5 and above 7.5.Lupine is susceptible to alkalinity due to free lime. In high alkaline, *L. pilosus* has shown high

resistanceto other white lupine varieties. This sensitivity is mainly due to the mechanisms of phosphorus and iron uptake by the roots (Gerke *et al*., 1994).Plant nutrition becomes impossible (Huyghe, 1997) as calcium citrate is destroyed in the presence of free lime (Dinkelaker *et al*., 1989).

Drought remains an abiotic stressor. It is important during the blooming and the formation of the pod to avoid droughtand to complete its flowering. Consequently, the completion of the biological cycle of lupine must be completed before drought period. The choice of autumn sowing and early flowering varieties contributes significantly to this. In Western Europe, the choice of such varieties results in reduced photosynthetic ability. However, temporary stresses may occur during the development of the pod (Rodrigues *et al.*, 1995; Chaves, 1994).

Conclusions

Lupine is a plant that has long been known for its nutritional potential and its cultivation is gaining ground throughout Europe.Lupine is a promising crop of livestock legumes for world livestock. It is a low cost crop with a protein content equal to that of soy (30-42%), making it one of the best animal nutrition options.

The process of converting raw materials into alternative animal feeds has become imperative because of global concern over genetically modified products.Lupinecould be a solution in GMOs problem by limiting the production of soybean, due to its advantages.

Finally, further research for the creation of new varieties as well as cultivation processing practices should be carried out in order to improve the efficiency of the lupine.

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ASSESSMENT OF CEREAL SPECIES BASED ON YIELD AND AGRO-PHYSIOLOGICAL PARAMETERS UNDER RAINFED CONDITION

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Abstract

Because of the various environment conditions yield, quality and other agro-physiological parameters in cereal species vary based on genotype, environment and its interaction. The experiment was carried out to assess bread wheat, durum wheat, barley and triticale cultivars for grain yield, agronomic parameters, physiological and quality parameters under rainfed conditions. In this experiment a total of 17 cultivars (5 bread wheat, 4 durum wheat, 4 barley, and 4 triticale) were evaluated during 2017-2018 growing season. The experiment was conducted in the randomized completely blocks design with four replications. Grain yield, days of heading, plant height, number of spike per square meter, aboveground biomass (NDVI), chlorophyll content (SPAD), canopy temperature, 1000-kernel weight, test weight, and protein ratio were investigated. Analysis of the variance revealed that there were significant differences among cereal species and cultivars for the parameters tasted. Mean grain yield for bread wheat was 6643 kg ha^{-1}, for barley 5588 kg ha^{-1}, for durum wheat 5193 kg ha^{-1} and for triticale 4914 kg ha^{-1}. According to results, bread wheat cultivars had higher grain yield and aboveground biomass, barley cultivars had higher number of tiller per square meters, and chlorophyll content. Triticale cultivars had higher plant height and barley had short plant height. Barley cultivars had higher 1000-kernel weight followed by bread wheat. Bread wheat had higher test weight and durum wheat had higher protein ratio. Result of the study showed that bread wheat had higher grain yield and desirable parameters tested than durum wheat, barley and triticale cultivars, so bread wheat has more advantageous than the other cereals crops.

Keywords: *Cereals species, cultivars, yield, agro-physiological traits*

Introduction

Almost all breeding programs in the world aim to improve varieties with stable yields. The yield stability is generally grouped as static or dynamic stability (Pfeiffer and Braun, 1989). Cereals crops play an important role in the agricultural system. Its grain yield and quality varies based on environment factors, agronomic practices and cereals crops. Various factors are considered responsible for better yield. Selection of improved and high yielding genotypes of different cereals having a wide range of adaptation to agro climatic conditions is essential to increase the yield (Shah *et al*., 2002). Different varieties of cereals respond differently to agro-climatic conditions of a particular area (Behara, 1994). Apparently, morpho-physiological traits of growth and development have the greatest impact on the adaptation of plants to the target environments with the aim of achieving a maximum productivity. Selection criteria based on morphological, physiological and biochemical traits have been suggested for screening drought tolerance in wheat (Araus *et al*., 2002). The adaptation strategies of the plants to drought stress include drought escape, drought avoidance and drought tolerance. Among these strategies, escaping drought involves the completion of the life cycle before the onset of the drought period. Therefore, early maturity has been known as a major drought escaping mechanism, particularly in terminal drought stresses (Levitt, 1980; Chaves *et a*l., 2002).

Bread wheat (*Triticum aestivum* L.) and barley are the most important and widely producing cereal crops throughout the Thrace region of Turkey (Öztürk and Korkut, 2017). Although the amount of the rainfall during growing season is enough for wheat production, the distribution of this rainfall is not regular. This fluctuation of rainfall causes reducing grain yield and quality in cereal crops (Öztürk and Korkut, 2018). Therefore, the objective of this study was to evaluate the performance and compare of the cereal crops cultivars to investigate their yield quality and agro-physiological character under rainfed condition in Trakya region, Turkey.

Material and Methods

The experiments were conducted during 2017-2018 growing years in Edirne locations, Turkey. Five bread wheat, four durum wheat, four barley and four triticale cultivars were studied in a randomized complete block design (RCBD) with four replications. Each plot was 6 meter long and had 6 rows, spaced 0.17 meters apart. A planting rate of 500 seeds m^2 was used. In this experiment a total of 17 cultivars were evaluated during 2017-2018 growing cycle. The experiment was conducted in randomized completely blocks design with four replications. Grain yield, days of heading, plant height, number of spike per square meter, 1000-kernel weight, test weight, and protein content were investigated. The Zadoks Decimal Code (Z) was used to describe plant growth stages (Zadoks *et. al*., 1974).

Grain yield, days of heading, plant height was taken from plot in each replication. In the experiments, 1000-kernel weights and test weight (Blakeney *et al*., 2009), protein ratio, (Köksel *et al*., 2000; Perten H, 1990; Pena, 2002) were determined. In the research; biomass (Gutierrez-Rodriguez *et al*, 2004), canopy temperature (Babar et al, 2006; Blum 2000; Jackson et al, 1981; Reynolds et al., 2001), chlorophyll content (Babar *et al*, 2006; Adamsen *et al*., 1999) were investigated. Physiological characters canopy temperature (CT), chlorophyll content (SPAD), and aboveground biomass (NDVI) were measured at heading stage of the plant development. Canopy temperature measurements were taken when the sky was clear and there was little or no wind and plant surfaces are dry. Canopy temperature measurements were made between 11:00h to 14:00h (Pask *et al*., 2012).

The analysis of variance for each character was measured followed by LSD to test significance difference between means (Steel and Torrie, 1980), and simple correlation coefficient and path analysis, which is the direct and indirect effects of each character was performed as per method of Dewey and Lu (1959). Data were analysed statistically for analysis of variance following the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test.

Results and Discussion

Trakya region has various environment conditions, so mainly issue for plant breeders in improving yield across variable environments. The analysis of variance for yield and quality components was performed and given in Tables 1 to 6. The results of variance analyses showed that there were significant differences ($P<0.01$) among genotypes based on locations. Grain yield in bread wheat ranged from the highest 6948 kg ha^{-1} to the smallest 6535 kg ha^{-1} Table 3. The mean grain yield was 6645 kg ha^{-1}. The highest grain yield performed by cultivar Gelibolu. Mean aboveground biomass of the cultivars was 0.74, 0.79 and 0.75 at Z30, Z45 and Z55 growth stages, respectively. Mean chlorophyll content of the cultivars was 46.9, and cultivar Tekirdağ had higher chlorophyll content. Canopy temperature was measured at Z55 growth stages and cultivar Gelibolu had the lowest canopy temperature under rainfed condition.

Table 1. Combined analysis of variance for bread and durum wheat cultivars for yield

Source of variation	Bread wheat			Durum wheat		
	DF	MS	F value	DF	MS	F value
Replication	3	1433.50	1.09	3	6007.58	12.29**
Cultivars	4	1217.64	0.93	3	2946.40	6.03*
Error	12	1310.55		9	488.62	

Note: *, ** Significance at respectively 5% and 1% level probability. Bread wheat: CV (%): 5.4, Durum wheat CV (%): 4.2

Table 2. Combined analysis of variance for barley and triticale cultivars for yield

Source of variation	Barley			Triticale		
	DF	MS	F value	DF	MS	F value
Replication	3	2051.44	1.55	3	6881.08	3.24
Cultivars	3	9472.99	7.17**	3	6333.46	2.98
Error	9	1321.58		9	2123.57	

Note: *, ** Significance at respectively 5% and 1% level probability. Triticale: CV (%): 9.3, Barley CV (%): 6.5

Selimiye was the early cultivars, while Pehlivan was late. Cultivars Gelibolu and Pehlivan had higher tillering capacity. Cultivars Selimiye and Pehlivan had higher thousand kernel weight and test weight (Table 3). Grain protein content is among the key determinants affecting of both end use and market value in wheat (Pena, 2002) also, protein quality and quantity is the most important components of wheat grains governing end-use quality (Pena, 2008; Niu et al., 2010). In the research cultivar Saban had higher protein ratio.

Table 3. Mean performance of physiological and quality parameters in bread wheat

No	**Cultivars**	**GY**	**NDVI Z30**	**NDVI Z45**	**NDVI Z55**	**SPAD Z55**	**CT Z55**
1	Selimiye	6535 a	0.78 a	0.79 ab	0.74 bc	47.7 a	29.3 ab
2	Pehlivan	6628 a	0.77 a	0.80 ab	0.75 b	44.3 a	28.2 ab
3	Gelibolu	6948 a	0.67 c	0.78 b	0.72 c	47.9 a	26.6 b
4	Tekirdağ	6549 a	0.73 b	0.82 a	0.81 a	48.7 a	30.6 a
5	Saban	6553 a	0.77 a	0.77 b	0.74 bc	45.9 a	29.3 ab
	Mean	6643	0.74	0.79	0.75	46.9	28.8
No	**Cultivars**	**PH**	**DH**	**SNM**	**TKW**	**TW**	**PRT**
1	Selimiye	96.5 b	112.5 d	415.5 a	41.8	83.1	9.9
2	Pehlivan	101.8 a	116.5 a	429.0 a	45.7	82.0	9.8
3	Gelibolu	87.3 c	112.8 cd	429.5 a	37.5	81.6	8.4
4	Tekirdağ	84.3 c	114.5 b	391.8 a	38.0	77.9	9.5
5	Saban	82.5 c	114.0 bc	411.5 a	40.1	78.4	10.1
	Mean	90.5	114.1	415.5	40.6	80.6	9.5

Note: **: P<0.01, *: P<0.05, ns: not significant, GY: Grain yield (kg ha^{-1}), NDVI: Aboveground biomass, SPAD: Chlorophyll content, CT: Canopy temperature (^{o}C), PH: Plant height (cm), DH: Days of heading, SNM: Spike number per square meter, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%)

Grain yield in durum wheat ranged from the highest 5589 kg ha^{-1} to the smallest 4990 kg ha^{-1}. The mean grain yield was 5193 kg ha^{-1}. The highest grain yield performed by cultivar Ç1252. Mean aboveground biomass of the cultivars was 0.64, 0.68 and 0.57 at Z30, Z45 and Z55 growth phase. Mean chlorophyll content of the cultivars was 46.9, and cultivars Eminbey and Mirzabey had higher chlorophyll content. Canopy temperature was measured at Z55 growth stages and cultivar Mirzabey had the lowest canopy temperature. With regard to genotypic effects, cultivar Ç1252 exhibited the highest plant height (95.3 cm), on the other hand, Mirzabey, which was showed the lowest plant height (88.3 cm). Cultivar Kızıltan had higher spike number per square meter and Ç1252 had higher 1000-kernel weight (TKW) and test weight (TW) (Table 4).

Table 4. Mean performance of physiological and quality parameters in durum wheat

No	Cultivars	GY	NDVI Z30	NDVI Z45	NDVI Z55	SPAD Z55	CT Z55
1	Kızıltan	4990 b	0.65 a	0.67 a	0.54 a	45.2 a	30.0 a
2	Eminbey	5143 b	0.64 a	0.70 a	0.58 a	48.8 a	30.2 a
3	Mirzabey	5050 b	0.63 a	0.65 a	0.59 a	48.6 a	29.5 a
4	Ç1252	5589 a	0.66 a	0.72 a	0.57 a	44.9 a	29.7 a
	Mean	5193	0.64	0.68	0.57	46.9	29.8
No	**Cultivars**	**PH**	**DH**	**SNM**	**TKW**	**TW**	**PRT**
1	Kızıltan	93.0 ab	124.5 ab	308.0 a	38.2	79.5	9.4
2	Eminbey	89.8 b	124.3 b	295.0 a	34.5	78.1	10.7
3	Mirzabey	88.3 b	125.3 a	271.8 a	38.6	78.3	10.5
4	Ç1252	95.3 a	122.5 c	289.3 a	41.5	82.7	10.3
	Mean	91.5	124.1	291.0	38.2	79.7	10.2

Note: **: P<0.01, *: P<0.05, ns: not significant, GY: Grain yield (kg ha^{-1}), NDVI: Aboveground biomass, SPAD: Chlorophyll content, CT: Canopy temperature (^{o}C), PH: Plant height (cm), DH: Days of heading, SNM: Spike number per square meter, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%)

Grain yield in barley cultivars ranged from the highest 6256 kg ha^{-1} to the smallest 5199 kg ha^{-1}. The mean grain yield was 5589 kg ha^{-1}. The highest grain yield performed by cultivar Hasat. Cultivar Hasat had also higher biomass (NDVI) at Z30, Z45 and Z55 growth stages. Mean chlorophyll content of the barley cultivars was 50.4, and cultivars Sladoran had higher chlorophyll content. Cultivars Hasat and Bolayır exhibited the highest plant height and, also Cultivar Bolayır and Hasat had higher spike number per square meter. Among cultivars 1000-kernel weight ranged from 40.0 g to 45.7 g and, mean was 42.0 g. Cultivar Harman had the highest 1000-kernel weight. Test weight varied among cultivars from 72.9 kg to 74.9 kg and the highest test weight was determined in cultivar Harman (Table 5).

Table 5. Mean performance of physiological, yield and quality parameters in barley

No	Cultivars	GY	NDVI Z30	NDVI Z45	NDVI Z55	SPAD Z55	CT Z55
1	Sladoran	5199 b	0.56 b	0.58 b	0.58 a	51.9 a	26.0 a
2	Bolayır	5257 b	0.60 b	0.61 ab	0.63 a	48.3 b	26.3 a
3	Harman	5643 b	0.63 ab	0.63 ab	0.63 a	50.8 ab	26.0 a
4	Hasat	6256 a	0.71 a	0.72 a	0.67 a	50.8 ab	25.9 a
	Mean	5589	0.62	0.63	0.63	50.4	26.1
No	**Cultivars**	**PH**	**DH**	**SNM**	**TKW**	**TW**	**PRT**
1	Sladoran	80.5 b	107.5 a	385.0 b	41.0	74.1	8.8
2	Bolayır	88.8 a	106.8 a	498.3 a	40.0	74.4	9.4
3	Harman	87.0 a	105.3 b	413.5 ab	45.7	74.9	10.4
4	Hasat	92.0 a	105.3 b	496.3 a	41.3	72.9	9.5
	Mean	87.1	106.2	448.3	42.0	74.1	9.5

Note: **: P<0.01, *: P<0.05, ns: not significant, GY: Grain yield (kg ha^{-1}), NDVI: Aboveground biomass, SPAD: Chlorophyll content, CT: Canopy temperature (^{o}C), PH: Plant height (cm), DH: Days of heading, SNM: Spike number per square meter, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%)

Based on grain yield in triticale cultivar MİKHAM had the highest (5312 kg ha^{-1}) yield and followed by Tatlıcak-97. The mean grain yield was 4913 kg ha^{-1}. The highest biomass was performed by cultivar Tatlıcak-97. Cultivar MİKHAM had the highest chlorophyll content and lower canopy temperature. Cultivars Presto and Tatlıcak-97 had the highest spike number per square meter. The highest thousand kernel weight was determined in cultivars MİKHAM (Table 6).

Table 6. Mean performance of physiological, yield and quality parameters in triticale

No	Cultivars	GY	NDVI Z30	NDVI Z45	NDVI Z55	SPAD Z55	CT Z55
1	Tatlıcak-97	5199 ab	0.75 a	0.79 a	0.59 a	48.9 bc	27.5 a
2	MİKHAM	5312 a	0.71 ab	0.76 ab	0.48 b	52.6 a	26.6 b
3	Presto	4578 ab	0.65 b	0.74 b	0.50 b	50.8 ab	27.5 a
4	Truva	4564 b	0.71 ab	0.73 b	0.52 b	47.3 c	28.2 a
	Mean	4913	0.70	0.75	0.52	49.9	27.5
No	**Cultivars**	**PH**	**DH**	**SNM**	**TKW**	**TW**	**PRT**
1	Tatlıcak-97	118.3 a	119.3 b	372.8 a	29.1	70.5	10.5
2	MİKHAM	116.8 a	109.8 c	337.0 b	33.4	69.1	9.6
3	Presto	118.3 a	109.5 c	383.8 a	30.4	68.2	9.1
4	Truva	113.8 a	120.8 a	308.0 c	29.7	65.4	9.3
	Mean	116.8	114.8	350.4	30.7	68.3	9.6

Note: **: P<0.01, *: P<0.05, ns: not significant, GY: Grain yield (kg ha-1), NDVI: Aboveground biomass, SPAD: Chlorophyll content, CT: Canopy temperature (^{o}C), PH: Plant height (cm), DH: Days of heading, SNM: Spike number per square meter, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%)

Table 7. Coefficients of correlation between grain yield and tested parameters in bread wheat, durum wheat, barley and triticale cultivars

Traits	Bread wheat	Durum wheat	Barley	Triticale
	GY	GY	GY	GY
NDVI (Z30)	-0.875*	0.683	0.977**	0.640
NDVI (Z45)	-0.314	0.833	0.979**	0.812
NDVI (Z55)	-0.536	0.155	0.858	0.188
SPAD (Z55)	0.144	-0.456	0.180	0.509
CT (Z55)	-0.891*	-0.226	-0.650	-0.770
PH	-0.085	0.679	0.745	0.384
DH	-0.259	-0.931*	-0.840	-0.142
SNM	0.617	-0.129	0.453	0.113
TKW	-0.372	0.614	0.213	0.469
TW	0.289	0.872	-0.691	0.776
PRT	-0.930**	0.287	0.368	0.717

Note: **: P<0.01, *: P<0.05, GY: Grain yield (kg da^{-1}), NDVI: Aboveground biomass, SPAD: Chlorophyll content, CT: Canopy temperature (^{o}C), PH: Plant height (cm), DH: Days of heading, SNM: Spike number per square meter, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%)

Correlation coefficients based on the investigated parameters for bread wheat, durum wheat, barley and triticale were determined by Pearson's correlation analysis (Table 7). In bread wheat, it was found significant negative correlation between grain yield and NDVI at Z30 growth stage (r=-0.875*), canopy temperature at Z55 (r=-0.891*) and protein ratio (r=-0.930**). Grain yield was positively correlated with number of spike per square meter and slightly correlated with test weight. Correlation coefficients for durum wheat cultivars showed that, it was found positive correlation between grain yield and NDVI at Z30 growth stage, and Z45 growth stage. There was a negative relation between grain yield and days of heading (p=-0.931*) in durum wheat. Grain yield was positively correlated with plant height, TKW and TW. In the research, in barley cultivars it was found positive association between grain yield and biomass (NDVI) at Z30 (r=0.683) and Z45 (r=0.833). Grain yield was slightly positively correlated with plant height, 1000-kernel weight and test weight. There was a negative relation with grain yield and days of heading (r=-0.931*). In triticale cultivars, it was found positive association between grain yield and biomass (NDVI) at Z30 (r=0.640) and Z45 (r=0.812). Grain yield was positively correlated with chlorophyll content at Z55, 1000-kernel weight and test weight. There was a negative relation with grain yield and canopy temperature (r=-0.770).

Conclusions

Because of the various environment conditions yield, quality and other agro-physiological parameters varied in cereal crops based on genotype, environment and its interaction. Analysis of the variance revealed that there were significant differences among cereal crops and among cultivars for the parameters tasted. Bread wheat had the highest grain yield than other cereal crops. According to results, bread wheat cultivars had higher biomass, barley cultivars had higher number of tiller per square meters, and chlorophyll content. Triticale cultivars had higher plant height and barley had short plant height. Barley cultivars had higher 1000-kernel weight followed by bread wheat. Bread wheat had higher test weight and durum wheat had higher protein ratio. Result of the study showed that bread wheat had higher grain yield and desirable parameters tested than durum wheat, barley and triticale cultivars. Results also showed that physiological parameters such as biomass and canopy temperature could be used in cereal breeding programs for selection of the higher yielding genotypes.

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EFFECT OF YEAR AND FERTILIZATION ON WINTER BARLEY QUALITY

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Abstract
The experiment was established at the experimental field of the Small Grains Research Centre in Kragujevac (Serbia) during the two growing seasons. The objective of the research was to evaluate the effect of fertilization and the environment on the yield of winter barley. The following characteristics were analysed: grain yield, 1000 grain weight and test weight. The average grain yield and 1000 grain weight of all treatments in 2010/11 growing season was significantly greater than in 2009/10, mostly as the result of highly favourable weather conditions at major stages of plant development. The grain yield of the barley was significant lower in control (treatment without fertilizer). Barley yield was the highest in the NP_1K and NP_2K (4.199 and 4.290 t/ha) treatments. Variance analysis showed statistically very significant differences for grain yield, 1000 grain weight and test weight between the vegetation seasons and very significant differences for grain yield and 1000 grain weight between the effects of fertilization. Variance analysis showed very significant differences for 1000 grain weight between the interaction of the vegetation seasons and variants of fertilization.

Keywords: *barley, fertilization, nitrogen, yield*

Introduction
Barley is one of the oldest agricultural species, which has gone through genetic alterations during the process of domestication. The basic purpose of the barley usage has been changed during thousands of years of the cultivation, i.e. from the main grain in the human diet to the very important animal nutrient. The grain quality of barley is affected by genetic and environmental factors as well as their interaction. Because of this, the same cultivar in different years can behave like brewing or feeding barley, according to the content of grain nutrients, which determines end-use (Đekić *et al.*, 2017). It is known that individual or mutual influence of abiotic stress factors (high and low temperatures, drought, acidic and saline soil) in different barley growth stages limited expression of the maximum grain yield potential (Đekić *et al.*, 2019). The length and intensity of the stress period vary from the environment to the environment, as well as between years in the same environment (Bratković *et al.*, 2018).
Cultivars of the new generation exhibit the high degree of the tolerance against temperatures shocks during the phase of the forming and filling of grain as well as against drought. To achieve high and stable grain yields, newly cultivars are demand more precisely and more complex NPK nutrition (Jelić *et al.*, 2015). The role of the fertilization before basic tillage become more important in the global warming condition. Furthermore, a good soil supply with P_2O_5 and K_2O is very important, since barley need to absorb the same or higher amounts of these elements for a short period of time (Jelić *et al.*, 2014). Efficacy of the nitrogen utilization from mineral fertilizers is decreasing with increasing of the nitrogen fertilizing level (Đekić *et al.*, 2014; Jelić *et al.*, 2015; Terzic *et al.*, 2018). Nutrient utilization from fertilizers and yield forming are under the important influence

of weather conditions and specific characteristics of the location (Popović *et al.*, 2011; Đekić *et al.*, 2014, 2019; Pržulj *et al.*, 2014; Bratković *et al.*, 2018; Terzic *et al.*, 2018).
Because of appearance of new demanded cultivars at permanent changes in soil fertility level and environmental conditions, still exist need to researches fertilization of barley, as well as determine optimal rates and balanced nutrition ratios in concrete agro ecological conditions. The main goal of this research was to investigate the effect of long-term applications of identical amounts and ratios of nitrogen, phosphorus and potassium on the yield components of winter barley cultivar „Grand".

Material and methods

Meteorological conditions

Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by month. Data in Table 1 for the investigated period (2010-2011) clearly indicate that the years in which the researches were conducted differed from the typical long-year average for Kragujevac region, regarding the meteorological conditions. The average air temperature in 2009/10 growing season was higher by 0.37°C and 2010/11 was higher by 0.16°C than with the perennial average. The sum of rainfall precipitation in 2009/10 growing season was higher by 612.1 mm, where the sum of rainfall in 2010/11 growing season was 86.2 mm lower than the perennial average and with a very uneven distribution of precipitation per months. During the April and May in 2009/10 growing season it was 142.2 mm and 116.7 mm of rainfall, what was 90.3 mm and 59.1 mm more compared with the perennial average. During the June in 2009/10 it was 196.7 mm of rainfall, what was 126.3 mm more compared with the perennial average.
In addition to the necessary reserve for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil (Jelic *et al.*, 2015; Đekić *et al.*, 2015; Terzic *et al.*, 2018).

Table 1 *Precipitation sum and average monthly temperature in Kragujevac, Serbia*

Months	Mean monthly air temperature (^oC)			The amount of rainfall (mm)		
	2009/10	2010/11	Average	2009/10	2010/11	Average
X	11.7	10.2	12.5	102.6	86.9	45.4
XI	8.8	11.4	6.9	77.5	27.9	48.9
XII	2.6	2.4	1.9	194.2	50.1	56.6
I	0.9	0.9	0.5	57.0	29.1	58.2
II	3.2	0.5	2.4	150.5	48.5	46.6
III	7.2	7.2	7.1	43.3	20.4	32.4
IV	12.1	12.0	11.6	142.2	20.8	51.9
V	16.5	15.8	16.9	116.7	65.8	57.6
VI	20.2	20.9	20.0	196.7	32.3	70.4
Average	9.24	9.03	8.87	1080.1	381.8	468.0

Experimental design and statistical analysis

Effects of mineral nutrition efficiency of barley have been studied at the stationary field trial of the Small Grains Research Centre in Kragujevac (Serbia) for two growing seasons (2009/10 and 2010/11). The experiment was laid out in a randomised block design with five replications and a plot size of 10 m^2 (5 m x 2 m). In all years, winter barley was sown in the second half of October at a row spacing of 12.5 cm. The rate of nitrogen application were 80 kg/ha N. The barley cultivar used in the experiment was Grand. Six variants of mineral nutrition: control, N (80 kg/ha N), NP_1 (80 kg/ha N and 60 kg/ha P_2O_5), NP_2 (80 kg/ha N and 100 kg/ha P_2O_5), NP_1K (80 kg/ha N, 60 kg/ha P_2O_5 and 60 kg/ha K_2O) and NP_2K (80 kg/ha N,

100 kg/ha P_2O_5 and 60 kg/ha K_2O). The following traits were analysed: grain yield, 1000 grain weight and test weight. Grain yield was measured for each plot and calculated as grain yield in t/ha at 14% grain moisture. Then, a sample was taken for 1000 grain weight and test weight determination.
The soil used in the trial was vertisol having a very acid reaction (pH in KCI: 3.92-4.27), the content of total nitrogen was medium (0.12-0.15%), while the content of affordable phosphorus (26.9 mg P_2O_5/100 g soil) and potassium (21.0 mg of K_2O/100 g of soil) is high.
On the basis of achieved research results the usual variation statistical indicators were calculated: average values, standard error and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Results and discussion

The average grain yield, 1000-grain weight and test weight of all treatments was significantly higher in 2010/11 growing season (Table 2).

Table 2 *Grain yield, 1000 grain weight and test weight of winter barley*

Fertilization	Years								
	2009-2010			2010-2011			Average		
	x	S	Sx	x	S	Sx	x	S	Sx
	Grain yield, t/ha								
C	0.575^{c}	0.124	0.055	0.909^{d}	0.055	0.024	0.742^{c}	0.198	0.063
N	1.980^{b}	0.889	0.397	3.928^{c}	0.697	0.312	2.954^{b}	1.273	0.403
NP_1	2.122^{b}	0.377	0.168	3.660^{c}	0.768	0.343	2.891^{b}	0.991	0.313
NP_2	2.322^{b}	0.501	0.224	4.060^{bc}	1.117	0.500	3.191^{b}	1.227	0.388
NP_1K	3.407^{a}	0.660	0.295	4.991^{ab}	0.118	0.053	4.199^{a}	0.947	0.299
NP_2K	3.378^{a}	0.163	0.073	5.202^{a}	0.880	0.393	4.290^{a}	1.131	0.358
	1000 grain weight, g								
C	38.74^{b}	1.529	0.684	40.34^{c}	0.615	0.275	39.54^{c}	1.385	0.438
N	40.22^{b}	1.267	0.567	41.48^{c}	0.873	0.390	40.85^{bc}	1.222	0.386
NP_1	39.82^{b}	1.43	0.639	41.62^{c}	1.266	0.566	40.72^{bc}	1.587	0.502
NP_2	40.22^{b}	1.083	0.484	43.02^{b}	1.443	0.645	41.62^{b}	1.904	0.602
NP_1K	42.52^{a}	1.126	0.503	44.50^{a}	0.561	0.251	43.51^{a}	1.339	0.423
NP_2K	43.56^{a}	1.004	0.449	45.64^{a}	0.472	0.211	44.60^{a}	1.323	0.418
	Test weight, kg/hl								
C	61.62^{c}	2.081	0.931	61.62^{c}	0.383	0.171	61.62^{b}	1.411	0.446
N	66.15^{a}	1.419	0.634	65.85^{b}	1.621	0.725	66.00^{a}	1.445	0.457
NP_1	63.66^{bc}	1.912	0.855	65.93^{b}	1.830	0.818	64.79^{a}	2.131	0.674
NP_2	64.26^{ab}	1.538	0.688	65.33^{b}	1.136	0.508	64.79^{a}	1.393	0.441
NP_1K	62.28^{bc}	1.192	0.533	66.06^{b}	2.039	0.912	64.17^{a}	2.539	0.803
NP_2K	63.84^{abc}	1.926	0.861	68.41^{a}	1.166	0.521	66.12^{a}	2.836	0.897

The highest grain yield had application of NP_2K in a quantity of 80 kg/ha N, 100 kg/ha P_2O_5 and 60 kg/ha K_2O (4.290 t/ha). The grain yield of the barley was significantly lower in control (treatment without fertilizer). Average grain yield of treatments ranged from 0.575 t/ha (control) to 3.407 t/ha (NP_1K) in 2009/10 growing season and 0.909 t/ha (control) to 5.202 t/ha (NP_2K) in 2010/11 growing season. In all years, NP_1K and NP_2K treatments produced significantly higher grain yields compared in the other treatments. Considerable variation in yield depending on years of research have established Popović *et al*. (2011), Jelić *et al*. (2014), Madić *et al*. (2014) and Đekić *et al*. (2019).

The average 1000 grain weight of all treatments in the 2010/11 growing season was significantly greater than in the 2009/10 growing season, mostly as the result of highly favourable weather conditions at major stages of plant development. Averaged across years, significantly higher values for 1000 grain weight were found in NP_1K and NP_2K treatments (43.51 g and 44.60 g).
During the 2009/10 growing season, test weight was significantly greater in N treatment (66.15 kg/hl) than in the other treatments. During the 2010/11 year, NP_2K treatment (68.41 kg/hl) had significantly higher values for test weight compared to the other treatments.
Table 3 shows the impact of the year, fertilization and interaction of year x fertilization on yield, 1000 grain weight and test weight. Analysis of variance was found highly significant effect of year on the grain yield ($F=18.358^{**}$), 1000 grain weight ($F=13.237^{**}$) and test weight ($F=10.234^{**}$). Based on the analysis of variance, it can be concluded that there are very significant differences in grain yield, 1000 grain weight and test weight regard the treatments of fertilization (Table 3).

Table 3 *The analysis of variance for the traits analyzed in Kragujevac, Serbia*

Effect	df	Mean sqr Effect	Mean sqr Error	F	p-level
The analysis of variance for grain yield					
Year, (Y)	1, 58	33.494	1.824	18.358	0.0002
Fertilization, (F)	5, 54	16.480	1.054	15.635	0.0000
Year x Fertilization, (YxF)	5, 48	0.865	0.398	2.173	0.0726
The analysis of variance for 1000 grain					
Year, (Y)	1, 58	55.296	4.177	13.237	0.0006
Fertilization, (F)	5, 54	35.945	2.183	16.468	0.0000
Year x Fertilization, (YxF)	5, 48	0.678	1.233	0.550	0.7376
The analysis of variance for test weight					
Year, (Y)	1, 58	53.941	5.271	10.234	0.0022
Fertilization, (F)	5, 54	26.835	4.175	6.427	0.0001
Year x Fertilization, (YxF)	5, 48	9.961	2.536	3.928	0.0046

The grain yield was in a positive correlation with the 1000 grain weight as well as with the test weight (Table 4). Barley yield in 2009/10 growing season was positively and highly significant correlated with 1000 grain weight (0.718^{**}), but in the 2010/11 vegetation season grain yield in was positively and highly significant correlation with the 1000 grain weight (0.720^{**}) and test weight (0.666^{**}). The present results confirm the statement of many authors that the traits analyzed and their correlations are genetically determined but are strongly modified by the nutrient status of the environment and weather conditions (Popović *et al.*, 2011; Madić *et al.*, 2014; Jelic *et al.*, 2015; Đekić *et al.*, 2017; Jamil *et al.*, 2017; Terzic *et al.*, 2018).
Table 5 shows the correlation coefficients between the studied fertilization treatments and analysed traits. Positive correlations were observed between grain yield and thousand grain weight in all treatments. Positively and strong correlations were observed between grain yield and thousand grain weight in the NP_2 ($r=0.725^{*}$), in the NP_1K ($r=0.836^{**}$) and treatment NP_2K ($r=0.711^{*}$). Positively and medium strong correlations were observed between thousand grain weight and test weight in the unfertilized control ($r=0.669^{*}$), in the NP_1K ($r=0.698^{*}$) and treatment NP_2K ($r=0.649^{*}$).

Table 4 *Correlations between the traits analyzed*

	Grain yield	1000 grain weight	Test weight
Correlations between the traits analyzed in 2009-2010			
Grain yield (t/ha)	1.00	0.718^{**}	0.098^{ns}
1000 grain weight (g)		1.00	0.041^{ns}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in 2010-2011			
Grain yield (t/ha)	1.00	0.720^{**}	0.666^{**}
1000 grain weight (g)		1.00	0.666^{**}
Test weight (kg/hl)			1.00

^{ns}-non significant; *-significant at 0.05; **-significant at 0.01

Table 5 *Correlation coefficients for the traits analyzed across treatments*

	Grain yield	1000 grain weight	Test weight
Correlations between the traits analyzed in the unfertilized control			
Grain yield (t/ha)	1.00	0.507^{ns}	-0.055^{ns}
1000 grain weight (g)		1.00	0.669^{*}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the N			
Grain yield (t/ha)	1.00	0.496^{ns}	0.153^{ns}
1000 grain weight (g)		1.00	0.583^{ns}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the NP_1			
Grain yield (t/ha)	1.00	0.349^{ns}	0.122^{ns}
1000 grain weight (g)		1.00	0.198^{ns}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the NP_2			
Grain yield (t/ha)	1.00	0.725^{*}	$0. 020^{ns}$
1000 grain weight (g)		1.00	-0.032^{ns}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the NP_1K			
Grain yield (t/ha)	1.00	0.836^{**}	0.589^{ns}
1000 grain weight (g)		1.00	0.698^{*}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the NP_2K			
Grain yield (t/ha)	1.00	0.711^{*}	0.842^{**}
1000 grain weight (g)		1.00	0.649^{*}
Test weight (kg/hl)			1.00

^{ns}-non significant; *-significant at 0.05; **-significant at 0.01

Conclusion

As the result of favourable weather conditions i.e. sufficient amounts of precipitation at major stages of plant development and moderate temperatures at the end of the growing season, the average grain yield of all treatments was significantly higher in 2010/11 than in 2009/10. Averaged across treatment, thousand grain weight and test weight were significantly greater in 2010/11 than in the previous year. Averaged across years, grain yield and 1000 grain weight was significantly greater in NP_1K and NP_2K than in the other treatments. Regardless of year, NP_1K and NP_2K treatments had significantly higher values for 1000 grain weight compared to the other treatments. Across years, there was considerable inconsistency in differences in traits among the treatments (fertilization x year interaction).

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GRAIN YIELD AND QUALITY OF WINTER WHEAT CULTIVARS

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Abstract

The experiment was established at the experimental field of the Small Grains Research Centre in Kragujevac (Serbia) during the two growing seasons. The objective of the research was to evaluate the effect of genotype and the environment on the grain yield of winter wheat cultivars (Takovčanka, Kruna, Planeta and Vizija). The following characteristics were analysed: grain yield, 1000 grain weight and test weight. The average grain yield of all cultivars in the 2010/11 growing season was significantly greater than in the 2009/10 year, mostly as the result of highly favourable weather conditions at major stages of plant development. Takovčanka and Kruna gave significantly higher grain yields in all years compared to Planeta. Averaged across years, significantly higher values for 1.000 grain weight were found in Planeta and test weight was found in Takovčanka. Different responses of cultivars to variable agroenvironmental conditions in terms of grain yield and 1.000 grain weight and test weight require the use of a number of cultivars in the crop structure.

Keywords: *cultivars, grain yield, wheat, quality characteristics*

Introduction

Wheat productivity and grain quality in Central Serbia are governed by a range of factors, notably climate, soil, genetics and crop nutrition. Soil acidity in wheat fields in Central Serbia has become a severe problem that leads to a significant decline in grain yield and quality of wheat (Đekić *et al*., 2013, Jelic *et al*., 2015). The yield per unit area is the result of the action of factors of genotypic factors and their interaction with environmental factors. Therefore, yield is a relative term and is determined by the variety, environmental conditions and the level of applied technology. Yield is largely dependent on the genetic potential, which could be defined the yield of variety which was grown in conditions on which it had been adapted, with adequately amounts of water and nutrients and efficient control of pests, diseases, weeds and other stresses (Đekić *et al*., 2014; 2019). Yields considerably vary primarily as a result of agro-ecological conditions during the growing season (Hristov *et al*., 2011; Đekić *et al*., 2012; Jelić *et al*., 2014; Djuric *et al*., 2018; Jordanovska *et al*., 2018; Terzić *et al*., 2018b).
In the production of wheat, the correct reonization (regional distribution) of varieties is very important, and it can contribute to a lesser variation in realized yields and achieving better average results (Dodig *et al*., 2008; Luković *et al*., 2014; Jordanovska *et al*., 2018; Terzić *et al*., 2018b; Đekić *et al*., 2019). Bearing all this in mind, it is necessary that climatic conditions are in accordance with the biological requirements of the plants. In the last few years, extreme temperatures and disturbances in the amount and distribution of precipitation have significantly affected the reduction in the total production of organic matter and yield reduction (Jelic *et al*., 2015; Perišić *et al.* (2016). In the continental climate, winter wheat has long been exposed to the influence of weather conditions, and hence the climate extremes.
Production of winter wheat with high grain yield and appropriate quality is possible only by choosing varieties of good quality with appropriate cultivation conditions and appropriate

production technology. The aim of this study was to determine the cultivars and the influence of ecological environmental factors on differences in stability and adaptability of cultivars regarding the grain yield, 1.000 grain weight and test weight of tested winter wheat cultivars, for the production conditions of Serbia.

Material and methods

Meteorological conditions

Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by month. Data in Table 1 for the investigated period (2009-2011) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average for Kragujevac region, regarding the meteorological conditions.

Table 1 *Precipitation sum and average monthly temperature in Kragujevac, Serbia*

Months	Mean monthly air temperature (^oC)			The amount of rainfall (mm)		
	2009/10	2010/11	Average	2009/10	2010/11	Average
X	11.7	10.2	12.5	102.6	86.9	45.4
XI	8.8	11.4	6.9	77.5	27.9	48.9
XII	2.6	2.4	1.9	194.2	50.1	56.6
I	0.9	0.9	0.5	57.0	29.1	58.2
II	3.2	0.5	2.4	150.5	48.5	46.6
III	7.2	7.2	7.1	43.3	20.4	32.4
IV	12.1	12.0	11.6	142.2	20.8	51.9
V	16.5	15.8	16.9	116.7	65.8	57.6
VI	20.2	20.9	20.0	196.7	32.3	70.4
Average	9.24	9.03	8.87	1080.1	381.8	468.0

The average air temperature in 2009/10 was higher by 0.37°C and 2010/11 was higher by 0.16°C than the average of many years. The amount sum of rainfall precipitation in 2009/10 was higher by 612.1 mm, whereas the respective rainfall in 2010/11 was 86.2 mm lower than the average of many years and with a very uneven distribution of precipitation per months. During the April and May in 2009/10 there were 142.2 mm and 116.7 mm of rainfall, and this was 90.3 mm and 59.1 mm higher compared to the perennial average. During June in 2009/10 it was 196.7 mm of rainfall, and this was 126.3 mm higher compared to the perennial average.

The total amount of precipitation is reflected on the multi annual average, but the distribution, especially at critical stages of development, is significantly disturbed in the 2009/10 year. In addition to the necessary reserve for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil (Đekić *et al*., 2014; Jelic *et al*., 2015; Grčak *et al*., 2018; Milivojević *et al*., 2018; Terzić *et al*., 2018a).

Experimental design and statistical analysis

During the 2009/10 and 2010/11 cropping seasons, four cultivars of winter wheat (Takovčanka, Kruna, Planeta and Vizija) grown at the experimental field of the Small Grains Research Centre in Kragujevac (Serbia) were studied.

The soil used in the trial was vertisol having a very acid reaction (pH in KCI: 3.92-4.27), the content of total nitrogen was medium (0.12-0.15%), the content of affordable phosphorus was high (26.9 mg P_2O_5/100 g soil), and the content of affordable potassium was high ranging from 19.5 to 21.0 mg of K_2O/100 g of soil. The climate of the region was characterised by variable precipitation and an uneven distribution across months. A randomised block design with three replications and the plot size was 10 m^2 (5 m x 2 m) was used. In all years, winter wheat was sown in the second half of October at a row spacing of 12.5 cm. Along with

primary tillage, 400 kg/ha complex NPK (15:15:15) was incorporated into the soil, while during the spring fertilization the soil was supplemented with 300 kg/ha (KAN 27%N). During the growing season, common cultural operations were applied, without irrigation. The following traits were analysed: grain yield, 1000 grain weight and test weight. Grain yield was measured for each plot and calculated as grain yield in t/ha at 14% grain moisture. Then, a sample was taken for 1000 grain weight and test weight determination.
On the basis of achieved research results the usual variation statistical indicators were calculated: average values, standard error and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Results and discussion

Table 2 shows the impact of the year, cultivar and interaction of year x cultivar on yield, 1.000-grain weight and test weight. The analysis of variance revealded a highly significant effect of year on the grain yield (F=17.913**) and 1.000-grain weight (F=60.540**). Based on the analysis of variance, it can be concluded that there are very significant differences in grain yield and 1.000-grain weight regarding the year of investigation, while among the investigated wheat cultivars the differences were not significant (Table 2).

Table 2 *Analysis of variance of the tested parameters*

Sources of variation	df	Mean squares		
		Grain yield, t/ha	1.000-grain weight, g	Test weight, kg/hl
Year	1	17.913**	60.540**	0.0002^{ns}
Cultivar	3	0.787^{ns}	0.132^{ns}	0.989^{ns}
Year x Cultivar	3	9.340**	1.216^{ns}	0.440^{ns}

** F –test significant at 0.01; * F –test significant at 0.05; ^{ns} –non-significant

As the result of favourable weather conditions i.e. sufficient amounts of precipitation at major stages of plant development and moderate temperatures at the end of the growing season, the average grain yield of all cultivars was significantly higher in 2010/11. Significantly lower yields were obtained in 2009/10 (Table 3).

Table 3 *Mean values for the tested parameters at winter wheat cultivars*

		GY (t/ha)	TW (kg/hl)	1.000GW (g)
Years	2009/10	3.897^{b}	71.20^{b}	37.63^{a}
	2010/11	4.602^{a}	77.36^{a}	37.64^{a}
Cultivar	Takovčanka	4.347^{a}	75.10^{a}	36.93^{a}
	Kruna	4.480^{a}	74.02^{a}	37.50^{a}
	Planeta	4.075^{a}	73.76^{a}	38.25^{a}
	Vizija	4.095^{a}	74.23^{a}	37.87^{a}

*Means within columns followed by different lowercase letters are significantly different according to the LSD test

The average grain yield of wheat cultivars ranged from 3.897 t/ha in 2009/10 to 4.602 t/ha in 2010/11. In all years, Takovčanka and Kruna produced significantly higher grain yields compared to Planeta. The average two-year value of test weight in Takovčanka cultivars was 75.10 kg/hl. The 1.000-grain weight of winter wheat varied across cultivars, from 36.93 g in cultivar Takovčanka to 38.25 g in cultivar Planeta. Thousand grain weight and test weight were significantly greater in 2010/11 than in the previous year.
The differences in the yields that were observed in the tested varieties in our experiment are the result of varietal specificities, which are mostly genetically conditioned. Thus, by

analysing the obtained results it can be concluded that there is a significant dependence of grain quality components on the genotype, and this is in agreement with the results of Đekić *et al.*, (2014), Perišić *et al*. (2016) and Terzić *et al*. (2018b).

Table 4 *Mean values for the tested parameters at winter wheat cultivars in two vegetation seasons*

		Grain yield (t/ha)	Test weight (kg/hl)	1.000-grain weight (g)
2009/10	Takovčanka	4.273[b]	72.52[a]	36.63[a]
	Kruna	4.007[a]	71.38[a]	37.10[a]
	Planeta	3.967[a]	71.08[a]	38.57[a]
	Vizija	3.340[a]	69.82[a]	38.23[a]
2010/11	Takovčanka	4.420[bc]	77.68[a]	37.23[a]
	Kruna	4.953[a]	76.67[a]	37.90[a]
	Planeta	4.183[c]	76.44[a]	37.93[a]
	Vizija	4.850[ab]	78.64[a]	37.50[a]

[*]Means within columns followed by different lowercase letters are significantly different according to the LSD test

Averaged across years grain yield was higher in Kruna and Takovčanka than in the other cultivars (Table 4). In two cropping seasons the average grain yield of the wheat cultivars ranged from 4.273 t/ha to 4.420 t/ha in Takovčanka, 4.007 t/ha to 4.953 t/ha in Kruna, 3.967 t/ha to 4.183 t/ha in Planeta and 3.340 t/ha to 4.850 t/ha in Vizija. Regardless of year, Takovčanka had higher values for test weight compared to the other cultivars. The 1.000 grain weight was higher at Planeta than in the other cultivars.

Table 5 *Correlation coefficients by studied environments in wheat*

	Grain yield	Test weight	1.000 grain weight
Correlations between the traits analysed in the 2009/10			
Grain yield	1.00	0.487	-0.366
Test weight		1.00	-0.073
1.000 grain weight			1.00
Correlations between the traits analysed in the 2010/11			
Grain yield	1.00	0.127	0.179
Test weight		1.00	0.202
1.000 grain weight			1.00
Correlations between the traits analysed in the 2009/11			
Grain yield	1.00	0. 708[**]	-0.121
Test weight		1.00	0.001
1.000 grain weight			1.00

[*] $p<0.05$; [**] $p<0.01$

The average values of the Pearson's coefficient of correlation (*r*) of investigated winter wheat traits are presented in Table 5. The established correlation coefficients between the grain yield and test weight in both vegetation seasons as well as during the two-year research were positive. The established correlation coefficients between the grain yield and the 1000 grains weight in both vegetation seasons as well as during the two-year research were negative, except in the second year of research in which a positive coefficient of correlation was noticed. The importance of these components in the formation of grain yield depends on the climatic conditions during the critical phases of growth and development, the applied agro-technology and the various combinations and relationships of NPK nutrients (Hristov *et al.*, 2011; Đekić *et al*., 2014; Jelić *et a*l., 2014; Djuric *et al*., 2018; Terzic *et al*., 2018a). Therefore, it is important to know the effect of these properties, i.e. yield components, as well as their interdependence on grain yield.

Conclusion

Based on the results during the two-year investigation in four Kragujevac's winter wheat cultivars, it can be concluded that the grain yield of wheat ranged from 4.075 t/ha (Planeta) to 4.480 t/ha (Kruna). The highest two-year average value of test weight was found in the cultivars Takovčanka (75.10 kg/hl). Winter wheat cultivars had test weight greater than 70 kg/hl. The highest 1.000 grain weight of investigation in winter wheat Planeta cultivar (38.25 g). During 2010/11 cropping season, statistically significantly higher grain yield per area unit, as well as 1.000 grain weight was obtained, compared to the 2009/10 cropping season.

On the base of studied parameters the wheat cultivars Takovčanka and Kruna were more tolerant to adverse chemical soil characteristics (low pH) and can be recommended as a suitable genotypes for wheat production on acid soils, especially after liming of soil. Different responses of cultivars to variable agroenvironmental conditions, particularly in terms of major grain quality indicators, require the use of a number of cultivars in the crop structure.

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PRODUCTIVITY AND BIOCHEMICAL COMPOSITION OF SOYBEAN GRAIN, DEPENDING ON THE MEANS OF PROTECTION

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Abstract

The study was conducted in 2015-2017 in order to obtain experimental data on the impact of soybean protection technologies on the productivity and biochemical composition of soybean grain. Biological and chemical agents (TMTD, Immunocitofit, Zircon, Bisolbifit, Extrasol, Nutri-Veit with water softener Spartan, liquid humic fertilizer Optimo) were studied for plant protection on the example of *Ivan Karamanov* soybean cultivar. The experimental plots were laid in cereal-soybean crop rotation on meadow-brown heavy loamy soil on the base of the Far Eastern Agricultural Research Institute (Khabarovsk, Russia). The application of pesticides and biological agents in soybean crops contributed to a statistically significant increase in soybean yield compared to control (without treatments): 1.2, 1.03 and 0.8 tons ha^{-1} on the plots where seeds and crops were treated by Zircon, Extrasol and TMTD with Optimo, respectively. The highest protein contents were observed on plots with the application of TMTD (35.75 %); TMTD with Optimo (35.72 %); Zircon (35.56 %); Extrasol (35.47 %). The largest fat content was in grain obtained from plots where seeds and crops were treated by Zircon (16.5 %).

Keywords: *Soybean, productivity, protein, fat, plant protection.*

Introduction

"No other plant can compete with soybean by the amount of valuable nutritional products and substances produced per sowing area unit" – the words of V.A. Zolotnitskiy. Soybean uniqueness, versatility of use is determined by its chemical composition – the content of organic and inorganic matters in seeds and green mass (Vashchenko *et al*., 2010).

This crop plays a special role because of the need of producing high-protein food for the population. High nutrition value of soybean products is due to the presence of high-grade protein (30-50 % and more) and oil (15-26 %) in seeds (Dospekhov, 1985). Besides proteins and lipids, soybean seeds are rich in mineral elements: Fe, I, Cu, Zn, Mn, Co, Mo, Se, Cr, Ni, Sn, Si, F, V (Shchegorets, 2002).

The use of soybean and its products is carried out in three directions worldwide:

1. The improvement of nutrition and its therapeutic and preventive effects on humans. Currently, in many countries soybean becomes the main source of protein for the food industry. Soybean oil takes the first place in global production of all vegetable oils, and soybean products are classified as "healthy" having a preventive or therapeutic effect on a number of diseases (Shchegorets, 2002). It is also possible to produce full-fat or low-fat flour, margarine, mayonnaise, soy milk, tofu, dry soy base, soy sauce, soy mince, complex of soy milk products from soybean. From soybean seeds of small-seed cailtivars could be done seedlings, rich in biologically active substances (Shchegorets, 2002).
2. The improvement of productivity and efficiency of animal husbandry. It has been established that with regular feeding of animals with soybean the average daily gain in live weight doubles; feed consumption per unit of livestock production is reduced by 30-35 %; quality of livestock products rises (Shchegorets, 2002).

3. Industrial production. Construction plates, artificial fabrics and materials, fertilizers and other goods are produced from raw soybean materials that are not used in the food industry and animal husbandry (Shchegorets, 2002).

Natural and climatic conditions of the Russia's Far East are comparatively favorable for the cultivation of soybean. But the yield in recent years was 1.2-1.5 tons ha^{-1}, with a potential of 3.0-3.5 tons ha^{-1}. One of the main reasons of low grain yield of soybean is high fungal diseases occurrence. To reduce this impact we develop and test new strategies for the protection of this culture.

Materials and methods.

The study was conducted in 2015-2017 in the grain-soybean crop rotation on the experimental fields of the Department of breeding and seed production (Far Eastern Agricultural Research Institute, Russia). The soil of experimental plots was meadow-brown, heavy loamy, and has the following characteristics: humus content (by Tyurin) – 4.8; P_2O_5 (by Kirsanov) – 4.3 mg per 100 g of soil; K_2O (by Maslova) – 20 mg per 100 g of soil. The experiment was held according to the Methodology of field trials (Dospekhov, 1985) to determine the main indicators of biochemical composition of soybean (protein, fat). Soybean grain was sent to the analytical laboratory.

The objects of research were: chemical seed treater TMTD (thiram, 400 g per 1 litre); biological agents – Immunocytofit (arachidonic acid ethyl ester, 20 g per kg); Zircon (hydroxycinnamic acid 0.1 g per 1 litre); Bisolbifit and Extrasol (strain of *Bacillus subtilis* H-13, 100 millions of CFU per ml); liquid foliar fertilizer Nutri-Veit (28 % of phosphorus in form of PO_3 and 26 % of potassium) with water softener Spartan; liquid humic fertilizer (LHF), as well as the chemical fungicide Optimo (pyraclostrobin, 200 g per 1 litre). The implementation of different protective schemes was performed by dressing seeds and spraying plants during the growing season.

The experiment was arranged in a randomized complete block design with four replications on plots 50 m^2 in size, with the cultivar of soybean "Ivan Karamanov". Crops were grown according to conventional technology generally accepted in a region. Seeds were treated with water moistening 10 litres per ton, according to the experimental design, the day before sowing (Table 1).

Soybean seeds were sown on experimental plots in the first decade of June at a rate of 300,000 plants ha^{-1} (70 kg of seeds ha^{-1}) in moist well prepared soil to the depth of 3-4 cm on a profiled surface (ridge 140 cm).

Table 1. Experiment design (treatments of seeds and plants)

Variant	Seeds treatment	Plants treatment during growing season
1. Control (without treatment)	Water moistening 10 l/t	
2. Standard - TMTD	TMTD 6 l/t	
3. TMTD + Immunocytofit	TMTD 3 l/t + Immunocytofit 1 tab./t	
4. Immunocytofit	Immunocytofit 1 tab./t	Immunocytofit 1 tab./ha during flowering stage
5. Spartan + Nutri-Veit	TMTD 6 l/t	Spartan 0,1 l/ha + Nutri-Veit 0,75 l/ha during 4-6 trifoliolate leaves and flowering stages
6. Zircon	Zircon 40 ml/t	Zircon 10 ml/ha during flowering stage
7. Bisolbifit	Bisolbifit 2 kg/t	

8. Extrasol	Extrasol 2 l/t	Extrasol 2 l/ha during trifoliolate leaves and flowering stages
9. LHF	LHF 0,4 l/t	LHF 0,4 l/ha during trifoliolate leaves and flowering stages
10. TMTD + Optimo	TMTD 6 l/t	Optimo 0,5 l/ha with disease symptoms appearance

During the years of study hydrothermal conditions varied tremendously. During the first period of growing season of 2015 the weather conditions were unfavorable. Heat supply in this period was insufficient. Due to the low temperature regime, heat accumulation was slow. Soybean plants were stunted. Hydrothermal conditions of 2016 were close to average long-term indicators, but were not evenly distributed during the growing season. The accumulation of heat was slow, heat supply of plants was insufficient. The weather conditions of June 2017 were unfavorable for the growth and development of soybean plants. Further vegetation of plants took place under favorable conditions.

Results and discussion

Studied remedies combined fungicidal properties with growth regulating and anti-stress activity, which had a positive effect on growth, development, structural elements of yield and productivity of soybean.

Significant increase in yield by 1.2; 1.03 and 0.8 t ha^{-1} comparing to control was obtained by seed dressing and spraying of growing plants with Zircon, Extrasol, Spartan with Nutri-Veit (Table 2).

Table 2. The influence of protective measures on the certain structural elements of yield and the productivity of soybean plants (average for 2015-20107)

Variant	Beans per plant	Seeds mass		Biological yield, t ha^{-1}
		Per plant	1000 seeds	
1. Control (without treatment)	23.4	9.1	164.5	2.68
2. Standard - TMTD	24.1	9.5	166.7	2.86
3. TMTD + Immunocytofit	25.4	10.4	172.1	3.12
4. Immunocytofit	24.8	10.2	169.7	3.02
5. Spartan + Nutri-Veit	29.8	12.1	175.2	3.62
6. Zircon	31.4	12.9	173.6	3.88
7. Bisolbifit	25.9	10.6	170.1	3.18
8. Extrasol	26.8	12.4	179.6	3.71
9. LHF	24.6	10.4	172.6	3.13
10. TMTD + Optimo	26.8	11.3	177.4	3.48
$LSD_{0,5}$	4.4	1.5	12.9	0.42

It is not a secret that agrometeorological conditions of the growing season, system of treatments and protective measures have the greatest impact on the yield development. Under favorable weather conditions and high-quality agricultural technology, soybean plants grow and develop accumulating nutrients, which are later converted into protein and fat. The use of biological and chemical means of protection smoothed the negative impact of the adverse meteorological conditions and contributed to an increase in assimilation surface of soybean plants, which in turn affected the protein and oil content in grain.

Table 3. The influence of tratments on the protein and lipid content of soybean cultivar "Ivan Karamanov" (average for 2015-2017)

Variant	Protein, %	Fat, %
1. Control (without treatment)	35,37	14,8
2. Standard - TMTD	35,75	15,1
3. TMTD + Immunocytofit	33,92	15,0
4. Immunocytofit	35,42	14,7
5. Spartan + Nutri-Veit	34,40	15,2
6. Zircon	35,56	16,5
7. Bisolbifit	35,27	15,0
8. Extrasol	35,47	15,1
9. LHF	35,36	14,7
10. TMTD + Optimo	35,72	14,8

The highest protein content was observed in variants treated with: TMTD – 35.75 %; TMTD with Optimo – 35.72 %; Zircon – 35.56 %; Extrasol – 35.47 %. The highest fat content was in grain obtained from dressing seeds and spraying plants with Zircon – 16.5 %.

Conclusion

Among the diversity of cultivated crops, soybean exceeds others by its versatility of use (Dega, 2012). As a bean crop, soybean nutritionally surpasses all crops and feeds of natural meadows and pastures (Burlaka, 1965). The biochemical composition of its grain is unique. It contains 30-50 % easily digestible and balanced in amino acids protein, 16-26 % of oil, favorable in fatty acids composition, 20-25 % of carbohydrates, 5-6 % of salts and vitamins. With the improvement of advanced soybean processing technologies, the products made from it are widely used in bakery, confectionary, canning, meat and dairy, textile, paint and varnish, soap making, pharmaceutical, automotive, aviation and several other industries (Dega, 2012).

According to results of our research, significant increase in yield of soybean by 1.2; 1.03 and 0.8 t ha^{-1} was obtained in variants with seeds and plants treatments with Zircon, Extrasol, Spartan with Nutri-Veit. The highest protein and fat contents were observed in grain from plants treated with TMTD, TMTD with Optimo, Zircon, Extrasol; and Zircon respectively.

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FORMATION OF SPRING TRITICALE GRAIN YIELD IN CONDITIONS OF MONSOON CLIMATE

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Abstract

The Far Eastern region is characterized by difficult soil and weather conditions, which have no analogues in the Russian Federation and neighboring countries. Currently, only 18 cultivars of spring triticale have been entered into the State Register of Protected Breeding Achievements and Approved for Use in the Russian Federation, therefore the expansion of the cultivated biodiversity of this crop in the area of high risk farming is undoubtedly an urgent task. In this regard, the purpose of research is to study the formation of spring triticale productivity in a monsoon climate. Field experiments conducted in the 2015-2018 year period. The object of research is testing of 10 cultivars of spring triticale of various ecological and geographical origins. The soil of experimental fields is a meadow-brown podzolized-gley heavily loamy. It was revealed that in the conditions of a monsoon climate, the need for heat and moisture in certain periods of organogenesis in spring wheat and spring triticale are diametrically opposed. The correlation dependences between the grain yeld of triticale cultivars and the parameters of environmental factors are established: the sum of the temperatures of the surface air layer and the amount of precipitation. Under the conditions of a monsoon climate, productive and promising genetic sources and donors of various ecological and geographical origins were identified for their involvement in the breeding process in order to expand the biological diversity of grain crops and increase the efficiency of rational use of natural agro-resources.

Keywords: *Far East region, crop productivity, crop cultivars, Russia*

Introduction

The most promising way of providing the world's population with a sufficient amount of healthy food can be done by the introduction of highly productive plants with valuable biochemical composition, which is especially important for Russia, where the variety of crops available for growing is limited (Kochetov *et al*., 2012). One of the most important reserves for increasing gross grain harvest is the use of uncommon for Russia culture in grain production - spring triticale. Triticale, occupying a certain niche in the structure of cultivated areas, expands biodiversity, thereby increasing the sustainability of crop production (Tylenko and Skatova, 2015). It is believed that the growth potential of its yield is significantly higher than that of wheat. Already at the present time, triticale ranks first in yield among cereal crops (Aydiev *et al*., 2016). Studies on the biological basis of triticale productivity and the creation of high-yielding forms of this promising culture are currently among the most promising areas in grain breeding (Emelyanova et al., 2018). Due to the specific genome, which includes the components of wheat and rye, triticale has a high potential for increasing productivity and is promising in the context of a lack of means of intensifying agricultural production.
Since 2009, triticale has been included in the list of grain crops in the final data of Rosstat. Amount triticale produced in Russia is insignificant if compared to other types of crops. According to the data, the share of this crop in the structure of the sown area of the country is 0.2-0.3% and the crops occupy an area of 165 to 251 thousand hectares. The average grain yield of triticale in the Russian Federation for 2009-2018 period reached 1.8-2.9 t/ha. Currently, 18 varieties of spring triticale have been entered into the State Register of Protected

Breeding Achievements and Approved for Use in the Russian Federation, which is not enough for use in various regions of the country, therefore expanding the varietal biodiversity of this crop in a monsoon climate is undoubtedly an urgent task.
The climate of the Far East sharply differs from the climate of the main agricultural regions of the Russian Federation and adjacent states. At the corresponding latitudes of the globe there are no analogues to it. According to the combination of soil and climatic factors, this region belongs to the zone of risk farming. Spring in all areas is late, long and cold. Rainfall in the spring and early summer periods, as a rule, is not enough. The second half of summer, on the contrary, abounds in precipitation and heat, and the relative humidity of air in this period reaches 100%. High humidity of the air and soil, combined with a rather high temperature during the period of ripening of cereal crops, contributes to the mass spread of diseases, including loose smut, Fusarium, dark brown spot and other diseases. All this has a negative effect on the realization of the genetic potential of productivity, especially in varieties of grain crops of foreign and foreign selection, which do not withstand water logging and high infectious load.
In this regard, the purpose of this research is to study the formation of spring triticale productivity in a monsoon climate.

Materials and Methods

The field experiments were conducted in the 2015-2018 year period. The object of research is 10 cultivars of spring triticale of various ecological and geographical origin. The standards – the zoned cultivar of spring soft wheat Khabarovchanka and the cultivar of spring triticale Ukro recommended for cultivation in this ecological zone. The soil of experimental fields is a meadow-brown podzolized-gley heavy loamy. Agronomyis common for the conditions of this region and they include: late autumn plowing, spring cultivation, harrowing in two wakes and sowing. Sowing of grain crops was carried out by the SSFC-7M seeder on plots with an area of 4 m^2 and randomized 3-fold with a seeding rate of 5.5 million viable seeds/ha. Accounting for the collection of spring triticale samples was carried out by the method of sub-section continuous threshing with the Hege-125 combine, followed by weighing and reduction to standard humidity and purity.
Field observations, measurements and biochemical analysis were carried out in full compliance with the field case methodology (Dospekhov, 1985), the state crop testing method (1985) and the methods of plant biochemical studies (Ermakov, 1987). Statistical processing of experimental data was performed using analysis of variance, correlation, and regression analysis using the Statistics v 10.0 software (Stat Soft, Inc., USA).

Results and Discussion

The Far Eastern region of Russia has sufficient natural and climatic resources for growing crops such as spring triticale. A main feature of meteorological conditions in recent years is a shortage of heat in the month of June with sharp amplitude of fluctuations in day and night temperatures of the surface air layer and rainy weather (Figure 1). Thus, the hydrothermal conditions during the years of research were diverse, rather fully reflecting the particularities of the monsoon climate, which made it possible to evaluate with a high level of confidence the influence of environmental factors on the growth, development and productivity of the samples of spring triticale under study.

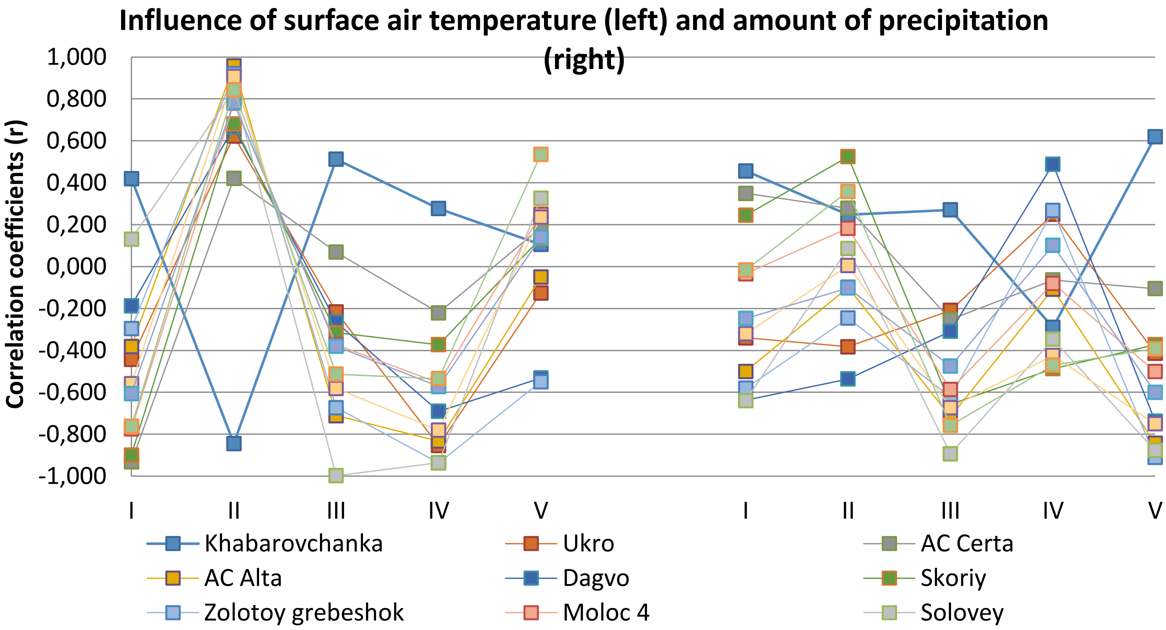

Figure 1. Agrometeorological conditions during research.

Main criterion of the economic value of a specific cultivar is grain yield. Overall grain yield of spring triticale cultivars AC Certa, Dagvo, Zolotoy grebeshok, Losinovske, Zgurivsky, exceeded the yield of standard varieties Khabarovchanka (spring wheat) and Ukro (spring triticale) by 0.1-0.6 t/ha (Table 1). Under the conditions of a monsoon climate, cultivar differences in productivity, according to the data in the table, were significant for triticale plants.

Table 1. Efficiency of spring triticale cultivars samples.

Cultivar	Productivity, t/ha		The protein content in grain, %		R	Linear regression equation	R^2
	X	lim	X	lim			
Khabarovchanka	2.2	1.5-2.9	15.5	13.9-16.2	0.80*	y=10.135+0.232*x	0.64
Ukro	2.2	1.4-2.6	15.0	13.3-15.9	-0.94*	y=18.665–0.183*x	0.88
AC Certa	2.8	1.7-3.6	14.9	14.1-15.6	-0.84*	y=16.511–0.059*x	0.70
AC Alta	1.9	1.5-2.4	15.0	13.1-17.0	-0.63*	y=20.504–0.289*x	0.40
Dagvo	2.3	1.1-2.9	15.0	12.9-18.1	0.04	y=14.767–0.011*x	0.01
Skoriy	2.2	1.9-2.3	15.5	13.9-16.9	-0.36	y=20.435–0.231*x	0.13
Zolotoy grebeshok	2.7	1.6-3.8	14.9	14.0-15.7	-0.28	y=15.521–0.024*x	0.08
Moloc 4	1.7	1.1-2.6	15.2	13.2-16.4	-0.62*	y =17.134–0.116*x	0.38
Solovey	1.9	1.1-2.8	14.9	13.7-16.9	-0.84*	y=21.231–0.287*x	0.71
Losinovske	2.6	1.5-4.1	14.9	14.1-16.4	-0.60*	y=16.388–0.056*x	0.36
Zgurivskiy	2.4	1.6-3.2	14.9	14.1-15.5	-0.62*	y=16.358–0.057*x	0.38
Brio	2.1	0.8-3.6	15.3	14.1-16.4	-0.69*	y=16.466–0.056*x	0.48

Note: X is the average value; lim is the minimum and maximum value; R is the correlation coefficient between yield and protein content in spring triticale grain.

A grain of valuable triticale class I according to GOST 34023-2016 (Russia) must contain at least 12% protein. A comparative assessment of quality indicators in the same environmental conditions shows that the protein content of spring triticale samples averaged 14.0-15.5%, which is at the level of the standard cultivar of spring wheat Khabarovchanka. Leadership in protein content in grain was noted in the cultivars AC Alta and Dagvo, in which the accumulation of protein substances in the grain in optimal hydrothermal conditions reached 17.0-18.0%. Varietal differences in protein content in the grain of spring triticale were revealed – higher quality grains were formed by the cultivars Skoriy and Brio. The variability of weather and climatic conditions is the main cause of instability in the quality of grain of cereals. In cool and wet conditions, the protein in the spring triticale grain accumulates less than in drier and warmer years. With an increase in yield, the protein content of the grains significantly decreases (R= -0.52), while the realization of the productive potential depends on the degree of compliance of the biological characteristics of an individual cultivars of agroclimatic and weather conditions of the region (Figure 2).

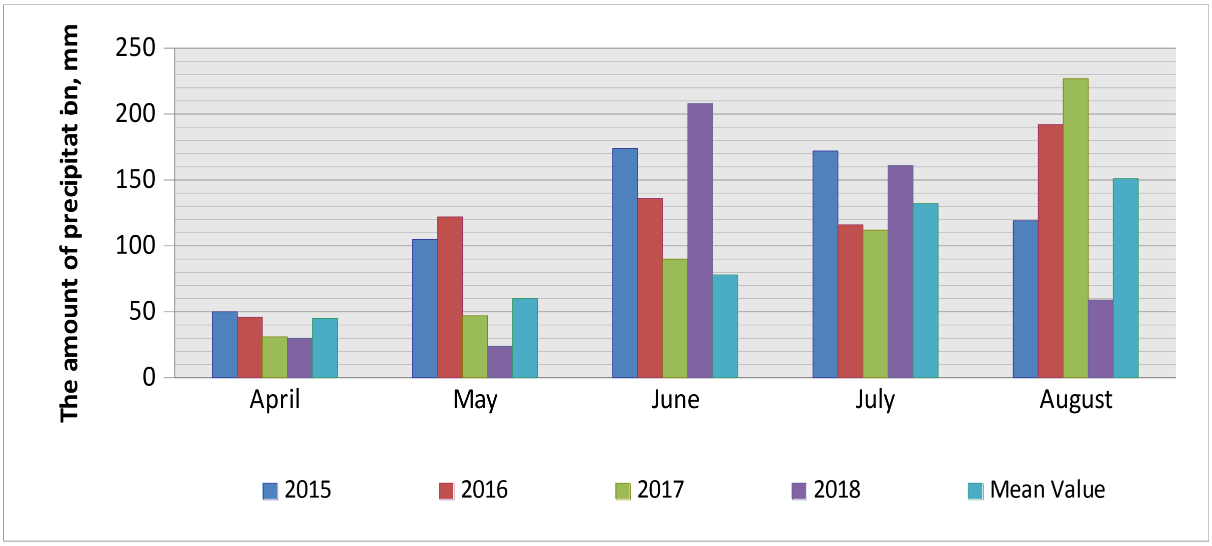

Fig. 2. The relationship between the yield of triticale and hydrothermal environmental conditions at different stages of organogenesis (I – Planting-sprouting, II – sprouting-tillering, III – tillering-jointing, IV – jointing-heading, V – heading-ripeness)

High temperature regime during the period of sprouting of spring triticale contributes to an increase of the tillering and the density of the productive stalk, while having a negative effect on the formation of spring wheat plants. Excessive water logging and the high temperature of the surface air layer during the period of jointing leads to a further loss of spikelets, higher levels of sterility and lower amount of grain per ear. Increased summary of temperatures and abundant rainfall in the reproductive period reduce the yield of triticale , but have a positive effect on the grain yield of spring wheat plants.

The magnitude of the multiple correlation coefficient indicates a close dependence of the yield and protein content in the grain of spring triticale on the agro-ecological conditions of the monsoon climate (R=0.78 and R=0.91, respectively). Combined effect of the temperature of the surface air layer, the amount of precipitation and moisture supply during the growing season determines the 61% of the yield variation and 83% of the variation in protein content in the grain of spring triticale. The remaining 39% and 17%, respectively, are attributed to factors unaccounted for in the regression model.

Conclusions

Thus, it has been established that in the conditions of a monsoon climate, different cultivars of spring triticale use different mechanisms in the formation of productivity. Cultivation of spring triticale in this ecological zone allows you to successfully harvest 2-3 t/ha of grain, but when exposed to stressful environmental factors, the yield varies from the average value by 2 times. According to the results of research, it has been established that the need for heat and moisture in certain periods of plant growth and development in spring wheat and spring triticale are diametrically opposed. At the same time, the productivity of triticale cultivars is depends on the cumulative effect of the temperatures of the surface air layer, the amount of precipitation and the level of moisture supply during the entire growing season.

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FEATURES OF THE INFLUENCE OF CLIMATIC FACTORS ON THE CROP AND QUALITY OF GRAIN OF SPRING OATS

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Abstract

The soil and climatic resources of the Far East of Russia from all grain crops, to the maximum extent corresponds to the biological characteristics of oats. Right now soybean prevails in the structure of sown areas of this ecological zone (72%), while grain crops occupy only 17%, which leads to possible environmental disaster of the natural environment with constant monoculture growing. The purpose of this research is to study the influence of climatic factors on the yield and grain quality of spring oats. The soil of is a meadow-brown podzolized-gley heavily loamy. The standard – zoned cultivar of spring oats Express in this zone was used. According to the economically valuable characteristics, 10 cultivars were selected and analyzed. As a result of research, the correlation between grain quality of different cultivars, meteorological conditions of the growing season and main phenological phases was determined in order to improve the adaptability of oats to environmental conditions. Correlation analysis of the data obtained in the study showed the dependence of the protein content in the grain of the hydrothermal conditions of the growing season. It was revealed that in most of the studied cultivars protein content decreases, while overall grain yield increases, as evidenced by the correlation coefficient ($r = –0.99$). Most cultivars demonstrated a significant positive correlation between completeness and grain size ($r = 0.95$). Grain inner hull in oat cultivar samples is a genetically determined trait with an indirect contribution of environmental conditions.

Keywords: *Spring oat, grain yield, grain quality, correlation analysis, Russia*

Introduction

An adaptive assortment of cultivated crops largely determines the competitiveness of a particular region and the country as a whole in the world grain market. The soil and climatic resources of the Far East of Russia from all grain crops, to the maximum extent correspond to the biological characteristics of oats. The cultivation of grain crops in the region is insignificant and in the last decade there has been a tendency to increase the acreage of soybeans. For 2018, soybean prevails in the structure of sown areas (72%), while grain crops occupy only 17%, which leads to incorrect type of crop rotations and, consequently, to an environmental disaster of the natural environment. The hydrothermal conditions of the cultivation zone have a decisive impact not only on the realization of the of the variety, but also on the quality of the harvest. A distinctive feature of the meteorological conditions in the Far East of Russia, including in the Khabarovsk Territory, is due to the monsoon nature of the climate, which is characterized by a significant amount of precipitation in the summer period. The winter in the region is harsh and long, with little snow, the average January temperature is -16 to -24 ^{0}C, which is why the soil freezes to 2.5-3.0 m. The summer is dry in the first half and rainy in the second. Over the warm period, the amount of precipitation varies by year over a wide range, 360-1048 mm. The sum of active temperaturesfor the period varies from 2460 to 2850 $^{\circ}$C. Accordingly, the air humidity in summer is much higher than in winter. On this basis, the study of the influence of the surface layer of air and the amount of precipitation in the main periods of organogenesis on the formation of yield and grain quality of spring oats is especially important in this ecological zone. In this regard, the purpose of this research is to study the influence of climatic factors on the yield and grain quality of spring oats.

Materials and Methods

Studies were conducted in the 2015-2018 year period in the Far Eastern Scientific Research Institute of Agriculture of Russia. The soil of crop rotation is a meadow-brown podzolized-gley heavy loamy. *Standard* - regionalized the cultivar of spring oats Express. The source material was taken as 100 samples of oats from the world collection of the Russian Institute of Plant Genetic Resources. N.I. Vavilova (VIR, Russia, St. Petersburg). According to the economically valuable attributes, 10 variety samples were isolated and analyzed: 14505 – Express, 14583 – Flamingsgelb, 15018 – Pg 17, 15053 – Nein, 15065 – Irtysh 22, 13717 – Alden, 14040 – Aurea 603, 14433 – Orpale, 14271 – Gallop, 14584 – Praefekt. All surveys and observations during the growing season were carried out in full accordance with the methodology of the field experiment (Dospekhov, 1985), methods of state variety testing of agricultural crops (1985) and the International Classification of the CMEA of the genus Avena (1984). Grain quality was determined using existing methods of biochemical studies of plants (Kazakov, 1987). Statistical analysis were performed using the "Statistics v. 10.0" software, to assess the influence of climatic factors on the grain quality of oats, a Spearman and Pearson correlation analysis was used.

Results and Discussion

The agrometeorological conditions in the years of research were varied, rather fully reflecting the characteristics of the region, which made it possible to evaluate with a high degree of certainty the influence of hydrothermal conditions on the growth, development and productivity of the cultivars. With the average long-year period norm of the amount of heat in April-August 2301.4 ^{0}C during the years of research, the amount of heat varied within 2291.2-2505.1 ^{0}C, the amount of precipitation - within 417-620 mm with a norm – 466 mm. A characteristic feature of meteorological conditions in recent years is a shortage of heat in the month of June with a sharp amplitude of fluctuations in day and night temperatures of the surface air layer and rainy weather. Under current hydrothermal conditions, the yield of the studied varieties during the years of research varied within wide limits – 1.6-6.7 t/ha and averaged 2.5-4.8 t/ha (Table 1). The highest realization of the productivity potential was observed in 2017 for the the cultivars Praefekt and Galop– 6.6 and 6.7 t/ha, respectively.

Table 1. Comparative characteristics of oat cultivars by yield and quality indicators.

Catalog Number VIR	Yield (t/ha)			Protein, %			Filminess, %			1000 grains weight, g			Test weight, g/l		
	min	max	X	min	max	X	min	max	X	min	max	X	min	max	X
14505	2.8	5.4	3.9	11	13	12	25	26	25	28	35	32	412	492	422
14583	2.6	5.3	3.9	12	13	13	25	26	26	28	35	31	442	492	468
15018	2.2	5.4	3.5	12	14	13	25	26	25	27	32	30	396	480	427
15053	1.6	3.9	2.5	11	13	12	26	28	27	26	31	29	400	500	489
15065	2.4	5.1	3.4	11	13	12	23	26	24	27	31	29	398	440	417
13717	3.3	6.3	4.6	11	13	11	26	29	28	28	34	31	448	490	469
14040	3.1	6.3	4.6	10	12	11	25	28	27	31	36	33	456	478	469
14433	3.1	6.3	4.6	10	12	11	23	26	24	27	36	31	428	510	471
14271	3.2	6.7	4.8	11	12	11	24	29	27	34	41	37	456	499	470
14584	3.1	6.6	4.7	10	12	11	25	27	26	28	34	31	462	525	483

Note: min is the minimum value of the attribute, max is the maximum value of the attribute, X is the average value of the attribute.

As a result of our previous studies, a close relationship was found between the yields of grain crops and the hydrothermal conditions of the Far Eastern region of Russia not during the growing season, but during the main periods of growth, development and yield formation (Aseeva and Melnichuk, 2017). According to Sazonova and Sartakova (2006), the quality parameters of oat grains are varietal hereditary traits, which makes it possible to further improve them, but they are subject to strong variability and under the influence of environmental conditions.
Correlation analysis between the quality indicators of oats and environmental conditions in certain periods of growth and development revealed a high degree of dependence between them. It was found that the majority of the studied cultivars, the protein content in the grain decreases with increasing yield, as evidenced by the correlation coefficient (r= -0.991*).
The varietal specificity of the dependence of the protein content in grain on the hydrothermal conditions of the growing season (Table 2) was revealed.

Table 2. The relationship of the protein content in the grain of oats with hydrothermal conditions at different stages of phenological phases

Catalog Number VIR	Sowing – Sprouting		Sprouting – Tillering		Tillering – Anthesis		Anthesis – Harvest Ripe		Sowing – Harvest Ripe	
	I	II	I	II	I	II	I	II	I	II
14505	0.13	0.85	-0.99	-0.13	0.94	-0.88	-0.42	-0.46	0.72	-0.34
14583	0.99*	0.53	0.16	0.99*	-0.11	0.54	-0.90	0.89	0.98	0.94
15018	-0.99*	-0.53	-0.16	-0.99*	0.19	-0.59	0.47	-0.82	-0.90	-0.92
15053	-0.38	0.47	-0.93	-0.61	0.98*	-0.99	-0.03	-0.99*	0.75	-0.92
15065	-0.38	0.47	-0.93	-0.61	0.82	-0.96	0.09	-0.97	0.86	-0.87
13717	0.13	0.85	-0.99	-0.13	0.99*	-0.48	-0.86	-0.45	0.81	-0.25
14040	-0.38	0.47	-0.93	-0.61	0.96	-0.99*	-0.45	-0.86	0.76	-0.75
14433	-0.98	0.78	-0.18	0.89	0.29	0.52	-0.85	-0.64	0.64	0.79
14271	0.61	0.99*	-0.78	0.38	0.32	-0.71	-0.63	-0.15	-0.43	0.09
14584	0.84	0.96	-0.53	0.66	0.74	0.70	-0.99*	0.37	0.86	0.64

Note: * – 95% significance level, ** – 99% significance level, I – the sum of the temperatures of the surface air layer, II – the amount of precipitation.

In most samples, high temperatures of the surface air layer during the periods from sowing to tillering and at the stage of seed loading and ripening cause a decrease in protein in the grain. A negative relationship was also found with the amount of precipitation in the period from sowing to tillering. Only in the period of tillering - heading, high temperatures of the surface air layer contribute to the accumulation of protein. In the same period, precipitation, on the contrary, restrain this process.
All studied cultivars have an inverse relationship between the amount of heat and moisture in the period of sowing-seedlings (Table 3). For several numbers, this relationship persists until the heading stage. Only during the period of grain ripening, the increase in heat contributes to the increase in grain weight. Excessive moisture in fact throughout the growing season reduces this figure.

Table 3. The relationship of the content of the natural weight of the grain of oats with hydrothermal conditions at different stages of development.

Catalog Number VIR	Sowing – Sprouting		Sprouting – Tillering		Tillering – Grain Formation		Grain Formation – Harvest Ripe		Sowing – Harvest Ripe	
	I	II	I	II	I	II	I	II	I	II
14505	-1**	-0.63	-0.04	-0.97	0.23	-0.38	-0.95	-0.83	-0.26	-0.90
14583	-0.75	0.03	-0.68	-0.90	0.64	-0.92	0.50	-0.99*	-0.39	-0.92
15018	-0.99*	-0.60	-0.07	-0.98	0.10	-0.52	0.55	-0.76	-0.86	-0.88
15053	-0.94	-0.86	0.32	-0.82	-0.15	0.12	0.99*	-0.15	0.69	-0.44
15065	-0.89	-0.23	-0.47	-0.98	0.25	-0.90	0.72	-0.89	0.32	-0.98
13717	-0.94	-0.87	0.33	-0.81	-0.60	-0.54	0.86	-0.55	-0.91	-0.73
14040	-0.12	0.68	-0.99*	-0.38	1**	-0.94	-0.67	-0.70	0.91	-0.55
14433	-0.90	-0.92	0.42	-0.75	-0.53	-0.29	0.96	-0.42	-0.82	-0.61
14271	-0.99	-0.50	-0.19	-0.99*	0.69	-0.29	0.98	-0.80	0.99*	-0.93
14584	-0.99*	-0.53	-0.16	-0.99*	-0.12	-0.99*	-0.71	-0.89	-0.31	-0.99

Note: * – 95% significance level, ** – 99% significance level, I – the sum of the temperatures of the surface air layer, II – the amount of precipitation.

The mass of 1000 grains is one of the most important components of the productivity and technological value of grain (Batalova *et al*., 2017). Oat grain inner hull have a low nutritional value. Therefore, the cultivars of the new generation should combine high particle size and low amount of grain hull. Most cultivars revealed a significant correlation between the test weight of the grain and a mass of 1000 grains (r=0.95). The filminess of oat variety samples is a genetically determined trait with an indirect contribution of environmental conditions. The content of hull in the Pg 17 cultivar is low (V=0.5%) and the lack of correlation with hydrothermal conditions indicates intravarietal uniformity.

Conclusions

The formation of qualitative indicators of oat grain, along with genotypic features, is significantly influenced by climatic conditions of the environment. The characters of correlation on the formation of quality in the grain of the analyzed cultivars of different ecotypes with the weather conditions of the vegetation period and the phases of development with the aim of improving the varietal potential of oats are described. The varietal specificity of the dependence of the protein content in the grain on the hydrothermal conditions of the growing season was revealed. Most cultivars revealed a significant correlation between the test weight of the grain and a mass of 1000 grains. The hull of oat cultivar samples is a genetically determined trait with an indirect contribution of environmental conditions.

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GENOTYPE BY ENVIRONMENT INTERACTIONS ON NINE COTTON FIBER TRAITS

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Abstract

Genotype × Environment interactions for cotton fiber traits are of great importance affecting quality. The purpose of this study was to evaluate fiber quality traits across four diverse environments by Advanced Fiber Information System (AFIS) instrument. The five most cultivated commercial upland cotton cultivars were used and each cultivar was sown in 16 fields (80 fields in total for all cultivars). Four samples from each field were collected for analysis of nine fiber traits. Many fiber traits exhibited statistically significant differences for both factors environments and cultivars (genotypes), indicating differences in environmental conditions and cultivar behavior. Genotype × Environment interactions were not present except for two traits that were very close to 0.05 significance level. Finess showed the greatest mean squares, while in most cases, mean squares of the factor environment were greater than for genotypes (except for IFC and Finess, where genotypes contributed the most in total variability). Thrace followed by Macedonia, proved to be the best regions in Greece to cultivate cotton for all fiber traits, except for trait SFC (percent of short fibers by weight). Cultivar CELIA showed the best measurements for almost all fiber traits across all Greek regions.

Key words: *AFIS, quality, Greek regions*

Introduction

Genotype × Environment interactions are of major concern to plant breeders for ensuring heritability during the process of developing improved cultivars (Cheng and Cheng, 2002; Kang, 2004; Campbell *et al.*, 2012). Upland cotton (*Gossypium hirsutum* L.), is one of the main cultivations in Greece, in four main regions with special environmental characteristics (Baxevanos *et al.*, 2013; Greveniotis and Sioki, 2017). Improvement in fiber quality is the primary objective, based on reliable methods that measure fiber traits. Cotton industry needs suggest breeders to adopt market expectations. More accurate measurements are now available to handle quality data (Hequet *et al.*, 2007). Now, Advanced Fiber Information System (AFIS, Uster Technologies, Inc.), is used by textile mills for extensive cotton fiber quality control (Hardin et al., 2018). Fiber quality is depended on various traits (Poehlman and Sleper, 2006) that depend on both genetic (cultivars) and environmental factors (Bradow and Davidonis, 2000). Ghosh et al. proposed at least three specimens for better assensing quality parameters (1992). McAlister *et al.* (2003) used AFIS for assessing fiber qulity. They stated that that the high micronaire cottons exhibited more convolutions and longer fibers exhibited higher frictional. Kelly and Hequet (2018), implemented a novel statistical model for better describing quality data retrieved by AFIS measurements. They also used comparisons to HVI measurements. The purpose of this study was to evaluate fiber quality traits across diverse environments by AFIS instrument which is used for more accurate quality estimations. This could lead to both an environment selection for cotton cultivations and also best cotton cultivars for a specific environment.

Materials and methods

Four main cotton-production Greek regions were selected because of their different environments, i.e. Thessaly, Sterea Ellas, Macedonia and Thrace. The five most cultivated commercial upland cotton (*Gossypium hirsutum* L.) cultivars were used, i.e. DP332 and DP377 (Monsanto Co), ST402 (Pioneer Hi-Bred), CELIA and ELSA (Bayer Crop Science). These cultivars varied in origin and maturity class. Four fields in each of four regions were chosen for sowing each cultivar separately, in order to evaluate different soil types and environmental conditions. During experimental years' environmental data were monitored and showed significant differences. Each cultivar was sown in 16 fields and 80 fields in total were used for all cultivars. Rows spaced 96 cm apart and plant density was on average 15 plants per m^2. Crop management practices for each location were consistent with typical agronomic practices. Four samples from each field were collected in random to analyze fiber quality traits at the accredited laboratory of National Center of Cotton, in Karditsa, Greece. Before testing cotton samples were conditioned for at least 24 hours at 65 ±2% RH and 21 ±1 ºC (ASTM, 2015).

The cotton traits were measured by AFIS instrument (Advanced Fiber Information System – AFIS by Uster Technologies AG, Uster, Switzerland) as follows: L-w: Average fiber length by weight of all the cotton fibers in the sample. L-n: Average fiber length by number of all cotton fibers in the sample. UQL-w: Upper Quartile Length by weight: length exceeded by 25% of the fibers. SFC-w: Percent of all fibers in a cotton sample that are shorter than 12.7 mm (0.5 in.) by weight. SFC-n: Percent of all fibers in a cotton sample that are shorter than 12.7 mm (0.5 in.) by number. L(n) 5%: 5%-Fiber Length by number IFC: Immature Fiber Content (%), Percentage of immature fibers. FINE (MTex): Fineness (MTex= in mg/m), fiber weight per length, estimated from fiber shape and form. MAT: Maturity Ratio, Ratio (%) based on degree of fiber wall thickness. Factor analyses (ANOVA) for each fiber trait across environments were performed according to Steel and Torrie (1981). Factors were both environments (the Greek regions) and genotypes (the five cultivars). Experimental Coefficient of Variation (CV%) was also computed. Separations of means were based on Duncan's test.

Results and discussion

As shown in Table 1, many fiber traits exhibited statistically very significant differences for both factors environments and cultivars (genotypes), indicating differences in environmental conditions and cultivar behavior (Bradow and Davidonis, 2000; Greveniotis and Sioki, 2017; Greveniotis *et al.*, 2017). Only fiber traits IFC and MAT showed small differences, statistically significant for cultivars but not for environments. Genotype × Environment interactions were not present except for traits UQL-w and Finess, which were found close to 0.05 level. Finess showed the greatest mean squares (up to 770.45), while in general, mean squares of the factor environment were greater than for genotypes (except for IFC and Finess, where genotypes contributed the most in total variability). Experimental CV(%) was high for IFC and SFC, indicating a rather unstable behavior of these fiber traits (Greveniotis *et al*,. 2018).

In Table 2, Thrace followed by Macedonia, proved to be the best regions in Greece to cultivate cotton for all fiber traits, except for SFC which is in diverse expression to other fiber traits by definition (Jin et al., 2016), favored by Thessaly and Sterea Ellas regions. This was in agreement with the findings of Greveniotis *et al.* (2018) for other measurements of fiber traits. Table 3 presents measurements and comparisons between cultivars. Cultivar CELIA showed the best measurements for almost all fiber traits across Greek regions, followed by ELSA and ST402. This was also in agreement with the findings of Greveniotis *et al.* (2018) for other measurements of fiber traits.

Table 1. Factor analyses (ANOVA) for each fiber trait (L-w, L-n, UQL-w, SFC-w, SFC-n, L(n) 5%, IFC, MTex, MAT) mean squares and degrees of freedom, as well as experimental CV(%) are presented

Source of variation	df	L-w	L-n	UQL-w	SFC-w	SFC-n	L(n) 5%	IFC	FINE (MTex)	MAT
Environments (E)	3	10.29**	14.69***	8.23**	7.19***	49.82**	11.16**	0.91 ns	172.95*	0.001 ns
Replications/E	36	1.09	1.35	1.17	0.59	5.25	1.26	0.77	83.33	0.001
Genotypes (G)	4	5.99***	7.08***	9.18***	1.83***	14.68***	12.73***	3.19*	770.45***	0.003**
G x E	12	0.89 ns	0.94 ns	1.46 †	0.31 ns	1.90 ns	1.49 ns	0.99 ns	107.80 †	0.001 ns
Error	48	0.55	0.59	0.81	0.30	2.53	1.56	1.39	61.20	0.001
CV (%)		2.77	3.24	2.88	15.60	13.10	3.06	23.53	3.06	2.89

(*) Significant at $p<0.05$, (**) Significant at $p<0.01$, (***) Significant at $p<0.001$
(ns) non-significant, (†)non-significant and close to 0.05 significance level (<0.07)

Table 2. Means of all fiber across the four different regions (Thessaly, Thrace, Macedonia and Sterea Ellas)

Variety	L-w	L-n	UQL-w	SFC-w	SFC-n	L(n) 5%	IFC	FINE (MTex)	MAT
Thessaly	26.22 b	22.66 b	30.73 b	4.14 a	13.89 a	34.63 b	5.12 a	182.8 b	0.95 a
Macedonia	27.02 a	23.89 a	31.39 ab	3.16 b	11.40 b	35.27 b	5.11 a	187.6 ab	0.95 a
Thrace	27.68 a	24.50 a	32.06 a	2.82 b	10.37 b	36.19 a	5.14 a	187.3 ab	0.95 a
Sterea Ellas	26.19 b	22.92 b	30.72 b	3.83 a	12.99 a	34.58 b	4.70 a	189.8 b	0.96 a

Table 3. Means of all fiber traits for the five cultivars (DP332, DP377, ST402, CELIA and ELSA)

Variety	L-w	L-n	UQL-w	SFC-w	SFC-n	L(n) 5%	IFC	FINE (MTex)	MAT
DP 332	25.79 c	22.38 c	30.08 c	4.06 a	13.74 a	33.90 c	5.37 a	184.8 bc	0.94 b
DP 377	26.59 b	23.41 b	30.92 b	3.48 b	11.77 b	34.79 b	5.33 a	189.6 b	0.96 ab
CELIA	27.04 ab	23.86 ab	31.32 ab	3.19 b	11.36 b	35.13 b	4.51 a	197.4 a	0.96 ab
ELSA	27.15 a	23.74 ab	31.89 a	3.43 b	12.38 b	35.92a	4.65 a	179.1 c	0.98 a
ST 402	27.31 a	24.06 a	31.89 a	3.27 b	11.57 b	36.10 a	5.27 a	183.6 c	0.95 b

Conclusions

Many fiber traits exhibited statistically significant differences for both factors environments and cultivars (genotypes), indicating differences in environmental conditions and cultivar behavior. Genotype × Environment interactions were not present except for two traits (IFC and MAT) that were very close to 0.05 significance level. Finess showed the greatest mean squares; while in general, mean squares of the factor environment were greater than for genotypes (except for IFC and Finess, where genotypes contributed the most in total variability). Thrace followed by Macedonia, proved to be the best regions in Greece to cultivate cotton for all fiber traits, except for SFC a rather unstable trait. Commercial cultivar CELIA showed the best measurements for almost all fiber traits across Greek cotton regions.

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ASSESSING YIELD OF WINTER CEREALS UNDER EXTREME CONDITIONS IN GREECE

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Abstract

The purpose of the present research was to analyze yields of 24 different genetic materials (cultivars) of winter cereals under extreme conditions for years (2011 and 2012). For this to be done, seven breadwheat cultivars (Yecora E, Acheloos, Centauro, Nestos, Vergina, Irnerio, Yenerozo E.), five durum wheat cultivars **(**Fenix, Simeto, Bob, Mexicali 81, Athos), five barley cultivars **(**Cannon, Plaisant, Sonora, Zotis, Konstantinos) five triticale cultivars **(**Catria, Ariti, Niobi, Vrodi, Vrito) and two oat cultivars (Pallini, Flega) were used. The experimental design was a Randomized Complete Block (RCBD) with four replications. Bread wheat cultivars exhibited the greater mean values 4.3 Mg/Ha, exceeding the mean value of the Greek bread wheat cultivars. The best cultivar was Yenerozo E. Reversely to this, durum wheat cultivars exhibited low yields about 2.7 Mg/Ha, compared to 3 Mg/Ha of the Greek mean. The best cultivars were Finix, Mexicali 81 and Simeto. The yields for barley were also low, about 3.8 Mg/Ha, compared to 4 Mg/Ha of the Greek mean. The best barley cultivars were Zotis and Cannon. Triticale cultivars showed unexpectedly very low yields. Oat had also very low yields. A Genotype x Year interaction was found statistically significant, indicating different response of the cultivars in relation to the environmental conditions.

Key words: *Wheat, barley, oat, triticale*

Introduction

Commercial cultivars in order to be useful to farmers must incorporate high yield potential and stability across environments (Stratilakis and Goulas, 2003). Inbred cultivars like wheat or barley are easily maintained by farmers in Florina, by keeping part of the harvest seed for the next season but this may result in off-types and reduced field yield (Khan *et al.* 2007; El-Kalla *et al.* 2010). Josephides (1993) analyzed various cultivars of bread and durum wheat together with barley and triticale, concluding that barley is a more suitable cereal for low yielding environments. Bread wheat and durum wheat are better performers in medium to high yielding environments respectively. Lopez-Castaneda and Richards (1994) compared bread and durum wheat together with barley, oat and triticale for grain yield and biomass. They concluded that barley was the best yielding cereal among all species. Geveniotis *et al.* (2009) compared and analyzed yield components between barley cultivars, in order to reveal the genetic basis of yield factors.

Florina (Greece) has extreme environmental conditions from October until April especially during winter, resulting in low yields for many cereals as reported by farmers and local authorities. That was the reason for the present research, in order to analyse yields of 24 different genetic materials (cultivars) of winter cereals under extreme conditions.

Material and methods

The 24 different genetic materials of winter cereals were sown in November in years 2010 and 2011 in the farm of the Technological Educational Institute (TEI) of Western Macedonia in Florina ($40^o46'$ N, $21^o22'$ E; 705 m a.s.l.); the soil was sandy loam: 61.2% sand, 27.6% silt, 11.2% clay, and pH 6.25. Environmental data are presented in Figure 1. For the purpose of the study, seven bread wheat (*Triticum aestivum* L.) **(**Yecora E, Acheloos, Centauro, Nestos, Vergina, Irnerio, Yenerozo E.), five durum wheat (*Triticum durum* L.) **(**Fenix, Simeto, Bob, Mexicali 81, Athos), five barley (*Hordeum vulgare* L.) **(**Cannon, Plaisant, Sonora, Zotis, Konstantinos), five triticale (x *Triticosecale* Wittmack) **(**Catria, Ariti, Niobi, Vrodi, Vrito) and two oat (*Avena sativa* L.) cultivars (Pallini, Flega) were used.

A Randomized Complete Block (RCBD) experimental design with four replications was established. Each plot was 1.5x5 m. Plant rows were 6 in each plot with conventional plant spacing. All the proper field activities were applied.

Measurements were based on mean grain yield (in Mg/Ha) fixed for moisture, after weighing by an electronic balance. Factor analysis (ANOVA) was performed according to Steel and Torrie (1981). Factors were both years (the local environment) and genotypes (five species – 24 cultivars). Experimental Coefficient of Variation (CV%) was also computed. Ranking of means were based on Duncan's test.

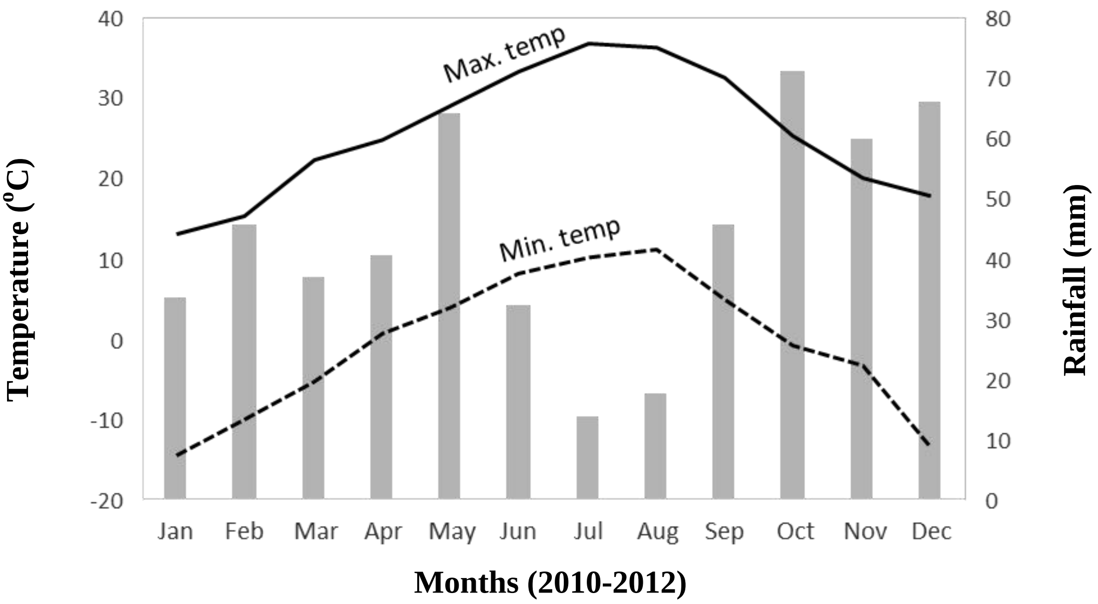

Fig. 1. Environmental conditions in Florina, Greece (temperatures and rainfall)

Results and discussion

The different genetic materials showed statistically significant differences within years and over years (Table 1). Bread wheat cultivars exhibited the greater mean value, that exceeded the corresponding Greek mean of (4.3 Mg/Ha and 4.0 Mg/Ha respectively, Tavoularis, 2012). The best cultivar was Yenerozo E with 4.8 Mg/Ha, followed by Irnerio and Centauro. In the opposite side, durum wheat cultivars exhibited lower yields compared the the Greek mean (2.7 Mg/Ha and 3 Mg/Ha respectively, Tavoularis, 2012). The best cultivars were Finix, Mexicali 81 and Simeto. A similar view was noticed in barley (3.8 Mg/Ha, compared to the 4 Mg/Ha of the Greek mean, Tavoularis, 2012). The best cultivars were Zotis and Cannon (over the Greek mean), while Konstantinos had very low yield and that was one of the reasons for the low barley mean yields. Triticale cultivars showed unexpectedly very low yields, about 3.9 Mg/Ha, compared to the 5 Mg/Ha of the Greek mean (Tavoularis, 2012) with Catria and

Niobi to performe better. In oat the view was similar with the mean yield to be lower than the corresponding Greek mean (2 Mg/Ha, and 4 Mg/Ha respectively Tavoularis, 2012). Cultivar Flega performed better than Pallini.

Table 1. Mean yield of the 24 cultivars (genotypes), within years and over years

	Genotypes	**2010-2011**	**2011-2012**	**Over years**
1	Yecora	3.4 defgh	3.8 cdef	3.6 ef
2	Acheloos	4.2 bc	4 cde	4.1 bcd
3	Centauro	4 bcd	4.9 a	4.4 ab
4	Nestos	4.1 bc	4.6 ab	4.3 bc
5	Vergina	4.5 ab	3.9 cdef	4.2 bcd
6	Irnerio	4.3 ab	4.6 ab	4.5 ab
7	Yenerozo E	4.9 a	4.7 a	4.8 a
8	Fenix	2.1 jk	3.7 efg	2.9 gh
9	Simeto	2.8 hi	2.8 i	2.8 h
10	Bob	2.7 ij	2.5 ij	2.6 hi
11	Mexicali 81	3.1 ghi	2.8 hi	2.9 gh
12	Athos	2.1 jk	2.2 jk	2.2 j
13	Cannon	3.8 bcdef	4.2 bcd	4.0 cd
14	Plaisant	3.5 cdefg	3.4 fc	3.5 ef
15	Sonora	4 bcd	3.7 defg	3.9 de
16	Zotis	4.1 bc	4.2 bc	4.2 bcd
17	Konstantinos	3.2 fghi	3.2 gh	3.2 fg
18	Catria	3.9 bcde	4.6 ab	4.3 bcd
19	Ariti	3.6 cdefg	3.6 efg	3.6 e
20	Niobi	4.4 ab	4.1 cde	4.2 bcd
21	Vrodi	3.3 efghi	3.9 cde	3.6 e
22	Vrito	3.9 bcdef	4.2 bc	4.0 bcd
23	Pallini	1.6 k	2 k	1.8 k
24	Flega	2.1 jk	2.4 ijk	2.2 ij
	CV %	11.8	8.2	10.1
F test	**Years**	-	-	**ns**
	Genotypes	***	***	***
	GxY	-	-	***

(***) Significant at p<0.001, (ns) non-significant
Letters determine the statistical differences between means

It is obvious that the extreme conditions in Florina decreased yields except in bread wheat mean yield. Zotis and Cannon barley cultivars are fully recommended for the area of Florina, since they produced very satisfactory yields. Oat and triticale are not recommended for the specific environmental conditions. Factor genotypes found significant indicating differences between species and cultivars. Genotype x Year interaction was also significant, indicating different response of the cultivars in relation to the environmental conditions (Greveniotis et al., 2009). These findings are not in fully agreement with Lopez-Castaneda and Richards (1994) reports, but they are similar in some cases to Josephides (1993) reports. Mediterranean conditions are not the same everywhere and, in case of Florina the extreme environmental conditions favoured two barley cultivars and bread wheat cultivars.

Conclusions

Bread wheat cultivars exhibited the greater mean values among all cereals. The mean is above the Greek mean for bread wheat. The best cultivar was Yenerozo E. Reversely to this, durum wheat cultivars exhibited lower yields, about 2.7 Mg/Ha, compared to the 3 Mg/Ha of the Greek mean. The best cultivars were Finix, Mexicali 81 and Simeto. Barley also performed lower than the Greek mean (3.8 Mg/Ha, compared to the 4 Mg/Ha of the Greek mean). Zotis and Cannon barley cultivars are fully recommended for environment of Florina, since they exhibited very satisfactory yields. Oat and Triticale are not recommended for the specific environmental conditions. Extensive research in many diverse environments will enrich our conclusions**.**

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THE INFLUENCE OF AGRO-ECOLOGICAL CONDITIONS ON THE QUALITY OF FIELD PEA

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Abstract

Two forage pea varieties (Saša and NS Junior) and three grain varieties (NS Javor, Baccara and NS Dukat) of field pea were cultivated in 2016 and 2017. The research was conducted at the experimental field of the Agricultural Institute of Republic of Srpska in Banja Luka and in the experimental field of the Agricultural Faculty in East Sarajevo to determine the influence of agro-ecological conditions on the protein content, fat content and ash content of pea grains. The grain quality of the pea was significantly influenced by the locality, while the interaction of the variety/location and the year/location significantly influenced the ash content and the fat content and had a significant effect on the protein content of the pea grain. In the experiment conducted in Banja Luka, there was significantly higher content of protein (26.89%) , fat (2.468%) and ash (3.504%) in the pea grain, compared to the quality of the pea grain produced in East Sarajevo, where the content of protein was (24.88%), fats (2.312%) and ash (3.10%). The largest amount of protein and ash was found in the variety Saša, in the area of Banja Luka. These results can influence the improvement of breeding work at the Agricultural Institute of Republic of Srpska, stimulating the production of peas and increasing the area on which this crop will be grown. Special attention should be paid to hilly and mountainous areas where field peas can be important in providing sufficient quantities of plant proteins to feed domestic animals.

Keywords: *pea, locality, proteins, fats, ashes.*

Introduction

Grain pea is an economically important agricultural plant species that is primarily cultivated due to the high content of protein in the grain. Favorable biological features, including short vegetation, modest requirements for production conditions, variety of use, high and stable yield, as well as the quality of biomass and grains, make the field pea significant in resolving the deficit of plant proteins (Čupić et al., 2010; Čupić et al., 2013). In the organized production of forage, pea provides rational use of land with relatively small investments (Ćupina et al., 2000; Ćupina *et al*., 2005). Despite the great importance of field pea in the production of fodder and grain, production in our country is very small and official statistics do not cover the area of forage pea production and seed yield production. Peas is primarily grown as a spring plant for grain and for green mass (Ćota & Čamdžija, 2010).

For the production of green forage production, most commonly used are winter peas varieties, but there is a need for a new assortment of pea's grain, which can have a dual purpose: the use of grain, but also the whole plant (Gantner et al., 2014). Spring field pea is usually produced for the usage of grains and represents a highly concentrated protein feed of high-quality nutritive values. In comparison to other raw materials, such as soybean, soybean meal, fish meal, it is a significant source of "cheaper" proteins.

Therefore, the biological value of peas as a forage crop is very similar to other protein crops (Mihailović et al., 2004; Marohnić, 2006). The protein content of pea seed types depends on the variety, the year and the region in which it is grown. The content of starch, fat, proteins

and constituent elements in pea grain vary, and are significantly influenced by variety and ecological conditions (Santalla et al., 2001; Wang & Daun, 2004).
The aim of this research was to determine the influence of agro ecological conditions and varieties on the quality of spring field pea. Since the Agricultural Institute of the Republic of Srpska has an acknowledged variety of common peas (Saša) it is included in this research.

Materials and methods

The field experiments were cultivated in 2016 and 2017 at two localities: locality 1- in the vicinity of Banja Luka (experimental field of the Agricultural Institute of the Republic of Srpska), altitude of 163 m (17°49'19 " latitude and 44°48'13" longitude), and locality 2 - in the vicinity of East Sarajevo (experimental field of the Faculty of Agriculture in East Sarajevo), altitude 550 m (43°49'01" latitude and 18°20'57" longitude). The basic soil treatment was carried out in the autumn, at a depth of 25 cm, and the pre-sowing preparation at a depth of 10 cm. Basic fertilization with 350 kg of ha^{-1} $N_8P_{24}K_{24}$ was done together with the basic cultivation of soil. The sowing was done at 12.5 cm spacing and a interspace of 8 cm in the main plot of 5 m^2. For sowing, inoculated seed of five spring fodder pea varieties were used: NS Javor (G1), Baccara (G2), NS Dukat (G3), NS Junior (G4) and Sasa (G5). Three varieties were cultivated at the Institute for Field and Vegetable Crops in Novi Sad - Republic of Serbia (NS Dukat, NS Junior and NS Javor), Baccara is a variety cultivated in France while Sasa is a homemade variety cultivated at the Agricultural Institute of Republic of Srpska in Banja Luka. In the group of genotypes for combined production (biomass and grain) we included: NS Junior and Sasa, while NS Javor, Baccara and NS Dukat were used for grain production. In 2016, the sowing was done on 15 April and harvest on 15 July, while in 2017 the sowing was on 23 March, and harvest on 6 July. In 2016, sowing was late because of bad weather conditions. Long rainy period did not allow pre-season preparation and the mechanization to enter the field.
The parameters of the pea's grain quality (chemistry composition) were determined by the following methods: the crude proteins were determined by the micro-Kjeldahl method, or the crude protein multiplied by a factor of 6.25; crude fats in plant material, using the Soxhlet method, and crude ash content in the plant material by annealing at 550°C to a constant weight. The analyzes were done inat the laboratory of the Faculty of Agriculture in East Sarajevo. The obtained data were processed by the variance analysis method (MANOVA) and tested by the LSD test (STATISTICA 7.1 for Windows) (Stat Soft 2005).
The distribution of total monthly rainfall and average monthly air temperature for research years and the perennial average for Banja Luka and East Sarajevo are shown in Table 1. Average monthly air temperatures for the locality Banja Luka in both years were higher than the perennial average, and only in May 2016 the average monthly temperature was slightly lower than the perennial average. In the period of vegetation, the precipitation varied from year to year and from month to month. In 2016, the extreme month was April whith only 0.5 mm of precipitation, while in 2017 dry months were June and July. The uneven distribution of the precipitation affected the yield and the quality of the fodder pea. There were higher average monthly temperatures, compared to perennial average at the locality East Sarajevo in 2016. The exception was May when the average monthly temperature was lower for 0.9 °C. The amount of rainfall was lower than the perennial average in April and May. In April and May 2017, the temperatures were lower than the averages for the given months, while in June and July they were significantly above the perennial average. The amount of rainfall was significantly lower than the perennial average for January, March, April, May, June and July. Analyzing the years, 2017 was evidently more unfavorable. Analyzing the meteorological conditions during the performance of the experiments in Banja Luka and East Sarajevo, we

found significantly more favorable meteorological conditions in Banja Luka, especially when it came to the quantity and distribution of precipitation.

Table 1. Average monthly air temperature (°C), monthly rainfall (mm) and perennial average for Banja Luka and East Sarajevo

Month			I	II	III	IV	V	VI	VII
Banja Luka	2016.	Temperature (°C)	2.3	7.6	8.0	13.5	16.2	21.5	23.3
		Precipitation (mm)	109.7	108.5	122.2	0.5	100.6	117.8	125.9
	2017.	Temperature (°C)	-3.6	5.5	9.7	11.8	17.5	22.9	24.4
		Precipitation (mm)	87.2	100.4	124.0	148.4	92.1	35.1	38
	Average 1981-2010	Temperature (°C)	0.6	2.3	6.8	11.5	16.5	19.8	21.8
		Precipitation (mm)	69.7	59.1	87.5	84.3	89.4	112.4	81.4
East Sarajevo	2016.	Temperature (°C)	1.2	7.4	7.1	12.9	13.9	19.5	21.1
		Precipitation (mm)	46.6	87	131.7	60.5	82.1	96.4	104.5
	2017.	Temperature (°C)	-4.8	5.2	7.9	8.6	14.6	19.3	21.2
		Precipitation (mm)	57.9	69.1	43.6	132.4	73.8	55	66.5
	Average 1961-1990	Temperature (°C)	-0.8	1.7	5.5	10	14.8	17.7	19.7
		Precipitation (mm)	74	63	73	76	85	94	83

Soil in Banja Luka where the experiments were set has good physical characteristics with a depth of 35 cm. According to agrochemical analysis done at the Agricultural Faculty in East Sarajevo, it contains 0.13% nitrogen, 2.05% humus, 40 mg/100 g phosphorus and 38.48 mg/100g potassium. The soil reaction is neutral, pH in nKCl is 6.97. According to the agrochemical analyzes conducted at the Agricultural Faculty in East Sarajevo, the soil at the experimental field of the Faculty of Agriculture (Eastern Sarajevo) contains 0.27% nitrogen, 4.12% humus, 40.40 mg/100g phosphorus and 36.41mg/100g potassium. The soil reaction is moderately acidic, pH in nKCl is 6.39.

Results and Discussion

The locality significantly influenced pea grain quality (Table 2). In the experiments in Banja Luka, there was significantly higher protein content (26.89%) , fat (2.468%) and ash (3.504%) in pea grain, compared to the quality of pea grain produced in East Sarajevo, where the pea grain had a protein content of (24.88%), fats content of (2.312%) and ash content of (3.10%).
Protein content of the pea seed depends on the genotype and the environmental factors during the filling of the seed (Lhuillier-Soundélé et al., 1999). Research by Santalla et al. (2001) have shown that there is a significant interaction between the variety and environment that influenced the protein content. Wang&Daun (2004) determined the protein content varied depending on the ecological conditions and the variety, the protein content ranged from 20.2% to 26.7%. In extensive researches by Mihailović (1994), the protein content of the grains of the pea varieties varied from 23.0% to 26.4%. The protein content, depending on the locality, varied from 24.26% to 27.90%. The domestic variety Saša had the highest protein content in the Banja Luka area, while the lowest varieties of proteins at the locality of East Sarajevo were found in the French variety Baccara. In all varieties, significantly lower protein content in pea grains grown in East Sarajevo was determined, compared with the content of protein in pea grain in all varieties grown in Banja Luka. In both years of research, the protein content was significantly higher in pea grain grown in Banja Luka.

Table 2. Quality of grain varieties of peas depending on cultivation location

		% of protein	% of fat	% of ash
Locality (L)	Banja Luka	26.89a	2.468a	3.504a
	East Sarajevo	24.88b	2.312b	3.100b
Sort (S)				
NS Dukat	Banja Luka	26.30b	1.943d	3.519a
	East Sarajevo	24.93c	2.298c	3.116c
NS Junior	Banja Luka	27.33ab	2.420c	3.344b
	East Sarajevo	25.07c	1.478e	3.136c
Baccara	Banja Luka	26.17bc	2.609b	3.525a
	East Sarajevo	24.26c	3.044a	3.053c
Saša	Banja Luka	27.90a	2.741b	3.573a
	East Sarajevo	25.30c	2.390c	3.044c
NS Javor	Banja Luka	26.77ab	2.629b	3.560a
	East Sarajevo	24.85c	2.351c	3.151c
Year (G)				
2016	Banja Luka	26.91a	3.349a	3.624a
	East Sarajevo	24.50c	2.645b	3.113c
2017	Banja Luka	26.88a	1.588d	3.384b
	East Sarajevo	25.27bc	1.979c	3.087c
L		**	**	**
LxS		*	**	**
LxG		*	**	**
LxSxG		ns	ns	ns

Values marked by different lowercase letters per column are significantly different at the level P 0.05 according to LSD¬test; * F test significant at P 0.05; ** F test significant at P P0.01; The ns¬F test is not significant.

The lowest fat content in pea grains had the variety NS Dukat at the locality of Banja Luka, and the highest content the variety Baccara at the locality of East Sarajevo (interaction of variety/locality), which is in agreement with the results of Wanga&Dauna (2004), which found significant influence of varieties and environmental conditions on the content of starch, ADF, NDF and fats. The highest variations in fat content, depending on the locality, were found in the variety NS Junior, and the least in the variety NS Javor. A highly significant year/locality interaction was also established. The highest fat content had the grain pea produced in Banja Luka in 2016, while the pea produced at the same location in 2017, had the smallest fat content.

The location had a significant influence on the ash content of pea grains in all the varieties studied (interaction of variety/locality), since all varieties grown in Banja Luka had more ash content in pea grain, in comparison withcompared to East Sarajevo. For the NS Junior varieties, these differences were significant, but minor compared to other varieties.

Compared to other varieties, this variety had the highest ash content in pea grains (Banja Luka), but at the site in East Sarajevo, the lowest content of ash was found in this variety, when compared to other varieties. Significant differences in ash content of the pea grain during the interaction of years / locality were also found. The pea grain produced in 2016 at the locality of Banja Luka had significantly higher ash content than grain pea produced in Eastern Sarajevo in both 2016 and 2017. The season did not have an impact on the ash content of pea grains produced at the location in East Sarajevo, which is in agreement with the results of Wang & Daun (2004).

Conclusion

In two years of quality research of five varieties of peas in the vicinity Banja Luka and East Sarajevo, we can conclude:

- The locality in the vicinity of Banja Luka has significantly more favorable agro-ecological conditions compared the locality in the vicinity to East Sarajevo, which has significantly influenced the better quality of field pea grain;
- Meteorological conditions (temperatures and precipitation schedule) were more favorable in 2016 compared to 2017, which is why pea grains produced in 2016 had higher protein, fat and ash content compared to 2017;
- The domestic variety Saša had the highest protein and ash content at the Banja Luka locality, while the Baccara variety at the East Sarajevo locality had the highest fat content;
- Production of field peas, should be stimulated, due to its characteristics, as well as the work on pea varieties breeding, at the Agricultural Institute of Republika Srpska, in order to obtain new varieties that can be grown in different agro-ecological conditions of Republika Srpska (Bosnia and Herzegovina).

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EFFECT OF THE 1BL.1RS WHEAT-RYE CHROMOSOMAL TRANSLOCATION IN BREAD WHEAT CULTIVARS ON PHYSIOLOGICAL TRAITS

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Abstract

In order to study the effect of the 1BL.1RS wheat-rye chromosome translocation on yield and physiological traits, three Hellenic spring wheat varieties with (cvs. Acheron, Elissavet and Orfeas), six cultivars without the translocation (cvrs. Apollonia, Acheloos, Vergina, Doirani, Nestos and Strymonas) and the Russian cultivar KVZ/Cgn as a check, were evaluated for three successive years under low input conditions. Total chlorophyll content, chlorophyll fluorescence, CO2 assimilation rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate were measured. Significant differences were recorded in yield and in two traits, i.e. total chlorophyll content and transpiration rate. Regarding yield, despite the existing variability between cultivars with and without the translocation, no effect of the translocation was noticed. The two cultivars with (Elissavet and Acheron) carrying the translocation performed almost equally with two of the top yielding varieties without the translocation (Apollonia, and Achellos). Also, the same two wheat cultivars without the translocation showed high total chlorophyll content but they did not differ significantly from cv. Elissavet one of the translocation carriers. No positive effect of the translocation was observed on transpiration rate with the top yielding cultivar Apollonia without the translocation differing significantly from the top yielding cultivar Elissavet with the translocation. When the studied physiological traits were compared to the yield of the corresponding cultivars it was concluded that the 1BL.1RS chromosome translocation did not give any significant advantage on the yield potential of the genotypes. Further research is needed under different environments to confirm the results of the present study.

Keywords: *Yield potential, Drought resistance, Chlorophyll content, Transpiration rate.*

Introduction

One of the main problems in releasing new cultivars is that this new germplasm will probably be grown on marginal environments due to the irrational waste of territorial resources noticed in previous years. The drought conditions prevailing in spring especially in the last decades, is the main obstacle of agricultural production in the southern regions of Europe (Yau and Saxena, 1997) complicating the problem of food sufficiency. Thus, one of the most decisive factors in all breeding programs is the identification and integration of genes into cultivated varieties that confer resistance or tolerance to drought (Blum, 1988) and avoid mistakes of the past (Acevedo and Fereres, 1994). This led breeders and especially those working on wheat, to look for new gene pools to face the problem (Fehr, 1987). According to various reports bread wheat *(Triticum aestivum* L. em Thell) cultivars carrying the 1BL.1RS wheat-rye chromosome translocation are characterized among other by high yield potential (Kim *et al.*, 2004; Xynias *et al.,* 2007) and resistance to drought (Hoffmann, 2008). The 1BL.RS translocation is originated from cv. Kavkaz/Cgn (Schlegel and Meinel, 1994) and it's unique traits are attributed to genes located on the short arm of the first chromosome of rye (Schlegel and Meinel, 1994; Xynias *et al*., 2007). According to Acevedo and Fereres (1994) one crucial mistake in developing new germplasm was the non-sufficient exploitation of certain physiological traits, rendering cultivated plants more competitive to weeds. Common physiological traits used to recognize increased stress tolerance are gas exchange parameters such as assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (c_i), chlorophyll content and chlorophyll fluorescence (Hura *et al.,* 2007; Živčák *et al.,* 2008). The above physiological traits have exhibited a good correlation with tolerance to stresses and yield parameters with a high heritability and repeatability (Fotovat *et al.*, 2007; Sayar *et al.*, 2008). In a preliminary report Pankou et al. (2017) stated that no evidence was obtained to confirm the positive effect of the translocation on relevant physiological traits.

The aim of the present study was to further investigate the effect of the 1BL.1RS wheat-rye chromosomal translocation on certain physiological traits and elucidate how they affect yield and drought resistance.

Materials and Methods

Plant material

For the purpose of the study, nine Hellenic bread wheat cultivars (eg. Acheron, Elissavet, Orfeas, Apolonia, Acheloos, Vergina, Doirani, Nestos and Strymonas) that were developed at the Cereal Institute of Thessaloniki (Anonymous, 1985) were used. The Russian cultivar Kavkaz/Cgn, one of the donors of the 1BL.1RS wheat-rye chromosome translocation (Xynias *et al.,* 2006; Weng, 2007) was used as check. Three of the Hellenic cultivars were found to carry the 1BL.1RS wheat-rye chromosome translocation (cvs. Acheron, Elissavet and Orfeas) whereas the other six cultivars, were lacking the specific translocation (Xynias *et al*., 2006; Peros *et al.,* 2015).

Methods

The experimentation applied for three growing seasons. The examined cultivars and the control were sown in early November 2015 and at the same time in November 2016 and November 2017, in a field of the University of W. Macedonia Farm in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content 14.0 g kg^{-1}, N-NO_3 100 mg kg^{-1}, P (Olsen) 50.3 mg kg^{-1} and K 308 mg kg^{-1} and water holding capacity 21.8% (0 to 30 cm depth). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P_20_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary. The Randomized Complete Blocks (RCB) experimental design (fixed model) was applied, with four replications. The plots were consisted of five rows (plot area 3 m^2) of which the three inner were threshed (harvest area 1.8 m^2).

The mechanically harvested grain was weighed and a sample of grain was dried in an oven at 105° C for 24 h to determine grain moisture content. Grain yield was referred to 12% grain moisture. Chlorophyll content readings were taken with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot six fully expanded flag leaves per plot were used when the plants were at physiological maturity with six measurements per leaf, a total of 36 readings per plot. A portable photosynthesis system (LI-6400 XT, Li-Cor, Lincoln, Nebraska, USA) equipped with a 2X6 (12 cm^2) open top narrow leaf chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance to water vapour (g_s), and intercellular CO_2 concentration (C_i) during grain filling period. Leaf gas exchange was measured on the fully expanded flag leaf during the grain-filling period on six plants from each plot from 09:00-12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday. The quantum

photosynthetic yield of photosystem (PS) II or Y was measured with the portable OS5p Chlorophyll Fluorometer (Opti-Sciences Inc. Hudson, NH, USA).
The means were compared according to the L.S.D. method. The data obtained were analyzed statistically with Mstat-C (Freed and Eisensmith, 1986).

Results and Discussion

Significant differences were recorded between the examined cultivars in yield (significant differences at p=1%, Table 1). Regarding of the physiological traits studied significant differences were revealed in total chlorophyll content, CO_2 assimilation rate and transpiration rate (in the last at p=5%). No differences were observed in the other physiological traits between the examined cultivars regardless of the presence of the translocation (Table 1).

Table 1. Analysis of variance of bread wheat with and without the 1BL.1RS wheat-rye chromosomal translocation regarding yield and six physiological traits.

Source	df	Yield (g/plot)	Total chlorophyll content	Chlorophyll fluorescence	CO_2 assimilation rate	Stomatal conductance	Intercellular CO_2 concentration	Transpiration rate
Years (Y)	2	ns	**	**	ns	**	ns	**
Cultivars (A)	9	**	**	ns	**	ns	ns	*
(Y) x (A)	18	**	* *	ns	**	ns	ns	ns
Error	81							
CV		17.35	10.71	45,73	15,06	74,17	6,01	19,00

Table 2. Ranking of the bread wheat cultivars according to chlorophyll content.

Cultivar	Yield (g/plot)	Total chlorophyll content	CO_2 assimilation rate	Transpiration rate
Acherontas*	A	BC	AB	AB
Elissavet*	A	AB	CD	CD
KVZ/Cgn*	A	CD	BC	AB
Orfeas*	E	CD	AB	BCD
Apollonia	AB	AB	A	A
Acheloos	A	A	AB	ABC
Yecora E	DE	D	D	D
Doerani	BC	A	AB	AB
Nestos	CD	BC	BC	ABCD
Strymonas	BC	AB	A	ABC
LSD	79.56	3.327	1.503	0.6056

*Cultivars carrying the 1BL.1RS wheat-rye chromosomal translocation

The cultivars were classified according to their yield performance (Table 2). Three cultivars with the translocation (Acheron* Elissavet*, KVZ/Cgn*) and one without (Acheloos) were ranked in top yielding places, (marked with A). They were followed by three cultivars without the translocation (Apollonia, Doerani, and Strymonas (marked with AB, and BC) and the last group was consisted of the cultivars Nestos, Yecora E, and Orfeas* (marked with CD, DE and E respectively). It is obvious from the above results that there was no particular yield advantage of the presence of the translocation on the varieties studied. This is in disagreement with the conclusion of Kim *et al*. (2004), who reported a positive effect of the translocation on yield performance. Sufficient yield can be produced by elite cultivars despite the effect of the translocation. This yield performance supports the view that it is possible that the presence of the translocation gives some advantage to the host cultivar. However, this advantage is not so crucial because two other cultivars without the translocation performed equally well. The results presented in this study are in agreement with the respective stated by various authors

that in order the translocation to express its valuable genes, the genetic background of the host cultivar is important (Lazaridou *et al.*, 2017; Xynias *et al.*, 2018).
The insufficient yield performance of cv. Yecora E could be attributed to the very low temperatures prevailing in Florina during winter. However, there is no explanation for the inferior performance of cvrs. Orfeas* and Nestos, which are supposed to be resistant to low-temperatures.
Similar results were recorded and in the ranking of the physiological traits (Table 2). The cultivars were classified in four groups according to total chlorophyll content and transpiration rate and in five groups in CO_2 assimilation rate. For total chlorophyll content, four cultivars without (cvs. Aheloos and Doerani, Appolonia, Strymonas) and one with the translocation (Elissavet*) were ranked in the first places. A similar view was found in assimilation and transpiration rates, where Apollonia was ranked first. Thus, it could be stated that there is no obvious effect of the translocation on the traits studied. The aforementioned results support the view of Acevedo and Fereres (1994) who stated that exploitation of certain physiological traits could be beneficial in producing high yield varieties. Cvs. Apollonia and Acheloos which were ranked in first places not only in yield but also in all physiological traits studied are good examples of the former statement.

Conclusions

The results obtained in the present study, supported previous statements that the 1BL.1RS chromosome translocation does not give any significant advantage regarding the physiological traits of the host cultivar. The presence of the translocation seems to be beneficial in the cold area of Florina. However, further research is needed to confirm the results of the present study.

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COMPARISON OF SPECIES COMPOSITION OF VASCULAR PLANTS IN AGROFORESTRY SYSTEM

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Abstract

Agroforestry systems combine two close areas of land use, namely agriculture and forestry. Agroforestry can provide certain ecological benefits, such as increased biodiversity, reduced soil erosion, increased land use efficiency and production, and increased carbon sequestration. The aim of this contribution is the evaluation of species composition of vascular plants in two woody strips on a field under organic farming. The experiment was established on two fields near to Starnberg (Germany) in 2014. In May 2019, the evaluation of the vegetation was carried out using phytosociological plots of size 4 m^2. The percent coverage was estimated in each plot. A total of 36 plant species belonging to 17 plant families occurred in the monitored area. Woody strips can provide shelter and food for animals, but they can also be a potential source for weeds spreading to the surrounding land. Our results show whether plants are able to spread to the surrounding land and whether they are able to affect the vegetation in agricultural lands.

Keywords: *short rotation coppice, agriculture, energy crops, phytosociological methods*

Introduction

Humans have altered earth's landscape more than any other species, with agriculture representing the largest anthropogenic use of land area (Foley *et al*., 2005; Ramankutty *et al*., 2008). Only in Europe is about one half of land is farmed (Kanianska, 2016). Agriculture is a multifunctional system. It provides economic (private) production and ecosystem services for public goods. Agriculture characterized appearance of agriculture landscape (Wiggering *et al*., 2006). Information on the effects of management on ecosystem processes is consequently necessary to the development of predictive management models for major land-use change (Forup *et al*., 2008). Climate change induced warming is expected to impact plant diversity and distribution at all levels starting from single species to biomes (Parmesan, 2006). It will also result in alterations in population dynamics of native species that may enhance climate mediated biological invasion, alter community interactions and structure, and ecosystems functions (Walther *et al*., 2002). Biologically-diversified farming can contribute to ecological intensification of agriculture by providing multiple ecosystem services, such as the promotion of biocontrol services (e.g., a decrease in pest abundance and an increase in the abundance of natural enemies of pests) (Harvey *et al*., 2014). The widening focus from the traditional tree-based land use practice to more advanced landscape scale agroforestry approach creates a stronger link between agroforestry and biodiversity conservation (Sanchez, 1995). Agroforestry practices are often part of strategies to improve natural resource management (Ong and Kho, 2015). Short Rotation Coppice (SRC) is a technique for the cultivation of biomass, which lies midway between agriculture and forestry. Fast-growing species of trees (such as poplar and willow) are densely planted in rows and then harvested at certain intervals. They are planted at a distance of 0.5 - 1 m and are harvested at intervals of 2 to 8 years (Vanbeveren and Ceulemans, 2019). Changes in species composition resulting from the transition from arable land to increased cultivation of SRC may have a wider impact on ecosystem processes and ecosystem services (Rowe *et al*., 2013). The objective of the present contribution is evaluation of species composition of vascular plants in two woody strips on a field under organic farming.

Material and Methods

The experiment was established on two fields near to Starnberg (Germany) in 2014. Trees (willows, poplars) were planted on the edge of the plot in four double rows. Rows of trees were spaced 3 meters and trees were planted with a spacing 50 cm. In the first plot, the planting of willow and poplar alternated every 50 m. In the second plot, the planting of willow and poplar alternated every 60 m. In May 2019, the evaluation of the vegetation was carried out using phytosociological plots of size 4 m^2. The percent coverage was estimated in each plot. Three phytosociological relevés were recorded in the woody strips (willows, poplars) with 10 meter spacing. In addition, four phytosociological relevés were recorded in the field with a distance of 4 m from each tree species (see Figure 1.). Phytosociological relevés or also vegetation plots serve to describe the species composition and diversity of the vegetation of a given site. It is a list with information about certain habitat species and other relevant data (for example density, frequency, cover, exposure of the given site…). Another four phytosociological relevés were placed on the opposite edge of the field without tree rows. The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and canonical correspondence analysis CCA. A total number of 999 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 5.0 (Ter Braak and Šmilauer, 2012).

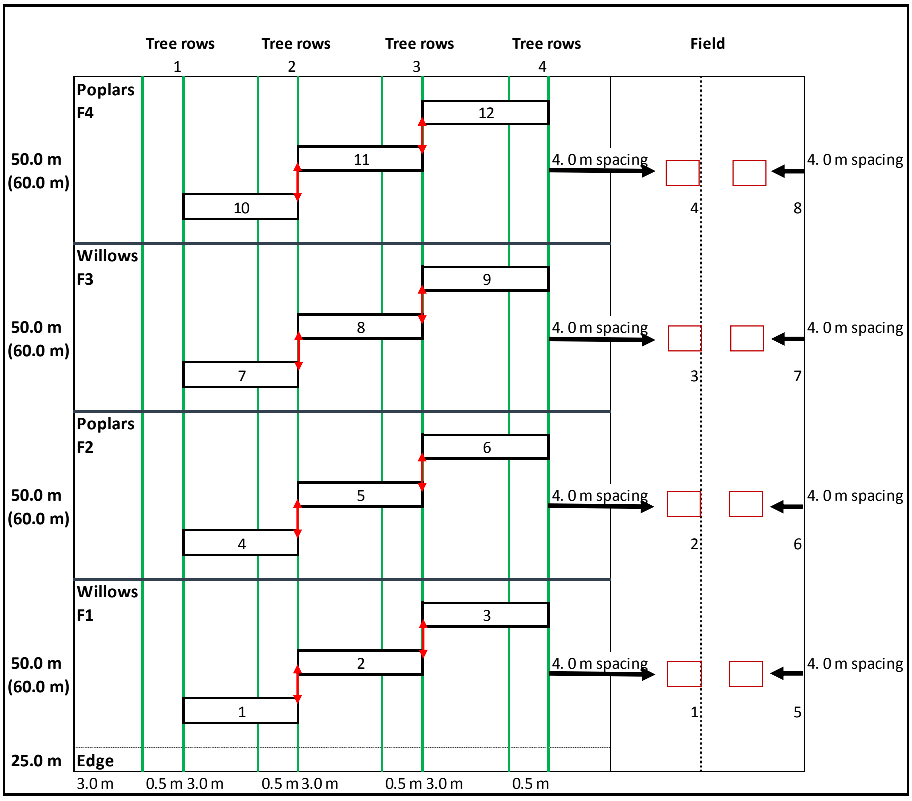


Figure 1 Schema of tooking phytosociological relevés

Results and Discussion

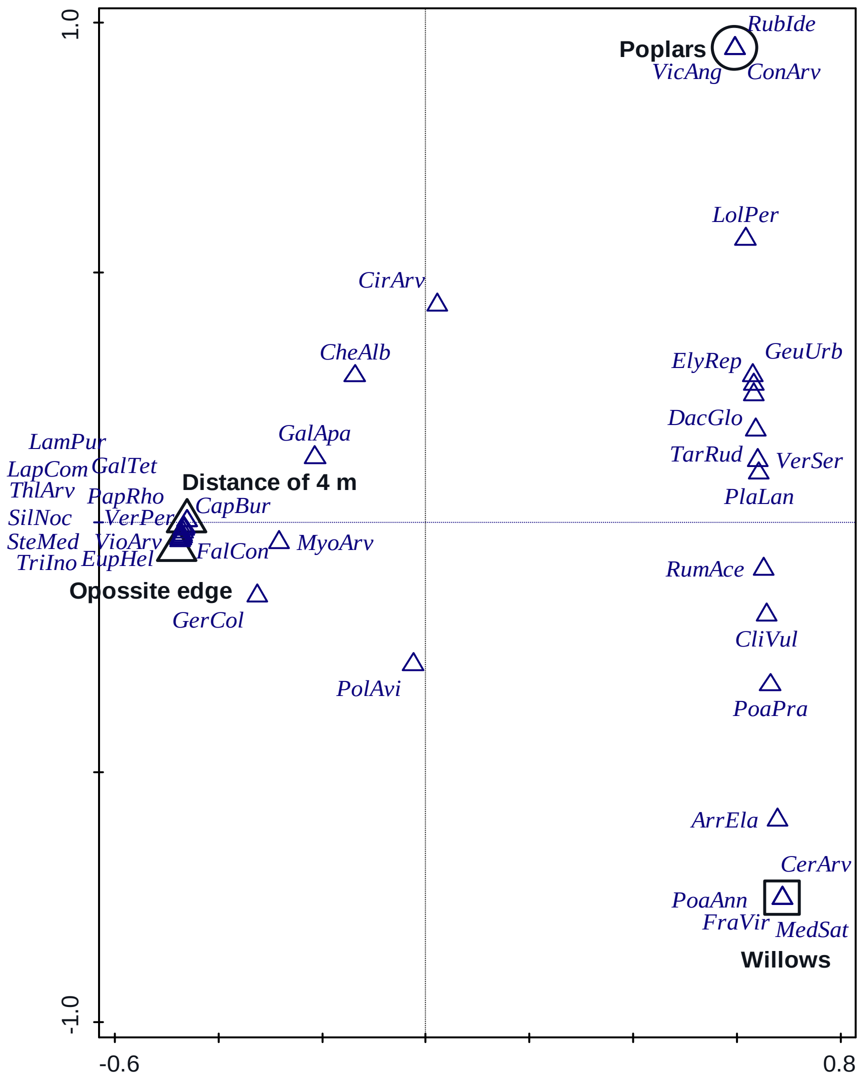
A total of 36 plant species belonging to 17 plant families occurred in the monitored area (see Table 1). Among the poplars 18 species have grown, among the willows 20. Of these, four species were also found on the areas at a distance of 4m from the stripes. In comparison, 13 species were found on the opposite side of the field. Only four of them grown on the areas with poplars and willows.

Table 1 Number of found species according to their family

	Willows	Poplars	Distance of 4 m	Opposite edge of the field
Family	Number of found species			
Amaranthaceae	0	1	0	1
Asteraceae	1	2	2	3
Boraginaceae	1	1	1	1
Brassicaceae	0	0	2	2
Caryophyllaceae	1	0	1	2
Convolvulaceae	0	1	0	1
Euphorbiaceae	0	0	1	1
Fabaceae	1	1	0	0
Geraniaceae	1	0	1	0
Lamiaceae	1	2	1	2
Papaveraceae	0	0	0	1
Plantaginaceae	2	2	1	1
Poaceae	6	5	0	0
Polygonaceae	2	1	1	1
Rosaceae	2	2	0	0
Rubiaceae	1	1	1	1
Violaceae	0	0	1	1

The obtained data about evaluation of species composition were initially processed by the DCA analysis which determined the length of the gradient, and it was 7.0. Based on this calculation for further processing was selected canonical correspondence analysis CCA. Analysis CCA defines the spatial arrangement of plant species and selected environmental factors. This is subsequently graphically expressed by the ordination diagram (see Fig. 2) Species and monitored factors are shown by points of different shape and colour. The species found can be divided into five groups: species that only occurred in willows, occurrence only in poplars, occurrence in poplars and willow, species that only occurred in the field and species that occurred at all sites. The only species found in willow were *Poa annua, Cerastium arvense, Fragaria viridis* and *Medicago sativa*. To species found only in poplars belong: *Convolvulus arvensis*, *Rubus idaeus* and *Vicia angustifolia*. The ordination diagram shows that the species from the family *Poaceae* were found only in poplars and willows. These species also had the highest coverage in the stripes of trees. The ordination diagram also shows that the number of found species was higher in the field, at different distances from woody strips. For example in willow and poplar plantations grown in southern and central Sweden, species numbers decreased with distance from the edges (Gustafsson, 1987; Weih *et al.*, 2003). Only four species were found at all sites, namely: *Cirsium arvense*, *Chenopodium album*, *Galium aparine* and *Polygonum aviculare*. These species can be considered as common weeds and results show that sites not influence occurrence of these species. These species belong to species with C, R and CR strategists and according to the Ellenberg indicator values for nutrients (value 7 and 8 for these species) belongs to species occurring at nutrient-rich sites.The found species *Chenopodium album* is listed as the world′s 10th most serious weed and is found in Asia, Europe and North America; as it is adapted to grow vigorously in many different climates and soils (Holm *et al.*, 1977). This species could be problematic for the future weed infestation; however, its occurrence is mainly dependent on the seeds in the soil seed bank.

There are many factors influencing species cover, species richness and the type of species occurring in SRC plantations (e.g. light climate and the tree age) (Baum *et al*., 2009).
Changes in species composition resulting from the transition from arable land to increased cultivation of SRC may have a wider impact on ecosystem processes and ecosystem services (Rowe *et al*., 2013). Depending on the location, SRC plantations can have positive as well as negative effects on biodiversity (Weih *et al*., 2003).
According to Baum *et al*. (2012) is species richness highest in SRC than in agricultural land and species composition correlated to SRC tree cover, SRC age and coppicing. There are a several studies assessed influence of a SRC. For example, Vanbeveren and Ceulemans (2019) conducted overview of the publications. They found out, that more than half relevant studies (33) assessed biodiversity of a willow SRC, while poplar was the second most studied SRC genus (20) other SRC genera were only occasionally reported on. The largest number of studies was performed on SRCs in, and equally spread between, the UK (22), the US (22) and Sweden (20). The remaining SRCs were mainly situated in Germany, Belgium and Italy.



Pseudo-F= 6.2, P= 0.001

Figure 2 Ordination diagram showing the relationship of found species and different sites

Legend for abbreviations: *Arrhenatherum elatius ArrEla, Capsella bursa pastoris CapBur, Cirsium arvense CirArv, Cerastium arvense CerArv, Clinopodium vulgare CliVul, Convolvulus arvensis ConArv, Dactylis glomerata DacGlo, Elytrigia repens ElyRep, Euphorbia helioscopia EupHel, Fallopia convolvulus FalCon, Fragaria viridis FraVir, Galeopsis tetrahit GalTet, Galium aparine GalApa, Geum urbanum GeuUrb, Geranium columbinum GerCol, Chenopodium album CheAlb, Lamium purpureum LamPur, Lapsana communis LapCom, Lolium perenne LolPer, Medicago sativa MedSat, Myosotis arvensis MyoArv, Rumex obtusifolius RumAce Rubus idaeus RubIde, Papaver rhoeas PapRho, Plantago lanceolate PlaLan, Poa annua PoaAnn, Poa pratensis PoaPra, Polygonum aviculare PolAvi, Silena noctiflora SilNoc, Stellaria media SteMed, Thlaspi arvense ThlArv Taraxacum sect. Ruderalia TarRud, Tripleuospermum inodorum TriIno, Vicia angustifolia VicAng, Veronica persica VerPer, Veronica serpyllifolia VerSer, Viola arvensis VioArv*

Conclusions

During botanical monitoring altogether 36 plant species belonging to 17 plant families occurred in the monitored area were found. Most representatives of the *Poaceae* family were found in woody strips. Only four species found at all sites, namely: *Cirsium arvense*, *Chenopodium album*, *Galium aparine* and *Polygonum aviculare*. These species can be considered as common weeds and results show that sites not influence occurrence of these species. Some of the found plant species are able to enrich the soil seed bank (*Chenopodium album*, *Capsella bursa-pastoris*). Other plant species can spread by anemochoria (*Cirsium arvense*). These four species can cause a weed infestation of the area in the coming years. The results also indicate that areas with fast growing trees (willows, poplars) increase the diversity of vascular plants and can thus support the diversity of agricultural landscapes. However, SRC is a relatively new way of management and its influence on vegetation are not yet well known. Therefore, this topic offers an interesting area for research.

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THE EFFECT OF DROUGHT STRESS ON FRUIT AND SEED COLOR IN PUMPKIN (Cucurbita pepo L.) GENOTYPES

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Abstract

Due to climate change, today, many agricultural areas are affected by drought. It is known that drought has many effects on plants, morphologically, physiologically and phenologically. In the study, the effects of drought stress on the fruit and seed color of the pumpkin genotypes were investigated. For this purpose, in 2017 and 2018, 48 pumpkin genotypes, consisting of 44 inbred lines and 4 commercial varieties, were grown in irrigation and non-irrigation conditions. The study was conducted by using a randomized block design with three replicates. Each parcel has an area of 20 m^2 and consists of 40 pumpkin plant. In this study, drip irrigation system was used and irrigation was applied ten times with seven days interval. We recorded fruits and seed color values (L *, a *, b *) with Chroma Meter CR-400, every two years. As a result of the study, it was determined that drought did not effect on fruit color values of L * and b *, but it had a significant effect on fruit color value of a* in pumpkin genotypes. Therefore, as the drought increases, the fruit colors shifted from green to red. Moreover, pumpkin seed color, which has economic value, was not affected by drought. When examined on the basis of genotypes, drought has important effects on fruit and seed colors.

Keywords: *drought, pumpkin, fruit color, seed color.*

Introduction

In the world, a total of 26 486 616 tons of pumpkin is produced in an area of 1 992 003 ha. China (7 838 809 tons) takes the first place in this production, while India (5 073 678 tons), America (3 315 909 tons), Russia (1 224 711 tons) and Indonesia (663 325 tons) make up the top five countries. Turkey is in area 764 458 da, 489 999 tons with production accounted for 1.84% of world production, it ranks 8th among the world countries (FAO, 2016). Confectionary pumpkin production in Turkey is approximately 737 891 in the area of 55 043 tons. The production of pumpkin is widespread in the inner regions of Turkey and Kayseri (16 751 tons), Nevşehir (16 403 tons), Konya (8 982 tons), Eskişehir (3 196 tons) and Karaman (1 205 tons) are the five provinces that produce the most (TÜİK, 2018).

Pumpkin seeds, in addition to being consumed as an appetizer in human nutrition, are used as additives for bread, salami, sausage, mayonnaise and many food products because of their high protein content (Rangahau, 2002). Some researchers have reported that pumpkin seeds improve the immune system (Chew and Park, 2004), and reduce the risk of stomach, breast, lung and colon cancer due to they have significant amount of antioxidants (Lelley *et al.*, 2009). It is also reported that phytosterols in pumpkin seeds play an important role in lowering cholesterol levels and in the treatment of advanced prostate (Fruhwirth and Hermetter, 2007).

Pumpkin seeds are among the oilseeds with a protein content of 28-40% (Kırnak *et al.*, 2019) as well as 35-50% fat content (Seymen *et al.*, 2016). In addition, it is rich in minerals such as potassium, phosphorus, calcium, magnesium, iron, which are important in human nutrition

(Erdinç *et al.*, 2018) and it is known as a good source of vitamin A, C and E (Eleiwa *et al.*, 2014; Kırnak *et al.*, 2019).

Climate change, rapid population growth, indiscriminate and excessive consumption and a shortage of fresh and clean water resources have led to irrigation problems (Rijsberman, 2006). Drought is currently one of the most common environmental factor limiting crop production in the world. Therefore, it is a necessity to make development of species and varieties which are tolerant to drought or where economic efficiency is achieved with less water.

With the increase of arid and semi-arid areas in the world, there is an increasing tendency to grow species such as confectionary pumpkin that can use water efficiently and economically. Its production has recently increased, due to the reasons such as less water requirement compared to other agricultural crops, lack of irrigation in some areas with sufficient precipitation, growing in rotation with field crops, ease of harvesting, the existence of mechanized cultural processes, the ability to be relatively resistant to diseases and pests, and the available for storage. Despite the increasing production, reaching commercial varieties in the market is an important constraint. Due to these reasons, a lot of research is being carried out, especially on drought studies in confectionary pumpkin, in Turkey (Yavuz et al., 2015a; Yavuz et al., 2015b; Ünlükara and Bakır, 2018; Seymen et al., 2019; Kırnak et al., 2019). Confectionary pumpkin often exhibits a higher economic yield in arid and semi-arid regions compared to other agricultural crops such as wheat, barley and chickpeas (Seymen et al., 2019).

It is well-known drought is cause many morphological and physiological changes in plants.

The objective of the present study was to determine the fruit and seed color through an experimentation conducted for two years in irrigated as well as non-irrigated conditions with 44 inbred pumpkin lines (G), 2 commercial hybrid cultivars (G1: Mert Bey F1 and G2: Sena Hanım F1), and 2 local cultivars (G3: Hatun Tırnağı and G4: Çerçevelik).

Materials and Methods

The present study was conducted during the May and Sept. in 2017 and 2018, at the Faculty of Agriculture, Selcuk University, Konya, located at 38°05'N, 32°36'E at an altitude of 1006 m, in central Anatolia, Turkey. Konya, which accounts for approximately 8% of the total agricultural area in Turkey and covers an area of 1.9 million hectares of arable land. Konya Plain has an arid and semi-arid climate, according to long-term climate data. In this region, the vegetation period (May-September) occurs only 90-100 mm of rainfall, and total annual precipitation is 320 mm. The soil of the study area has a silty–clayey–loamy texture. The organic matter content present in 0–90 cm of the soil profile, pH, and the bulk density values of the soil in the study area ranged from 1.43% to 2.16%, 7.44 to 7.50, 1.28 to 1.32 g/cm^3, respectively.

In this study used plant materials were collected from different regions of Turkey. The 44 confectionary pumpkin genotypes which were purified to the S7 stage by self-pollinated were used. In addition, two-hybrid (G1: Mertbey F1 and G2: Senahanim F1) and two local cultivars (G3: Hatuntırnağı and G4: Cercevelik) with high commercial value in the market were used to serve as a control group.

The present study was conducted by using a randomized block design with three replicates, in full-irrigated and complete-stress (drought) conditions. Each parcel has an area of 20 m^2 and consists of 4 rows of plants. A total of 40 pumpkin seeds were sown by hand with 1 m row spacing and 0.5 m inter-row spacing within each parcel. In this study, drip irrigation system was used and irrigation was applied ten times with seven days interval.

On the basis of soil analyses, 10, 10, and 12 kg ha^{-1} of N, P_2O_5, and K_2O were applied before sowing the seeds, respectively. Throughout the developmental period of the plants, cultural

practices such as cultivation, weed control, pest and disease management were conducted regularly. Twenty days prior to the harvest time, irrigation was discontinued in the fully-watered plots. Post complete drying of the plants, fruits were harvested and mature seeds were extracted from these fruits.
In both years, 4 fruits obtained from 20 other plants, except edge effects in the plots, were harvested at harvest maturity stage and L, a *, b * were recorded by chroma Meter CR-400. In addition, L, a *, b * values from each genotype were determined from a total of 5 seeds.The fruit and seed color parameters were subjected to analysis of variance, and the results were considered statistically significant at 5% significance level according to Duncan's test (Düzgüneş *et al.,* 1987). The analysis of variance and correlation tests were performed by using the SPSS 22.0 packaged software.

Results and Discussion

Fruit color ''L*''

The L * value ranges from 0 to 100 and describes the coloration between black (0) and white (100). In the study, it was found that irrigation and drought conditions had a statistically significant on the average L * value in different genotypes in 2017 and 2018 at 5 % significance level (Table 1).In respect to the average L* values of 2017-2018, it was determined that the drought conditions caused a change in L * values by % according to the irrigation conditions (Table 1). When the changes were examined, L* values increased in some genotypes in drought conditions but caused decreases in some genotypes. The average L* values of the genotypes were found to increase by 1.8 % under drought conditions. Genotypes 3 (%8.58), 6 (%4.87), 12 (%4.52), 20 (%4.37), 28 (%4.73), 29 (%5.16), 38 (%4.74), 39 (%6.79) and 40 (%4.17) were the highest L* values under drought conditions. On the other hand, genotypes 11 (5.75%) and 31 (3.31%) had lower L * values under drought conditions than irrigation conditions.

Fruit color ''a*''

Positive values of ''a*'' refer red-purple colors while negative values define blue-green colors. In the study, it was found that full irrigation and full drought conditions had a statistically significant effect on average "a*'' value of 2017, 2018 and average of the years according to 5% significance level in different genotypes (Table 1). Considering of the average ''a*'' values of the different pumpkin genotypes in 2017-2018, it was determined that the stress conditions caused % change in the ''a*'' values according to the irrigation conditions (Table 2). When the changes were examined, '' a*'' values increased in some genotypes in drought conditions. ''a*'' value decreased by 14% under drought conditions. 9 (31%), 13 (88%), 39 (18%), 42 (62%) and 45 (25%) genotypes gave higher fruit color ''a*'' values under stress conditions. On the other hand, 5 (48%), 6 (72%), 21 (66%), 31 (50%) and 41 (48%) genotypes gave lower''a*'' values under drought conditions compared to irrigation conditions.

Fruit color ''b*''

In b *, positive values are yellow and negative values are blue. In the study, it was found that the full irrigation and full drought conditions had a statistically significant effect on the average ''b*'' values of 2017, 2018 and average of the years in different genotypes at 5% significance level (Table 1). It has been determined that the drought conditions cause a variation in ''b*'' values according to the irrigation conditions (Table 1). ''b*'' values increased in some genotypes and decreased in some genotypes under drought conditions. ''b*'' values of genotypes were found to increase by 2.64 % under drought conditions. When genotypes were examined, 3 (10.86%), 4 (10.56%), 10 (11.28%), 25 (11.40%), 29 (14.89%), 37 (11.04%) and 43 (17.14%) had higher ''b*'' values under drought conditions. On the other hand, genotype 28 (7.93%), 31 (10.17%), 35 (6.39%) and 39 (5.10%) gave lower ''b*'' values under drought conditions than irrigated plots.

Table 1. Fruit color ''L*, a*, b*'' under irrigation and drought conditions in 2017 and 2018.

Gen	''L''			''a''			''b''		
	Average 2017-2018			Average 2017-2018			Average 2017-2018		
	Irrigation	Drought	% variation	Irrigation	Drought	% variation	Irrigation	Drought	% variation
1	**77.30 ab**	**79.21 abc**	2.47	4.13 s-y	2.49 q-u	-39.71	57.72 c-k	59.29 b-h	2.72
2	73.50 b-k	74.39 f-m	1.21	7.60 j-o	5.88 ı-p	-22.63	56.19 e-m	57.53 b-l	2.38
3	70.18 ı-m	76.20 b-j	8.58	9.86 e-k	4.80 k-t	-51.32	55.19 e-m	**61.20 abc**	10.89
4	**76.99 ab**	**77.90 a-g**	1.18	2.36 xy	3.57 o-u	51.27	53.21 ı-n	58.83 b-ı	10.56
5	71.20 f-m	70.93 l-r	-0.38	12.07 b-f	6.21 ı-o	-48.55	56.95 d-k	57.53 b-l	1.02
6	**74.40 a-ı**	**78.02 a-f**	4.87	5.33 o-u	1.47 u	-72.42	**60.44 a-e**	58.35 b-k	-3.46
7	**77.25 ab**	**80.14 a**	3.69	2.62 wxy	2.40 r-u	-8.40	**59.49 a-g**	**61.69 ab**c	3.70
8	74.04 b-j	73.71 h-o	-0.45	4.13 s-y	4.13 m-u	0.00	50.50 mno	53.55 ı-l	6.04
9	73.27 b-k	71.91 k-q	-1.86	7.49 k-p	9.83 c-h	31.24	58.30 b-j	57.53 b-l	-1.32
10	71.20 f-m	73.18 j-p	2.78	8.91 h-m	7.44 g-l	-16.50	**59.49 a-g**	**66.28 a**	11.28
11	69.71 k-o	65.72 u	-5.75	9.64 f-k	5.88 ı-p	-39.00	**59.39 a-h**	54.16 e-l	-8.81
12	70.12 ı-m	73.29 ı-p	4.52	9.86 e-k	7.02 g-m	-28.80	55.19 e-m	57.53 b-l	4.24
13	**78.55 a**	**78.58 a-d**	0.04	1.89 y	3.57 o-u	88.89	**59.66 a-g**	**62.11 ab**	4.11
14	68.52 l-p	67.70 r-u	-1.20	10.69 d-ı	**11.83 abc**	10.66	**58.54 a-ı**	57.53 b-l	-1.73
15	62.86 q	68.65 q-u	9.21	**16.14 a**	**14.45 a**	-10.47	56.19 e-m	58.69 b-j	4.45
16	**76.81 abc**	**77.94 a-g**	1.47	4.37 r-y	3.74 n-u	-14.42	57.72 c-k	**61.19 abc**	6.01
17	**75.34 a-f**	76.03 c-j	0.92	4.13 s-y	2.96 p-u	-28.33	55.19 e-m	55.30 d-l	0.20
18	**77.45 ab**	**78.58 a-d**	1.46	2.79 v-y	1.94 stu	-30.47	55.19 e-m	**60.68 a-d**	9.95
19	64.58 pq	65.84 tu	1.95	12.28 b-e	10.70 b-f	-12.87	54.07 g-n	54.12 f-l	0.09
20	68.90 l-o	71.91 k-q	4.37	8.91 h-m	7.94 e-k	-10.89	57.72 c-k	57.53 b-l	-0.33
21	**76.34 a-d**	**77.90 a-g**	2.04	4.91 q-w	1.66 tu	-66.19	57.72 c-k	54.12 f-l	-6.24
22	70.43 h-m	73.18 j-p	3.90	11.00 c-h	9.83 c-h	-10.64	57.72 c-k	58.35 b-k	1.09
23	70.02 j-n	71.16 l-r	1.63	9.26 g-l	6.87 h-n	-25.81	56.19 e-m	57.53 b-l	2.38
24	65.75 n-q	66.17 stu	0.64	13.34 bc	**12.56 abc**	-5.85	52.36 k-n	54.12 f-l	3.36
25	**74.71 a-h**	73.71 h-o	-1.34	6.34 n-s	5.15 j-r	-18.77	52.38 k-n	58.35 b-k	11.40
26	**76.56 a-d**	**76.88 a-j**	0.42	4.64 q-x	5.09 k-s	9.70	**59.07 a-ı**	**61.30 abc**	3.78
27	69.71 k-o	74.09 g-n	6.28	**14.13 ab**	**11.39 a-d**	-19.39	**63.00 abc**	**62.17 ab**	-1.32
28	**76.73 abc**	**80.36 a**	4.73	2.29 xy	2.96 p-u	29.26	**60.06 a-f**	55.30 d-l	-7.93
29	74.08 b-j	**77.90 a-g**	5.16	3.59 t-y	2.96 p-u	-17.55	52.52 j-n	60.34 b-d	14.89
30	**76.81 abc**	**77.12 a-ı**	0.40	4.21 s-y	3.57 o-u	-15.20	54.07 g-n	59.44 b-f	9.93
31	70.02 j-n	67.70 r-u	-3.31	11.85 b-g	5.88 ı-p	-50.38	57.72 c-k	51.85 l	-10.17
32	70.98 g-m	71.16 l-r	0.25	9.64 f-k	8.57 d-ı	-11.10	56.42 d-m	58.35 b-k	3.42
33	72.40 d-l	73.29 ı-p	1.23	10.08 e-j	8.57 d-ı	-14.98	**58.36 a-j**	**61.06 abc**	4.63
34	70.02 j-n	70.93 l-r	1.30	8.28 ı-n	7.77 f-l	-6.16	50.77 l-o	52.95 jkl	4.29
35	72.67 c-l	75.41 c-k	3.77	6.78 n-r	4.66 l-u	-31.27	**63.98 ab**	59.89 b-f	-6.39
36	69.71 k-o	68.65 q-u	-1.52	11.85 b-g	11.00 b-e	-7.17	56.19 e-m	55.30 d-l	-1.58
37	68.52 l-p	68.87 q-u	0.51	8.91 h-m	7.02 g-m	-21.21	48.74 no	54.12 f-l	11.04
38	66.93 n-q	70.10 o-r	4.74	10.69 d-ı	**11.37 a-d**	6.36	54.48 f-m	58.87 b-ı	8.06
39	65.42 opq	69.86 o-s	6.79	7.00 l-q	8.32 d-j	18.86	56.45 d-l	53.57 g-l	-5.10
40	71.97 e-l	74.97 d-l	4.17	5.33 o-u	3.57 o-u	-33.02	53.52 h-n	58.35 b-k	9.02
41	**75.01 a-g**	**80.04 ab**	6.71	3.00 t-y	1.55 u	-48.33	56.19 e-m	59.38 b-g	5.68
42	69.71 k-o	70.21 n-r	0.72	5.28 o-v	8.57 d-ı	62.31	54.07 g-n	58.69 b-j	8.54
43	**76.04 a-e**	**78.32 a-f**	3.00	2.86 u-y	2.03 r-u	-29.02	45.18 o	52.83 kl	17.14
44	69.71 k-o	69.93 p-t	0.32	10.20 e-ı	9.97 c-g	-2.25	56.19 e-m	55.36 d-l	-1.48
45	71.20 f-m	69.86 o-s	-1.88	8.91 h-m	11.15 bcd	25.14	**58.81 a-ı**	58.35 b-k	-0.78
46	**75.40 a-f**	**77.22 a-h**	2.41	4.99 p-w	3.57 o-u	-28.46	53.52 h-n	53.02 jkl	-0.93
47	69.71 k-o	68.65 q-u	-1.52	13.01 bcd	**13.65 ab**	4.92	**62.24 a-d**	**62.30 ab**	0.10
48	**74.79 a-g**	74.63 e-m	-0.21	5.44 o-t	6.21 ı-o	14.15	**64.29 a**	**62.39 ab**	-2.96
Ave.	**72.13**	73.46	1.84	7.56	6.48	-14.29	56.30	57.79	2.64
LSD	I: 4.29	D: 3.92		I: 2.52	D: 3.19		I: 5.94	D: 5.74	

In many studies, ''L *, a *, b *'' values of harvested fruits were determined by using Chroma meter. In our study, vide range of variations in ''L *, a *, b *'' color values were observed on the basis of genotypes. The drought did not have much effect on the ''L*'' and ''b*'' color values, but caused a significant change ~~on~~ in the color ''a*''. As drought increased, fruit colors turned from green to red. In other words, the drought caused the fruit color to become red. Ermiş (2010) determined the fruit colors in different pumpkin genotypes and different locations. He obtained from ''L*'' values between 63-74, ''a*'' values between 3.42-19.17 and ''b*'' values between 35-62. As a result of the study, it was reported that fruit colors are not affected by locations but there are significant differences between genotypes. Different researchers have reported that genotype, growing conditions, ripening period and cultural practices have an effect on fruit colors (Itle and Kabelka, 2009; Gajewski *et al.*, 2008). The values we obtained were similar to the previous studies, and it was observed that drought had a significant effect especially on the ''a*'' value.

Seed color ''L*''

In the study, it was found that the full irrigation and full drought conditions had a statistically significant effect on the average seed color ''L*'' value of the 2017, 2018 and years average according to 5% significance level in different genotypes (Table 2). Pumpkin genotypes give an ''L*'' value average of 78.88 in 2017 and 2018 under irrigation conditions. Under stress conditions, the average ''L*'' value was 78.88 in 2017 and 2018. It was determined that the drought conditions caused the seed color ''L*'' values change according to the irrigation conditions ''L*'' values of different pumpkin genotypes in 2017-2018 (Table 2). When the changes were examined, seed color ''L*'' values increased in some genotypes in drought conditions but caused some genotypes to decrease. When the average color ''L*'' values of the genotypes were examined, no difference was found. Genotypes 7 (8%), 11 (4%) and 40 (4%) gave higher seed color ''L*'' values under drought conditions. On the other hand, genotypes 2 (4%), 4 (4%), 18 (5%), 31 (5%), 43 (4%) and 45 (4%) had lower seed color ''L*'' values under drought conditions.

Seed color ''a*''

In the study, it was found that the full irrigation and full drought conditions had a statistically significant effect on the average seed color ''a*'' value of the 2017, 2018 and years average according to 5% significance level in different genotypes (Table 2). It has been determined that the drought conditions caused by the seed color ''a*'' values of different pumpkin genotypes in the 2017-2018 year caused a change in % of seed color ''a*'' values according to the irrigation conditions (Table 2). When the average color ''a*'' values of genotypes were examined, it was found that it decreased by about 3.92% under drought conditions. Genotypes 2 (77%), 5 (90%), 6 (103%), 10 (105%), 26 (98%), 28 (82%) and 43 (114%) had higher seed color ''a*'' values under stress conditions. On the other hand, 7 (52%), 16 (39%), 25 (42%), 29 (44%), 34 (54%), 39 (58%), 40 (57%) and 48 (53%) genotypes gave lower seed color ''a*'' values under stress conditions.

Seed color ''b*''

In the study, it was found that the full irrigation and full drought conditions had a statistically significant effect on the average seed color ''b*'' value of the 2017, 2018 and years average according to 5% significance level in different genotypes (Table 2). It has been determined that the drought conditions caused by the seed color ''b*'' values of different pumpkin genotypes in the 2017-2018 year caused a change in % of seed color ''b*'' values according to the irrigation conditions (Table 6). When the seed color ''b*'' values of the genotypes were examined, it was found that it increased by 2.91% under drought conditions. Genotypes 4 (11%), 6 (14%), 17 (12%), 23 (15%), 25 (12%), 31 (30%), 35 (11%) and 46 (11%) gave higher seed color ''b*'' values under drought conditions. On the other hand, genotypes 7 (14%), 15 (10%), 40 (10%) and 48 (15%) gave lower seed color ''b*'' values under drought conditions.

Using Chroma Meter to determine the color of the seeds, ''L*, a*, b*'' values give important information. In this study, although drought did not have much effect on ''L*, a* and b*'' values, significant changes in ''a* and b*'' values occurred on the basis of genotypes. It is preferred to have cream-colored and bright seeds in appetizer pumpkin. Turgut (2015), obtained from different pumpkin genotypes ''L*'' value between 73-82, ''a*'' value between

-0.84-1.1 and ‘’b*’’ values between 10-17. Our results are consistent with the study. Ermiş (2010), reported that ecology has no significant effect on seed color. In the study, it was observed that drought did not show differences in seed color in general.

Table 2. Seed color ‘’L*, a*, b*’’ under irrigation and drought conditions in 2017 and 2018.

Gen	‘’L’’			‘’a’’			‘’b’’		
	Average 2017-2018			**Average 2017-2018**			**Average 2017-2018**		
	Irrigation	**Drought**	**% variation**	**Irrigation**	**Drought**	**% variation**	**Irrigation**	**Drought**	**% variation**
1	76.44 h-l	76.71 d-ı	0.35	0.74 ı-l	0.72 f-j	-2.70	15.50 op	16.45 qr	6.13
2	79.65 b-k	76.09 f-ı	-4.47	0.40 l	0.71 f-j	77.50	18.20 j-m	17.57 n-r	-3.46
3	77.63 f-k	74.68 hı	-3.80	0.67 ı-l	0.98 b-j	46.27	17.81 k-n	18.47 j-r	3.71
4	**81.31 a-f**	77.57 c-ı	-4.60	0.76 h-l	**1.30 a-g**	71.05	17.88 k-n	21.01 c-ı	17.51
5	77.48 f-k	78.35 b-ı	1.12	0.75 h-l	**1.43 a-f**	90.67	18.83 h-l	19.91 d-o	5.74
6	77.87 e-k	**80.78 a-f**	3.74	0.61 jkl	**1.24 a-h**	103.28	18.03 k-n	20.59 c-l	14.20
7	73.12 l	**79.45 a-h**	8.69	1.34 c-h	0.64 g-j	-52.24	21.55 b-f	18.40 j-r	-14.62
8	**83.38 ab**	**81.24 a-e**	-2.57	0.91 f-l	0.70 f-j	-23.08	15.14 p	16.10 r	6.62
9	77.61 f-k	**79.95 a-g**	3.02	0.88 g-l	0.62 g-j	-29.55	16.37 m-p	17.62 n-r	7.64
10	78.79 c-k	**80.73 a-f**	2.46	0.68 ı-l	**1.40 a-f**	105.88	17.80 k-n	19.51 d-p	9.61
11	76.15 jkl	**79.22 a-h**	4.03	**2.15 ab**	**1.39 a-f**	-35.35	20.30 d-ı	19.27 e-p	-5.07
12	75.83 kl	76.13 f-ı	0.40	**1.79 abc**	**1.24 a-h**	-30.73	**22.46 abc**	22.65 bc	0.85
13	79.88 b-j	**79.24 a-h**	-0.80	0.80 h-l	0.54 hıj	-32.50	20.45 d-ı	20.24 c-m	-1.03
14	**82.23 abc**	**82.01 abc**	-0.27	0.47 kl	0.39 j	-17.02	16.51 m-p	17.83 m-r	8.00
15	77.03 g-l	76.61 e-ı	-0.55	1.52 cde	1.12 b-j	-26.32	21.50 b-f	19.19 f-p	-10.74
16	77.08 g-k	76.54 e-ı	-0.70	1.25 c-ı	0.76 e-j	-39.20	20.38 d-ı	18.50 j-r	-9.22
17	**81.81 a-e**	**78.69 a-ı**	-3.81	0.45 kl	0.56 hıj	24.44	18.99 h-l	21.31 c-g	12.22
18	80.28 b-h	75.62 ghı	-5.80	1.02 e-k	0.86 d-j	-15.69	19.49 g-k	20.82 c-j	6.82
19	77.74 f-k	78.40 b-ı	0.85	**2.20 a**	**1.60 abc**	-27.27	**23.66 a**	**24.59 ab**	3.93
20	77.92 e-k	77.73 c-ı	-0.24	0.99 e-l	**1.31 a-g**	32.32	20.07 e-ı	19.71 d-p	-1.79
21	80.64 b-g	**81.95 abc**	1.62	0.71 ı-l	0.97 b-j	36.62	17.79 k-n	17.97 m-r	1.01
22	77.67 f-k	78.16 c-ı	0.63	1.16 d-j	**1.16 a-h**	0.00	19.04 h-l	19.99 d-n	4.99
23	78.04 d-k	78.20 c-ı	0.21	1.52 cde	1.13 b-ı	-25.66	21.03 b-g	**24.33 ab**	15.69
24	78.99 c-k	**78.87 a-ı**	-0.15	0.75 h-l	1.14 b-ı	52.00	19.02 h-l	19.14 g-p	0.63
25	76.43 h-l	77.56 c-ı	1.48	1.41 c-g	0.81 d-j	-42.55	19.31 g-k	21.69 cde	12.33
26	78.70 c-k	**79.73 a-g**	1.31	0.61 jkl	**1.21 a-h**	98.36	20.36 d-ı	20.67 c-k	1.52
27	80.16 b-ı	**81.76 abc**	2.00	0.81 h-l	0.74 f-j	-8.64	18.79 ı-l	18.67 ı-q	-0.64
28	**84.81 a**	**83.35 ab**	-1.72	0.47 kl	0.86 c-j	82.98	15.81 op	18.85 h-q	19.23
29	80.50 b-g	**81.34 a-e**	1.04	0.75 h-l	0.42 ıj	-44.00	20.64 c-h	19.98 d-n	-3.20
30	76.27 ı-l	75.53 ghı	-0.97	1.55 cde	**1.41 a-f**	-9.03	19.19 g-k	19.88 d-o	3.60
31	80.54 b-g	76.45 e-ı	-5.08	1.55 cde	**1.88 a**	21.29	20.29 d-ı	**26.56 a**	30.90
32	78.09 d-k	**79.70 a-g**	2.06	1.56 b-e	1.02 b-j	-34.62	**21.91 a-e**	21.29 c-h	-2.83
33	80.65 b-g	**83.48 a**	3.41	0.73 ı-l	0.69f-j	-5.48	18.81 h-l	18.36 k-r	-2.39
34	78.04 d-k	**80.99 a-f**	3.78	1.12 d-j	0.51 hıj	-54.46	**21.93 a-d**	20.95 c-ı	-4.47
35	79.34 c-k	**79.21 a-h**	-0.16	**1.69 a-d**	**1.49 a-e**	-11.83	19.40 g-k	21.61 c-f	11.39
36	78.49 c-k	78.25 c-ı	-0.31	0.73 ı-l	0.97 b-j	32.88	20.45 d-ı	19.74 d-o	-3.47
37	79.61 b-k	**80.48 a-g**	1.09	0.73 ı-l	0.74 f-j	1.37	20.53 d-ı	20.65 c-k	0.58
38	79.50 b-k	76.72 d-ı	-3.50	1.01 e-k	**1.67 ab**	65.35	20.47 d-ı	21.73 cd	6.16
39	79.71 b-k	**79.46 a-h**	-0.31	1.48 c-f	0.61 g-j	-58.78	19.45 g-k	19.92 d-o	2.42
40	77.44 f-k	**81.10 a-f**	4.73	**1.72 a-d**	0.73 f-j	-57.56	**22.58 ab**	20.25 c-m	-10.32
41	**81.92 a-d**	**82.52 abc**	0.73	0.82 g-l	0.94 b-j	14.63	20.02 f-j	20.99 c-ı	4.85
42	78.79 c-k	**79.68 a-h**	1.13	0.72 ı-l	0.98 b-j	36.11	19.36 g-k	19.61 d-p	1.29
43	79.45 b-k	75.57 ghı	-4.88	0.71 ı-l	**1.52 a-d**	114.08	16.29 nop	17.51 o-r	7.49
44	78.46 c-k	**78.35 a-ı**	-0.14	0.70 ı-l	0.62 f-j	-11.43	17.28 l-o	17.94 pqr	3.82
45	77.17 g-k	73.99 ı	-4.12	0.85 g-l	0.59 g-j	-30.59	20.53 d-ı	20.21 c-m	-1.56
46	77.33 g-k	77.87 c-ı	0.70	0.79 h-l	0.86 c-j	8.86	17.81 k-n	19.77 d-o	11.01
47	**83.30 ab**	**81.71 a-d**	-1.91	0.84 g-l	**1.18 a-h**	40.48	16.65 m-p	18.14 l-r	8.95
48	77.12 g-k	**78.83 a-ı**	2.22	1.24 c-ı	0.58 g-j	-53.23	19.63 g-k	16.54 qr	-15.74
Ave.	78.88	78.88	0	1.02	0.98	-3.92	19.27	19.83	2.91
LSD	I: 3.95	D: 5.02		I: 0.59	D: 0.73		I: 5.94	D: 5.74	

Conclusion

In this study, the effect of drought on fruit and seed color of different pumpkin genotypes was determined. According to the results, important fruit and seed colors were found among genotypes. In addition, it was observed that drought did not have any effect on seed color, while fruit color had a significant effect on ’’a* ’’ value. It was thought that fruit color became redder in drought conditions and color substances were secreted more in drought conditions. This is explained as a physiological response of the plant to drought.

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THE COMPOSITION OF POLLENIZERS FOR SWEET CHERRY (*Prunus avium* L.) CULTIVARS RELEASED IN THE REPUBLIC OF SERBIA

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Abstract

As the result of national breeding programmes, three sweet cherry cultivars have been named and released so far in the Republic of Serbia – 'Asenova Rana' and 'Čarna', developed by planned hybridization at Fruit Research Institute (FRI), Čačak, and 'Canetova', selected from natural cherry population at Faculty of Agriculture, University of Belgrade. In addition to the inadequate assortment structure, lacking in well-adapted domestic and introduced cultivars, sweet cherry production in Balkan region is generally accompanied by insufficient knowledge on gametophytic self-incompatibility, the fertilization relationship among the cultivars belonging to certain incompatibility groups, as well as phenological characteristics and synchronization during the flowering phenophase of the main cultivars and pollenizers. The assessment of an adequate pollenizer composition for 'Asenova Rana', 'Čarna' and 'Canetova' in this research is based on determined *S*-allele constitution, parameters of pollen tube growth *in vivo* in the pistils of these cultivars, and their perennial average flowering characteristics in relation to the introduced cultivars – potential pollenizers. The research was conducted in the main sweet cherry growing regions in the Republic of Serbia (West Serbia/Šumadija and region of Belgrade). It resulted in the development of a pollination scheme for in total 28 national and introduced cultivars, its application in the plant material production, and dissemination of quality national sweet cherry cultivars with adequate pollenizers. The results could also influence sweet cherry breeding work on development of genotypes with improved biological and productive characteristics, and application of modern molecular and reproductive biology methods in this research area.

Keywords: *sweet cherry, cultivars, S-allele constitution, flowering, pollination scheme*

Introduction

Sweet cherry (*Prunus avium* L.) flowers express a self-incompatibility system which is controlled by the polymorphic *S*-locus with gametophytic action. The interaction between the components in the style (*S*-allele specific ribonuclease – product of the *S-RNases* gene) (Bošković and Tobbut, 1996) and in the pollen (*S* specific F-box protein, product of the *SFB* gene) (Yamane *et al.*, 2003), determines the outcome of fertilization. Furthermore, fertilization will be prevented if a pollen expresses the same *S*-allele as one of the two *S*-alleles present in the style (Marchese *et al.*, 2007). The other aspects of the reproductive process, which also affect optimal fruit set and yield are characteristics of flowering and stable overlap in the full bloom between the main cultivars and pollenizers from year to year, pollen quality and its transfer, stigma receptivity, ovule longevity etc.

In recent decades, at FRI, Čačak, many research in this area have been done – determination of *S*-allele constitution of introduced sweet cherry cultivars (Radičević *et al.*, 2013, 2015; Marić and Radičević, 2014; Marić *et al.*, 2015); evaluation of sweet cherry flowering characteristics in the Republic of Serbia (Radičević *et al.*, 2012, 2015); investigation of

climate change effect on flowering characteristics, in sense of earlier flowering onset, the shortening of the phenophase duration (Wenden *et al.*, 2016; Drkenda *et al.*, 2018), and ovule longevity (Radičević *et al.*, 2018); evaluation of pollen viability *in vitro* in domestic and introduced sweet cherry genotypes (Cerović *et al.*, 2005; Radičević *et al.*, 2012). The previous research has also revealed the importance of ensuring full-flowering overlap of at least five days (Cerović *et al.*, 2005) accompanied by a short range of the flowering beginning, up to 2–3 days (Radičević *et al.*, 2015), as well as the advantage of one or two days earlier flowering pollenizer, which is better than later-flowering (Radičević *et al.*, 2016).
Up to now, three sweet cherry cultivars have been released in the Republic of Serbia – 'Asenova Rana' and 'Čarna', developed by planned hybridization at FRI, Čačak, and 'Canetova', selected from natural cherry population at Faculty of Agriculture, University of Belgrade. The aim of this paper is to determine the best pollenizers for these national cultivars, which are recommended for commercial growing in the Republic of Serbia, due to their good pomological and productive characteristics (Radičević *et al.*, 2017).

Material and Methods

Twenty-eight national and introduced sweet cherry cultivars were used in this study viz. , 'Asenova Rana', 'Bigarreau de Schrecken', 'Bigarreau Jaboulay', 'Burlat', 'Napoleon', 'Bing', 'Canetova', 'Colney', 'Čarna', 'Ferrovia', 'Drogans Gelbe Knorpelkirshe', 'Emperor Francis', 'Germersdorfer', 'Hedelfinger', 'Junska Rana', 'Karina', 'Kordia', 'Lambert', 'Lapins', 'Merchant', 'Regina', 'Souvenir', 'Stark Hardy Giant', 'Stella', 'Summer Sun', 'Summit', 'Sunburst' and 'Van'. The trial was performed at two sites – West Serbia/Šumadija region (sweet cherry collection orchard of FRI, Čačak, Ljubić facility; 43°54' N, 20°21' E, 224 m above the sea), and region of Belgrade (orchard of an individual grower, Grocka; 44°40' N, 20°43' E, 116 m above the sea), which are typical areas for fruit growing.
Young leaves of 'Asenova Rana', 'Čarna' and 'Canetova' were frozen in liquid nitrogen and stored at –80°C until use for the extraction. Genomic DNA was isolated using the CTAB method (Doyle and Doyle, 1987), with addition of 1% ß-mercaptoethanol and 2% polyvinylpyrrolidone (PVP 40) in the buffer. To identify the *S-RNase* alleles, polymerase chain reactions (PCR) were performed with the consensus primers specific for the first and the second intron (PaConsI-F + -R and PaConsII-F + -R, respectively) and primers specific for alleles S_3 to S_6, as well as for S_1 and S_9 alleles (Sonneveld *et al.*, 2003). PCR products were separated by electrophoresis in a 1.5% agarose gel (70 V for 3–4 h), visualised by ethidium bromide staining and sized by comparison with a 1 Kb plus DNA ladder (Invitrogen).
Flowering phenophase was investigated according to Wertheim (1996), in the period of 2000–2014 (Ljubić), i.e. 2004–2015 (Grocka).
Pollination experiment was carried out according to the method described by Radičević *et al*. (2016). Aniline blue staining was used (Kho and Baër, 1968; Preil, 1970), and pollen tube growth *in vivo* of the following pollenizers: 'Asenova Rana', 'Burlat', 'Čarna', 'Stella' and 'Van' (in the pistils of 'Asenova Rana' and 'Čarna'), i.e. 'Bing', 'Burlat', 'Bigarreau Jaboulay', 'Emperor Francis' and 'Van' (in the pistils of 'Canetova') was monitored under UV light on the Olympus BX61 microscope, and analysed by AnalySIS software, using Multiple Image Analysis. A total of 30 pistils of each treatment (15 combinations) was observed.

Results and Discussion

The results of investigation include: 1) developing of pollenizer composition schemes, in the main sweet cherry regions in the Republic of Serbia – West Serbia/Šumadija, and region of Belgrade; 2) defining the general rules for their aplication; 3) creating the list of pollenizers by priority (the categories of mandatory pollenizers, pollenizers of the second priority, pollenizers of the last choice, and unsuitable pollenizers), for each national cultivar.

♂ Cultivar ♀	‘B. Jaboulay’	‘Souvenir’	‘Lapins’	‘Burlat’	‘Asenova Rana’	‘Junska Rana’	‘Merchant	‘E. Francis’	‘Napoleon’	‘Van’	‘Stella’	‘Čarna’	‘S. H. Giant’	‘Summit’	‘Sunburst’	‘Kordia’	‘Summer Sun’	‘Germersdorfer I’	‘Bing’	‘Hedelfinger’	‘Karina’	‘Germersdorfer II’	‘Colney’	‘Ferrovia’	‘Lambert’	‘Regina’	‘D. G. Knorpelk.’
‘B. Jaboulay’	⊗	†	‡	†	†	†	†	‡	‡	‡	‡	‡	‡	‡	‡	†	†	‡	‡	‡	‡	‡	†	‡	‡	‡	‡
‘Souvenir’	†	⊗	‡	⊗	⊗	⊗	†	†	†	†	†	‡	‡	‡	†	†	†	†	†	†	†	†	‡	†	†	†	‡
‘Lapins’	‡	‡	†	‡	‡	‡	†	†	†	†	‡	⊗	†	†	‡	‡	†	†	†	‡	†	‡	‡	‡	†	†	†
‘Burlat’	†	⊗	‡	⊗	⊗	⊗	†	†	†	†	†	‡	‡	‡	†	†	†	†	†	†	†	†	‡	†	†	†	‡
‘Asenova Rana’	†	⊗	‡	⊗	⊗	⊗	†	†	†	†	†	‡	‡	‡	†	†	†	†	†	†	†	†	‡	†	†	†	‡
‘Junska Rana’	†	⊗	‡	⊗	⊗	⊗	†	†	†	†	†	‡	‡	‡	†	†	†	†	†	†	†	†	‡	†	†	†	‡
‘Merchant’	†	†	‡	†	†	†	⊗	†	†	‡	‡	†	‡	‡	‡	‡	⊗	†	†	‡	†	‡	‡	‡	†	‡	‡
‘E. Francis’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Napoleon’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Van’	‡	†	†	†	†	†	‡	†	†	⊗	†	†	†	†	†	†	‡	†	†	†	†	†	‡	†	†	⊗	†
‘Stella’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	‡	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Čarna’	‡	‡	†	‡	‡	‡	†	†	†	†	‡	⊗	†	†	†	‡	†	†	†	‡	†	‡	‡	‡	†	†	†
‘S. H. Giant’	‡	‡	†	‡	‡	‡	‡	‡	‡	†	‡	†	⊗	⊗	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	†	†
‘Summit’	‡	‡	†	‡	‡	‡	‡	‡	‡	†	‡	†	⊗	⊗	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	†	†
‘Sunburst’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Kordia’	†	†	‡	†	†	†	‡	†	†	†	†	‡	‡	‡	†	⊗	‡	†	†	†	†	†	†	†	†	†	‡
‘Summer Sun’	†	†	‡	†	†	†	⊗	†	†	‡	‡	†	‡	‡	‡	‡	⊗	†	†	‡	†	‡	‡	‡	†	‡	‡
‘Germersdorfer I’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Bing’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Hedelfinger’	‡	†	‡	†	†	†	‡	†	†	†	†	‡	‡	‡	†	†	‡	†	†	⊗	†	†	†	†	†	†	†
‘Karina’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Germersdorfer II’	‡	†	‡	†	†	†	‡	†	†	†	†	‡	‡	‡	†	†	‡	†	†	†	†	⊗	‡	⊗	†	†	‡
‘Colney’	†	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	†	‡	‡	⊗	‡	‡	‡	†
‘Ferrovia’	‡	†	‡	†	†	†	‡	†	†	†	†	‡	‡	‡	†	†	‡	†	†	†	†	⊗	‡	⊗	†	†	‡
‘Lambert’	‡	†	‡	†	†	†	†	⊗	⊗	†	†	†	‡	‡	†	†	†	⊗	⊗	†	⊗	†	‡	†	⊗	†	‡
‘Regina’	‡	†	†	†	†	†	‡	†	†	⊗	†	†	†	†	†	†	‡	†	†	†	†	†	‡	†	†	⊗	†
‘D. G. Knorpelk.’	‡	‡	†	‡	‡	‡	‡	‡	‡	†	‡	†	†	†	‡	‡	‡	‡	‡	†	‡	‡	†	‡	‡	†	⊗

♀ – female cultivar; ♂ – pollenizer
□ – flowering time overlap
▒ – insufficient overlap in certain seasons
■ – insufficient overlap

Graph. 1. The scheme of pollenizer composition for sweet cherry cultivars ‘Asenova Rana’ and ‘Čarna’ in agroecological conditions of West Serbia/Šumadija

Besides the research conducted, developing the schemes (Graph. 1, 2) was also based on the previous results of *S*-genotyping for introduced sweet cherry cultivars (Bargioni, 1996; Bošković and Tobutt, 2001; Wiersma *et al.*, 2001; Kappel, 2002; Sonneveld *et al.*, 2003; Andersen *et al.*, 2003; Schuster *et al.*, 2007; Schuster, 2012; Radičević *et al.*, 2013; Marić and Radičević, 2014; Radičević *et al.*, 2015).

Cultivar (♀ / ♂)	'B. Jaboulay'	'Souvenir'	'Lapins'	'Burlat'	'Canetova'	'E. Francis'	'Napoleon'	'Van'	'Stella'	'Sunburst'	'Kordia'	'Germersdorfer I'	'Germersdorfer II'	'Bing'	'Hedelfinger'
'B. Jaboulay'	⊗	†	‡	†	†	‡	‡	‡	‡	‡	†	‡	‡	‡	‡
'Souvenir'	†	⊗	‡	⊗	‡	†	†	†	†	†	†	†	†	†	†
'Lapins'	‡	‡	†	‡	‡	†	†	†	‡	‡	‡	†	‡	†	‡
'Burlat'	†	⊗	‡	⊗	‡	†	†	†	†	†	†	†	†	†	†
'Canetova'	†	‡	‡	‡	⊗	‡	‡	‡	‡	‡	†	‡	‡	‡	†
'E. Francis'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Napoleon'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Van'	‡	†	†	†	‡	†	†	⊗	†	†	†	†	†	†	†
'Stella'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Sunburst'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Kordia'	†	†	‡	†	†	†	†	†	†	†	⊗	†	†	†	†
'Germersdorfer I'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Germersdorfer II'	‡	†	‡	†	‡	†	†	†	†	†	†	†	⊗	†	†
'Bing'	‡	†	‡	†	‡	⊗	⊗	†	†	†	†	⊗	†	⊗	†
'Hedelfinger'	‡	†	‡	†	†	†	†	†	†	†	†	†	†	†	⊗

♀ – female cultivar; ♂ – pollenizer
☐ – flowering time overlap
☐ – insufficient overlap in certain seasons
■ – insufficient overlap

Graph. 2. The scheme of pollenizer composition for sweet cherry cultivar 'Canetova' in agroecological conditions of Belgrade region

The research indicates the following:
- For 'Asenova Rana' (S_3S_9), the mandatory pollenizers are 'Lapins', 'Bigarreau Jaboulay', 'Merchant', respectively; pollenizers of the second priority – 'Emperor Francis', 'Napoleon', 'Van', 'Stella', 'Čarna', respectively; pollenizers of the last choice – 'Stark Hardy Giant' and 'Summit', respectively; the unsuitable pollenizers are: 'Souvenir', 'Burlat' and 'Junska Rana', as well as the cultivars from category 'insufficient overlap', regardless of incompatibility group (Graph. 1);
- For 'Čarna' (S_1S_4), the mandatory pollenizers are 'Stella', 'Van', 'Napoleon', 'Emperor Francis', 'Stark Hardy Giant', 'Summit', 'Sunburst', 'Kordia', 'Summer Sun', 'Germersdorfer I', respectively; pollenizers of the second priority – 'Merchant', 'Junska Rana', 'Asenova Rana', 'Burlat', 'Lapins', 'Souvenir', 'Bigarreau Jaboulay', respectively; pollenizers of the last choice are 'Bing', 'Hedelfinger', 'Karina', 'Germersdorfer II', 'Colney', 'Ferrovia', respectively; the unsuitable pollenizers are cultivars from category 'insufficient overlap', regardless of incompatibility group (Graph. 1);
- For 'Canetova' (S_5S_6), the mandatory pollenizers are 'Burlat', 'Lapins', 'Souvenir', 'Bigarreau Jaboulay', 'Emperor Francis', 'Napoleon', respectively; pollenizers of the second priority are 'Van' and 'Stella', respectively; the unsuitable pollenizers are cultivars from category 'insufficient overlap', regardless of incompatibility group (Graph. 2).

Conclusions

Determination of pollenizers for sweet cherry cultivars released in the Republic of Serbia – 'Asenova Rana', 'Čarna' and 'Canetova', and developing pollination scheme for in total 28 national and introduced sweet cherry cultivars, are directly applicable in dissemination of quality national and introduced cherry cultivars, with adequate pollenizers. The results are also important for developing new sweet cherry cultivars, as well as for further application of molecular and reproductive biology methods in fruit trees breeding work.

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MOLECULAR CHARACTERISATION OF MAIZE HYBRIDS

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Abstract
Despite the huge diversity of maize germplasm, modern maize breeding programme and agricultural practices decrease the diversity of modern hybrids. Genetic characterization of maize hybrids allows knowledge of the genetic relationship among them, thus preventing the risk of increasing uniformity. Because of their high reproducibility, informativeness and easey application of microsatelites are the most frequently used molecular markers in maize genetic diversity studies. The aim of our work was to evaluate genetic diversity of maize hybrids by SSR markers and compare results with their pedigre information. Sixteen polymorphic SSR (*Simple Sequence Repeats*) markers were used to characterize 14 maize (*Zea mays* L.) hybrids belonging to different breeding programs and FAO groups (from 300 to 800). A total of 53 alleles were found, ranging from two to four alleles. Genetic similarities were calculated in NTSYSpc2 program package using Jaccard's coefficient based on binary data (presence or absence of alleles). The highest value of genetic similarity was 0.80 between H1 and H2, while the lowest value (0.26) was found between H12 and H13. Cluster analysis was done by unweighted pair group method (UPGMA) on the basis of genetic similarity matrix. Dendrogram analysis grouped maize hybrids in one cluster (most of the analyzed genotypes), one smaller cluster and one branch. The results revealed genetic heterogeneity between analyzed maize hybrids.

Keywords: *maize hybrids, genetic similarity, SSR markers, Zea mays L.*

Introduction
Maize, wheat and rice are the most important cereal crops grown in the world. Maize is used mainly for food, feed, as feedstock for food processing and in chemical industry. Due to huge genetic and phenotypic variability maize is adapted to different agroecological environments, edaphic and climatic conditions.

Maize has an important role in the field of scientific research such as the application of molecular genetic techniques and identifying genes and their functions. Maize is biological model system for genetics, evolution and domestication of cereals of the highest public interest (Wei et al., 2007). The main characteristic of maize is based on its variability in morphological traits and the high polymorphism of the DNA sequence (Matsuoka et al., 2002). During the breeding programs, maize hybrids have narrowed the genetic basis, leding to a significant reduction in diversity, so maize in commercial use contribute around 5% of the available germplasm (Carena et al., 2009).

In order to prevent genetic erosion, i.e. loss of individual genes and their combinations, as well as further narrowing of maize diversity, it is necessary to characterize existing elite lines, modern varieties and hybrids. Many methodologies are used for the assessment of genetic diversity in maize such as pedigree data, morphological traits and molecular markers. Compared with morphological variation, molecular polymorphism is generally considered to be independent of the environment and they are able to detect differences on DNA level on different individuals. (Ghebru et al., 2002; O'Neill et al., 2003).

Molecular diversity analyses can be performed using various kinds of methods including fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms

(AFLPs), randomly amplified polymorphic DNA markers (RAPD), simple sequence repeats (SSRs) or single nucleotide polymorphisms (SNPs). SSR markers have been the marker of choice for assessing maize genetic diversity due to the high level of polymorphism, multi-allelic nature, random distribution throughout the genome and cost effectivness (Barcaccia et al., 2006; Mason, 2015). In plant genomes they show an extensive variation in different individuals and genotypes (Cömertpay et al., 2012). Single nucleotide polymorphism (SNP) are new marker technologies that could also be used for estimating genetic diversity, but they are still to be adjusted for studies of genetic relatedness in maize (Yang et al.,2011).
In the present work SSR fingerprinting of 14 maize hybrids was done for molecular identification and assessment of genetic diversity, as well as to compare their clasification with their pedigre information.

Materials and Methods

A set of fortheen maize hybrids from the Maize Research Institute „Zemun Polje" that belong to different breeding programs were analyzed with molecular markers to evaluate genetic diversity. These 14 hybrids are covering different FAO groups, in the range from 300 to 800 (Table 1).

Table 1. List of the analyzed maize hybrids and their FAO groups

Hybrid	FAO group	Hybrid	FAO group
H1	300	H8	500
H2	300	H9	600
H3	600	H10	700
H4	300	H11	800
H5	500	H12	600
H6	500	H13	400
H7	500	H14	400

Genomic DNA was isolated from kernel using the CTAB (cetyl trimethylammonium bromide) procedure according to Doyle and Doyle (1987). Simple sequence repeat (SSR) characterization was done with 18 markers from the maize germplasm bank (www.maizegdb.org) (Table 1). Polymerase chain reaction (PCR) was carried out in 25µL reaction volume containing: 50ng of DNA sample, 1xBuffer, 0.8mM dNTP, 0.5µM of each primer pair and 1U Taq polymerase. The PCRs were performed using the following touch-down program: an initial denaturation at 95ºC/5min. by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5/1min (-0.5ºC/cycle) and extension at 72 ºC /1min; and another 22 cycles of 95 ºC /30 s, 56ºC/1min and 72ºC/1min. Final elongation was at 72°C for 4min. Using vertical electrophoresis (Mini Protean Tetra-Cell BioRad) the amplified PCR fragments were separated on 8% polyacrylamide gel, with 20 bp ladder as a marker. Gels were photographed under UV light on BioDocAnalyse Biometra after staining with 0.5µg/µL ethidium bromide. Data were assembled into a binary matrix after SSR profiles were scored as presence or absence (1/0) of fragments in each sample. Genetic similarities (GS) between maize hybrids were calculated in accordance to Jaccard (1908): $GSij = a/(a+b+c)$; where *a* is number of fragment shared by both individuals; *b* is number of fragments present in *i* but not in *j*; *c* is number of fragments present in *j* but not in *i*. For marker data analyses statistical NTSYSpc2 program package (Rohlf FJ, 2000) was applied.

Results and Discussion

The loss of maize diversity is considered one of today's most serious problem in maize production. Genetic biodiversity of maize is crucial for future breeding programmes in continuing advances in yield, grain quality improvement, disease and pest resistance.

In order to determine genetic diversity between 14 maize hybrids, molecular characterization was done using 18 SSR markers (Table 2). Two SSRs were not included in data analyses since they were monomorphic. Total of 53 alleles among the analysed 14 maize hybrids were identified. The number of alleles obtained with different primers varied from two (nc133) to four (bnlg1083, phi083, umc1448, umc1109, bnlg2235, umc1492 and umc1152) with the average value of 3.31 per locus. Similar number of alleles 3.33 was found in work with 38 Iranian maize hybrids (Shiri et al., 2015). Bantte and Prasanna (2003) reported slightly lower number of alleles (3.25) with 36 SSR loci. Some other works have shown considerably higher number of alleles. Nikkhoy and Shiri (2017) obtained 4.2 alleles per locus with 20 SSRmarkers on maize hybrids, while Lu and Bernardo (2001) found 4.9 alleles with 83 SSR loci, same as Warburton et al. (2002) with 85 SSR loci.

Table 2. List of 16 informative primers, with their chromosome position, repeat motif, number of alleles and allele range within analyzed maize hybrids

	Probe	Bin	Repeat motif	Number of alleles
1.	umc1282	1.00	(AT)6	3
2.	phi109275	1.03	AGCT	2
3.	bnlg1083	1.02	AG(29)	4
4.	umc1122	1.06	(CGT)7	3
5.	umc2047	1.09	(GACT)4	3
6.	phi083	2.04	AGCT	4
7.	umc1448	2.04	(GCT)5	4
8.	nc133	2.05	GTGTC	2
9.	umc1109	4.10	(ACG)4	4
10.	umc1153	5.09	(TCA)4	3
11.	phi452693	6.04	AGCC	3
12.	bnlg2235	8.02	AG(23)	4
13.	phi080	8.08	AGGAG	3
14.	umc1492	9.04	(GCT)4	4
15.	umc1152	10.01	(ATAG)6	4
16.	bnlg1526	10.04	AG(15)	3

Based on presense or absense of alleles in each sample coefficient of similarity was calculated by Jaccard. The genetic similarities were in range from 0.26 for H12 and H13 to 0.80 for two pairs of hybrids H1 and H2. Hybrids H12 nad H13 belong to specific types of maize, popcorne and sweet corn hybrids, respectively. On the other hand, single crosses hybrids H1 and H2 showed the higest similarity, because they have one same parental component and the other one are highly related inbred lines.

The cluster analysis using UPGMA method, based on Jaccard similarities distributed genotypes into two clusters (A and B) and one branch (c) showing good separation of hybrids and agrement with their pedigre data. Hybrids with very similar parental lines were grouped together within dendrogram. Cluster A contained most of the analyzed genotypes (11), dividing into subclusters A1 and A2. Subclaster A1 grouped hybrids with same genetic basis; hybrids H5 and H7 have one same parental component, and H4 and H6 are three way crosses and they have one parental line in common. This subclaster contained all hybrids from 300

FAO group, two from 500 FAO group and one from 600 FAO group. In subcluster A2 were grouped hybrids from 500 to 800 FAO groups, which have also same genetic basis. H9 and H10 have very similar parental lines, from te same source. Sweet corn hybrids (H13 and H14) formed cluster B, while branch c was formed of popcorn hybrid H12.

The average similarity of 0.54 among 14 maize hybrids showed satisfying variability between hybrids. The lower variability was reported in Bauer et al. (2005) with RAPD markers on maize hybrids, where Jaccard's coefficient of similarity ranged from 0.69 to 0.93. Also, Chen et al. (2008) reported higer genetic similarities with average value of 0.77 among 186 maize hybrids in China.

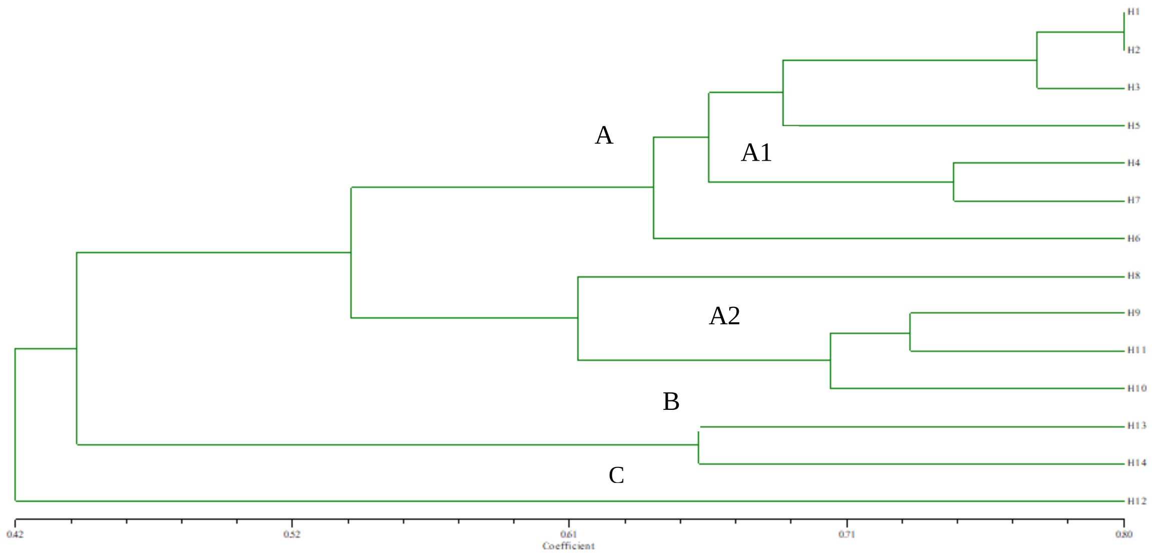

Figure 1. Dendrogram of 14 maize hybrids constructed using UPGMA cluster analysis of genetic similarity values (Jaccard, 1908) obtained from SSR data.

In his study SSR analysis determined variability among maize hybrids, as well as their grouping by genetic background. The dendrogram constructed from UPGMA method distinguishing hybrids to clusters according to their parental lines.

Conclusions

SSR markers is powerful tool for genetic characterization of maize hybrids and their clasification comparing with pedigree data. In this work 16 polymorphic SSR markers classified 14 maize hybrids into different groups in accordance to their pedigree data. Molecular marker could be successful and significant in evaluation of maize diversity assigned the most of the maize hybrids to their genetic background.

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THE UTILITY OF AGRO-MORPHOLOGICAL DESCRIPTORS IN UNIFORMITY AND STABILITY DETERMINATION OF MAIZE INBREDS

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Abstract

Testing of maize inbred lines distinctness, uniformity and stability was done according to the UPOV (Union Internationale pour la Protection des Obtentions Végétales) markers. In this study, three-year field experiment was conducted according to Complete Randomized Block Design (RCBD), in two densities and sowing data, in two replications. Eleven visual (VG) assessed traits, eight measured (MS) morphological traits and eight yield components, as well as grain yield, were evaluated. For this purpose, previously applied Ultra Thin Isoelectric Focusing marked five inbred lines (L1 – L5) differing in genetic purity, to be evaluated in field experiment for uniformity and stability. According to visual assessment, all inbred lines observed exhibited maximum uniformity in anthocyanin coloration of glumes of cob. Poor uniformity was achieved by all inbreds evaluated for anthocyanin coloration of glume excluding base and color of dorsal side of grain. L1 inbred expressed the highest uniformity and stability for most of the traits. Both inbreds with low genetic purity (i.e. L4 and L5) showed trend of segregation for almost all the traits. Three-way Analyses of Variance (ANOVA) was used for agro-morphological data analyzing. The best performing line was L3, as the most stable and uniform. Compared to pure inbreds, results of ANOVA showed more pronounced variations in morphological traits within lines with low genetic purity. Testing of inbreds differing in genetic purity by UPOV descriptors confirmed, to a certain extent, the results considering genetic purity obtained by IEF testing.

Keywords: *Genetic purity, Isoelectric Focusing, UPOV descriptors, Zea mays* L.

Introduction

Uniformity and stability of maize hybrids depend upon high and stable inbred lines performance. For both attaining good agronomic performances and encouraging investment and innovation in plant breeding, a high level of genetic purity in crop varieties have to be achieved and maintained *(Ipsilandis et al.*, 2005). The ability of a genotype to specify a phenotype consistently (i.e. the phenotype uniformity) is a key target for selective breeding in crops (Makumburage and Stapleton, 2011). Distinctness, uniformity and stability (DUS) testing is one of the important criteria to test inbred lines and it is essential to comprehend different modes of the traits expression. DUS testing of cultivars is one of the requirements for granting Plant Breeders Rights (PBR) and it is conducted according to national guidelines prepared on the basis of UPOV (Union Internationale pour la Protection des Obtentions Végétales) guidelines (Yadav and Singh, 2010). According to the environmental changes and low level of polymorphism, low heritability, late expression, limited discriminative power, and lower potential to measure relatedness and genetic similarity, morphological characterization has its own disadvantages, it is time-consuming and varies (Babic *et al.*, 2016). However, morphological traits are still very important in determination of the agronomic value and in taxonomic classification of plant species, including maize (Ortiz *et al.*, 2008). Traditionally, morphological comparisons have formed the basis for genetic purity evaluations. Biochemical assays, including isozymes, can distinguish varieties within species. Isozymes have been routinely used in checking seed-lot purity in maize. Ultra Thin Isoelectric

Focusing (UTLIEF) analysis is a standard reference method for testing the genetic purity of lines and hybrids (Dou *et al*., 2012). This method consists of extracting seed storage proteins (aqueous soluble – albumins and alcoholic soluble - zeins) from individual grains and their separation on a polyacrylamide gel. However, this method is limited due to many factors affecting isozyme expression, including development of plant tissue and the environment. Less loci and restricted polymorphism may also affect the utility of these markers. Because isozymes and genes affecting morphological traits are most usually coded by different and unlinked loci, a "clean" isozyme profile will not necessarily correlate with morphological homogeneity. For this reason, the aim of this study was to estimate the efficiency of morphologycal markers towards genetic purity determination.

Material and Methods

Based on previously conducted UTLIEF method (ISTA, 2005) regarding determination of electrophoretic patterns for storage proteins (albumines and zeins), five maize inbred lines (L1 – L5) differing in genetic purity were tested. Further on, the inbreds were subjected to distinctness, uniformity and stability evaluation according to the UPOV markers in three-year field experiment (from 2016 to 2018), carried out in Zemun Polje, Serbia (44°52´N, 20°19´E, 81 m asl). The experiment was set up according to Complete Randomised Block Design (RCBD), in two densities (i.e. D-30cm and D-40cm) and two sowing dates (in ten-day interval), in two replications. Inbreds of different maturity group L1 – L2 (maturity of FAO 300), L3 – L4 (maturity of FAO 400), and L5 (maturity of FAO 600), were sown in the four-rowed plot, with 10 hills per row and spaced 0.75 m apart. Four seeds per hill were sown and thinned to two plants per hill. Both visualy scored (VG) and measured (MS) morphological traits were observed on plants from two central rows, in a particular developmental stage. Eleven VG traits included eight quantitative (QN) traits (e.g. anthocyanin coloration of sheath, at base of glume, of glumes excluding base, of anthers, silks, of brace roots and of glumes of cob, as well as the angle between blade and steam), two pseudo-qualitative (PQ) traits (e.g. color of top and dorsal side of grain) and one qualitative (QL) trait – type of grain. In both replications, measuring of morphological traits – plant height (PH), plant hight to upper most *node (PHN)*, number of leaves above upper most ear (NLAE), leaf length (LL), leaf width (LW), ear height (EH), length of main axis above the lowest lateral (LALB) and the highest lateral (LAHB) branch, were conducted on ten representative plants per maize inbred. After manual harvesting and drying to 14% of moisture content, grain yield (GY) was calculated per plant basis. For each inbred line, yield components – ear length (EL), ear diameter (ED), cob diameter (CD), kernel length (KL), width (KW) and thickness (KT), number of kernels per row (NKR) and number of rows per ear (NRE), were recorded on ten randomly chosen ears per replication. Three-way analyses of variance (ANOVA) was used for agro-morphological data analyzing.

Results and Discussion

Genetic homogeneity refers to the presence of identical genotypes, whereas genetic stability refers to phenotypic uniformity (homeostasis) in different environments *(Živanović et al*., 2004). When a variety has been shown to be uniform, it can also be considered to be stable by UPOV. It has been shown that the uniformity threshold was never surpassed within a single trial, but discrepancy was observed between three annual scores (Gunjaca *et al*., 2008). In our study, all five observed inbreds exhibited maximum uniformity and stability for anthocyanin coloration of glumes of cob (ACGC). In all inbreds, poor uniformity was evidenced for anthocyanin coloration at base of glume (ACBG) and color of dorsal side of grain (CDG), respectively. According to VG traits, L1 expressed the highest uniformity and stability (Table 1). This line showed maximum uniformity (100%) in three observed traits: ACSh, ACBG and

GT, and close to this rating was ACBR. Lines L2 and L3 also were highly uniform and stabile. Similar trend in VG traits expression is observed between 2016 and 2017 in L1 and L2, and between 2017 and 2018 in L3. In L4 and L5, however, segregation was recorded for most of the observed traits, as well as their higher variation per year basis. Thus, results of visual assessment by UPOV descriptors confirmed, to a certain extent, the results obtained by UTLIEF.

Table 1. Segregation in VG traits according to particular UPOV descriptors scale level (given in brackets) for five (L1 – L5) maize inbred lines evaluated

VG trait	L1	L2	L3	L4	L5
	Quantitative traits (QN)				
ACSh	1:0 - (1)	5:1 - (3,5)	5:1 - (1,3)	4:1 - (1,3)	1:7:2:2 - (1,3,5,7)
ACBG	1:0 - (1)	1:5 - (1,3)	1:0 - (1)	5:1 - (1,3)	5:7 - (1,3)
ACGB	2:1 - (3,5)	1:3 - (7,9)	1:3 - (3,5)	5:7 - (1,3)	4:5:3 - (1,3,5)
ACA	1:5 - (1,3)	5:1 - (1,3)	1:3 - (5,7)	7:5 - (1,3)	1:2:1 - (1,3,5)
ACS	1:5 - (1,3)	1:3 - (3,5)	3:1 - (3,5)	1:4:1 - (3,5,7)	2:9:1 - (5,7,9)
ACBR	1:11 - (1,3)	1:3 - (7,9)	5:1 - (5,7)	1:1 - (1,3)	1:1 - (5,7)
ACGC	1:5 - (3,5)	1:0 - (5)	11:1 - (5,7)	2:1 - (5,7)	1:0 - (1)
ABS	5:7 - (3,5)	1:3 - (1,3)	1:11 - (3,5)	6:5:1 - (1,3,5)	7:5 - (1,3)
	Pseudo-qualitative traits (PQ)				
CTG	1:3 - (4,5)	11:1 - (5,6)	1:3 - (3,4)	1:1 - (3,4)	5:7 - (3,4)
CDG	1:3 - (3,4)	3:1 - (3,4)	7:5 - (5,6)	5:3:4 - (2,3,4)	6:2:3:1 - (1,3,4,5)
	Qualitative traits (QL)				
GT	1:0 - (1)	2:1 - (1,2)	1:5 - (3,4)	5:7 - (3,4)	3:1 - (2,3)

ACSh ─ anthocyanin coloration of sheath; ACBG ─ anthocyanin coloration at base of glume; ACGB ─ anthocyanin coloration of glumes excluding base; ACA ─ anthocyanin coloration of anthers; ACS – anthocyanin coloration of silks; ACBR ─ anthocyanin coloration of brace roots; ACGC ─ anthocyanin coloration of glumes of cob; ABS ─ angle between blade and steam; CTG ─ color of top side of grain; CDG ─ color of dorsal side of grain; GT ─ type of grain.

The plant morphology depends largely upon environmental conditions, as well as upon plants traits with their genetic variability (Tardieu, 2013). Since the experiment was conducted in three year, highly varying in environmental conditions, factor A had the most significant influence on observed morphological traits for all analyzed genotypes (Table 2). Moreover, higher plant density impose a variety of stresses on plants, including competition for light, water, and nutrients (Sher *et al.*, 2017). Factor B showed significant effect on two traits (NLAE and LW) only in inbred L5. Interaction A x B influenced L4 for PH, PHN and LL, while L5 varied for PHN. Although pure L1 inbred varied in five out of eight traits observed, inbreds L2 and L3 exhibited high stability. Inbred L4 was affected the most by factor C for LL, LW, EH, LALB and LAHB. Considering observed interactions, under A x C, L1 performed the best, however, pure L3 line varied in LW, LALB, and LAHB (the last two are the most variable traits). Genetically poor inbred L4 varied in NLAE, LW and LAHB, while L3 varied in PHN and EH. Our findings were in line with the study on four maize inbred lines under two-year experiment, that LALB and LAHB were considered relatively less stable traits as compared to other measurable characteristics (Akande and Lamidi, 2006; Olaoye, 2009). For all inbred lines tested, interactions B x C and A x B x C were insignificant. Similarly, in study on forage maize, Moosavi *et al.* (2012) reported insignificant effect of sowing date and plant density on morphological traits.

Table 2. Sagnificance for observed morphological traits according to ANOVA for inbred lines

Inbred	PH	PHN	NLAE	LL	LW	EH	LALB	LAHB
				Factor A (year)				
L1	***	***	ns	***	***	***	**	*
L2	***	***	**	***	***	***	ns	ns
L3	***	***	**	***	***	***	***	ns
L4	***	***	**	**	**	***	***	*
L5	***	***	**	***	***	***	***	***
				Interaction Factor B (plant density)				
L1	ns	ns	ns	ns	ns	ns	ns	ns
L2	ns	ns	ns	ns	ns	ns	ns	ns
L3	ns	ns	ns	ns	ns	ns	ns	ns
L4	ns	ns	ns	ns	ns	ns	ns	ns
L5	ns	ns	**	ns	**	ns	ns	ns
				Factor C (sowing date)				
L1	ns	ns	ns	ns	ns	ns	**	**
L2	ns	ns	ns	ns	ns	ns	ns	ns
L3	ns	ns	ns	ns	ns	ns	ns	ns
L4	ns	ns	ns	**	*	*	**	*
L5	ns	**	ns	ns	ns	ns	ns	ns
				Interaction A x B				
L1	ns	**	*	*	ns	ns	**	*
L2	ns	ns	ns	ns	ns	ns	ns	ns
L3	ns	ns	ns	ns	ns	ns	ns	ns
L4	*	*	ns	***	ns	ns	ns	*
L5	*	**	ns	ns	ns	*	ns	ns
				Interaction A x C				
L1	ns	ns	ns	ns	ns	ns	*	*
L2	ns	ns	ns	ns	ns	ns	***	ns
L3	ns	ns	ns	ns	**	ns	**	***
L4	ns	ns	*	ns	**	ns	ns	**
L5	ns	***	ns	ns	ns	**	ns	ns

P – plant height; *PHN* – plant hight to upper most *node;* NLAE – number of leaves above upper most ear; LL – leaf length; LW – leaf width; EH – ear height; LALB – lowest lateral; LAHB – length of main axis above the highest lateral branch; *,**,*** – significant at $p \leq 0.05$, 0.01 and 0.001 probability level, respectively; ns – non-significant.

In comparison with morphological traits, the results of ANOVA for yield components showed a slightly pronounced effect of observed factors and their interactions (data not presented). Effect of year (A) had the biggest impact on the observed parameters, similarly as observed for morphological traits. Factor B had the highest influence on pure L1 and L2 inbreds for most of the traits. Our results are in agreement with Testa *et al.* (2016), that plant density decreased cob diameter (-10.8%) and the number of kernels per row (-10%). Pure L2 inbred was highly affected by A x B interaction for KW, KT, NKR and CD as the most variable trait, except for L5. Effect of sowing date (factor C) was observed only for CD in L4 line, opposite to reported significant effects on yield components (Beiragi *et al.*, 2011). Interactions A x C and A x B x C affected pure L1 line for EL, ED, CD, KL and NRE. Compared to morphological traits, more pronounced effect of A x B x C interaction was observed for yield components. In all lines evaluated, interaction B x C was insignificant for all the traits observed, similar to morphological traits.

Grain yield and yield stability across multiple years (i.e. weather conditions) are some of the most important selection targets for plant breeding (Moose and Mumm, 2008). In our study, the results of ANOVA for grain yield, showed that factor A (year) had highly significant ($p \leq 0.001$) effect on all observed inbreds, being the highest in optimal 2018 and the lowest in dry 2017 (Table 3). Opposite trend for plant density (factor B) effect on grain yield was observed between pure inbreds (L1, L2 and L3) (i.e. $p \leq 0.001$, 0.001 and 0.01, respectively) and low genetic purity inbreds (L4 and L5) (insignificant). Sowing date (factor C) was highly significant for all the inbreds ($p \leq 0.001$), except for L4 ($p \leq 0.05$). For both L4 and L5 (low genetic purity inbreds), effect of all interactions on grain yield was insignificant, which was opposite to pure inbreds (L1, L2 and L3). This could be explain by the presence of genomic heterozygosity, which plays a significant role in stability level (Ertiro *et al.*, 2015).

Table 3. Three-way analyses of variance (ANOVA) for grain yield achieved by inbred lines evaluated

Inbred line	Source of variation	Repl.	Factor A	Factor B	Factor C	A x B	A x C	B x C	A x B x C
	df	1	2	1	1	2	2	1	2
L1	MS	44.6	7654.1	367.4	228.8	489.9	92.2	4.1	225.9
	F-value	4.7	806.5***	38.7***	24.1***	51.6***	9.7**	0.4ns	23.8***
	CV (%)	6.39							
L2	MS	15.8	7329.7	824.9	966.4	155.6	686.9	252.9	219.5
	F-value	0.5	239.7***	27.0***	31.6***	5.1*	22.5***	8.3*	7.2**
	CV (%)	14.4							
L3	MS	41.3	3450.0	215.4	668.9	121.7	87.6	25.8	131.2
	F-value	2.2	179.0***	11.2**	34.7***	6.3*	4.5*	1.3ns	6.8*
	CV (%)	11.14							
L4	MS	51.9	7318.6	201.3	742.6	32.4	241.6	275.4	197.7
	F-value	0.6	85.5***	2.4ns	8.7*	0.4ns	2.8ns	3.2ns	2.3ns
	CV (%)	19.3							
L5	MS	0.1	6469.5	63.4	821.340	67.6	129.9	0.4	20.4
	F-value	0.01	169.6***	1.7ns	21.5***	1.8ns	3.4ns	0.01ns	0.5ns
	CV (%)	16.03							

df ‒ degrees of freedom; MS ‒ mean square; CV ‒ coefficient of variation; Factor A ‒ year; Factor B ‒ plant density; Factor C ‒ sowing date; *,**,*** ‒ significant at $p \leq 0.05$, 0.01 and 0.001 probability level, respectively; ns ‒ non-significant.

Conclusions

Uniformity and stability largely differed among observed plant traits. High stability of visualy scored morphological traits was confirmed by estimated good performances of pure L1 inbred, as well as segregation trend for almost all VG traits in low genetic purity L4 and L5 inbreds. A certain level of variability observed in pure L1 inbred performances, followed by more pronounced variations in morphological traits within lines with low genetic purity, confirmed the low stability of MS agro-morphological traits. Opposite to pure inbreds, the presence of genomic heterozygosity contributed to higher level of yield stability in low genetic purity inbreds.

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THE INFLUENCE OF CONTINUOUS ANTHROPOGENIC PRESSURE ON FERTILITY AND BIOLOGICAL ACTIVITY OF SOILS

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Abstract

The paper compares agrochemical and microbiological parameters of arable land (for more than 50 years involved in agricultural activity) and natural ecosystem never exposed to anthropogenic impact. The study was conducted during the growing season of 2018 on meadow brown soil of experimental field of Far Eastern Agricultural Research Institute, Russia (48°31'05.9"N 135°16'25.6"E). Indicators of soil fertility and biological activity were determined. Hydrolytic acidity and pH values were measured potentiometrically; aluminium content was measured colorimetrically with xylenol orange; organic matter (humus), humic and fulvic acids – according to the methodology of M.M. Kononova and N.P. Belchikova. The total number of CFU was determined by method of serial dilutions with consequent spread plating on agar media (nutrient agar, starch-ammonia agar). A rise in exchange acidity provided an increase in the aluminium content (Al^{3+}) in arable horizon. Intensive pressure on the soil of experimental plots led to a decrease in humus content compared to the soil of natural ecosystem by 2.69-1.68 times and to an increase in the acidity of the arable horizon by 1.4-1.1. Anthropogenic pressure had a negative effect on the number of microorganisms inhabiting the soil of agrocenoses: the content of nitrifiers was 50.2 million units / 1 g abs. dry soil in the meadow versus 11.66 million units / 1 g abs. dry soil of agrocosystem; the content of ammonifiers - 21.2 million units / 1 g abs. dry soil versus 3.14 million units / 1 g abs. dry soil respectively.

Keywords: *Agrocenosis, Nitrifiers, Ammonifiers, Anthropogenic transformation, Soil fertility.*

Introduction

All the most important soil processes take place with the direct or indirect participation of organic matter. Nearly all soil organic matter is transformed by microorganisms. The final products of this activity are minerals. However, in the process of transformation of organic matter, intermediate complex and diverse organic compounds are formed, which are united by one common concept - humus. Fulvic and humic acids play a special role in soil formation. The quality of humus depends on the ratio of humic acids carbon to fulvic acids carbon. All soils with humate-fulvate (1.0-0.5) and especially with fulvate (<0.5) humus are characterized as acidic, they urgently need liming.

After the involvement of virgin soils in agricultural activity, the humus content in them noticeably decrease due to the strengthening of its decomposition processes. The loss of humus is greatest in the first years after involvement, and then a lower equilibrium content of humus is established in the soil.

Soil organic matter is a source of nutrition for both plants and microorganisms. Under their influence it is transformed with the formation of biologically active substances and releasing the large amounts of carbon dioxide, which is essential for activating photosynthesis in plants. (Basistiy, 2008)

The content and forms of nutrient compounds in the soil reflect the nature of the soil-forming process and serve as a diagnostic indicator of the fertility of soil (Sedykh et al., 2014). However, in modern conditions, natural factors affecting the soil, are combined with anthropogenic, affecting, as a rule, the level of soil fertility. Therefore, both natural and anthropogenic impacts are almost always the cause of changes in soil fertility. The anthropogenic factors may include (but not limited to) the application of chemicals (fertilizers, pesticides), soil and plants treatments using agricultural machinery (ploughing, cultivation, planting, harvesting etc.) with consequent fuel pollution, depletion of nutrients in soil as a result of continuous agricultural activities.

The anthropogenic pressure also has a significant effect on the soil microbial community, transforming important ecological functions in the biosphere associated with the cycling of nutrients, regulation of the atmospheric gas composition and the formation of the soil structure (Griffiths, 1965; Glazovskaya, 1984; Zvyagintsev, 1992; Conrad, 1996).

Soil microorganisms perform system-forming functions in such processes as soil formation, decomposition of soil organic matter, growth stimulation for plants and performing protection from pathogenic microflora (Morris et al., 1998; Raubuch, Beese, 1995).

The purpose of this study was to determine the effect of long-term anthropogenic stress on the transformation of physicochemical and microbiological properties of soils involved in agricultural activities.

Materials and Methods

The study was conducted in the fields of the long-term stationary experiments of the Far Eastern Agricultural Research Institute, Russia (48°31'05.9"N 135°16'25.6"E), which had been cultivated since 1963. The ecosystem that has never been exposed to anthropogenic pressure (the meadow) was chosen as a control. The region is characterized by meadow-brown, heavy loamy soils with an acidic soil pH. The sum of surface layer air temperatures >10℃ for the growing season (from sowing to harvesting) was 1793.5 ℃; the amount of precipitation for the period was 297.6 mm. The crop rotation system on experimental field includes the alternation of oats, soybean and wheat with perennial grasses. Soil treatment system is conventional for the region and includes ploughing, spring pre-sowing disking and harrowing in 2 tracks.

The studies were conducted in two directions: 1 – the comparison of the amount of exchangeable bases (calcium, magnesium), aluminium, hydrolytic acidity and pH values in soils of agroecosystem (with the application of different doses of mineral fertilizers) and the natural ecosystem never involved in agricultural activity (meadow); 2 – the comparison of the content of humus, humic and fulvic acids, their ratio, pH and number of nitrifying and ammonifying bacteria in soils of agroecosystem (with mineral fertilizers and with the aftereffect of organic fertilizers and liming) and natural ecosystem (meadow). Samples for microbiological analyses were taken from plots without any fertilizers.

Soil samples were taken before sowing crops, in the middle of the growing season and before harvesting. For microbiological analyses, samples were taken with observance of asepsis.

Hydrolytic acidity and pH values were measured potentiometrically; aluminium content was measured colorimetrically with xylenol orange; organic matter (humus), humic and fulvic acids - according to the methodology of M.M. Kononova and N.P. Belchikova.

The method of serial dilutions with consequent spread plating on agar media (nutrient agar, starch-ammonia agar) was used to determine the total number of colony-forming units per 1 gram of absolutely dry soil and quantitative characteristics of two main groups of microorganisms: nitrifying and ammonifying bacteria. Nutrient agar (NA) was used to count the ammonifying microorganisms that decompose the nitrogen-containing organic matter in the soil. Starch-ammonia agar (SAA) - to count the amylolytic microflora, capable of carrying out the destruction of oligo-, polysaccharides and immobilization of nitrogen.

Also, the coefficient of mineralization was determined as a ratio of the number of ammonifying microorganisms to the number of microorganisms that assimilate mineral nitrogen.
Based on the data on the quantitative composition of microorganisms on different nutrient media, the conclusion about the level of enrichment of soil with microorganisms was made.
The species composition of soil microorganisms was determined by molecular genetic methods: DNA was extracted from the most typical microorganisms, the part of 16S ribosomal RNA gene was amplified using PCR, and the obtained products were sequenced by Sanger.

Results and Discussion

Long-term use of meadow-brown soils in field crop rotation has led to levelling of zonal properties, as well as to their unidirectional degradation (Table 1), especially with increasing anthropogenic pressure. The rise of metabolic acidity provided an increase in the content of aluminum (Al^{3+}) in arable horizon, especially in the variants with high doses of nitrogen fertilizers. The acidity of the soil solution is an important factor in determining the availability of nutrients for plants.

Table 1. Transformation of soil properties in agricultural lands.

Variants	pH	Hydrolytic acidity	Ca^{2+}	Mg^{2+}	Ca^{2+}+ Mg^{2+}	Al^{3+}, mg / 100 g soil
		mEq. / 100 g soil				
1. Natural ecosystem (meadow)	5.6	7.1	8.4	11.4	19.8	0.06
2. Without fertilizers	4.9	3.7	5.4	11.4	16.9	0.09
3. $N_{16}P_{16}K_8$	4.8	5.5	6.1	11.8	17.9	0.10
4. $N_{32}P_{16}K_{16}$	4.7	6.8	8.1	10.7	18.8	0.29
5. $N_{48}P_{16}K_{24}$	4.8	6.0	9.2	10.0	19.2	0.62
6. $N_{16}P_{16}K_8$	4.8	3.4	8.4	10.2	18.6	0.10
7. $N_{16}P_{32}K_{16}$	4.8	3.0	6.8	12.8	19.6	0.33
8. $N_{16}P_{48}K_{24}$	4.7	5.0	7.3	10.3	17.6	0.94
9. $N_{56}P_{56}K_{28}$	4.7	5.1	6.1	11.0	17.0	0.94

On acidic soils, plants lack nitrates. The soil pH has a particularly strong influence on the phosphate regime of the soil, since as a result of the inhibition of the nitrification capacity, the binding of phosphates to trivalent forms of iron and aluminium inaccessible to plants occurs. On acidic soils, the negative effect of aluminium increases. In addition, aluminium forms insoluble phosphates with phosphorus that are poorly used by plants in an acidic environment (Sychev et al., 2010). In this case, an excess of aluminium and manganese has a toxic effect on plants. In addition, the alienation of the overwhelming majority of biomass with harvested crops violated the biogeochemical circulation, as a result of which the soil supply with biophilic nutrients decreased.

The total activity of humus in relation to the mineral part of the soil depends on the ratio and content of humic and fulvic acids. Due to the strongly acidic reaction (control variant) and good solubility in water, fulvic acids actively destroy the mineral part of the soil and reduce humus accumulation. In the variants with mineral fertilizers and with the aftereffect of organic fertilizers and liming, the ratio of humic acids carbon to fulvic acids carbon C_H/ C_F). grows, which has a favourable effect on the accumulation of humus in the arable horizon of the soil, and at a ratio of C_H/ C_F. more than 1, active humus accumulation occurs and the mineral part remains almost unchanged (Table 2).

Table 2 - Changes in the content of humus and humus acids with continuous anthropogenic impact.

Variants	Mass fraction of organic matter (humus), %	Humic acids, %	Fulvic acids, %	C_H/ C_F	pH
Natural ecosystem (meadow)	**5.48**	**1.17**	**1.85**	**0.63**	**5.6**
1. Control (without fertilizers)	2.79	0.24	0.74	0.32	4.2
2. Peat compost 100 t / ha (aftereffect)	3.15	0.45	0.78	0.58	4.3
3. $N_{32}P_{32}K_{16}$	3.80	0.71	0.72	0.99	4.5
4. Ca 2.25 GK (aftereffect)	3.70	1.07	0.76	1.41	4.5

Microflora of areas exposed and not exposed to anthropogenic influence, differed significantly in the number of microorganisms that decompose mineral nitrogen: an average of $50.2 \cdot 10^6$ in the meadow versus $4.01 \cdot 10^6$ in agrocenosis; as well as by the number of organic nitrogen destructors: $21.2 \cdot 10^6$ versus $3.14 \cdot 10^6$ (Table 3).

Table 3. The average number of microorganisms in soils affected and not affected by anthropogenic factor

Soil type	Number of soil microorganisms, million units / 1 g of absolutely dry soil	
	NA	SAA
Natural ecosystem (meadow)	21,20	50,20
anthropogenically modified soil	3.14	11.66

The number of microorganisms had been increasing during the growing season, but it was smaller than before the crop was planted. On average, there is a noticeable predominance of nitrifiers over ammonifiers in areas both influenced and not influenced by agricultural activity ($50.2 \cdot 10^6$ vs. 21.2 10^6 in the meadow; 11.66 10^6 against 3.14 10^6 under oats), the numbers of these groups of microorganisms differ by 2-3 times.
According to the scale of assessment of the level of soil enrichment with microorganisms (Table 4), meadow soils not involved in agricultural activity were very rich, while long-term

anthropogenic impact negatively affected the soil microflora in the experimental plots, thus transferring these soils into medium enriched group.

Table 4. Scale of assessment of the degree of soil enrichment with microorganisms (according to Zvyagintsev, 1980)

Level of soil enrichment with microflora		number of soil microorganisms determined by cultivation on various nutrient media, $\times 10^6$ units / 1 g absolutely dry soil.	
		NA	SAA
I	Very poor	<1	<2
II	Poor	1-2	2-4
III	Moderately enriched	3-5	5-10
IV	Rich	6-10	11-20
V	Very rich	> 11	> 21

The coefficient of mineralization shows the intensity of transformation of organic matter of plant and animal residues, as well as applied various organic fertilizers in the soil (Milashchenko, 1990). It is determined by the ratio of the number of microorganisms that characterize the process of conversion of ammonia nitrogen, to the number of microbes that characterize the transformation of protein substances in the soil. This coefficient represents the degree of development of the amylolytic part of the soil microflora and, accordingly, its activity in the transformation of soil carbohydrates and the binding of free nitrogen. The higher it is (> 1), the more immobilization processes take place, which indicates either very high ammonia nitrogen availability in soil (this may be due to the high amounts of ammonifiers), or the appearance of organic matter in the soil (straw, bark, etc.). The latter phenomenon, in turn, can activate the development of oligotrophic and autochthonous groups of microorganisms, which ultimately leads to an increase in the number of amylolytic microflora, since a certain amount of ammonia is released into the soil solution as a result of the activity of oligotrophs. In agrocenosis, an excessively large value of this coefficient (> 3-5) may indirectly indicate an increase in the rate of decomposition of the specific organic matter of the soil — humus (Schulz et al., 2013).

Calculated this coefficient for our soils, we obtained the following data: 2.34 for meadow and 3.56 for agrocenosis in average (Fig. 1).

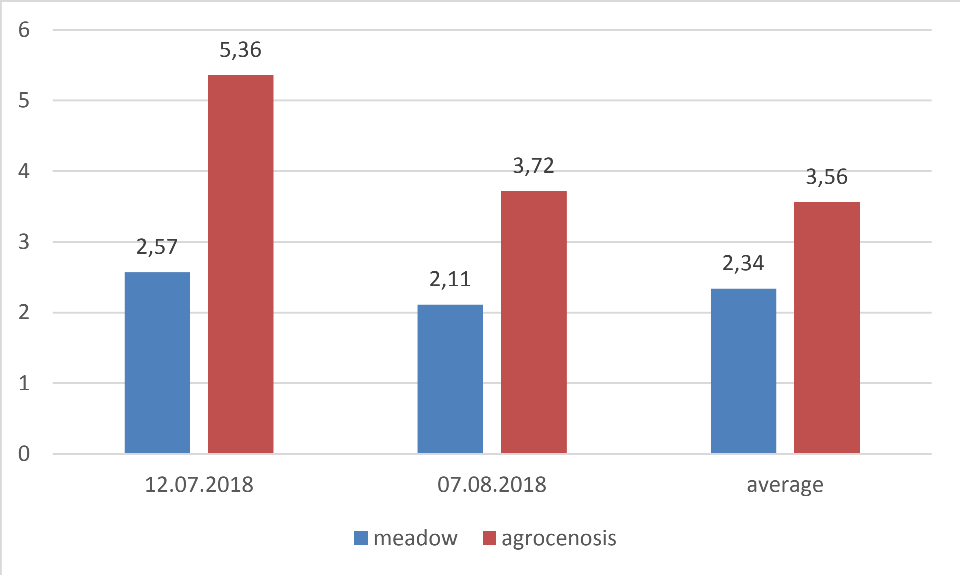

Figure 1. Dynamics of the mineralization coefficient

According to the sequencing data, the microorganisms most typical for the soils of the region were identified. The most common microorganisms were representatives of the genera *Flavobacterium, Pedobacter, Bacillus, Enterobacter, Arthrobacter, Streptomyces, Acinetobacter, Leifsonia, Luteibacter, Burkholderia, Stenotrophomonas, Massilia, Microbacterium, Aneurinibacillus.*

Conclusions

Long-term use of meadow-brown soils in field crop rotation has led to leveling of zonal properties, as well as to their unidirectional degradation. A rise in the exchange acidity provided an increase in the content of aluminium (Al^{3+}) in the arable horizon.

The involvement of the soil in agricultural activities led to a decrease in the percentage of humus in the upper arable layer, and it is necessary to consider not only the natural loss of humus, but also an increase of the treated arable layer with a general decrease in the humus content due the involvement of the low humus below lying horizons. Extensive pressure on the soil of experimental plots led to a decrease in the humus content compared to soil of natural ecosystem by 2.69-1.68 times and to an increase in the acidity of the arable horizon by 1.4-1.1.

In addition to decrease in soil fertility, active involvement in agricultural practices also significantly reduces the biological activity of the soil. Anthropogenic pressure had a negative effect on the number of microorganisms inhabiting the soil of agrocenosis: the content of nitrifiers was 50.2 million units / 1 g abs. dry soil in the meadow versus 11.66 million units / 1 g abs. dry soil of agroecosystem; the content of ammonifiers - 21.2 million units / 1 g abs. dry soil versus 3.14 million units / 1 g abs. dry soil respectively.

The value of the mineralization coefficient indicates that the rate of transformation of organic nitrogen in the soils affected by agricultural activity is about one and a half times higher than in meadow soils. The reason for this can also be the long-term waterlogging of the soil during the growing season (prolonged flooding contributed to the accumulation of large amounts of ammonia nitrogen).

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QUANTITATIVE PHENOTYPIC CHARACTERISTICS OF PARENT PLANTS OF SOYBEAN CULTIVAR BATYA

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Abstract

Soybean production contributes to the wide range of industries and is one of the main grain and oilseed crops in global agriculture. The task of increasing its production in Russia is of great importance. The paper analyzes the results of study of the productivity of soybean cultivar Batya plants, grown from seeds obtained from different nodes of the parent plant in Khabarovsk Kray (Russia's Far East). The yield of offspring, productivity of one plant, mass of 1000 seeds were considered. The following phenotypic characteristics were determined: plant height, number of nodes, number of productive nodes, number of pods, bottom pod attachment point. Parent plants of soybean cultivar "Batya" were characterized by high productive qualities. The average seed weight per plant was 11.7 grams, and the yield with the density of 50 plants m^{-2} was 4.67 tons ha^{-1}. With an average 1000 seeds mass of 163.8 grams, the largest mass of 1000 seeds was on 7-16 nodes, and the highest productivity was on 8-15 nodes. Studies have shown that soybean plants grown from the seeds of the lower layer of parent plants possessed the highest productive qualities. The average number of productive nodes on plants grown from lower layer seeds was 12.0, and the number of pods was 26.9. And, as a result, the average yield of plants grown from seeds of the lower layers was 4.86 tons per hectare, which is 0.67-0.7 tons per hectare higher than yield of plants grown from seeds of the middle and upper layers.

Keywords: *Soybean, Parent plants, Productivity, Plant node, Yield structure.*

Introduction

Soybean occupies the main areas in the agrocenoses of the Russian Far East. The prevalence and rapid growth of soybean production is mainly due to the unique biochemical composition of its grain, which determines its multifunctional (food, feed and technical) use, and the high profitability of its production (Benkin and Tomilina, 1985; Pavlovskaya, 2004). High and stable yield is the result of high cultivar adaptability to the conditions of cultivation that vary in a wide range. For stable maintenance of a sort, it is necessary to have a precise characteristic of the developmental rhythm due to specific environmental and climatic factors, and to identify the number of major and related biotypes in it, as well as those that give the full range of features of this sorting population during reproduction. In order to properly conduct seed production, it is necessary to know and consider the structure and genetic features of a particular cultivar, its degree of ecological stability. It should be noted that the potential achievable productivity of all soybean cultivars, including the most precocious, is realized only by 30-70% (Ozyakova and Polzukhina, 2014). However, it cannot be denied that the mechanisms for maintaining high productivity of the cultivated varieties, their adaptation to growing conditions, and resistance to abiotic and biotic factors remain insufficiently studied (Belyaeva, 2007). The most important attributes that determine the yield of soybean varieties include, for example, the number of seeds per pod, the number of pods per plant, plant height, branching, seed weight per plant, height of attachment of the lower pod (Vashchenko *et al.*, 2014). A promising, highly productive soybean cultivar "Batya" - middle-ripe, belongs to the Manchurian type. The vegetation period is 108-118 days, indeterminate type, the degree of lateral shoots formation is average, the plant is compact. The height of the plants is 80-110 cm. The mass of 1000 seeds is 193-270 g. Due to the rapid growth in the period of germination - flowering and wide leaf plate this cultivar competes well with weeds. Seed yield is up to 5.4 t / ha. The purpose of this study was to compare the productivity of soybean plants of the "Batya" cultivar, grown from the seeds of different nodes of the parent plants.

Materials and Methods

The studies were conducted on the fields of the Far Eastern Agricultural Research Institute in 2017 and 2018. The soil of experimental plot is meadow-brown, heavy loamy, humus content – 4.1%, pH <4.5, hydrolytic acidity – 10-12 mEq/100 g of soil, the sum of exchangeable bases – 15-17 mEq/100 g of soil, the availability of mobile phosphorus is low, and availability of exchangeable potassium is high to very high. The object of the study was the soybean cultivar "Batya". Sowing seeds, treating crops and harvesting experimental plots were conducted manually. Soybean seeds for the research were taken from soybean plants, harvested in 2017. The experiment was laid on the ridges with a base of 70 cm. Seeds were sown in 2 lines, the distance between the lines was 8 cm, the density of standing of the stems is 50 pieces / m^2. The analysis of the yield structure of soybean plants grown from parent plants' seeds was performed according to the following parameters: plant height, seed weight from 1 plant, mass of 1000 seeds, number of productive nodes, number of pods per plant, the height of attachment point of the lower pod. The protein content in seeds of the plants grown in 2018 was also considered.

Results and Discussion

Parental plants of soybean cultivar "Batya" possessed high productive qualities. The average seed mass per plant was 11.7 g, and the yield with a density of 50 plants per 1 m^2 was 4.67 t / ha. With an average weight of 1000 soybean seeds of 163.8 g, the largest mass of 1000 seeds was at 7-16 nodes, and their highest productivity was in nodes 8-15 (table 1, figure 1).

Table 1 - Characteristics of parent soybean plants of the "Batya" cultivar

node number	Seed, pcs.	The mass of seeds in the node, g	The mass of 1000 seeds, g
1	-	-	-
2	0.50	0.07	140
3	1.18	0.18	152
4	1.92	0.32	167
5	2.98	0.51	171
6	4.4	0.77	175
7	5.36	**0.96**	**179**
8	6.09	**1.06**	174
9	6.74	**1.16**	172
10	6.56	**1.18**	**180**
11	6.67	**1.14**	140
12	5.61	**0.96**	152
13	5.81	**1.04**	167
14	5.65	**0.96**	171
15	4.01	0.69	175
16	2.56	0.42	**179**
17	1.34	0.20	174
18	0.30	0.04	172
19	0.16	0.02	**180**
20	-	-	-

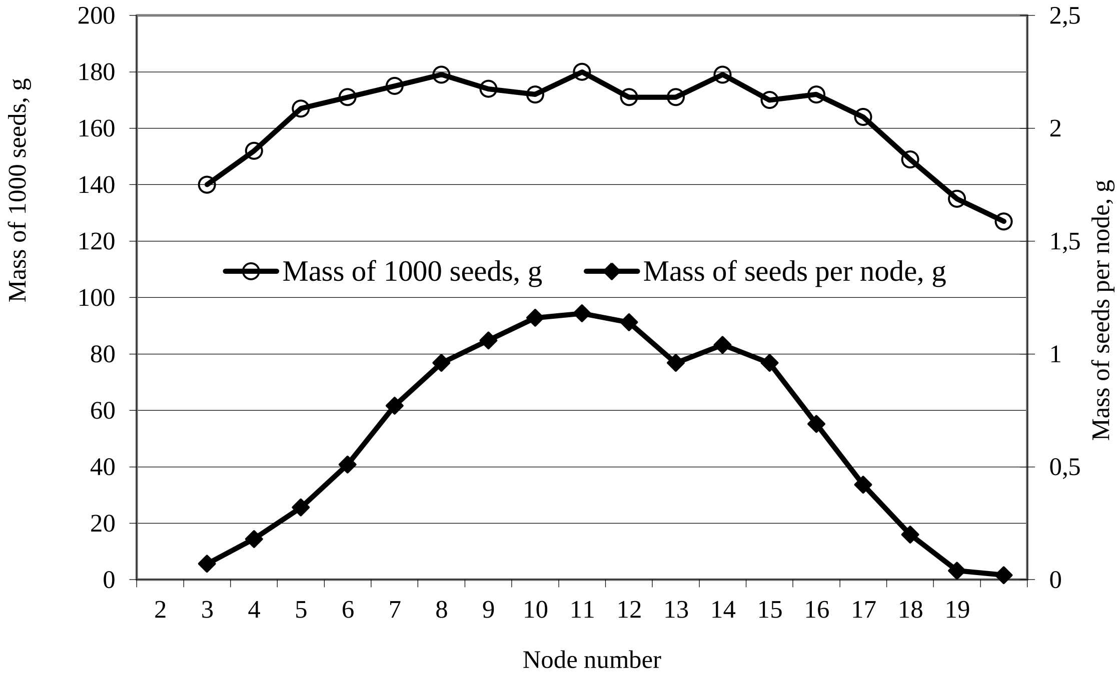

Figure 1 - Productive qualities of parent plants.

The number of productive nodes on the plant was the most stable feature. The average number of productive nodes on soybean plants grown from the seeds of the lower level was 12.0, and from the top - 10.4.

The number of pods on a plant is a varying characteristic and one of the most important elements of productivity, and depends on the biological parameters of the cultivar, soil, climatic and agrotechnical conditions of cultivation. On average, about 25 pods were formed on the plants during the study period. At the same time, the highest value of this indicator was observed in seeds grown from the lower layer – 26.9 pcs., and the lowest value - 23.7 pcs. - from the top layer. The average yield of plants grown from the upper layers is lower by 0.67-0.70 tons / ha compared to the yield of plants on the lower layer.

One of the main features in the structure of the plant, which determines the productivity, is the mass of seeds per plant. Analysis of the productivity indicators of soybean plants (Table 2), grown from seeds of various nodes of parent plants, showed that from the second to the nineteenth node, the productivity of soybean plants of the cultivar Batya decreased by 2 g, which is indicated by the trend line, obtained as a result of approximation and smoothing the graph and has a polynomial appearance (Fig. 2).

Elements of the yield structure of plants are important indicators. The height of the stem of soybean plant is genetically determined. Under the same growing conditions, the variability of this trait in different soybean cultivar samples is on 80–90% determined by the genotype. The height of plants grown from the lower layers of the parent plant reached 101-109 cm, and the average height of plants from seeds of the upper layers was 93 cm.

Table 2 - The productive qualities of soybean plants grown from the seeds of various nodes of the parent plants

Node / layer 2-7 / lower layer 8-13 / middle layer 14-19 / upper layer	Average productivity of soybean plants grown from seeds of various nodes of parent plants of soybean								
	Plant height, cm	Number of nodes, pcs.	Number of productive nodes, pcs.	Number of pods, pieces	Lower pod attachment point	Protein, %	Mass of grain per plant, g	Yield, t / ha	Mass of 1000 seeds, g
2	108.14	16.14	13.43	32.57	3.14	34.5	13.95	5.58	174.7
3	111.2	14.8	11.1	26.6	4.2	34.7	11.85	4.74	192.6
4	102.6	14.2	12.6	24.5	3.7	34.8	11.37	4.55	198.4
5	101	13.7	10.6	23.4	4	35.2	10.88	4.35	216.3
6	102.4	15.5	12.2	29.2	3.9	35.2	12.1	4.84	183.4
7	109	15.7	12	24.9	4.4	35.4	12.71	5.08	212
Average Layer	**106**	**15.0**	**12.0**	**26.9**	**3.9**	**35.0**	**12.1**	**4.86**	**196**
8	105.6	14.8	11	23.4	4.5	35.5	9.76	3.90	183.9
9	98.4	12.9	10.1	20.6	3.8	36.5	8.33	3.33	197.5
10	99	14.6	11.7	27.2	3.6	36.4	12.11	4.84	179.6
11	97.5	14.5	11.5	28.6	3.7	34.9	11.98	4.79	172.5
12	95.1	13.3	10.9	26	3.2	36.9	11.38	4.55	169.6
13	98.1	12.8	9.2	19.7	4.2	31.9	9.29	3.72	203.2
Average Layer	**99**	**13.8**	**10.7**	**24.2**	**3.8**	**34.9**	**10.5**	**4.19**	**184**
14	101	13.6	10.6	23.6	3.5	33.2	11.07	4.43	193.8
15	91.9	13.8	11.8	28.2	3.1	32.8	11.52	4.61	166.7
16	98.5	14.1	11	25.3	3.8	31.9	11.01	4.40	191.4
17	93.6	14	11.2	25.4	3.4	31.2	12.16	4.86	197
18	82.1	11.6	8.5	18.8	4	32.0	7.72	3.09	173
19	88.6	11.7	9.4	21	3.2	34.9	8.91	3.56	190.8
Average Layer	**93**	**13.1**	**10.4**	**23.7**	**3.5**	**32.0**	**10.4**	**4.16**	**185**

The height of the bottom pod attachment point is a technologically important attribute. The variability of this trait is only by 28% determined by hereditary factors, 72% fall on the conditions of cultivation of crop (Babich and Kokhanyuk, 2014). In our experiment, the attachment point of the lower pod was on the 3 and 4 nodes of the soybean plant.

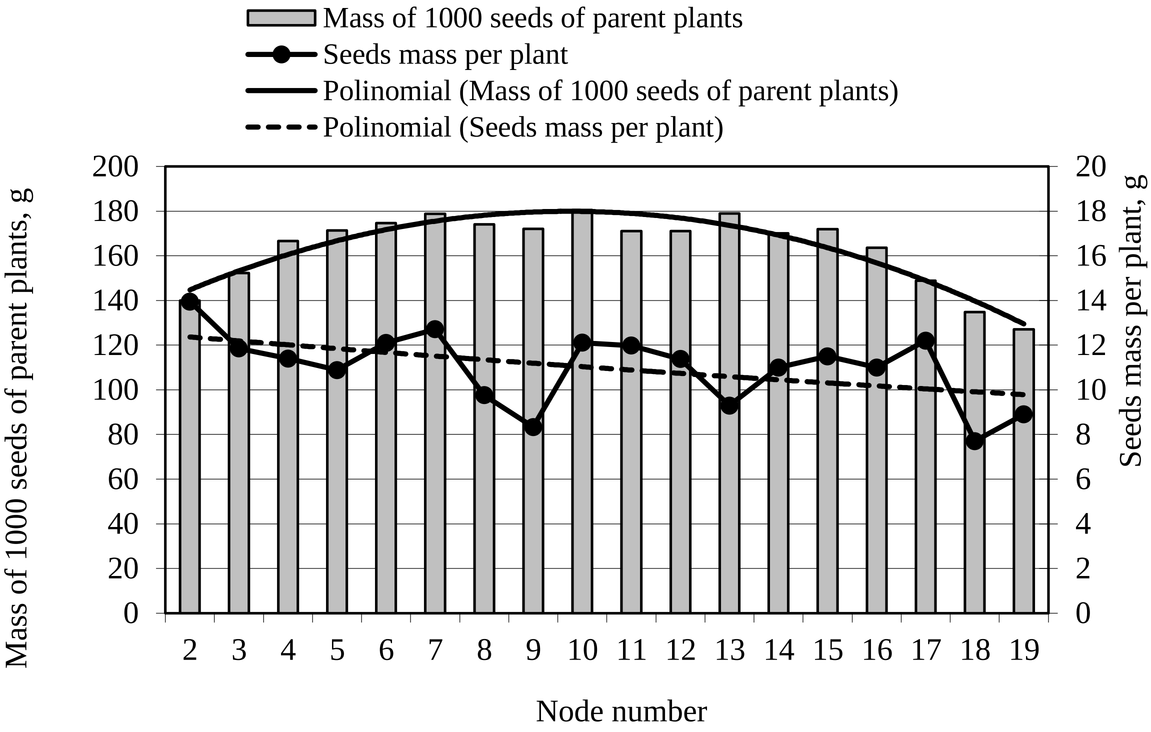

Fig. 2. The productivity of soybean plants grown from seeds of various nodes of parent plants.

The correlation coefficient between the mass of 1000 seeds of parent plants and the productivity of plants grown from these seeds is 0.19, which indicates a small association of indicators. A more significant connection (r = –0.5) occurs between the node numbers of the parent plants and the productivity of the offspring. This indicates that the seeds of the lower nodes of the parent plants are more productive and provide a higher yield than the seeds from higher layers (Table 2, Fig. 2).

A medium positive correlation was established (r = 0.37) between the mass of 1000 seeds of parent plants and the mass of 1000 seeds of offspring. There is no reliable connection between the numbers of the nodes of the parent plants and the mass of 1000 seeds of the offspring; the mass of 1000 seeds of offspring practically does not depend on which node of the parent plants the seeds are taken from. A very insignificant association (r = 0.19) was observed between the mass of 1000 seeds of soybean parent plants and the productivity of the offspring grown from these seeds. Comparison of the quantity and quality of the crop by the layers of parent plants showed a significant superiority of the productive qualities of soybean plants grown from the seeds of the lower layer. Plants grown from the seeds of other layers of parent plants had slightly different productive qualities, except for a mass of 1000 seeds and plants height. The mass of 1000 seeds of the upper layer, compared to the mass of 1000 seeds grown from seeds of the middle layer, decreased by 6 g.

The protein content is a quantitative trait characterized by polygenic inheritance and high sensitivity to changes in environmental conditions, which often exceed the influence of the genotype (Petibskaya, 2001). Under favorable conditions of humidity, soybean cultivars completely support themselves with biologically fixed atmospheric nitrogen, and therefore can realize their genetic potential - the protein content in the seeds rises to 48% (Posypanov *et al.,* 2006).

In the experiments, the protein content in the seeds of plants grown from the seeds of the lower and middle layers was almost the same (35 and 34.9%), and in the seeds of plants grown from the seeds of the parent plants of the upper layer was 32.0% (3% lower) . The correlation coefficient between the numbers of the parent plant nodes and the protein content in the seeds of the soybean offspring of the "Batya" cultivar was (- 0.87). This convincingly demonstrates the advantage in protein content of plants grown from seeds of the lower layers of parent plants.

Conclusion

Studies have shown that soybean plants grown from the seeds of the lower layer of parent plants possessed the highest productive qualities. The average number of productive nodes on plants grown from lower layer seeds was 12.0, and the number of pods was 26.9. And, as a result, the average yield of plants grown from seeds of the lower layers was 4.86 tons per hectare, which is 0.67-0.7 tons per hectare higher than yield of plants grown from seeds of the middle and upper layers. In this regard, during the primary seed production of the soybean cultivar "Batya" in nurseries of individual selection of initial plants, selection should be carried out and seeds from 2-7 nodes of the lower layer should be used for sowing.

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THE FIRST RECORD OF *RATTUS NORVEGICUS* ON THE ISLAND OF CYPRUS AND THE EXPECTED EFFECT ON AGRICULTURE AND LOCAL BIOTA

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Abstract

The island of Cyprus - a centre of endemism and a biodiversity "hotspot" - is located in the Eastern part of the Mediterranean Basin, which comprises one of the largest groups of islands in the world. This island system has been early and widely colonized by the black rat *Rattus rattus* since their spread from the Indian Peninsula. This species is considered one of the most damaging alien invasive predator to have been introduced on more than 80% of the world's major islands and known to negatively affect island biota and agriculture. Although across the Mediterranean Sea the brown rat *R. norvegicus* is present along with the black rat, on the island of Cyprus there were only *R. rattus.* This study reports the first documented record of *R. norvegicus* on the island of Cyprus from two localities, the city of Geri and Neo Chorio near Kythrea. The species identification was first carried out by morphological analysis, and then confirmed by molecular evidences inferred by the analysis of mitochondrial DNA sequences. The implications of the discovery of this new alien invasive species on agriculture and the local biota of Cyprus are examined.

Keywords: *Rattus norvegicus, brown rat, agricultural damages, alien invasive species.*

Introduction

According to CABI Invasive Species Compendium, (https://cabi.org.isc/datasheet/46829), the brown or Norwegian rat (*Rattus norvegicus*) is globally widespread and costs primary industry hundreds of millions of dollars per year. It has caused or contributed to the extinction or range reduction of native mammals, birds, reptiles and invertebrates through predation and competition. It restricted the regeneration of many plant species by eating seeds and seedlings, eats food crops and spoils food stores by urinating and defecating in them (ISSG, 2011; Donahoe, 2016; CABI, 2019). *R. norvegicus* is found on 36% of the world's island groups (Atkinson, 1985) and there is ongoing risk of invasion of new islands. Once an island group is colonised *R. norvegicus* is able to widespread throughout the archipelago rapidly through further hitch-hiking and natural dispersals, including swimming. It is capable of swimming 2.5 km (CABI, 2019). Cyprus is the third largest island in the Mediterranean Basin, however, until now there is no documented record of *R. norvegicus* on this island (Kryštufek and Vohralík, 2009; Nicolaou *et al.*, 2016; Hadjisterkotis, 2017). In January 2019 in the City of Geri (Nicosia district) we observed a female *R. norvegicus* feeding in a bird feeder, with all the external characteristics of brown rats described by Qumsiyeh (1996), Yiğit (1999), Nagorsen (2002), Kryštufek and Vohralík (2009). Several other rats were seen also in two other localities on the island. *Rattus* genus includes 66 species (Musser and Carleton, 2005), its taxonomy is complex and is further complicated by many synonyms for different species (Robins *et al.*, 2007). Identification of individuals, even by experts, is often difficult (Taylor *et al.*, 1982). The analysis of morphological traits combined with the genetic characterization of molecular markers is the most efficient system for identifying individuals at the species level (Musser and Carleton, 2005).

The primary aim of this report is to examine for the first time new molecular and morphological evidences to establish or reject the presence of *R. norvegicus* on the island of Cyprus. In addition, to examine the possible effects which this alien invasive species might have on agriculture, livestock and the local biota, and to recommend management measures for the protection of biodiversity, livestock and agriculture.

Materials and Methods

Examination of live specimens and carcasses

In January 2019 a female rat bred in a rock garden next to a bird feeder in the city of Geri in Nicosia district (Figure 1).

Figure 1. Female *Rattus norvegicus* in the city of Geri, Cyprus. Photo: E. Hadjisterkotis

Two of the younger animals were captured using snap traps in order to take body measurements and hairs for DNA analysis. A roadkilled rat with the external characteristics of *R. norvegicus* was found at Neo Chorio village near Kythrea, on the 5th of April 2019, and a hair sample was collected for DNA analysis. In addition, visual observations at the Mia Milia sewage treatment ponds indicated the presence of rats having the characteristics of *R. norvegicus*, i.e. compared with *R. rattus*, smaller ears, smaller eye, blunt nose, thick heavy body, tail shorter than head and body (Qumsiyeh, 1996; Nagorsen, 2002; Kryštufek and Vohralík, 2009). On the skull of *R. rattus* supraorbital ridges diverged posteriorly along parietals; close to occipital region they are slightly bent or curved. In *R. norvegicus* supraorbital ridges ran mainly parallel along parietals (Qumsiyeh, 1996; Kryštufek and Vohralík, 2009; Nagorsen, 2002). In older individuals, the skull ridges are characteristic in shape, well-defined, relatively straight and situated close to one another, which gives the impression of a relatively narrow braincase (Pimsai *et al.*, 2014).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from hair by means of the InstaGene™ Matrix (Bio-Rad) according to the manufacturer's protocol. Sample quality and DNA concentration were determined via spectrophotometry using a ND-8000 (NanoDrop Technologies, Thermo Fisher Scientific Inc., Wilmington, DE). Two pairs of primers were designed by means of the "Web Primer: DNA and Purpose Entry" bioinformatic tool available at http://www.candidagenome.org/cgi-bin/compute/web-primer (Skrzypek *et al.*, 2017) and used to amplify two regions of the *R. norvegicus* mitogenome encompassing the first 434 bp of the D-loop region (HVS-I domain) and the first 534 bp of the cytochrome c oxidase subunit I (CO I) gene, respectively. PCR products were purified and then sequenced on an ABI 3130 sequencer. Sequences were compared to GenBank sequence database using BLAST in order to estimate the statistical significance of matches. Sequences were also aligned with the homologous sequences of *R. rattus* (NC_012374) and *R. norvegicus* (KM114608) using Clustal X 2 (Larkin *et al.*, 2007). Further experimental details can be found in the Hadjisterkotis et al., 2019 (BioInvasion Records - submitted on 9 July 2019).

Results and Discussion

Observations on dead specimens

The preliminary body measurements and the shape of the skulls of the two juvenile rats collected in the city of Geri, and the rat found dead on the road in the village Neo Chorio indicated that all three specimens were *R. norvegicus* (Table 2). The tail length is less than head and body in both types wild (Geri) and laboratory (Neo Chorio).

Examining the cranial characteristics, it was observed that the braincase is narrow and elongated. The parietals and interparietal are bordered by well-defined ridges which are straight and almost parallel, a diagnostic characteristic of *R. norvegicus*. In the juvenile specimen from Geri the skull ridges were not so well-defined as in the older specimens.

For the Mia Milia sewage treatment ponds only visual observations were available, with the diagnostic characteristic of *R. norvegicus*, i.e. blunt nose, shorter ear, shorter tail than head and body, heavy thick body and smaller eyes.

Table 2. Body measurements (mm) of *R. norvegicus* from Geri, and one adult male from Neo Chorio, Kythrea.

	Geri 1	**Geri 2 (juvenile)**	**Neo Chorio Kythrea**
Head and Body	195	149	230
Tail	160	130	205
Hind foot	43.4	32.0	43.0
Ear	20.3	12.31	20.00
Ear bend forward	Just reaching the eye	Just reaching the eye	Not reaching the eye

DNA analysis

The sequences analysis of the D-loop region and the COI gene from two rat specimens allowed to assess that both samples were *R. norvegicus*, although belonging to two different genetic strain. Discrimination was possible due to the variability detected in the D-loop region which showed a 1.66% divergence between the two sequences. No differences were found at the level of the COI gene.

Competition with black rats

A problem that this new alien invasive species might face in its dispersal in Cyprus, is competition with the previously introduced black rat, which occupy just about every rural habitat (Kryštufek and Vohralík, 2009; Hadjisterkotis, 2017). For such a widespread and damaging pest, with the exception of its damage on carob trees, surprisingly little is known about its ecology and its impact on Cypriot ecosystems. This makes it even more difficult to predict and highlight the potential for impacts of *R. norvegicus* on humans, livestock, agriculture and wildlife in Cyprus. The black rat eats the eggs of ground nesting birds and the eggs of woodpigeons nesting on trees (Hadjisterkotis, 2000, 2017). They are a menace for carob trees (*Ceratonia siliqua*) plantations, almonds (*Prunus amygdalus*), pomegranate (*Punica granatum*), and other fruits and crops. They feed on carob pods and on the bark, eventually killing the trees (Hadjisterkotis, 2017). However, brown rats being larger in size, on other Mediterranean countries are described as more adaptable and much more aggressive than black rats (Canale, 2019). Black rats are well adapted to small islands, whereas Norway rats are mostly observed on the largest islands where humans also occur (Amori *et al.*, 2008; Canale, 2019). Considering that Cyprus is the third largest island in the Mediterranean, Norway rats are expected to adapt and to compete well with black rats and to exert a stronger impact on native species.

Destruction to agriculture, livestock and spreading of zoonoses

Rats are destructive pests particularly in and around farm facilities. This can be especially true during the winter months, as they seek food and refuge indoors. Rats consume and contaminate feed, gnaw on structural, mechanical, and electrical components, weaken concrete slabs and walkways with their burrowing activities and spread diseases to livestock as well as to wild animals (Donahoe, 2016). Are reservoirs and vectors of pathogens that can infect livestock, wildlife species and humans. Such infectious zoonotic diseases are the plague agent *Yersinia pestis*, hantavirus, leishmania, leptospirosis, scrub typhus, toxoplasmosis viral haemorrhagic fevers (Herbreteau *et al.*, 2012) and many other. A costly disease that was found to infect farm animals and rats, is paratuberculosis. This is a chronic, contagious granulomatous enteritis of farm ruminants characterized by persistent diarrhea (mainly in cattle and less in sheep), progressive weight loss, debilitation and eventually death. The etiologic agent is *Mycobacterium avium* ssp *paratuberculosis* (MAP). MAP infection results in significant economic losses for the farm industry with annual estimates of millions of dollars around the world. In one study it was estimated that paratuberculosis costs the U.S. dairy industry alone 200 to 250 million dollars annually (Ott, 1999). Viable MAP has been isolated from Cypriot cattle, sheep and goat populations and dairy food (Liapi *et al.*, 2011, 2015; Botsaris *et al.*, 2013). Several studies revealed MAP isolations from non-ruminant free-ranging wildlife, such as from tissue samples from foxes (*Vulpes vulpes*), Norway rats (Florou *et al.*, 2007) and feral cats (*Felis familiaris*; Palmer *et al.*, 2005). Infected rats may have the greatest effect on the epidemiology of *MAP* infection on farms that have eliminated all infected livestock from the premises or on *MAP*-free farms in the same geographic area as infected farms. Two of the predators of rats on Cyprus are red fox (*Vulpes vulpes*) which can be infected by feeding on rats, as well as domestic cats which are kept on farms for the control of rats and mice. These species may live for several years with home ranges that cover areas large enough to include more than one farm, spreading the disease from farm to farm, and reinfecting farms which eliminated all infected livestock.

Conclusions

This study reports the first documented record of an alien invasive species *R. norvegicus* on the island of Cyprus and the first breeding record in the wild. On other islands this species has caused or contributed to the extinction or range reduction of native mammals, birds, reptiles and invertebrates through predation and competition, is causing millions from damages in agricultural crops and the spreading of diseases to humans, livestock and wildlife. For the protection of agriculture, livestock and biodiversity, an extensive study for the dispersal and habitat selection of *R. norvegicus*, competition (both within the species and with *R. rattus*), predation, zoonoses and effect on agriculture must commence as soon as possible. These studies should take place in combination with an eradication program, with emphasis on farm land and livestock premises.

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INFLUENCE OF INTERCROPPING MAIZE WITH COWPEA AND FERTILIZATION WITH CLINOPTILOLITE ON FORAGE YIELD AND QUALITY

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Abstract

Maize forage is poor in protein content which leads to low quality and nutritive value. Regarding the high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content, and thus, provide cost savings. In this study, maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) were intercropped in different sowing densities and fertilization with naturale zeolite clinoptilolite and their monocropping equivalents were tested to determine the best intercropping system on forage yield and quality. Maize was cultivated alone (75 000 plants ha^{-1}) and intercropped with cowpea as follows: 75 000 plants ha^{-1} of maize and 37 500 plants ha^{-1} of cowpea (MC$_1$), 75 000 plants ha^{-1} of maize and 50 000 plants ha^{-1} of cowpea (MC$_2$) and 75 000 plants ha^{-1} of maize and 75 000 plants ha^{-1} of cowpea (MC$_3$), in rows alternating with maize. The highest dry matter yield was produced by MC$_3$ (23.2 t ha^{-1}), and the lowest by maize monocrop (20.3 t ha^{-1}) in fertilization with clinoptilolite. All intercropped systems had higher crude protein contents, MC$_1$ (99 g kg^{-1} DM), MC$_2$ (106 g kg^{-1} DM) and MC$_3$ (114 g kg^{-1} DM), than the maize monocrop (82 g kg^{-1} DM) in fertilization with clinoptilolite. Intercropping of maize with cowpea and fertilization with clinoptilolite reduced neutral detergent fiber, resulting in increased forage digestibility. Therefore, maize intercropping with cowpea and fertilization with clinoptilolite could substantially increase forage quantity and quality, and decrease requirements for protein supplements as compared with maize monocrop.

Keywords: *Intercropping, Natural Zeolite Clinoptilolite, Maize, Cowpea, Yield, Quality.*

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used in feeding cows and fattening cattle. Despite its high energy content, the protein content is low (88 g kg^{-1}) compared with legumes silage (Anil et al., 2000) and needs to be supplemented with proteins for better feed quality (Stoltz et al., 2013). Intercropping maize with legumes for silage is a feasible strategy to improving the level of crude protein (Contreras-Govea et al., 2009). Javanmard et al (2009), worked on intercropping of maize with different legumes, and showed that dry matter yield and crude protein yield of forage were increased by all intercropping compositions compared with the maize monocrop. Dahmardeh et al. (2009) concluded that intercropping of maize and cowpea resulted in more digestible dry matter and also crude protein content than maize mono-cropping. Physiological and morphological differences between intercrop constituents influence their ability to use resources; especially cereals with legumes, have several advantages such as higher overall yields, better soil utilization (Dhima et al., 2007), yield stability of the cropping system (Lithourgidis et al., 2006), better use of light, water and nutrients (Javanmard et al., 2009), improved soil conservation (Anil et al., 1998), soil fertility through biological nitrogen fixation, increases soil conservation through greater soil coverage as compared to sole cropping, and ensures better soil-susceptible crop in monoculture (Lithourgidis et al., 2006), and better control of pests and weeds (Vasilakoglou et al., 2008). Atmospheric nitrogen fixation using legumes plants can reduce nitrogen competition in the reciprocal intercropping system of legumes and

cereals enabling the cereals to use more nitrogen in the soil (Eskandari et al., 2009). This can affect the quality of the fodder intercrop components because the protein content is directly related to the content of nitrogen in the forage plants (Putnam et al., 1985). Nutrients use efficiency can also be achieved through theuse of clinoptilolite zeolite because of the unique physical and chemical properties of clinoptilolite zeolite coupled with their abundance in sedimentary deposits and in rocks derived from volcanic parent materials have made them useful in many agricultural applications (Ramesh et al., 2010). Clinoptilolite zeolite is widely used in cultivating different crops such as cereals, forage, vegetables, vine, and fruit crops due to their exceptionally high ion-exchange capacity (Butorac et al., 2002).
This study was designed to determine the influence of different patterns of maize-cowpea intercropping and fertilization with natural zeolite clinoptilolite on the yield and quality of forage.

Material and Methods

A field experiment was carried out during the 2018 growing season at experimental fields in Oborovo (45°40'54"N 16°15'12.5"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Mean monthly air temperature and rainfall during the 2018 growing season

Meteorological data	Month					
	April	May	June	July	August	September
Air temperature (°C)	15.7	19.2	20.9	22.4	23.0	17.2
Rainfall (mm)	51.6	50.5	144.4	94.4	66.1	36.4

The experiment was set up as a randomized complete block design with three replicates. Maize hybrid seed (KWS Kolumbaris) was obtained from Seed Company "KWS". Seed of the cowpea cultivar "Dolga vigna" was obtained from Company "Sjemenarna". The treatment comprising the individual plot size was 50 m × 2,8 m. The maize population 75 000 plants ha^{-1} (SM) were spaced at 70 cm × 19 cm and cowpea population 37 500 (MC_1), 50 000 (MC_2) and 75 000 plants ha^{-1} (MC_3) were spaced at 70 cm × 38.1 cm, 70 cm x 28.6 cm and 70 x 19 cm, respectively, in rows alternating with maize. Basic tillage was carried out by ploughing to 30 cm depth. Presowing preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 200 kg of K_2O ha^{-1} in variant of control and additionally in the vegetation of crops (stage six maize leaves) introduced supplementation 300 kg natural zeolite clinoptilolite ha^{-1}. Clinoptilolite used in this work originated from Slovakia, and the particles size of zeolite ranged in size from 0.5 to 2.0 mm. Maize and cowpea were sown to a depth of approximately 5 cm by maize drill in May 8, 2018. Herbicide Wing P (active substance 212.5 g/l dimethenamide-p and 250 g/l pendimethalin) was applied pre emergence in intercropping maize with cowpea at a dose of 4 l ha^{-1}. The soil of the research area has an acid pH 4.2 reaction (M-KCl), good humus (3.2%), poorly supplied with physiologically active phosphorous (4.6 mg P_2O_5/100 g soil), medium supplied with physiologically active potassium (20.0 mg K_2O/100 g soil) and richly supplied with total nitrogen amounting to 0.17%. The fresh fodders were manually harvested when the maize reached soft dough stage and cowpea at R8 stage and then chopped into 20 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein was measured according to Kjeldah, phosphorus was analysed by colorimetry (AOAC, 2000) and neutral detergent fibre according to (Van Soest et al., 1991).

Analyses of variance were made for fresh fodder and dry matter yield and forage quality parameters ($P<0.05$), and the Tukey test was used for comparing means ($P<0.05$). Data were analyzed using SAS statistical software (SAS Inst., 2002).

Results and Discussion

The diferences in the yield of forage are statistically significantly and yield of dry matter are not statistically significantly ($P<0.05$). The yield of forage and dry matter ranged from 77.4 t ha^{-1} (MC_3) to 60.1 t ha^{-1} (SM) and 23.2 t ha^{-1} (MC_3) to 20.3 t ha^{-1} (SM) in fertilization with naturale zeolite clinoptilolite (Table 2). The diferences in the yield of forage and dry matter in fertilization with naturale zeolite clinoptilolite were better than in control of the variant are statistically significantly ($P<0.05$). The use of natural zeolite with urea increased silage corn dry matter production and provided the best use of nitrogen at the higher doses of fertilizer (Bernardi et al., 2011). According to the results, when cowpea seed number and fertilization with naturale zeolite clinoptilolite increased in intercrop, forage and dry matter yields on parcels increased. Cowpea can be intercropped with maize (Dahmardeh et al., 2009) and sorghum (Azraf et al., 2007). for a higher yield and quality compared with sole cropping. Legume contribution to maize in mixtures was significant and increased the total biomass yield of mixtures (Geren et al., 2008). One of the main reasons of intercropping maize and cowpea is the increase crude protein level in silage.

Table 2. Fresh forage yield and dry matter yield of maize and maize-cowpea intercropped

Treatments	Fresh forage yield t ha^{-1}			Dry matter yield t ha^{-1}		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	53.5a	60.1c	56.8d	19.4a	20.3b	19.9a
MC_1	59.5a	67.3b	63.4c	20.0a	22.1ab	21.1a
MC_2	62.9a	71.5ab	67.2b	20.6a	22.9a	21.8a
MC_3	66.7a	77.4a	72.1a	21.0a	23.2a	22.1a
Mean	**60.7b**	**69.1a**		**20.3b**	**22.1a**	

Different letters in the column mean significant difference ($P<0.05$).

Since crude proteins are very important in cattle fodder, silage containing more crude proteins is desirable. In this study it was found that the value of crude proteins of intercropped forage MC_1, MC_2 and MC_3 was statistically significantly ($P<0.05$) higher than SM during a two treatments of fertilization (Table 3). According to the results, when cowpea seeds number and fertilization with naturale zeolite clinoptilolite increased in intercrops, the content of crude protein in the mixture increased. Cowpea fodder is a rich source of crude protein, giving up to 184 g kg^{-1} (Khan et al., 2010).

Table 3. Content and yield of crude protein of maize and maize-cowpea intercropped

Treatments	Content of crude protein g kg^{-1} DM			Crude protein yield t ha^{-1}		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	78c	82d	80d	1.51c	1.66d	1.59c
MC_1	93b	99c	96c	1.86b	2.19c	2.03bc
MC_2	99a	106b	103b	2.04a	2.43b	2.24ab
MC_3	106a	114a	110a	2.23a	2.65a	2.44a
Mean	**94b**	**100a**		**1.91b**	**2.23a**	

Results in the present study were in agreement with other studies where legumes also increased crude protein concentration when in a mixture with maize (Dawo et al., 2007). This could be due to higher nitrogen availability for maize in intercropping compared with the monoculture crop (Eskandari et al., 2009). In this study it was found that the yield of crude proteins of intercropped fodder MC_1, MC_2 and MC_3 was statistically significantly ($P<0.05$) higher than SM during a two fertilization treatments (Table 3). Treatment of MC_3 had the highest yield of crude protein 2.23 t ha^{-1} in control of the variant and 2.65 t ha^{-1} in variant fertilization with naturale zeolite clinoptilolite from other fodder mixtures (Table 3). From this point of view fodder produced in maize-cowpea intercrops is important not only to profit from the increase in the content of crude protein, but also from the reduction of the content of neutral detergent fibers. For this reason, the best option in maize-cowpea intercropping is the use of cowpea genotypes that provide forage with the greatest amount of pods at harvest. In addition, the level of neutral detergent fibers is associated with the stage of maturity of the fodder due to the level of the cell wall components, mainly cellulose, hemicellulose and lignin (Mugweni et al., 2000). The value of a neutral detergent fiber refers to the total cell wall and consists of an acid detecting fiber fraction plus hemicellulose. In this study it was found that the values of neutral detergent fibers of intercropped MC_2 and MC_3 were statistically significantly ($P<0.05$) lower than SM during two fertilization treatments (Table 4).

Table 4. Content of neutral detergent fiber and phosphorus of maize and maize-cowpea intercropped

Treatments	Content of neutral detergent fiber g kg^{-1} DM			Content of phosphorus g kg^{-1} DM		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	367a	339a	353a	2.2c	2.4d	2.3d
MC_1	355a	326ab	341ab	2.3bc	2.5c	2.4c
MC_2	342b	319bc	330bc	2.4ab	2.6b	2.5b
MC_3	327c	303c	315c	2.5a	2.7a	2.6a
Mean	**348a**	**322b**		**2.35b**	**2.55a**	

Different letters in the column mean significant difference ($P<0.05$).

According to the results, when cowpea seed number and fertilization with clinoptilolite increased in intercrop, the values of neutral detergent fibers in the mixture decrase. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh et al., 2009). Since smaller amounts of fiber components are used for better digestion, the cowpea intercropped plots to be superior to monocrop maize in terms of neutral detergent fiber. Result in the present study were in agreement with other studies where clinoptilolite decreased of neutral detergent fibers values in silage of maize (Bernardi et al., 2011). In this paper, the value of phosphorus of intercropped forage MC_1, MC_2 and MC_3 was statistically significantly ($P<0.05$) higher than SM during a two fertilization treatments (Table 4). According to the results, when the cowpea seed number and fertilization with naturale zeolite clinoptilolite increased in intercrop, the values of phosphorus in the mixture increased. Contribution of legumes with sweet sorghum in mixtures was significant increased phosphorus in fresh fodder (Basaran et al., 2017).

Conclusion

The conclusion of the present study is that intercropping of maize with cowpea at various planting densities and fertilization with natural zeolite clinoptilolite was shown to be an effective way to influence fresh biomass production, dry matter and crude protein yield to enhance nutrient quality of forage. Intercropping of maize with cowpea and fertilization with natural zeolite clinoptilolite increased values of crude protein, phosphorus and decreased values of neutral detergent fibre concentrations in forage. Finally, intercropping with 75 000 plants ha^{-1} of maize and 75 000 plants ha^{-1} of cowpea and fertilization with 300 kg natural zeolite clinoptilolite ha^{-1} was most suitable according to the nutrient composition in forage.

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UPTAKE OF PHOSPHORUS AND POTASSIUM IN SORGHUM PLANTS IN DEPENDENCE ON NUTRITIONAL LEVEL

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Abstract

A pot experiment was carried out to determine the uptake and distribution of phosphorus and potassium in sorghum plants depending on the level of mineral nutrition. The plants were grown on eight levels of nutrition – $N_0P_0K_0$, $N_0P_{200}K_{200}$, $N_{600}P_0K_0$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$, $N_{600}P_{200}K_{200}$, $N_{800}P_{200}K_{200}$, $N_{600}P_{400}K_{400}$. The different levels of nutrients were created by applying of NH_4NO_3, $Ca(H_2PO_4)_2$ and KCl dissolved in water. It was established that sorghum grown at N_{600} level in combination with elevated levels of phosphorus and potassium $P_{400}K_{400}$ had the highest concentration of phosphorus and potassium. Self-nitrogen fertilization $N_{600}P_0K_0$ lowered the concentration of the two elements in the grain to values close to the unfertilized control. The highest content of phosphorus 1.09 $g.pot^{-1}$ was obtained at $N_{600}P_{200}K_{200}$ level, which exceeded the control by 146.7%. Sorghum plants removed the most potassium in maturity at $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$ levels. Self-nitrogen fertilization $N_{600}P_0K_0$ reduced the total phosphorus and potassium uptake of the above-ground parts compared to triple combinations $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$. The phosphorus and potassium harvest indexes were higher in the control plants, at the lower levels of mineral nutrition ($N_{200}P_{200}K_{200}$ and $N_{400}P_{200}K_{200}$) and at the N_{600} level combined with higher $P_{400}K_{400}$ fertilization. Sorghum gown at $N_{600}P_0K_0$ had been shown to reduce the proportion of phosphorus and potassium absorbed in the sorghum grain. Compared to the control, the decrease was by 4.8% for phosphorus harvest index and by 10.0% for potassium harvest index. Sorghum plants grown at the higher $N_{800}P_{200}K_{200}$ level were characterized with the lowest phosphorous (65.2%) and potassium (17.0%) harvest index.

Keywords: *Phosphorus, Potassium, Uptake, Sorghum.*

Introduction

Sorghum (Sorghum bicolor L. Moench) is one of the five major crops in the world (Shehzad et al., 2009). It can be used as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and organic by-products (Fernandes et al., 2013). In Bulgaria the production of grain sorghum has increased in recent years and sorghum is one of the top ten grown crops in the country (Ivanov, 2006). Sorghum is a multipurpose crop belonging to the Poaceae family, which are C_4 carbon cycle plants with high photosynthetic efficiency and productivity (Tari et al., 2012). The modern varieties are hybrids with high productivity potential appropriate for application of intensive forage grain production technologies (Kertikov, 2007). Nitrogen, phosphorus, potassium and water are considered as the major limiting factors in crop growth, development and finally economic yield (Enchev and Kikindonov, 2015; Jones, 1983). Proper nitrogen nutrition is critical to meet crop needs and indicate considerable opportunities for improving nitrogen use efficiency (Murell, 2011). Many agricultural soils have a limited ability to supply available nitrogen for target yields and nitrogen is the most limiting nutrient for cereal crops, including sorghum production (Gerik et al., 2014). Nutrient uptake of sorghum precedes dry matter accumulation because nutrients are required for growth and dry matter accumulation (Soleymani et al, 2011). The level of mineral nutrition has greater effect on growth and yield of cereals plants (Raun and Johnson, 1999). The studies of sorghum genotypes in this connection are limited.

For better fertilizer management the study about the effects of different levels of nitrogen, phosphorus and potassium on the growth and nutrient uptake of sorghum is very crucial. The objective of this research was to investigate the effect of different nutritional levels on the accumulation and distribution of phosphorus and potassium in sorghum plants.

Material and Methods

The effect of different levels of mineral nutrition on the accumulation and distribution of dry mass and nitrogen of sorghum plants was studied in a pot experiment under conditions of growing installation. The experimental design consisted of eight levels of mineral nutrition and four replications of each variant. The studied treatments of soil nutritional levels were: $N_0P_0K_0$, $N_0P_{200}K_{200}$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$, $N_{600}P_{200}K_{200}$, $N_{800}P_{200}K_{200}$, $N_{600}P_0K_0$ and $N_{600}P_{400}K_{400}$. The levels of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) in the soil were created by adding of NH_4NO_3, $Ca(H_2PO_4)_2$ and KCl dissolved in a water. Three plants per pot of hybrid EC Alize were grown under optimal water regime in plastic pots of 10 L. Each pot contained 15 kg air-dry soil. The main agrochemical characteristics of the soil before sowing of the sorghum were: pH_{H2O} = 7.80 (slightly alkaline reaction); content of mineral nitrogen 27.6 mg $Nmin.kg^{-1}$ soil; content of available phosphorus (Egner-Ream) 158 mg $P_2O_5.kg^{-1}$; and content of exchangeable potassium 210 mg $K_2O.kg^{-1}$. These results showed low supply of the soil with mineral nitrogen and good soil supply with available phosphorus and potassium. A good water regime was maintained in the pots during the vegetation of the sorghum plants. The aboveground biomass was collected from the plants under all the treatments in a full maturity phase. The harvested samples were separated in a grain and stover (stems + leaves + chaff). The dry weight of grain and stover was determined after drying for 48 hours at 60 °C. The sub-samples of 0.5 g ground and dry plant material were mineralized using a wet digestion by H_2SO_4 and H_2O_2 as a catalyst (Mineev, 2001). The concentration of phosphorus and potassium in plant samples were determined by colorimetric methods and potassium concentration was analyzed by the flame photometer model PFP-7 (Tomov et al, 2009). The content of accumulated phosphorus and potassium was obtained by multiplying the dry mass of sorghum grain and stover by the concentration of nitrogen in each plant part. The differences in the accumulation and distribution of dry mass and nitrogen into sorghum plants among all levels of mineral nutrition were calculated by using the overall analysis of variance (ANOVA). Duncan's Multiple Range Test (Duncan, 1955) at $p < 0.05$ was used in order to determine the difference among the means.

Results and Discussion

The level of mineral nutrition had a significant effect on the percentage of phosphorus and potassium in the grain and sorghum stover in maturity (Table 1). The average phosphorus content of the grain was 0.97% P_2O_5 and 0.25% P_2O_5 in the stover. The results indicated that the concentration of phosphorus in the sorghum grain was nearly three times higher than in the stover. The lowest phosphorus concentration in the grain was obtained in the self-nitrogen fertilized variant and the control. The highest concentration of phosphorus in the grain, of plants grown at the level of N_{600} in combination with elevated levels of phosphorus and potassium $P_{400}K_{400}$. Self-nitrogen fertilization $N_{600}P_0K_0$ had a negative effect on the percentage of phosphorus in the grain and led to values close to the control. Excluding nitrogen fertilization and only added phosphorus and potassium slightly affected the percentage of phosphorus in the grain. The value of 0.97% P_2O_5 in this variant of $N_0P_{200}K_{200}$ did not differ significantly from that of the controls. The elevated N_{800} level of nitrogen had been shown to lower the phosphorus concentration in the sorghum grain compared to the fertilized variants with lower nitrogen levels N_{200}, N_{400}, N_{600} in combination with $P_{200}K_{200}$. The concentration of phosphorus in sorghum changed to a relatively narrow range of 0.23% to

0.33% P_2O_5. The highest concentration of phosphorus in sorghum stover was obtained in the self-phosphorus-potassium fertilization variant and at the $N_{600}P_{200}K_{200}$. The negative effect of increasing the level of phosphorus-potassium fertilization and its exclusion ($N_{600}P_{400}K_{400}$, $N_{600}P_0K_0$) on the percentage of phosphorus in sorghum stover compared to variant $N_{600}P_{200}K_{200}$ had been proven.

Table 1. Concentration of phosphorus and potassium of sorghum plants depending on the nutritional level

Nutritional level	% P_2O_5		% K_2O	
	Grain	Stover	Grain	Stover
$N_0P_0K_0$	0.90 de	0.29 bc	0.31 f	0.91 d
$N_0P_{200}K_{200}$	0.97 bcd	0.33 a	0.35 e	0.99 c
$N_{200}P_{200}K_{200}$	1.01 ab	0.27 c	0.37 de	1.05 bc
$N_{400}P_{200}K_{200}$	1.02 ab	0.30 b	0.41 bc	1.12 ab
$N_{600}P_{200}K_{200}$	0.99 abc	0.33 a	0.42 b	1.15 a
$N_{800}P_{200}K_{200}$	0.93 cde	0.29 bc	0.39 cd	1.12 ab
$N_{600}P_0K_0$	0.87 e	0.27 c	0.30 f	0.85 d
$N_{600}P_{400}K_{400}$	1.05 a	0.23 d	0.45 a	1.18 a
Average	*0.97*	*0.29*	*0.38*	*1.05*

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

In contrast to grain phosphorus concentration and mature stover, sorghum was characterized by approximately three times higher potassium concentration in the stover (1.05% K_2O average) compared to grain (0.38% K_2O average). The highest concentration of potassium in the above-ground portions of sorghum was found in variant N_{600} in combination with elevated levels of phosphorus and potassium $P_{400}K_{400}$, and the lowest in the control and self-nitrogen fertilized variant. $N_{600}P_0K_0$ nitrogen fertilized plants are characterized by low potassium concentration in maturity and the percentage of potassium in the grain and their stover did not differ significantly from plants in the control plants. No proven differences in potassium concentration in grain of sorghum grown at elevated $N_{800}P_{200}K_{200}$ and lower levels of $N_{200}P_{200}K_{200}$ and $N_{400}P_{200}K_{200}$ had been identified. The results reported indicated a tendency to increase the concentration of potassium in the stover in nitrogen fertilized plants. Plants grown only with nitrogen fertilizer $N_{600}P_0K_0$ are an exception to this trend. They had the lowest potassium concentration in stover 0.85% K_2O. Differences in potassium content in sorghum stover between variants $N_{600}P_{400}K_{400}$ and $N_{600}P_{200}K_{200}$ were not mathematically proven. This indicated a slight effect of the elevated $P_{400}K_{400}$ level on the potassium content of sorghum stover.

Accumulated amounts of phosphorus in the above-ground portions of sorghum in maturity varied widely depending on the level of mineral nutrition (Table 2). The average export of phosphorus to the sorghum grain was 0.55 g $P_2O_5.pot^{-1}$ and in the stover 0.24 g P_2O_5. All nitrogen fertilized variants export more phosphorus to the above-ground portions of the Sorghum accumulated the largest amount of phosphorus in the grain at N_{400} and N_{600} combined with phosphorus-potassium fertilization $P_{200}K_{200}$ or $P_{400}K_{400}$. In these two variants, $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$ the phosphorus exported to the grain were 0.72-0.73 g $P_2O_5.pot^{-1}$ which exceeds the neutral control by 132.6% and 135.1%, respectively. Self-nitrogen fertilization $N_{600}P_0K_0$ had been shown to reduce the absorbed phosphorus in the grain compared to the $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$ combinations.

Table 2. Phosphorus uptake of sorghum plants depending on the nutritional level, $g.pot^{-1}$

Nutritional level	Grain	% to $N_0P_0K_0$	Stover	% to $N_0P_0K_0$	Grain+Stover	% to $N_0P_0K_0$
$N_0P_0K_0$	0.31 d	100.0	0.13 f	100.0	0.44 f	100.0
$N_0P_{200}K_{200}$	0.38 cd	122.1	0.17 e	131.0	0.55 e	124.8
$N_{200}P_{200}K_{200}$	0.58 b	187.0	0.22 d	172.6	0.80 c	182.8
$N_{400}P_{200}K_{200}$	0.70 a	225.7	0.31 b	240.8	1.01 b	230.1
$N_{600}P_{200}K_{200}$	0.72 a	232.6	0.36 a	280.2	1.09 a	246.7
$N_{800}P_{200}K_{200}$	0.44 c	141.4	0.23 d	179.6	0.67 d	152.6
$N_{600}P_0K_0$	0.53 bc	170.6	0.25 c	195.7	0.78 c	178.1
$N_{600}P_{400}K_{400}$	0.73 a	235.1	0.25 c	192.9	0.98 b	222.6
Average	*0.55*		*0.24*		*0.79*	

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

Differences in the amount of phosphorus accumulated in the grain of the $N_{600}P_0K_0$ variant and nitrogen intake variant of 400 mg $N.kg$ $soil^{-1}$ combined with phosphorus-potassium fertilization $P_{200}K_{200}$ had been demonstrated. The lowest concentration of phosphorus in the sorghum grain was accumulated in variant $N_0P_{200}K_{200}$ and the control. The mineral nitrogen deposited at N_{200}, N_{400} and N_{600} levels significantly increased the amount of phosphorus accumulated in sorghum stover and increased by 72.6-180.2% relative to the non-fertilized plants. Control plants accumulated the lowest amount of phosphorus in the stover, and the most was accumulated in the $N_{600}P_{200}K_{200}$ variant compared to the other variants. The accumulated phosphorus in the stover had close values (0.22-0.25 g $P_2O_5.pot^{-1}$) at levels $N_{200}P_{200}K_{200}$, $N_{800}P_{200}K_{200}$, $N_{600}P_0K_0$ and $N_{600}P_{400}K_{400}$. The concentration of phosphorus in sorghum stover increased in parallel with an increase in the amount of nitrogen intake by 200, 400 and 600 mg N.kg soil-1 combined with phosphorus-potassium fertilization $P_{200}K_{200}$.

Table 3. Potassium uptake of sorghum plants depending on the nutritional level, $g.pot^{-1}$

Nutritional level	Grain	% to $N_0P_0K_0$	Stover	% to $N_0P_0K_0$	Grain+Stover	% to $N_0P_0K_0$
$N_0P_0K_0$	0.11 f	100.0	0.41 f	100.0	0.52 f	100.0
$N_0P_{200}K_{200}$	0.14 e	124.7	0.51 e	125.3	0.65 e	125.2
$N_{200}P_{200}K_{200}$	0.21 c	193.1	0.86 c	210.3	1.07 c	206.6
$N_{400}P_{200}K_{200}$	0.28 b	255.3	1.15 b	280.5	1.43 b	275.2
$N_{600}P_{200}K_{200}$	0.31 a	278.7	1.27 a	308.5	1.57 a	302.2
$N_{800}P_{200}K_{200}$	0.18 d	166.6	0.90 c	218.8	1.08 c	207.8
$N_{600}P_0K_0$	0.18 d	166.6	0.79 d	192.9	0.97 d	187.3
$N_{600}P_{400}K_{400}$	0.31 a	282.7	1.26 a	308.0	1.57 a	302.6
Average	*0.22*		*0.89*		*1.11*	

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The average export of potassium to the sorghum grain was 0.22 g of $K_2O.pot^{-1}$ and 0.89 g of $K_2O.pot^{-1}$ in the stover (Table 3). All fertilized variants exported more potassium to the grain and stover compared to the control. Sorghum accumulated the highest amount of potassium in the above-ground parts when grown at the N_{600} level combined with phosphorus-potassium fertilization $P_{200}K_{200}$ or $P_{400}K_{400}$. In these two variants $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$, the export of potassium to the grain was 0.31 g K_2O pot^{-1} in the stover was 1.26-1.27 g $K_2O.pot^{-1}$ which exceeded the untreated control by 178.7% in the grain and 208.5% in the stover, respectively. Concentration of potassium in stover and grain of sorghum increased in parallel with an increase in the amount of nitrogen intake of 200, 400 and 600 mg N.kg soil^{-1} combined with phosphorus-potassium fertilization $P_{200}K_{200}$, including variant $N_0P_{200}K_{200}$. $N_{600}P_0K_0$ self-nitrogen fertilization had proven to reduce the absorbed potassium in the above-ground portions of sorghum compared to the $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$ combinations. Differences in the amount of accumulated in the grain and stover potassium in variant $N_{600}P_0K_0$ and variant with nitrogen input 400 mg N.kg soil^{-1} combined with phosphorus-potassium fertilization $P_{200}K_{200}$ had been proven. The lowest content of potassium in the grain and sorghum stover accumulated in the control. The differences in the amount of potassium exported in the sorghum grain between variants $N_{800}P_{200}K_{200}$ and $N_{600}P_0K_0$ had not been proven. The mineral nitrogen deposited at N_{200}, N_{400} and N_{600} levels significantly increased the amount of accumulated potassium in the grain by 93.1 - 178.7%, and in the stover the increase was by 110.3 - 208.5%, compared to the control plants.

Table 4. Distribution of phosphorus and potassium into sorghum plants depending on nutritional level

Nutritional level	PHI, %	% to $N_0P_0K_0$	KHI, %	% to $N_0P_0K_0$
$N_0P_0K_0$	70.9 abc	100.0	20.9 ab	100.0
$N_0P_{200}K_{200}$	69.0 bcd	97.3	21.1 a	100.8
$N_{200}P_{200}K_{200}$	72.1 ab	101.7	19.8 abc	94.6
$N_{400}P_{200}K_{200}$	69.1 bcd	97.4	19.6 abc	93.9
$N_{600}P_{200}K_{200}$	66.4 cd	93.7	19.5 cd	93.3
$N_{800}P_{200}K_{200}$	65.2 d	92.0	17.0 d	81.2
$N_{600}P_0K_0$	67.5 bcd	95.2	18.8 c	90.0
$N_{600}P_{400}K_{400}$	74.4 a	104.9	19.8 abc	94.6
Average	*69.3*		*19.5*	

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan’s multiple range test.

The fraction of phosphorus and potassium absorbed in the grain at maturity represents 69.3% and 19.5% of the total accumulated phosphorus and potassium in the biomass at maturity (Table 4). The phosphorus harvest index for sorghum varied between 65.2 and 74.4%, and the potassium harvest index ranges from 17.0 to 21.1% depending on the level of mineral nutrition. The phosphorus and potassium harvest index were higher in the control, at the lower levels of mineral nutrition $N_{200}P_{200}K_{200}$ - $N_{400}P_{200}K_{200}$ as well as in the N_{600} in the background of increased $P_{400}K_{400}$ phosphorus-potassium fertilization. Sorghum gown at $N_{600}P_0K_0$ had been shown to reduce the proportion of phosphorus and potassium absorbed in the sorghum grain by 4.8% for PHI and by 10.0% for KHI compared to the control. No proven differences were found in the phosphorus harvest index for variants $N_0P_0K_0$, $N_0P_{200}K2_{00}$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$, $N_{600}P_0K_0$ and the obtained values were close 67.5 - 72.1%. The KHI values obtained for variants $N_0P_0K_0$, $N_0P_{200}K_{200}$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$ were very close 19.6 - 21.1%. Cultivation of the sorghum at N_{800} nitrogen level resulted in plants with the lowest phosphorous (65.2%) and potassium (17.0%) harvest index.

Conclusions

The sorghum grain had an average phosphorus concentration of 0.97% P_2O_5, which was nearly three times higher than the average phosphorus concentration 0.29% P_2O_5 in the stover. The average potassium concentration in the stover was 1.05% K_2O or approximately three times higher than in the grain. Cultivation of sorghum at the N_{600} level in combination with elevated levels of phosphorus and potassium $P_{400}K_{400}$ resulted in the grain with the highest

concentration of phosphorus and potassium. Self-nitrogen fertilization $N_{600}P_0K_0$ lowered the concentration of the two elements in the grain to values close to the control. Total phosphorus accumulated in maturity was highest at fertilization level $N_{600}P_{200}K_{200}$, which exceeded the control by 146.7%. Sorghum took up the most potassium in maturity at levels $N_{600}P_{200}K_{200}$ and $N_{600}P_{400}K_{400}$. Self-nitrogen fertilization $N_{600}P_0K_0$ reduced the export of phosphorus and potassium to the above-ground parts of the sorghum in maturity.
The harvested phosphorus index for sorghum varied between 65.2 and 74.4%, and the harvesting index of potassium ranged from 17.0 to 21.1% depending on the level of mineral nutrition. Cultivation of the sorghum at the increased N_{800} nitrogen level resulted in plants with the lowest phosphorous (65.2%) and potassium (17.0%) harvest index.

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STUDIES ON STABILITY OF GRAINS WEIGHT FROM PANICLE TO A COLLECTION OF OATS AUTUMN (*Avena Sativa* L.) GENOTYPES

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Abstract

The research tracked the behavior of the grain weights in the main panicle to a collection of autumn oat genotype. The purpose of the study was to assess the stability of this character under the influence of the climate conditions from the Western Plain of Romania. The studied collection comprised of 73 genotypes of different origins. Experimental data has been collected over three years. In statistical processing several methods of stability assessment, determining: the regression coefficients and stability, (Finlay-Wilkinson model), the variance of linear regression analysis (Hardwick-Wood model) and the variance heterogeneity (Muir model) were applied. The weight of grains in the principal panicle was influenced by the climatic conditions, the values differing from one year to another depending on genotype response. In the case of this character, 52.24% of the genotype x environment interaction was due to imperfect correlations, so that in appreciating its stability to study genotypes the results of both components could be used. Between the different models of stability assessment for this character, the studied material had a close concordance (very significant value for $\chi 2$ = 133.75). The estimates of used models, based on the ranks sum, showed that the highest stability of grain weight in the principal panicle occurred at: Carie, 4458, ARK 0151-61, 4480, Thonson genotypes. The analysis of the genotype x environment interaction revealed a high stability due to reduced interaction (less than 0.75% of total value) to: 4458, 4480, ARK-0151-61, Gerald genotypes. The studied collection includes valuable genotypes for breeding programs.

Key words: *autumn oats, stability, grains weight form panicle*

Introduction

Oats are grown on small surfaces although it has a special biochemical composition. A very important role is played by biologically grown material. The main parameters of productivity are the weight of grains from panicle, the grain size and grains number of the panicle. Against this, the stability of genotypes is the most important element for cultivators, because the risks due to unfavorable conditions are minimal (Brunava *et al.*, 2015). Productivity of oats panicle, in particular the number and weight of the grains of panicle are influenced by nitrogen fertilization and the precursor plant (Mantai Rubia Silva *et al.,* 2016). Production of grains of panicle is favored by the direct effect of the harvest index and indirectly by the size of the grain (Mantai Rubia Diana *et al.,* 2016). The characters of productivity and quality are also influenced by ecological conditions. Under the influence of annual conditions, the weight and the number of the grains of panicle are fluctuating characters with lower stability (Dumlupinar Ziya *et al.,* 2011). The variation of grain weights of panile depends also on the type of oats. The weight of the grains of the panicle is higher in the autumn sown forms than in the spring sown. Differences can be up to 80%. Much of these differences are due of differentiated development of primary and secondary grains (Crampton *et al.,* 1997). Among the productivity and quality characters, the productivity characters have the highest variability and lower stability under the influence of climatic conditions (Iannucci Anna *et al.,* 2011). Genotypic

correlations provide information on the genetic association between characters and indicates the most useful characters to be followed in the selection programs to achieve a certain objective. Grain production on the plant showed significant positive correlation coefficients in interdependence with the paniclea size, the weight of 1000 (Mushtaq *et al.,* 2013). In the realization of the weight of the grains of panicle and the stability of this character may intervene the competition between plants. The response to competition differs according to the variety's plasticity, the influence of the environment on productivity can range from 9% to 71%. Not found a negative association between production and the variety response to competition. Forms with high values will remain stable under conditions of competition with other plants (Sadras *et al.*, 2018). The study of varieties is necessary for their recommendation in culture, for each area, having to be recommended the best adapted variety to the conditions of that area. (Siloriya *et al.*, 2014). Assessment of the adaptability and stability of varieties is best done through quantitative characters. The oats genotypes have significant variations in the production of grains at the surface unit and all the characters that contribute to its realization. There are very stable genotype for(the weight of the panicle grains as well as for those quality. Through such studies can be found the genotypes adapted to specific conditions, to be cultivated or to be used in breeding programs for obtain high productivity varieties (Zeki *et al.,* 2018)
The present study aimed to evaluate the grains weight from main panicle stability in a collection of autumn oat genotypes.

Material and Methods

The study was conducted in Timisoara, under field conditions. The study was conducted over three years (2015-2017). The experimental soil was moderately cambicated chernozem. During the experiments, the climatic conditions were close to normal. Temperatures were within normal range. In the second year, in the spring was drought. In the third year, the drought was also in summer. The biological material harvested 73 genotypes of autumn oats. As a control, was used the Romanian variety Florina. The experimental data was obtained by determining the grain weights in panicle. From each genotype, representative samples were constituted.
In the processing of exprimental data, was applied a method of assessing the stability of genotypes under different environmental conditions, based on the fact that the components of the genotype x environment interaction are linearly linked to the effects of environmental conditions. These linkages are expressed by the average of genotype performances for the studied character. The genotype relative adaptability to different environmental conditions is appreciated through three parameters: the average of its performance, the response of genotype to different environmental conditions (regression coefficient), the stability of performance (deviations from regression) (Bernardo, 2002). There are several types of stability. According to the "static" concept, type I stability is when the performance of a genotype is constant in averages (coefficient of regression bi = 0). "Dynamic" concept, or Type II stability occurs if the response to the media is parallel to the average response of all genotypes studied. Type III stability is present when deviation from the regression line is small (Annicchiarico, 2002; Bernardo, 2002). For processing o experiemntal datas, were used different models: Finlay-Wilkinson, Hardwick-Wood, Wrike (Ciulca, 2006).For analyzing the genotype x environment interaction, are separated two components: one due to heterogeneity of genetic variants and another due to imperfect correlations. The association between the results of the different methods of assessing the stability of the studied characters was achieved using the coefficient of concordance (Muir *et al.,* 1992).

Results and Discussion

Table 1 shows that the highest Type I stability of production of the main panic during the experimental period was observed to genotypes: Solva, Le Conte, PA 725-4787, Gospodarski 48, Suergrain, to which this character has presented close values in the climatic conditions of the three years of experimentation. Also, the genotypes PA 621-3274, S Dak 40, 4482, 3378, Marettos Anderson, have achieved different values of this character from one year to the next exhibiting a reduced static stability. The lowest significant values of ecological valence, which indicates a high stability of the grain weight of the principal panicle were recorded in genotypes: Thonson, 3378, 4444, 4458, 4472, 4480. High variance values for genotype x environment interaction related to panicle productivity were recorded at: 4478, PA 621-3274, S Dak 40, Gospodarski 48, 4475, 4482, which shows a reduced stability of this character. The values of regression coefficient close to unity which shows a high dynamic stability have genotypes: Carie, 4458, ARK 0151-61, Gerald, CI 1908, where the weight of the main panicle beans was proportional to the favorable environmental conditions of the three experimental years. High values of median x genotype interaction were observed at the lines: S Dak 40, 4482, PA 621-3274, 3378. Minimum values of deviations from the right of regression, respectively high type III stability of this character was observed in genotypes: Norline, Tripolis, 4451, 4458, 4472, ARK 0151-61. Also, at 4478, PA 522-7, 5029, 4475 and Marys Quest, which exhibit reduced type III stability, the grain weights of the principal panicle in the three experimental years, shows great deviations from the right of regression. To the genotypes 4478, PA 621-3274, 4478, 3378, 4482, is observed a very high variability, associated with values of paniculum production above the average of experience.Between the results of different models of appreciation of the stability for the grain weight of the principal panicle in the varieties and lines studied there is a very close match, taking into account the very significant value χ^2 =133.75***. Given the estimates of the four used models, based on the amount of rank it is noted that the highest stability of that character presented the varieties and lines: Carie, 4458, ARK 0151-61, 4480, Thonson. (table 1)

Table 1.Stability of the grain weight/main panicle through linear regression (FINLAY-WILKINSON) and ecovalence (WRIKE) for winter oat genotypes studied

No	Genotype	Average	through FINLAY-WILKINSON				through WRIKE		Concordance between different stability models	
			Regression coefficient	Stability			Ecovalence value	Ecovalence stability rank		
				Type I (rank)	Type II (rank)	Type III (rank)			Amounts ranks	SS_R
0	1	2	3	4	5	6	7	8	9	10
1	Florina (control)	1.38	0.695	27	28	45	0.12	31	131	289
2	Norline	1.43	0.521	17	40	5.5	0.08	24	86.5	3782.25
3	Arlingthon	1.04	0.561	21	36	27	0.09	25	109	1521
4	Blamouth	0.92	0.304	7	54	41	0.23	51	153	25
5	CI 1908	1.09	0.937	38	5	62	0.17	38.5	143.5	20.25
6	Cimarron	0.99	1.490	57	41	67	0.32	58	223	5625
7	Crater	0.97	0.764	29	24	68	0.25	55	176	784
8	Earlygrain	0.91	0.456	15	45	13	0.11	28.5	101.5	2162.25
9	Excel	1.44	2.022	68	66	59	0.49	67	260	12544
10	Fergushon	1.50	1.763	63	58	35.5	0.24	52.5	209	3721
11	Fulwood	1.17	0.911	36	9	54.5	0.10	26.5	126	484
12	Jeferson	1.26	0.936	37	6	35.5	0.04	18.5	97	2601
13	Le Conte	0.98	0.055	2	64	48	0.40	62	176	784
14	Nortex	1.51	0.327	9	52	24.5	0.17	38.5	124	576
15	Suergrain	1.34	0.166	5	61	33.5	0.20	42	141.5	42.25
16	Thonson	1.38	0.905	34	11	17.5	0.01	7.5	70	6084
17	Walken	1.50	0.248	6	56	46.5	0.20	42	150.5	6.25
18	Compact	1.67	1.399	55	31	61	0.22	48.5	195.5	2256.25
19	Pennwin	1.32	0.586	23	34	49	0.15	36.5	142.5	30.25
20	2288	1.56	0.835	31	18	24.5	0.02	15.5	89	3481
21	3378	1.39	2.111	70	69	63	0.01	7.5	209.5	3782.25

22	834-4-1-3	1.06	0.423	13	47	23	0.13	33.5	116.5	992.25
23	3412	1.51	1.191	51	22	50.5	0.11	28.5	152	16
24	S Dak 40	1.85	2.393	72	72	26	069	71	241	8649
25	3868	1.35	0.529	18	39	37	0.12	31	125	529
26	Cocker 41-51	1.16	0.432	14	46	15.5	0.12	31	106.5	1722.25
0	1	2	3	4	5	6	7	8	9	10
27	4444	1.63	1.067	43	7	19.5	0.01	7.5	77	5041
28	4451	1.39	0.547	19	38	5.5	0.07	23	85.5	3906.25
29	4458	1.36	0.957	40	2	5.5	0.01	7.5	55	8649
30	4472	1.02	1.138	48	17	5.5	0.01	7.5	78	4900
31	4475	1.30	1.512	58	43	72	0.50	68.5	241.5	8742.25
32	4476	1.41	1.291	54	26	13	0.03	17	110	1444
33	4477	1.12	0.337	10	51	42	0.22	48.5	151.5	12.25
34	4478	1.65	0.671	25	30	73	2.23	73	201	2809
35	4480	1.17	1.075	44	8	5.5	0.01	7.5	65	6889
36	4482	1.35	2.111	71	70	44	0.50	68.5	253.5	11130.25
37	4483	1.25	1.203	52	23	31	0.05	21	127	441
38	4484	1.20	1.129	45	14	17.5	0.01	7.5	84	4096
39	4488	1.04	1.173	49	20	5.5	0.01	7.5	82	4356
40	4492	1.47	1.962	67	65	60	0.45	64	256	11664
41	5029	1.52	0.492	16	42	70	0.35	59	187	1521
42	5032	1.64	1.756	62	57	38	0.24	52.5	209.5	3782.25
43	Marrettos Anderson	1.46	2.029	69	67	54.5	0.40	62	252.5	10920.25
44	8276	1.38	1.526	59	44	57	0.21	45	205	3249
45	PA 522-7	1.17	0.564	22	35	71	0.48	66	194	2116
46	PA 522-23	1.29	1.713	61	55	29.5	0.21	45	190.5	1806.25
47	PA 621-3274	1.81	2.452	73	73	28	0.75	72	246	9604
48	PA 724-2580	1.39	0.548	20	37	43	0.14	35	135	169
49	PA 725-2154	1.37	0.833	30	19	21.5	0.02	15.5	86	3844
50	PA 725-4743	1.43	1.130	46	15	52.5	0.10	26.5	140	64
51	PA 725-4787	1.30	0.089	3	62	64.5	0.47	65	194.5	2162.25
52	PA 725-6113	1.20	0.418	12	48	29.5	0.15	36.5	126	484
53	PA 822-818	1.29	0.908	35	10	66	0.22	48.5	159.5	132.25
54	ARK 0151-61	1.32	1.054	42	3	5.5	0.01	7.5	58	8100
55	AR 104-18	1.33	0.897	32	13	21.5	0.01	7.5	74	5476
56	Marys Quest	0.95	0.898	33	12	69	0.25	55	169	441
57	Wodan	1.74	1.944	66	63	52.5	0.40	62	243.5	9120.25
58	Gospodarski 48	1.07	-0.133	4	71	58	0.57	70	203	3025
59	5183	1.60	1.182	50	21	32	0.05	21	124	576
60	Tripolis	1.86	1.413	56	33	5.5	0.01	7.5	102	2116
61	Krusevac	1.30	1.773	64	59	11	0.21	45	179	961
62	Boer	1.06	0.345	11	50	46.5	0.20	42	149.5	2.25
63	Algerian	1.29	0.682	26	29	64.5	0.22	48.5	168	400
64	Mirabel	1.24	0.322	8	53	50.5	0.25	55	166.5	342.25
65	Gerald	1.26	0.940	39	4	13	0.01	7.5	63.5	7140.25
66	Nuptiale	0.92	0.590	24	32	5.5	0.01	7.5	69	6241
67	Solva	1.23	-0.034	1	68	19.5	0.38	60	148.5	0,25
68	Valiant	1.53	1.597	60	49	40	0.18	40	189	1681
69	Barra	1.55	1.791	65	60	39	0.27	57	221	5329
70	Carie	1.25	1.031	41	1	5.5	0.01	7.5	55	8649
71	Krypton	1.08	1.279	53	25	56	0.13	33.5	167.5	380.25
72	Chamois	0.94	1.134	47	16	33.5	0.05	21	117.5	930.25
73	Emperor	1.11	0.705	28	27	15.5	0.04	18.5	89	3481
	Sum				2701	2701	2701	2701	10804	240836
									χ^2 =133.75***; $\chi^2_{0,1\%}$ =112.32	

High influence of genotype x environment interaction on the phenotypic expression of this character, respectively high values of the sum of the ranks according to the statistical models used, was observed at: 4482, Marettos Anderson, Wodan, 4475, Cimarron. In the case of this character, 52.24% of the genotype x medium interaction is due to imperfect correlations so in appreciating the stability of the grain weights of the principal panicle to the different varieties and lines from collection can be used efficiently the results of both components. Thus, taking into account imperfect correlations it is noted that the most stable values of principal panicle production were recorded by genotypes: Norline, Earlygrain, 4451, Nuptiale, Cocker 41-51. High values of deviations between ranks for primary panic production values during the experimental period they presented: 4478, PA 522-7, Gospodarski 48, PA 725-4787, 4475.In the case of varieties and lines Nortex, Suergrain, Norline, Walken, 4451, stability due to the reduced variation coefficients from one year to the next is correlated with a higher panic production than the average of the experience. To the genotypes 4478, PA 621-3274, 4478, 3378, 4482, is observed a very high variability, associated with values of paniculum production above the average of experience (Table 2) Of the plant morphological characters, some have greater variability and a lower stability to the interaction with growth factors. According to the analysis of correlation coefficients, the production of berries on the plant is positively correlated with the number of fertile tillers and the weight of the grains in the principal panicle. These characters should be used in the selection to improve the production capacity (Premkumar *et* al., 2017)

Table 2. Stability of the grain weight/ main panicle through (MUIR) heterogeneous variances (HV) and imperfect correlations (IC) for winter oat genotypes studied

No	Genotype	SS–HV (%)	SS-IC (%)	SS-GE (%)	No	Genotype	SS–HV (%)	SS-IC (%)	SS-GE (%)
1	Florina (mt.)	0.88	1.17	1.03	38	4484	0.69	0.76	0.72
2	Norline	1.53	0.33	0.92	39	4488	0.69	0.74	0.72
3	Arlingthon	1.27	0.66	0.96	40	4492	2.37	1.70	2.03
4	Blamouth	1.54	1.19	1.36	41	5029	0.74	2.66	1.72
5	CI 1908	0.69	1.66	1.18	42	5032	1.53	1.30	1.41
6	Cimarron	1.30	1.93	1.62	43	Marrettos Anderson	2.52	1.61	2.06
7	Crater	0.69	2.15	1.44	44	8276	1.15	1.47	1.32
8	Earlygrain	1.66	0.38	1.00	45	PA 522-7	0.70	3.43	2.10
9	Excel	2.58	1.71	2.13	46	PA 522-23	1.39	1.22	1.30
10	Fergushon	1.53	1.27	1.39	47	PA 621-3274	4.25	1.63	2.91
11	Fulwood	0.71	1.27	0.99	48	PA 724-2580	1.09	1.13	1.11
12	Jeferson	0.74	0.87	0.81	49	PA 725-2154	0.88	0.61	0.74
13	Le Conte	1.55	2.16	1.86	50	PA 725-4743	0.70	1.26	0.99
14	Nortex	1.93	0.48	1.19	51	PA 725-4787	1.04	3.07	2.08
15	Suergrain	1.96	1.08	1.51	52	PA 725-6113	1.52	0.76	1.13
16	Thonson	0.81	0.63	0.72	53	PA 822-818	0.69	1.97	1.35
17	Walken	1.43	1.59	1.51	54	ARK 0151-61	0.71	0.67	0.69
18	Compact	1.02	1.63	1.33	55	AR 104-18	0.81	0.64	0.73
19	Pennwin	0.98	1.27	1.13	56	Marys Quest	0.69	2.10	1.41
20	2288	0.87	0.64	0.75	57	Wodan	2.20	1.55	1.87
21	3378	3.08	1.90	2.47	58	Gospodarski 48	1.28	3.39	2.36
22	834-4-1-3	1.68	0.47	1.06	59	5183	0.69	0.96	0.83
23	3412	0.73	1.26	1.00	60	Tripolis	0.82	0.90	0.86
24	S Dak 40	3.92	1.58	2.72	61	Krusevac	1.47	1.14	1.30
25	3868	1.27	0.81	1.03	62	Boer	1.32	1.44	1.38
26	Cocker 41-51	1.71	0.39	1.03	63	Algerian	0.73	1.92	1.34
27	4444	0.70	0.74	0.72	64	Mirabel	1.30	1.56	1.43
28	4451	1.46	0.36	0.89	65	Gerald	0.78	0.64	0.71
29	4458	0.77	0.61	0.69	66	Nuptiale	1.36	0.39	0.86
30	4472	0.69	0.72	0.70	67	Solva	2.73	0.91	1.80

31	4475	1.92	2.89	2.42	68	Valiant	1.18	1.26	1.22
32	4476	0.72	0.85	0.79	69	Barra	1.63	1.34	1.48
33	4477	1.45	1.21	1.33	70	Carie	0.72	0.66	0.69
34	4478	5.09	9.36	7.28	71	Krypton	0.80	1.32	1.07
35	4480	0.70	0.68	0.69	72	Chamois	0.69	0.94	0.82
36	4482	2.79	1.59	2.18	73	Emperor	1.09	0.52	0.80
37	4483	0.70	0.97	0.84		TOTAL	48.76	5.,24	100.00
HV-Heterogenity variance; IC-Imperfect correlations GE-Genotype x environment interaction; SS – Sum square									

Conclusions

The weight of grains from the main panic to oats is a character that depends on the climatic and technological conditions. Creation of genotypes with good stability of this character is a major breeding target. The greatest variability of this character we found to the genotypes studied in climatic conditions of the first year of experimentation. The genotypes S Dak 40, PA 621-3274, Wodan, Tripolis, show values of main panicules production superior to the average of experience, which are strongly influenced by the genotype x environment interaction. The estimates of used models, based on the ranks sum, showed that the highest stability of grain weight in the principal panicle occurred at: Carie, 4458, ARK 0151-61, 4480, Thonson genotypes. The analysis of the genotype x environment interaction revealed a high stability due to reduced interaction (less than 0.75% of total value) to: 4458, 4480, ARK-0151-61, Gerald genotypes. The studied collection includes valuable genotypes for breeding programs.In the collections studied, there are few genotypes with good stability of the grain weight of the main panicle. However, there are some varieties that can be recommended for the breediongs programs of autumn oats in Western Romania.

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GENETIC ANALYSIS OF CHLOROPHYLL CONTENT IN BARLEY (*HORDEUM VULGARE* L.)

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Abstract

The aim of this study is to develop a selection approach to improve the adaptability of barley (Hordeum vulgare L.) to unfavorable environmental conditions based on chlorophyll content. A semicomplete diallel cross, including four barley genotypes, was analyzed for estimating variance of genetic effects, combining ability and heritability. Diallel analysis was used to estimate the genetic action of chlorophyll content in barley and provided an opportunity to get a quick and comprehensive view of the genetic control of a set of parents in early generation.The results revealed that the highest values of "cis" and "trans" heterosis were recorded in hybrids of the Andrew x DH260/18 combination, followed by hybrids of the Andrew x Djerbel combination. The greatest potential for amelioration of chlorophyll content is represented by the Andrew x Adi,combination, which allows for the identification of approximately 7,93 % recombinant lines with a chlorophyll content of at least 40 SPAD units in downstream generations. Achieving this 95% selection threshold requires the choice of at least 36 lines and 84 lines for an accuracy of 99.9%. High values of erytability in the broad sense and low values of erytability in a narrow sense confirms that a considerable part of the variability of this indicator is due to the genotype, due to the involvement of major genes in the determinism of this character.

Key words: *barley, chlorophyll content, genetic analysis, diallèl cross*

Introduction

Barley (*Hordeum vulgare*), first domesticated in the Near East, is a well-studied crop in terms of genetics, genomics, and breeding and qualifies as a model plant for *Triticeae* research (Nese *et al.*, 2008). Among the cereal crops, barley is a species with the greatest adaptability to a wide range of environments. Barley is cultivated from arctic latitudes to tropical areas, grown at the highest altitudes and adapted to specific sets of agro-ecological areas (Alemayehu *et al.*, 1997). It is adapted to a broad range of agro-ecological environments and tolerant to soil salinity, drought and frost to a considerable level. The crop grows successfully in the arid climates. In terms of the area and production worldwide, barley is the fourth most important cereal after wheat, rice and maize (Abebe *et al.*, 2010). In a 2007 ranking of cereal crops in the world, barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566.000 km²).The choice of an efficient breeding program depends to a large extent on the knowledge of gene action involved in the expression of the character. Diallel analyses were used to estimate gene action of yield components in barley and provide an opportunity to obtain a rapid and overall picture of genetic control of a set of parents in the early generation. Besides genetic effects, breeders would also like to know how much of the variation in a crop is genetic and to what extent this variation is heritable, because the efficiency of selection mainly depends on additive genetic variance (Eshghi *et al.*, 2009).These authors reported additive genetic effects of tillers number and an over-dominance gene action for number of grains per spike. Drought tolerance is the ability of a plant to survive, grow, and produce yield with limited water supply. Great progress has been achieved in the last two decades in understanding the genetic control of drought tolerance

(Abou-Elwafa, 2016).Chlorophylls are associated with chlorophyll-binding proteins of the photosystem I (PSI) and II (PSII) complexes (Kim *et al.*,1994 , Croce *et al.*, 2012) and accumulate in tissues where PSI and PSII are produced. A number of studies have been reported on the genetic elements that control chlorophyll accumulation in photosynthetic tissues (Eckhardt *et al.*,, 2004, Croce *et al.*, 2012). The aim of this study is to develop a selection approach to improve the adaptability of barley (Hordeum vulgare L.) to unfavorable environmental conditions based on chlorophyll content. The method will be used during the growing season for field applications, or at any time during the year in laboratory applications. Our study emphasized the addition of some new explanations concerning the hydric stress on chlorophyll accumulation to sustain the applying that indirect method of testing in a quickly selection of tolerant shapes.

Material and methods

Four barley varieties with different genetic and ecologic origin, along with their 6 one-way crosses, were studied in the field of Breeding plant departamant, from Banat's University of Agricultural Sciences and Veterinary Medicine King Michael I from Romania Timisoara. The biological material was cultivated in drought conditions during 21 days. The measurement of the chlorophyll content was made in early stage of the plants using portable chlorophyll meter SPAD- 502 (Konica Minolta, Japan), which measures the absorbance at 650 nm, this being a non-destructive method. The evaluation of results from diallel was done based on the mathematical model described by Hayman (1954).

Results and discussion

According to the data presented in table 1, it is observed that most of the hybrids, cultivated under stress conditions, showed positive values of the heterosis index, respectively a higher chlorophyll content than the average of the parental forms. The highest values of the heterosis index associated with a higher amount of chlorophyll were made by the hybrids, Andrew xDjerbel (4.09), Andrew x Dh 260/18 (2.94), combinations in which there were large differences between the parental forms in terms of this character. The lowest values of the heterosis index, correlated with a lower chlorophyll content to the average parent on the background of close values between parental forms, were recorded in the hybrids: Dh260 / 18 x Djerbel (-1.43); Adi x Djerbel (-0.16).

Table 1. Heterosis Index for chlorophyll content in F1 Hybrids cultivated in stres conditions

Genitors	Andrew	Adi	DH 260-18	Djerbel
Andrew	-	0.75	2.94	4.09
Adi		-	0.42	-0.16
DH 260-18			-	-1.43
Djerbel				-

In terms of chlorophyll content in F1 hybrids, some of the hybrids were inferior to parental forms. Compared to parents' average, the hybrids of this generation showed average values of *cis* heterosis of 5.60% and the *trans* heterosis of 1.40%.The highest values of *cis* heterosis (19.26%) and trans (15.47%) were recorded in hybrids of the Andrew x DH260/18 combination, followed by hybrid of Andrew x Djerbel 8.59% for 7.46% for heterosis trans and hybrid Andrew x Adi 7.20% for cis and 2.31% for trans heterosis.

Table 2. Average value for chlorophyl content in F1 Hybrids and their parents

No	Hybrid Combination	F_1	Genitors			Value F_1 from ♀			Value F_1 from de ♂		
		x+sx	♀	♂	p	%	Difer.	t	%	Difer.	t
1.	Andrew x Adi	34.56±1.29	30.70	33.78	32.24	12.57	3.86	4.77***	2.31	0.78	0.69
2.	Andrew x DH 260-18	35.45±0.34	30.70	28.75	29.73	15.47	4.75	15.08***	23.30	6.70	13.20***
3.	Andrew x Djerbel	32.99±1.28	30.70	30.06	30.38	7.46	2.29	2.85*	9.75	2.93	2.82*
4.	Adi x DH 260-18	33.40±1.10	33.78	28.75	31.27	-1.12	-0.38	-0.36	16.17	4.65	5.77***
5.	Adi x Djerbel	31.31±0.66	33.78	30.06	31.92	-7.31	-2.47	-2.73^0	4.16	1.25	1.56
6.	DH 260-18 x Djerbel	27.53±1.30	28.75	30.06	29.41	-4.24	-1.22	-1.34	-8.42	-2.53	-2.41^0

Table 3.Heterosys values and potency report for the chlorophyl content at F1 hybrids in stres conditions

No	Hybrids	Average (SPAD)	Parents average (cis)		Superior parents(trans)		Potency
			H (%)	Diferenţa	H (%)	Diferenţa	
1	Andrew x Adi	34.56a	7.20a	2.32ab	2.31abc	0.78abc	1.51abc
2	Andrew x DH 260-18	35.45a	19.26a	5.73a	15.47a	4.75a	5.87ab
3	Andrewx Djerbel	32.99a	8.59a	2.61ab	7.46ab	2.29ab	8.16a
4	Adi x DH 260-18	33.40a	6.83a	2.14ab	-1.12bc	-0.38bc	0.85abc
5	Adi x Djerbel	31.31ab	-1.91b	-0.61b	-7.31bc	-2.47c	-0.33bc
6	DH 260-18 x Djerbel	27.53b	-6.38b	-1.88b	-8.42c	-2.53	-2.86c
	Media	***32.54***	***5.60***	***1.72***	***1.40***	***0.41***	***d/a=2.20***
	LDS 5%	*5.15*	*16.26*	*4.86*	*14.86*	*4.62*	*7.42*
	LDS 1%	*7.33*	*23.13*	*6.92*	*19.77*	*6.15*	*10.56*
	LDS 0.1%	*10.61*	*33.48*	*10.01*	*25.71*	*8.00*	*15.29*

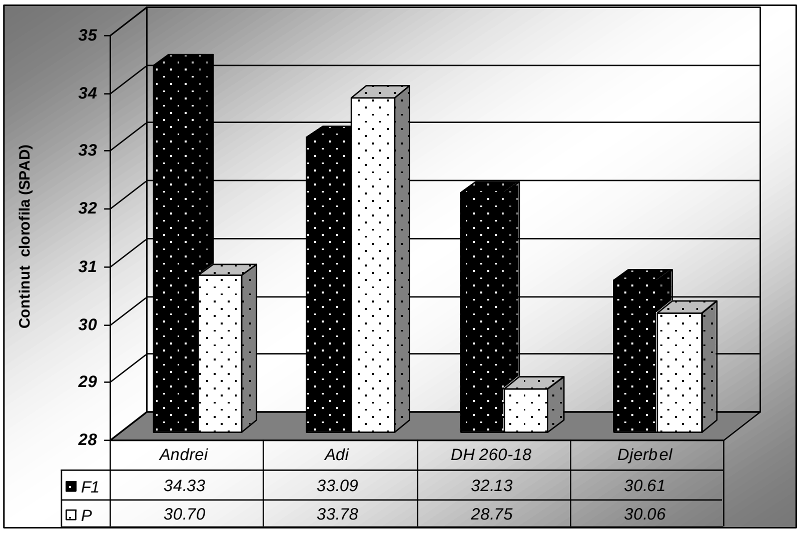

Fig .1. The content of chlorophyll for the F1 hybrids groups with the same recurrent parent (author elaboration based on the obtained results)

Considering hybrids with the same recurrent parent, the limits range from 30.61 to the hybrids of the Djerbel variety and 34.33 to the hybrids of the Andrew variety. Significant differences are observed between the Andrew hybrids, which have a higher chlorophyll content than the hybrids of the other varieties.Based on the data of fig. 1, it is observed that in most cases the hybrids showed a higher chlorophyll content than the recurrent parent, with the exception of the Andrew variety, whose hybrids registered an increase in this aspect.The highest amelioration potential of chlorophyll content is represented by the Andrew x Adi combination, which allows the identification in downstream generations of about 7.93% recombinant lines of at least 40 SPAD units for the chlorophyll content. Achieving this selection threshold in a proportion of 95% assume choosing a minimum 36 lines, respectively 84 lines for an accuracy of 99.9%.

Table 4. The amelioration potential of hybrid combinations in the terms of the recombinant lines percentage (LR %) with a minimum chlorophyll content of 40SPAD units and with the number of required lines to be selected (n)

Combination	F_1 (SPAD)	*s*	*LR %*	$n_{5\%}$	$n_{1\%}$	$n_{0.1\%}$
Andrew x Adi	34.56	3.86	7.93	36	56	84
Andrew x DH 260-18	35.45	1.01	0.01	29956	46049	69074
Andrew x Djerbel	32.99	3.83	3.36	88	135	202
Adi x DH 260-18	33.40	3.29	2.28	130	200	300
Adi x Djerbel	31.31	1.97	0.01	29956	46049	69074
DH 260-18 x Djerbel	27.53	3.91	0.07	4278	6577	9865

Based on the data presented in table 4, it is observed that the most significant contribution to the variability of the chlorophyll content was the additive variant (a), which is significant. Dominance variance (b) presents a significant value with a lower contribution to the variability of this character than the additive variance.Regarding the dominance and the way it acts in the genetic determinism of the character, it is found that the subcomponent of the variant b1 dominance which indicates the average deviation of the hybrids from the parental values, respectively the directional effects of the dominance, presents a statistically assured value. Since in most cases the average of this character in the F1 hybrids is superior to the average of the parents, the significance of b1 indicates in most cases the dominance of the parents with higher values of the chlorophyll content, but the differences are reduced.The sub-component of the dominance b2 variance through its statistically assured values indicates an asymmetry of the distribution of alleles with positive and negative effects that influence this character in the parental forms. Subcomponent b3 shows the existence of differences between hybrids due to dominance.

Table 5. Analysis of alternative for the chlorophyll content on the F1 hybrids in stress conditions

Cause of variability	SP	GL	PM	Test F
Repetition	89.00	2	44.50	7.72**
a	83.15	3	27.71	4.81*
b	99.64	6	16.61	2.88*
b_1	21.28	1	21.28	3.69
b_2	58.06	3	19.35	3.36*
b_3	20.31	2	10.15	1.76
Eroare	103.73	18	5.76	
Total	375.52	29		

Considering the distance between the right regression line and the parable and also the position of the parental genotypes versus the right regression line results that mainly the effects of dominance and additivity have an important role in the genetic determinism of this character, according to genetic variance analysis. Among the studied genitors Dh 260/18 and Djerbel varieties show the highest proportion of recessive alleles, while Andrew (76.6%) and Adi (69%) have the highest proportion of dominant alleles (table 6).

Table 6. The average values (Yr), alternative (Vr), covariance (Wr) and the proportion of dominant alleles of the parents used to obtain F1 hybrids in terms of the chlorophyll content under stress conditions

No	Genitors	Parents Average Yr	Variance Vr	Covariance Wr	Vr + Wr	Proportion of dominant allele
1	Andrew	30.70	3.301	-0.478	2.823	0.766
2	Adi	33.78	0.890	0.445	1.335	0.690
3	DH 260-18	28.75	13.103	4.215	17.318	0.025
4	Djerbel	30.06	4.260	2.446	6.706	0.391

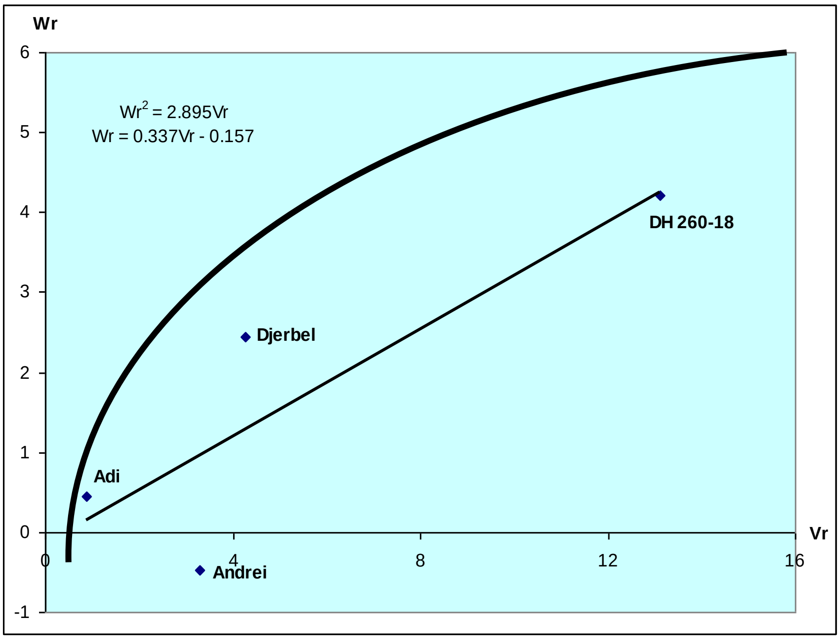

Fig. 2. The regrssion chart Wr/Vr for the chlorophyll content on the F_1 hybrids (author elaboration based on the obtained results)

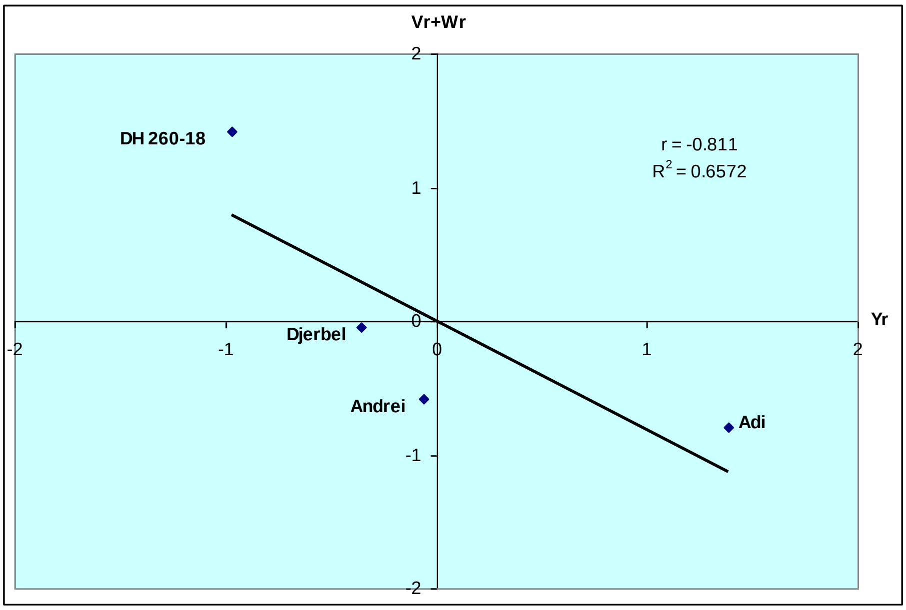

Fig. 3. Standard deviation chart (Wr + Vr) and yr for the chlorohyll content on the F_1 hybrids(author elaboration based on the obtained results)

The distribution of parental forms for this character can be assessed on the basis of standard deviations yr (Clorophila SPAD) and Wr + Vr, indicating a high proportion of recessive alleles at the genotype DH 260/18.

Conclusions

The highest values for the heterosis index associated with a higher amount of chlorophyll were accomplished by Andrew x Djerbel (4.09), Andrew x Dh 260/18 (2.94) hybrids.
Among the varieties studied the Dh 260/18 and Djerbel varieties show the highest proportion of recessive alleles, while Andrew (76.6%) and Adi (69.00%) have the highest proportion of dominant alleles.

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BREEDING VEGETABLE ROOTSTOCKS TOWARD COPING WITH BIOTIC AND ABIOTIC STRESSES

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Abstract

In Palestine, cucumber (the second important vegetable crop after tomato), is usually grown sexually using their own seeds. Recently, its cultivation is facing some serious problems such as soil-borne diseases, salty soil conditions, drought, etc. To alleviate such problems, grafting is highly recommended. Toward this end, an experiment was conducted to study the effects of different squash rootstocks on growth, yield and quality of cucumber. Cucumber (var. Kareem) was grafted on four squash rootstocks (A-113, A-184, USA and Salah-2) using tube grafting technique (with 20 replicates), in addition to the control. The trial was set-up in a single-span greenhouse located at Hebron University campus, supported by drip irrigation system. All of the necessary cultural practices (irrigation, fertilization, spraying, ventilation, etc) were made. Different growth parameters, yield component and quality were regularly measured. In general, Rootstock A-148 presented significantly higher production (kg) and acceptable appearance (dark green color, symmetrical, regular), comparing to the other examined rootstocks and the control as well. Furthermore, Rootstock A-148 and the control (Kareem) presented earlier flowering and accordingly earlier production by 15 days compared to the other examined rootstocks. Dry weight of the total rooting system of A-113 rootstock was significantly higher than the other evaluated treatments, followed by USA and A-148, respectively. In contrast, the control revealed the lowest value. In conclusion, A-148 rootstock could be efficiently used as a promising rootstock for cucumber cultivation in Palestine.

Keywords: *Cucumber, breeding, grafting, biotic & abiotic stresses.*

Introduction

Cultivation of cucumber (*Cucumis sativus* L.) is considered the second important vegetable crop (after tomato) for greenhouse production in Palestine in terms of both areas covered as well as economic returns. Commonly, it is propagated sexually by seeds produced by specialized commercial companies. Due to its intensive and continuous cultivation (3 cycles per year); its cultivation is suffering from many damaging effects of different biotic (soil-borne diseases, soil nutrient imbalances, salty or alkaline soil conditions, etc., (Farhadi et al., 2016), and abiotic stresses (mainly drought), resulting thereby to severe crop loss as well as restrict its plantation and prevent its future expanding. Unfortunately, chemical pest control is expensive, not always effective, and harms the environment (Davis et al., 2008). Furthermore, drought and water scarcity as a direct result of global and regional climate change seems to be the most devastating stress affecting the overall Palestinian agriculture including vegetable production with no exceptional (Basheer-Salimia and Sayara, 2017). Indeed, detectable climate change has been observed as lower average precipitation rate, more marked changes in the distribution of precipitation from one year to the next, with winter getting shorter and extensive (Basheer-Salimia and Ward, 2014). Unluckily, a worst climate change scenario is expected to get more pronounced in the future. According to a realistic emissions scenario for the region, the IPCC predicts that warming over the 21st century will be larger than global annual mean warming – ranging between 2.2-5.1ºC. To overcome many of these biotic problems and to cope with drought, one promising approach could be breeding vegetables using grafting on different rootstocks that adapted to non-suitable factor/s for the desired cultivars. Though, choosing the proper rootstocks will give the scion the best chance to survive and produce good economical yield. The main goal of the present research is to study the effects of different squash species (*Cucumis maxima x Cucurbita moschata)* rootstocks on growth, yield and quality of cucumber.

Materials and Methods

In a well-controlled commercial nursery, cucumber (var. Kareem) was grafted on four squash rootstocks (A-113, A-184, USA and Salah-2), using tube grafting technique with 15 replicates each in addition to the control. After two weeks, seedlings were transplanted in med-autumn 2018, and accordingly maintained under a single-span greenhouse (12-18C^{o}) located at Hebron University campus using larger pots (9 liter volume) with a mixture-media of soil and peat-moss (1:1). The trial was set-up in a completely randomized design, and seedlings were supported with drip irrigation system. All necessary cultural practices irrigation, fertilization, spraying, ventilation, etc) were accomplished.

Different growth parameters (flowering date and fruiting, dry biomass of the rooting system), yield component and quality, were regularly measured.

Obtained data were statistically analyzed using one-way analysis of variance (ANOVA) and means were separated using the Tukey's pairwise comparisons at a significance level of $P \leq 0.05$ using the MINITAB package system.

Results and Discussion

Many vegetable species including squash are used as successful rootstock to overcome several biotic and abiotic stresses. In Palestine, unfortunately vegetable grafting is not yet commercialized and we still so beginners in this field. Here, is the first conducted pilot study. The combination of the grafted Kareem (commercial cucumber variety) on A-148 (Rootstock) presented earlier flowering and accordingly fruiting (similar to the control) by 15-20 days comparing to the remaining examined combinations. However, all examined combinations were under the same environment, therefore the existing variations are likely depends, to a great extent, on the rootstock–scion physiological interaction rather than the environment (Cohen et al., 2007). Furthermore, dry biomass of the total rooting system of A-113 rootstock was significantly higher than the other evaluated combinations, however the control revealed the lowest value (Table 1). Similar authors confirm this finding who stated that grafting is generally enhancing the vegetative growth and increase the vigorous root system (Goreta-Ban et al., 2014; Ferriol and Picó 2008). However, fibrous roots (control) are usually shorter, densely packed fine, and lighter.

Table 1. Mean production per plant (kg) and mean roots dry biomass (gr) of own rooted cucumber (var. Kareem) and grafted Kareem on four squash rootstocks (Mean* ± StDev).

Parameters / Treatments	Production per plant (Kg)	Roots Dry Biomass (gr)
Kareem (Control)	3.98 ^{b} ± 0.13	8.06 ^{c} ± 1.52
Kareem on A-148	4.93 ^{a} ± 0.15	17.33 ^{b} ± 2.14
Kareem on A-113	3.95 ^{b} ± 0.15	24.23 ^{a} ± 3.55
Kareem on USA	3.05 ^{c} ± 0.16	17.51 ^{b} ± 2.20
Kareem on Salah-2	4.00 ^{b} ± 0.16	16.49 ^{b} ± 3.42
P-value	0.000	0.000

*: Means within columns using different letters are differ significantly at the P≤0.05 level (using one way ANOVA analysis).

Regarding yield, Rootstock A-148 presented significantly higher production values of 4.93 kg per plant (=10.84 tones per dunum based on using 2200 plants per dunum) comparing with the control (8.76 tone) as well as the other examined treatments (Table 1). Both Salah-2 and A-113 rootstocks revealed similar and medium production with 8.80 and 8.69 tones respectively. However, Rootstock USA exhibited significantly the lowest production value with only 6.71 tones (Table 1). In addition, the rootstock A-148 presented acceptable overall appearance of the fruits (dark green color, symmetrical, regular), comparing to the other examined rootstocks as well as the commercial (Kareem) cultivar. Similar findings were also registered by Oda, 2002; Rivero et al., 2003; Davis et al., 2008; Koundinya & Kumar, 2014; and Kyriacou et al., 2017, who stated that using rootstocks improve plant ontogeny via avoiding damages caused by different biotic stresses; contributing to enhance tolerance to a biotic stresses; increase the vigorous root system; improve resistant to cold and warm; optimize water use, enhance of nutrient uptake; and improve fruit yield and quality. Based on the obtained primarily results with cucumber, introducing rootstocks to the other common cultivable vegetable crops would be a great interest and an added value to the Palestinian agricultural economy especially with tomato since it is considered the main bulk of the Palestinian vegetable cultivation in terms of area covered as well as economical returns.

Conclusion

A-148 rootstock could be efficiently used as a promising rootstock for cucumber cultivation in Palestine, whereas USA rootstock should be executed from any further studies.

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EFFECT OF DIFFERENT DOSES OF NITROGEN FERTILIZER AND VARIETY ON THE YIELD AND GRAIN QUALITY OF WINTER TRITICALE

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Abstract

The effect of different doses of nitrogen fertilizers and variety on the yield and grain quality of winter triticale is given in this paper. The study was carried out in the vicinity of Bijelo Polje (Montenegro) during three growing seasons (2009/12.). The research included five varieties of triticale (Odissey, Kg-20, Triumph, Rtanj and Tango) and five variants of fertilization: control (without fertilization), N_{60}, $N_{60}P_{80}K_{80}$, $N_{90}P_{80}K_{80}$ and $N_{120}P_{80}K_{80}$. The experiment was set up as randomized block system in three replications. The grain yield, hectoliter weight and 1000 grain mass were monitored. The results of the study showed that both the variety and the different amounts of nitrogen, in combination with phosphorus and potassium, had a significant impact on grain yield and its quality. The highest average grain yield and a 1000 grains mass had the variety Tango (5304.0 kg ha^{-1}) or 47.9 g, while the lowest values of these characteristics were noted in the variety Kg-20 (4366.2 kg ha^{-1} and 32.7 g). On average, for all tested varieties, the highest grain yield was obtained in fertilization with 120 kg ha^{-1} nitrogen, but it was significantly higher only in relation to the control and the variant of fertilization where the minimum nitrogen (60 kg ha^{-1}) was used. The highest average hectoliter weight had the variety Triumph (72.1 kg), and the lowest variety Kg-20 (66.5 kg). Also, it was noted that the use of fertilizers caused an increase in hectoliter weight in the tested varieties. The $N_{60}P_{80}K_{80}$ rate was found to be economically optimal for these varieties of winter triticale in this area.

Key words: *Triticale, Nitrogen, Variety, Yield*

Introduction

The triticale, a species produced by crossbreeding of wheat and rye, was created with the idea of combining positive traits of parental species. The hope was that triticale would combine the high yield potential and good grain quality of wheat, and the resistance/tolerance to the biotic and abiotic stresses of rye (Mergoum et al., 2009; Fraś et al., 2016). The great possibility of using triticale for different purposes, as well as the emphasized varietal differences, imposes the need and importance of more complete study of new varieties, with the aim of their more efficient exploitation in commercial production. Triticale as the species is characterized by very rapid growth and development, adaptable to different cultivation technologies, and it achieves high and stable grain yields. In addition to high tolerance to soils with poor physical and chemical properties, triticale has also been shown to be suitable for cultivation at higher altitudes with pronounced resistance to biotic and abiotic stress (Villegas et al., 2010; Zečević et al. 2010). Also, it is often pointed out that triticale is a world-class species and that its growth and development depend largely on weather conditions and it is of great importance to understand the impact of climate change on the success of its production (Márton et al., 2007; Kádár et al., 2008 ; Đekić et al., 2010; Jamil et al., 2017). Within the technology of growing grain cereals, the special significance for forming yields is: sowing time, sowing density, variety characteristics, mineral nutrition and crop protection measures. According to the opinion of Claudio et al., (2010) and Kendal and Sayar (2016) those varieties which with high yields and good quality have the potential to produce high genetic potential for yield under different conditions of cultivation. In addition to the triticale genetics

growing season parameters affect the growth and yield of crop (Lobell and Asner, 2003) and cause yield variations. Jelić et al. (2004) and Popović (2010) state that the type and quantity of mineral fertilizers necessary for the formation of high yield and quality as well as the time of their application are different, depending on the fertility of the soil. In the opinion of Lestingi et al. (2010) and Zečević et al. (2010) mineral fertilization, especially nitrogen nutrition is one of the major agrotechnical factors which affect grain yield and enable farmers to take advantage of the high production potential of cereals. Similarly, Nefir and Tabără (2011) point out that the increased yield can best be achieved by using mineral fertilizers, and among the elements of mineral nutrition, nitrogen plays the most important role in increasing the yield (Kastori et al., 2005). Alzueta et al. (2012), and Wang et al. (2017) noted that there are two growing period when cereals take up nitrogen, first at tillering stage and second for spike formation stage. On the other hand, Nikolić et al. (2012) emphasize that high yield and good grain quality can be expected only if plants are able to adopt nitrogen during the entire vegetation period.
The aim of the conducted research was to determine the effect of the different doses of nitrogen fertilizers and variety on the yield and grain quality of winter triticale.

Materials and Methods

In order to monitor the influence of different quantities of nitrogen and varieties on the yield and grain quality of winter triticale, the experiment was set in the period from 2009 to 2012 in the vicinity of Bijelo Polje (Montenegro). The soil on which the experiment was carried out belongs to the type of Eutric Cambisol on alluvial coating. The outdoor experiment was set by the random block system in three repetitions. The plot size was 6 m^2 (3m x 2m). The research included five varieties (factor A) from different breeding houses and five variants of fertilization (factor B).
Varieties: Odyssey, Kg-20, Triumph, Rtanj and Tango.
Variants of fertilization: control (with no fertilization); N_{60} (60kg ha^{-1} N); $N_{60}P_{80}K_{80}$ (60kg ha^{-1} N, 80kg ha^{-1} P_2O_5, 80kg ha^{-1} K_2O); $N_{90}P_{80}K_{80}$ (90 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O); $N_{120}P_{80}K_{80}$ (120 kg ha^{-1} N, 80 kg ha^{-1} P2O5, 80 kg ha^{-1} K2O).
The sowing was done manually in the second decade of October in pre-drawn furrows with a distance of 12 cm. The other technology of production which was applied in the experiment was standard. Phosphorus and potassium in equal amounts of 80 kg ha^{-1} were applied prior to sowing, while nitrogen was introduced to a small extent (20 kg ha^{-1}) before sowing, and the rest to the envisaged amount was given as the nutrient, in the second decade of March. Harvest was done manually in a phase of full maturity. The yield is calculated per hectare at 14% moisture. After harvest, samples were taken to determine a 1000 grain weight and hectoliter weight. The obtained results were statistically processed using method of variance analysis using statistical softwer WASP 1.0, whereby the significance of the average treatments were tested with LSD test, with significance threshold of 1 and 5%.
Soil and climatic conditions.
Soil samples were collected prior to winter triticale sowing, to a depth of 0-30 cm and 30-60 cm. Based on the results, it was found that the soil has slightly acid reaction with total carbonate content of 5.62 to 5.63%, it is humus rich (3.35-3.96%) and poor in available phosphorus (5.12-4.24 mg 100g $^{-1}$) and potassium (7.5-3.8 mg 100g $^{-1}$).
The data in Table 1. for the researching period (2009-2012) clearly indicate that the years in which the study was carried out in meteorological conditions differed from the period average for the given area. In all three years of research, the average air temperature was higher in relation to the perennial average, while the amount of precipitation in 2009/10 was 98 mm higher, and lower in 20010/11 and 2011/12 by 18 mm and 231 mm compared to the perennial average. Higher rainfall in the autumn months in the first two years of the study compared to

the third year created more favorable conditions for germination, emergence and preparation of crops for the winter period. On the other hand, the crops in the third year of the research were exposed to abundant snowfall with long retaining of snow cover, which slowed down the vegetation in the spring, due to which we had later sowing and flowering crops. A significant amount of precipitation and their favorable distribution in the spring months of the first year of the study enabled unhindered pouring of the grain, which had a positive effect on the yield.

Table 1. Average monthly air temperature and precipitation amount

Year	Months										Average
	X	XI	XII	I	II	III	IV	V	VI	VII	
	Monthly rainfall (mm)										
2009-10	135	94	94	101	80	70	78	80	63	86	881
2010-11	65	131	147	36	76	31	46	121	33	79	765
2011-12	36	7	55	79	183	57	47	46	34	8	552
1961-90	80	115	91	87	68	60	70	76	72	64	783
	Average monthly temperatures (°C)										
2009-10	9.77	5.95	4.06	1.31	2.4	6.39	10.93	15	18.11	20.95	9.5
2010-11	10.12	8.54	2.05	-0.65	0.94	6.03	10.54	14.5	18.9	21.23	9.2
2011-12	9.3	3.25	2.17	-1.72	-3.52	5.96	10.8	15.02	20.67	24.63	8.7
1961-90	9.4	4.7	0.2	-1.3	0.7	4.9	9.0	13.3	16.3	17.9	7.5

Results and Discussion

Grain yield, from an economic point of view, is the most important property, and it represents the end result of the vegetative and generative life cycle of the plant. On the other hand, a 1000 grain weight and hectoliter weight are grain quality parameters, which are largely determined by the variety, but at the same time they are significantly influenced by environmental factors and applied agrotechnology. Three-year average values of grain yield, a 1000 grain mass and hectoliter weight in the examined varieties of winter triticale, depending on the variation of fertilization are shown in Table 2.

Table 2**.** Effect of different doses of nitrogen on properties of winter triticale varieties

Winter triticale properties (3-year means: 2010-2012)						
Cultivar (A)	Nitrogen fertilization (kg ha^{-1})* - the factor B					
	a	b	c	d	e	
	N 0 A	N60	N60P80K80	N90P80K80	N120P80K80	Average
	Grain yield (kg ha^{-1})					
Odissey	3632,2	4428,9	5274,4	5357,8	5626,7	4864,0
Kg-20	3232,9	4010,1	4840,0	4865,1	4882,8	4366,2
Trijumf	3667,8	4647,8	5584,4	5573,2	5598,3	5014,3
Rtanj	3614,4	4677,7	5490,4	5597,8	5821,7	5040,4
Tango	3845,0	4837,3	5883,3	5863,3	6091,1	5304,0
Average B	3598,5	4522,3	5414,5	5451,4	5604,1	4917,8
		LSD	A	B	AB	
		0,05	240,9	240,9	541,1	
		0,01	341,2	341,2	767,3	
	1000 grain weight (g)					
Odissey	39,9	42,0	41,5	42,9	45,3	42,3
Kg-20	28,8	32,3	34,2	33,3	35,1	32,7
Trijumf	40,2	42,9	43,4	43,1	45,0	42,9
Rtanj	42,1	45,8	45,5	45,5	46,1	45,0
Tango	44,4	47,6	47,8	49,2	50,3	47,9
Average B	39,1	42,1	42,5	42,8	44,4	42,2
		LSD	A	B	AB	
		0,05	1,01	1,01	2,27	
		0,01	1,44	1,44	3,22	

	Hectoliter weight (kg)					
Odissey	65,9	72,2	71,5	70,8	70,2	70,1
Kg-20	65,2	65,9	66,7	65,9	68,6	66,5
Trijumf	70,3	72,9	71,9	72,7	72,8	72,1
Rtanj	67,0	68,3	67,3	67,8	68,3	67,7
Tango	68,4	70,0	69,2	68,3	70,4	69,3
Average B	67,4	69,9	69,3	69,1	70,1	69,1
		LSD	A	B	AB	
		0,05	1,54	1,54	3,44	
		0,01	2,18	2,18	4,87	

The results of the study showed that different amounts of nitrogen in combination with phosphorus and potassium had different influence and intensity of action on the grain yield of the tested varieties of triticale. The data in the Table 2. show that the lowest grain yields for all varieties involved in the research were achieved on the control variant (3598.5 kg ha^{-1}) and that the application of fertilizers caused a significant increase in the grain yield relative to the control. The average grain yield of winter triticale, observed for all varieties and varieties of fertilizers in the three-year study period was 4917.8 kg ha^{-1}. On average for all tested varieties, the highest grain yield (5604.1 kg ha^{-1}) was obtained in fertilization with 120 kg ha^{-1} nitrogen, but it was statistically significantly higher only in relation to the control and variant where only nitrogen in the amount of 60 kg ha^{-1} was applied. From data in Table 2. it can be seen that on average, the highest grain yield was recorded with the variety Tango (5304.0 kg ha^{-1}), and the lowest with variety Kg-20 (4366.2 kg ha^{-1}).
These results are in great agreement with the results of Popović and Malešević (2011) and Lalević and Biberdžić (2016). The results of the study show that there was a significant difference in the values of a 1000 grain weight among the tested varieties, but also that the use of fertilizers significantly influenced the observed trait. Accordingly, the highest 1000 grain weight of the tested triticale varieties were achieved on the fertilization variant where nitrogen was used in the amount of 120 kg ha^{-1} (44.4 g) and was significantly higher than the same achieved on other variants of fertilization. The lowest value of the aforementioned trait was observed on the control (39.1 g). The values of the three-year average show that the highest 1000 grain weight was achieved with the Tango variety (47.9 g), while the lowest value of this characteristic was recorded in the Kg-20 variety (32.7 g). Our results are in accordance with the results of Jaćimović et al. (2008) who found that a 1000 grain mass was significantly higher with more intensive fertilization treatments, especially in cases when nitrogen was used. Also, our results are in agreement with Jelic et al. (2013), Lalević et al. (2016) who pointed out that a 1000 grain mass is a property that is largely determined by the variety, due to which, among rare varieties, there is a greater variation in a 1000 grain mass than among the variants of mineral nutrition.
The average values of hectoliter weight in the tested triticale varieties ranged from 66.5 kg in the Kg-20 to 72.1 kg in the Triumph variety. The research of Milovanović et al. (2004) showed that the variety Triumph, besides high and stable yields, is characterized by good quality parameters. The results show that the use of fertilizers led to a very significant increase in the value of this property in relation to control, but also that among the different variants of fertilization there were no significant differences in the value of the hectoliter weight which is in agreement with the results of and Lalević et al. (2012) who pointed out that despite the fact that the application of mineral fertilizers leads to an increase in the hectoliter weight, the variation between the different variants of fertilization is low.

Conclusions

The differences in terms of yield and grain quality were determined by the variety and the applied fertilizer variant. The application of mineral fertilizers had led to a very significant increase in grain yield in all tested varieties of winter triticale in relation to control. However, as between the use of the minimum and maximum nitrogen in combination with phosphorus and potassium, there were no statistically significant differences in grain yield. In terms of economy we can recommend the variant $N_{60}P_{80}K_{80.}$ The value of the 1000 grain weight on all variants of fertilization was significantly higher compared to control. The highest increase in the 1000 grain weight was achieved on a plot where nitrogen was used in the amount of 120 kg ha^{-1}. The hectoliter weight was changed depending on the variety and the amount of nutrients used. However, despite the fact that the highest average value of hectoliter weight was recorded in the use of the highest amount of nitrogen, there were no statistically significant differences between the different variants of fertilization. The most varied varieties in our research were Tango and Triumph. The obtained results provide the possibility to give reliable recommendations when choosing a variety of winter triticale for cultivation in the northern part of Montenegro, but also to popularize and expand this variety in production.

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CHANGE IN ACIDITY AND MOBILE ALUMINIUM LEVELS IN FOREST, MEADOW AND ARABLE LAND PSEUDOGLEY SOILS

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Abstract

Acidic soils are present in over 60% of the total arable land in Central Serbia. On such land, agricultural production takes place under unfavorable conditions, where beside acidity, the high content of mobile aluminum limits the maximum genetic potential of new varieties and hybrids. Researches in this paper were carried out on pseudogley lands of Čačansko-kraljevačka basin with different purposes (meadow, forest and field vegetation). Within the forest vegetation we oppened 13 profiles 16 meadow profiles and 8 field profiles, where the content of the active and substitution acidity of the soil and the content of mobile aluminum were tested at the depth of humus-accumulative (A_h), eluvial-pseudogley (E_g) and iluvial (B_{tg}) horizon. The results of the analyzes showed that the active acidity of the soil varied in the range of pH 4.45 to 6.10 in all open profiles, regardless of the manner of use. The state of substitution acidity of the examined pseudogleys in all three horizons (A_h, E_g and B_{tg}) showed an acidic and highly acidic reaction, excluding the Ah and Eg horizons at the location of Jarčujak. The influence of the manner of use largely affected the substitution acidity, most notably in forest profiles. Of the 13 profiles, all showed an extremely acidic soil reaction (pH/KCl<4.5), only one profile in the A_h horizon had a pH/KCl value of 5.36. Under meadow and field vegetation, 87.5% of the profile showed a very acidic reaction in the A_h horizon, which pointed to the tendency of reduction with depth. The content of mobile Al in the soil showed a large interval of variation from 0.14 to 53.33 mg/100 g of soil, depending on the pH, location and manner of use.

Keywords: *Pseudogley, Soil profiles, Soil acidity, Mobile aluminium.*

Introduction

Serbia characterised by a real mosaic of soil types, subtypes, varieties and forms, which is a consequence of a very complex geological and lithological base, diversity of relief and climate in some of its regions, as well as of anthropogenic activities. Acidic soils are widespread (over 60% of a total territory), and due to their low productivity have become a limiting factor of the successful plant production (Stevanović et al., 1995). Approximately 71% of extremely acidic soils are located under forest and grassland vegetation. A total of 27% of extremely acidic soils are distributed over total agricultural land, while 23% of strongly acidic soils cover plough field areas, gardens and perennial plantations (Čakmak et al., 2009). The majority of acidic soil areas are located in central and western Serbia, with a large presence of pseudogley soils. Unfavourable physical and chemical traits are the principal properties of pseudogley. Active acidity (pH/H_2O) of these soils varies from pH 5 to 6, while their humus content is low (1.5-3%). These soils are poorly supplied with available nitrogen forms due to unfavourable conditions for the processes of ammonification and nitrification and strongly pronounced de-nitrification. The content of available phosphorus is very low, predominately 1-3 mg/100 g of soil, which is a result of the phosphate ion binding to Fe, Al and Mn into hardly soluble phosphates. Pseudogleys are fairly supplied with available potassium forms (Dugalić, 1998).

Aluminium is one of the most abundant elements in the Earth's crust, and although it is not an essential element, its toxicity is the most common limiting factor of the plant production on acidic soils. Al exists in the form of insoluble alumosilicates and oxides in neutral and weakly acidic soils (Jelić and Milivojević, 2015), while small amounts are present in a soluble form that can affect biological systems (May and Nordstrom, 1991). The Al mobility in the soil increases with the intensification of soil acidification caused by leaching of Ca, Mg, Na and K ions (Matsumoto, 2000). The content of mobile Al in pseudogley soils in the Čačak-Kraljevo Basin is varies to a great extent depending on the soil pH and soil profile (Dugalić and Gajić, 2002).

Regarding the proportion of H^+ and Al^{3+} ions in the total exchangeable acidity, it is very important to determine which proportion is greater and especially to establish the content of Al ions. Namely, due to the fact that Al ions at the increased concentration are much more toxic to plants than H^+ ions, plants will, at the same value of the total exchangeable acidity of the soil, more suffer if the proportion of Al ions is greater. It was established that Al ions with the content of mobile Al ions of 6-10 mg/100 g of soil, quite unfavourably affect the growth and development of the majority of arable crops, which is a consequence of not only the immobilisation of phosphate ions in the soil, but also of a poorer development of the root system, as well as, disturbance of metabolism of carbohydrates, nitrogen and phosphorus in plants.

Considering the importance of soil acidity and the content of mobile Al in the process of nutrient absorption, and consequently the growth and development of plants and the achievement of stable, high-quality yields, the aim of the present study was to observe their vertical dynamics in the Ah, Eg and Btg horizons of pseudogley soils of the Čačak-Kraljevo Basin under various utilisation methods.

Material and methods

Field studies encompassed pseudogley soils of the Čačak-Kraljevo Basin that have been differently used. A total of 31 soil pits were dug including the following soil profiles: 11 forest, 14 meadow and 6 field profiles. Soil samples were separately drawn form the humus-accumulative (Ah), eluvial (Eg) and illuvial (Btg) horizons, during 2016 year. Samples were then dried to an air-dry status, finely ground and sieved through 2-mm mesh sieve.

Laboratory tests encompassed the determination of active (pH/H_2O) and exchangeable (pH/KCl) soil acidity by the application of the potentiometric method in suspension with water and KCl (1:2.5). The titrimetric method was used to determine the Al^{3+} ions in the extracts of 1 mol L^{-1} KCl solution, 1:10 (v/v) soil/solution ratio. The exchangeable acidity (Al^{3+} + H^+) was determined by titrations of 25 mL KCl extract with 0.025 mol L^{-1} phenolphthalein as an indicator. Then, the concentration of mobile Al was obtained by back-titration of the same KCl extracts, previously used, after the acidification with a drop of HCl and addition of 40 g L^{-1} NaF, with 0.025 mol L^{-1} HCl.

Results and discussion

Obtained results of active and exchangeable soil acidity, as well as the content of mobile Al show great variations in their magnitudes over both, various profiles and horizons Table. 1).

Active acidity in forest pseudogley soils, in the Ah horizon, was higher than 6, 5 and 4 in one, three and six locations, respectively, while it was less than 4 in one location. The greater depth in the Eg horizon was the greater active acidity was, but it decreased from the Btg horizon. The analysis of exchangeable acidity performed in the Ah horizon show that pH/KCl > 5 i.e. 4 was recorded in one i.e. three locations, respectively, while pH/KCl<4 was detected in seven locations. The utilisation method greatly affected exchangeable acidity in Eg and Btg horizons, most notably in the forest profiles, as pH/KCl was lower than 4 (3.53-3.87) in all profiles of the Eg horizon, while this range varied from 3.47 to 3.96 in the Btg horizon.

Table 1. Active and exchangeable acidity and the content of mobile Al in the soil

No. of profiles	Horizon	Forest profiles			Meadow profiles			Field profiles		
		pH/H_2O	pH/KCl	Mobile Al	pH/H_2O	pH/KCl	Mobile Al	pH/H_2O	pH/KCl	Mobile Al
1	Ah	4.80	4.00	11.24	5.03	4.01	8.72	4.55	3.74	14.93
	Eg	4.50	3.53	27.52	5.05	3.77	16.36	4.63	3.77	14.12
	Btg	5.00	3.56	22.66	5.36	3.81	11.96	4.68	3.58	22.93
2	Ah	4.78	3.72	12.22	5.98	5.38	0.18	5.24	4.48	1.66
	Eg	4.82	3.82	12.32	5.70	5.22	0.14	5.55	4.58	0.28
	Btg	5.22	3.96	7.87	5.30	4.12	5.16	5.46	4.42	2.73
3	Ah	4.45	3.67	33.19	5.50	4.53	1.08	5.69	4.58	1.17
	Eg	4.81	3.63	27.34	5.24	3.89	9.44	5.13	3.80	15.38
	Btg	5.34	3.64	20.05	5.36	3.84	12.41	5.18	3.68	22.21
4	Ah	4.82	3.64	24.55	5.70	4.77	0.99	5.05	4.16	3.51
	Eg	5.09	3.60	28.10	5.44	4.11	3.51	5.24	3.92	9.17
	Btg	5.36	3.67	18.75	5.65	3.95	7.64	5.69	3.79	16.72
5	Ah	4.68	3.82	27.34	5.17	4.23	3.51	5.50	4.52	1.26
	Eg	4.80	3.64	34.89	5.04	3.67	19.74	5.67	4.33	2.88
	Btg	5.19	3.66	38.04	5.64	3.73	12.45	5.66	4.16	5.04
6	Ah	4.72	3.83	27.16	4.97	3.90	13.31	5.36	4.34	1.98
	Eg	4.79	3.54	50.81	5.22	3.73	18.89	5.40	4.12	5.39
	Btg	5.17	3.47	25.85	5.37	3.70	29.50	5.63	4.17	3.69
7	Ah	5.17	4.35	1.08	5.35	4.26	2.25	-	-	-
	Eg	4.84	3.60	32.80	5.47	4.00	7.91	-	-	-
	Btg	5.24	3.70	13.76	5.40	3.66	36.42	-	-	-
8	Ah	5.03	4.09	4.32	4.78	3.94	15.20	-	-	-
	Eg	4.85	3.74	15.11	4.87	3.65	38.18	-	-	-
	Btg	4.85	3.70	23.65	5.22	3.80	18.30	-	-	-
9	Ah	5.26	4.42	0.89	5.24	4.18	2.79	-	-	-
	Eg	4.89	3.70	20.77	5.17	3.92	12.41	-	-	-
	Btg	5.24	3.71	18.35	5.53	3.77	28.60	-	-	-
10	Ah	6.10	5.36	0.36	5.06	3.94	9.35	-	-	-
	Eg	5.10	3.87	9.13	5.00	3.62	28.96	-	-	-
	Btg	4.92	3.63	23.38	5.40	3.59	43.89	-	-	-
11	Ah	4.83	3.87	9.80	5.18	4.18	2.43	-	-	-
	Eg	5.04	3.56	27.97	5.23	3.79	16.95	-	-	-
	Btg	5.07	3.47	58.01	5.31	3.84	11.78	-	-	-
12	Ah	-	-	-	5.27	4.22	2.61	-	-	-
	Eg	-	-	-	5.23	3.75	17.04	-	-	-
	Btg	-	-	-	5.33	3.83	12.14	-	-	-
13	Ah	-	-	-	5.07	4.28	1.98	-	-	-
	Eg	-	-	-	5.11	3.83	16.51	-	-	-
	Btg	-	-	-	5.42	3.84	13.98	-	-	-
14	Ah	-	-	-	4.95	3.91	1.42	-	-	-
	Eg	-	-	-	4.96	3.74	17.27	-	-	-
	Btg	-	-	-	5.52	3.91	10.57	-	-	-

The values of the mobile Al content changed over horizons in accordance with changes in soil acidity. Namely, in one location where pH/KCl>5 in the Ah horizon, the content of mobile Al was 0.36 mg/100 g of soil, while in locations where pH/KCl>4, the content of Al ions was below 5 mg/100 g of soil, which can be considered as values tolerable for cultivated plants. In all other locations, in Ah, Eg and Btg horizons, the content of Al^{3+}ions was within toxic concentrations (above 10 mg/100 g of soil, Jakovljević et al., 1991). In addition to the pH value, the content of organic matter, the composition of soil minerals and other soil properties significantly affect the content of mobile Al in the soil (Naramabuye and Haynes, 2007).

The basic characteristic of active and exchangeable acidity in meadow soils was the greater uniformity among observed locations (Table 1). The $pH/H_2O<5$ was recorded in three out of 14 dug profiles in the humus-accumulative horizon, while it varied from 5.03 to 5.98 in remaining profiles. The values of exchangeable acidity varied from 3.90 to 3.94 in four locations, while it was above 4 in nine locations. This value amounted to 5.38 only in the estate of the Agricultural School in Kraljevo, due to anthropogenic activities. Exchangeable acidity increased over the soil depth, and its average value in Eg and Btg horizons amounted to 3.91 and 3.81, respectively. The content of mobile Al was directly dependent on soil acidity, because the content of Al^{3+} ions in the Ah horizon with profiles where pH/KCl ranged from 3.90 to 3.94 varied from 9.35 to 15.20 mg/100 g of soil. In remaining profiles in the Ah horizon, the content of mobile Al was below 5 mg/100 g of soil, except in the location Jarčujak where pH/KCl amounted to 4.01 and the content of Al^{3+} ions was 8.72 mg/100 g of soil. The greater soil acidity in Eg and Btg horizons was the greater participation of Al^{3+} ions in exchangeable acidity was (Eg - 15.95, Btg - 18.20 mg Al^{3+}/100 g of soil).

The toxic content of Al^{3+} ions (14.93 mg/100 g of soil) was recorded in just one of six dug field profiles in which active acidity of the soil was below 5 (pH/KCl - 3.74) (Table 1). The $pH/H_2O>5$ and pH/KCl >4 were recorded in all remaining dug profiles, while the average content of mobile Al amounted to 4.08 mg/100 g of soil, which was approximately three-fold lower than the average content of Al^{3+} ions in forest profiles in pseudogleys. Exchangeable acidity in Eg and Btg horizons was increased (Eg – 4.09 and Btg – 3.97 pH units), which resulted in the increased content of Al^{3+} ions (Eg -7.87 and Btg – 12.22 mg/100 g of soil).

Gained results show that the distribution of soil acidity and the content of mobile Al were strongly dependent on the soil profile, and that the content of Al^{3+} ions in pseudogley soils of the Čačak-Kraljevo Basin highly varied over the method of soil utilisation and the profile depths (Table 2).

Table 2. Average values of pH/KCl and the content of mobile Al over soil profiles

	Forest profiles	Meadow profiles	Field profiles
pH/KCl	3.79	4.00	4.12
Mobile Al	21.49	12.95	8.06
Ah- pH/KCl	4.07	4.27	4.30
Ah-mobile Al	13.83	4.70	4.08
Eg- pH/KCl	3.65	3.91	4.09
Eg-mobile Al	26.07	15.95	7.87
Btg- pH/KCl	3.65	3.81	3.97
Btg-mobile Al	24.58	18.20	12.22

Soil acidity has the greatest impact on changes in contents of Al^{3+} ions ($r = -0.74$). The highest average soil acidity was recorded in forest soils (pH/KCl 3.79), then in meadow (pH/KCl 4.00) and arable field soils (pH/KCl 4.12). The soil acidification was accompanied by high average values of mobile Al (21.49, 12.95 and 8.06 mg/100 g of forest, meadow and arable field soils, respectively), which had been previously established by Dugalić and Gajić (2002) and Dugalić et al. (2008). Furthermore, these studies confirmed conclusions made by Nikodijević (1964), who had studied the content and dynamics of Al^{3+} ions of pseudogley soils of western Serbia and had determined that soils with pH/KCl<4, pH/KCl 4-4.5 and pH/KCl>4.5 had contained 13-45, 3-13 and less than 3 mg of Al^{3+} ions/100 g of soil. High values of mobile Al in pseudogleys were established by Osaki et al. (1997) and Mládková et al. (2005).

Table 3. Analysis of variance of the tested parameters (ANOVA)

		n	pH/H_2O $\overline{X} \pm S\overline{x}$	pH/KCl $\overline{X} \pm S\overline{x}$	Al $\overline{X} \pm S\overline{x}$
Lokacija					
1		33	4,99±0,049 b	3,79±0,056 b	21,49±1,773 a
2		42	5,29±0,044 a	3,99±0,050 a	12,95±1,572 b
3		18	5,29±0,067 a	4,11±0,076 a	8,06±2,401 b
Dubina					
1		31	5,14±0,054 b	4,21±0,062 a	7,54±1,948 b
2		31	5,11±0,054 b	3,88±0,062 b	16,63±1,948 a
3		31	5,32±0,054 a	3,81±0,062 b	18,33±1,948 a
Lokacija x Dubina					
1	*1*	11	4.97±0.086	4,07±	13,83±3.071
1	*2*	11	4,87±0.086	3,66±	26,07±3.071
1	*3*	11	5,14±0.086	3,65±	24,58±3.071
2	*1*	14	5,23±0.076	4,27±	4,70±2.722
2	*2*	14	5,21±0.076	3,91±	15,95±2.722
2	*3*	14	5,44±0.076	3,81±	18,20±2.722
3	*1*	6	5,23±0.116	4,30±	4,08±4.159
3	*2*	6	5,27±0.116	4,09±	7,87±4.159
3	*3*	6	5,38±0.116	3,97±	12,22±4.159
Anova		***df***			
Lokacija		2	**	**	**
Dubina		2	*	**	**
Lokacija x Dubina		4	ns	ns	ns

The same small letters in column with average values indicate non-significant differences (P>0.05)
*F-test: N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01;*

The content of soil organic compounds (humic and citric acids) affects the content and distribution of Al^{3+} ions in a sense that they reduce acidity, and therefore the content of free Al ions. Binding of soil Al into organic complexes reduces the Al toxicity, which reduces its accessibility in the soil and its absorbability by plants (Parker et al.,1988). In his previous studies, Dugalić (1998) determined a low humus content in these soils, which reduced the ability of Al ions to bind to humic acid, which additionally affected the increase of its mobility in the soil.

Conclusion

The extensive studies of the vertical dynamics of active and exchangeable soil acidity and the content of mobile Al in forest, meadow and arable field pseudogley soils of the Čačak-Kraljevo Basin point out to a great variability of these parameters depending on the method of soil utilisation and the profile depth. The highest average soil acidity was established in forest soil profiles (pH/KCl 3.79), and then in meadow (pH/KCl 4.00), and field soil profiles (pH/KCl 4.12). The average content of mobile Al amounted to 21.49, 12.95 and 8.06 mg/100 g of forest, meadow and filed soils. The lowest acidity, and therefore the lowest content of mobile Al was determined in the Ah horizon, then in Eg horizon, while the highest acidity was recorded in the Btg horizon.

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THE USE OF WASTEWATER FROM THE RECIRCULATING AQUACULTURE SYSTEM FOR BASIL CULTIVATION AND ITS EFFECTS ON THE ESSENTIAL OIL COMPOSITION

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Abstract

The wastewater resulting from the recirculating aquaculture systems (RAS) is rich in nitrogen and phosphorus, which is why it could be used for plant cultivation, as a sustainable disposal management. The aim of this study was to test the effects of RAS wastewater on basil yield and essential oil quality. A green basil cultivar 'Genovese' was used for this study. Plants were divided into two groups, a control group watered with distilled water (50 ml H_2O / pot / day) and one treated with RAS wastewater (50 ml H_2O / pot / day). Chlorophyll content, plant height, lateral stems number, and the fresh biomass were assessed. The essential oil was also extracted and its composition determined by GC/FID and GC/MS techniques. The results showed that fresh biomass of basil was higher (+13%) under RAS wastewater treatment compared with control. The chlorophyll content was higher in the same group (+13%). Regarding the composition of the essential oil, 26 compounds were identified, including 8 monoterpenes, 16 sesquiterpenes and 2 phenylpropanoids. The main determined compounds were eucalyptol, β-linalool, eugenol, methyl eugenol, α-trans bergamotene, germacrene D. RAS wastewater treatment influenced the production of the main compounds as followes: -12% eucalyptol, +11% β-linalool, +1,5% eugenol, -1.9% methyl eugenol, -4.7% α-trans bergamotene and + 22% germacrene D, compared with control. This study demonstrates that the use of RAS wastewater can improve the fresh yield of basil and it can also stimulate the production of specific compounds in the essential oil.

Keywords: *Ocimum basilicum* L., chlorophyll content, fresh mass, essential oils

Introduction

Basil essential oil EO is utilized in numerous industries due to a wide range of bioactivities such as antibacterial, antioxidant, or chemoprotective (Pandey *et al.*, 2014). Generally, the constituent compounds of basil EO are terpenes and phenylpropanoids, and the proportion of compounds belonging to one of these classes depends on both genetic and environmental factors (Zheljazkov *et al.*, 2008). Due to the growing demand on the pharmaceutical and perfumery markets for certain compounds, such as linalool, eugenol, camphor, eucalyptol, etc. numerous studies have been aimed at stimulating the synthesis of a desired compound by various techniques of elicitation, fertilization and selection (Onofrei *et al.*, 2018).
Water is a very important factor for optimal growth and development of plants; consequently, there is currently a tendency to reuse water from different activities such as aquaculture, for irrigation. Irrigation of plants with water resulting from a recirculating aquaculture system (RAS) could be a preferred solution because it is nutrient rich. RAS is an important branch of aquaculture. This technology is based on the reuse of water using mechanical, biological and UV filtration. Due to the fact that water is reused in RAS systems, it results in reduced water consumption, so RAS systems are environmentally friendly. Depending on the species and the

growing technology, up to 36% of the feed is excreted by fish in the form of organic residues. Furthermore, 75% of nitrogen, 85% of phosphorus and 80-88% of carbon of aquaculture feed are released into wastewater (Endut *et al*., 2011).

Numerous studies have been carried out in order to re-use the aquaculture wastewater for production of valuable cultures such as Bell Peppers (Zheljazkov and Horgan, 2011), water spinach (Endut *et al*. 2009; Effendi *et al*. 2015), lettuce (Effendi *et al*. 2017; Wahyuningsih *et al*. 2015). According to these studies, positive results on plant growth are owed to the dissolved nutrients such as nitrogen and phosphorus, specific organic and inorganic compounds, and total suspended solids, which results from uneaten feed and metabolic wastes from the fish. Accumulation of high nutrients concentration in water can became harmful to fish, which is why, research for new technologies for water treatment and water reusing represent a preferred solution for both the welfare of fish and for the environment (Effendi *et al*. 2015).

The overall objective of this study was to evaluate the effect of RAS wastewater utilization on the plant morph-physiology and the quality of essential oil of basil.

Materials and methods

Plant growth conditions: sweet basil (*Ocimum basilicum* L.) was cultivated under controlled environmental conditions: 1 L black plastic pots; natural light was supplemented by florescent tubes 12 h/day; temperature 25°C; the soil was Florisol® (N-0.41%; P_2O_5-0.44%; K_2O-1.16; organic matter – 41%); plants were watered daily with 50 ml H_2O per pot.

After 4 weeks, plants were divided into two groups: group 1 - control variant (labeled V1 - control) that was watered with distilled water; and group 2 - watered with water from RAS (labeled V2 - RAS) (pH 7.9; dissolved oxygen 8.79 mg/l; electrical conductivity 1405 S/cm3; total dissolved solids 782 mg/l; NO_3^- 44 mg/l; NO_2^- absent; NH_3^+ absent; NH_4^- absent; phosphorus 0.9 mg/l). Each group consisted of 3 repetitions of 4 pots (1 plant/ pot).

After 2 weeks plants were harvested and fresh mass (g/plant), plant height (cm) and total chlorophyll content (CCI) were assessed.

Chlorophyll content index (CCI): was measured with a portable device CCM-200 plus (Opti-Sciences, USA).

Essential oil extraction: fresh plant material from the variants (10.0 g in 100 mL H_2O) was used for the volatile oil extraction by hydrodistillation for 2 h in a Clevenger type apparatus.

Essential oil characterization: the EO samples were analyzed by GC/FID and GC/MS techniques. The GC/MS analysis was carried out with an Agilent 5975C MSD system coupled to an Agilent 7890A GC (Agilent Technologies Inc., Santa Clara, CA). An Agilent J&W HP-5MS column, 30m, 0.32mm, 0.25µm was used with helium (purity 99.99%) as a carrier gas. Operating conditions were as follows: oven temperature 60 °C (3 min), 1°C/min to 80 °C (3 min); 5°C/min to 280°C (5 min); flow rate of 1.2 ml/min (He); injector T=260°C; FID T=270°C; 1.0µl injection volume at split ratio 20:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230°C, transfer line temperature 280°C, solvent delay 4.00 min and mass range: 50-500 m/z. The MS was operated in scan mode. 1.0 µL of EO diluted with n-hexane (10%, v/v) was injected into GC/MS system. The GC analysis was carried out using an Agilent 7890A GC system. In order to obtain the same elution order with GC/MS, simultaneous triplicate injections were done using the same column and operational conditions.

Identification of the components present in the EOs was made by comparing mass spectra of components in EOs with those from NIST'08 and Adams mass spectra library, by AMDIS (Automated Mass Spectral Deconvolution and Identification System), and by comparing with literature data and estimated Kovats (retention) indices. These were determined using a mixture of homologous series of normal alkanes from C_8 to C_{40} in hexane, under the same

above-mentioned conditions. The percentage ratio of EO components was computed by the normalization method of the GC/FID peak areas, and average values were taken into further consideration (n=3).
Statistical analyses: results are expressed as means ± standard errors. Descriptive statistics, analyses of variances (ANOVA) were performed for fresh biomass, plant height and chlorophyll content index.

Results and discussion

Morph-physiological parameters
In order to test the effect of RAS wastewater on morph-physiology of basil, fresh mass, plant height, and chlorophyll content were analyzed (Table 1). Fresh mass and plant height were higher with 10% and 11%, respectively, in plants that were treated with wastewater from the RAS, compared with control (distilled water) (Table 1). The differences between treatments were statistically significant according to the ANOVA test ($p<0.05$) only for the fresh biomass.
Chlorophyll content increased by 13% in RAS treated plants compared with the control variant, the differences being statistically significant ($p = 0.03$). These changes in the photosynthetic apparatus may also explain the increase of fresh biomass in plants treated with water from the RAS (Table 1).

Table 1. Morpho-physiological parameters of basil treated with RAS wastewater

Parameter	Treatment	Mean ± standard error	Minimum	Maximum	F	p
Fresh mass (g/plant)	Control water	24.33±0.51	23.66	25.33	10.170	0.030
	RAS wastewater	26.89±0.62	25.66	27.66		
Plant height (cm)	Control water	15.17±0.84	13.76	16.67	2.840	0.160
	RAS wastewater	16.86±0.55	15.76	17.50		
Chlorophyll content CCI	Control water	6.23±0.22	5.83	6.60	10.610	0.030
	RAS wastewater	7.04±0.11	6.90	7.26		

The Research and Development Station for Aquaculture and Aquatic Ecology from Iasi is one of the largest RAS in Romania that is used for growing of sturgeons. In order to meet the objective of blue growth and circular economy, there is a need to reuse the wastewater from the RAS for various activities such as plant irrigation. RAS wastewater contains nitrates and phosphates that may explain the positive results in this study. The presence of nitrates and phosphates in RAS wastewater is resulted from the use of fish feed with up to 45% protein content (Effendi et. *al*., 2017). Fish excretes most of the nitrogen in form of urine through the gills, thus this element is completely dissolved in water. In the biofilter of RAS, nitrogen is transformed in nitrate, the preferred form for plants. Basil plants react positively to nitrogen fertilization, by increases of the mass and morphological parameters (plant height, number of lateral branches, number of leaves, etc.) (Bufalo *et al*., 2015; Burducea *et al*., 2018). Similarly, in our study, the use of RAS wastewater has led to the increase of both the fresh biomass and height of basil plants. Nitrogen plays an essential role in plant growth and development being used in protein synthesis. Likewise, phosphorus is another essential element that is used in the energy processes (Marschner, 1995). At the same time, the greater availability of nitrogen and phosphorus in RAS wastewater may be responsible for an improved physiological state of basil, as was estimated by the quantification of chlorophyll content. This is due to the fact that nitrogen enters the composition of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and ribulose 1,5-bisphosphate (RuBP), molecules which together with carbon dioxide and in the presence of light leads to the synthesis of organic substances in the photosynthesis process (Marschner, 1995).

Essential oil composition

In the present study, 26 compounds were identified in the EO of basil (Table 2). The compounds belongs to two chemical classes: phenylpropanoids (eugenol and methyl eugenol), and terpenoids (8 monoterpenoids and 16 sesquiterpenoids). RAS wastewater treatment influenced the metabolic pathway for synthesis of terpenes. Accordingly, increases of 11.4% for linalool, 8.3% for β-caryophyllene, 22.8% for germacrene D were obtained for RAS wastewater treated plants compared with control. The variations of phenylpropanoid compounds were small (+1.5% eugenol and -1.9% methyl eugenol) of the essential oil belonging to the RAS wastewater group compared with the control group.

In our study, the main identified compounds (linalool, eucalyptol, eugenol and methyl eugenol) recorded quantitative changes depending on the water used as treatment (RAS or distilled water). Generally, the species belonging to the genus Ocimum sp. may be rich in terpenoids (40-70%) or phenylpropanoids (Zheljazkov et al., 2008). Terpenoids are formed through the metabolic pathway of mevalonic acid (MVA) in the cytoplasm and via the methylerythritol phosphate (MEP) pathway in plastids. The metabolic pathway for the formation of phenylpropanoid compounds starts with phenylalanine - the amino acid from which phenylpropanoids (eugenol and methyl eugenol) are formed, and intermediates used in the biosynthesis of lignin, anthocyanins, rosmarinic acid, etc. are also formed. These metabolic pathways can be influenced by internal factors: differentiated gene expression, microRNA, transcription factors, promiscuity of enzymes, as well as external factors such as light, radiation, temperature, growth substrate composition. Both terpenoid and phenylpropanoid compounds have a great utility in various industries such as food, cosmetics and pharmaceutics. Terpenes are used as dyes, flavorants and medicinal purposes. For example, linalool, which is a major compound in the volatile oil of many basil chemotypes, has a series of bioactivities such as: antimicrobial, anti-inflammatory, anticancer, antioxidant (Pandey et *al*., 2014). Furthermore, linalool is used in domestic products such as soaps, detergents and shampoos (Kamatou and Alvaro, 2008). Phenylpropanoid compounds such as methyl eugenol are known to have antifungal and insecticidal properties. An important constituent of the phenylpropanoids identified in our study is methyl eugenol (44-45%). Methyl eugenol is found in approximately 450 plant species, its role being protection against herbivores attack (Tan and Nishida, 2012).

Table 2. The composition of basil essential oil

No.	Name	Compound group	RT (retention time)	RI (retention index)	Control	RAS
1	β-Pinene	monoterpene	6.11	979	1.26	1.06
2	Eucalyptol (1.8-Cineole)	monoterpene	7.13	1031	9.81	8.55
3	β-cis-Ocimene	monoterpene	7.31	1040	0.89	0
4	Terpinolene	monoterpene	8.33	1086	0.37	0
5	β-Linalool	monoterpene	8.66	1095	8.93	9.99
6	Camphor	monoterpene	10.07	1141	1.16	1.08
7	α-Terpineol	monoterpene	11.34	1188	1.07	1.19
8	Bornyl acetate	monoterpene	13.65	1254	1.18	1.35
9	β-Elemene	sesquiterpene	16.54	1389	0	0.11
10	Eugenol	phenilpropanoid	15.69	1354	8.79	8.85
11	α-Copaene	sesquiterpene	16.22	1376	0.14	0.12
12	β-Elemene	sesquiterpene	16.54	1389	1.05	1.13
13	Methyl eugenol	phenilpropanoid	16.81	1403	45.39	44.49
14	β-Caryophyllene	sesquiterpene	17.48	1417	0.24	0.23
15	α-trans-Bergamotene	sesquiterpene	17.68	1434	8.05	7.68
16	α-Guaiene	sesquiterpene	17.79	1436	0.13	0.12
17	(E)-β-Farnesene	sesquiterpene	18.1	1456	1.22	1.19
18	Humulene (α-Caryophyllene)	sesquiterpene	18.45	1454	1.38	1.38
19	trans-Muurola-4(14).5-diene	sesquiterpene	18.61	1466	0.16	0.15
20	Germacrene D	sesquiterpene	19.12	1481	2.52	3.09
21	Bicyclogermacrene	sesquiterpene	19.49	1500	0.43	0.38
22	α-Bulnesene	sesquiterpene	19.6	1509	0.41	0.24
23	γ-Cadinene	sesquiterpene	19.92	1513	1.18	1.15
24	β-Sesquiphellandrene	sesquiterpene	20.1	1522	0.34	0.36
25	1.10-di-epi-Cubenol	sesquiterpene	22.62	1618	0.25	0.89
26	1-epi-Cubenol	sesquiterpene	23.18	1627	2.67	4.02

Conclusion

In this study, the fresh biomass and chlorophyll content of basil increased as a result of RAS wastewater treatment. Analyzing the composition of the essential oil, 26 compounds were identified, of which 24 are terpens and 2 are phenylpropanoids. Two of the main compounds (linalool and methyl eugenol) recorded an increase in the essential oil extracted from the plants treated with RAS wastewater. Our results indicate that the water treatment and water reusing represent a preferred solution for both the welfare of fish and for the environment.

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COMPARISON OF COMMON FERTILIZERS AND FERTILIZERS WITH NITRIFICATION INHIBITOR TO YIELD AND PROTEIN CONTENT ON TRITICUM DURUM

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Abstract

One of the most important winter cereals cultivated in Greece is *Triticum durum*. In this research we studied the effect of different fertilizer types containing nitrification inhibitor and conventional fertilizers at 4 different N-levels (0, 70, 140 and 210 kg ha^{-1})on two different durum wheat varieties at the Experimental Farm of the Technological Educational Institute of Thessaly in Greece (TEI; Larissa plain). The sowing of the crop took place on November 2018. Nitrification inhibitor ensured crops N-nutrition for longer period compared to conventional fertilizers and may lead to higher yields with higher protein content. There were not found any statistical significant differences on yield between the tested factors for both varieties but only numerically superiority of the fertilizers containing nitrification inhibitor. On the other hand, there were found statistically significant differences in protein and starch content for both varieties. It was found that the N-levels of 140 & 210 kg ha^{-1} produced seeds of higher protein content, ranging 15.87-16.03 and 15.48-16.20 %, for both varieties, respectively. Furthermore, protein and starch content have a linear relationship with nitrogen supply, where R^2 is very high (0.93 and 0.97), respectively.The above results were found through the first year of experimentation, and therefore safer conclusions expected to arise after the repetition of the experiments in the same place for a second and a third year.

Keywords: *Triticum, nitrification inhibitor, fertilizers, yield, Greece.*

Introduction

Durum wheat is grown on over 3.7 million ha in the European Union, largely concentrated in the Mediterranean countries (Italy, Greece and Spain), where prevailing the ideal growing conditions for producing quality grains (Nachit, 1998). This area accounts for82%of total EU production; however, grain yields are lower than those obtained in Northern European countries (European Commission, 2002).

The most influencing factor for the quality of the grains is N fertilization, although the degree of influence is governed by annual weather conditions and by residual soil N (L´opez-Bellido et al., 2001). It has been reported (Campbell et al., 1993) that there is a strong interaction between soil water use and crop fertilizer response in semi-arid conditions. Progressive design of nitrogen fertilizer program is essential to optimize wheat yield and quality (Grant et al., 2001).

Quality grain durum wheat is generally dependent on the protein content, at least in the range of protein percentages encountered in commercial wheat cultivars (Grant and Flaten, 1998; Anderson, 2000; Clarke, 2001). Carefully managing of nitrogen N application, may lead to higher yields while protein may be maintained or increased (Chaney, 1990; Gate, 1995).

It is therefore important to conduct research to determine the crop response to N fertilization and to develop rational practices for more efficient N use in this crop (Knowles et al., 1991; Anderson, 2000), which requires a higher level of management (Impligia and Anderson, 1998).

Farmers apply different N fertilizers such as urea, ammonium nitrate, ammonium sulphate and potassium nitrate to increase yields. However, this increase in N use, with N-response efficiency reported to be between 33 and 50%, is contributing to higher worldwide N losses via NH_3 volatilization and NO_3^-leaching that impact air and water quality (Raun and Johnson, 1999; Howarth and Marino, 2006; Nosengo, 2003).

Nitrogen is an important component of proteins and chlorophyll-building cells and plant tissues (Vickery and Vickery, 1981). It is also an important determinant of the rate of basic physiological processes in plants, such as photosynthesis and respiration (Lewis et al, 2000; Takashima et al., 2004). Nitrogen is often limited to most agricultural ecosystems, therefore, nitrogen fertilizers (ie, chemical or organic) are often applied to meet the N-requirements of growing plants and improve soil fertility.

Improvement of fertilizers that are already used is done through appropriate product design (Bröckel and Hahn, 2004). The product profile is determined by chemical and physical properties, environmental safety and stability against mechanical stress, hygrometry and temperature. The design of new solid fertilizers products is mostly done to improve handling properties. Increasing the efficiency of inorganic N in fertilizers is not easy, because plants occupy N normally at nitrate or ammonium form through their roots from the soil solution. However, ammonium-N, as opposed to N-nitrate, can be retained as soil components, so that soil and plants compete for ammonium-N, whether it is already available on the soil or applied (Amberger, 2006).

Urea is the basic type of chemical fertilizer. Since 50% of the world's demand for N is met by the use of urea, this large use of urea is due to a number of factors, including high levels of N (46% N by weight), high water solubility, easy transportation, handling and application.

Nowadays there are new N-fertilizers types using many inhibitors. One of these types is also the fertilizer that has a substance that inhibits the biological oxidation of ammonium-N with nitrate-N and it calls nitrification inhibitor.

This study was conducted in the main agricultural plain (Thessaly) to evaluate the effect of new N fertilizer types containing nitrification inhibitor on the yield of durum wheat in Greece.

Material and Methods

For the purpose of the study, two field experiments were established in a typical soil-climatic environment of Thessaly plain, in central Greece. The experimental site is located at the Experimental Farm of the Technological Educational Institute of Thessaly in Greece (TEI; Larissa plain, coordinates: 39.626100, 22.381363).

Field management and experimental design

Sowing was occurred using a modern cereal seeding machine (on November 2018), applying 225 kg ha^{-1} of seeds in a row-distance of 12.5 cm.

Basic fertilization (250 kg ha^{-1} of 20-10-0) applied one-two days before sowing using a dispenser and then the fertilizer was incorporated using a rotary cultivator. There was performed post-emergence herbicide application to control weeds.

The experimental design for both tested varieties (2 varieties were selected V_1: *Iride* and V_2: *Simeto*) was a split-plot design with major factor the fertilizer type (2 fertilizer types, F_1: conventional and F_2: fertilizer using nitrification inhibitor), and sub-factor the N-level (4 N-levels, L_1: 0, L_2: 70, L_3: 140 and L_4: 210 kg ha^{-1}) and three replications (blocks).

Soil characteristics

The soil of the experimental site is characterized as a calcareous (pH = 7.8), with low organic matter content (0.9) and low salinity (0.11) at a depth of 30 cm (Table 1).

Table 1. Soil characteristics of the field experiment.

Property	Soil depth (0-25) cm
Texture	Loam
pH	7.81
EC (dSm^{-1})	0.11
Organic matter (%)	0.93
N-inorganic (mg kg^{-1})	44.8
K-exchangeable (mg kg^{-1})	373.3
P –Olsen (mg kg^{-1})	13.1

Plant measures

Total biomass and seed yield measured by harvestsampling (according to Zadocks scale: ripening stage 91-99). To avoid any border effect, 1 m^2 in the inner plot was harvested in each sampling above the ground. The samples were weighed at the field and then a sub-sample was taken for further laboratory measurements.

Protein, starch and gluten content was determined in dry seeds of each sample by near-infrared reflectance (NIR) spectroscopy technique using the DA 7250 NIR analyzer (Perten Instruments, Hägersten, Sweden).

The statistical package GenStat (7th Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982).

Results and Discussion

Yield and Quality wheat characteristics

The results have shown none statistical significant differences between the tested factors for both varieties but only numerically superiority of few treatments (Table 2), especially in case of seed yield which is the product of economic value.

The fertilization using nitrification inhibitor have a numerically superiority for both tested varieties. The difference in seed yield between the different fertilizer types is 262 and 227 kg ha^{-1}, in case of Iride and Simeto, respectively. Moreover, it was found that the 3rd N-level for the fertilizer with nitrification inhibitor produced the same yield with the higher fertilization level of the conventional fertilizers (Table 2).

Table 2. Biomass, straw (hay) and seed yield of two different durum wheat varieties, under different fertilization application.

Characteristics / Treatments	Iride			Simeto		
	Biomass	Straw	Seed Yield	Biomass	Straw	Seed Yield
	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
F_1	8323	3856	4467	8265	4664	3601
F_2	8757	4027	4729	8942	5113	3828
$LSD_{.05}$	ns	ns	ns	ns	ns	ns
L_1	8427	3790	4637	8343	4712	3631
L_2	8267	3564	4702	8510	4940	3570
L_3	8387	4075	4312	8763	4926	3837
L_4	9080	4338	4742	8797	4977	3820
$LSD_{.05}$	ns	ns	ns	ns	ns	ns
F_1L_1	8380	3729	4651	8493	4948	3545

F_1L_2	7820	3370	4450	8287	4882	3405
F_1L_3	8200	4063	4137	8467	4609	3858
F_IL_4	8893	4262	4631	7813	4219	3594
F_2L_1	8473	3851	4622	8193	4476	3717
F_2L_2	8713	3759	4954	8733	4999	3735
F_2L_3	8573	4086	4488	9060	5244	3816
F_2L_4	9267	4414	4853	9780	5735	4045
$LSD_{.05}$	ns	ns	ns	ns	ns	ns
CV (%)	14.6	15.0	14.3	13.2	13.1	13.3

Unlike the quantitative characteristics, the quality characteristics have statistically significant differences. Particularly there were found differences in protein and starch content for both varieties. The results are presented in Table 3, where it is shown that N-levels of 140 & 210 kg ha^{-1} produced seeds of higher protein content, ranging 15.87-16.03 and 15.48-16.20 %, for both varieties respectively.

On the other hand, in case of starch content, the zero fertilization level is the treatment with the higher starch content indicating that seeds of higher protein content have lower starch content. Zou et al (2015) mentioned that proteins can have some obvious effects on enzymatic degradation of starch, such as by physically hindering access of degradation enzymes to starch, while Wenwen et al. (2017) concluded that the protein content significantly and negatively correlated with starch molecular structures in a study for barley cultivation, which is in agreement with our findings.

Table 3. Protein and starch % content of two different durum wheat varieties, under different fertilization application.

Characteristics / Treatments	Iride		Simeto	
	Protein dry basis	Starch dry basis	Protein dry basis	Starch dry basis
	%	%	%	%
F_1	14.84	69.75	14.06	70.51
F_2	13.81	69.68	14.03	71.83
$LSD_{.05}$	*0.156*	*ns*	*ns*	*0.521*
L_1	12.65	70.57	10.68	74.92
L_2	12.75	71.10	13.80	71.55
L_3	16.03	68.27	15.48	70.22
L_4	15.87	68.93	16.20	67.98
$LSD_{.05}$	*0.097*	*0.302*	*0.133*	*0.578*
F_1L_1	11.37	73.10	10.37	74.63
F_1L_2	14.77	69.60	14.27	69.80
F_1L_3	17.30	67.20	14.63	70.27
F_IL_4	15.93	69.10	16.97	67.33
F_2L_1	13.93	68.03	11.00	75.20
F_2L_2	10.73	72.60	13.33	73.30
F_2L_3	14.77	69.33	16.33	70.17
F_2L_4	15.80	68.77	15.43	68.63
$LSD_{.05}$	*0.144*	*0.464*	*0.192*	*0.744*
CV (%)	*0.5*	*0.3*	*0.8*	*0.6*

Protein and Starch content relation with N fertilization level

Plotting the protein (%) content according to the nitrogen supply (kg ha^{-1}) results in the protein- nitrogen supply relation illustrated in Figure 1.

It can be seen that a linear relationship apply that might explain 93% of the existing variation (R^2= 0.93), largely independent of fertilizer type and variety, where the higher nitrogen supply the higher protein content is achieved.

In a study in Mediterranean conditions (Abad et al., 2004), it was found that seed protein ranged from 14.1 to 16.4% with the increased content found at higher fertilization levels, which is in agreement with our findings.

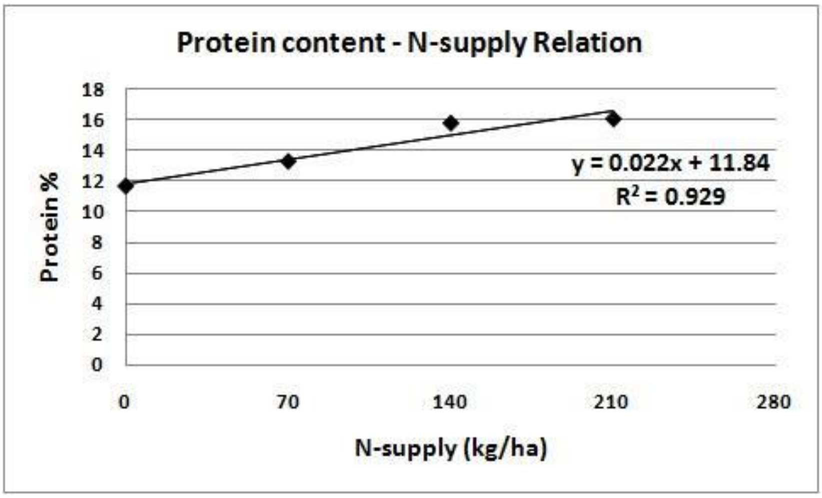

Figure 1. Protein % content – N-supply relation.

Plotting the starch (%) content according to the nitrogen supply (kg ha^{-1}) results in the starch-nitrogen supply relation illustrated in Figure 2.

It can be seen that a linear relationship apply that might explain 97% of the existing variation (R^2= 0.974), largely independent of fertilizer type and variety, where the higher nitrogen supply the lower starch content is achieved.

In a study (Wenwen et al., 2017) was concluded that starch has a negatively correlation with protein content. Therefore, if starch has a negatively correlation with protein, and protein has a positively correlation with nitrogen supply, then it is verified the negatively relation of starch content with nitrogen supply.

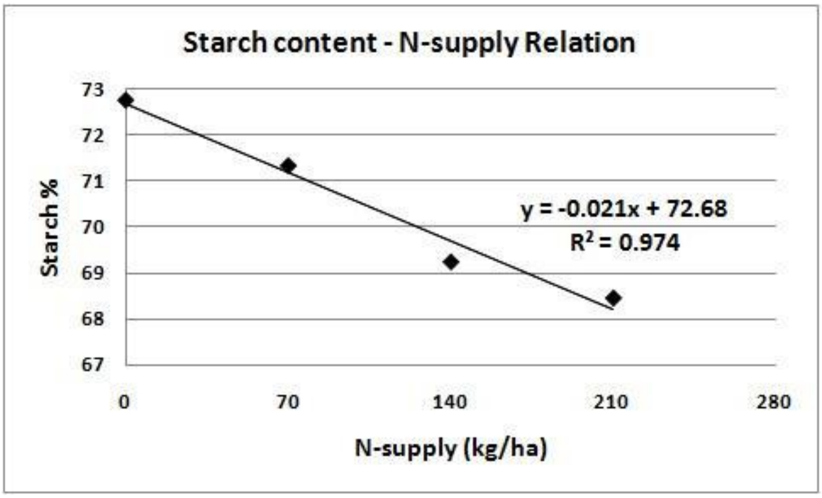

Figure 2. Starch % content – N-supply relation.

Conclusions

In both durum wheat varieties *Iride* and *Simeto*, there were not found significant differences for the quantitative characteristics but only numerically superiority of the fertilizers with nitrification inhibitor. On the other hand, statistically significant differences were found in case of the quality characteristics (protein and starch % content).Protein and starch content have a linear relationship with nitrogen supply, where R^2 is very high (0.93 and 0.97), respectively.Due to the fact that the above results were found through the first year of experimentation, safer conclusions expected to arise after the repetition of the experiments in the same place for a second and a third year.

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VARIABILITY OF LENGTH OF SPIKE AND NUMBER OF SPIKELETS PER SPIKE IN WHEAT (*Triticum aestivum* L.)

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Abstract

Variability of length of spike and number of spikelets spike^{-1} have share in forming of grain yield of wheat. The aim of this study was estimation of variability of length of spike and number of spikelets spike^{-1} in 20 genetically divergent wheat cultivars grown in different environmental conditions. The experiment was set up as a randomised block design in three replications. Obtained results indicated differences in average values of length of spike and number of spikelets spike^{-1} among tested cultivars in both years of experiment. In average for all cultivars length of spike was higher in the second year than in first year of experiment. Also, average value of number of spikelets spike^{-1} was higher in second year at the analysed wheat cultivars. The wheat cultivar Dejana expressed the longest spike (12.50cm) in the second experimental year while the wheat cultivar Sumadinka had the shortest spike (8.91cm) in the first year. On the base of results were established variability of both analysed spike traits in wheat cultivars. Also, this results showed significant differences among wheat cultivars according to length of spike and number of spikelets spike^{-1}, which are determined by genetic and environmental factors.

Keywords: *wheat, variability, spike length, spikelets, cultivars.*

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops as a source of food for the people worldwide. Increasing of wheat grain yield is the main task of breeders which require effort in improving characteristics of spike traits, grain, stem, leaf and root traits. The long and fertile spike potentially can contribute to improvement of grain yield of wheat (Zečević *et al*., 2008; Knezevic *et al*., 2014). Spike length together with number of spikelets and number of florets per spike represent great potential for yield improvement (Zečević, *et al*., 2004; Dimitrijević *et al*., 2011) through developing grain number spike^{-1} and as a source of assimilate in grain filling period as well as forming grain yield. Increasing of number of spikelets potentially related to increasing of number of grains (Álvaro *et al*., 2008). Spike length had positive relationship with number of spikelets spike^{-1} at both genotypic and phenotypic levels (Akram *et al*., 2008). Floral development is an important part of the pre-anthesis stage. Anther and ovary growth as well efficient pollination connected to grain number per spike, grain size and grain weight (Guo *et al*., 2015). Grain number per spike is related to floret survival (Gonzalez *et al*., 2011; Sreenivasulu and Schnurbusch, 2012). Spike structure has advantages in utilizing light in compare to other parts of plant and contribute to increasing of yield. Also, spike together with awns contribute to longer stay green area duration. All these characteristics of spike contribute to accumulate in average 20-30% of dry matter depends of genetic and environmental factors as well as their interaction (Knežević *et*

al., 2015: Branković *et al*., 2015). The effect of genetic and environmental factor at the length of spike and development of number of spikelets per spike need further investigation. Increasing of genetic capacity of spike traits is a potential direction of increasing grain yield of wheat (Knezevic *et al*., 2012).
The aim of this paper was investigation of variability of length of spike and number of spikelets spike^{-1} in genetically divergent wheat cultivars grown in different environmental conditions.

Materials and Methods

The twenty geneticaly divergent winter wheat cultivars were used for study of length of spike and number of spikelets spike^{-1} during two season of vegetative growth year (2015/16 and 2016/17). The experiment was performed in randomized block design in three replications on the field in Kraljevo, Serbia. The seeds of varieties were sown at the distance of 0.05m in rows of 1m length among which was the distance of 0.2m. For analysis of length of spike and number of spikelets spike^{-1} were used 60 plants in full maturity stage (20 plants per replication). For statistical analysis used MSTAT C version 5.0. The significant differences between the average values were estimated by F-test values. The analysis of variance was performed according to a random block system with one factor and significant differences were tested by means of test value of $LSD_{0.05}$ and $LSD_{0.01}$.

Weather conditions

In the first year experiments 2015/206, the average temperature was 9.9 °C and the total amount of precipitation was 651mm. In the second year of experiment 2016/17 average temperature was 13.0 °C and the total amount of precipitation was 523 mm. The average rainfall in the first year (651mm) was significantly higher than in the second year (523.1 mm), and than for ten years - 417.8 mm (Table 1). For plants growth in the second year was more favorable regime of temperature and precipitation. During October-November, a greater amount of water residue in the second year (161.7 mm) was higher than in the first year (120.8 mm) but in both years weather condition in this period was favorable for seed germination and development of plants to be in good condition for survive in the future winter period. Also, in the two months (October-November) the average temperature values were similar in both year of experiment. During the February-April amount of precipitation in the first year (250.5 mm) was higher than in the second (174.0 mm), although the distribution of rainfall was more favorable in the second year experiment (Table 1).

Table 1. Average monthly temperature and total monthly precipitation in Kraljevo

	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
Temperature °C	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	
Temperature °C	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	**8.74**	
2000-2010		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	**8.5**	
Precipitatin (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6	72.3	**651.0**
Precipitatin (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0	41.1	**523.1**
2000-2010		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	**417.8**

Results and Discussion

The length of spike in the first year of experiment varied in range of 8.91-11.11 cm with average value 9.92 cm, while in second year varied from 10.03 to 12.50 cm with average value 11.09 cm. Number of spikelets spike^{-1} in the first year of experiment varied in interval of 20.85 - 24.38 with mean value 22.79, while in second year number of spikelets spike^{-1} varied between 21.23 and 25.0 with average value 23.53 (Table 2). The obtained results showed significant differences in the average values of length of spike and number of spikelets spike^{-1} per year, that indicating diversity of studied cultivars.

Similar results were reported in previous investigation of Serbian wheat (Zečević *et al.*, 2008; Knezevic *et al.*, 2012) as well as for Italian and Spanish wheat cultivars (Álvaro *et al.*, 2008).

Table 2. Variability of length of spike and number of spikelets spike-1

	length of spike (cm)			*Number of spikelets spike^{-1}*		
Cultivars	First year 2015/16	Second year 2016/17	Average	First year 2015/16	Second year 2016/17	Average
Evropa 90	11.09a	12.07b	11.58	22.83cde	23.00defg	22.92
Dejana	11.11a	12.50a	11.80	24.38a	24.87ab	24.62
Sila	9.95bcd	10.54ijk	10.24	22.40de	24.38abc	23.39
Omega	10.06 bcd	11.27cd	10.66	22.80cde	23.50cdefg	23.15
Lasta	10.07 bcd	11.23cd	10.65	22.00ef	23.33cdefg	22.66
Milica	10.05 bcd	10.83efghi	10.44	23.63abc	23.65cdef	23.64
Partizanka	10.43abc	11.53c	10.98	23.67abc	24.00abcde	23.83
Pobeda	9.50de	10.73ghij	10.12	23.67abc	24.00abcde	23.83
Dična	9.99bcd	11.14cdef	10.56	23.00bcde	24.00abcde	23.50
NSR-5	9.96bcd	10.03l	10.00	23.10bcd	22.90efg	23.00
Alfa	9.59cde	11.07defg	10.33	24.00ab	25.00a	24.50
Rodna	9.50de	11.30cd	10.40	22.67cde	23.33cdefg	23.00
Agrounija	9.56cde	10.76fghij	10.16	22.27de	22.83fg	22.55
Zadruga	10.50ab	10.30kl	10.40	20.85g	21.23h	21.04
KG -75	10.55ab	12.35ab	11.45	21.08fg	23.20defg	22.14
Šumadinka	8.91e	10.97defgh	9.94	22.48de	22.40g	22.44
Levčanka	9.56cde	10.65hijk	10.10	23.67abc	23.73bcdef	23.70
Oplenka	8.98e	10.36jkl	9.67	23.08bcd	23.92abcdef	23.50
Gruža	9.56cde	10.93defghi	10.24	22.00ef	23.35cdefg	22.67
KG-56	9.41de	11.21cde	10.31	22.39de	24.05abcd	23.22
Average	**9.92**	**11.09**	**10.50**	**22.79**	**23.53**	**23.16**

The significant differences among the investigated wheat cultivars were established for the length of spike (Table 3). Also, the values of length spike of analysed genotypes were significantly different between first and second experimental years (Table 2). Generally, in average all studied wheat cultivar in both year expressed higher values in relation to average value of length of spike in the first year. This indicates response of genotypes to environmental conditions.

Differences among cultivars according to value of spike length are affected more by genotype than by relationships to the geographic origin (Dotlačil *et al.*, 2003). The length of spike controls by additive and nonadditive gene with prevalence of additive gene effects (Ljubičić *et al.*, 2014). Also, the sensitivity of length of spike under environmental variation noticed (Zečević et al., 2008; Knezevic *et al.*, 2014) and represent important components of wheat yield. The environmental factors as well temperature values, precipitation, nutrition have influence on increasing of capacity of spike (Petrović *et al.*, 2008; Knežević *et al.*, 2016) and grain yield (Marijanović *et al.*, 2010).

Table 3. Components of phenotypic variance for length of spike (cm) of wheat – in 1st and 2nd year

	First Year						Second Year					
Source of variance	DF	SS	MS	F	LSD		DF	SS	MS	F	LSD	
					0.05	0.01					0.05	0.01
Repetitions (R)	2	0.500	0.250	0.9270^{ns}	-	-	2	0.111	0.056	0.9978^{ns}	-	-
Genotypes (G)	19	20.606	1.085	4.0247**	0.886	1.212	19	23.938	1.260	22.6335**	0.404	0.553
Error	38	10.240	0.269	-	-	-	38	2.115	0.056	-	-	-
Total	59	31.345	-	-	-	-	59	26.165	-	-	-	-

The significant differences among the tested wheat cultivars, in both year of experiment, were established for the number of spikelet spike^{-1} (Table 4). The number of spikelets spike^{-1} at the analysed wheat cultivars variate and were significantly different among the cultivars and between the years of experiment. Generally, the all tested cultivars had higher number of spikelet spike^{-1} in second year than in first year of experiment (Table 2).

Table 4. Components of phenotypic variance for number of spikelets spike^{-1} in wheat

	First Year						Second Year					
Source of variance	DF	SS	MS	F	LSD		DF	SS	MS	F	LSD	
					0.05	0.01					0.05	0.01
Repetitions (R)	2	1.265	0.632	1.7152^{ns}	-	-	2	0.709	0.355	0.7949^{ns}	-	-
Genotypes (G)	19	47.646	2.508	6.8009**	1.038	1.419	19	41.244	2.171	4.8668**	1.141	1.560
Error	38	14.012	0.369	-	-	-	38	16.949	0.446	-	-	-
Total	59	62.922	-	-	-	-	59	58.902	-	-	-	-

The investigated trait highly depended to genetic and environmental factors (Zečević *et al.*, 2004; Dodig *et al.*, 2008). The spike length is yield components which highly positively correlated to number of spikelets spike^{-1} (Akram *et al.*, 2008). The spike length has strong indirect influence on yieald through number of spikelets spike^{-1} and futher on number of grain and size and weight of grain (Zečević *et al*., 2004). Improvements in the number of grains per spikelets related with increasing the number of grains spike^{-1}.

Conclusions

In this investigation the differences were determined among wheat genotypes according to values of length of spike and number of spikelets spike^{-1}. The highest values of length of spike expressed wheat cultivar Dejana (12.50cm) in the first experimental year while the least in wheat Šumadinka (8.91cm) in first experimental year, too. Breeding programs in the aim of improvement of spike traits of wheat need develop on the base of use wide germplasm resources and conduct in the different environments. Increasing of wheat grain yield is achievable through improving of all morphological, physiological traits of spike as well other organs of wheat.

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PISTACHIO KERNEL CONTENT OF MINERAL ELEMENTS, PROTEIN AND TOTAL OIL UNDER RAIN-FED CULTIVATION IN SYRIA

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Abstract

This investigation was conducted at Sweida Agricultural Research Center and farmers' fields at altitude of 900–1,100 m above the sea to study the chemical content of 13 pistachio genotypes including kernel dry oil content, protein, micro and macro-elements. The results showed the precedence of Syrian genotypes concerning their content of dry oil (58.43%) in comparison with introduced genotypes (one Iranian and two Turkish genotypes). Protein content differed among all studied genotypes and it ranged between 15.18%–29.59% indicating high nutritional values of local genotypes in parallel with introduced ones. Potassium content was virtually low (0.54–0.82%) in all genotypes, whereas phosphorus concentration was widely variable in the samples (0.3–0.64%). Indeed, nitrogen content in pistachio kernels was by some means in an intermediate level (2.942–5.192%). Micro-elements concentration was extremely low in pistachio kernels concerning several elements (Cu, Zn and Mn). Cupper level ranged 3.45-8.3 ppm. The genotype Ash.6"Iranian genotype" was recognized by its high level of zinc element comparing with all other studied genotypes (30.05 ppm). The content of Fe was at high concentrations in all samples (81.6- 191.5 ppm). The recent outcomes indicated a negligible content of calcium which was assigned to the low soil content. Mg content was likewise low in all studied genotypes (0.0179- 0.0994%). Hence, it is fundamental to project fertilization programs accompanied with proper irrigation regulations appropriate to different soil granular structures particularly concerning the content of Ca as a vital structural element.

Keywords: *Pistachio, proteins, dry oil, mineral elements.*

Introduction

Pistachio tree is recognized by many economic and environmental features which makes it as an important species (Hadj-Ibrahim *et al.*, 1998). This tree occupies the fifth grade among the most important commercially crops all over the world (Surucu and Demirkiran, 2013). Many wild species of the genus *Pistacia* native to Syria as a natural habitat, some of which are used as rootstocks, whereas others serve as pollinators for different pistachio cultivars: *P. atlantica*, *P. terebinthus*, *P. palaestina*, *P. khinjuck*. Alternate bearing phenomena is considered as undesirable behavior in pistachio tree which affects the absorption process of the main minerals from the soil according to on-off-bearing years. Farther more, it effects the distribution of mineral elements among all plant parts. Previous studies indicated that the range of root growth decreased in the on-bearing years in comparison with off years, but there is not a clear correlation between the growth of the root system and the absorption of some elements from the soil, consequently nut's demands of mineral elements control the absorption and nutritional system (Rosecrane *et al*., 2002). Pistachio nuts content of different mineral elements was affected by fertilization applications, cultivation practices, soil structure, and environmental circumstances (Siahnouri *et al*., 2013). Ghaseminasab Parizi *et al*. (2016) mentioned that the chemical composition of pistachio relies on cultivar, rootstock, maturity at harvest, and moisture content. The type and concentration of nutrients taken up by plant affect its development and eventually the quality and quantity of the fruits (Aznarte-Mellado *et al*., 2016). In most cultivated areas in Syria, pistachio trees are exposed to the

severe deficiency symptoms of mineral elements which lead to a hole leaves disrobe on the brunches, and in harder conditions it causes leaves disrobe of all the tree leaving the panicles exposed to the direct sunshine and that directly effects the bearing in the recent and coming years. Nutrition requirements of the plant differ according to the growth stage, yet there are few previous studies which discussed the content of mineral elements at ripening stage which considers as an important indicator about the deficiency of applied fertilization in the soil.
This research aimed to determine the chemical content of different pistachio genotypes and cultivars including the ratio of oil content as estimated to the dry weight, protein content and macro and microelements.

Material and methods

This research was conducted in the General Commission for Scientific Agricultural Research and in pistachio fields at the green belt area (950–1150 m.a.s.).
Thirteen different genotypes and cultivars of pistachio were examined: Ash. 1, Ash. 2, Ash. 3, Ash. 4, Ash. 5, Ash. 6, Ajam. 1, Ajam. 2, Bat. 1, Bat. 2, Bead. 1, Turk. 1, and Turk. 2.
Pistachio fruits were collected at full ripening stage (August and September), then the kernels were dried and grinded into powder.
Nitrogen content in both of soil and the grinded kernels was estimated by Kjeldahel method. Phosphorus content was calculated using spectrophotometer according to Olsen (1954), and potassium content was estimated by flame-photometer.
Oil content/ dry weight was estimated according to Horwitz (2000).
Raw protein content was estimated according to the nitrogen percentage and nutritional protein indicator of plant material.
- the Nitrogen percentage ratio of the crushed kernels was calculated according to the following formula (Rain *et al*., 2003):

$$\%\mathrm{N} = \frac{(\mathrm{V} - \mathrm{B})\mathrm{x\ NxR\ 14.01x\ 100}}{\mathrm{Wt\ x\ 1000}}$$

R: The ratio between the total volume of the digested sample and the consumptive volume of distillation;
B: The calibrated volume of the digested instance (ml);
Wt: Dry plant weight (g);
V: The consumptive volume of sulfate acid solution (0.01 N);
N: Molarity of sulfate acid solution;
The extraction of microelements in the soil was taken place using DTPA method according to (Jones, 2001), and all of microelements in both of soil and grinded kernels were estimated by atomic absorption.
The experiment was designed as randomized complete blocks (3 replications of each genotype, and 3 samples of each replicate), and the mean values were compared at LSD 5% level using Gen Stat program (ver. 12).

Results and Discussion

The analysis of soil including physical, chemical and mechanical characters of the studied locations are elucidated in Table (1).

Table 1: Soil physically and mechanically properties in the areas of pistachio.

Region	pH	EC	CaCO3	Organic material	K	P	Mechanic analysis (%)		
							Sand	Clay	Celtic
Loc. 1	7.64	0.31	7.94	0.74	528.75	25.87	21.83	60.67	17.33
Loc. 2	6.72	0.13	1.2	0.87	195.00	34.15	19.67	49.67	30.33
Loc. 3	6.83	0.12	0.43	0.94	110.00	20.55	20.50	31.00	48.50

Total dry oil content

The content of dry oil of pistachio kernels ranged between 48.16% in Ash. 4 genotype to 58.43% in Ash. 2 genotype which means that the variance between Ashouri's genotypes was larger than the difference among the investigated cultivars (Figure-1). Mahmoodabadi *et al*. (2012) indicated to close oil percentages in the Iranian cultivars (54.93- 55.40%). The lowest content of total oil was in Turkish cultivars Turk. 2 (49.68%) of significant differences in parallel to all other studied genotypes except Beadi cultivar (50.78%). In comparison with Okay (2002) the total percentage of oil content of Turkish cultivars ranged from 55.85 to 59.73%.

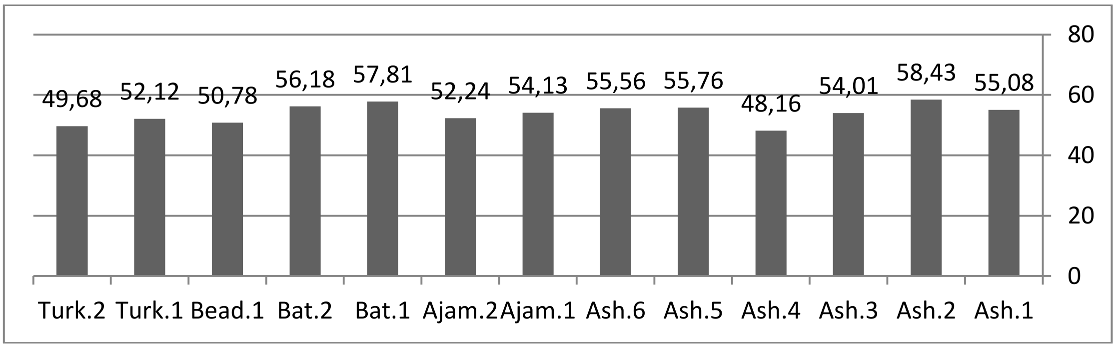


Figure-1: Dry oil content in all investigated genotypes

The content of protein in the genotype Ash. 2 was exceeded all other genotypes 29.5944 %. Two genotypes of Ashouri cultivar (Ash. 3 and Ash. 6) were adjacent to each other of their protein content (Figure-2). In parallel to previous studies Abdoshahi *et al*. (2011) mentioned that the protein content of different pistachio cultivars ranged between 16.265–20.703%. Similarly, Franz *et al*., (2002) pointed that the constituent of 100g kernel consisted of 15.0-21.2% protein.

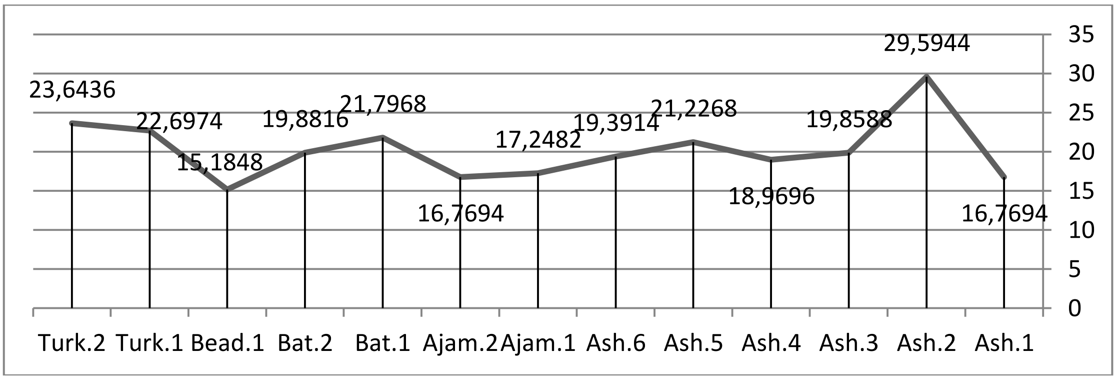
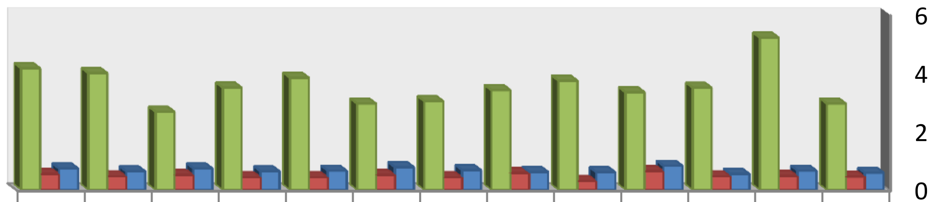


Figure. 2: Protein content in all investigated genotypes

The content of potassium was ranged between 0.54% in Ash. 3 genotype to 0.82% in Ash. 4 genotype. The contents of potassium in Beadi cultivar (Bead.1) and one Turkish genotype (Turk.2) were identical 0.73% (Figure 3). Tavallali and Rahemi (2007) indicated to high levels of potassium content in pistachio nuts in comparison with our results (1.28–2.2%).

The highest percentage of phosphorus showed significant difference in Ash. 4 genotype (0.64%) comparing with other studied genotypes except Ash. 6 genotype (Figure 3). Surucu and Demirkivan (2013) mentioned that the percentage of phosphorus content ranged between 0.08–0.13% in pistachio leaves. The standard content of P in pistachio kernel according to National Nutrients Database USDA (2018) is 0.490 %.

The highest nitrogen content was up to 5.19% in Ash. 2 genotype of significant differences with all examined genotypes except Turk. 2 genotype (4.15%), whereas the lowest percentage of nitrogen content was 2.66% in Bead.1 (Figure 3). Our results were in comparable percentages with Davarynejad *et al*. (2012) that the nitrogen content in *Pistcia atlantica* nuts ranged between 2.2– 4.5%.



	Tur k.2	Tur k.1	Bea d.1	Bat. 2	Bat. 1	Aja m.2	Aja m.1	Ash. 6	Ash. 5	Ash. 4	Ash. 3	Ash. 2	Ash. 1
% K بوتاسيوم	0,73	0,63	0,73	0,63	0,65	0,77	0,67	0,61	0,6	0,82	0,54	0,65	0,58
% P فوسفور	0,54	0,46	0,51	0,43	0,43	0,51	0,43	0,57	0,3	0,64	0,46	0,47	0,45
% N آزوت	4,15	3,98	2,66	3,49	3,82	2,94	3,03	3,4	3,72	3,33	3,48	5,19	2,94

Figure 3: N P K contents of investigated genotypes

Two Ashouri genotypes (Ash.2 and Ash.5) significantly exceeded all other investigated genotypes of their content of copper (8 and 8.3 ppm). In parallel to previous studies, Mohammadabadi *et al*. (2012) referred to high amounts of copper in pistachio nuts ranged between 15.05–17.07 ppm. National Nutrients Database USDA (2018) referred to standard content of Cu in pistachio nuts 13 ppm.

Zink content analysis showed that the genotype Ash. 6 was significantly exceeded all other genotypes 30.05 ppm (Table.2). However, non-significant differences were detected between Batouri's genotypes Bat.1 and Bat. 2 (22.45 and 24.4 ppm, respectively). In comparison with previous studies our results were almost low, Siahnouri *et al*. (2013) obtained high zink contents in pistachio nuts (upward to 44.5 ppm).

The genotype Ash. 2 significantly exceeded all investigated genotypes of iron content (191.5 ppm), followed by Ash.3 genotype (170.45 ppm) which was also of significant differences at 5% level in comparison with the concentrations in all other investigated genotypes (Table- 2). Afshari *et al*. (2008) indicated to lower iron content in pistachio nuts ranged between 43–49 ppm.

Manganese content extensively differed among genotypes and ranged between 14.5ppm in two genotypes Bat. 2 and Bead.1 to 17.7 ppm in Turk.1 genotype, while Turk.2 genotype was too low of its content (6.55ppm). National Nutrients Database USDA (2018) referred to a standard content of Mn in pistachio nuts 12ppm.

The highest amount of Mg was in Bat. 2 genotype (0.0994%) of significant differences in comparison with all other studied genotypes, whereas the lowest content was 0.017% in Ash. 5 genotype (Table2). Evoli *et al*. (2015) indicated to an average content of pistachio nuts 0.121%. Same results were obtained by Bullo *et al*. (2015) whereat the Mg content in pistachio raw nuts was also 0.121 %.

All genotypes were of excessive subminiature ratios of calcium content due to the low calcium percentage in all locations. The highest ratio of calcium element was 0.3562% in Bat. 1 genotype of significant differences in comparison with all other studied genotypes, whereas the lowest ratio was extremely exiguous (0.0367%) in Turk.1 genotype (Table2). In comparison with Ling *et al*. (2016) the content of Ca in partially defatted unroasted flour was 0.154.01±0.00427%. Accordingly, serious determination of fertilization programs should be applied for pistachio trees in such poor soils including classical amounts of calcium carbonate or lime stones to supply the normal demands of calcium element as an essential structural element in all plant parts.

Table 2. The content of Cu, Zn, Fe, Mn, Mg and Ca in investigated genotypes

Genotype	Cu ppm	Zn ppm	Fe ppm	Mn ppm	Mg %	Ca %
Ash.1	5 EFG	24.35 CDEF	82.4 F	6.75 CD	0.0552 C	0.0819 BC
Ash.2	8 AB	23.8 EFG	191.5 A	11.9 B	0.0231 E	0.0441 D
Ash.3	6.75 BC	21.9 G	170.45 B	7.2 C	0.0237 E	0.0403 D
Ash.4	4 GH	24.25 CDEF	125.6 D	7 CD	0.0181 E	0.0506 CD
Ash.5	8.3 A	26.3 BC	81.6 F	5.8 CD	0.0179 E	0.0415 D
Ash.6	4.25 FGH	30.05 A	52.35 H	5.5 CD	0.0374 D	0.0973 B
Ajam.1	5.2 EFG	19.35 H	67.85 G	5.45 CD	0.0244 E	0.0399 D
Ajam.2	6.3 CE	24.15 DEF	155.75 C	6.25 CD	0.0248 E	0.0474 D
Bat.1	6.2 CE	22.45 FG	123.05 D	5.35 CD	0.0389 D	0.3562 A
Bat.2	5.65 CEF	24.4 CDEF	85.65 F	4.15 D	0.0994 A	0.0826 B
Bead.1	4.3 FGH	25.05 CDE	156.75 C	4.15 D	0.0744 B	0.0832 B
Turk.1	3.9 GH	27.4 B	129.5 D	17.7 A	0.022 E	0.0367 D
Turk.2	3.45 H	26.2 BCD	108.1 E	6.55 CD	0.0233 E	0.0417 D
LSD 5%	**1.503**	**2.073**	**11.104**	**3.002**	**0.0124**	**0.032**

Conclusion

All Syrian pistachio genotypes were rich of their content of total dry oil, in addition to high levels of protein content especially the genotype Ash.2. the basic rocky material and climatic factors "basically rainfall rates" play an important role of determining the nature of pedological activities in the region, also it regulates the distribution of calcium carbonate and the variation of soil content of different elements and determining the quality and the quantity of all minerals and their reflects on the viability usage to be well absorbed. From nutritional point of view, plant analysis is built on the base that the concentration of any nutritional element inside the plant is a result of the total interaction of all factors that work on together to be achieved. Consequently, the lack of rainfall ratios besides to non-applying of the complimentary irrigation for fruit trees widely affected the convenience of macro and micro mineral elements to be easily absorbed especially concerning potassium availability. This investigation revealed that it is of paramount important to apply fertilization programs which is congruous with the chemical and the granulated structure of different soil types in expanding areas of pistachio cultivation. Moreover, the recent results confirm the importance of adding calcium carbonate (soil fertilization or foliar spray) as calcium supplementary for pistachio trees due to its indispensable role as a structural element in the plant.

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GENOTYPE ENVIRONMENT (GXE) INTERACTION AND ASSESSMENT OF BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES UNDER RAINFED CONDITION

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Abstract

Due to the various environment conditions yield and quality in bread wheat varies and GGE biplot analysis provides an easy and comprehensive solution to genotype by environment and it allows effective assessment of the genotypes and the target environments. In this experiment a total of 17 bread wheat genotypes were evaluated during 2008-2009 cycle at 4 environments under rainfed condition. The experiment was conducted in randomized completely block design with four replications. Grain yield, 1000-kernel weight, test weight, protein ratio, gluten value, gluten index, hardness and sedimentation were investigated. Mean grain yield across locations varied from 4742 kg ha^{-1} to 6601 kg ha^{-1}. Tekirdağ was the highest yielding location. Graphical result from PCI showed that the first principal component PC1, explained 42.85% of square interaction while the second principal component, PC2 explained 22.45% of square interaction. The result of PCA revealed that 2 principal components (PC1, PC2) contributed 65.30% of the total variability. The highest test weight and 1000-kernel weight in Tekirdağ location, protein ratio and gluten value in Edirne location were obtained. Cultivar Aldane was the best as it was more stable than the other genotypes based on sedimentation value, hardness, protein ratio and gluten value. The results of the study revealed that there was considerable variation among locations that could be used in selection of bread wheat genotypes for the development of cultivars. Cultivar Aldane was very stable for quality parameters and could be used in a breeding program to obtain quality characters of the genotypes.

Keywords: *Bread wheat, environment, quality parameters, GxE interaction.*

Introduction

Bread wheat (*Triticum aestivum* L.) is the most important and widely producing cereal crop throughout the Trakya region of Turkey. Although the amount of the rainfall (589.1 mm) during growing season is enough for wheat production, the distribution of this rainfall is not regular. This fluctuation of rainfall causes reducing grain yield and quality (Öztürk and Korkut, 2018a). Because of the various environment conditions yield and quality in wheat varies and GGE biplot analysis provides an easy and comprehensive solution to genotype by environment data analysis and it not only allows effective evaluation of the genotypes but also allows a comprehensive understanding of the target environment and the test environments (Öztürk and Korkut 2018b). Superior genotypes must be evaluated on the basis of multi-environment trials (MET) and multiple traits to ensure that the selected genotypes have acceptable performance in variable environments within the target region. For this reason, MET are conducted throughout the world for major crops every year in which multiple traits and characteristics are usually recorded (Yan and Rajcan, 2002). To develop varieties for different environments, very essential for breeders to evaluate their genotypes based on many years and several locations. Environmental variations are important in determining performance of elite materials. Genotype ranks consistently across different tested location has less response for highly unstable environment (Solomon et. al., 2018). Most breeding programs face complex mega-environments with unpredictable GEI and genotype evaluation based on mean performance and stability has been a perennial problem and challenge (Yan and Kang, 2003). Performance trials have to be conducted in multiple environments because of the presence of GE. Variety trials provide essential information for selecting and recommending crop cultivars. However, variety trial data are rarely utilized to their full capacity. Although data may be collected for many traits, analysis may be limited to a single trait (usually yield) and information on other traits is often left unexplored (Yan and Tinker, 2006). The objective of the study was to evaluate the performance of the advance genotypes and to investigate their yield stability and genotype-by-environment interactions under rainfed environment conditions.

Material and Methods

The experiment was conducted to assess genotype x environment interaction and diversity by biplot analysis for yield, and quality parameters. In this experiment a total of 17 bread wheat genotypes were evaluated under rainfed condition, during 2008-2009 cycle at four locations of Trakya Region, Turkey. Each plot was 6 meter long and had 6 rows, spaced 0.17 meters apart. A seed rate of 500 seeds m^2 was used. The experiment was conducted in randomized completely blocks design (RCBD) with four replications. Grain yield and quality parameters were investigated. In the study, 1000-kernel weights and test weight were determined by Blakeney et al., 2009, protein ratio, grain hardness, gluten value, gluten index, and sedimentation by Anonymous, 2002; Köksel et al., 2000; Perten, 1990, and Pena, 2008. The quality analysis of Zeleny sedimentation test and wet gluten content were determined according to ICC standard methods No. 116/1 and 106/2, respectively (Anonymous, 1972; Anonymous, 1984). In order to visually display relations of tested parameters and genotypes multivariate biplot analysis (genotype by trait biplot), described by Yan and Rajcan, (2002), Yan and Tinker (2006) and Yan and Kang (2003) was used. A positive correlation between two traits is represented by an acute angle between them, while obtuse angle represents a negative correlation. Correlations between all characteristics were calculated. Data were analysed statistically for analysis of variance the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test (Kalaycı, 2005).

Results and Discussion

Trakya region has various environment conditions. A genotype having stabile grain yield across the environment condition is very important in wheat. Genotypes (G) x Environment (E) interaction is a mainly issue for plant breeders in improving high yield across variable environments. Seventeen advanced genotypes were tested at four locations to investigate yield and quality components based on Principal Component Analysis (PCA). The analysis variance for yield and quality components was performed and given in Table 1, and 2. The results of variance analyses showed that there were significant differences ($P<0.01$) among genotypes based on locations. Mean grain yield across four locations ranged from the highest 6601 kg ha^{-1} to the smallest 4742 kg ha^{-1}, and mean grain yield was 5903 kg ha^{-1}. The highest grain yield performed by lines G12 and followed by G19 and G11 (Table 2). Because of the favorable environment condition Tekirdağ was the highest yielding location. The mean grain yield for tested genotypes was lower relative in Lüleburgaz location.

Table 1. Combined analysis of variance for wheat genotypes across four environments.

Source of variation	DF	SS	MS	F value
Environment	3	520031.86	173.34	51.87**
Genotype	16	127343.90	7.96	2.38*
Error	48	160406.59	3341.80	
Total	67	807782.35		

Note: *, ** Significance at respectively 5%, and 1% level probability.

There were significant differences among genotypes based on TKW and TW. 1000-kernel weights ranged from 28.5 g to 44.8 g among genotypes and mean was 36.9 g. Line G2 had the highest TKW and followed by Pehlivan, Aldane and Bezostaya cultivars. TW ranged in genotypes from 75.4 kg to 82.4 kg (Bezostaya) and mean value of the test weight was 78.9 kg.

Table 2. Mean performance of quality parameters at four environments conditions.

No	Genotype	GY	TKW	TW	PRT	GLT	IND	HARD	SED
1	Bezostaya	4742 d	42.3 abc	82.4 a	12.0 ab	32.4 ab	86.6 bcd	51.5 ab	41.8 bc
2	G2	6130 ab	44.8 a	81.2 ab	11.2 bc	30.4 a-d	90.6 abc	51.3 ab	41.3 bc
3	G3	5888 abc	36.9 def	76.6 fg	11.5 abc	29.8 a-d	90.5 abc	46.8 c	37.8 cd
4	G4	5682 bc	32.3 gh	77.0 d-g	11.5 abc	31.9 abc	83.7 cd	49.3 abc	38.5 cd
5	Pehlivan	5881 abc	43.8 a	80.4 abc	10.7 bc	30.5 a-d	78.5 de	48.5 bc	36.5 cd
6	G7	5594 bc	28.5 h	75.4 g	11.4 abc	31.3 a-d	85.3 cd	50.5 ab	40.5 bcd
7	G8	5297 cd	33.3 fg	76.9 efg	11.3 bc	27.7 b-e	94.6 ab	49.3 abc	40.3 bcd
8	Flamura85	5852 abc	39.2 bcd	80.9 abc	11.7 abc	29.0 b-e	96.2 a	49.0 abc	42.3 bc
9	G11	6374 ab	31.1 gh	79.3 bcd	11.1 bc	27.0 c-f	86.4 bcd	50.0 ab	34.5 de
10	G12	6601 a	31.0 gh	78.0 def	11.3 bc	26.3 def	97.3 a	51.8 a	45.0 ab
11	Kate A-1	6242 ab	34.9 efg	80.4 abc	10.8 bc	32.1 abc	71.9 e	51.3 ab	36.5 cd
12	G16	5989 abc	38.5 cde	77.6 d-g	10.4 c	21.8 f	94.2 ab	37.0 e	30.0 e
13	G19	6398 ab	31.3 gh	77.6 d-g	11.1 bc	29.6 bcd	86.8 bcd	50.3 ab	38.3 cd
14	Gelibolu	6100 abc	36.7 def	79.2 bcd	10.9 bc	24.0 ef	96.8 a	41.5 d	37.5 cd
15	G22	5666 bc	38.4 cde	80.3 abc	11.6 abc	31.8 abc	83.7 cd	39.8 de	39.0 bcd
16	Aldane	5748 bc	42.8 ab	80.6 abc	12.7 a	35.0 a	92.0 abc	50.0 ab	51.0 a
17	Tekirdağ	6174 ab	41.9 abc	78.9 cde	11.3 bc	30.8 a-d	84.6 cd	49.5 abc	39.0 bcd
Mean		5903	36.9	78.9	11.3	29.4	88.2	48.1	39.4
LSD (0.05)		81.74	3.98	2.27	1.34	5.26	8.66	3.18	6.06
F value		2.38*	13.09**	5.81**	1.21ns	3.11**	4.99**	15.07**	4.38**

Note: **: P<0.01, *: P<0.05, ns: not significant, TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten value (%), IND: Gluten index, HARD: Hardness, SED: Sedimentation (ml)

Grain protein content is among the key determinants affecting of both end use and market value in wheat. Protein quality and quantity is the most important components of wheat grains governing end-use quality (Pena, 2008). Table 2 shows mean and range of variations for protein ratio in all genotypes were evaluated and found protein ratio varied from 10.4% to 12.74% in the genotypes. The mean protein content was 11.3% and the highest protein ratio was determined in Aldane and Bezostaya. Gluten value of genotypes ranged from 21.8% to 35.0% and mean was 29.4%, the highest gluten value was determined in cultivar Aldane. In addition, there was significant difference for sedimentation and cultivar Aldane had highest sedimentation value.

Figure 1a showed that to rank the genotypes based on performance in any environment, and to rank environments on the relative performance of any genotype. To develop varieties for different environments, very essential for breeders to evaluate their genotypes based on many years and several locations. Based on grain yield graphical result from PCI showed that the first principal component PC1, explained 47.09% of interaction some of square while the second principal component, PC2 explained 35.52% of some of square interaction (Figure 1a). The result of PCA revealed that the 2 principal components (PC1, PC2) contributed 82.61% of the total variability.

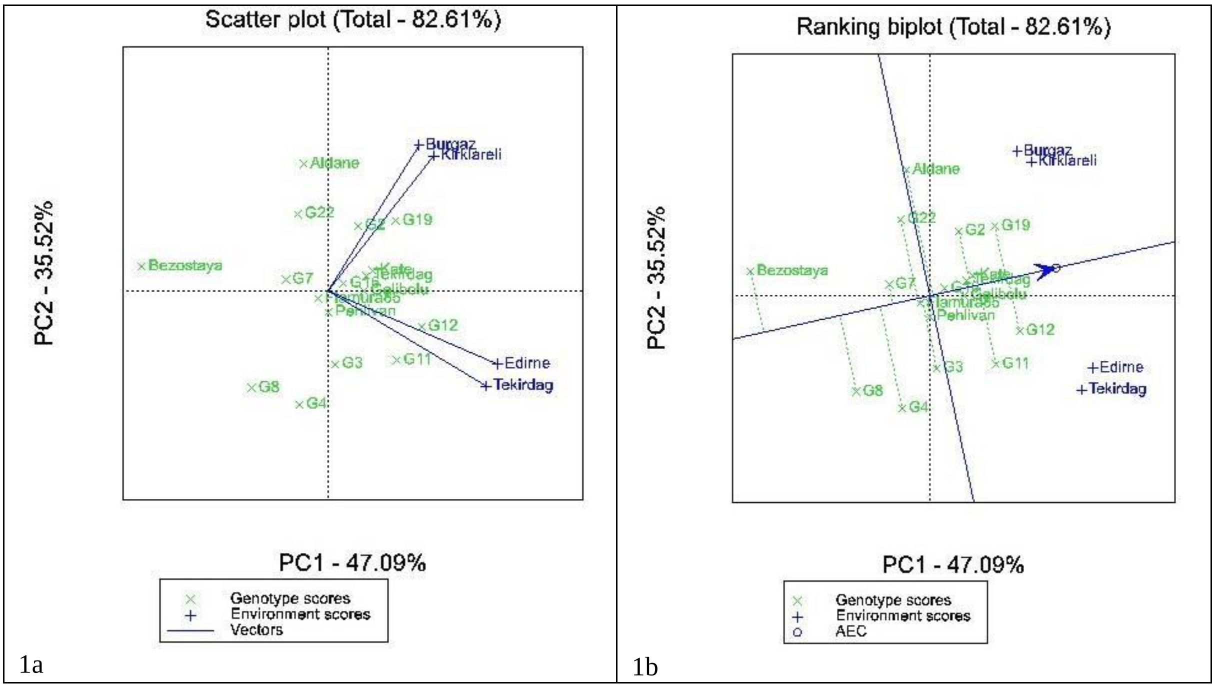

Figure 1. The GGE biplot showing the performance of each genotype in each environment (1a). Ranking genotypes based on performance across four environments (1b)

To rank the genotypes based on their performance across environment, a line is drawn that passes through the biplot origin and the environment. This line is called the axis for this environment, and along it is the ranking of the genotypes. Figure 1b ranks the genotypes based on performance across environments. Genotypes Bezostaya, G8, G4, and G7 had lower than average yield, cultivars Pehlivan, and Flamura85 had near average yield, and all others had higher than average yields. The highest yielder across environments was G12 line, and the lowest yielder cultivar Bezostaya.
A genotype by trait biplot can help understand the relationships among traits and can help identify traits that are positively or negatively associated, traits that are redundantly measured, and traits that can be used in indirect selection for another trait (Yan and Tinker, 2006; Yan and Kang 2003). The biplot in Figure 2a presents data of 17 genotypes determined for seven parameters. Across the 17 tested genotypes, sedimentation with protein ratio, protein ratio with gluten value, and test weight with 1000-kernel weight was positively associated. Gluten index with test weight and 1000-kernel weight was negatively correlated (Figure 2a).
The vertices of the polygon were the genotype markers located farthest away from the biplot origin in various directions, such that all genotype markers were contained within the resulting polygon. The vertex genotype in each sector represented the highest quality genotype in the environment that fell within that particular sector. The polygon view of the GGE-biplot analysis helps one detect cross-over and non-crossover genotype-by-environment interaction and possible mega environments in multi-location yield trials (Yan et al., 2007). Based on the GGE analysis the first two principal components explained about 65.30% of the total interaction variation. Aldane was the best genotype based on gluten, protein ratio, sedimentation value, and hardness. According to Figure 2b, the vertex genotypes were Aldane, G7, G16, Pehlivan, and Bezostaya. These genotypes were the best or the poorest genotypes based on quality parameters in some or all of the test environments since they had the longest distance from the origin of the biplot (Figure 2b).

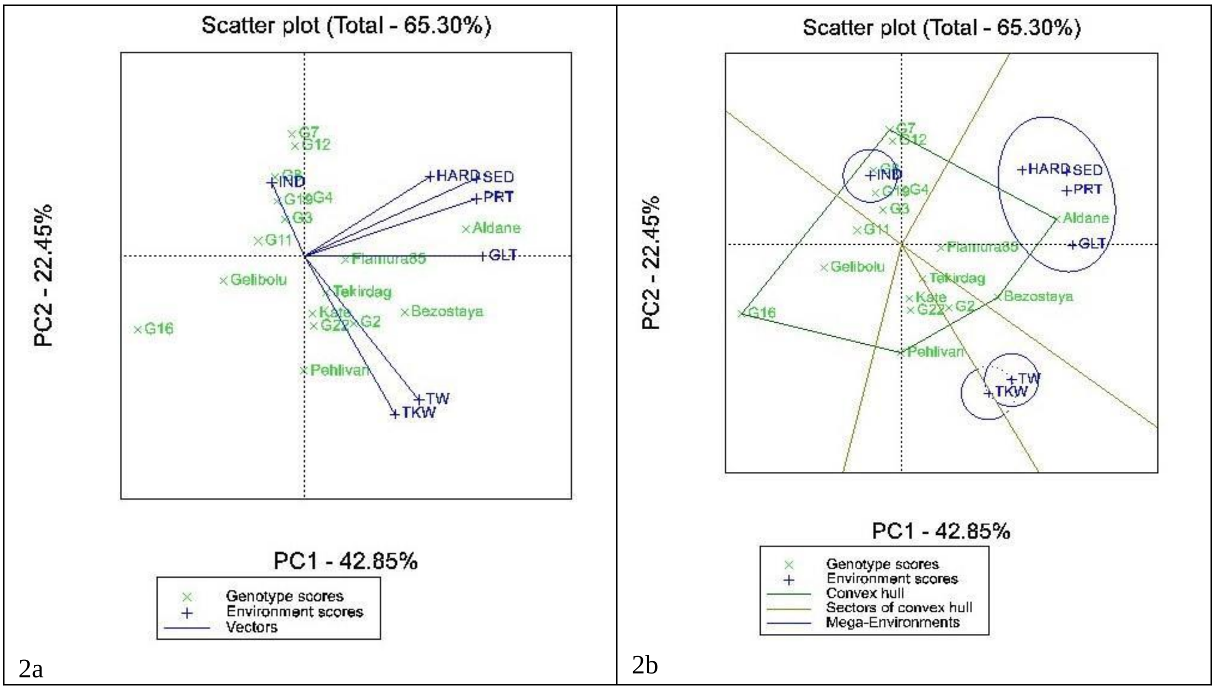

Figure 2. The GGE biplot to show which genotypes performed bets by trait biplot representing 17 winter wheat genotypes measured for 11 parameters (2a). Polygon view of the GGE biplot based on environment and investigated parameters and genotypes (2b)

Correlation coefficients based on parameters were determined by Pearson's correlation analysis (Table 3). It was found highly significant positive correlation between test weight and 1000-kernel weight (r=0.722**). There was a negative slightly relation with grain yield and TKW, TW, protein ratio and gluten value. In the study, no correlation was found between grain yield with gluten index and hardness. Protein ratio was highly significant positively correlated with gluten value (r=0.669**), and sedimentation (r=0.843**). The positive correlation between sedimentation with gluten value (r=0.566*) and hardness (r=0.514*) were significant. Gluten value was negatively associated with gluten index (r=-0.548*).

Table 3. Coefficients of correlation between grain yield and tested parameters.

Traits	GY	TKW	TW	PRT	GLT	IND	HARD
TKW	-0.249						
TW	-0.155	0.722**					
PRT	-0.459	0.200	0.255				
GLT	-0.359	0.252	0.307	0.669**			
IND	0.024	-0.024	-0.163	0.187	-0.548*		
HARD	0.015	-0.122	0.110	0.346	0.540*	-0.231	
SED	-0.188	0.176	0.249	0.843**	0.566*	0.280	0.514*

Note: **: P<0.01, *: P<0.05, GY: Grain yield (kg da^{-1}), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten (%), IND: Gluten index (%), HARD: Hardness, SED: Sedimentation (ml)

Conclusions

Trakya region has various environment condition so genotype x environment interaction is a mainly issue in improving high yielding genotypes across variable environments. The results of analyses indicated that wheat grain yield and other quality parameters were highly affected by environmental effect followed by the magnitude of GEI. GGE biplot analysis permitted estimation of interaction effect of a genotype in each environment. To develop varieties for different environments, very essential for breeders to evaluate genotypes based on many years and several locations. The highest yielder genotype was G12, and the lowest yielder

Bezostaya. According to results, sedimentation with protein ratio, protein ratio with gluten value, TW and TKW was positively associated. Gluten index with TW and TKW was negatively correlated. Cultivar Aldane was the best genotype as it was more stable than the other genotypes based on sedimentation value, hardness, protein ratio and gluten value. The results of the study revealed that there was considerable variation among locations that could be used in selection of bread wheat genotypes for the development of cultivars. Cultivar Aldane was very stable for quality parameters and could be used in a breeding program to obtain quality characters of the genotypes.

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INVESTIGATION OF THE EFFECT OF CALCIUM APPLICATIONS ON PLANT DEVELOPMENT OF PEPPER PLANT IN SALT STRESS

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Abstract

The Study was carried out in the climate room where normal atmosphere was provided in the Plant Physiology Laboratory. The main purpose of the normal atmosphere in the experiment is ensure that the effects of salt stress occur as normal circumstance. Thus, the stress level to which the plants are exposed in the outdoor environment will be measured with the least margin of error and the results obtained as a result of the application of the results obtained will show greater consistency with the study results. Morphological effects of calcium (Ca) applied in different doses to pepper plant under salt stress were investigated in this study. The study was carried out under controlled conditions in 16/8 hour light / dark photoperiod, 25^{o}C and 70% humid climate room. Root weight, stem weight, leaf weight, number of leaves, plant height and total plant weight were measured. In addition, salt resistance scales of plants were determined. In the growth parameters, the 1st (150 ppm) and 2nd (200 ppm) doses of Ca applications showed the highest decrease compared to the control. As the dose increased, the values approached the control but started to decrease again at the 5th (350 ppm) dose of calcium. In the scale evaluation, which was a morphological observation, the most damaged plants were observed at the 1st (150 ppm), 2nd (200 ppm), 3rd (250 ppm) and 5th (350 ppm) doses of calcium respectively, the least damage was the 4th (300 ppm) dose of calcium.

Keywords: *Pepper (Capsicum annum), Calcium, NaCl, Salt stress*

Introduction

Salinity problem arises naturally in arid and semi-arid areas where rainfall is low and evaporation is high. The main causes of soil salinity are the accumulation of soluble salts in soil layers and ground water, or transport of salts to the soil surface due to the rise of existing ground water, poor water quality in irrigation water, the presence of excess soluble salts in irrigation waters, and insufficient drainage in the root zone (Epstein et al., 1980; Stushnoff and Quamme, 1983).

Munns and Termaat (1986), Snap and Shennan (1992), Yasar et al. (2007a), Karanlık (2001) reported that the development of plants under salt stress in their studies adversely affected.

Salt stress is affected by growth and development, photosynthesis, protein synthesis, energy and lipid metabolism. In essence, the first response to salt stress manifests itself as a decrease in the growth of leaf surface area (Üzal, 2009).

Calcium (Ca + 2), an important macro nutrient, acts as the central regulator of plant growth and development. There is competition and exchange between calcium (Ca), potassium (K) and sodium (Na) in the regions of cell membranes. Ca in the form of pectins in the cell wall, cell walls and tissues undertook the basic task of strengthening (Kacar and Katkat, 2006). In plants under salt stress, it has been reported that potassium is a cofactor for many enzymes and may reduce the harmful effect of NaCl by external application of Ca (Hasegawa et al., 2000). High salt concentrations reduce the calcium uptake and transport of the plant and cause calcium deficiency (Cramer et al., 1986; Huang and Redmann, 1995). Calcium is an element that has a positive effect on salt stress. High doses of exogenous calcium reduce the permeability of the cell membrane to Na + ion. In this way, the accumulation of sodium in the cell and plant is prevented by passive uptake (Hoffman et al., 1989; Whittington and Smith,

1992). The common ideas of researchers trying to explain the role of calcium in the protection of salt stress through various mechanisms; calcium to strengthen the cell membrane and ion selectivity in the uptake and transport. Ca2 + ion, cross-linking the cell membrane with negatively charged basic groups and thus preserving the structural integrity of the cell membrane is also made in the description (Cramer et al., 1986; Lauchli, 1990). Rengel (1992), the calcium membrane of the cell membrane by controlling the permeability of the cell, the calcium in the cell prevents the release of the proposed.
In this study, morphological aspects of calcium (Ca) effects in pepper plant under salt stress were investigated. In accordance with this purpose, the effects of the application on the salt stress were determined by applying calcium compounds to pepper plant exposed to salt stress.

Material and Methods

This research was carried out in the climatic room of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Horticulture, Physiology Laboratory. Demre sharp pepper variety was used in the study.
The experiment was carried out in a split air-conditioned climate room with normal atmosphere and water culture. The main objective of the experiment is to ensure that the effects of salt stress occur as normal under normal conditions. In this way, the stress level to which the plants are exposed in the outdoor environment will be measured with the least margin of error and the results obtained as a result of the application of the results obtained will show greater consistency with the study results.
In the study, first of all, pepper seeds were sown in 40x25x5 cm plastic germination cups filled with pumice sieved and then watered with fountain water. This irrigation method has been used since it is not possible to use normal irrigation water or water in the environment where pepper is grown for internal irrigation. The bottom surface of the germination vessels has 9 holes with a diameter of 0.5 cm and the irrigation water is drained by the plants. After the pumice was thoroughly wetted and the irrigation water was drained, the germination pots were placed in the climate room with 25±2^{o}C temperature 70% humidity, covered with A4 paper and the containers were regularly checked and the pumice was continued to be irrigated with tap water. For the better development of the seedlings, the cotyledon leaves coming horizontally and the first true leaves (3-4) began to be seen, irrigation was started with Hoagland nutrient solution. (Hoagland and Arnon, 1938).

Table 1. Contents of the nutrient solution used (ppm).

Elements	App. 1 Control (ppm)	App. 2 Ca1+ NaCl (ppm)	App. 3 Ca2+ NaCl (ppm)	App. 4 Ca3+NaCl (ppm)	App. 5 Ca4+ NaCl (ppm)	App. 6 Ca5 + NaCl (ppm)
Nitrogen (N)	186	186	186	186	186	186
Phosphorus (P)	31	31	31	31	31	31
Potassium (K)	167	167	167	167	167	167
Magnesium (Mg)	49,28	49,28	49,28	49,28	49,28	49,28
Calcium (Ca)	**200**	**150**	**200**	**250**	**300**	**350**
Sulfur (S)	66	66	66	66	66	66
Iron (Fe)	3.3	3.3	3.3	3.3	3.3	3.3
Manganese (Mn)	0.031	0.031	0.031	0.031	0.031	0.031
Boron (B)	0.205	0.205	0.205	0.205	0.205	0.205
Copper(Cu)	0.015	0.015	0.015	0.015	0.015	0.015
Zinc (Zn)	0.023	0.023	0.023	0.023	0.023	0.023

The nutrient solution used was prepared according to (Hoagland and Arnon, 1938).
Seedlings, which also formed the 2nd true leaves in the pumice environment, were taken to water culture in 25x25x18 cm size plastic cuvettes filled with Hoagland nutrient solution.

Specially prepared and perforated for each seedling in plastic trays pepper seedlings were placed by wrapping with small sponge pieces. The trays were placed on the trays with the plant roots in the nutrient solution.
The aeration was done by immersing the thin plastic hoses connected to the aquarium pump into the nutrient solution. Seedlings were grown in water culture for two weeks and salt application was started on seedlings having 4-5 real leaves. The experiment was designed according to the exact chance design with three replications and 15 replicates per repetition. NaCl was added to the nutrient solution (1/2 Hoagland) with a salt concentration of 75 mM. During the regeneration of the repeating solutions every week, the same concentration of salt applications was maintained. Pepper seedlings with salt (NaCl) 5 different doses (150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm) Ca was added. As a result, 6 different applications were performed as control, salt + Ca (150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm). The ppm values of all nutrients in the nutrient solution are given in Table 1.
Sampling for measurements and analyzes was performed twice before the salt application (day 0) and on the 20th day of salt application. In these samples, some basic growth parameters (root weight (g), stem weight (g), leaf weight (g), total plant weight (g), plant height (cm), number of leaves (number), salt resistance scale were determined.
Determination of root weight, stem weight, leaf weight and total plant weight were weighed in 3 replicates with 1 / 10,000 precision digital balance. Plant height was measured in cm with ruler. The number of leaves is indicated in pieces.
A scale was established to determine the degree of damage that occurs morphologically in plants. For this purpose, plants were scored between 1-5 according to the degree of damage. Salt stress test pepper plants according to the following symptoms from 1 to 5 points were given (Üzal, 2009).
1: Plants are not affected by salt stress at all (control plants)
2: Local yellowing and curling of leaves
3: Yellowing of leaves and 25% necrotic spotting
4: 50-75% necrotic stain on leaves and deaths
5: Severe necrosis of 75-100% in leaves and complete death of plant
According to the experiment design randomized parcels with 3 replications were established as 15 plants per repetition. In order to evaluate the data obtained as a result of the study, Statgraphics was subjected to variance analysis in statistical analysis package program. Duncan multiple comparison test ($P < 0.05$).

Results and discussion

Table 2.Some growth and development parameters in the samples taken after the applications.

App..	Root weight (g)	Stem weight (g)	Leaf weight (g)	Total plant weight (g)	Plant height (cm)	number of leaves. (piece)
Control	3,804 A	3,497 A	10,362 A	17,664 A	15,4 A	14,0 A
Ca 1 +Salt	1,251 D	1,264 E	4,581 D	7,096 E	9,1 D	9,8 C
Ca 2 + Salt	1,046 D	2,087 D	5,269 D	8,402 D	14,9 AB	12,0 B
Ca 3+ Salt	2,454 C	3,008 B	6,743 C	12,206 C	14,1 B	11,4 B
Ca 4+ Salt	2,865 B	3,432 A	7,819 B	14,117 B	11,8 C	14,0 A
Ca 5+ Salt	2,724 B	2,703 C	6,653 C	12,081 C	12,6 C	12,4 B
P Value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

The difference between the means taking the same capital letter in the same column is insignificant according to $P \leq 0.05$.

The root weight (g), stem weight (g), leaf weight (g), total plant weight (g), plant height (cm) and number of leaves (pieces) of the Demre pepper plants subjected to salt stress at the end of the 20th day were measured and obtained values were given in Table 2.

As a result of 20-day salt stress, statistically significant decreases were observed in root weights (g) of all other salt treatments compared to the control group. The highest values of root weight among salt treated plants were measured in control (3.804) group, while the closest values to the control group were measured in Ca 4 + Salt (2.865) and Ca 5 + Salt (2.724) applications. The lowest values were measured in Ca 2 + Salt (1.046) and Ca 1 + Salt (1.251) applications in the same group. The highest values of body weight were measured in the control group (3,497), while the closest value to the control group was measured using Ca 4 + Salt (3,432) in the same group. The lowest value was determined in Ca 1 + Salt (1.264) application. It was seen that there were differences between salt applications in terms of body weights. The highest value in terms of leaf weight was measured in the control group (10.362 g), while the closest value to the control group was measured in Ca 4 + Salt (7.819) application. The lowest values of the same statistical group in the Ca 1 + Salt (4.581) and Ca 2+ Salt (5.269) were measured in the applications. There were differences between the salt applications in terms of leaf weight. The highest value in terms of total plant weight was measured in the control group (17.664), while the closest value to the control group was measured in Ca 4 + Salt (14.117) application. The lowest value was determined in Ca 1 + Salt (7,096) application. Ca 3+ Salt and Ca 5 + Salt were in the same group. There were generally differences between salt applications in terms of plant weight. The highest value in terms of plant height was measured in the control (15.4) group, while the closest value to the control group was measured in Ca 2 + Salt (14.9) application. The lowest value was measured in Ca 1 + Salt (9.1) application. Ca 4+ Salt and Ca 5+ Salt were in the same statistical group. There were differences between salt applications in terms of plant height. When the number of leaves was examined, the highest value was obtained from the plants in the control group (14.0), while the lowest value was measured in Ca1 + Salt (9.8) application. It was determined that the application having the same number of leaves as control plants was Ca 4+ Salt (14.0), Ca 2+ Salt, Ca 3+ Salt and Ca 5+ Salt were included in the statistical group. It was noteworthy that there were generally statistical differences ($P \leq 0.05$) between salt applications in terms of all plant growth parameters.

Scale values according to leaf symptoms

Seedlings were given a score of 1 to 5 as indicated in the scale-forming method used to demonstrate the degree of damage that occurs morphologically in plants (Table 3).

Table 3. Salt resistance scale according to the symptoms in the leaves (score)

Application	Scale Values
Control	1
Ca 1+ NaCl	4,5
Ca 2+ NaCl	3
Ca 3+ NaCl	2,5
Ca 4+ NaCl	1,5
Ca 5+ NaCl	2

High scoring values are the most affected by salt.

When the scale values are examined, it is seen that the least affected plants are in Ca 4+ NaCl application. This is followed by Ca 5+ NaCl, Ca 3+ NaCl and Ca 2+ NaCl applications respectively. The most morphologically damaged application is Ca 1 + NaCl application.

75 mM NaCl salt treated pepper plants were given nutrient solution containing Ca element in different doses. On the 20th day of stress application, growth parameters such as root, stem and leaf weight, plant height, number of leaves and total plant weight of pepper plants showed the highest decrease in the 1st and 2nd doses of Ca applications compared to the control. However, as the dose increased, the values approached the control but started to decrease

again at the 5th dose of calcium. Yaşar (2003) and Yaşar et al. (2007a, 2007b, 2013, 2016) stated similar results in plant growth parameters in their salinity stress studies with different species and stated that total plant weights were an important parameter in determining the response to salt stress. It reduces calcium uptake and transport in plants with high concentrations of salt, thus causing calcium deficiency and ion imbalance in the plant (Cramer et al., 1986; Huang and Redmann, 1995). Due to calcium is an element having a positive effect on salt stress, high dose exogenous calcium decreases the permeability of the cell membrane against Na + ion, prevents the accumulation of sodium in the cell and in the plant by passive uptake and provides ion balance (Hoffman et al., 1989; Whittington and Smith, 1992), therefore, that calcium protects plants against salt stress and is therefore less susceptible to salt stress. The results of our study showed that due to the deterioration in the ion balance of the plants in saline environments, growth and development of plants decreased due to the slowing of the respiration of the plants. The deterioration of the respiratory system affects the entire metabolic system, especially slowing down in the photosynthesis system of the plant, and consequently a decrease in the formation of assimilation occurs and decrease in plant growth and development (Çakırlar and Topçuoğlu, 1985; Yasar 2003; Yasar, 2007).
In our study, in order to provide control of all growth we examined in the scale evaluation, which is a morphological observation, by applying salt in our study, the most damaged plants were observed at the 1st dose of calcium according to the degree of damage. It was seen in 2nd, 3rd and 5th doses respectively. The least damage was the 4th dose. Aktaş (2002) in pepper, Yaşar (2003) in eggplant, Öztaş (2018) benefited from the scale they created in their studies in pepper. Both of these researchers indicated that the scale value had a very high correlative relationship with the total plant weights and especially the ratios of Ca / Na and K / Na ions.

Conclusions

Appropriate doses of calcium can protect the plant from the toxic effect of salt by maintaining ion balance in the cell, even under salt stress. As a result, Ca kept the plant at a level that could control the plant by limiting plant growth in order to control metabolic activity. In terms of both growth parameters of plants and scale values indicating the degree of damage of salt in the plant; It was concluded that the most appropriate Ca dose to be applied to the plants under salt stress conditions is the 4th dose (250 ppm).

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INFLUENCE OF TWO APPLE ROOTSTOCKS ON LEAVES AND FRUITS MINERAL CONTENT OF STARKRIMSON CULTIVAR UNDER RAINFED CONDITIONS

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Abstract

This investigation was conducted at Pome and Grapevine Division- Horticulture administration- GCSAR in Sweida governorate under rainfed conditions during 2014-2015 to study the influence of two apple rootstocks (*Malus domestica* Borkh and MM106 grafted by Starkrimson cultivar) on K, Ca and Mg mineral concentrations in leaves and fruits during the growth season. The results showed that *M. domestica* significantly revealed higher K concentration than MM106 rootstock of leaves and fruits in general, whereas MM106 revealed higher concentration of Ca in leaves and fruits. Mg concentration revealed discrepant rate among two rootstocks during the growth season. The highest leaves and fruits K concentration was in August in *M. domestica* (1.57%),while in MM106 rootstock (1.30%)it was in June. On the other hand, the highest leaves content of Ca was in October in two rootstocks which was below the sufficient range. Two rootstocks exposed the same rate of fruit Mg and Ca mineral concentrations which were decreased through the growth season. The K/Ca and K+Mg/Ca ratios in leaves and fruits "as an indicator for biter pit physiological disorder" were calculated for two rootstocks, which varied depending on growth stage analysis. Consequently, the results led to establishing an efficient strategy for fertilization management of apple orchards depending on the fertilizers requirements and nutrient accumulation track during growth season.

Key words: *apple, rootstock, Starkrimson, mineral concentrations*.

Introduction

Rootstocks are the main factor affecting tree growth and show different responses to nutrient (Kucukyumuk and Erdal, 2011), and effect on the concentration of leaf scion minerals of apple trees (Tagliavini, 1992). However, the ability of mineral uptake of rootstocks and cultivars should be considered for plant growth, yield and to get better fruit quality (Campeano *et al*., 2009). The different nutrient uptake capacity of apple rootstocks can be illuminated as a genetic effect on the nutrient concentration of trees (Jimenez *et al*., 2007, Kucukyumuk and Erdal, 2009). Somehow, the effects of rootstocks are not stable from site to site (Yahia *et al*., 2004) and depend on the structure and root growth which influence mineral uptake (Jimenez *et al*., 2004; Jobir *et al*., 2017). The analysis of growth parts in apple trees was taken into account to determine the nutrient requirements especially in fruits to provide sufficient information about fruit quality depending on previous known about adequate and critical nutrient content, then build the fertilization strategies to prevent mineral deficiencies and physiological disorders in fruits (Ernani *et al.*, 2002; Nachtigall and Dechen, 2006).The nutrient concentration rate during growth season is an important indicator of nutrient demand in each development stage in apple trees, many studies were achieved to evaluate the leaves and fruit nutrient seasonality accumulation of apple cultivars ((Nurzynski *et al*., 1990; Cheng and Raba, 2009; Jivan and Sala, 2014) .

In Syria, the nutrient concentration of vegetative growth in apple trees was evaluated in few sites and included the two main cultivars Golden Delicious and Starking Delicious (Muzher *et al*., 2016; Wassouf *et al*. 2017), furthermore the distribution of new economic apple cultivars and rootstocks and the application of new strategies of foliar fertilization on apple trees require enough information about the seasonality accumulation of nutrients especially where apple production is concentrated. Therefore, the current investigation aimed to study the influence of the two apple rootstocks *M. domestica* and MM106 which grafted by Starkrimson cultivar on seasonality accumulations of K, Ca and Mg mineral nutrients in leaves and fruits in Sweida governorate at the south of Syria which considered as the main important state of apple production.

Material and Methods

The current research was conducted at Pome and Grapevine Division- GCSAR which located 1,525 m altitude in Sweida governorate at the south of Syria under rainfed conditions. The soil is clay with low content of organic material (1.5%) and nitrogen, high concentration of phosphorous 60 $mg.kg^{-1}$ (Olsen et al., 1954), and moderate content of potassium (245 $mg.\ kg^{-1}$), pH 6.5-6.8.The annually rainfall 525 mm.

Plant material

Malus domestica Borkh is a seedling rootstock considered as vigorous rootstock with deep roots, tolerant to frost and drought, suitable for different soil types (Colett, 2011).

MM106 is a semi- vigorous vegetative rootstock, derived from the hybridization between M1 rootstock and Northern spy cultivar which has the resistance to woolly apple aphid, MM106 considered as moderate rootstock, high productive capacity, resistant to woolly apple aphid, moderate tolerant to spring frost and drought, susceptible to ring root, and compatible with most commercial apple cultivars (Preston, 1966).

Starkrimson cultivar is a bud mutation derived from Starking Delicious cultivar found in America in 1870, with moderate to vigorous growth, fruits are conical with dark red color and big size, successful cultivation in areas exceeded 1300m altitude in the studied area(Muzher and AlHalabi, 2012).

Methods

Leaves and fruit nutritional content: Potassium (K), calcium (Ca) and magnesium (Mg) mineral contents were studied in the leaves and fruits of 15 years old trees of Starkrimson cultivar which grafted on *M. domestica* and MM106 rootstocks during the growth season at 50 days alternate periods, under rainfed conditions. K was estimated at humid digestion method of plant material, and then K content measurement using flame photometer apparatus (Benton, 2001). Calcium and magnesium were estimated after digesting plant samples with concentrated perchloric acid nitrogen by 9:4 then determined by atomic absorption (Tandon, 2005).

- leaves were sampled from each replicate (30 x number of replication) in three alternate periods (June, August and October).Samples were washed two times with distilled water to remove surface residues, then dried enough at 68° c and grinded into fine powder for nutrient analysis.
- Fruits from each tree (20 fruits) in each replicate were collected in three alternate periods (June, August and October). Samples were washed in fountain water followed by distilled water to remove surface residues, then prepared as very thin slices and dried on 68° c until weight stability and powdery grinded for nutrient analysis.

Analysis of variance was performed using two way ANOVA to compare means of obtained data at the level of significance (LSD at $P< 0.05$). in completely randomized block design having four replicates was used.

Results and discussion

The results showed that K concentration was significantly varied in the two studied rootstocks during growth season. As seen in Table 1, the seedling rootstock *M. domestica* had significantly higher K concentration in comparison with the semi vigor rootstock MM106 at the three alternate growth periods except the first period. The highest leaf K concentration of *M. domestica* was in August (1.57%) which was at normal concentration according to Neilsen and Neilsen, (2003), whereas the highest K concentration was in June for MM106 rootstock (0.99%), then decreased until October, somehow, it was less than the sufficient amount in leaves during the growth season, which ranged between 1.5–2.5% after 110-125 days of full bloom (Neilsen and Neilsen, 2003). The variance of K concentration between the two rootstocks could be described as a genetic variance of which leads to different nutrient uptake and absorption capacities through roots (Kucukyumuk and Erdal, 2011).

Leaf concentration of Ca was significantly decreased during the growth season in the two rootstocks, which can explained due to the Ca immobility in leaf tissues, and no redistribution to other plant organs (Kucukyumuk and Erdal, 2011) . *M. domestica* revealed the highest leaf Ca value in October (0.75%) in significant variance compared with the other studied growth period. Likewise, MM 106 revealed the highest significant leaf Ca concentration in October (0.79%). In addition, MM106 revealed significant higher leaf Ca concentration in comparison with *M. domestica* rootstock during all studied growth periods (Table, 1). Leaf Ca concentration was significantly less than the optimal concentration during the growth season, which ranged between 1.2–1.6% according to (Joseph,2004). Velemes *et al*. (1999) stated that the leaf Ca sufficiency of Starkrimson cultivar was 0.8–1.2%.

Mg concentration revealed discrepant track among the two studied rootstocks, leaf Mg concentration was significantly increased in *M. domestica* that revealed the higher concentration in October (0.35%), whereas MM106 rootstock revealed continues significant reduction of Mg accumulation during the growth season, the highest Mg concentration was in June (0.29%) then decreased until October (0.18%). In general, the concentration of Mg in leaf was in the sufficient level (0.2-0.4%) according to Uepmnht, (1990), while Steve *et al*. (2004) mentioned that the sufficient threshold varied depending on the rootstocks and cultivars and ranged between 0.35–0.50 % .

Table 1. Leaf K, Ca and Mg concentrations (%) of leaf in *M. domestica* and MM106 grafted by Starkrimson cultivar.

Rootstock	K				Ca				Mg			
	June	Aug.	Oct.	LSD 0.05	June	Aug	Oct	LSD 0.05	June	Aug.	Oct.	LSD 0.05
M. domestica	1.05	1.57	0.8	0.044	0.45	0.57	0.75	0.009	0.26	0.29	0.35	0.009
MM106	0.99	0.78	0.62		0.72	0.74	0.79		0.29	0.28	0.18	
LSD.0.05	0.038			0.066	0.006			0.011	0.006			0.011

The ratio of K+Mg/Ca differed according to the rootstock, it was 2.91 in June then increased to the highest ratio in August (3.27) to decrease again in October (1.54) in *M. domestica*. Whereas, MM106 revealed different track, the highest K+Mg/Ca was in June (1.67) to decreases continuously until October (1.02). Buchloh and Bangerth, (1974) stated that the development of bitter pit is associated with K+Mg/Ca and differed from organ to the other in the tree and should be (1–2/1) in leaves. Likewise, K/Ca ratio significantly revealed the same track from June until October in the two rootstocks, and *M. domestica* revealed significant higher K/Ca ratio compared with MM106 rootstock (Figure 1). Nagy and Holb (2006) pointed that the optimal value of K/Ca is 0.87 in apple leaves, and the probability of bitter pit disorder is unpredicted when this ratio less than 1 (Derugen and Koluken, 1988).

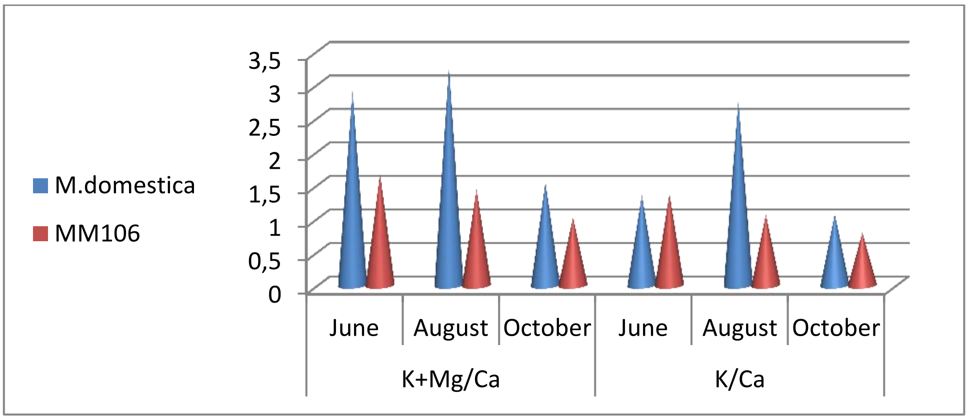

Figure 1. K+Mg/Ca and K/Ca ratios in leaves of *M. domestica* and MM106 rootstocks

In general, K concentration in apple fruit was higher in M. *domestica* and showed different track than MM106 rootstock,. As shown in Table 2, the two rootstocks revealed the same K accumulation (0.79%) in June, then increased in *M. domestica* until August (1.57%) to decrease again in October (0.61%), while the concentration of MM106 continuously decreased significantly until October (0.51%). Our results are in agreement with Kucukyumuk and Erdal (2011 **),** they found that the vigor rootstocks revealed higher fruit K accumulation than the semi vigor rootstocks, and K concentration varied depending on the apple varieties.
Concentrations of Ca and Mg in fruit were the same in the two rootstocks through the alternate growth stages except Mg concentration in October. The highest concentrations were in June, then decreased until October (0.03% for Ca) and (0.02 and 0.01% for Mg of *M. domestica* and MM106, respectively). However, Ca and Mg of Starkrimson fruit on the two studied rootstocks was too low in comparison with previous studies (Kucukyumuk and Erdal, 2011).

Table 2. Fruit K, Ca and Mg nutrient concentrations (%) in *M. domestica* and MM106 grafted by Starkrimson cultivar.

Rootstock	K				Ca				Mg			
	June	Aug.	Oct.	LSD 0.05	June	Aug	Oct	LSD 0.05	June	Aug.	Oct.	LSD 0.05
M. domestica	0.79	1.57	0.61	0.02	0.05	0.03	0.03	0.009	0.03	0.02	0.02	0.008
MM106	0.79	0.78	0.51		0.05	0.03	0.03		0.03	0.02	0.01	
LSD.0.05	0.017			0.029	-			-	-			-

According to the mean values of fruit nutrients, the two rootstocks showed different K+Mg/Ca ratios during the growth season. *M. domestica* revealed the highest ratio in August (53/1) then significantly decreased in October (21/1). Likewise, MM106 showed the highest ratio in August (26.67/1), then significantly decreased in October (17.3/1). Buchloh and Bangerth (1974) mentioned that the normal K+Mg/Ca ratio is correlated to the rootstocks and varieties and ranged between (20–50/1). Depending on the previous study, the fruit K+Mg/Ca ratio was in the normal track for *M. domestica* more than MM106 rootstock, which lead to establish sufficient nutrient management in MM106 orchards. Also, K/Ca ratio showed the same track among the two rootstocks during the growth stages, and the two rootstocks revealed the highest ratio in August (52.3/1 and 26/1 for *M. domestica* and MM106 respectively) then decreased until October (Figure 2).

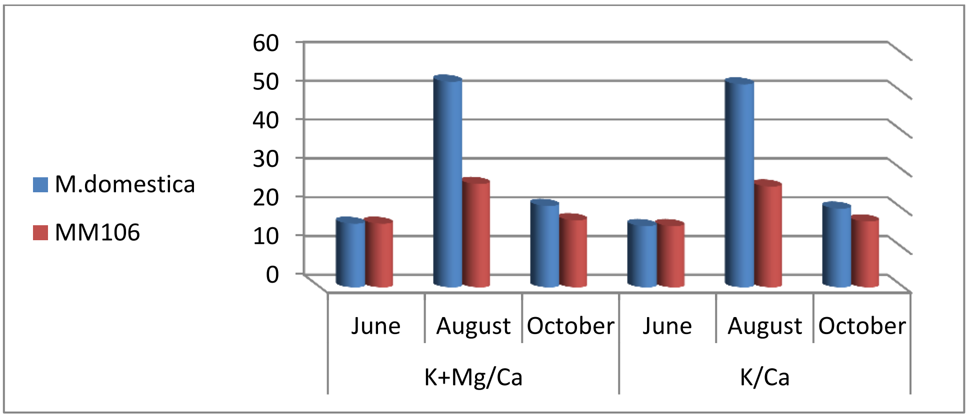

Figure 2. K+Mg/Ca and K/Ca ratios in fruits of *M. domestica* and MM106 rootstocks

Conclusion

The results showed that the nutrient concentration was particularly less than the sufficient nutrient level especially for Ca and Mg in October, and MM106 reveled less accumulation compared with *M. domestica* which may due to the surface distribution of its roots and prevent nutrient absorption under rainfed condition and high temperature in the second part of Summer. Since, the application of foliar spray of K, Ca and Mg should be taken into account in the fertilization management program at appropriate periods during growth season.

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EFFECT OF DIFFERENT SEED TREATMENTS ON DORMANCY BREAKING AND GERMINATION OF ACER CAPILLIPES MAXIM.

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Abstract

Snake-bark maple is a small, deciduous tree that grows up to 10-15 m tall, with arching branching and a decorative bark and leaves. It is native in the mountain forests of Japan. Kyushu Maple is tolerant of frosts, smoke and urban sites. It is not an invasive species and it should be planted in green spaces in Serbia, especially as an alternative to invasive plant species. For this reason, we decided to investigate possibility of rapid and easy generative propagation of this species which has endogenous seed dormancy. Seeds were collected in the Arboretum of Swedish University of Agricultural Sciences in Alnarp. During the experiment, the seeds were cold stratified in a perlite or in bags *without* a *substrate* for 15 and 30 days. Parameters of seed germination were determined according to ISTA rules. The best results were achieved with seeds cold stratified in a perlite, for both treatments (15 or 30 days stratification). The highest germination rate and germination energy both 94% ,were achieved after the 30 days treatment.

Keywords: *Snake-bark maple, seed dormancy, cold stratification, generative propagation*

Introduction

Acer capillipes Maxim. (Red Snake-bark maple, Kyushu maple) is a deciduous small tree, usually low-branched, with a moderately slow growth rate and could reach up to 9 m high and 7 m wide. It has decorative leaves and vertically striped bark with green and white strips (Dirr and Warren, 2019). Foliage emerge redish, becoming dark green in the summer, followed by yellow, orange and red shades in autumn. It is native in the mountain forests of Japan. Snake-bark maple belongs to zones 5 – 7, and it is tolerant of frosts, heat, smoke and urban sites, but it does not like reflected heat and drought (Dirr and Warren, 2019; Rushforth, 1999). According to the DAISIE, a database of alien and invasive species in Europe *A. capillipes* is not an invasive species in Europe, and it deserves to be planted more often in Serbian green spaces. The common propagation method of this species is by seed (Dirr and Warren, 2019), but there is no much available data about its propagation. There is a recommendation that seed should be cold stratified for 3 months on 5° C, but there is no data about expected germination rate (Arnoldia, 1954). In more recent available publications there is no sufficient data about generative propagation of this species.

The majority of the other species from genus *Acer*, including *A. campestre*, *A. monspessulanum*, *A. opalus*, *A. platanoides*, *A. pseudoplatanus*, *A. glabrum*, *A. ginnala*, *A. cincinatum* and *A. palmatum*, requires cold stratification treatment before they will germinate, which is necessary in order to overcome internal dormancy in the seed (Tóth and Garrett, 1989; Suszka et al., 1996; Piotto and Di Noi, 2003; Zasada and Strong, 2006, Ylmaz 2007). Besides those species, for the *A. rubrum* and *Acer saccharinum* a pretreatment is not required for germination (Ellis et al., 1985; ISTA, 1996). The aim of this study was to establish a rapid and easy generative propagation of this species, investigating the possibility of reducing the stratification period from recommended three months to a shorter time period.

Material and Methods

Seeds were collected in the Arboretum of Swedish University of Agricultural Sciences in Alnarp. The seeds were stored in a plastic bags, at temperature 5°C before testing. Four pre-sowing chilling treatments were made. The seeds were stratified at 3-5°C for 15 or 30 days. There were two groups of treatments. The first one was chilling in plastic, transparent bags which were opened each week and moisture was controlled regularly (naked stratification). The second group of seeds was put in the chilling in perlite. Before stratification, the seeds were treated with Captan fungicide (0.6 %). The seeds in control treatment were tested without chilling pretreatments. After stratification, the seeds were examined and seeds that germinated during stratification were recorded and removed. The remaining seeds were placed on the top of two layers of filter paper in petri dishes. Humidity was controlled daily by adding distilled water if necessary. Temperature during germination was 21°C. Germination was carried out in long day conditions (light/dark period 16/8h). The germination was tested according to ISTA (1996) rules for the genus *Acer*. The first count was the 7th day (germination energy) and the last count was the 21th day after placing the seeds. In this period, the number of germinated seeds was recorded daily. Besides germination rate and germination energy, real germination rate was also calculated as a percentage of sound (viable) seeds that germinate (Grbić, 2003). Each treatment consisted of four replicates with 50 seeds per repetition. The obtained data were statistically analyzed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

In the control treatment the germination rate was low, only 8% of seeds germinated (Table 1), thus indicating that a chilling pretreatment is necessary for *A. capillipes* generative production. However, considerably better results and high germination rate were obtained after stratification thus indicating that endogenous dormancy of *A. capillipes* is not deep. Germination rate was influenced by both the treatment duration and stratification substrate and better results were obtained after 30 days of stratification (85.9 % - chilling in plastic bags and 94.0% - chilling in perlite). The germination energy was very high, mostly the same as germination rate, which means that almost all seeds germinated during the first 7 days after placing them for germination. This high germination energy has a practical importance, because during a nursery production, we can expect that the seeds will germinate rapidly and uniformly. Real germination rate or the percentage of sound (viable) seeds that germinate was as same as the germination rate after 30 days of stratification in perlite, but higher than germination rate after 30 days of naked stratification (Table 1). This finding suggests that all the seeds put on stratification in perlite were viable, and the difference between germination after 30 days of naked stratification was caused by the certain amount of non-viable seeds. For that reason, we can consider that both stratification in perlite and naked stratification would give similarly good results, depending on the quality of the seeds.

Table 1. Germination of collected seeds

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
Control	8.0[d*]	1.0[c]	8.0[d]
15 days stratification in perlite	80.5[bc]	79.0[b]	82.1[c]
15 days naked stratification	75.4[c]	75.4[b]	87.5[bc]
30 days stratification in perlite	94.0[a]	94.0[ab]	94.0[ab]
30 days naked stratification	85.9[b]	85.9[a]	94.9[a]

*Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Some *Acer* species have a much deeper endogenous dormancy and the seeds of *Acer pensylvanicum* L. should be soaked for 48 h, followed by moist chilling for 16 weeks, for successful germination (92%) (Burgoin and Simpson, 2004). Germination of *A. opalus* after 3 months of cold stratification was only 50%, and even in wild condition, the seeds of this species needs 2 years (winters) to germinate (Gleiser et al. 2004). The other problem with some *Acer* species, including *A. saccharinum* and *A. pseudoplatanus,* is recalcitrant seed (seeds that can not survive if desiccated) so it is very important to store these seeds in the appropriate humid conditions (Dickie and Pritchard, 2002; Hong and Ellis, 1996). To the contrary, the species *A. campestre*, *A. platanoides* and *A. pensylvanicum* have orthodox seeds (seeds that can tolerate water loss during storage) (Suszka et al., 1996; Greggains et al., 2000; Connor and Bonner, 2001; Bourgoin and Simpson, 2004).
Besides cold stratification treatments, a treatment with gibberellic acid can also have a positive effect on germination of *Acer* spp. (Stejskalová et al. 2015, Marshall et al. 2000).

Conclusions

Selected genotype of *A. capillipes* can be successfully propagated by seeds. The seeds do not have a deep endogenous dormancy and they requires cold stratification pretreatment for only 30 days, in perlite, at the temperature of 3-5°C. Good results can also be obtained with the naked stratification of seeds, in plastic bags. Germination rate obtained in this research was high (94%). However, there is a possibility that a treatment of seeds with gibberellic acid can improve the germination rate of this species, which should be further investigated by additional research.

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MORPHOLOGICAL PROPERTIES OF DIRECTLY SOWED AND TRANSPLANTED SWEET CORN PLANTS CULTIVATED WITH COVERING TECHNOLOGIES

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Abstract

Aim of experiment set up in 2014 was, to investigate shortening of sweet corn growing period with application of some technological elements: propagation time, propagation method, floating row cover. The choosen variety was a conventional, very early ripening sweet corn hybrid, 'Spirit'. The following growing technologies were compared: 1. direct sowing of plants with floating row cover, early period, 2. plants transplantation with floating row cover, early period 3. plants transplantation with floating row cover, normal period and 4. direct sowing of plants without row cover, normal period (regarded as control). The influence of above mentioned technological elements on some important morphological properties of sweet corn plant was studied such as: evolution of phenological phases, plant height, tassels length, ear weight, height of ear insertion.

Key words: *earliness, sweet corn, fleece covering.*

Introduction

Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary. After dates of Hungarian Fruit & Vegetable Interprofessional Organization from 2005 the growing area was about 30,000 hectares. Sweet corn (Zea mays L. convar. saccharata), considered a vegetable, is a special type of corn destined exclusively for human consumption, less in fresh form or more in processed (canned or frozen) foods (Riverti *et al*., 2018). Near 50% of world sweet corn production came from US (Slezák *et al.*, 2012).

In order to promote fresh consumption, as well as to maintain and increase the sweet corn exports, it is necessary to promote investigations so as to be able to ensure a further increase in the growing area and yields of sweet corn with the help of the experiences. The literature mainly is concerned with maize growing technology, but a lot of dates could be used by sweet corn. Of the production technology elements, a number of researchers studied or are currently studying the sowing time of sweet corn.

As early as at the ending of 19^{th}- and the beginning of the 20^{th} century some researchers (Cserháti, 1901) highlighted the importance of the sowing date. Ripening can occur earlier when sowing earlier and using high quality seeds as compared to normal or late sowing. I'só (1969) and Pásztor (1966), after their multi-year sowing date trial, concluded the following: in the case of an earlier sowing seed germination will be more protracted, but silking and harvesting occur sooner than by lately sowing time. After multi-year trial Berzsenyi et al (1998) had studied the effect of different sowing times on maize development.

Because transplanting provides optimal environmental conditions for seed germination, and earlier development stage, sweet corn has been transplanted, as majority of other vegetables, experimentally in an attempt to improve stands and hasten maturity (Di Benedetto and Rattin, 2008, El Hamed et al., 2011). About the covered early sowing as a technological variation Aylswirth (1986) mentioned, that from an early sowed crop, made in first week of April, arranged in twin rows (42 cm) and covered by plastic, we could harvested marketable cobs by the fourth of July. In case of direct sowing, as propagation method, another earliness

increasing solution is the temporary covering with plastic or vlies, used in different combinations (Hodossi and Kovács, 1996).
The most widespread method of seedling production is the use of soil blocks (Pereczes, 1999) which can also significantly increase earliness. The combined application of seedling growing and floating row cover can advance harvest by three weeks as compared to the traditional technology and can give farmers a three to four times greater income (Kurucz, 1998). Rattin et al., (2006) compared direct sowed and transplanted sweet corn varieties, without covering and concluded no difference, in ear weight and ear length, between transplanted treatments plant's, in comparison to direct sowing treatments.

Materials and methods

The experiment was set up in 2014 on an area eqiupped for irrigation at Tg-Mures, Mures County, situated in Central part of Romania. Conventional, reliable and sufficiently known among growers sweet corn variety, Spirit, was used as a reference variety in the variety comparison trials of the Central Agricultural Office. Hybrid has short growing period of 85 days and yellow kernels. Average height of plants is 159 cm, average ear height is 37 cm, ear length 19.6 cm and average ear weight is 245 g. The variety was granted official recognitions in 1988 and has been the dominant variety of the early ripening category till now. In the year prior to the experiment the area was under wheat.
The following treatments, each with four replications, were applied during the experiment:
P1 = direct sowing, with floating row cover (April 8th)
P2 = plants transplantation with floating row cover (April 8th)
P3 = plants transplantation, with floating row cover (April 25th)
P4 = uncovered direct sowing (April 25th), (regarded as control).
For the frame structure of the treatments with cover we used ø 4.2 mm zinc coated wire coils. The fleece, 60 cm in width, was stretched over a small tunnel of 40 cm in height and then its edges (25-25 cm, respectively) were covered with soil using a hoe and the its ends were tied to the stakes hammered down. The construction of the frame structure and the setting out of the fleece cover were carried out at the same day as direct seeding and out planting.
At the two propagation times the treatments P1 and P3 were covered with Novagryl floating row cover, having a weight of 19 g/m^2, (using the small tunnel technique) in order to enhance earliness. The stand was created to contain 60,607 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of 110+40x22 cm in twin rows. Each plot had an area of 6x3.5m (8 parallel rows and 16 seeds sown in each row). Sowing depth was 3 cm. The edge was the respective outer rows of the 4 twin rows of the plot.
In October 2013, 35 t/ha of farmyard manure was worked into the soil with ploughing on area. Nitrogen fertilizer (120 kg/ha) was applied at the 6-7 leaf stage, the form of top dressing. The fertilizer application was worked into the soil with a rotary hoe.
During the experiment, we studied plant growth rates and recorded the time of the occurrence of the major phenological stages. For this purpose, we carried out regular observations (every 3 to 5 days) according to the following:
appearance of tassels (by 50% of the plants),
beginning of tasseling (pollen shed has begun on the axes of tassels),
50% silking (silks have reached a length of 2 cm on half of the ears) "milky stage" (harvest).
highness of plants (cm),
length of tassels (cm),
highness of ear insertion (cm).
For measurements, were selected 10 plants/treatment/replication, from the four central (two twin) rows.

The statistical analysis of the results was carried out by using the programme *RopStat 1.1*. When the standard deviations were identical the mean values were compared by pairs using the *Tukey-Kramer* test, while in the case of the non identical standard deviations the means were compared using the *Games-Howell* test (Vargha, 2007).

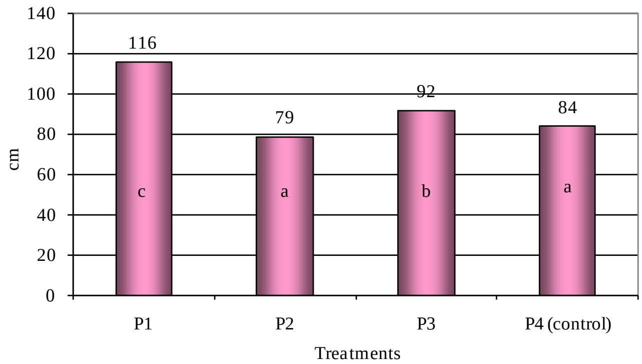
Results and discussion

Table 1. illustrates the times in days elapsed to different phaenological stages.

Treatments (sowing, outplanting day)	Tassels appearance by 50% (days)	Beginning of male flowering (days)	Stigma appearance by 50% (days)	Starting harvest (days)
P1 (IV. 8)	50 (V.28.)	64 (VI.11.)	70 (VI.17.)	85 (VII.2.)
P2 (IV. 8)	41 (V. 19.)	55 (VI.2.)	60 (VI. 7.)	76 (VI. 23.)
P3 (IV. 25)	28 (V. 23.)	43 (VI. 7.)	49 (VI. 13.)	64 (VI.28.)
P4 (control) (IV. 25)	57 (VI. 21.)	67 (VII. 1.)	71 (VII. 5.)	85 (VII. 19.)

Table 1: Rhytm of generative phaenophases

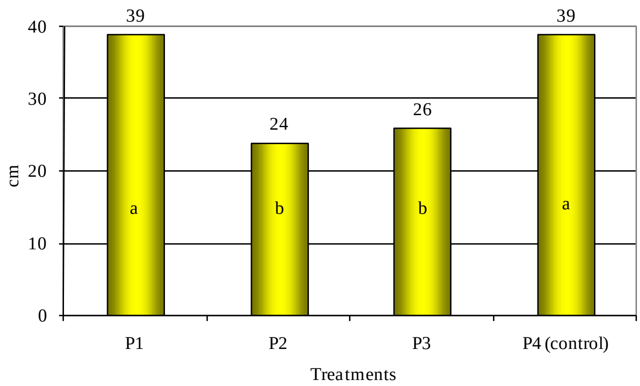
Covering helped in earlier appearance of generative phaenophase (tassels) P1treatment by 7 days respectively, in case of harvesting by 17 days earliness, compared to P4 (control). In the case of the plants sown at the second sowing date the emergence could be considered practically normal as common. According to our results of experiment, the male flowers (tassels) appeared in the shortest time by transplanted treatments. The transplant growing, at this stage of development, resulted in 9 days earliness as considers the treatment P2, compared to the treatments P1 which had been direct seeded at a similar time. Compared to the control (P4), the P3 treatment transplanted at the same date, tassels and stigma appearance started earlier by 29, respectively 22 days and could be harvested 21 days earlier.
The highness of plants indicator of general condition is represented by Figure1.



F(4;176)=40,356; Sd=99%

Fig. 1. Highness of plants (cm).

The highest value of plant highness was registered by plants of earlier sowed (P1) covered treatment, difference was significantly (at $p<0.01$ level), compared to all other treatments. Covering (P1) had favourable effect on plant highness in normal time outplanted treatment P3 compared to uncovered, earlier sowing time (P4, control) treatment. Among all covered (P1, P2, P3) treatments plant highness resulted significantly (at $p<0.01$ level) differences.
Evolution of tassels length, supposed to carry more male flowers and implicite better pollination, is summarised on Figure 2.

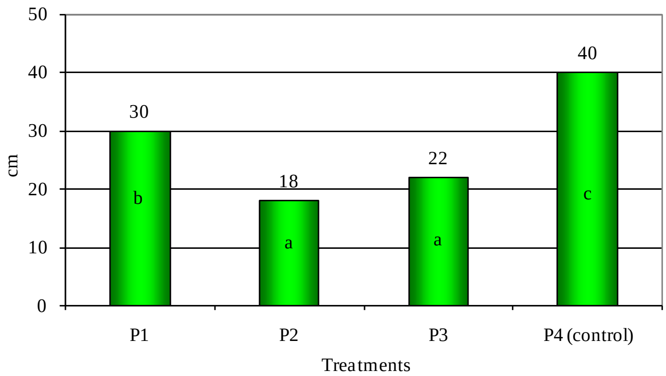


F(4;174)=7,561; Sd=99%

Fig. 2. Tassels length (cm).

In case of tassels length propagation method of sowing (earlier, normal) had higher influence. Plants from earlier sowed P1 treatment and later sowed uncovered, control (P4) treatment had significantly (at p<0.01 level) longer tassels compared to earlier transplanted (P2) and normal time transplanted (P3) treatments. Covering had no effect on tassels length evolution.

Highness of ear insertion is a very important morphological plant property in case of mechanical harvesting, because adapters of usual harvesting machines can cut only de sweet corn ears inserted higher than 40 cm, is illustrated on Figure 3.



F(4;174)=10,473; Sd=99%

Fig. 3. Highness of ear insertion (cm).

Obtained data confirmed significantly (at p<0.01 level) higher insertion of ears in case of earlier and later sowed, covered and uncovered (P1 and P4) treatments compared to earlier transplanted P2 and normal time transplanted P3 treatments. Covering on earlier sowed (P1) treatment produced favourable effect on highness of ear insertion. P4 (control) treatment offers significantly (at p<0.01 level) higher insertion of ears compared to all other covered treatments. Only P4 (control) treatment seems to offer possibility applying mechanical harvesting on sweet corn crops in open field production. Mechanical harvesting by P1 (earlier sowed) and P2 (earlier transplanted), P3 normal time transplanted treatments can not be used.

Conclusions

Based on the results of the 2014 year experiment, the following conclusions can be made:

In case to compare the earlier sowed P1 and normal time sowed P4 (control) treatments, covering shortened with 7, respectively 1 days, appaerance of generative organs (tassels and stigmina) and no difference in growing period, 85 days in both treatments.

The growing period was significantly shortened with transplantation of sweet corn plants, combined with covering compared to direct seeded. Harvest time occurred 9 days earlier in the case of transplantation and floating row cover, earlier transplanted (P2) application compared to direct sowed, uncovered, control (P4) treatment, and earlier to direct sowed, covered P1 treatment.

At the same time the floating row cover produce 11 days shortening in growing season between P2 (earlier transplants with floating row cover) and P3 (normal time transplants with floating row cover) treatments.

In case of earlier, direct sowed treatment (P1) the effect of covering had positive effect on plants highness, tassels length and highess of ear insertion.

Insertion point of ear, higher than 40 cm, offer possibility, in case of normal time sowed, uncovered P4 (control) treatment. of sweet corn crop, to use mechanical harvesting.

Covering has no significantly positive effect on highness of ear insertion.

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THE INFLUENCE OF ABSORBENT AND SOIL TYPE ON GERMINATION, GROWTH AND DEVELOPMENT OF PEA GRAINS

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Abstract

The pea germination process begins when the seeds absorb a certain amount of water relative to the seed mass. The lack of water in the germination phase inhibits the growth of plants and reduces the production of crops. By solving the problem of providing soil moisture during vegetation we are aming to create technologies for maximum savings and efficient water supply for the plants. The main goal of this research is to determine the influence of the "Tverdaya Voda" on germination, as well as on the length and mass of germs and roots, in laboratory conditions. The experiment involved six treatments: control variant (A_0), super absorbing agent "*Tverdaya Voda*" (A_1), super absorbing agent "*Tverdaya Voda*" enriched with growth stimulator (A_2), super absorbing agent "*Tverdaya Voda*" enriched with microorganisms (A_3), super absorbing agent "*Tverdaya Voda*" enriched with microelements (A_4) and super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements (A_5), and two soil types (alluvial soil and humofluvisol). The experiment was performed in the laboratory of the Faculty of Agriculture in East Sarajevo. For all examined characteristics, the best results were found in the use of super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements (germination energy, germination, mass and length of the hypocotyls), while the control variant had the lowest results.

Keywords*: absorbent, soil, germination, hypocotyl, root.*

Introduction

Pea (*Pisum sativum* L.) is annual legume plants, of winter and spring type. It grows from 20° to 67° north latitude, and it has a large growing area, thanks to its good adaptability, a large number of varieties, short vegetation and diverse use (Gagro, 1997). Pea is a multipurpose crop, with the main purpose in the human nutrition and nutrition of domestic animals, and can be used in the form of green fodder, dry matter, forage fluor, unripe grain, ripe grains and straw, and green manure as well (Mikić et al., 2006). It is known that seed quality affects germination, as well on the production and profitability of crops (Finch-Savage and Bassel, 2015). Germination of seeds is one of the most important components of seed quality (Van Gastel et al., 2007), which is determined by the standard method of ISTA (The International Seed Testing Association) germination testing. Ecological factors such as temperature, light, pH and soil moisture affect the germination of seeds (Canossa et al., 2008; Ikeda et al., 2008; Rizzardi et al., 2009). Availability of water is a basic prerequisite for the start of the germination process, that begins with its adoption. The process of pea germination begins when the seeds absorb a certain amount of water relative to the seed mass, about 115% (Jevtić et al., 1986). Drought stress is one of the main environmental factors that limits the growth of plants, and the most common cause of it, is the increase in temperature and the reduction of available water to the seed or plants (Nazar et al. 2015). The lack of soil water in the germination phase inhibits plant growth and reduces crop production. (Yan, 2015). By solving the problem of moisture protection during vegetation, we are aming to create technologies for maximum savings and efficient water supply for the plants. In this regard, it is interesting to use environmentally friendly biodegradable sorption materials that can retain moisture in the root zone of plants. In this way, plants can be provided with sufficient amounts of water and the influence of stressful weather conditions during vegetation can be reduced (Lukin et al., 2018). The agent with qualities of super absorbing agent, was synthesized in accord with traditonal methods (Bellamy, 1975). During the last phase of polymerization to the reaction mass (concentration ratio 4: 1), organic and inorganic matter are added (Kuznetsov et al., 2016a; Kuznetsov et al., 2016b). Super absorbing agent can be actively used in agriculture to improve soil structure and preserve moisture in arid areas, but it is also used as an additional source of mineral fertilizers. The main goal of this research is to determine the influence of different compositions of the super absorbing agent "*Tverdaya Voda*" on germination, as well as on the length and mass of germs and roots, in laboratory conditions.

Material and methods

To examine the influence of super absorbing agent "*Tverdaya Voda*" on pea germination, a control variant (A_0) was used, together with other variants: super absorbing agent "*Tverdaya Voda*" (A_1), super absorbing agent "*Tverdaya Voda*" enriched with growth stimulants (A_2), super absorbing agent "*Tverdaya Voda*" enriched with microorganisms (A_3), super absorbing agent "*Tverdaya Voda*" enriched with microelements (A_4) and super absorbing agent "*Tverdaya Voda*" enriched with stimulants growth, microorganisms and microelements (A_5).

We took soil samples from experimental parcels in East Sarajevo and Bijeljina After laboratory analysis it was established that alluvial soil (B_1) from East Sarajevo has pH_{H_2O} 6.63, it contains 3.62% humus, 0.23% N, and in 100 g^{-1} soil, it has 14.75 mg of soluble P_2O_5 and 15.59 mg of soluble K_2O, while humofluvisol (B_2). from Bijeljina had pH_{H_2O} 7.16, containing 4.12% humus, 0.27% N and 100 g^{-1} of the soil had a 40 mg soluble P_2O_5 and 36.41 mg of soluble K_2O. Soil samples (alluvial soil and humofluvisol) dried were in an electric oven at 105°C to constant mass.

After that, the land was put in 1 m^2 containers and mixed with various combinations of super absorbing agent (2g per m^2). We took soil samples to the depth of 15 cm from containers, using a plastic cylinder with a diameter of 5 cm, then a gauze is placed on the lower opening of the cylinder, which leaks the surplus of the soil dilution and retains the soil. Distilled water was poured in the cylinders. The surplus of soil solution that passed through the cylinders, was collected in glass containers and used to treat quartz sand during peas germination.

The germination energy and germination analyzes were carried out according to the standard method for laboratory germination of pea seed, germination in quartz sand, which was sterilized before it was used. The sand is placed in the ventricle container for germination, filling 2/3 of its height. The seed that was previously washed with distilled water, was placed in the dishes properly, dispersed to the same depth, and it moistened soil dilution up to 80% of filed water capacity. The containers were placed in a thermostat at a temperature of 20° C, and the humidity of 90%. To test the energy of germination and germination, 100 seeds are used in 4 replications (Haramija, 2007). Germination and energy of germination are expressed in percentages. It takes 4 days to determine the germination energy, and 7 days for determining germination.

When calculating the energy of germination and germination, normally developed germs were count after each repetition separately. The examination of the influence of super absorbing agent "*Tverdaya Voda*" with different composition on the length and weight of the germs, as well as on the length and mass of the roots, was also performed according to the standard method for laboratory pea testing. Each plant was segregated from the sand separately, length of the germ, mass of the germ, and the length of the main germ root were measured. The length of the hypocotyl is measured from the point where root crosses to hypocotyl (the root's last hair) to the top of the hypocotyls (basal part of the cotyledon). The root length is

measured from the crossing of the root into the hypocotyl, to the longest part of the root. The length of the germ and root is expressed in centimeters (cm), and the weight in grams (g).
The obtained results are analyzed by the twofactorial reflection variance analyze (super absorbing agent, soil types), and the individual differences tested by the LSD test, using the SAS / STAT program (SAS Institute, 2000).

Results and discussion

The influence of different combinations of absorbents and soil types on germination, germination energy of pea seeds in laboratory conditions, as well as length and mass of germ, length and root mass are shown in Table 1.
The use of microorganisms as biofertilizers and/or biopesticides in the production of different plants allows for better utilization of growth potential both plants and microorganisms (Frame, 2005; Avis et al., 2008; Hayat et al., 2010). In the researches of Shaukat et al. (2006) bacterialization (Azospirillum, Pseudomonas and Azotobacter) of sunflower and wheat seeds had a positive effect on germination and length of germ, which is in accordance with our researches, because the application of the super absorbing agent "*Tverdaya Voda*" enriched with microorganisms, gave better results compared to the control variant, and variant where only the super absorbing agent "*Tverdaya Voda*" was applied. The use of microelements, especially in combination with growth stimulators, can increase plant growth up to 10% (Chau et al., 2019), which is in accordance with our results. More attention is now devoted to alternative methods, where biopreparations, created on the basis of natural substances of biological origin, are used to stimulate seeds. Their application is not only allowing seed protection from microorganisms, but also facilitating the adoption of biogenic substances in plants (Mukhrjee et al., 2007).
In the treatment of super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements, a significantly higher germination energy (70.62%) was found compared to other treatments, while the control variant had the lowest germination energy (51.00%). The use of humofluvisol , used in the surveys had a significantly higher energy of pea grain (65.62%) compared to alluvial soil (58.54%). For both soil types, in average, super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements compared to other treatments had significantly higher germination energy, while the control variant had the lowest energy of germination.
By analyzing the effects of different combinations of absorbents on pea grain germination, it was found that in the treatment of "*Tverdaya Voda*" super absorbing agent enriched with growth stimulators, microorganisms and microelements, higher germination (94.50%) was significantly higher compared to other treatments, while the control variant had the least germination of pea grain (79.12%).
The land from humofluvisol used in the surveys had a significantly higher pea grain germination (89.92%), compared to alluvial soil (84.75%). For both soil types, in average, super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements

Table 1. Influence of super absorbing agent and soil types on germination energy, germination, length and mass of germs, length and mass of roots

		Germination energy (%)	*Germination* (%)	Germ (hypocotyl) length (cm)	*Root length* (cm)	Germ (hypocotyl) mass (g)
Super absorbing agent (A)	A_0	51.00f	79.12f	2.42e	4.39e	1.160d
	A_1	57.38e	84.75e	4.20cd	6.53cd	1.325c
	A_2	67.88b	90.50b	4.70c	6.19d	1.624b
	A_3	59.12d	86.50d	4.13cd	6.59cd	1.681b
	A_4	66.50bc	88.62c	5.38b	7.12bc	1.536b
	A_5	70.62a	94.50a	6.28a	8.17a	1.990a
Soil types	(B_1)	58.54b	84.75b	4.43	6.04b	1.319b
	(B_2)	65.62a	89.92a	4.61	6.95a	1.787a
AxB Alluvial soil	A_0	46.00gh	75.75g	1.89fg	4.41d	0.925f
	A_1	48.25g	81.25ef	4.20cd	5.95bc	1.187ef
	A_2	66.00cd	87.25cd	4.33cd	5.07cd	1.440cd
	A_3	56.00f	83.50e	3.37de	5.97bc	1.032ef
	A_4	66.00cd	88.00c	6.30a	7.94ab	1.662cd
	A_5	69.00bc	92.75b	6.48a	6.89bc	1.665cd
Humofluvisol	A_0	56.00f	82.50e	2.96ef	4.37d	1.395de
	A_1	66.50cd	88.25c	4.20cd	7.12bc	1.463cd
	A_2	69.75b	93.75b	5.07b	7.30ab	1.807bc
	A_3	62.25e	89.50c	4.89bc	7.20ab	2.330a
	A_4	67.00c	89.25c	4.46bc	6.29bc	1.410cd
	A_5	72.25a	96.25a	6.07ab	9.45a	2.315a
ANOVA	A	**	**	**	**	**
	B	**	**	ns	*	**
	AxB	**	**	*	*	**

Mean values designated with the same lowercase letter are not significantly different at the 95% level according to the LSD test

*** F-test significant at 0.01; * F-test significant at the 0.05 level; ns non-significant*

compared to other treatments had significantly higher level of germination, while the control variant had the lowest germination of pea seed. The peas had the longest hypocotyl (6.28 cm) in the treatment with super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements, and the shortest hypocotyl had the peas treated with the control variant (2.42 cm). The observed differences were statistically highly significant. The influence of soil types on the length of the hypocotyls was not statistically significant, but on both localities a significant effect of the super absorbing agent "*Tverdaya Voda*", enriched with growth stimulators, microorganisms and microelements was determined.

The length of the root was significantly influenced by the use of different combinations of absorbents, while the soil types, as well as the interaction of the absorbent x soil types, had a statistical significance. The longest root (7.12 cm) was determined in variant A_5, and the smallest (4.39 cm) in control variant. At the soil types B_2, the root (6.95 cm) is significantly longer compared to the soil types B_1 (6.04 cm). At the soil types B_1, the longest root had the application of super absorbing agent "*Tverdaya Voda*" enriched with microelements, and at the soil types B_2 the application of super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements, while the control variant had the smallest roots at both l soil types.

The examined factors, as well as their interaction, exhibited a high influence on the mass of the hypocotiles. The highest mass of hypocotil was in treatment A_5 (1.990 g), and the smallest in the control variant (1.160 g). The B_1 soil types (1.319 g) had a lower weight of the hypocotil compared with the soil types B_2 (1.787 g). At the alluvial soil, the hypocotil had the highest mass in the use of the absorbents A_4 and A_5, while at the humofluvisol the highest mass of the hypocotyl was when absorbents A_3 and A_5 were applied. The examined factors exhibited a high influence on the mass of the hypocotile, while their interaction had a significant effect. The highest root weight was in treatment of A_5 (1.286g), and the smallest one was in control variant.

The B_1 soil types (0.819 g) had a lower weight of the hypocotyl compared to the B_2 soil types (1.250 g). At the alluvial soil, the root had the highest mass when absorbent A_4 was applied, while at the humofluvisol the highest mass of root was by using absorbent A_5.

Conclusion

Based on the research taken in the laboratory of the Faculty of Agriculture in East Sarajevo, the following can be concluded:

- The application of the absorbent has exhibited a very significant influence on the examined pea grain characteristics. For all examined properties, the best results were when super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements was applied, and the the lowest germination had the control variant (no absorbent applied). Better results were obtained by applying enriched absorbent (super absorbing agent "*Tverdaya Voda*" + growth stimulants, super absorbing agent "*Tverdaya Voda*" + microorganisms, super absorbing agent "*Tverdaya Voda*" + microelements and super absorbing agent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements) in comparison with pure super absorbing agent "*Tverdaya Voda* ".Highly significant differences between soil types were recorded for germination energy, germination, hypocotil mass, and root mass, while the soil types had a significant influence on the length of the root.
- By using super absorbing agent "Tverdaya Water" enriched with growth stimulators, microorganisms and microelements at both localities, significant differences in germination energy, germination and mass of the germs and significant differences in the length of the hypocotil were found.
- After finishing the experiments in the laboratory, they should be continued in the experimental field of the Faculty of Agriculture and the acquired results should be presented to the agricultural producers.

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YIELD OF SELECTED GENOTYPES OF SPRING PEAS (*Pisum sativum* L.) DEPENDING ON TOP DRESSING AND AGROECOLOGICAL CONDITIONS

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Abstract

In two years of research (2016-2017), five varieties of peas (NS Javor, Baccara, NS Dukat, NS Junior and Sasa) were examined at two localities: 1) field in the vicinity of East Sarajevo (experimental field of the Faculty of Agriculture in East Sarajevo), and 2) field in the vicinity of Banja Luka (experimental field of the Agricultural Institute of Republic of Srpska), to determine the effect of agroecological conditions and mineral nutrition on the mass of 1000 seeds and the yield of peas. In order to determine optimal needs for mineral nutrition, three variant of fertilizer were applied: control variant - without fertilizer use, basic fertilization with 350 kg ha^{-1} $N_8P_{24}K_{24}$ and basic fertilization with 350 kg ha^{-1} $N_8P_{24}K_{24}$ + top dressing 27 kg ha^{-1} N (KAN 27% N). The following yield components were determined: the mass of 1000 seeds and the yield of pea grain. The average mass of 1000 seeds was 194.4 g. The largest mass of 1000 seeds was found in the genotype NS Javor (232.09 g), and the smallest mass was found in the genotype NS Junior (139.57g). The average yield of pea seeds was 4014.5 kg ha^{-1}. The seeds yield was under significant influence of the genotype and varied within the limits of 2561.9 kg ha^{-1} in the genotype NS Junior, which we consider to belong to a group of combined genotypes, while the highest yield was found in the protein pea genotype NS Javor (4666.5 kg ha^{-1}).

Key words: *peas, genotype, yield, the mass of 1000 seeds, locality, fertilization.*

Introduction

The cultivation of annual fodder legumes, such as fodder peas (*Pisum sativum* L.) and their use in feeding domestic animals, provides one of the best solutions for the lack of plant-based protein in livestock breeding (Mihailović and Mikić, 2014). Grain pea is an economically significant agricultural plant species, which iz primarily grown because of the high proteins content in the grain. Favorable biological features, including short vegetation, modest requirements regarding production conditions, versatility, high and stable yield, as well as the quality of biomass and grains, make fodder peas significant factor in the solution of plant protein deficiency (Čupić et al., 2010; Čupić et al, 2013). Despite the great importance of fodder peas in the production of fodder and grain, and favorable agro-ecological conditions, the production in our country is very small and official statistics does not cover the areas of production, production itself and yield of fodder peas or protein peas.

By examining various varieties of peas and using different amounts of mineral fertilizers at different localities, the selection of appropriate varieties for viable fodder production can be achieved.

The aim of this research was to determine the influence of genotype, mineral nutrition and agroecological conditions quantitative traits of fodder peas.

Material and method

Field experiments were set up in 2016 (Y_1) and 2017 (Y_2) at two locations: 1). locality in the territory of the city of East Sarajevo (experimental field of the Faculty of Agriculture in East Sarajevo), altitude of 550 m (43°49'01" NGW and 18°20'57" EGL) (L_1) and 2). locality on the territory of the city of Banja Luka (experimental field of the Agricultural Institute of Republic of Srpska), altitude of 163 m (17°4912'19" NGW and 44°48'13" EGL) (L_2). The basic soil treatment was carried out in the autumn, at a depth of 25 cm and the pre-sowing preparation at a depth of 10 cm. In order to determine optimal pea nutrition, three fertilizer treatment were applied:

- control variant - without fertilizer use (F_1),
- basic fertilization with 350 kg ha^{-1} $N_8P_{24}K_{24}$ (F_2) and
- basic fertilization with 350 kg ha^{-1v}$N_8P_{24}K_{24}$ + top dressing 27 kg ha^{-1}N (KAN 27% N) (F_3).

The sowing was done at row spacing of 12.5 cm and the distance in the row of 8 cm in the basic plot of the total area of 5 m^2. For sowing, inoculated seed of five spring fodder pea varieties were used: NS Javor (G_1), Baccara (G_2), NS Dukat (G_3), NS Junior (G_4) and Sasa (G_5). Three varieties were cultivated at the Institute for Field and Vegetable Crops in Novi Sad - Republic of Serbia (NS Dukat, NS Junior and NS Javor), Baccara is a variety cultivated in France, while Sasa is variety cultivated at the Agricultural Institute of Republic of Srpska in Banja Luka. The following yield components were determined:

- mass of 1000 grains (g) (based on the mass of 5x100 grains taken from the randomly selected samples) and
- Grain yield (kg ha^{-1}) was determined by weighing yield after harvest on elementary plot and its conversion to hectare.

Statistical analysis of experimental data was done by four-factor analysis of variance: Y-year, G-genotype; F-fertilization and L-locality.

The original experimental data were processed, analyzed and evaluated using the mathematical and statistical methods, factor impact on the plant characteristics (variance analysis) with the use of the statistical package STATISTICA 12 for Windows. Applied mathematical and statistical methods for the experimental data processing, analysis and evaluation of the research results enabled the correct understanding of all results. Significance was calculated based on LSD test for probability levels 0.05% and 0.01%. Stability tested traits were determined by the coefficients of variation (%).

Growth conditions

Soil in Banja Luka where experiments were set has good physical characteristics with a depth of 35 cm. According to agrochemical analyzes conducted at the Agricultural Faculty in East Sarajevo, it contains 0.13% total N, 2.05% organic matter, 40 mg 100 g^{-1} phosphorus and 38.48 mg 100g^{-1} potassium. The soil reaction is neutral, pH in nKCl is 6.97. According to the agrochemical analyzes conducted at the Agricultural Faculty in East Sarajevo, the soil at the experimental field of the Faculty of Agriculture (Eastern Sarajevo) contains 0.27% nitrogen, 4.12% humus, 40.40 mg 100g^{-1} phosphorus and 36.41mg 100g^{-1} potassium. The soil reaction is moderately acidic, pH in nKCl is 6.39. The distribution of total monthly rainfall and average monthly air temperature for research years and the long term average for Banja Luka and East Sarajevo are shown in Table 1. Average monthly air temperatures for the locality Banja Luka in both years were higher than the perennial average, and only in May 2016 the average monthly temperature was slightly lower than the long term average. In the period of vegetation, the precipitation varied from year to year and from month to month. In 2016, in April, there was only 0.5 mm of precipitation, while in 2017, precipitation in June (35.1 mm) and July (38 mm) was well below the long term average.

Table 1. Average monthly air temperature (°C), monthly rainfall (mm) and long term average for Banja Luka and East Sarajevo

Month			I	II	III	IV	V	VI	VII
Banja Luka	2016	°C	2.3	7.6	8.0	13.5	16.2	21.5	23.3
		mm	109.7	108.5	122.2	0.5	100.6	117.8	125.9
	2017	°C	-3.6	5.5	9.7	11.8	17.5	22.9	24.4
		mm	87.2	100.4	124.0	148.4	92.1	35.1	38
	Average 1981-2010	°C	0.6	2.3	6.8	11.5	16.5	19.8	21.8
		mm	69.7	59.1	87.5	84.3	89.4	112.4	81.4
East Sarajevo	2016	°C	1.2	7.4	7.1	12.9	13.9	19.5	21.1
		mm	46.6	87	131.7	60.5	82.1	96.4	104.5
	2017	°C	-4.8	5.2	7.9	8.6	14.6	19.3	21.2
		mm	57.9	69.1	43.6	132.4	73.8	55	66.5
	Average 1961-1990	°C	-0.8	1.7	5.5	10	14.8	17.7	19.7
		mm	74	63	73	76	85	94	83

At the locality East Sarajevo, in 2016, there were higher average monthly temperatures, compared to perennial average. The exception was May when the average monthly temperature was lower for 0.9 °C. The amount of rainfall was lower than the perennial average in April and May. In April and May 2017, the mean temperatures were lower than the averages for the given months, while in June and July they were significantly above the perennial average. The amount of rainfall was significantly lower than the perennial average for January, March, April, May, June and July.

Results and Discussion

Tables 2 and 3 show the average value of the 1000 grains mass and yield of examined pea genotypes per year, level of fertilization and localities. The mass of 1000 grains is the most stable yield component, because it depends, to a higher extent, on the genetic structure (Poggio al., 2005) and is highly inheritable trait. The average mass of 1000 grains ranged from 137.81 g in the second year in G_4 (NS Junior) to 232.81 g in the first year in G_1 (NS Javor). The mass of 1000 grains ranged from 137.61 g in control variant in the genotype G_4 (NS Junior) to 234.63 g in the third fertilization variant in the genotype G_1 (NS Javor). The genotype G_1 had the highest mass of 1000 grains at both localities compared to other genotypes. The genotype G_4 had the lowest mass of 1000 grains at both localities. In genotypes G_2 and G3, the mass of 1000 grains ranged from 214.38 g to 222.14 g. The highest difference between localities was observed in the genotype G_3, while the lowest difference was in the genotype G_1. The lowest variation was in the genotype G_2. Results of Tan et al. (2013) confirmed the existence of 1000 grains mass variations. They found huge variations of 63.3g (Balcesme) to 207.7 g (Incili) in the local populations in Anadolu (Turkey).

The average yield of the examined genotypes of peas was different. The highest values were recorded in the genotype G_1 (NS Javor), with the yield of 4666.5 kg ha^{-1}. The yield of 2561.9kg ha^{-1} was the lowest one found in the genotype G_4 (NS Junior). Examined genotypes of peas gave different yield regarding fertilization variants, shown in Table 3. High yields were achieved in genotypes G_1, G_2 and G_3, among which the genotype G_1 stood out with 4200.2 kg ha^{-1} in control variant, and 5126.6 kg ha^{-1} in the third fertilization variant. Low yield was found in the genotypes G_5 (Sasa) and G_4 (NS Junior), while the lowest average yield was found in the first fertilization variant in the genotype G_4 (NS Junior) of 2346.9 kg ha^{-1}, which was for 2779.7 kg ha^{-1} less than the highest achieved value for this characteristic. Regarding yield variations concerning different fertilization variants within the same genotype, the highest variation was observed in the genotype G_1 (NS Javor) with the difference of 926.4 kg ha^{-1} between the first and the third fertilization variant, while the lowest variations were found in the genotype G_5 (Sasa) with the difference of 108.3 kg ha^{-1} between the first and the third fertilization variant.

Table 2. Analysis of influence factors and their interaction on variability of 1000 grains mass pea

		Y_1			Y_2			Average
		L_1	L_2	Average	L_1	L_2	Average	
G_1	F_1	232.15	232.53	233.81	226.35	226.71	226.53	230.17
	F_2	235.08	235.23	236.36	229.19	229.34	229.27	232.82
	F_3	237.48	237.73	228.25	231.54	231.78	231.66	229.95
	Average	234.90	235.16	232.81	229.03	229.28	229.15	230.98
G_2	F_1	218.76	219.53	220.81	213.29	214.04	213.67	217.24
	F_2	222.09	222.78	223.47	216.53	217.21	216.87	220.17
	F_3	224.15	224.10	220.95	218.55	218.49	218.52	219.74
	Average	221.67	222.14	221.74	216.12	216.58	216.35	219.05
G_3	F_1	217.80	218.35	219.05	212.36	212.89	212.63	215.84
	F_2	219.75	220.83	221.46	214.26	215.30	214.78	218.12
	F_3	222.08	222.03	180.46	216.52	216.47	216.49	198.48
	Average	219.88	220.40	206.99	214.38	214.89	214.63	210.81
G_4	F_1	138.88	139.83	140.59	135.40	136.33	135.87	138.23
	F_2	141.35	141.73	142.39	137.82	138.18	138.00	140.19
	F_3	143.05	143.20	153.59	139.47	139.62	139.55	146.57
	Average	141.09	141.59	145.52	137.56	138.04	137.81	141.66
G_5	F_1	163.98	164.68	165.46	159.88	160.56	160.22	162.84
	F_2	166.23	166.15	166.63	162.07	161.99	162.03	164.33
	F_3	167.10	167.38	167.24	162.92	163.19	163.06	165.15
	Average	165.77	166.07	165.92	161.62	161.91	161.77	163.85
Average		196,662	197,072	194,596	191,742	192,14	191,942	193,27
$LSD_{0.05}$	Y	G	F	L	YxG	YxF	GxF	YxL
	0.2072^{**}	0.3277^{**}	0.2538^{**}	0.2072^{**}	0.4634^{**}	0.3589^{ns}	0.5675^{**}	0.2931^{ns}
	GxL	FxL	YxGxF	YxGxL	YxFxL	GxFxL	YxGxFxL	
	0.4634^{ns}	0.3589^{ns}	0.8026^{ns}	0.6553^{ns}	0.5076^{ns}	0.8026^{ns}	1.1351^{ns}	
$LSD_{0.01}$	Y	G	F	L	YxG	YxF	GxF	YxL
	0.2734^{**}	0.4323^{**}	0.3349^{**}	0.2734^{**}	0.6114^{**}	0.4736^{**}	0.7488^{**}	0.3867^{ns}
	GxL	FxL	YxGxF	YxGxL	YxFxL	GxFxL	YxGxFxL	
	0.6114^{ns}	0.4736^{ns}	1.0590^{ns}	0.8647^{ns}	0.6698^{ns}	1.0590^{ns}	1.4977^{ns}	

ns – factor or interaction is not significant; * - significance at $p<0.05$; ** - significance at $p<0.01$

The choice of locality influenced the yields of pea genotype (Table 3), which is in agreement with the results of Đurđić et al. (2017) Đurđić et al. (2018). The genotype G1 had the highest yield at the second locality, while the genotypes G_2, G_3, G_4 and G_5 had highest yield at the first locality. The highest yield was in the genotype G_1 of 4735.5 kg ha^{-1} at the second locality, while the lowest yield of pea grains was found in the genotype G4 of 2490.3 kg ha^{-1} at the second locality. The lowest difference between localities was observed in the genotype G_2 (36.8 kg), and the highest in the genotype G_5 (184.3 kg).

Table 3. Analysis of influence factors and their interaction on variability of yield pea

		Y_1			Y_2			Average
		L_1	L_2	Average	L_1	L_2	Average	
G_1	F_1	4385.2	4436.5	4410.9	3866.8	4112.0	3989.4	4200.2
	F_2	4759.0	4904.2	4831.6	4389.8	4637.8	4513.8	4672.7
	F_3	5133.2	5260.5	5196.9	5050.7	5061.9	5056.3	5126.6
	Average	4759.1	4867.1	4813.1	4435.8	4603.9	4519.8	4666.5
G_2	F_1	4202.0	4200.0	4201	4266.1	4120.4	4193.3	4197.2
	F_2	4400.8	4583.7	4492.3	4421.8	4379.3	4400.6	4446.5
	F_3	4700.0	4732.2	4716.1	4559.6	4527.2	4543.4	4629.8
	Average	4434.3	4505.3	4469.9	4415.8	4342.3	4379.1	4424.5
G_3	F_1	4211.7	4291.8	4251.8	4128.3	4017.0	4072.7	4162.3
	F_2	4550.0	4551.8	4550.9	4641.9	4353.7	4497.8	4524.4
	F_3	4746.0	4866.0	4806	4950.8	4651.4	4801.1	4803.6
	Average	4502.6	4569.9	4536.2	4573. 7	4340.7	4457.2	4496.7
G_4	F_1	2492.0	2297.0	2394.5	2360.6	2238.2	2299.4	2346.9
	F_2	2703.2	2530.7	2616.9	2578.6	2461.7	2520.2	2568.6
	F_3	2890.5	2760.5	2825.5	2775.7	2653.8	2714.8	2770.2
	Average	2695.2	2529.4	2612.3	2571.6	2451.2	2511.4	2561.9
G_5	F_1	3872.2	3964.2	3918.2	3753.6	3873.7	3813.7	3865.9
	F_2	4194.7	3832.5	4013.6	4123.0	3746.6	3934.8	3974.2
	F_3	4100.5	3823.1	3961.8	4044.3	3742.4	3893.35	3927.6
	Average	4055.8	3873.3	3964.5	3973.6	3787.6	3880.6	3922.6
Average		4089.4	4069	4079.2	3079.4	3905.1	3949.6	4014.4
$LSD_{0.05}$	Y 39.26^{**}	G 62.07^{**}	F 48.08^{**}	L 39.26^{**}	YxG 87.78^{**}	YxF 68.00^{ns}	GxF 107.51^{**}	YxL 55.52^{ns}
	GxL 87.78^{**}	FxL 68.00^{ns}	YxGxF 152.04^{ns}	YxGxL 124.14^{ns}	YxFxL 96.16^{ns}	GxFxL* 152.04^{ns}	YxGxFxL 215.02^{ns}	
$LSD_{0.01}$	Y 51.80^{**}	G 81.90^{**}	F 63.44^{**}	L 51.80^{**}	YxG 115.82^{**}	YxF 89.72^{ns}	GxF 141.86^{**}	YxL 73.25^{ns}
	GxL 115.82^{ns}	FxL 89.72^{ns}	YxGxF 200.61^{ns}	YxGxL 163.80^{ns}	YxFxL 126.88^{ns}	GxFxL 200.61*	YxGxFxL 283.71^{ns}	

ns – factor or interaction is not significant;; * - significance at $p<0.05$; ** - significance at $p<0.01$

In 2016, in Banja Luka, average monthly temperatures, except January, were higher than the average perennial values. In that year, for period Januar – July, there was 101.4 mm more precipitation. In 2017, the average monthly temperatures were lower than the average perennial values, compared with 41.4 mm higher precipitation in that year compared with the average perennial values. Favorable meteorological conditions in 2016 led to higher pea yield in that year, compared to 2017. In East Sarajevo, in 2016, the average monthly temperatures were higher than the perennial average, and in comparison with the perennial average values for the period January-July, there was 60.8 mm more precipitation. Compared with 2017, in 2016, rainfall in January-July was higher by 110.5 mm. January and April 2017 were colder than the perennial average and 2016. The yield of grain peas is influenced by the right choice of varieties, application of agrotechnical measures, and also the interaction of the genotype x environment have a significant influence (Lakić et al., 2018). Average grain yield in peas in this experiment was 4014.4 kg ha^{-1}, which is in accordance with the results of many authors analyzing grain yield achieved in agroecological conditions of Serbia (Mihailović et al., 2003; Erić et al., 2004; Karagic et al., 2008), and Croatia, Osijek, which is very similar to our agroecological conditions. Rapčan et al. (2010) achieved grain yield from 1780 to 3420 kg ha^{-}

[1], but also Spies et al. (2010) and Rasaei et al. (2012). Grain yield was under significant influence of genotype and ranged from 2561.9 kg ha^{-1} in the genotype NS Junior, which belongs to the group of combined genotypes, to 4666.5 kg ha^{-1}achieved in the genotype NS Javor which is a protein pea. In their research, Acikgoz et al. (2009) found significant interactions of genotype x years, genotype x locality and genotype x locality x years, and our research found significant interactions of years x genotype; genotype x fertilization; genotype x locality and genotype x fertilization x locality.

Conclusion

Significant interactions of year x genotype; genotype x fertilization; genotype x locality and genotype x fertilization x locality. In this experiment, the average mass of 1000 grains was 193.27 g. The average grain yield was 4014.4 kg ha^{-1}. The grain yield was under significant influence of certain genotype and varied from 2561.9 kg ha^{-1} in the genotype NS Junior, combined genotype, to 4666.5 kg ha^{-1} in the protein pea genotype NS Javor.

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DETECTING THE BERRY SIZE RESPONSES IN TWO STRAWBERRY CULTİVARS USING DIFFERENT IRRIGATION LEVELS AND BIO-ACTIVATOR

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Abstract

The Mediterranean region is one of the regions most affected by global warming. Therefore, optimizing applied irrigation levels and finding alternative cultivation practices are vital for the plant species including strawberry. In this study, the responses of strawberry cultivars 'Rubygem' and 'Kabarla' to irrigation levels and bio-stimulant use were investigated by evaluating the berry size parameters (diameter, length and weight) under high tunnel conditions in this region throughout the growing season of 2016-2017. The amounts of irrigation water applied were 0.50 (IR 50), 0.75 (IR 75), 1.00 (IR 100) and 1.25 (IR 125) times the water surface evaporation. Application of IR 50 was found to be significantly different from the other 3 applications, which partaking in the same group with the lowest fruit diameter, length and weight values in both cultivars. While it was determined that bio-activator applications did not provide significant increases in fruit diameter and weight values in 'Kabarla'; cultivar 'Rubygem' fruit diameter was increased 4.7% and weight by 9.0%. It was also detected that bio-activator applications did not provide significant differences in fruit length in both cultivars, and 'Rubygem' produced wider, lengthier and heavier fruits than the 'Kabarla'. It can be concluded that permanent damage in the diameter, length and weight of the fruits representing the quality are dependent on the level of water stress and strawberry cultivars.

Keywords: *Mediterranean region, Water stress, Fruit weight, Fruit quality*

Introduction

Strawberries are produced mostly in China, USA, Mexico, Turkey and Spain. Turkey provides about half of the production of strawberries from the Mediterranean region. Drought that more frequent and long-term are expected worldwide due to climate change, and the Mediterranean region is thought to be one of the most affected regions. Farmers generally uses of experiences on weather and plant conditions to determine the irrigation water amount. But they uses excessively or inadequately applied water, which can have a negative impact on berry size (Liu et al., 2007; Ghaderi et al., 2015, Sardas et al., 2017). It is known that during the growing period, products are often exposed to water stress. However, water stress, especially during certain stages of growth, such as seedling and flowering, causes a number of damages to product development, leading to major losses in yield and quality (Pessarakli, 1999; Çeliktopuz., 2019). Optimizing applied irrigation levels and finding alternative cultivation practices such as bio-activators are vital for the whole plant species including strawberry, due to fact that it is clear there will be reductions in product yield and quality under these conditions. Bio-activators are environmentally friendly applications that increase abiotic tolerance such as water stress and help increase yield and fruit quality (Spinell et al., 2010; Bulgari et al., 2015). Although there has been a large number of studies on water stress tolerance for numerous plants (Bota et al., 2001; Herralde et al., 2001), there are lack of information on determining changes in fruit quality parameters under conditions that water availability of soil is reduced or increased for different strawberry cultivars. Understanding the effects of water stress on fruit quality characteristics and developing strategies to minimize the damages caused by water stress are essential for the future of agriculture. The aim of this study was to determine the effects of excessive and inadequate irrigation on the diameter, length and weight of the fruit in order to achieve fruits with greater physical characteristics.

Material and Methods

The trial was conducted inside the high tunnel at the experimental farm of the Çukurova University in Turkey. Strawberry frigo seedlings (*Fragaria x ananassa Duch. Kabarla and Rubygem cv.*) were planted from 20th September in 2016 until 15th June in 2017. Fertilization and spraying processes were carried out in accordance with our previous studies in line with the requests of plants and soil. The study was planned in 4x2x2 factorial order (irrigation levels, bio-stimulants, cultivar), and in split-split plot randomized block design with 3 replicates, totally 48 plots. Bio-activator applications were designed as main plot and different irrigation levels were arranged as sub plot.

The content of the bio-activator which was named ComCat was certified by the BCS Öko-Garantie GMBH, Nurnberg, Germany as seaweed extract. The composition of bio-activator was organic matter (67%), K_2O (1.5%), alginic acid (18%) and gibberellic acid (250 ppm). The bio-activator was applied by foliar spraying on strawberry 4 times as 40 gr extract in 30 L water per decare. Moreover, fertilizer was applied uniformly to all treatments during the trial by drip irrigation and foliar spraying. After planting, equal amount water (179 mm) was applied to all plants until the plants were reached trifoliate and then 4 different irrigation levels were introduced. The water quantities were calculated by using Eq (1) as 0.5 (IR50), 0.75 (IR75), 1.00 (IR100) and 1.25 (IR125) times and the pan evaporation measured by the Class A pan.

$$t = (A \times Ep \times Pc \times Kcp) / (q \times n) \qquad (1)$$

where, t is the irrigation time (hours), A: the area of plot (m^2), Ep: the cumulative free surface water evaporation at irrigation interval (mm), Pc: the plant cover (%), Kcp: the crop-pan coefficient which is taken 1.25, 1, 0.75, 0.5 throughout the trial, q: the flow rate of emitters and n: the number of emitters in the plot.

The effects of different irrigation levels and bio-activator applications on fruit diameter, length and weight during the active harvest period (March–May) were determined for 'Rubygem' and 'Kabarla' strawberry cultivars. While fruit diameter and length were measured by a digital caliper, fruit weight was measured by precision scales (precision of 0.1 g). The obtained data were analyzed with the statistical program JMP version 5.0.1.

Results and Discussion

Total irrigation amount were applied as 451, 397, 343 and 288 mm to IR125, IR100, IR75 and IR50 from beginning of the trial to the end, respectively. In previous studies, they reported that irrigation water applied to strawberries vary from 250 mm to 825 mm (Kanber, 1986, Yuan, 2004; Kumar, 2011; Lozano, 2016, Kapur, 2018). The fruit diameter, length and weight values for both cultivars were presented in Table 1 and Table 2.

Differences due to irrigation x month interactions on fruit diameter, length and weight values were statistically significant for both cultivars. While IR 125 (40.7 mm and 33.1 mm) had the highest, IR 50 (36.3 mm and 29.5 mm) had the lowest fruit diameter values for both cultivars. Whereas in the 'Rubygem' cultivar IR 125 (42.3 mm) had the highest, IR 50 (40.0 mm) had the lowest fruit length value, in the 'Kabarla' cultivar IR 100 (36.0 mm) had the highest, IR 50 (40.0 mm) had the lowest fruit length value. While in the 'Rubygem' IR 125 (32.4 g) had the highest, IR 50 (26.1 g) had the lowest fruit weight value, in the 'Kabarla' IR 100 (20.1 g) had the highest, IR 50 (14.6 g) had the lowest fruit weight value. Besides IR 50 application in both cultivars, the smallest fruit diameter, length and weight values were significantly different from the other three applications in the same group. Giné Bordonaba and Terry (2016) reported that deficit irrigation on strawberries caused reductions in fruit length. Kapur and Şahiner (2019) found that water stress significantly reduced fruit size in the 'Fortuna' strawberry cultivar. Also, Çeliktopuz et al. (2019) determined that IR50 irrigation application caused significantly decreased (11 %) on fruit weight in 'Fortuna' cultivar.

Table 1. Effects of different irrigation levels and bio-activator applications on fruit diameter, length and weight of 'Rubygem' strawberry cultivar

	Irrigation level	Applications	Months March	Months April	Months May	Irrigation X Application	Irrigation Average
Fruit Diameter (mm)	**IR50**	**Control**	43.3	32.0	32.8	*36.0*	**36.3 B**
		Bio-activator	42.2	34.7	33.1	*36.7*	
	IR75	**Control**	43.9	36.0	31.6	*37.2*	**39.0 A**
		Bio-activator	47.4	40.0	35.2	*40.9*	
	IR100	**Control**	45.9	36.7	35.7	*39.4*	**40.1 A**
		Bio-activator	45.7	38.4	38.0	*40.7*	
	IR125	**Control**	47.0	38.2	34.6	*39.9*	**40.7 A**
		Bio-activator	48.4	40.9	35.2	*41.5*	
	Months Average		***45.5 a***	***37.1 b***	***34.5 c***		
	Applications Average			**Control**		38.1 b	
				Bio-activator		39.9 a	
	LSD month * = 1.60 LSD app *** = 1.31 LSD irr *** = 1.85 LSD irr x month=N.S**						
	LSD irr x app= N.S LSD irr x app x month = N.S						
	Irrigation level	**Applications**	**March**	**April**	**May**	**Irrigation X Application**	**Irrigation Average**
Fruit Length (mm)	**IR50**	**Control**	45.8	40.0	35.0	*39.4*	**40.0 B**
		Bio-activator	44.3	38.9	35.9	*40.6*	
	IR75	**Control**	45.7	41.9	34.1	*40.6*	**42.1 A**
		Bio-activator	49.2	43.4	38.3	*43.7*	
	IR100	**Control**	48.0	38.7	36.4	*41.0*	**41.9 A**
		Bio-activator	45.8	42.2	40.5	*42.8*	
	IR125	**Control**	47.4	42.3	35.6	*41.7*	**42.3 A**
		Bio-activator	46.4	44.2	38.0	*42.8*	
	Months Av.		***46.6 a***	***41.5 b***	***36.7 c***		
	App. Av.			**Control**		41.0	
				Bio-activator		42.2	
	LSD month * = 1.56 LSD irr * = 1.80 LSD app = N.S LSD irr x month=N.S**						
	LSD irr x app= N.S LSD irr x app x month = N.S						
	Irrigation level	**Applications**	**March**	**April**	**May**	**Irrigation X Application**	**Irrigation Average**
Fruit Weight (g)	**IR50**	**Control**	38.8	19.4	18.7	*25.6*	**26.1 B**
		Bio-activator	37.0	24.5	18.1	*26.5*	
	IR75	**Control**	41.0	26.1	17.2	*28.1*	**31.3 A**
		Bio-activator	50.8	32.3	20.6	*34.6*	
	IR100	**Control**	45.0	27.4	22.3	*31.6*	**31.5 A**
		Bio-activator	39.3	29.1	25.8	*31.4*	
	IR125	**Control**	42.8	28.9	20.5	*30.8*	**32.4 A**
		Bio-activator	43.2	36.6	22.3	*34.0*	
	Months Av.		***42.3 a***	***28.0 b***	***20.7 c***		
	App. Av.			**Control**		29.0 b	
				Bio-activator		31.6 a	
	LSD month * = 3.07 LSD irr *** = 3.54 LSD app * = 2.51**						
	LSD irr x month=N.S LSD irr x app= N.S LSD irr x app x month = N.S						

(1). Differences between the means were showed with different letters
(2). N. S.: Not Significant, ***: p<0.01; *: p<0.05

It was determined that in cultivar 'Rubygem' in which applied bio-activators produced 1.8 mm wider, 1.2 mm longer and 2.6 g heavier fruits than the control plants. However,in cultivar 'Kabarla', bio-activator application did not significantly affect the diameter, length and weight values of the fruit. The lowest average fruit diameter, length and weight values for both cultivars were obtained from the fruits harvested in May on the end of the growing period. As the season progresses, factors such as increasing evapotranspiration and temperatures, decreasing plant growth and increasing vegetative structure are thought to adversely affect fruit diameter, length and weight. Similarly, Bogunović et al. (2015) reported that the average fruit diameter of strawberry cultivars decreased towards the end of the season. Moreover, Wang and Camp (2000), and Kruger et al. (2012) reported that fruit length and weight decreased at high temperatures.

Cultivars were differed in their response to different irrigation levels and bio-activator applications. While cultivar 'Rubygem' produced average of 39.0 mm fruit diameter, 41.6 mm fruit length and 30.3 g weight, 'Kabarla' produced 32.0 mm, 34.8 mm and 18.4 g weight. Cultivar 'Rubygem' was found to produce wider, longer and heavier fruits than cultivar 'Kabarla'. Giné Bordonaba and Terry (2016) reported difference in fruit size of strawberry cultivars under water stress.

Table 2. Effects of different irrigation levels and bio-activator applications on fruit diameter, length and weight of 'Kabarla' strawberry cultivar

	Irrigation level	Applications	Months March	April	May	IrrigationX Application	Irrigation Average
Fruit Diameter (mm)	**IR50**	**Control**	37.3 bcd	25.8 ıj	27.9 hı	*28.6*	**29.5 B**
		Bio-activator	36.8bcd	26.6 hıj	22.4 j	*30.3*	
	IR75	**Control**	35.6cde	28.5 ghı	31.1 e-h	*31.7*	**32.5 A**
		Bio-activator	41.5 ab	29.6 f-ı	28.9 f-ı	*33.3*	
	IR100	**Control**	43.9 a	29.9 f-ı	27.2 hıj	*33.7*	**32.7 A**
		Bio-activator	36.3 cd	30.3 f-ı	28.8 ghı	*31.8*	
	IR125	**Control**	39.4 abc	31.3 e-h	30.4 f-ı	*33.7*	**33.1 A**
		Bio-activator	33.8 def	33.2 d-g	30.4 f-ı	*32.4*	
	Months Average		***38.1 a***	***29.4 b***	***28.4 b***		
	Applications Average			**Control**		32.4	
				Bio-activator		31.5	
	LSD month* = 1.75 LSD irr*** = 2.02 LSDapp= N.S LSD irr x month=N.S LSD irr x app= N.S LSD irr x app x month * = 4.96**						
Fruit Length (mm)	**Irrigation level**	**Applications**	**March**	**April**	**May**	**IrrigationX Application**	**Irrigation Average**
	IR50	**Control**	38.6	26.3	27.8	*30.9*	**32.3 B**
		Bio-activator	41.2	29.2	30.2	*33.6*	
	IR75	**Control**	42.1	29.0	34.0	*35.0*	**35.2 A**
		Bio-activator	44.9	30.9	30.2	*35.3*	
	IR100	**Control**	46.6	32.6	29.9	*36.4*	**36.0 A**
		Bio-activator	40.4	35.5	30.7	*35.5*	
	IR125	**Control**	42.8	35.0	32.0	*36.6*	**35.7 A**
		Bio-activator	36.6	36.8	31.2	*34.9*	
	Months Average		***41.6 a***	***31.9 b***	***30.8 b***		
	Applications Average			**Control**		34.7	
				Bio-activator		34.8	
	LSD month * = 2.20 LSD irr * = 2.54 LSD app= N.S LSD irr x month=N.S LSD irr x app= N.S LSD irr x app x month= N.S**						
Fruit Weight (g)	**Irrigation level**	**Applications**	**March**	**April**	**May**	**IrrigationX Application**	**Irrigation Average**
	IR50	**Control**	22.4 bcd	9.6 hı	9.6 hı	*13.9 e*	**14.6 B**
		Bio-activator	25.9 bc	11.1 ghı	9.3 ı	*15.4 de*	
	IR75	**Control**	25.4 bc	14.0 f-ı	15.5 e-h	*18.3 bcd*	**20.0 A**
		Bio-activator	34.6 a	15.9 efg	14.3 f-ı	*21.6 ab*	
	IR100	**Control**	37.4 a	15.0 e-ı	13.4 ghı	*21.9 a*	**20.1 A**
		Bio-activator	24.8 bc	16.7 d-g	13.4 ghı	*18.3 bcd*	
	IR125	**Control**	27.4 b	17.2 d-g	14.1 f-ı	*19.6 abc*	**18.7 A**
		Bio-activator	20.7 cde	19.9 c-f	13.2 ghı	*17.9 cd*	

Months Average	***27.3 a***	***14.9 b***	***12.8 b***	
Applications Average	**Control**			18.3
	Bio-activator			18.4
LSD month * = 2.16**	**LSD irr *** = 2.49**	**LSD app= N.S**	**LSD irr x month=N.S**	
LSD irr x app * = 3.53	**LSD irr x app x month *** = 6.11**			

(1). Differences between the means were showed with different letters
(2). N. S.: Not Significant, ***: $p<0.01$; *: $p<0.05$

Conclusions

It was thought that the application of IR 50, where water stress was most frequently, reduces the sweating by closing the stomata and consequently disturbing the active water and nutrient carrier mechanism, decreasing fruit diameter, length and weight values. Moreover, application of more water than the need for plants (IR 125) in both cultivars, had not been found to have a positive effect on fruit quality parameters such as diameter, length and weight. Bio-activator applications significantly affected on the fruit diameter and weight for cultivar 'Rubygem' in this study. It can be said that the permanent damages in diameter, length and weight representing the quality of the fruits depend on the level of water stress and cultivar. Although less irrigation water was applied, it is suggest that IR 75 irrigation application should be used together with higher dose bio-activator application in future studies in order to produce fruits with good physical characteristics.

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VARIABILITY OF CAROTENOIDS AND TOCOPHEROLS CONTENT IN MAIZE INBRED LINES

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Abstract

Maize assumes worldwide significance owing to its utilization as a human food and livestock feed. Besides high yield as the most important goal, production of hybrids with better nutritional properties is also of great importance. Developing maize varieties with improved grain quality traits can be achieved through breeding, which involve the use of natural genetic variation existing in the breeding pool. The aim of this study was determination of carotenoids and tocopherols content as well as insights into genetic diversity in set of maize inbred lines. Twenty-one maize inbred lines with different kernel type (dent, flint, sweet corn, popcorn) and kernel color (yellow and orange) were evaluated under RCB design with two replications in Zemun Polje during 2017 and 2018 and their carotenoid and tocopherol contents in grain were determined by high-performance liquid chromatography. Analyses of maize inbreds revealed wide genetic variation for lutein and zeaxanthin (14.94–39.21/14.30-38.19 μg/g), β-carotene (1.95–15.34/1.40-14.03 μg/g), α tocopherol (3.95-18.0/ 3.72-17.70 μg/g) and γ tocopherol (18.25-88.05/20.97-88.60 μg/g) in 2017 and 2018, respectively. The highest value of β-carotene had inbred line P21, L+Z one sweet corn inbred line and α – tocopherol inbred GR-9. Also, cluster analysis based on 21 SSR primers polymorphism was done. A total of 134 alleles were detected with average number of alleles 6.3. The dendrogram consisted of two clusters, each cluster was composed of several sub-clusters. Most sub-clusters comprised genotypes belonging to different kernel type/color groups. The lines with high content of particularly micronutritients may be used in breeding program to improve nutritional value.

Keywords: *carotenoids, genetic diversity, maize, tocopherols.*

Introduction

The carotenoids present in maize grain are classified into carotenes (*β*-caroteno e *α*-caroteno) and xanthophylls (lutein, zeaxanthin and *β*-criptoxanthin), with higher concentrations of lutein and zeaxanthin compared to other carotenoids. These compounds have great importance for human health, acting in mechanisms related to cancer, cardiovascular diseases and macular degeneration prevention in humans (Fraser and Bramley 2004). Also, maize kernels are rich in total tocopherol consisting α-, β-, γ- and δ- isoforms. Tocopherols, which are vitamin E compounds, play an important role in maintaining human health. The deficiency leads to age-related macular degeneration, neurological disorders, cancer, cataracts, Alzheimer's-, cardiovascular-, and inflammatory-diseases (Bramley *et al*. (2000)). The efforts thus directed to increase carotenoids and tocopherols in maize will have positive impact on the health and well-being of humans. Food fortification has been used worldwide to alleviate micronutrient deficiencies, but not had been found to be viable in the long run (Tanumihardjo *et al.* 2007). On the other hand, development of micronutrienten riched plant through biofortification holds promise for sustainable and cost-effective food-based solutions to combat micronutrient deficiencies (Pfeiffer and McClafferty 2007).

For breeding purposes and biofortification improvement in maize, it is necessary to have naturally occurred variability, and the first step is to identify concentration of micronutrient of interest, e.g. carotene, xantophylls, tocopherol etc. Information pertaining to the extent of variation for kernel carotenoids among diverse maize germplasm and the effect of environment on the performance of the genotypes, if any, assume tremendous significance to formulate an effective breeding strategy. Intensive efforts are currently being undertaken to develop maize inbreds enriched with carotenoids especially provitamin A (Babu *et al*. 2013; Gupta *et al*. 2013; Frano *et al*. 2014).

Maize exhibits great natural variation considering carotenoids and tocopherol contents. Provitamin A carotenoids (β-cryptoxantin, α-and β-carotene) usualy represent 10-20%, whereas zeaxantin and lutein are dominant carotenoids with 30- 50% of total content in maize grain (Ortiz- Monasterio *et al*., 2007). Compared with other staple foods, maize grains contain high level of tocopherols. α-tocopherol is the major vitamin E in nearly all green plants, in non-green plant parts, such as seeds and fruits, γ-tocopherol is preferentially found instead (Matea *et al*., 2008). Several studies have shown significant differences among maize inbreds for carotenoid and tocopherol levels (Quackenbush *et al*., 1963; Egesel, 1997).

Maize is one of the most important food, feed, and industrial crops globally. It can be classified based on endosperm and kernel constitution as flint, dent, floury, waxy, sweet, and pop corn; kernel colour (white, yellow, orange, blue) and maturity. Maize Research Insitute „Zemun Polje" gene bank contains maize genotypes with enormous genetic variability which could be potential sources of beneficial alleles for various traits of interest.

SSR markers have been utilized extensively in maize to characterize the genetic structure and diversity, to construct phylogenetic trees, to define potential heterotic groups, and to identify unique sources of allelic diversity. Carneiro Vieira *et al.* (2016) disscussed importance and wide application of SSR markers in plant genotyping and stated that over the period from 2010 to 2015, the 930 articles applying this technique in the genetic analysis of cultivated plants were published according to Web of Science. This report proves the relevance of SSRs in plant genotyping in spite of the fact that SNPs are stated as improved marker technique with many advantages relative to other types of molecular markers.

Material and methods

Twenty-one genotypes from Maize Research Institute gene bank with different kernel type (sweet corn L7-L11, popcorn L12-L14) and kernel color (orange L1-L6, and yellow L15-L21) was analyzed for carotenoids and tocopherols content by high performance liquid chromatography (HPLC). Maize grain, from selfed ears, from field trial conduct in Zemun polje in 2017 and 2018, were milled into flour (particle size less than 500μm). Standards for carotenoids (lutein, zeaxantin and β-carotene) and tocopherol (α-, γ+β and γ) were produced by Sigma Aldrich (Germany). Carotenoid content and composition was determined using HPLC, slightly modified method proposed by Rivera and Canela, (2012). Tocopherols (α-, γ+β and γ) were extracted using method described by Gliszczyńska-Świgło *et al.* (2007).

Twenty-one genotypes from Maize Research Institute gene bank with different kernel type and color were selected for molecular characterization using 21 highly informative SSR markers. For each group of kernel type/colour, leaf samples were prepared for DNA isolation taking tissue from five individual plants for each tested line. Genomic DNA extraction was performed applying modified Dorokhov and Klocke (1997) protocol. Total volume of each PCR reaction was 25 μl and final concetrations of components were as follows: 1xbuffer, 0.5μM primers, 0.8mM dNTPs each, 1U of Taq Polymerase (Thermo Scientific), 2 μl of 25 μg/μl genomic DNA and sterile water. PCR amplificaton was done using following program: 5 min intial denaturation step at 94°C, followed by 35 cycles of 94°C for 30s, 1 min of annealing at temerature specific for each primer, 2 min of extension at 72°C and final exension for 10 min at the same temperature. Polyacrylamide electrophoresis was done for PCR products separation (8% gels, 1 hour at 60mA, BioRAD tetra cell) and results were documented by photographing the gels under UV light using Biometra BioDocAnalyze Live gel documentation system. Cluster analysis was done with MEGA 7 using matrices of genetic distances calculated in PowerMarker V3.25 applying Rogers' coefficient and UPGMA method.

Results and disscussion

The estimation of carotenoids and tocopherols using HPLC revealed the existence of considerable variation among the maize inbreds. Analyses of 21 maize inbreds revealed wide genetic variation for lutein+ zeaxantin (14.94–39.21/14.30-38.19 μg/g) and β-carotene (1.95–15.34/1.40-14.03 μg/g), α tocopherol (3.95-18.0/ 3.72-17.70 μg/g) and γ tocopherol (18.25-88.05/20.97-88.60 μg/g) in 2017 and 2018, respectively. The mean lutein + zeaxantin concentration was 26.08 μg/g, while the same for β-carotene was 6.70 μg/g. The orange lines have the highest average lutein+zeaxantin content 29.60 μg/g and 10.34 μg/g for β –carotene, folowing with yelow inbreds (25.43/5.56 μg/g), sweet corn (24.06/5.11 μg/g) and popcorn have the lowest (23.98/4.74 μg/g). Among the lines included in our diverse maize panel, β carotene levels reached as high as 15.34 μg/g in L6 (P21). The sweet corn inbred L7 (38.7 μg/g), and two orange inbreds L3 (37.47 μg/g) and L1 (36.65 μg/g) were found promising for lutein+zeaxantin, while four orange inbreds L6 (P21, 14.69 μg/g), L3 (10.80 μg/g), L4 (10.63 μg/g) and L5 (10.68 μg/g) were identified with high β-carotene (Table 1). The observed variation for carotenoids is in agreement with the reports of Egesel *et al*. (2003), Menkir and Maziya-Dixon (2004) and Menkir *et al*. (2008). Chander *et al*. (2008) also observed a similar trend of variation while evaluating a set of Chinese germplasm, and found less provitamin A concentration and more of lutein and zeaxanthin. Promising inbred P21 with >15 μg/g of provitamin A showing potential for use in breeding programme (Zhang *et al*.2012; Babu *et al*. 2013). The content of lutein+zeaxantin (26,54 μg/g /25,64 μg/g) as well as β-carotene (6,83 μg/g /6,57 μg/g) was higher in 2017 than in 2018, respectively.

Table 1. Lutein+zeaxantin, β-carotene, α tocopherol and γ tocopherol content in maize inbred lines from 2017 and 2018.

Inbred line		L+Z 2017	L+Z 2018	β-carotene 2017	β-carotene 2018	α tocopherol 2017	α tocopherol 2018	γ tocopherol 2017	γ tocopherol 2018
L1	orange dent	37,22	36,08	7,12	6,47	10,73	12,76	18,25	20,97
L2	orange flint	29,24	26,92	8,45	8,51	13,74	16,25	28,16	32,46
L3	orange flint	38,20	36,74	11,42	10,18	8,67	8,70	47,51	52,91
L4	orange dent	33,82	32,30	10,45	10,81	12,16	14,87	33,35	38,02
L5	orange flint	22,62	21,69	10,98	10,38	18,00	18,70	63,77	78,24
L6	orange semi flint	20,93	19,38	15,34	14,03	7,86	9,88	50,11	53,71
L7	sweet corn	39,21	38,19	9,44	9,19	9,02	12,11	65,83	67,93
L8	sweet corn	21,24	18,81	3,73	3,33	10,63	11,47	88,05	88,60
L9	sweet corn	28,99	28,59	3,44	3,13	14,39	14,05	74,44	79,53
L10	sweet corn	14,94	14,30	1,95	1,40	9,65	9,74	72,28	79,72
L11	sweet corn	18,94	17,40	8,09	7,41	10,68	9,57	75,26	80,15
L12	popcorn	27,09	25,17	4,54	4,62	13,13	14,79	53,45	65,22
L13	popcorn	25,58	24,46	4,92	4,80	14,24	16,78	46,60	48,26
L14	popcorn	21,22	20,38	4,92	4,64	13,24	13,08	41,76	50,71
L15	yellow dent	22,41	19,25	6,18	6,34	5,92	6,02	30,85	38,04
L16	yellow dent	25,42	29,36	4,21	4,43	3,95	4,72	34,19	36,84
L17	yellow dent	27,75	28,28	6,28	6,83	4,14	4,29	39,19	40,91
L18	yellow dent	19,59	17,64	3,92	3,49	16,84	17,68	44,00	50,25
L19	yellow dent	22,20	27,39	4,80	4,53	15,61	16,43	30,80	36,47

L20	yellow dent	31,21	31,12	4,62	4,90	14,26	14,60	30,55	39,56
L21	yellow dent	29,53	24,91	8,72	8,56	10,89	10,96	70,54	72,38

As it was previously shown (Combs and Combs, 1985), the most abundant form of tocopherols in maize kernels were γ+β. The content varies from 18,25 to 88,05 μg/g in 2017 and from 20,97 μg/g to 88,60 μg/g in 2018 (Table 1). Average content of α tocopherol was 11.79 μg/g and for γ tocopherol 52.14 μg/g. The content of α tocopherol (12,26 μg/g /11,32 μg/g) as well as γ+β tocopherol (54,80 μg/g /49,47 μg/g) was higher in 2018 than in 2017, respectively. The popcorn lines have the highest average α tocopherol content 14,21 μg/g, folowing with orange inbreds (12,69 μg/g), sweet corn (11,13 μg/g) and yelow inbred lines have the lowest (10,45 μg/g). While, sweet corn lines have the highest average γ+β tocopherol content 77,17 μg/g, folowing with popcorn inbred lines (51,00 μg/g), orange inbreds (43,12 μg/g), and yellow inbred lines (42,47 μg/g). Two inbred lines, one with orange kernal (L5) and one with yellow kernal (L18) have the higest content of α tocopherol (18,0/18,7 μg/g and 16,84/17,68 μg/g, respectively). The high γ+β tocopherol content have three swet corn inbred lines L8 (88,65/88,60 μg/g), L11 (75,16/80,15 μg/g) and L9 (74,44/79,53 μg/g). The level of most important, α-tocopherol is about 20% of total tocopherols in maize and increased α-tocopherol is commercially desirable because of its higher anti-oxidant activity than γ-tocopherol (Rocheford *et al.*, 2002).

A panel consisted of 21 maize inbreds differed according to kernel type (dent, flint, popcorn, sweet maize) and kernel color (orange, white, yellow) was tested using 21 microstelite markers. All of the genotypes studied could unequivocally be distinguished with the combination of the SSRs used. In total, 134 SSR alleles were identified, with a mean of 6,3 alleles per locus. PIC values ranged from 0.56 to 0.89, average 0.73. Wende *et al.*, (2013) analysed 20 inbreds using 20 microsatellites and detected 108 allels with an average of 5.4. This result corroborates the finding of Liu *et al.* (2003) that 9–12 SSR markers are sufficient to genotype maize inbred lines. In order to gain further insight into the genetic diversity among different groups of the maize inbreds, a neighborjoining tree based on Roger's genetic distance was constructed. The dendrogram (Figure 1) consisted of two clusters (I and II). Each cluster was composed of several subclusters. Most subclusters comprised genotypes belonging to different kernel type/color groups. Cluster I included two subclusters. One of them, Ia was divided in two smaller groups. One group consisted of four swet corn inbred lines and one orange and the other consisted of two orange and one yellow inbred lines. The second group have four yellow inbred lines. Ib cluster have also two groups. First group comprised three popocorn and two yellow inbred lines and second one three orange and one swet corn inbred lines.

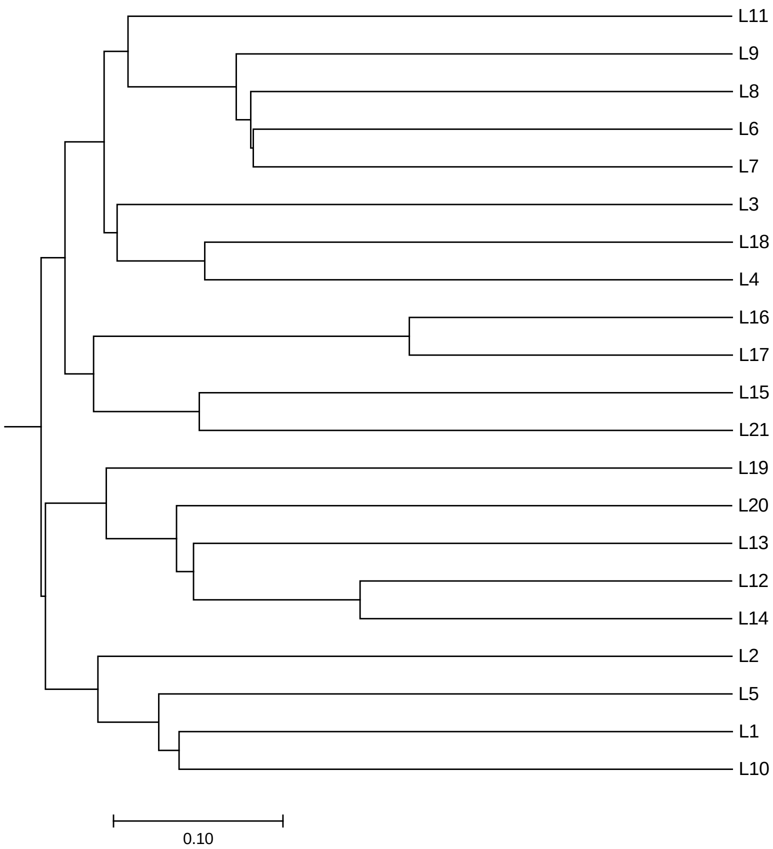

Figure 1. Dedongram of 21 maize inbred lines based on 21 SSR markers

Conclusion

The great natural variation for carotenoids and tocopherolos are presented in maize inbred lines with different kernal type (normal, sweet corn, popcorn) and kernal color (white, yellow, orange**).** The sweet corn inbred L7 and two orange inbreds L3 and L1, promising for lutein+zeaxantin, orange inbreds L6 (P21) with high β-carotene as well as two inbred lines, one with orange kernal (L5) and one with yellow kernal (L18) with high content of α tocopherol have potential for use in breeding programme.

Thus, inbreds with high lutein and zeaxanthin identified in the study can be used in developing hybrids specifically for poultry industry, and inbred identified with high β-carotene would be useful in eveloping provitamin A enriched hybrids.

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MINERAL COMPOSITION OF TWO PEPPER CULTIVARS (*CAPSICUM ANNUUM* L) FROM SERBIA AT THREE RIPENING STAGES

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Abstract

The aim of this study was to evaluate mineral composition of two commonly cultivated pepper varieties from Serbia (cultivars *Kalifornijska* and *Slonovo uvo*), at three ripening stages. Concentration of macro (K, Ca, Mg, Na) and micro elements (Zn, Fe, Mn, Cu, B, Cr, Mo, Se, Li, Al), and heavy metals (Pb, Hg, Cd, As, Ni) was determined, using AAS and ICP-MS, after microwave-assisted digestion. Relative standard deviations of AAS and ICP-MS measurements, for the most of analyzed elements, were between 0.08 - 9.28 %, indicating that precision was satisfactory. Potassium was the most abundant element in all samples, followed by Mg and Ca. Among the investigated micro elements the average content of zinc was the highest, followed by iron, for most of analyzed samples. Zinc and iron were followed by copper, manganese and boron. Semi-mature peppers of cultivar *Kalifornijska* were richer in K, Ca, Mg, Fe and Zn compared to other maturity stages. For cultivar *Slonovo uvo* results were different compared to cultivar *Kalifornijska*. The content of K and Ni increased with ripening of fruit, while the content of magnesium decreased with ripening of fruit, which was not case for cultivar *Kalifornijska*. The higher values of Mg, Fe, Zn; lower values of Ca and higher values of Cu in cultivar *Slonovo uvo* were reported by different authors for other pepper varieties.

Keywords: *pepper, mineral composition, maturity stage, AAS, ICP-MS.*

Introduction

Sweet red pepper (*Capsicum annuum* L.) is an important vegetable crop in the world and Serbia (Tebić, 2010; Bhandari, 2016). Many varieties that are cultivated differ in shape, size and aroma (Todorova, 2017; Ilić, 2013). Peppers are good sours of minerals, vitamins C, A, E, phenols and carotenoids and have important antioxidant properties (Kolton, 2011; Martinez, 2007; Guil-Guerrero, 2006). Composition of peppers is influenced by growing conditions, variety, maturity, harvest period, pre-harvest and post-harvest handling (Martinez, 2007; Guil-Guerrero, 2006). Peppers are mostly consumed as fresh or dried, but also as frozen, smoked and fermented (Berke, 2012). Pepper fruits are consumed in different stages of maturity, green and red ones are the most used. Due to intensive respiratory and metabolic processes green (immature) peppers have shorter shelf-life than the mature ones (Silva, 2013; Martinez, 2007).

Pepper fruits are rich in macro elements (potassium, phosphorus, magnesium, calcium, sodium) and some micro elements such as iron and zinc, while content of other micro elements vary significantly (Angeles Botella, 2017; Lopez, 2013; Rubio, 2002).

Trace elements or micro elements are those that are needed in small quantities, less than 100 mg/day, for normal functioning of plants and human body. They include Co, Mo, Mn, Zn, Cr, Fe, Cu, and Se. Nickel, B and Al are also trace elements, but their biological role in human is still unknown (Rattanachaiwong, 2019; Fillipini, 2019). Beside their essential role, some of them may be toxic at higher concentration (Cu, Cr, Mo, Ni, Se, Al and Zn) (Tokalioglu, 2018; Tokalioglu, 2019).

On the other hand, heavy metals, such as cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As) are of more concern, because they are toxic and have cumulative properties. These elements mostly accumulate in liver, kidneys, lungs, fat depots and muscles and can cause many damages and problems to the human body (Papadomichelakis, 2018; Tokalioglu, 2018). Contamination of vegetables is mainly caused by irrigation water, environment, fertilizers and pollutants from industry and vehicle (Li, 2018; Tokalioglu, 2019).
The aim of this study was to determined mineral composition and heavy metal content of two pepper varieties commonly cultivated in Serbia, at three maturity stages.

Material and Methods

Two pepper cultivars, at three ripening stages: green, semi-mature and mature were obtained from local farmers in Serbia. Samples were collected in September 2018. After collection, samples were washed, dry with paper towel, milled to pepper pulp and then frozen in thin layer until analysis.
Nitric acid (65% w/w) and hydrogen peroxide (30% w/w) were purchased from Sigma Aldrich (St. Louis, USA). Distilled water was used for dilution of digested mixture, while HPLC grade water was used for preparation of standard solutions. Individual single-element stock solutions of K, Na, Ca and Mg, with concentration of 1000 mg/dm^3 were supplied by AccuStandard (New Haven, USA). Multi-element standard solution for ICP-MS, with concentration of each element 10 μg/cm^3, was supplied by AccuStandard (New Haven, USA).
Water content was determined by gravimetric method after drying to constant weight at 105^{o}C.
Microwave-assisted digestion was performed in closed-vessels (PTFE vessel). Peppers were weighted (0.5 g), placed in vessels and mixed with 5 ml HNO_3 and 2 ml H_2O_2. After that vessels were closed and placed in microwave oven. Digestion lasted 35 minutes with following regime: 10 min to 150^{o}C and hold 5 min; then 15 min to 190^{o}C and hold for 15 minutes. After cooling of vessels, obtained mixtures were diluted to 50 cm^3. One blank solution was run with set of 12 pepper samples. The concentrations of micro elements and heavy metals were determined by ICP-MS (iCAP Q, Thermo Scientific, UK), while the concentrations of macro elements were determined by AAS (Agilent Technologies, 200 Series AA, USA). In this study concentrations of following macro elements were investigated: K, Na, Ca and Mg; concentrations of following micro elements : Zn (64), Cu (65), Se (77), Mo (95), Li (7), Al (27), B (11), Cr (52), Fe (57), Mn (55), and heavy metals: Pb (206), Hg (200), Cd (111), As (75), Ni (60). The wavelengths selected in measurements of Ca, Mg, Na and K were 422.7 nm, 285.2 nm, 589.0 nm and 404.4 nm; respectively.
Results are expressed as mean ± SD (standard deviation). The significance of differences between mean values was determined by an analysis of variance using MINITAB software package (Release 15, Minitab Inc., State College, PA). Differences were considered significant at $p < 0.05$.

Results and Discussion

The moisture contents were 91.3%, 92.9% and 93.4% for green, semi-mature and mature peppers, respectively of cultivar *Kalifornijska*, and 92.7%, 93.7% and 91.9% for green, semi-mature and mature peppers of cultivar *Slonovo uvo*, respectively.
In Table 1 are given the mean values of minerals and heavy metals, expressed on fresh weight, in pepper *Kalifornijska* at three ripening stage. In pepper *Kalifornijska* at all three ripening stages there was no detection of Mo, Li, Al, Cr and Hg. Potassium was the most abundant mineral in all three ripening stages, which content ranges from 135 mg/100g fresh weight to 162 mg/100 g fresh weight, followed by Mg, Ca and Na. Such order of the content of macro elements is in agreement with research of Rubio et al (2002) and Martinez et al

(2007), while the content of magnesium is higher than the content of calcium for other pepper varieties and researches of other authors (Bernardo, 2008; USDA database; Bhandari, 2016). Macro elements are followed by microelements. The most abundant micro element was Zn at all three ripening stages, followed by Fe, Cu, Mn and B. The content of Fe and Zn in analysed samples was higher compared to data of other authors, while the content of Cu and Mn were similar (Rubio, 2002) or lower compared to Martinez et al (2007). At all three ripening stages heavy metals Pb and Ni were detected, while Cd and As was detected in green and mature pepper. The lead content is significant and ranges from 173 μg to 289 μg per 100 g of fresh weight samples.

Table 1. Mineral and micro element content of pepper *Kalifornijska* in different ripening stages, per 100 g of fresh sample

Metal	Ripening stage		
	Green	Semi-mature	Mature
	mg/100g		
K	$135 \pm 0.02^{b,c}$	$162 \pm 0.02^{a,c}$	$144 \pm 0.03^{a,b}$
Na	$2.10 \pm 0.06^{b,c}$	$1.60 \pm 0.30^{a,c}$	$3.47 \pm 0.14^{a,b}$
Ca	$10.9 \pm 0.02^{b,c}$	$13.7 \pm 0.20^{a,c}$	$10.7 \pm 0.03^{a,b}$
Mg	$33.3 \pm 0.38^{b,c}$	$37.0 \pm 0.25^{a,c}$	$32.6 \pm 0.33^{a,b}$
	μg/100g		
Fe	949 ± 41.8^{b}	1686 ± 207^{a}	1277 ± 393
Zn	1515 ± 418	2499 ± 466	2465 ± 1767
Cu	$74.9 \pm 3.30^{b,c}$	$55.3 \pm 4.96^{a,c}$	$186 \pm 2.88^{a,b}$
Mn	$54.8 \pm 0.42^{b,c}$	$50.4 \pm 1.39^{a,c}$	$47.0 \pm 1.09^{a,b}$
B	17.5 ± 0.18^{c}	16.1 ± 0.95^{c}	$18.8 \pm 0.13^{a,b}$
Ni	$164 \pm 1.72^{b,c}$	$51.1 \pm 1.57^{a,c}$	$65.2 \pm 1.89^{a,b}$
Pb	$173 \pm 0.38^{b,c}$	$289 \pm 11.1^{a,c}$	$238 \pm 5.28^{a,b}$
Cd	$2.68 \pm 0.33^{b,c}$	n.d.	0.81 ± 0.50^{a}
As	0.94 ± 0.65	n.d.	0.40 ± 0.60

Mean value ± SD. a-significantly different from green; b-significantly different from semi-mature; c- significantly different from mature.

The highest content of K, Ca, Mg, Fe and heavy metal Pb was obtained in semi-mature pepper. Due to the fact that the size of the fruit and water content, for this pepper cultivar, increased with the degree of ripeness, it can be concluded that peppers fruits of this cultivar adopted minerals from soil to a certain stage of maturity, after which the content of the minerals decreased due to further maturation, as it is likely to continue adoption of water, while the adoption of minerals stops.

The content of Mn and Ni is higher in green peppers, which means that these metals are accumulated in pepper fruit while it is green, and with further growth their content decreased due smaller absorption and dilution with further water adsorption. The content of Na and Cu was the highest in mature peppers, which indicates that these minerals were still absorbed from soil until full maturity.

The mean values for minerals and heavy metals, expressed on fresh weight of *Slonovo uvo* cultivar are listed in Table 2. In pepper fruits of cultivar *Slonovo uvo* at all three ripening stages there was no detection of Mo, Li, Al, Cr and Pb. The order of the levels of the macro elements was the same as for cultivar „*Kalifornijska*", at all maturity stages: K>Mg>Ca>Na. The most abundant micro element in semi-mature and mature pepper was Fe, while in green pepper it was Zn. These minerals are followed by Cu, Mn and B. The concentration of K and

Ni increased with ripening of pepper fruit, while the concentration of Mg decreased with the ripening of pepper fruit.

Table 2. Mineral and micro element content of pepper *Slonovo uvo* in different ripening stages, per 100 g of fresh sample

Metal	Ripening stage		
	Green	Semi-mature	Green
	mg/100g		
K	161 ± 0.005^{b,c}	170 ± 0.02^{a,c}	197 ± 0.002^{a,b}
Na	0.57 ± 0.03^{b,c}	0.95 ± 0.06^{a,c}	0.74 ± 0.00^{a,b}
Ca	12.9 ± 0.02^{b,c}	8.47 ± 0.01^{a,c}	14.7 ± 0.18^{a,b}
Mg	36.8 ± 0.23^{b,c}	17.9 ± 0.62^{a,c}	16.3 ± 0.18^{a,b}
	μg/100g		
Fe	377 ± 118^{b}	1171 ± 71.7^{a,c}	524 ± 118^{b}
Zn	573 ± 73.7^{b,c}	306 ± 13.2^{a}	411 ± 16.8^{a}
Cu	144 ± 4.94^{b,c}	98.8 ± 4.70^{a,c}	127 ± 2.32^{a,b}
Mn	60.7 ± 1.01^{b}	55.6 ± 1.85^{a,c}	64.1 ± 2.06^{b}
B	20.4 ± 0.49^{b}	32.1 ± 0.93^{a,c}	23.3 ± 0.74^{b}
Ni	44.9 ± 2.53^{b,c}	56.3 ± 3.03^{a,c}	150 ± 5.62^{a,b}
Hg	n.d.	n.d.	31.5 ± 3.9^{a,b}
Cd	n.d.	0.08±0.77	0.47 ± 0.88
As	0.29 ± 0.88	1.80 ± 1.16	n.d.

Mean value ± SD. a-significantly different from green; b-significantly different from semi-mature; c- significantly different from mature.

Zinc and Cu are the most abundant in green peppers, which indicated that these minerals are probably most absorbed while the fruit is green, and with further ripening theirs contents decreased due to dilution (semi-mature fruits). The fact that the concentrations of these minerals were higher in mature then the semi-mature fruits was in agreement with the change of water content for this cultivar. Mercury was detected in mature fruits, while the concentration of Cd in semi-mature and mature peppers; and As in green and semi-mature peppers were low and closed to instrumental limits of detections.
Contents of K and Mn, in fruits of cultivar *Slonov uvo*, are comparable with results reported by Rubio et al (2002). Bernardo found similar values for iron and zinc, higher values for Cu and lower for K and Mn (Bernardo, 2008).

Conclusion

It can be concluded that mineral composition of cultivars *Kalifornijska* and *Slonovo uvo* was influenced by maturity stage, and also by cultivar. The most abundant mineral of analyzed elements was potassium in all samples, followed by magnesium and calcium. Cultivar *Kalifornijska* had higher concentrations of micro elements Fe and Zn, and lead was detected in all samples at different ripening stages, which wasn't case for cultivar *Slonovo uvo*. For cultivar *Kalifornijska* content of K, Ca, Mg, Fe and Zn was the highest in semi-mature fruits. This analysis showed that concentration of K and Ni increase with fruits maturation of cultivar *Slonovo uvo*. When compared to bibliography, data the concentrations of Mn, Fe and Zn in this two pepper varieties were higher and the concentration of Ca was lower in all investigated samples.

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EFFECT OF DIFFERENT STORAGE TEMPERATURES ON PHYSICAL STATE AND SOME INNER CONTENTS OF SWEET POTATO (*IPOMOEA BATATAS* L.) BULBS

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Abstract

In China and African countries, the production of the root vegetables especially sweet potato is facing several factors concerning the environmental storage conditions and the need for increased shelf-life of the bulbs. In fact, the storage of the bulbs is considered as one of the critical points of the production of the sweet potato crop around the world. Some of the vintage goals of the Department of Vegetable and Mushroom Growing at the Szent Istvan University were to inspect the influence of storage temperature on the quality change of the stored sweet potato bulbs and detect the weight loss changes during cold and ambient storage. In addition, non-destructive texture measurement of the stored bulbs was carried out using the acoustic response technique. This experimental work consists of two parts: the first part stores the washed and the unwashed bulbs in cold storage at two different temperatures (6 °C and 10 °C) in relative humidity of 85-90 % and determines the weight loss during the storage for washed and unwashed bulbs. The second part is carried out at ambient storage conditions at 20-25 °C and relative humidity of 60-70 %. This experiment proceeds in controlled environmental conditions in laboratories of the Department of Postharvest Science and Sensory Evaluation at Szent Istvan University for cold storage period, while the ambient storage was carried out in the Department of Vegetable and Mushroom Growing lab. According to our results, as it was expected, the percentage of the weight loss during cold storage has increased in different ways. After removal to ambient conditions (shelf-life), mass loss and negative textural changes (softening) became more extent and rapid. However, it was different in case of the examined varieties as well.

Keywords: *Sweet potato, Ipomoea batatas, Washed bulbs, Cold storage, Ambient storage.*

Introduction

Sweet potato (*Ipomoea batatas* L.) belongs to the family *Convolvulaceae* (morning Glory). It is an herbaceous dicotyledonous plant. It is originated in Central and South America (Steinbauer and Kushman, 1971; Yen, 1982; Thurston, 1984; Otterdijk, 1999). It was introduced to Europe in the 16th century and gradually in the 18th and 19th century became an essential food crop. Sweet potatoes are now widely grown throughout the temperate and warm tropical regions, where an adequate water supply is available to support their growth (Tai-Hua and Peng-Gao, 2019).

The nutritional value of the sweet potato is 50% higher than potato (Ofori et al., 2005), while the edible part is not only the bulb, but also the leaves. Concerning the storage of the bulbs, one of the major limitations is its postharvest perishability and the need for increased of the shelf-life (Mpagalile et al., 2007). Also, there are many traditional methods used to store the sweet potato such as heap storage, in-ground storage, platform and pit storage. These methods have been practiced in Nigeria and across African countries by farmers, but the most common traditional method is the pit storage. Pit storage can generally be considered as a cheap

method for the rural communities since it requires minimum materials and it has been reported in many countries for example Indonesia, Malawi and Zimbabwe (Woolfe, 1992). The pit storage method is considered as the best traditional method to store the tubers considering decay, such as sprouting, moisture loss and pathological losses, if compared to other possible storage methods (Yakubu, 2005). According to an experiment concerning storage temperature carried out by Cushman and Smart (1954) earlier, the results showed that the storage of the bulbs at 12-15 °C is advisable (in a fixed relative air humidity at 85 %). Later this was confirmed by Maynard and Hochmuth (2007), who defined the temperature and air humidity in 12-15 °C and 85-95 % for 4-7 months. According to Thompson and Scheuerman (1993) short exposures to temperatures as low as 10 °C may not cause damage.
Quality of bulbs depends on firmness of flesh, however during the storage the weight is reduced due to the loss of water, but this change is closely related to the loss of sugars in the respiration process in the tubers, and in the end the product can soften. The transformation of the carbohydrates especially the decrease in starch and increase in sugar contents (Kimbrough, 1942). However, according to Thompson and Kelly (1957) the taste and the consistency in the cooked product is different. Also the soft product is not marketable, so it is advisable to follow the rate of postharvest softening.
This experimental work objective was also to investigate the influence of different storage temperatures on the physical and the inner contents change during storage, especially focusing on the loss of weight and increase of softening by the use of non-destructive measuring methods.

Materials and methods

The measured parameters focused on physical state and inner contents as well. In this article, the percentage of the weight loss, textural change determined as acoustic stiffness coefficient change, and change of water-soluble dry material content (Brix %, Anthocyanin and β-carotene) were analyzed. The samples of sweet potato were grown at the Experimental and Research Farm of Szent Istvan University at Soroksar, Hungary. The experimental and research farm is sited on the Danube casting site, the physical properties of sandy soil. During the experiment, two varieties were used as the orange variety ('Ásotthalmi-12') and ' Purple' variety (Fig. 1). The propagating material was ordered from Asotthalom, Southern Hungary, and 'mother-bulbs' was propagated and grown in the experimental farm in 2017 in 300 m^2-s.

Figure 1. (A) 'Ásotthalmi-12' Orange Sweet Potato Variety (B) Purple Sweet Potato variety (Photos: author).

This experiment was carried out in a small range space with two varieties of sweet potato bulbs. Two temperature-controlled household fridges were used to store the sweet potato bulbs under two different temperature conditions at the Department of Postharvest Science and Sensory Evaluation, Faculty of Food Science, Szent Istvan University. The relative humidity and temperature were collected by a relative humidity and temperature meter and data logger (Trotec Bl-30 climate data logger). 80 bulbs were stored in two fridges with 5 bulbs in 4 repetitions in 4 treatments. Markings of our treatments and storage conditions are shown in Table 1.

Table 1. Treatments and environmental conditions during the cold and ambient storage.

Variety	**Treatments**		**Rel. Humidity (%)**	**Storage temperature**	**Sign**	
Control (Orange and Purple variety)	-			-	O	P
Ásotthalmi-12' orange fleshed and **Purple fleshed**	Cold storage	Unwashed	80-90%	6 °C	AO	AP
				10 °C	BO	BP
		Washed		6 °C	CO	CP
				10 °C	DO	DP
	Ambient storage		60-70%	20-25 °C	all above	

The cold storage of the bulbs lasted from 2nd of November until 12th of December. After this, the bulbs were transferred to shelf-life (ambient storage) from 13th of December until 15th of January in order to notice the effect of different environmental conditions on quality and weight loss during the storage and to detect the convenient method to store the sweet potato bulbs. For weight-loss determination laboratory scales and rulers were used in order to collect the individual weight (g) and length (cm) data of the bulbs.

Acoustic measurements: Nondestructive acoustic stiffness analysis was carried out at the Department of Postharvest Science and Sensory Evaluation at Szent Istvan University, Faculty of Food Science. The purpose-built measuring device (developed at the Department of Physics and Control, Szent Istvan University) consists of a data acquisition hardware and software in order to detect and analyze the characteristic sound response related stiffness of the bulbs. Measurements were carried out according to the measurement of carrots reported by Zsom-Muha and Felföldi (2007). The vertically located sample was excited for its sound response by a gentle hit on its top. The characteristic sound response was collected and recorded by a microphone located under the bulb in the sample holder. Then it was transformed and analyzed by a special software called Stiffness. All points were marked, in this case the second time exciting (knocking) point was the same. 10 sound samples were collected per every bulb, so 800 samples were evaluated. The acoustic stiffness coefficient of the bulbs (S, m^2s^{-2}) was calculated as $S = f^2 * l^2$, where the ‘f’ is the characteristic frequency of the sound response (Hz), and the ‘l’ is the length of the bulb (m) (Zsom-Muha and Felföldi, 2007).

Inner content parameters: Sweet potatoes Brix (%) was measured by Hanna HI 96801 type refractometer. Quality of vegetables relates to the quantity of dissolved solids in plant sap (fresh juice to detect the sugar contents as well).

β-carotene content was measured by spectrophotometric analysis using color absorption levels, while Anthocyanin content of purple sweet potatoes was measured by spectrophotometric analysis based on method of Jungmin et al. (2005).

Statistical Analysis: The results were expressed according to basic statistic rules, average ± st. deviation with significance level 5% (α=0.05). Basically, Microsoft 365 ProPlus Excel with Statistic ToolPak and IBM SPSS Statistics v25 software were used for Analysis of Variances (ANOVA).

Results and Discussion

From weekly measurements we calculated the ratio of bulb weight loss (in percentage compared to initial weight). Table 2 includes that from 2nd of November until 15th of January. According to our results, the weight loss increase speeded up especially after the bulbs’ removal from cold to ambient storage conditions (note the change between 13th Dec. and 15th

Jan.). There was a difference between the varieties ('O' - orange and 'P' - purple colored variety). Additionally, difference was also found between washed (marked with C, D) and unwashed (marked with A and B) bulbs. Unwashed bulbs lost their weight earlier with higher intensity. All of 6 °C samples (marked with A and C) suffered faster reduction of weight than samples stored at 10 °C (marked with B and D) especially after removal to shelf-life at ambient conditions due to its susceptibility to too low storage temperature caused physiological disorder under 12 °C (chilling sensitivity and injury), to prove statements of Cantwel an Suslow (2001).

Table 2. Reducing of Weight loss (%) of the bulbs during storage

	Weight loss (%)								
Treatments	**30th Oct.**	**02nd Nov.**	**10th Nov.**	**16th Nov.**	**23rd Nov.**	**30th Nov.**	**08th Dec.**	**13th Dec.**	**15th Jan.**
AO	0,0	0,5	11,1	12,0	16,4	23,8	24,9	28,9	73,1
BO	0,0	0,5	1,8	5,1	22,9	24,0	32,8	36,0	69,6
CO	0,0	0,4	6,1	14,1	15,3	20,7	23,9	28,2	66,0
DO	0,0	0,4	1,3	7,2	9,1	10,0	10,8	11,6	42,9
AP	0,0	0,3	1,4	7,4	8,7	9,8	10,4	11,6	54,5
BP	0,0	0,3	0,7	1,9	12,7	13,1	14,7	16,0	39,9
CP	0,0	0,5	1,1	3,0	4,7	5,4	16,6	17,2	63,2
DP	0,0	0,3	1,3	2,2	3,4	4,3	5,6	6,4	31,2

From the 13th of December until the 15th of January, the environmental conditions of storage changed from cold to ambient storage conditions (shelf-life) providing a clear effect on the percentage of weight-loss as (Fig. 2 and Fig. 3). These results are in close connection to the results of mass loss change vs. storage time and temperature as mentioned earlier.
In our figures, connection could be observed as the bulbs lost their weight, the textural change as acoustic stiffness follows the decrease, however the difference of decrease is not linear, and because of the high standard deviations of weight values, significance cannot be stated.
In case of water-soluble dry material content (Brix %), the connection could be observed as well (Figure 4), as a connection between the Brix value decreasing and the change in carotene and anthocyanin content (mg l^{-1}). Unfortunately, data is missing in case of orange variety (O) from January measurement.
The applied storage method affects the quality and the inner content of the bulbs. It has an influence on shelf-life properties also, and additionally, on the postharvest susceptibility of the bulbs to physiological disorders and/or infections caused by many pathogens related rotting diseases.

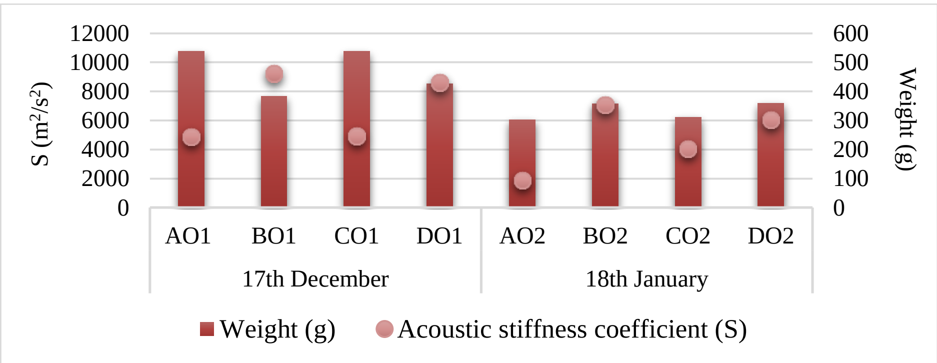

Figure 2. The connection between the orange bulbs weight loss and acoustic stiffness during ambient storage.

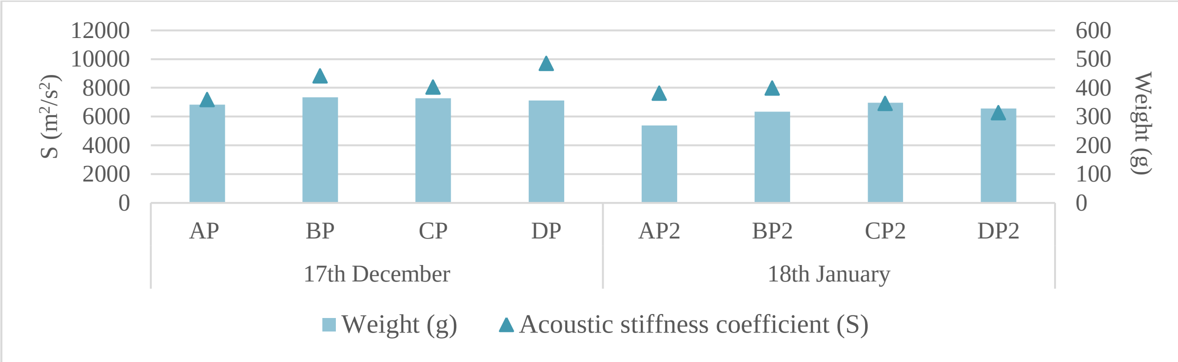

Figure 3. The connection between the purple bulbs weight loss and acoustic hardness factor in ambient storage time.

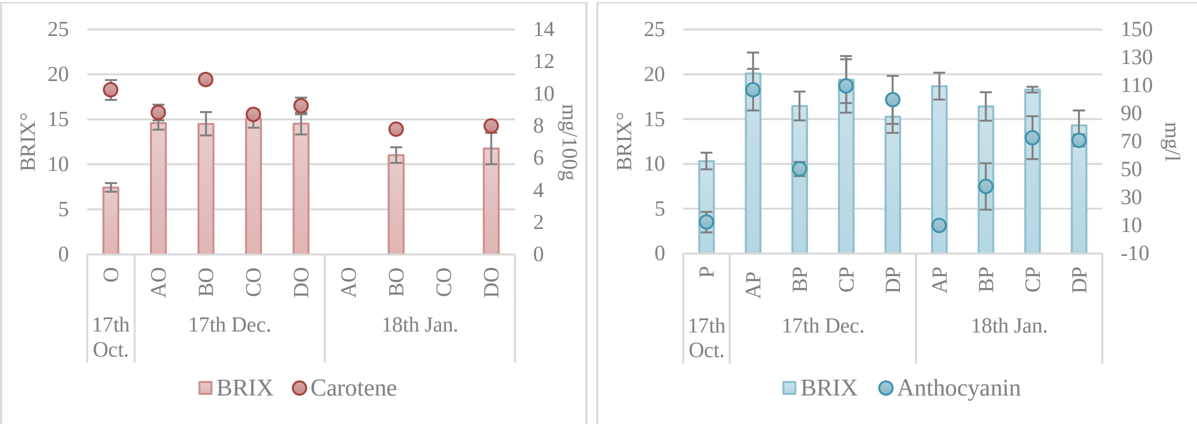

Figure 4. Change of Brix (%) and color contents (carotene and anthocyanin) of flesh in case of Orange (left) and Purple (right) type of sweet potato.

Conclusion

Several important facts were gained during cold storage time and ambient storage time. Effect of different storage environments caused different weight loss. During the cold storage the percentage of the weight loss increased faster in case unwashed orange bulbs on 10 °C, unwashed purple bulbs on 10 °C and washed purple variety on 6 °C. After ambient storage weight loss increased faster in all treatments. While in case of orange variety (O) the weight loss speed seemed independent on the washing treatment, in case of washed purple bulbs the weight loss was slower. Also, the weight loss was slower in every cases of treatments of purple variety, where the water-soluble dry material content was higher. In this case we can state that dry material content has higher effect to weight loss like storage temperature or washing of bulbs during storage time. Moreover, our results agree with Zainalabidin et al. (2019), where reported as the minimum safe temperature to store the sweet potato at low temperature is 13 °C and the chilling injury symptoms occur as the bulbs decline between 0 °C and the safe temperature of storage. However according to Cantwel and Suslow (2001) the recommended environment is dry air humidity and 12,5-15 °C temperature for long term (6-10 mths) storage, in short exposure the 10 °C temperature may not cause damage (Thompson and Scheuerman, 1993).

The non-destructive acoustic stiffness measurement showed interesting results, providing the possibility to follow the weight loss related overall quality and textural changes. Further experiments are needed to be carried out in order to get more information concerning any connections between some of the inner contents' change and textural changes, and with use destructive and non-destructive methods parallel to gain more accurate results.

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EFFECT OF DRYING ON THE CHANGE OF SUGAR CONTENT IN PLUM FRUITS

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Abstract

Drying is one of the oldest methods of preserving fruit. In the course of drying process, there is a change in some components of chemical composition to a degree depending on food type, its composition, and drying process itself. The testing was performed in three replications using plum fruits of the 'Čačanska Lepotica', 'Mildora', 'Čačanska Rodna' and 'Stanley' cultivars, at the optimum ripening stage required for drying, based on soluble solid contents. Drying was performed at the experimental dryer using the convective (streaming) drying process, at two constant air temperatures, 90 °C and 70 °C, until attaining 75% of total dry matter in the prunes. In addition to the control, the fruits were subjected to a pretreatment consisting of dipping in boiling water. This paper reviews the values of total sugars, invert sugars and sucrose in fruits of fresh and dried plums (calculated in grams per 100 grams of total dry matter), as well as the change of these parameters in prunes in relation to the fresh fruits (expressed in percentages). Drying temperature affected the change of total sugars, invert sugars and sucrose content in fruit of the tested plum cultivars. On the other side, dipping as an applied pretreatment had no effect on the change of these parameters except in the cultivar 'Čačanska Rodna', which dipped fruits are found to have higher decrease of sucrose compared with non-treated fruits. During drying, hydrolysis of sucrose occurred, manifesting in a dramatic decrease of its content in dried fruits, in relation to the starting raw material in all of the tested cultivars. Intensity of changes was conditioned by varietal characteristics.

Key words: *prune, drying temperature, dipping, invert sugar, sucrose.*

Introduction

Plum (*Prunus domestica* L.) is considered a national fruit species ranking first in the fruit growing of Serbia by both number of trees and by its annual production. Although a major part of plum yield in Serbia is processed into brandy (Popović et al., 2006), the most significant product coming from plum, in terms of nutritional value, is prune. Prune represents significant energy source for human body though is more commonly classified in high nutritional food with special dietetic and physiological importance, primarily due to its protective and therapeutic effect on digestive tract of consumers (Zlatković, 2000; Piga et al., 2003).

Drying is defined as a process of moisture loss resulting from simultaneous existence of the heat and mass transfer phenomena. Plum drying is a slow and long-term process because whole fruits are dried, hence, there are real conditions for various chemical transformations, primarily carbons, since they make up over 90% of the total dry matter of fruit (Friedman, 1996). In plum fruit, the most predominant are sugars, i.e. fermentable sugars (fructose, glucose and sucrose). During plum drying, in addition to oxidation reaction and acid hydrolysis (Dikerman et al., 2004), non-enzymatic browning reactions with sugars participating, such as caramelization and Maillard reaction (Manzocco et al., 2001; Kim end Lee, 2008), can be conducted too. Depending on the intensity of these reactions, characteristic colour and flavour of prune appears but on the other hand, they may lead to degradation of

product aroma and appearance (Dikerman et al., 2004; Sanz et al., 2001). Appropriate drying technology and raw material can affect the flow of these reactions in order to minimize the change of product quality, which is an ultimate goal of each processing.
The aim of the work was to examine the effect of drying temperature and dipping on the change of sugar content, both total and invert sugars as well as sucrose content in fruits of the plum cultivars 'Čačanska Lepotica', 'Mildora', 'Čačanska Rodna' and 'Stanley'.

Material and Methods

For the purpose of testing, plum fruits of the cultivars 'Čačanska Lepotica', 'Mildora', 'Čačanska Rodna' and 'Stanley' were obtained from the plantations of the Fruit Research Institute Čačak, in which standard agro- and pomotechnical practices were performed. Fruits for drying were selectively picked at full maturity stage for the appropriate cultivar, with the total dry mass content 15.77%, 25.50%, 22.67% and 19.59%, with average mass of about 41 g, 23 g, 36 g and 42g, respectively.
Drying of fruits was carried out in an experimental dryer for testing convective drying process (Kandić et al., 2006). An air streaming drying procedure was used at two constant air temperatures, 90 °C and 70 °C. Control fruits (without pretreatment) and dipped fruits (plum fruits which are, in laboratory conditions, immersed into boiling water for 20 seconds) were dried. Fresh plum fruits were placed in a single layer on an inox tray and 6 trays were placed in a drying chamber. Dipped and control fruits were dried simultaneously in the same experiment on the trays symmetrically placed in the drying chamber (3 trays with the control and 3 trays with dipped fruits). Through the trays with plum fruits, vertical heated air stream with predefined characteristics (temperature, flow) was introduced. Direction of vertical streaming during the process of drying was changed alternately and periodically for 60 minutes, thus achieving the same drying conditions on all of the trays. Fruit drying was completed once the fruits achieve 75% of total dry mater.
Fresh fruits have been kept in plastic bags until chemical analyses at –18 °C, while the dried fruits have been kept in plastic bags at room/ambient temperature (20 °C) until chemical analyses, for conditioning. By testing of fruit chemical composition of the investigated cultivars, the following was determined: content of dry matter (DM), obtained by drying at the laboratory dryer "Sutjeska" (Srbija) at 105 °C until constant mass, and the content of total and invert sugars and sucrose (by Luff-Schoorl's method). Results were expressed in grams per 100 gram of total dry matter (g/100 g DM).
Results were shown as arithmetical mean of three replications ± standard deviation and were processed statistically using analysis of variance (ANOVA). For testing of the difference significance of mean values of the tested parameters, Dunkan's multiple grading test for significance threshold was used $P \leq 0,05$.

Results and Discussion

Table 1 shows the content of total sugars, sucrose, invert sugars in the fresh and dried fruits of the plum cultivars, expressed in g/100 g DM for more accurate and clearer comparison. Percentage of the total sugars, invert sugars and sucrose in dried fruits compared to fresh fruit is shown in Graphs 1, 2 and 3, respectively, with the content of these parameters in fresh fruit shown by a line representing 100% of its content. Percentage of contents of the tested parameters depending on the drying temperature (a) and pretreatment influence (b) are shown separately on Graphs.
Analyzing the contents of total sugars in fresh fruit of the tested cultivars (Table 1), leads to an interesting information that the cultivar 'Čačanska Lepotica' had the highest value of this parameter (68.44 g/100 g DM). This could be explained with the fact that this cultivar, due to the lowest contents of total dry matter (15.77%), compared to the other tested plum cultivars,

has a greatest share of total sugars. In 'Mildora', the content of total sugars was 67.48 g/100 g DM, which was higher in comparison with the cultivars 'Čačanska Rodna' and Stanley, as expected, having in mind that this cultivar is known for the high content of total dry matter (Ogašanović and Ranković, 1996) and the total sugars (Mitrović et al., 2006).

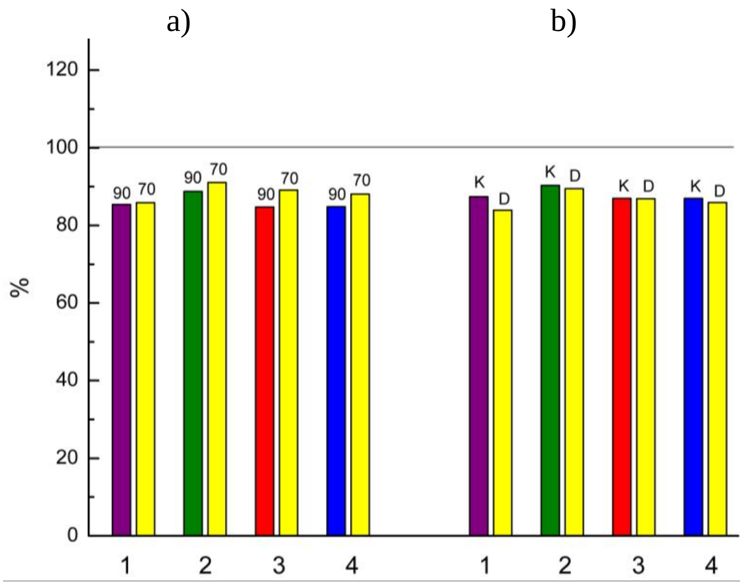
Tab.1. Content of total sugars, invert sugars and sucrose (g/100 g DM) in fresh and dried fruit of cultivars

Cultivar	Sample		Total sugars	Invert sugars	Sucrose
Čačanska Lepotica	Fresh plum		68.44 ± 3.17 a	47.11 ± 2.84	20.26 ± 1.55 a
	Prune				
	90 ^oC	control	59.68 ± 1.56 b	51.61 ± 0.88	7.67 ± 1.56 b
		dipping	57.28 ± 3.84 b	49.66 ± 5.04	7.24 ± 2.35 b
	70 ^oC	control	59.98 ± 3.25 b	54.12 ± 0.10	5.57 ± 3.06 b
		dipping	57.60 ± 4.90 b	52.40 ± 6.26	4.93 ± 1.29 b
Mildora	Fresh plum		67.48 ± 5.08	41.36 ± 3.92 b	24.82 ± 1.58 a
	Prune				
	90 ^oC	control	59.58 ± 2.88	52.07 ± 2.70 a	7.13 ± 0.95 b
		dipping	60.25 ± 4.45	51.21 ± 3.01 a	8.59 ± 1.61 b
	70 ^oC	control	62.31 ± 3.26	55.31 ± 1.54 a	6.62 ± 1.74 b
		dipping	60.56 ± 3.05	54.67 ± 2.34 a	5.60 ± 0.88 b
Čačanska Rodna	Fresh plum		65.57 ± 1.66 a	39.27 ± 2.76 b	24.97 ± 3.71 a
	Prune				
	90 ^oC	control	55.84 ± 2.59 b	47.52 ± 4.41 a	7.90 ± 1.92 b
		dipping	55.33 ± 2.95 b	48.07 ± 2.88 a	6.89 ± 1.63 b
	70 ^oC	control	58.23 ± 2.47 b	47.54 ± 2.81 a	10.15 ± 2.87 b
		dipping	58.67 ± 3.51 b	50.19 ± 2.47 a	8.06 ± 2.78 b
Stanley	Fresh plum		64.80 ± 0.99 a	39.67 ± 1.77 b	23.86 ± 2.20 a
	Prune				
	90 ^oC	control	55.71 ± 3.29 b	48.53 ± 1.74 a	6.83 ± 1.67 b
		dipping	54.18 ± 2.43 b	48.78 ± 1.94 a	5.13 ± 0.48 b
	70 ^oC	control	57.00 ± 1.26 b	48.35 ± 1.55 a	8.22 ± 0.49 b
		dipping	57.20 ± 1.33 b	48.50 ± 1.71 a	8.26 ± 1.56 b

Data followed by different letters within each column and cultivar are significantly diferent according to Duncan's multiple range test at P ≤ 0.05

In dried fruits of all tested cultivars, except in 'Mildora', a lower content of total sugars was found, compared to initial fresh fruit regardless of the applied drying temperature and dipping procedure (Table 1, Graph 1). That loss is about 10-15%, although there was no juice leakage during the drying process. This was probably because of the participation of sugars in non-enzymatic browning reactions. Wilford et al. (1997), investigating the kinetics of sugar change during the drying of the plum cultivar d'Agen at three drying temperatures using convective drying, concluded that the Maillard reaction starts at about 45-50% of moisture loss, and the proof for that is profile change in the presence of some sugars.

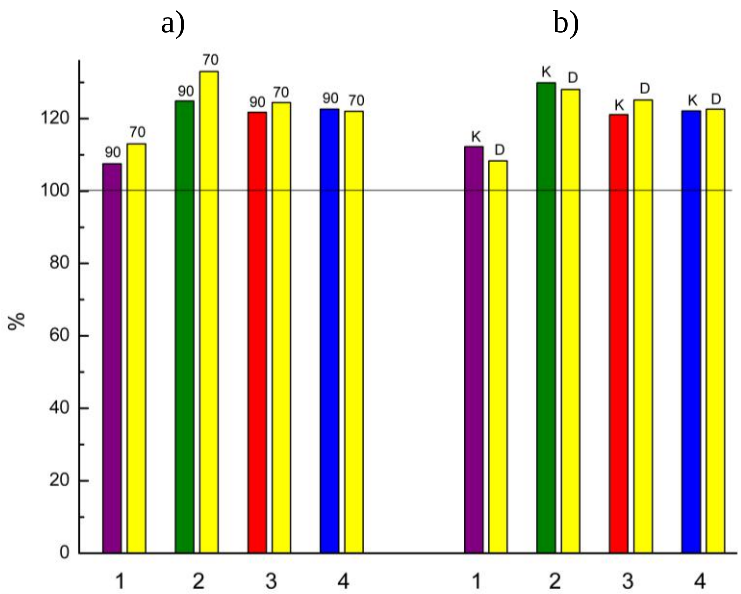
During the drying process, there comes to the hydrolysis of sucrose in plum fruit and the increase in the contents of invert sugars. Analyzing Graph 2, dried fruit of all tested cultivars were found to act similarly regardless of the drying temperatures and dipping procedure applied, i.e. the increase is about 25-30%. Exception is the plum cultivar 'Čačanska Lepotica', in which a minimum increase in the content of invert sugars was observed, of no statistical importance, and which was lower in fruits dried at higher temperatures (7.5% in total) than in dipped fruits where the increase is only 8.3%.



Graph. 1. Total sugars content in prunes compared to the frush plums, expressed as percentages

Legend:

- Cultivar: 1- Čačanska Lepotica; 2- Mildora; 3- Čačanska Rodna; 4- Stanley
- Influence of the drying temperature: 90 °C; 70 °C
- Influence of the dipping pretreatment: K- control; D - dipping



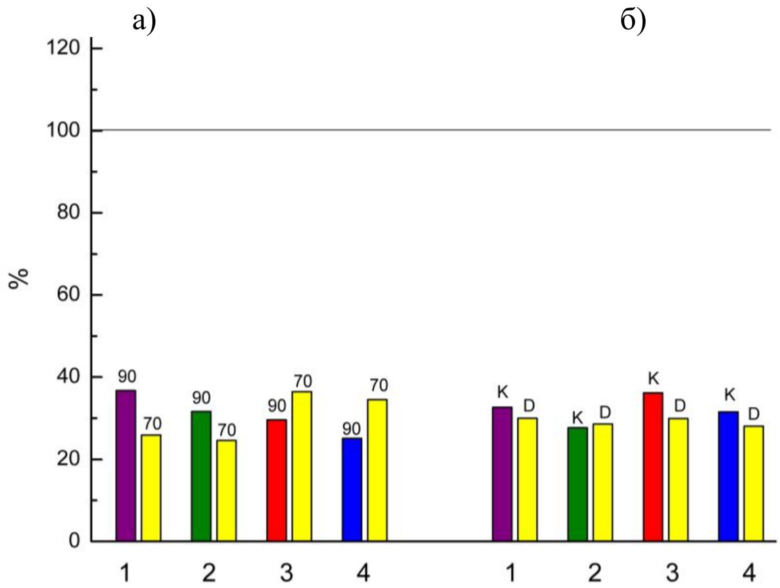
Graph. 2. Invert sugars content in prunes compared to the fresh plums, expressed as percentages

Legend:

- Cultivar: 1- Čačanska Lepotica; 2- Mildora; 3- Čačanska Rodna; 4- Stanley
- Influence of the drying temperature: 90 °C; 70 °C
- Influence of the dipping pretreatment: K- control; D - dipping

Examining the carbohydrate content of prunes and their products, Dikeman et al. (2004) reported a significant increase in the content of glucose and fructose, the two most important monosaccharides in plums, in relation to fresh fruit as a starting material. Cinquanta et al.

(2002) found the increased content of glucose and fructose in dried fruits of all the plum cultivars, whereas in the cultivar 'Stanley' differences in the increased content of these simple sugars among fruits dipped in different manners were noted to be of no statistical significance, compared with non-treated fruits.
As formerly stated, results of the sucrose content in fresh and dried fruit of the investigated plum cultivars are shown in Table 1. In all tested cultivars, dramatically lower sucrose content in dried plum was found compared to fresh fruit.
Analyzing Graph 3, drying temperature is found to have greater impact on the reduced sucrose content in dried fruit of all tested cultivars in comparison with the applied pretreatments. Dried fruits of the cultivars 'Čačanska Lepotica' and 'Mildora' acted in similar way, sucrose content in fruits dried at 90 °C was about 3 times lower than in fresh fruits, while in fruits dried at 70 °C, the content was 4 times lower. In cultivars, 'Čačanska Rodna' and 'Stanley' the opposite was found, and a lower sucrose loss was observed by a lower drying temperature.



Graph. 3. Sucrose content in prunes compared to the frush plums, expressed as percentages
Legend:
- Cultivar: 1- Čačanska Lepotica; 2- Mildora; 3- Čačanska Rodna; 4- Stanley
- Influence of the drying temperature: 90 °C; 70 °C
- Influence of the dipping pretreatment: K- control; D - dipping

Dipping as an applied pretreatment, had much less effect on the reduced sucrose content in dried fruit compared with non-treated fruits, i.e. control. A slight difference was observed between dipped and control fruits in decreased sucrose content in the cultivars 'Čačanska Lepotica' and 'Stanley', whereas in 'Mildora', which dried fruits contain about 3.5 times less sucrose compared to the starting raw material, sucrose loss is the same regardless of whether the fruits are dipped or not. Only in the plum cultivar 'Čačanska Rodna', effect of dipping on reduced sucrose content was recorded so that the dipped fruits contain 3.3 times less sucrose than the fresh fruits and in non-treated fruits, representing control, sucrose amount was reduced 2.7 times.
During the process of plum drying, the hydrolysis of sucrose occurs, thereby generating glucose and fructose. Analyzing the kinetics of carbon hydrate change during drying of the plum cultivar d'Agen, Wilford et al. (1997) found that, during the drying process, sucrose is completely hydrolysed. According to them, low pH value and high moisture of fruit in early stages of drying favour the hydrolysis of sucrose generating glucose and fructose. In their

investigations, at the drying temperature of 70 °C, sucrose was completely vanished in 6-7 hours, while at 90 °C, sucrose loss was noted after 2 hours of drying. Authors Di Matteo et al. (2003) also noted a complete loss of sucrose in dried fruit of the plum cultivar Angeleno. Cinquanta et al. (2002) recorded the complete sucrose loss in dried fruit of the cultivars Angeleno and Empress too, while on the other side in the cultivar 'Stanley' only reduced content of sucrose in dried fruit compared with fresh fruit was noted, thus emphasizing that 'Stanley' is an excellent cultivar for drying. Decrease in sucrose content in the course of drying is not a plum characteristic only as a fruit species. Moreover, in the course of banana drying, the hydrolysis of sucrose occurs, i.e. there is a decrease in content of sucrose in comparison with the starting raw material. (Leite et al., 2007).

Conclusion

Based on the investigation results of the drying temperature and dipping effect on the change of total sugars, invert sugars and sucrose content in fruit of the plum cultivars 'Čačanska Lepotica', 'Mildora', 'Čačanska Rodna' and 'Stanley', the following conclusions can be drawn:

- Drying temperature affected the change of total sugars, invert sugars and sucrose content in fruit of the investigated plum cultivars. Change intensity was conditioned by varietal characteristics.
- Dipping, as a pretreatment applied, had no effect on the change of these parameters content, except in the cultivar 'Čačanska Rodna' in which greater decrease of sucrose compared to non-treated fruits was noted.
- In dried plum fruits of the cultivars 'Čačanska Lepotica', 'Čačanska Rodna' and 'Stanley', a lower content of total sugars was noted, based on the calculation of the total dry matter in relation to initial fresh fruit, regardless of the drying temperatures and dipping procedure applied. The only exception was the cultivar 'Mildora' in which there were no statistical differences in the total sugar content in dried fruit compared to fresh fruit.
- During the drying process, an increase of invert sugars content occurred in all tested cultivars, except in 'Čačanska Lepotica', in which there was no statistically significant increase in the content.
- In the course of plum drying, the hydrolysis of sucrose occurred, which was manifested in a dramatic decrease of its content in dried fruits compared to starting raw material in all tested cultivars.

Acknowledgements

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VARIABILITY OF QUALITY AND RHEOLOGICAL PROPERTIES IN WINTER WHEAT UNDER THE INFLUENCE OF ECOLOGICAL FACTORS

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Abstract

This research involved analyses of six genotypes of the winter bread wheat (Toplica, Takovčanka, Perfekta, Vizija, KG-56S, and Aleksandra) on the experimental field of Centre for Small Grains in Kragujevac during two growing seasons (2011/2012 and 2012/2013). The most important parameters of bread-making quality were analysed (wet gluten content and rheological flour and dough properties). Grain samples were milled using a Brabender Quadrumat Junior laboratory mill. The gluten content was determined by the standard method and rheological flour and dough properties by Brabender Farinograph. Wet gluten content of analyzed wheat genotypes varied depending on the genotype and the year. The cultivar Aleksandra had the highest value of wet gluten content in both years of investigation (35.48% and 39.03%). The analyses of variance showed highly significant differences of wet gluten content between cultivars, investigated years as well as their interaction. The lowest water absorption in the first year of study was found in Takovčanka cultivar (59.2%), while in the second year in KG-56S (63.8%). The cultivar Vizija showed the highest water absorption in both years of studying (62.13% and 67.3%). According to the analysis of variance, highly significant differences for the water absorption were determined between genotypes, examined years and their interaction. Farinograph properties showed that flour of analyzed cultivars belonged to B_1 and C_1 quality groups in the first year, and B_1 and B_2 in the second year. The analysis of phenotypic variance indicated that the highest impact of variance for wet gluten content belonged to year, while for water absorption belonged to cultivar × year interaction.

Keywords: *wheat, quality, gluten, rheological properties.*

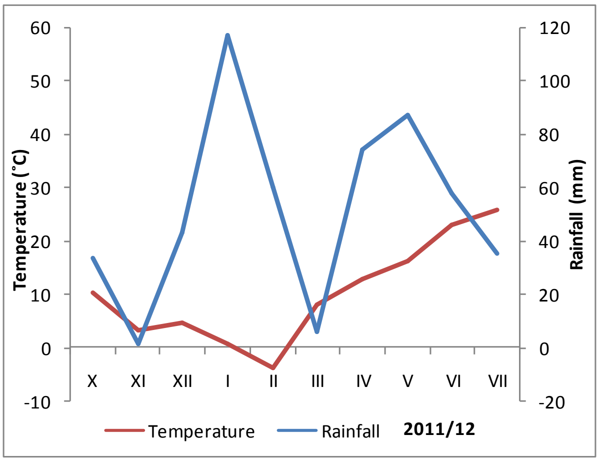
Introduction

The technological quality of wheat is primarily determined by the genetic potential of the variety, which is realized to a greater or lesser extent, depending on the agroecological conditions. The grain quality of wheat mostly depends on the quantity and quality of gluten. Gluten is formed in the early stages of ripening (milk) but its quality changes during maturity to the final characteristics (Lookhart et al., 2001). Temperature and precipitation in the filling phase and grain maturation has a great influence on the quality and content of the protein as well as on the rheological properties of the test (Đurićet al., 2010; Hurkman and Wood, 2011; Torbica et al., 2011). Water absorption is an important indicator of the quality of the flour, which is in a positive correlation with the protein content (Koppel and Ingver, 2010; Abbasi et al., 2011; Al-Saleh and Brennan, 2012). It represents one of the most important parameters in assessment of flour strength and it is directly related to the yield of finished bakery product (Dapčević et al., 2011). The quality properties associated with protein content are found under much greater influence of ecological factors and interaction of the genotype x environment, in contrast to the properties associated with the quality of the protein, the rheological characteristics of the test and the characteristics of the starch, where the influence genotype is significantly higher (Williams et al., 2008). It is known that one and the same genotype

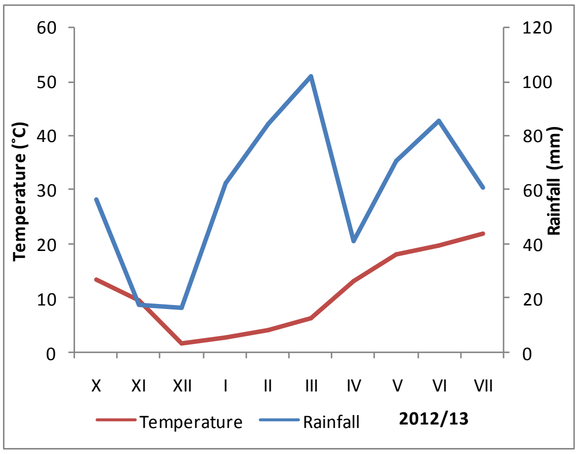
cultivated in different environmental conditions can belong to different quality classes and quality groups (Zečević et al., 2007). The cause of the variation in quality traits wheat is the consequence of genetic differences in cultivars, ecological factors, as well as their interactions, that are determined in research by a large number of authors (Zhang et al., 2004; Finlay et al., 2007; Vázquez et al., 2012; Zečević et al., 2013; Laidiget al., 2017).
The goal of this research is to investigate the influence of the ecological factors, genotypes and their interactions on some quality and rheological properties in winter wheat.

Material and Methods

In these studies, six wheat varieties (Toplica, Takovčaka, Perfekta, Vizija, KG-56S and Aleksandra) were analyzed. Varieties were grown in the experimental field of the Center for Small Grains Kragujevac (Serbia) during two vegetation season (2011/12 and 2012/13).
The rheological properties of grain quality were analyzed: wet gluten content, water absorption, quality number and quality group. Grain samples were milled using a Brabender Quadrumat Junior laboratory mill. The gluten content was determined by standard method (ICC standard methods 106/2, 1992). Farinograph was used to determine rheological properties of flour and dough. The results of the research were studied by Analysis of Variance (ANOVA) according to completely randomized block design with two main factors (genotype and year) using SPSS Statistics 22 statistical program. Evaluation of the importance of difference between average values of studied characteristics was tested by *Duncan* test. Components of variance (genetic, environment and interaction) were calculated by FALCONER (1981).
The average values of mean monthly air temperatures and precipitation amounts per month are shown on the graph1 and 2 (Republic Hydrometeorological Service of Serbia).



Graph. 1. Average monthly air temperatures and total amount of precipitation in production 2011/12 year



Graph. 2. Average monthly air temperatures and total amount of precipitation in production 2012/13 year

The first year of research, in November, was characterized by a dry period with extremely low precipitation. The average air temperature as well as total precipitation during October and November in the second year, was higher compared to the same months in the first year, which had a more favorable effect on seed germination and plant growing. Winter was mild with significantly lower precipitation in December and January in the second year compared to the first (78.8 mm compared to 160.5 mm). The stem elongation period and heading of wheat, during April and May, took place in both vegetation season in similar temperature conditions, with the amount of rainfall being higher in 2011/12 (74.5 mm, 87.3 mm compared to 41.2mm, 70.8 mm). However, in June, the filling phase of the grain was carried out at a higher air temperature (23 ˚C compared to 19.8 ˚C) and a lower amount of precipitation in 2012 compared to 2013 (85.4 mm compared to 66.9 mm), which affected the accelerated maturation of cereals.

Results and Discussion

In the first year of the research, the highest value of wet gluten content had the cultivar Aleksandra (35.48%) and KG-56S (34.6%), and in the second year the cultivar KG-56S variety (45.2%). On average, the wet gluten content was higher in 2012/2013 (39.14%) compared to 2011/12 year (31.56%), Table 1.

Table 1. Mean values for wet gluten content of wheat cultivars

Genotype	**Wet gluten content (%)**		
	2011/12	2012/13	Average
Toplica	31.75ab	38.28b	35.02
Takovčanka	29.49a	33.96a	31.72
Perfekta	29.05a	38.76b	33.91
Vizija	28.99a	39.59b	34.29
KG-56S	34.6bc	45.2c	39.9
Aleksandra	35.48c	39.03b	37.26
Average	31.56	39.14	35.35

Distinct letters in the row indicate significant differences according to Duncan test ($P \leq 0.05$).

Analysis of variance showed highly significant differences among investigated genotypes ($F=18.337$; $p<0.01$), years ($F=193.563$; $p<0.01$) and their interactions G × Y ($F=5.606$; $p<0.01$). Components of variance for wet gluten content have shown that the most variability belonged to year (69.16%), and a significantly smaller genotype (14.05%) and interactions genotype x year (10.17%), Table 2.

Table 2. Analysis of variance for wet gluten content of wheat cultivars

Source	**DF**	**MS**	**F**	**Components of variance**	
				σ^2	**%**
Repetition	2	5.892	2.206^{ns}	-	**-**
Genotype (G)	5	48.974	18.337^{**}	5.667	**14.05**
Year (Y)	1	516.956	193.563^{**}	27.89	**69.16**
G×Y	5	14.971	5.606^{**}	4.1	**10.17**
Error	22	2.671	-	2.671	**6.623**
Total	**35**	**-**	**-**	**40.328**	**100**

** Significant at P = 0.01 level; ^{ns} Non significant

The results of these studies are consistent with the results of Kaya et al. (2014) and Luković et al. (2017) who found that quality traits were largely dependent on environmental factors while the impact of the genotype was lower, suggesting that breeders' quality objectives should be adapted to the targeted environments.
Water absorption is the amount of water that needs to be added to the flour to obtain the dough with optimum consistency. This indicator depends primarily on the content of damaged starch grains, the content of protein and non-polysaccharide arabinoxylane (Rakszegiet al., 2014). In both years of research, the highest water absorption was established at cultivar Vizija (62.3%, 67.3%, respectively), with all varieties on average having a higher water absorption capacity in the second year compared to the first year of testing. Using the Farinograph the quality number and quality group for analyzed cultivars was determined. In 2011/2012, the best quality, expressed through a quality number, showed Aleksandra (61.3/B_1). The analyzed genotypes, in 2012/2013, showed the quality of the flour at the level of the B_1 quality group, except for the cultivar Vizija (B_2), Table 3.

Table 3.Mean values for water absorption (%), quality number and quality group of wheat cultivars

Genotype	Water absorption (%)			Quality number/ quality group	
	2011/12	2012/13	Average	2011/12	2012/13
Toplica	60.33b	65.47bc	62.9	47.6/B2	66.2/B1
Takovčanka	59.2a	66.2c	62.7	21.5/C1	62.2/B1
Perfekta	60b	64.33a	62.17	21.8/C1	63.3/B1
Vizija	62.13c	67.3d	64.72	31.9/C1	54.4/B2
KG-56S	61.07bc	63.8ab	62.43	46.8/B2	65.2/B1
Aleksandra	61.27bc	65.37bc	63.32	61.3/B1	61.5/B1
Average	60.67	65.41	63.04	-	-

Distinct letters in the row indicate significant differences according to Duncan test ($P \leq 0.05$)

The analysis of variance showed a significant influence of all sources of variation on the expression of the water absorption of the of studied wheat varieties.

Table 4. Analysis of variance for water absorption (%) of wheat cultivars

Source	DF	MS	F	Components of variance σ^2	Components of variance %
Repetition	2	0.081	0.071^{ns}	-	-
Genotype (G)	5	10.631	9.336^{**}	0.651	**12.01**
Year (Y)	1	175.563	154.176^{**}	1.771	**32.66**
G×Y	5	6.723	5.904^{**}	1.861	**34.32**
Error	22	1.139	-	1.139	**21.01**
Total	**35**	**-**	**-**	**5.422**	**100**

** Significant at P = 0.01 level, ^{ns} Non significant

By performing analysis of variance it was determined that the most significant contirbution of variance belongs to the interaciton G × Y (34.32%) as well as the year of (32.66%), while substanitally lower for the genotype (12.01%), Table 4. The results of these investigations agree with the results of Zečević et al. (2007) and Rozbicki et al. (2014) who emphasize that water absorption was more affected by the environment than by the genotype. Also, Laiding et al. (2017), studying the genetic improvement in quality of winter wheat varieties in the last

32 years in Germany, found that traits influencing baking quality (protein sedimentation, water absorption and bread volume), most impact of genetic factors (more than 60% of total variability belongs to the genotype), emphasizing that the years are more important than locations to explain variation.

Conclusion

In these studies, a significant influence of ecological factors on the quality of analyzed wheat varieties was established. The analysis of phenotypic variance indicated that the highest impact of variance for wet gluten content belonged to year, while for water absorption belonged to cultivar × year interaction. The year of 2012/2013 was a milder winter with more accurate rainfall during the wheat growing season. The period of heading and blooming in the month of May lasted with enough rainfall in both years. However, the grain filling phase in the second year of study was carried out at a slightly lower air temperature and higher precipitation compared to the first year, which caused the wheat varieties to be studied to achieve better quality of grain and flour.

Acknowledgement

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VARIATION IN MORPHOLOGICAL AND CHEMICAL TRAITS IN RED AND YELLOW MINI WATERMELON

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Abstract

Watermelon is an annual vegetable crop from *Cucurbitaceae* family. Five mini watermelon cultivars (red-fleshed ′Faerie′ F1, ′Golden crown′ F1, ′Bonanza′ F1 and yellow-fleshed ′Sureness′ F1, ′Yellow baby′ F1) were grown in open field experiment from May to July 2018. Plants were grown on a fertile soil, covered with black mulch film, on a distance 75x120 cm. During vegetation period regular agricultural practices were applied (fertirrigation, weeding and preventive protection against diseases and pests). Fruits were harvested after 60 days from planting. The aim of this study was to examine morphological traits of five cultivars in full physiological maturity. Fruit weight ranged between 1.57-2.65 kg with the highest value in cultivar ′Sureness′. Number of fruits ranged from 20 (′Yellow baby′, ′Sureness′) to 35 (′Faerie′). Rind thickness ranged between 5.22-11.91 mm with the highest value in ′Yellow baby′. Total soluble solids ranged from 9.5-10.95 °Brix and randman between 37-49%. Among all cultivars, red-fleshed ′Bonanza′ showed the highest randman and total soluble solids (49% and 10.95 °Brix, respectively). Opposite to that, the lowest total soluble solids showed yellow-fleshed cultivars ′Sureness′ and ′Yellow baby′. Generally, yellow cultivars showed higher fruit weight and rind thickness, whereas red cultivars showed higher number of fruits per plant, total soluble solids and randman. The present study outlines that ′Bonanza′ and ′Faerie′ showed the highest quality (soluble solids content) among tested varieties.

Keywords: *Watermelon, Fruit weight, Rind thickness, Randman, Total soluble solids.*

Introduction

Watermelon is an annual vegetable crop from *Cucurbitaceae* family. Nutrition quality of watermelon fruits originate from lycopene, flavonoids, phenolics, vitamin C, citrulline and fibers. According to FAO (2017) total production in European Union was 3.216.037 t with the highest production in Spain (1.112.192 t), Greece (647.000 t) and Italy (570.762 t).

Watermelon production depends on genotype selection, environmental conditions, growing practices and consumer habits. Breeding programs allowed accomplishing different market demands for fruit size, shape and colour, flesh colour, rind thickness and taste. Fruit size of 4-6 kg, round or oval fruits, the maximum rind thickness 1.5 cm, high randman and total soluble solids 8-10% (Gvozdanovic-Varga et al., 2011). Guner and Wehner (2003) reported the existence of genes responsible for flesh colour (red, orange, white and yellow). The same authors emphasized importance of rind colour in breeding programs. Rind thickness is a quantitative trait (*Sharma* and Choudhury, *1988*) with preference in thick rind and higher randman. Total soluble solids can describe sweetness degree and level of ripening (Sabeetha et al., 2017). Watermelon studies confirmed positive correlation between total soluble solid and sugar content (Soteriou et al., 2014).

New type of watermelons with reduced fruit size, mini watermelon, came to market in 2003 with round fruit shape, thin rind and average fruit weight 1.5-4 kg (Gusmini and Wehner, 2007). Mini watermelons are *characterized* as sweet, crisp, juicy, seed or seedless, from pink to red flesh colour. According to Magda (2016) they have more lycopene and beta-carotene (6.700-9.600 μg per 100 grams) compared to large watermelons (3.700-6.900 μg per 100 grams).

The objective of this study was to investigate five different mini watermelon cultivars, their morphological and chemical properties in conventional agriculture practice.

Material and Methods

Five mini watermelon cultivars were examined (′Faerie′ F1, ′Golden crown′ F1, ′Sureness′ F1, ′Yellow baby′ F1- Known-You Seed Co., Ltd and ′Bonanza′ F1- Syngenta). ′Faerie′ has a globe shaped fruit, average size 3 kg with light yellow rind and pinkish flesh. ′Golden crown′ is an early watermelon, average size 2.5 kg, oblong, icebox-sized with golden yellow rind and red flesh. ′Bonanza′ has a round fruit, average weight 1.5-3 kg, with thick light green rind and red flesh. ′Sureness′ has oval fruit with green rind and yellow flesh, sized 4-5 kg. ′Yellow baby′ has a globe shaped fruits, sized 3.5-4.5 kg with thin dark green rind and bright yellow flesh. An open field experiment was conducted during April-July 2018 at the company Iceberg Salat Centar, Surcin, Serbia. Before experiment chemical analysis of the soil showed sufficient levels of major nutrients and humus (nitrogen-0.18 %; phosphorus-66.6 mg/100g; potassium-27.2 mg/100g and humus-2.9 %).

Watermelon seedlings were grown in a peat cubes, size 6 cm, from the substrate Potgrond H (Klasmann-Deilmann) in a controlled glasshouse conditions. Seedlings were sown on April 28 and their production lasted for 30 days. Watermelon plants were transplanted on May 29, in a black marsh soil, covered with black mulch film. During growing cycle regular cultivation practices were carried out (fertirrigation, protection against diseases and pests, weeding). Soluble commercial fertilizers were applied with drip irrigation system, 7 times, during growing cycle. During vegetation period air temperature, air relative humidity and precipitation were collected from meteo station in Surcin. Average air temperature, air relative humidity and total precipitation are presented in Table 1.

Table 1. Climate conditions during watermelon growing cycle

	Average temperature (°C)	Average humidity (%)	Minimum temperature (°C)	Maximum temperature (°C)	Total precipitation (mm)
April 2018	16.6	63.1	5.0	29.0	14.4
May 2018	19.9	64.4	10.1	30.3	13.4
June 2018	21.4	71.9	10.2	34.5	57.8
July 2018	21.9	73.5	10.7	31.1	138.3

The experiment was organized in a complete block design. Each plot consisted of 13 plants and distance was 75x120 cm. Fruits were harvested 60 days after planting, at full physiological maturity. For morphological and chemical analysis, we used 5 plants. All morphological traits were measured using scale, digital caliper and ruler. Total soluble solids were measured using refractometer (Brix/Specific Gravity Refractometer w/ATC, Vee Gee Scientific, USA) and results are presented in degrees Brix (°Brix).

Statistical analysis was performed using DSAA STAT (2011) and Microsoft Office Excel 2007. One-way ANOVA was used to examine the effect of genotype with LSD test for comparation. All tests were performed at a significance level α of 0.05.

Results and Discussion

Fruit weight, rind thickness, randman and total soluble solids were statistically affected by genotype.

Table 2. Different watermelon parameters affected by genotype

Cultivar	Fruit weight (kg)	Rind thickness (mm)	Randman (%)	Total soluble solids (°Brix)
Faerie	1.62	5.22	49	10.95
Bonanza	2.12	7.56	49	10.95
Golden crown	1.57	11.00	37	10.50
Sureness	2.65	10.67	47	9.50
Yellow baby	2.25	11.91	46	10.15
Min	1.57	5.22	37	9.50
Max	2.65	11.91	49	10.95
Average	2.04	9.27	46	10.41
CV (%)	13.59	15.51	8.12	5.39
LSD 0.05	0.3664	1.8977	4.9083	0.7404
LSD 0.01	0.4998	2.5885	6.6952	1.0099

Fruit weight ranged between 1.57-2.65 kg and the average weight was 2.04 kg (Table 2). Cultivar ′Sureness′ showed the highest fruit weight (2.65 kg) and the lowest ′Golden crown′ (1.57 kg). Among red-fleshed cultivars, the highest fruit weight showed ′Bonanza′. Generally, yellow-fleshed cultivars showed higher fruit weight and there was significant difference between yellow-fleshed and red-fleshed cultivars with exception between cultivars ′Yellow baby′ and ′Bonanza′. Our results were similar to those found by Proietti et al. (2008) on ungrafted mini watermelon plants (2.27 kg). Experiments with black mulch film showed the highest watermelon yield and fruit weight (White, 2003) and it is recommended to cultivate watermelon on mulch film to decrease water and fertilizer loss from the soil, prevent weeds and contact between fruit and soil.

Total number of fruits ranged between 20-35. Yellow-fleshed cultivars, ′Sureness′, ′Yellow baby′, showed the lowest total number of fruits (20) compared to red. The highest total number of fruits showed cultivar ′Faerie′ (35). Fruit number per plant was similar to results Proietti et al. (2008) with 74.7%, 49.4% and 54.5% increased fruit number per plant in red (′Faerie′, ′Golden crown′ and ′Bonanza′, respectively) compared to yellow (data not presented in Table 2).

Rind thickness ranged between 5.22-11.91 mm and the average rind thickness was 9.27 mm (Table 2). Cultivar ′Yellow baby′ showed the highest rind thickness (11.91 mm) and the lowest cultivar ′Faerie′ (5.22 mm). Yellow-fleshed cultivars showed thicker rind compared to red-fleshed. Significant difference was found between yellow and red-fleshed cultivars with exception between two yellow fleshed cultivars ′Yellow baby′ and ′Sureness′ with ′Golden crown′. Gusmini et al. (2004) separated watermelon fruits into 3 groups according to rind thickness (higher than 19 mm, 10-19 mm, less than 10 mm). According to this classification cultivars ′Faerie′ and ′Bonanza′ belong to first group (less than 10 mm) and ′Golden crown′, ′Sureness′ and ′Yellow baby′ belong to second group (10-19 mm). Our results are in agreement with Proietti et al. (2008) on ungrafted mini watermelon plants (8.9 mm). On contrary, results of White (2003) showed higher values of rind thickness (16.7-22.3 mm).

Watermelon randman ranged between 37-49 % and the average randman was 46 % (Table 2). Cultivar ′Bonanza′ showed the highest randman (49 %) and the lowest cultivar ′Golden crown′ (37%). Cervenski et al. (2008) studied randman of two watermelon genotypes and showed higher randman compared to our results (′Danka′ 62.74 %, ′Crimson Sweet′ 53.38 %).

Mainly, red-fleshed cultivars showed higher randman compared to yellow but this difference wasn't significant. Cultivar ′Golden crown′ showed statistically lower randman compared to all cultivars.
Total soluble solids ranged between 9.5-10.95 °Brix and the average total soluble solids were 10.41 °Brix (Table 2). Cultivars ′Bonanza′ and ′Faerie′ showed the highest total soluble solids (10.95 °Brix) and the lowest ′Sureness′ (9.50 °Brix). Red-fleshed cultivars showed significant higher total soluble solids compared to yellow with exception between ′Golden crown′ and ′Yellow baby′. Within cultivars in red-fleshed group and yellow-fleshed there wasn't significant difference. Our results were in the range for red and yellow-fleshed watermelons (Pardo et al., 1997; Sabeetha et al., 2017) or even higher (Proietti et al., 2008). Sabbetha et al. (2017) reported lacking information of total soluble solids in yellow-fleshed watermelons. Their research showed no significant difference between red and yellow-fleshed watermelons (10.46 and 9.91 °Brix). Results of Davis et al. (2008) showed positive correlation between flesh colour (orange, yellow, red, pink) and total soluble solids. Their results indicated lower total soluble solids in yellow-fleshed compared to red (8.8 and 11.5 °Brix, respectively). According to Vasquez et al. (2005) 8-10 °Brix is a market tolerable value. All our results are in this range and compared to previous results are in agreement with lower total soluble solids in yellow compared to red-fleshed cultivars. Total soluble solids depend on environmental factors and maturity (harvest). Watermelon requires temperature 23-28 °C, relatively low air humidity, long day (Costa and Leite, 2007). During vegetation period average air temperature (16.6-21.9 °C) and air relative humidity (63.1-73.5 %) were almost optimal for watermelon production. During last month of their production total precipitation was higher and that could influence on lower total soluble solids. Even though it was a rainy period (especially July) we obtained satisfactory value of total soluble solids.

Conclusions

Mini watermelons have a round fruit shape, thin rind and average fruit weight 1.5-4 kg. The present study indicated that genotype (cultivar) affected fruit fresh weight, rind thickness, randman and total soluble solids. Yellow-fleshed cultivar ′Sureness′ showed the highest fruit weight. The highest rind thickness showed yellow cultivar ′Yellow baby′. Mainly, yellow-fleshed cultivars showed higher fruit weight and rind thickness compared to red-fleshed. Red-fleshed cultivars showed higher total number of fruits, fruit number per plant, randman and total soluble solids. Among tested cultivars, we can recommend ′Faerie′ and ′Bonanza′ as a source of sweetness and for the highest quality parameter (total soluble solids), as well as the highest randman.

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INFLUENCE OF CULTIVAR, MICROBIOLOGICAL FERTILIZERS AND GROWING SEASONS ON NITRATE CONTENT IN LETTUCE

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Abstract

Lettuce belongs to a group of leafy vegetable crops with special importance in human nutrition. Along with healthy compounds lettuce can accumulate nitrate in leaves. The purpose of this study was to examine the effects of genotype, microbiological fertilizers and season on plant weight and nitrate content in outer and inner leaves. Green cultivars (′Kiribati′, ′Aquino′, ′Aleppo′) were cultivated in a fertile soil, in a greenhouse trial at the company Iceberg Salat Centar, during three successive seasons (autumn, winter and spring). Microbiological fertilizers (EM Aktiv, Vital Tricho and combination of EM Aktiv and Vital Tricho) were applied in the soil before trials and foliar. In spring cultivar ′Aquino′ showed the highest head fresh weight (360.3 g). All microbiological fertilizers led to increased head fresh weight in ′Aquino′ during autumn trial. Mainly fresh weight was higher in spring and winter compared to autumn. Cultivar ′Aquino′ showed the highest nitrate content (985.4 mg/kg, autumn) in outer leaves with application of Vital Tricho. Opposite to that, the lowest nitrate content was found in inner leaves of cultivar ′Aleppo′ (35.4 mg/kg, spring) with combination of fertilizers. Generally, microbiological fertilizers significantly increased nitrate content in outer and decreased in inner leaves. In both leaves the lowest nitrate level was measured in spring season. During all trials nitrate content remained under allowed level of European Commission Regulation (563/2002) for protected lettuce.

Keywords: *Lettuce, Microbiological fertilizers, Season, Plant weight, Nitrate.*

Introduction

Lettuce is an annual, cool weather vegetable crop from *Asteraceae* family. Generally, lettuce has short vegetation period and in temperate climate it is feasible to accomplish multiple harvests all year-round. According to Food and Agriculture Organization (FAO) in 2017, total area harvested in European Union was 118.508 ha with leading countries Spain (34.508 ha), Italy (34.069 ha) and Germany (15.096 ha). Lettuce is consumed as a fresh vegetable and it is a component of fourth range products which are processed and packaged in modified atmosphere and come to market as ready for consumption (Borghi, 2003). It is rich is beneficial phytochemicals important in human diet (minerals, fiber, antioxidative compounds).

Leafy vegetables, including lettuce, tend to accumulate nitrate. Various factors like genetic, environmental (photoperiod, irradiance, humidity) and growing practice (use of fertilizers and pesticides) influence on nitrate accumulation in plants (Maynard et al., 1976; Reinink and Eenink, 1988). Major sources of human nitrate intake are from vegetables, drinking water and meat products (Wolff and Wasserman, 1972). Excessive use of nitrate in agriculture can have a negative effect on human health (transformation of nitrate into nitrite and its interaction with haemoglobin to form methaemoglobin). European Commission regulated maximum levels of nitrate that can be found in vegetables. It is allowed for lettuce grown under cover (excluding

'iceberg' type) harvested between 1 October to 31 March, 4500 mg/kg nitrates and between 1 April to 30 September 2500 mg/kg nitrates (EC regulation No 563/2002).
Effective microorganisms are a group of naturally found beneficial and compatible microorganisms that can be used as microbial inoculants in different preparations (fungi, lactic acid bacteria, photosynthetic bacteria, yeasts and actinomycetes). Application of effective microorganisms can have a positive effect on soil physical and chemical properties, improve plant growth, yield and quality (Babalola, 2010). Fungus genus *Trichoderma* spp. includes avirulent, cosmopolite, opportunistic species that can be used as biofertilizer, biopesticide and soil improver. They can colonize plant roots without evoking defense response in plants, ameliorate root growth and enhance nutrient uptake, improve plant growth and yield, enhance quality of vegetables and alleviate impact of different stresses (Lopez-Bucio et al., 2015). Effective microorganisms and *Trichoderma* preparations can be used in conventional, organic and integrated agriculture. With their application we can decrease the usage of inorganic fertilizers and synthetic pesticides and preserve natural environment (soil, water and air) from further pollution.
The aim of this study was to investigate the effect of genotype, microbiological fertilizers and season on plant weight and nitrate content in outer and inner lettuce leaves.

Material and Methods

Three green lettuce cultivars (oak ′Kiribati′, multi-leaf butterhead ′Aquino′ and lollo ′Aleppo′-Rijk Zwaan) were studied. Seedlings were grown in peat cubes, made from substrate Potgrond H (Klasmann - Deilmann) in a controlled glasshouse conditions. After transplanting plants were cultivated in the greenhouse without additional heating, in a fertile soil, covered with black mulch film. The experiments were conducted during three consecutive growing seasons autumn (11 October-7 December 2016), winter (27 December 2016-5 April 2017) and spring (27 April-3 June 2017) at the company Iceberg Salat Centar, Surcin.
Before experiments chemical analysis showed sufficient level of macronutrients and humus (nitrogen-0.22 %; phosphorus-58.35 mg/100g; potassium-32.45 mg/100g and humus-5.02 %) and all experiments were carried out without application of inorganic fertilizers. Two different microbiological fertilizers and their combination were examined. EM Aktiv (EMA; Candor) is a liquid preparation of different groups of beneficial microorganisms isolated from natural surroundings: *Aspergillus oryzae, Azotobacter chroccooum, Bacillus subtilis, Bacillus megaterium, Lactobacillus plantarum, Lactobacillus casei, Rhodopseudomonas palustris, Rodobacter sphaeroides, Saccharomyces carevisiae, Streptomyces albus, Streptococcus lactis, Streptomyces griseus*. Vital Tricho (VT; Candor) is a powder preparation of two *Trichoderma* species (*Trichoderma* ***asperellum*** and *Trichoderma viride*). The experiments were organized in a complete block design with four treatments (control - without fertilization (C), EM Aktiv (EMA), Vital Tricho (VT) and combination of EM Aktiv and Vital Tricho (EMA+VT)) and 3 replications. Each plot consisted of 32 plants with 25x25 cm density. Microbiological fertilizers were applied in the soil before planting (150 ml/10 l H_2O EMA, 21 g/10 l H_2O VT and 150 ml + 21 g/10 l H_2O EMA+VT) and four times foliar during vegetation period (30 ml/6 l H_2O EMA, 12 g/6 l H_2O VT and 30 ml + 12 g/6 l H_2O EMA+VT). Regular agricultural practices were applied for lettuce greenhouse production (ventilation, irrigation, weeding and preventive protection against diseases and pests).
During vegetation period air temperature and air relative humidity were measured for 24 hours using RC-4HC Data Logger. Monthly reviews of average air temperature and air relative humidity with minimum and maximum temperatures are represented in Table 1.

Table 1. Climate conditions during three growing seasons

	Average temperature (°C)	Average humidity (%)	Minimum temperature (°C)	Maximum temperature (°C)
October 2016	12.4	85.4	1.2	26.2
November 2016	8.0	87.7	-6.2	26.1
December 2016	2.3	89.3	-7.9	26.4
January 2017	-1.7	85.2	-16.6	20.8
February 2017	6.5	82.0	-9.8	38.0
March 2017	13.4	78.4	-2.1	38.8
April 2017	15.8	67.5	3.1	34.6
May 2017	21.1	74.5	5.0	40.4
June 2017	26.0	70.1	15.9	40.3

Plants were harvested when they achieved marketable size, by hand, same day. Rosette fresh weight was measured on scale. After that lettuce leaves were divided into two groups, outer and inner. Fresh leaves were dried for 72 h at 70 °C to constant weight to obtain dry weight, results are represented in grams (g). Nitrate content was determined colorimetrically by nitration of salicylic-acid described by Cataldo et al. (1975) with modifications (Jana and Moktan, 2013). Absorbance was recorded at 410 to 420 nm. Nitrate content in outer and inner leaves is represented in ppm FW. Three-way ANOVA with Tukey's test for post-hoc comparation was used in order to test effects of genotype, treatment and season. All tests were performed at a significance level α of 0.05. Statistical analysis was performed using software SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp) and Microsoft Office Excel 2007.

Results and Discussion

Results of rosette (head) fresh weight are represented in Table 2. Rosette (head) fresh weight varied between 90.7-171.7 g in autumn, 188.7-324 g in winter and 229-360.3 g in spring.

Table 2. The effect of genotype, microbiological fertilizers and season on rosette (head) fresh weight (g FW)

			Treatment			
Parameter	Season	Cultivar	C	EMA	VT	EMA+T
Rosette (head) fresh weight (g FW)	Autumn	Kiribati	110.5±2.0 aAx	117.7±4.1 a,bAx	133.3±19.8 aAx	150.0±10.6 a,bAx
		Aquino	102.3±10.7 aAx	148.3±5.2 bBx	147.0±11.6 aBx	171.7±2.7 bBx
		Aleppo	90.7±12.1 aAx	104.0±14.2 aAx	119.7±14.08 aAx	131.0±8.6 aAx
	Winter	Kiribati	261.5±0.9 bAy	266.0±1.2 bAy	290.0±21.6 aAy	270.7±18.0 aAy
		Aquino	275.0±8.4 bAy	261.3±11.4 bAy	324.0±38.0 aAy	286.3±16.6 aAy
		Aleppo	188.7±21.9 aAy	197.0±17.3 aAx,y	274.3±41.4 aAy	276.7±18.1 aAy
	Spring	Kiribati	328.0±22.3 aAz	294.0±4.6 aAz	299.7±30.4 aAy	287.7±7.8 aAy
		Aquino	360.3±15.2 aBz	313.3±17.5 aA,By	290.3±16.3 aA,By	249.0±27.7 aAx,y
		Aleppo	321.7±6.3 aAz	261.0±30.4 aAy	257.3±22.3 aAy	229.0±13.2 aAy

Values followed by the same letter aren't significantly different at the 0.05% level of probability according to Tuckey's test. Symbols are a,b - differences between cultivars; A,B - differences between treatments; x,y,z - differences between seasons

Cultivar ′Aquino′ showed the highest head fresh weight in spring (control treatment, 360.3 g) and with application of VT in winter (324 g). In all treatments cultivar ′Aleppo′ showed the lowest rosette fresh weight in autumn (control, 90.7 g; EMA, 104 g). Literature data indicate positive impact of different effective microorganisms and *Trichoderma* preparations on yield, fresh and dry weight of different vegetables (Björkman et al., 1998; Tošić et al., 2016). Effective microorganisms can synthesize vitamins, hormones, organic acids, enzymes, siderophores and other compounds that can stimulate plant growth. They contribute to enhanced nutrient availability and uptake, improve plant growth and yield, ameliorate soil structure, contribute to microbial diversity in the soil and suppress plant pathogens. In our study, all microbiological fertilizers significantly increased head fresh weight in cultivar ′Aquino′ in autumn. On contrary, in spring combination of microbiological fertilizers led to significantly decreased head fresh weight in aforementioned cultivar. Some experiments didn't show positive effect on yield of different crops with application of microbiological fertilizers (Poldma et al., 2001; Van Vliet et al., 2006). The effectiveness of microbiological fertilizers, including *Trichoderma*, depends on genotype, potential root colonization by different *Trichoderma* strains, soil characteristics, method and frequency of application. Our results showed that in autumn and winter season microbiological fertilizers led to increased rosette fresh weight in different cultivars but this wasn't statistically significant. This can be probably explained with particular interaction of genotype and effective microorganisms (*Trichoderma*) or inadequate quantity of applied fertilizers. Season significantly affected rosette (head) fresh weight in all cultivars and treatments. The present study pointed out to increased rosette (head) fresh weight in spring and winter compared to autumn season. This can be probably linked to influence of day length (autumn-short day 11-9 h, winter-growing day 9-13 h, spring-long day 14-15 h) and temperature. Optimal temperature for lettuce planting is 21-23 °C (day) and 15-18 °C (night). Our study confirmed previous reports in lettuce (Pavlou et al., 2007; Konstantopoulou et al., 2012) regarding to reduced plant weight and yield in autumn compared to spring and winter production.

Results of nitrate content in outer and inner leaves are represented in Table 3. Results of nitrate content varied between 202.7-985.4 ppm in autumn, 210.0-932.0 ppm in winter and 35.4-316.0 ppm in spring. In control treatment, the highest nitrate content in outer and inner leaves showed cultivars ′Aquino′ and ′Aleppo′ (autumn 908.2 ppm, winter 701.5 ppm). Siomos et al. (2002) found higher nitrate content in butterhead type compared to leaf lettuce. In both leaves, the lowest nitrate content was found in spring in cultivars ′Kiribati′ and ′Aleppo′ (control 62.4 ppm, 35.4 ppm with combination of fertilizers). According to Sorensen et al. (1994) nitrate accumulation in lettuce depends on genotype (variety) and environmental factors. Application of EMA and combination of fertilizers led to significantly enhanced nitrate content in outer leaves of cultivar ′Kiribati′ in autumn and spring, VT in cultivar ′Aquino′ in spring and EMA in cultivar ′Aleppo′ in spring. On contrary, combination of fertilizers led to significantly decreased nitrate content in cultivar ′Aquino′ in winter. In spring, application of all microbiological fertilizers led to significantly increased nitrate content in inner leaves in cultivar ′Kiribati′ and application of EMA in cultivar ′Aleppo′. In autumn fertilizer VT significantly decreased nitrate content in cultivars ′Kiribati′ and ′Aquino′ and in winter in cultivar ′Aleppo′. Generally, in outer leaves application of microbiological fertilizers led to increased nitrate content as well in spring in inner leaves. These results probably indicate contribution of effective microorganisms to increased nutrient availability. Experiments with lettuce and rocket showed enhanced nitrogen uptake from the soil using *Trichoderma* fertilizers (Fiorentino et al., 2018). Season significantly affected nitrate content in both leaves of all cultivars and treatments with exception of cultivar ′Aquino′ with application of VT in inner leaves. The lowest nitrate content in both leaves was measured in spring. Short day and low light intensity can influence on increased nitrate content

(Govedarica-Lučić and Perković, 2013). In all seasons nitrate content varied 35.4-985.4 ppm and remained under allowed level for protected lettuce (EC regulation No 563/2002).

Table 3. The effect of genotype, microbiological fertilizers and season on nitrate content in outer and inner leaves (ppm FW)

			Treatment			
Parameter	Season	Cultivar	Control	EMA	VT	EMA+T
Nitrate content outer leaves (ppm FW)	Autumn	Kiribati	315.2±39.2 aAy	552.7±15.8 aBy	507.8±36.2 aA,By	705.9±77.5 aBy
		Aquino	908.2±127.8 bAy	887.0± 120.8 aAy	985.4±34.5 bAy	751.9±115.8 aAy
		Aleppo	651. 3±14.9 a,bAy	854.6± 76.6 aAy	781.4±123.0 a,bAy	956.4±139.8 aAy
	Winter	Kiribati	380.8±25.0 aAy	495.5± 50.5 aAy	518.6±51.4 a,bAy	385.11±20.1 bAx
		Aquino	451.97±25.7 aB,Cx	529.8±34.9 aCx	395.7±14.6 aBx	210.0±27.2 aAx
		Aleppo	866.7±63.0 bAz	932.0±14.4 bAy	775.1±89.0 bAy	859.4±13.0 cAy
	Spring	Kiribati	62.4±8.7 aAx	90.1±2.3 aAx	94.2±11.0 aAx	211.9±36.5 a,bBx
		Aquino	216.9±16.1 bAx	287.9±17.1 bA,Bx	316.0±16.0 bBx	271.8±23.2 bA,Bx
		Aleppo	98.7±5.1 aA,Bx	249.1±8.6 bCx	63.4±1.2 aAx	117.2±18.1 aBx
Nitrate content inner leaves (ppm FW)	Autumn	Kiribati	441.4±58.1 aBy	477.4±73.4 aBy	202.7±23.7 aAy	462.5±39.4 aBy
		Aquino	593.7±45.9 aBz	410.8±44.4 aA,By	309.1±37.0 aAx	476.1±74.0 aA,By
		Aleppo	409.11±14.1 aAy	462.4±72.9 aAy	574.1±55.3 bAz	488.3±75.9 aAy
	Winter	Kiribati	296.6±39.7 aAy	315.8±9.1 aAy	212.1±28.3 aAy	225.0±33.5 aAx
		Aquino	305.4±19.6 aAy	254.5±42.2 aAx,y	276.4±11.2 a,bAx	232.0±18.2 aAx
		Aleppo	701.5±36.1 bBz	650.1±54.8 bBy	347.9±22.2 bAy	560.0±93.0 bA,By
	Spring	Kiribati	54.8±7.6 aAx	114.6±2.1 aBx	103.3±7.9 aBx	109.4±15.0 bBx
		Aquino	166.9±22.8 bAx	246.9±14.4 bAx	213.8±31.0 bAx	226.9±7.8 cAx
		Aleppo	72.4±8.9 aAx	178.8±24.9 a,bBx	64.3±0.1 aAx	35.4±3.1 aAx

Values followed by the same letter aren't significantly different at the 0.05% level of probability according to Tuckey's test. Symbols are a,b,c - differences between cultivars; A,B,C - differences between treatments; x,y,z - differences between seasons

Conclusions

The study showed that genotype, microbiological fertilizers and season significantly affected rosette (head) fresh weight and nitrate content in both leaves. Cultivar ′Aquino′ showed the highest head fresh weight in spring. In autumn season, all microbiological fertilizers significantly enhanced head fresh weight in cultivar ′Aquino′. The lowest nitrate content was measured in cultivar ′Aleppo′, in spring, using combination of fertilizers. Mainly, in outer leaves microbiological fertilizers led to increased nitrate content whereas in inner leaves microbiological fertilizers led to significantly decreased nitrate content. Application of VT and combination of fertilizers had a great influence on tested parameters. In all seasons nitrate content stayed bellow maximum allowed level for protected lettuce. Authors can recommend spring season to obtain the highest rosette fresh weight and the lowest nitrate content.

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IMPACT OF PASTERIZATION ON THE QUALITY OF POMEGRANATE JUICE FROM "HICAZ" VARIETY FROM THE REGION OF NORTH MACEDONIA

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Abstract

Originating from Iran and the surrounding area, pomegranates have been cultivated for thousands of years yet are still considered emerging crops although well noted for ancient uses and cultural importance. Pomegranates contain a number of functional compounds that are responsible for beneficial health properties. Polyphenolic compounds are primarily responsible for these benefits and include phenolic acids, flavonoids (e.g. anthocyanins), and tannins. These compounds have shown effects in many studies including those related to cardiovascular, anti-inflammatory, anticancer, and antidiabetic conditions. The object of our study was to investigate the impact of pasteurization on the quality of pomegranate juice from "Hicaz" variety from the region of North Macedonia. The pH value and total acidity of the juice were not affected by pasteurization. There were no significant differences between those two parameters in fresh and pasteurized juice. However, all other parameters were significantly affected. The level of citric acid was almost double in fresh juice from "Hicaz" variety of pomegranate (781 mg/L) in comparison with pasteurized juice (402 mg/L). The amount of total anthocyanins was higher in fresh juice (598 mg/L) in comparison to pasteurized juice (537 mg/L). In addition, total phenols were higher in fresh then pasteurized juice (3367 mg/L and 3196 mg/L respectively). Opposite to this tendency, the total catechins were higher in pasteurized (50.1 mg/L) than fresh juice (44.9 mg/L). The pasteurization strongly affected the color of juice. The intensity of the color, the hue and the yellow color were higher in fresh juice. Opposite, the red and blue colors were more intense in pasteurized juice. The results from our study lead us to conclusion that pasteurization influenced significantly the quality of pomegranate juices from "Hicaz" variety.

Key words: *pasteurization, organic acid, total phenolic compounds, anthocyanins, catechins.*

Introduction

Pomegranate (*Punica granatum* L.) is one of the major sources of polyphenolic phytochemicals, such as anthocyanins and catechins (Mphahlele *et al*., 2014; Jaiswal et al., 2010). However, the processing and storage of pomegranate juice had a decisive impact on the degradation of anthocyanin compounds and the consequent formation of brown pigments (Vegara et al., 2013). Total anthocyanin content and antioxidant capacity were substantially and significantly influenced by the heat treatment applied according to the working group of Mena (Mena et al., 2013). The results from study of Tezcan *et al*. showed that commercial pomegranate juices had markedly high total phenolic contents and antioxidant capacity. In the six commercial pomegranate juices studied in their work, in comparison to fruit juices reported in the literature, much higher total phenolic content and antioxidant capacities were observed, with increased health benefits for the consumers (Di Nunzio *et al*., 2013). The antioxidant activity of pomegranate aril juice, attributed to a great extent to total phenols and anthocyanins by cyanidin-3,5-diglucoside was the major antocyanin in pomegranates. (Cassano *et al*., 2011; Turfan *et al*., 2011). Furthermore, excellent review of Kalaycıoğlu and Erim quantitatively established the antioxidant activity, total phenolic content, anthocyanins,

organic acids, sugars and other important ingredients in pomegranate juices obtained from cultivars from different regions (Kalaycıoğlu and Erim, 2017). The late-pomegranate fruits were rich in phytochemicals and could be of great interest to the juice industry (Galindo *et al.*, 2014; Nag and Sit, 2018). In the work of Mena et al., 15 pomegranate cultivars was studied in order to demonstrate the wide diversity among the quality of Spanish pomegranates. According to their findings, "Wonderful" juices displayed large antioxidant activity and a polyphenol content with very high acidity. In contrast, 'Mollar de Elche' showed fewer anthocyanins although it had very superior organoleptic properties. In addition to a high content in ellagitannins, 'Valenciana' juices had exclusive colour parameters (Mena *et al.*, 2011). Regarding total phenol content, rutin was predominant flavonoid from the pomegranates peel (Mphahlele *et al.*, 2017).
To the best of our knowledge, there is no published results for the impact of the pasteurization on the quality of pomegranate juices from the region of North Macedonia. Therefore, the objects of this study was impact of pasteurization on freshly squeezed juice from "Hicaz" pomegranate variety as well as determination of chemical parameters such total polyphenolics, total anthocyanins, the level of organic acids as well and intensity of color and hue.

Materials and Methods

Sample preparation

The sampled fruits were harvested from the region of Kavadarci and Valandovo (East region of North Macedonia in October 2018. The samples were selected randomly in order to separate three replicates for analysis, using 10 kg per replicate and cultivar. Pomegranates were weighed, cut in halves, and arils were handseparated from thepith avoiding contamination by components in membranous walls (septum). Juices of each cultivar were obtained by pressure of arils and were weighed to determine the juice yield. Samples of freshly prepared juice were stored frozen (−20 ◦C) until analyzed (O'Grady *et al.,* 2014). The process of pasteurization was performed on 65◦C for 20 min.

Determination of titratable acidity, total soluble solids, pH

All analyses for the quality of pomegranate juices as well as the process of pasteurization were performed at the department of food control at UNILAB, Faculty of Agriculture, University "Goce Delčev"-Štip.
For determination of titratable acidity (TA), 2 ml of fresh juice was diluted with 70 mL of distilled water and titrating with 0.1 M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). All measurements were made on triplicate.

Determination of organic acids

For determination of organic acids, A Chromatograph Agilent technologies 1200 series, with Jasco AS-950 sampler, an auto injector (10 μl injection volume) and refractive index detector was used for analyses of organic acids. Separation of organic acid was performed on AMINEX HOX-87 (H 300 x 7.8) column. The eluent was prepared by mixing of 75μl H_2SO_4 in 250 mL H_2O. The mobile phase flow rate was 0.6 mL/min and the temperature was 55°C.

The percentages of organic acids were measured by following calculation:

$$mg\ organic\ ac./100ml = \frac{At}{As}\ x\frac{Cs}{Ct}x100$$

where:

- **At** is the area of test sample
- **As** is the area of standard
- **Cs** is the concentration of standard in mg/mL and
- **Ct** is the concentration of test in mg/mL

*1.1.*Determination of total phenolic compounds (TPC), colour, total catechins and total antocynanins

The total phenolic content of pomegranate juices was determined with Folin–Ciocalteau reagent (O'Grady et al., 2014). Determination of total phenolics was performed by the colorimetric method of Singleton and Rossi (Singleton and Rossi, 1965). For determination of total catechins, the modified method of Atamossa and Gholap was used. In brief, the pomegranate juices from both varieties were dissolved in water (1:5) and measurements of total catechins was performed using UV-VIS spectrometer in the spectral range of 200 to 500 nm (Atamossa and Gholap, 2015). The measurements were performed in triplicate.

1.2.Statistical analyses

The level of significance in differences between anthocyanin content and total phenolic content was determined by 5% by a one-way ANOVA using Tukey's test. The results from statistical analyses were classified using letters (different letters means significant differences among results). The letters are a,b,c,d,e and f according to the decrease of the result values. SPSS v.16.0 software, IBM corporation, USA was used for the applied statistical treatment.

Results and Discussion

The chemical parameters of the samples of fresh and pasteurized pomegranate juice from "Hicaz" variety are presented in table 1.

Table 1. Chemical parameters of pomegranate fresh and pasteurized juice

Samples of "Hicaz" juice	Brix	pH	Total acids g/L	Malic acid g/L	Citric acid g/L	Total phenolics	Total catechines	Total antocynains
Fresh	16.0±1.1[a]	3.04±0.0[a]	29.9±2.3[a]	0.18±0.10[b]	0.28±0.03[b]	2422±211[b]	25.5±4.3[b]	362.2±28.2[b]
Pasteurized	16.8±0.8[a]	3.04±0.1[a]	24.3±1.1[b]	0.36±0.52[a]	0.78±0.09[a]	3196±207[a]	50.1±5.0[a]	537.6±31.8[a]
	IC	**Hue**	**A_{420}**	**A_{520}**	**A_{620}**			
Fresh	2.87±0.12[b]	0.23±0.07[a]	15.7±0.12[b]	72.9±5.98[a]	9.9±0.5[a]			
Pasteurized	4.14±0.27[a]	0.26±0.05[a]	19.6±0.22[a]	70.5±4.77[a]	9.8±1.4[a]			

*Source: Author s' elaboration based on the obtained results.

As results show, there is significant difference between all measured parameters with exception of pH of the juices and brix. The level of malic and citric acid was significantly lower in comparison to commercial pomegranate juices from Turkey. However, the amounts of total phenolic compounds for fresh "Hicaz" juice was very similar to the same samples (Di Nunzio *et al*., 2013). The level of monomeric anthocyanins were almost double for pasteurized in comparison to fresh juice. Furthermore, the amount of monomeric anthocynains for fresh juice was very similar to the results published for pomegranate juices from the working group of Jaiswal (Jaiswal *et al*., 2010). The intensity of the color was higher for pasteurized juice, which was expected, due to the higher level of total phenolic compounds and monomeric anthocyanins (Table 1). Moreover, the higher intensity of red color can be linked to the dominance of cyanidin-3-glucoside as the major anthocyanin in pomegranate juice (Turfan *et al*., 2011; Cassano *et al*., 2011; Varasteh *et al*., 2012). Furthermore, citric and malic acids were the predominant organic acids in the sample of pasteurized juice. The acids attributed to the formation of ester upon the reaction of predominant citric acid with some juice compounds (Gundogdua and Yilmaz, 2012).

The results for amounts of total phenolic compounds obtained from Macedonian variety of "Hicaz" are similar to some varieties published by working group of Mena. (Mena *et al*., 2011). According to their findings, total phenolic content in WSN sample had similar value as Macedonian "Hicaz" variety. He stated that Folin–Ciocalteu values were not the sum of polyphenols which can be detected by HPLC (EA derivatives, punicalagins and anthocyanins) as other compounds, such as flavanols, flavonols, phenolic acids, proanthocyanidins, and hydrolysable tannins that are different to punicalagins, also contribute to the phenolic profile (Mena *et al*., 2011). The pomegranate juice from Macedonian "Hicaz" variety had higher value for total phenolic components than Valencia variety but significantly lower value than Akko, Hershkovitz and Wonderful varieties (Jaiswal *et al*., 2010). The amount of total phenolic component for fresh juice is similar to total phenolic content in Spanish pomegranate juice which was in range from 170-270 mg/mL gallic acid equivalent (Galindo *et al*., 2014).

Generally speaking, we assumed that higher level of anthocyianins, catehines, total phenolic compounds and intensity of the color in pasteurized juice can be results of enzymes inactivation and microbial destruction (Reddy *et al*., 2007). Results from our study showed that pH 3 is insufficient to inactivate enzymes and microbiota in degradation of phenolic compounds in fresh juice. However, changes of the color can be linked by the degradation of anthocyanin compounds and the consequent formation of brown pigments (Vegara *et al*., 2013).

Conclusion

The purpose of the present study was to evaluate the overall quality of freshly squeezed and pasteurized juice from "Hicaz" pomegranate variety from the region of North Macedonia. Based on the explanation above, it can summarize that pasteurized pomegranate juice from "Hicaz" variety is richer source with polyphenolic compounds in particular monomeric anthocyanins and total phenolic compounds. The effect of pasteurization gives improvement in terms of the enzymes inactivation and microbial destruction, which affects to the chemical parameters and quality of the juice.

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NUTRITIVE CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF PSEUDOGRAINS

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Abstract

Amaranth (*Amaranthus sp.,* Amaranthaceae), quinoa (*Chenopodium quinoa,* Amaranthaceae) and canihua (*Chenopodium pallidicaule,* Amaranthaceae) are pseudograins originating from South America. They are widely used in the diet as well as chia seeds (*Salvia hispanica,* Lamiaceae) from South America and wheat (*Triticum aestivum*, Poaceae) and millet (*Panicum miliaceum,* Poaceae), characteristic for our climate. The aim of the study was to determine the nutritive characteristics, phenolic content and antioxidant activity of commercial samples of pseudograins (amaranth, quinoa and canihua) from our market, as well as a comparative analysis to chia seeds, common and khorasan wheat and millet. Basic nutritive value parameters and mineral contents were assessed for pseudograins and chia seeds. The content of total phenolic compounds (TPC) and total flavonoid compounds (TFC), antioxidant activity by DPPH and FRAP tests were determined for all samples. The results of the nutrient composition indicates that analyzed pseudograins are good sources of proteins (13.39%) and unlike chia seeds are characterized by relatively low fat content (5.97%). Analyzed pseudograins contain calcium, magnesium, zinc and iron in a significant amount. The highest TPC content was observed in chia seeds (395.7 mg GAE/100 g), following canihua (327.4 mg GAE/100 g), quinoa (161.9 mg GAE/100 g), common wheat (61.8 mg GAE/100 g), Khorasan wheat (51.1 mg GAE/100 g), while the lowest content was identified in millet (32.9 mg GAE/100 mg). TFC content was in a range from 0.001 % (common wheat, khorasan wheat) to 0.099 % (canihua). Antioxidant properties of pseudograins were lower compared to chia seeds, but similar to common wheat and khorasan wheat while even higher comparing to millet.

Keywords: *pseudograins, chia, millet, wheat, phenolic compounds, antioxidant activity.*

Introduction

Amaranth (*Amaranthus sp.*), quinoa (*Chenopodium quinoa* Willd.) and canihua (*Chenopodium pallidicaule* Aellen) from Amaranthaceae are usually called pseudograins (pseudocereals) because they belong to the different family than the usual cereals like wheat, oat or rise (Poaceae). They are native to South America and have been used for thousands of years (Repo-Carasc-Valencia et al., 2010). Chia presents edible seeds of *Salvia hispanica* L., (Lamiaceae), annual plant from Central America (Da Silva et al., 2017). Pseudocereals as gluten-free grains, and chia seeds, contain various biologically active compounds like polyphenols of growing interest regarding their potential health benefits (Vollmannova et al., 2013; Valdivia-López and Tecante, 2015; Tang and Tsao, 2017).
Millet (*Panicum miliaceum* L., Poaceae) originates from India and represents one of the oldest cereals. It is traditionally used in European and Asian countries and just like pseudocereals,

millet is also gluten-free and is convenient for patients suffering from celiac disease (Alverez-Jubete et al., 2010). Moreover, the proteins in millet grains have higher biological values compared to wheat grain, due to the higher content of essential amino acids (leucine, isoleucine, methionine) (Kalinova and Moudry, 2006).
Another nutritionally valuable grain is an ancient khorasan wheat, kamut or oriental wheat (*Triticum turanicum* Jakubz.). Whole grain consumption can have the significant role in the prevention of cardiovascular disease, diabetes and other metabolic diseases. It has also been found that khorasan wheat products promotes the growth of probiotic strains in the gastrointestinal tract and improves metabolic, lipid and antioxidant status in healthy subjects and in patients with acute coronary syndrome (Sofi et al., 2013; Whittaker et al., 2015)
The aim of the study was to evaluate the nutritive characteristics, phenolic content and antioxidant activity of commercial samples of pseudograins (amaranth, quinoa and canihua) from our market, as well as a comparative analysis to chia seeds, common and Khorasan wheat and millet.

Material and methods

Commercial samples of amaranth, quinoa, canihua, chia, millet, khorasan and common wheat were purchased from the market in Belgrade, Serbia. Three independent samples from different suppliers were used for the research. All samples were grounded, homogenized and stored at room temperature until the analyses.

Nutritional analysis

Determination of moisture (water, loss on drying), ash and crude fiber in the samples was performed by the recommended methods by the Association of Official Analytical Chemists (AOAC 930.04; AOAC 930.05; AOAC 930.09; AOAC 977.02). Proteins were estimated from the nitrogen content by multiplying by factor 6.25. The analysis of fat content was done by Soxhlet extraction after acid hydrolysis and carbohydrates were calculated by subtracting the total sum of proteins, lipids, crude fiber and ash from 100% sample. The contents of nutrients was expressed in percentages. Total energy values were calculated in kcal multiplying the amount of fat by factor 9 and protein and carbohydrate by factor 4.
After dry ashing mineralization (AOAC method 999.11 B), the content of sodium, calcium, magnesium, phosphorus, iron, zinc and manganese were determined by the ICP-OES, Vista-PRO Simultaneous ICP-OES (Varian Inc.). The mineral content was expressed as miligrams per 100 g.

Total phenolic content (TPC)

The total phenolics were determined in methanol (80% *V/V*) extracts of investigated samples spectrophotometrically by *Folin–Ciocalteu* method. Gallic acid was used for construction of calibration curve and the results were expressed as mg of galic acid equivalents/100 g of sample (Singleton et al., 1999).

Total flavonoid content (TFC)

The content of the total flavonoids in methanol extracts was determined according to the procedure given in the monograph of Ph. Eur. 7.0. (Ph. Eur. 7,0)

Antioxidant activity determination by DPPH scavenging activity

For the DPPH scavenging activity determination the solution of DPPH (0.0147 g DPPH in 20 ml of ethanol) was prepared *ex tempore*. The standard solution was Trolox in ethanol (2 mM/l) in the range 0.2-0.7 mM/l. The colour change was measured spectrophotometrically at 525 nm after 1 hour of incubation at the dark place. The results are presented as mM Trolox equivalents/100 g of sample (Brand-Williams et al., 1995).

Antioxidant activity determination by FRAP test

Ferric reducing antioxidant power (FRAP) assay is a method for determination of total reduction ability. During analysis the antioxidant donates electrone to the [Fe^{3+}-*TPTZ*] complex (Fe^{3+}-2,4,6-tris(2-pyridyl)-*s*-triazine), that is subsequently reduced to blue [Fe^{2+}-*TPTZ*] at low pH values. After the incubation at 37˚C for 40 minutes, the absorbance is measured at 593 nm. The Trolox was used for the construction of the calibration curve (0.1-0.8 mM/l). The results are presented as mM Trolox equivalents/100 g of sample (Benzie IFF, Strain, 1999).

HPLC analysis

HPLC analysis of flavonoids was performed on the Agilent 1100, using the Zorbax Eclipse XDB-C18 analytical column (4.6 × 250 mm, 5 μm particle size), with the photodiode array detector set to 370 nm. Dried methanol extracts were dissolved in methanol (10 mg/ml). The samples were gradiently eluted with a two phase system: phase A = water/phosphoric acid (99.97:0.03), pH=2.75; and phase B = 10% A in acetonitrile, flow rate of 0.8 ml/min, at 25ºC. The identification of components was performed by comparing the spectra with reference standards and quantification of rutin by constructing the calibration curve.

All analyses were done in triplicate and results were expressed as average mean.

Results and discussion

The results of nutritional analysis of commercial samples of pseudograins, chia seeds, millet and khorasan wheat are given in Table 2. The amount of water was in the range 7.89-12.03% that was in accordance with the previous data (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017). The highest content of total ash was determined in the chia seeds (4.16%) and the lowest in the millet (0.7%). The results of total ash were also in agreement with former investigations (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017).

Pseudograins are important source of valuable proteins (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017; Kalinova and Moudry, 2006; Villa et al., 2014). Amaranth (19.91%) and chia (18.38%) contained the highest content of proteins, while lower contents were obtained in quinoa (8.31%) and millet (9.63%). However, obtained protein contents for quinoa (8.31%) and canihua (10.5%) were lower, while the amaranth (19.91%) protein content was higher than previously published data (Repo-Carrasco-Valencia et al., 2010).

Relatively low content of total lipids is one of the main nutritive characteristics of pseudograins. As expected, the obtained lipid content was 3.89-9.59%. On the contrary, chia seeds are characterised with high lipid content. So, obtained average content (30.09%) of total lipids in chia seeds is in accordance with literature data (Da Silva et al., 2017; Coates and Ayerza, 2009). Importantly, chia seed lipids contain very high amounts of polyunsaturated fatty acids, especially essential omega-3 alpha-linolenic acid (Coates and Ayerza, 2009). Observed higher total lipid content in chia seeds compared to other samples, corresponded the higher energy value than in other analysed samples.

Table 1. Nutritional values of commercial samples of pseudograins, chia seeds, millet and khorasan wheat

Sample	Water [a]	Ash	Proteins	Lipids	Available carbohydrates	Crude fiber	Energy value kcal/100 g
	Content, %						
Amaranth	10.06	1.94	19.91	3.89	63.63	0.57	369.17
Quinoa	12.03	2.15	8.31	6.29	70.92	0.3	373.53
Canihua	11.76	2.13	10.5	9.59	65.84	0.27	390.86
Chia	7.89	4.16	18.38	30.09	38.53	0.95	498.45
Millet	11.27	0.7	9.63	4.11	74.18	0.11	372.23
Khorasan	10.94	2.12	14.4	2.21	67.13	3.2	346,01

[a] loss on drying;

The mineral content of analyzed samples is given in Table 2. The highest average content of calcium was observed for chia seeds (597.15 mg/100g). The obtained value was about three to four times higher than in amaranth (166.71 mg/100 g), about ten times higher than in quinoa (60.52 mg/100g), and even one hundred times more than in millet (5.25 mg/100g). Also, noticed variations in phosphorus contents were in the range 37.26 mg/100 g (millet) to 820.61 mg/100 g (chia seeds). Similarly, the lowest (51.73 mg/100 g) and the highest (337.06 mg/100 g) magnesium contents were recorded in the millet and chia seeds, respectively. Opposite to other samples, the quinoa had a relatively higher concentration of sodium (average 47.11 mg/100 g). Variations in iron content were in the range 0.93-4.04 mg/100g. The highest levels of iron were noticed for amaranth and chia, while the quinoa had the highest average content of manganese.

Table 2. The content of minerals in commercial samples of pseudograins, chia seeds and millet

Sample	Calcium	Phosphorus	Magnesium	Sodium	Iron	Manganese	Zinc
	mg/100g						
Amaranth	166.71	499.20	256.91	2.00	4.04	3.92	4.24
Quinoa	60.52	378.98	193.42	47.11	2.33	5.39	2.74
Canihua	41.23	184.37	117.59	8.53	1.47	3.67	2.29
Chia	597.15	820.61	337.06	5.70	3.85	3.61	3.89
Millet	5.25	37.26	51.73	2.38	0.93	0.21	2.79

The total phenolic content (TPC) in investigated samples was in the range 32.90-395.75 mg GAE/100 g and total flavonoid content (TFC) 0.91-98.90 mg/100 g (Table 3). The highest content of phenolic compounds was in chia (395.75 mg GAE/100 g) and in canihua (327.36 mg GAE/100 g) samples, while the lowest in millet (32.90 mg GAE/100 g). Although the chia seed contained the highest value of phenolic compounds the obtained results were lower than previously described (757 mg GAE/100 g) (Hirose et al., 2010). The total phenolic content in amaranth (65.55 mg GAE/100 g) was similar to the Khorasan (51.05 mg GAE/100 g) and common wheat (61.82 mg GAE/100 g) samples. The highest amounts of total flavonoids were measured in canihua (98.90 mg/100 g) and quinoa (86.41 mg/100 g) samples.

Additionally HPLC method was used to identify the phenolic compounds in ethyl acetate extracts obtained after the hydrolysis of bounded phenolics and glycosides. Identified compound was present in very small amounts. In the ethyl acetate extracts of canihua and quinoa seeds quercetin aglycone was identified at 370 nm.

Table 3. Total phenolic and flavonoid content and antioxidant activity of commercial samples of pseudograins, chia seeds, millet, khorasan and common wheat

Sample	TPC [a]	TFC [b]	FRAP	DPPH
	mg GAE/100g	mg/100 g	mM TE/100g	
Amaranth	65.55	2.06	5.8	2.55
Quinoa	161.92	86.41	3.0	0.85
Canihua	327.36	98.90	5.5	1.70
Chia	395.75	4.14	13.7	3.65
Millet	32.90	2.48	1.7	- [c]
Khorasan	51.05	0.91	6.4	9.06
Wheat	61.82	1.08	5.2	10.38

[a] total phenolic content; [b] total flavonoids content; c no antioxidant effect

The antioxidant activity of pseudograins, chia seeds, millet, khorasan and common wheat was not pronounced (Table 3). The total reduction ability measured by FRAP assay was in the range 1.7-13.7 mM TE/100 g and the DPPH radical scavenging activity 0-10.38 mM TE/100 g. The highest antioxidant activity in FRAP assay was obtained with chia seed (13.7 mM TE/100 g) and the lowest with millet sample. On the contrary khorasan and common wheat samples showed the highest scavenging activity in DPPH test (9.06 and 10.38 mM TE/100 g).

Conclusion

Despite its growing popularity, this study is one of the scarce that offers insight in nutritive value of commercial samples of pseudograins on our market. Opposite to nutritional composition, there are observed differences in total polyphenol content and consequently in antioxidant activities among amaranth, quinoa and canihua samples. Also, there are prominent differences in all analyzed parameters among pseudograins and chia seeds, compared to millet. No differences were observed between khorasan and common wheat. In conclusion, obtained results of favorable nutritive value support the importance of pseudograins and chia seeds intake as part of our variety and balanced diet.

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BIODIVERSITY OF CHICKPEA: BLACK CHICKPEA

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Abstract
Sustainability of agriculture includes; conservation of natural resources in long term besides using agricultural technologies that are non-destructive to environment and living creatures. Providing diversity of products supplies protection of soil, using local seeds, decreasing of pesticide usage, information transfer to farmers about chemical fertilizers etc. components. Local plant species are developed by selection method in traditional agriculture that are well adapted to ecology, provides the cultural demands and traditions. Local seeds are one of the main sources for conventional agriculture and a great effort for social, environment, economic output amongst farmers over the World. Otherwise, expansion of industrial agriculture caused to pressure on conventional methods under narrow area and difficulty on product sales. Therefore, farmers prefer growing the seeds which are desired by markets which causes a serious danger of distinction of the local genetic resources. Black chickpea is a type of chickpea which has a rich content of nutritional value and taste, contains more protein ratio and some other minerals than common chickpeas. Ideal for losing weight due to higher fiber content, a good vegetable energy source, health care statues especially for diabetes and cholesterol, a well iron source, provides oxygen demand for body, higher antioxidant content. The present paper describes some features of the less known legume – black chickpea and offers some proposals about farming systems depending on biological diversity which could provide more employment, more food production, better quality, effective usage of the local facilities, food security, controlling of production costs, more income to families and prevention of migration.

Keywords: *Biosecurity, Climate change, Conservation, Sustainable agriculture.*

Introduction
Plant genetic sources are the main sources which came up by development of historical process. Genetic materials are ancestral live material which are genetically protected, used for development of new plant varieties and called as modern varieties present day. Production of goods and services was increased after Second World War for almost all the sectors in addition to changes in technology, consumption and production molds. However, pressures on natural resources were increased more than 10% ratio between 1947 and 1970 years (Aruoba, 1997). During 1970s, the "ecological foot print" that means request of human for sources was more than yearly biological renovation capacity of world. By other words, human population consumed more than renovation capacity of source ecosystem (WWF, 2010). Today, legumes are essential plant species for both human health and soil features. Black chickpea; a species of legumes is a less known plant which is promising for various works especially for adaptation mechanism of plants and well balanced nutrition projects in plant breeding programs.

Applications of agricultural industry, agricultural policies that supporting the seed usage that are released by private sector, changes in legislation related with seed, increasing dominance of super markets instead of local production and consumption, pesticides and their relative seed production activities by companies that are providing higher yield but unhealthy production methods, asymmetric knowledge of farmers and customers about those products etc. main factors caused to concern about protection and production of local seeds.

The present paper reviews the importance of biodiversity by means of plants and introduces "black chickpea" that is a less known edible legume species by view of its nutritional value, health care statues in addition to potentials by mean of sustainable agriculture.

Agricultural Sustainability and Biodiversity of Local Genotypes

The term of agricultural sustainability means that; protection of natural resources in long term as well as application of agricultural technologies that are not harmful for environment and other living organisms. There are many applications together with sustainable agriculture. Providing of product diversity and hence protection of soil, using of local seeds, decreasing of chemical usage and giving information to producers and extension works, preservation of natural areas and forests etc. factors are all components of the subject. As human invited the power of seed; agriculture was started on earth. Since that time, a lot of effort had been made about seed, plant growing, yield, taste, nutritional and other quality components.
Importance of plant genetic resources by view of sustainability may be summarized as following; possess various characteristics belong to different ecologies, high level of allele, developing of new varieties by agricultural activities, preserving of rich genetic characteristics by generations. From this perspective, determination and defining of plant varieties is an essential topic for various plant species that will provide to main data about plant biotechnology and agricultural breeding works. Increasing population and so demand for food, climatic changes, urbanization and industrialization, deforestation etc. events caused to serious problems for sustainability of life. In this point, evaluation of genetic resources – especially local plant genetic resources are quite important.
Industrial agriculture is increasing over the world in addition to migration from village to city. Most of the populations in villages are consisted from old people. These factors cause ton increasing of extinction on local seeds. The best way to protection of seeds is; production and preservation. Day by day, human being is more sensible for importance of the situation especially for sustainability of environment and human health.
Local ecotypes of plant seeds which are also called as "ecotype" also called as "landrace" are developed by traditional agricultural production systems that are including selection method, well adapted to the ecology, providing cultural demands and tradition. Present day, agricultural production depends on less species (Schmidt et al., 2010; Padulosi, 2011). According to the FAO reports, only 120 of plant species are produced for human food while 75% of plant based energy is realized by only 9 plant species while more than half is provided by wheat, rice and corn (Padulosi, 2011). The spread of industrial agriculture limited traditional agriculture to narrow areas, causing many difficulties especially in the marketing of those products. Therefore, farmers are obliged to higher valued commercial species instead of traditional species leading to the extinction of plant biological richness. For all the above mentioned factors, existence of traditional agricultural systems and traditional seed species and their protection will provide to sustainability of life.

Black Chickpea

Common chickpea (*Cicer arietinum* L.) on the dinner table is consumed for a long time and usually yellow colored while its seed color changes from white to black and from green to brown. Black chickpea has less oil, sugar and starch while more fiber than yellow chickpeas (Anonymous, 2019a). Three natural benefits of black cihckpeas may summarized by; clean digestive tract, cure bloating and belching, threat diarrhea naturally (Koradia, 2019) On the other hand, white and yellow chickpeas have more calories, potassium, calcium and iron than black chickpea (Quora, 2019). As the most important components of protein, amino acid compositions of yellow and black chickpea genotypes are presented on Table 1 (Rossi et al., 1984). Black chickpea is called by various local names; "gara nohut, siyah nohut, kara nohut, mukaşer" depending on local accents. Due to its dark color, it is not usually preferred by customers while it has better nutrition and health statues. In general, black chickpea is a kind of anti-oxidant store, seed coat is a little thicker than yellow types, so it should be wait in water around 24-36 hours.

Nutritional value and taste of black chickpea is better than the common chickpeas. Both protein concentration and the other nutritional components are quite higher. Higher ratio of fiber is ideal for the person who wants to diet. Additionally, it is a good plant energy source besides a wonderful legume crop for diabetes and cholesterol. Black chickpea is also an excellent source of iron. It helps to meeting of body oxygen need. On the other hand, it has more anti-oxidant matters (total phenolic components) than other legumes. It provides to avoid from cancer. It also has skin beautifying effects in case of regular consumption. Due to containing vitamin B6 and zinc, it is also quite effective on hair loss (Anonymous, 2019b).

Table 1. Amino Acid Composition (Milligrams per Gram of N) of Samples (Rossi et al., 1984)

	YELLOW										*BLACK*									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
Asp	699	708	717	683	711	692	685	683	789	679	705	691	683	694	694	692	699	694	680	697
Thr	218	214	215	224	217	225	216	207	231	217	218	218	211	220	219	210	210	215	188	213
Ser	309	291	294	312	303	300	305	301	308	302	309	309	298	310	307	311	296	304	261	305
Glu	1180	1265	1231	1284	1271	1265	1226	1206	1312	1297	1229	1253	1227	1211	1261	1263	1340	1228	1365	1191
Pro	247	265	263	239	249	356	245	278	238	247	275	235	246	278	236	251	250	243	339	245
Gly	206	205	209	209	204	210	207	199	211	202	201	204	235	203	210	202	195	204	200	208
Ala	235	234	235	243	225	236	233	223	238	228	219	227	215	224	229	223	211	227	253	224
Cys	47	52	57	42	55	49	61	56	43	49	43	66	44	64	47	54	53	63	40	52
Val	250	261	274	250	259	257	268	261	253	249	266	247	253	262	256	260	239	293	258	247
Met	62	51	67	60	56	63	68	67	49	64	57	61	54	64	64	59	58	55	51	63
Iso	266	258	275	263	268	261	276	260	258	256	261	257	251	253	265	260	247	271	264	266
Leu	474	469	477	476	458	459	477	465	471	474	471	464	456	450	471	460	439	455	463	476
Tyr	139	125	139	98	128	92	116	124	88	137	91	134	119	139	116	118	137	133	93	141
Phe	374	345	358	355	339	325	350	357	340	369	339	340	346	340	339	346	347	345	355	358
His	166	147	142	168	138	160	164	164	128	160	163	151	201	162	166	165	163	163	138	172
Lys	412	440	428	439	413	437	437	438	414	428	455	458	459	426	453	424	423	430	443	442
Arg	514	502	513	458	478	413	466	485	411	442	443	478	500	499	467	486	554	503	408	520
Try	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54

Some of the other benefits of black chickpea is summarized as it following; it is good for back pain, increases virility and so it is used on camels, resolves to congestion on spleen and hepatic, makes the bowels soften, fixes the toothache, complementary for cereals (Anonymous, 2018).

Conclusions

Farming systems depends on biological diversity provides more employment, foods as better quality, assurance for product quality that are desired by various consumers. Additionally, traditional production systems are realized by available local possibilities. Production costs are under control and so the farmer families will gain more. Protection of local seeds and transfer to the next generations is an urgent issue. Therefore, farmers should protect them as the first step for their own usage. As a member of legume crops, there is need for depth research on black chickpea by view of its features related with sustainability concept.

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SUSTAINABLE SOIL USAGE: LEGUMES AND HEAVY METALS

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Abstract

Industrialization and increasing of population caused effects on the whole ecosystem and environmental problems especially for heavy metal pollution of air, water and soil. There is not a distinct definition of heavy metals in literature but known as more than 20 for atom number (such as; Ag, As, Cd, Cu, Fe, Hg, Ni, Pb, Zn) and they have versatile damages particularly on soil. Some of the heavy metals in nature join to food chain and presenting toxic effects even very small amounts. Most of the heavy metals accumulate on biological systems. Some are necessary for plant growing but more quantities than requirement are toxic. Furthermore, some of the hyper accumulator plants are used for medical and aromatic purposes. So, those kinds of plants should be studied for negative and positive sides as well. Cleaning of soil from heavy metals is called as phytoremediation and the topic is quite essential in the world. Researches on hyper accumulator plants that are able to accumulation of metals reported 400 plant species in total. These plants are gathered from the following plant families; *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Lamiaceae*, *Violaceae*, *Euphobiaceae*, *Poaceae* and *Fabaceae*. It is fair that; legumes (*Fabaceae*) are the main protein sources for both animal and human. On the other hand, these crops are known as "absolute crops" for rotation programs. Present paper focused on; heavy metals in soil, their accumulation by legumes and statues in legume based foods.

Keywords: *Healthy food, Hyper accumulation, Phytoremediation, Pulses, Sustainable agriculture.*

Introduction

Industrialization and increasing of population brings many environmental problems by affecting the ecosystem wholly. Components such as air and water, soil are also an important creature. Because, soil is the first essential component to human survive which is including most of the minerals. Industrialization causes to an increasing heavy metal accumulation of soil. Some of the heavy metals especially mercury, cadmium and lead is threatening to food chain and all the world. Most of the metals are also spread to environment by industry (Urano, 2010). Content of heavy metal in soil ranges from 1 to 100.000 ppm (mg kg^{-1}) values. Higher content of them causes to deterioration of soil organic structure, decrease of yield and quality (Long et al., 2002) and so human and other living organisms are under the adverse effects (Blaylock and Huang, 2000).

Disposal of heavy metals from soil is realized by trenching, chemical stabilization, soil washing, bury etc. techniques. But, application of these techniques is hard and expensive so they are not practical. Some of the plants that are growing on the contaminated soils are accumulating the heavy metals by higher values on their tissues. Hyper accumulator plants are able to collection of heavy metals from 1000 ppm to 10.000 ppm values. Accumulation is realized on their roots, stem and leaves. Hyper accumulator plants are also used for organic pollutants and radioactive elements (Yalçuk et al., 2004).

Cleaning of heavy metal contaminated soils (phytoremediation) by hyper accumulator plants is quite important and so, determination of those plants is essential as well. Hyper accumulation defines that; collection of heavy metals in its own structure by higher values.

Present paper describes; heavy metal pollution, hyper accumulation and role of legumes by view of sustainable soil usage.

Heavy Metals

There is not a distinct definition of heavy metals in literature while their specific weight is 5 and atom number is more than 20 (Ag, As, Cd, Cu, Fe, Hg, Ni, Pb, Zn etc.) and they have versatile damage. In some cases, their quite less values are toxic that are take part of food chain in the nature. Most of the heavy metals are accumulated in biological systems and excessive values are cause to death in some cases (Kahvecioğlu et al., 2007). In general, zinc, copper and nickel include most of the heavy metals. Definiation of heavy metals and their structure is quite important (Paschke et al., 2005). Table 1 presents ecological classification of some heavy metals (Avcil, 2018).

Table 1. Ecological classification of some heavy metals (Avcil, 2018)

Element	**Spesific weight (g/cm^3)**	**Requirement (R) for plant and animal**	**Pollutant (P) or non- pollutant**
Copper (Cu)	8,9	R	P
Mercury (Hg)	13,6	-	P
Zinc (Zn)	7,1	R	P
Iron (Fe)	7,9	R	P
Silver (Ag)	10,5	-	P
Cadmium (Cd)	8,5	-	P
Stannous (Sn)	7,3	-	P
Cobalt (Co)	8,9	R	P
Chrome (Cr)	7,2	R	P
Lead (Pb)	11,3	-	P
Manganese (Mn)	7,4	R	-
Molybdenum (Mo)	10,2	R	P
Nickel (Ni)	8,9	R	P
Platinum (Pt)	21,5	-	-
Thallium (Tl)	11,9	-	P
Tungsten (W)	19,3	R	P
Uranium (U)	19,1	R	P
Vanadium (V)	6,1	R	P
Zircon (Zr)	6,5	-	-

Hyperaccumulation

Pollution of soil is emerged by human effects and causes to structural corruption. Main activities that are cause to soil pollution is summarized in the below (Karpuzcu, 2010):
- Garbage deposition areas,
- Transfer of solid wastes by many of the industrial establishments,
- Muds formed on refinement facilities,
- Unloading of sewer contents,
- Transfer of liquid wastes to soil,
- Accumulation of agricultural chemicals (pesticides),
- Fertilizer using in agriculture (chemical fertilizers),
- Accumulation of particles and aerosol air pollutant on soil,
- Increasing of salt content by irrigation.

Cleaning of the heavy metal contaminated soils is really hard, expensive, needs long time as in mentioned formerly. Plant are used both macro nutrients (N, P, K, Ca, Mg, S) and micro nutrients (Fe, Mn, Zn, Ni, Cu, Mo) while some of the plants are collecting As, Co, Cd, Se while they are not using them (Alford et al., 2010; Lasat, 2000). Those plants are called as hyperaccumulator. According to a former research, almost 400 plant species are able to metal accumulation. Some the plant families are; *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Lamiaceae*, *Violaceae*, *Euphobiaceae*, *Poaceae* and *Fabaceae*. Amongst these, *Brassicaceae* is the widest family by 7 genus and 72 species (Thompson, 1997).

Legumes and Sustainable Usage of Soil

Among angiosperms, some of the hyper accumulator families are: *Brassicaceae*, *Caryophyllaceae*, *Cunoniaceae*, *Cyperaceae*, *Fabaceae*, *Flacourtiaceae*, *Lamiaceae*, *Poaceae*, and *Violaceae* (Prasad, 2001). Legumes are reported as one of the common plant family which is presented on metal enriched soils on temperate zone that is located in tropics while it is not obviously evolved metal tolerance but infrequently formed naturally in metal enriched soils (Macnair and Baker, 1994; Wu, 1990). It is noticed that; *Fabales* contains homo-phytochelatins that are metal binding peptides of homo-glutathione (Grill et al., 1986). In another research, *Brassicaceae* and *Fabaceae* families are defined as generally including hyper-accumulator species. Nature of hyper accumulation is still not fully understood. The featured hypotheses may be summarized as following 3 titles: a) Hyper accumulation is related with hyper tolerance that is resulted by chelation and vacuolar classification. b) In some cases, metal content of shoots may be more than root content. a c) Hyper accumulator are tolerant to drought stress and competes with plants in the same area. d) Hyper accumulators are effective to combat with fungi and insect. Accumulation of Nickel showed protection against fungal and bacterial pathogens and insects as well. Similarly, zinc presented anti-herbivory effect (Martens and Boyd, 1994; Pollard and Baker, 1997).

In addition the those features, as the legumes have symbiotic living mechanism by *Rhizobium* sp. bacteria, they are the essential choice for crop rotation systems that are presenting natural nitrogen fixation mechanism that is an eco-friend type of nitrogen in addition to many advantages on soil reforming features.

Conclusions

Some of the hyper accumulator plants exist in same area with medical and aromatic plants. So, human have to take care about the risk. On the other hand, increasing human activities causes to metal pollution on the environment and its risk is rising rapidly. Therefore, there is need to more studies on the phytoremediation to find issues and providing sustainability concepts.

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NITROGEN FIXATION IN LEGUMES AND CHANGES IN CLIMATE

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Abstract

Increase in the world population is directly related with requirement of fossil energy and production of fertilizer as well. The truth in this condition is economic difficulty and environmental damage. Intensive agriculture systems cause to huge amount of nitrogen fertilizer usage which led to concern on N cycle over the soil-water nitrate accumulation and nitrogen oxide in atmosphere. Change in climate over the world is an essential issue for humankind. Scientific researches on plant response to environmental factors are a topic which increasingly takes attention due to concern on resources of plants, biodiversity and global food security. Legume family known as eco-friend and health-care plants which are fundamental crops for sustainability besides presenting second major crop of agricultural importance over the world. Depending on environmental changes, response of legume nitrogen fixation varies by view of drought, salinity, heat stress, carbon dioxide concentration and soil acidity. Present paper describes the main environmental factors which are directly connected with climatic changes and their major effects on N_2 fixation and legume production worldwide. Additionally, suggestions for adaptation of symbiotic legumes to climatic variability on production quantity are given depending on recent studies by view of molecular and biotechnological efforts over the world. Therefore, aim of the paper is improvement of healthy food supply consider to sustainable agricultural system.

Keywords: *Eco friendly, Global warming, Grain legumes, Rhizobium, Sustainable agriculture.*

Introduction

According to NASA records for 137 years (from 1880 to 2016), 9 of 10 highest years was realized after 2001 year. Additionally, 2016 presented the highest temperature. Agriculture in the world is developing day by day in accordance with providing the personal and public requirements. Global competition is also increasing while trade of agricultural products are developing, agricultural supports showing a declining trend, profit margin is shrinking, China and India countries are important factors for global agricultural factors; increasing income causes to more consumption of meat, fish, vegetables and fruit, cereal production is decreasing as the advantage of vegetables and fruit, demands of China causes to increase in prices for raw materials.

Nitrogen based chemical fertilizer are the most used types over the world. One of the most important points in this is the negative effects of nitrogen to environment and climate as well. Legumes are the most valuable crops by mean of their symbiotic nitrogen fixation mechanism. Present paper describes climatic changes and its agricultural effects in addition to why we need to take a part to legumes in crop rotation programs.

Climate Change and Agricultural Production

Climate changes will also cause to; periods with warmer and less precipitation, increasing of extreme meteorological events such as flood, overflowing and vigorous storms, erosion, decrease in water sources, increase in drought intensity, corruption of soil and water quality, decrease in ecosystem and biological diversity, increase in disease and insects, decrease in agricultural productivity, narrowing and/or extinction of ecological habitat, problems in

sustainable food security. On the other hand, changes in climate will give rise to some other problems for agricultural activities such as; sowing-planting, harvest and thrash, soil tillage, fertilization, drainage, spraying, cultural practices (hoeing- pruning), yield and quality, providing of irrigation water, increasing of vegetation period, diversity of plants, increased CO_2 concentration will cause to increase in plant growing. Water consumption of several food sources are quite different from each other (Gleick, 2000, Guy et al., 2004; Hoekstra et al., 2011, Pegram et al., 2014).
There are some scenarios for climatic changes and its effects on agriculture. According to a considerable report (RPCC, 2016), the negatively affected places would be; Africa, South America, South Asia, Middle East and Australia while positively effects on; North America, Europa and North Asia. Some of the risk factors are summarized as following: In global scale, climatic fluctuations will cause to more than 30% loss of products (Lobell and Field, 2007). Only each 1^oC increase in temperature, CO_2 fertilization will cause to yield decrease almost 6.0% of wheat, 3.2% of rice, 7.4% of corn, 3.1% of soybean if an effective adaptation and genetic improvement will not be realized (Zhaove et al., 2017).

Legumes - Nitrogen Fixation and Global Climate Change

Legumes are the third plant group by mean of production that is following to cereals and oil seeds (Graham and Vance, 2013). Due to wonderful symbiotic nitrogen fixation mechanism with *Rhizoubium* bacteria species, they are minimizing the environment and socio-economic dangers of chemical nitrogen fertilizers. Increasing of CO_2 concentration is an expected result of increasing of temperature and drought (Feller, 2016). Global climate change present variable effects by increasing of decreasing of temperature in different regions (Bhandari et al., 2017). Higher temperature has more effects on nodule and bacterioid formation than other parts of the plants (Aranjuelo et al., 2014). Drought stress causes to deformation of cells, less nitrogenase activity, protein less nitrogen for biosynthesis of protein and decrease in seed yield (Farooq et al., 2016). Legumes are sensible to salinity while *Rhizobium* species are more tolerant. But as a consequence, salinity causes to decreasing of nodule formation in *Rhizobium* due to osmotic stress and/or ion toxicity besides decreasing of nitrogenase activity in legumes (Hanumantha Rao et al., 2016). Similarly, increasing of CO_2 will give rise to decreasing of nitrogen content in plants (Hikosaka et al., 2011) while some times increasing of root secretion and thus encouragement of microbial developing and activity (Haase et al., 2007).
It is fair that, changes in climate will cause to increasing of carbon dioxide and temperature in atmosphere and hence drought and salt. These changes will negatively effect to legumes that are grown in arid and semi-arid climates. Increase in CO_2 will increase nodule developing and biological nitrogen fixation. However, drought stress by 2-4^oC increasing of temperature will give rise to negative effects on; root hair infection, number of nodules, size – growing and activity of nodule and so fixation mechanism. Additionally, salinity problem will reduce to respiration of nodule and content of hemoglobin (Yavaş and Ünay, 2018).
Agriculture is an important sector for both economy and social concepts. On the other hand, fields are a kind of factory which has no roof, so; it has a very sensitive structure by view of main components such as; ecosystem, and also sensible and unprotected for climatic changes. Therefore, changes in climate are a topic which should be paid more attention, faster and accurate estimations. According to "Global Framework for Climate Services (World Meteorological Organization) - GFCS", agriculture and food security are within priority sectors. Similarly, "Sustainable Development Goals (SDGs)" identified the first two topics as "no-poverty" and "zero hungry" respectively. Increasing of atmospheric CO_2 and longer plant vegetation period are caused to positive effects while changes in climate have negative impacts on higher temperature and so changes in quantity and regime of precipitation, increased frequency and violence of excessive weather events, and hence many unfavorable

effects on agricultural activities. Agricultural systems are forced to provide increased demands for food and renewable energy. Change in climate, scarcity of water, pollution, erosion and corruption of soil are more than threats on food security and quantity. Velocity of climatic changes and its effects are estimated more and more in following years. Short term plans are not solution for the problem. Main goals should cover; energy projects, irrigation programs, consolidation of fields, developing of climatic adaptable plant species etc. climatic based projects. All the mentioned effects of climate will effect to; animal husbandry, fishery and agricultural production by main negative effects on; yield and quality reduction, increased demand for water-pesticide-fertilizer, changes in sowing and planting times. Therefore, there is need for pay more attention about effective usage and reservation of agricultural inputs that are mainly composed by water and fertilizers. Herein, legumes are essential plants for crop rotation programs that are promising for the following statues related to combat with global climate changes; effective production planning, diversity of agricultural production, protection of biological diversity – soil and water resources, increased yield and producer profit, balance of supply and demand, reduction of public finance welded by purchase, increasing of international competition power and production planning, healthy agriculture inventory, better producing plan, demand projection for future, supports will be rational – router – effective and so; natural resources will be protected and all the factors will provide sustainability.

Conclusions

Agricultural production of the world should be increased. World population is growing as much population of Turkey every year. For the sustainability of agriculture, we need to take care the following issues; usage efficiency should be increased by the least source and input consumption, increasing of yield by low cost, the least intervention to nature and minimum environment damage, possible least period and a small number of process, independent from climate as much possible. Nitrogen - as an important and essential component of agriculture is able to be fixed by legumes – bacteria symbiotic live mechanism, naturally. So, there is need for increased legumes production for sustainability of healthy nutrition, agriculture, ecosystem and food security.

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SUSTAINABLE PESTICIDE USAGE IN FARMING LEGUMES

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Abstract

Pesticides are used in agriculture commonly including insecticides, fungicides and herbicides. Today, almost a total of 900 pesticide products and 600 active pesticide ingredients accessible in the markets. Millions of tons of pesticides are used in agriculture while only 5% of them are selective which means rest of it (95%) stored in soil, non-targeted systems, mix to water and atmosphere as well. Plants that are exposed by pesticides response in various amounts depending on physicochemical features, genotype, soil and environmental conditions. Pesticide application can realized by roots and other upper organs of the plants. A part of the pesticides are reasonably persistent and non-biodegradable. Pesticides which are taken by plants can change the locations through xylem or phloem while only by phloem in roots. Their mobility affected from water solubility characteristics. Therefore, oxidation of pesticides is important for their activity. For all that, many of pesticides have long half-life and remains in plants. Legumes are the secondary important food for human following to grains. Yield and quality of legumes reduces owing to pesticides (aldrin, chlorpyrifos, chlordane etc.). Additionally, pesticides also have negative effects on symbiotic nitrogen fixation by affecting the rhizobium bacteria. Nevertheless, bacteria which have pesticide tolerant strains may grow under pesticide stress. As the main principle of sustainable agricultural systems, legumes are essential crops in rotation. Present paper describes effects of the pesticides which effects growing, yield, photosynthetic activity, nitrogen fixation and tolerance in legumes.

Keywords: *Agricultural pollution, Biodegradation, Pesticide effect, Sustainable agriculture, Toxicity.*

Introduction

Legumes are valuable food sources due to higher nutritional statues, environment friend crops and have wide adaptation ability over the world. Farming legumes provides many advantages on economy, social life, husbandry, soil, sustainable agriculture, food safety, human health, environment and so it means a kind of essential investment for human and future. Additionally, increasing of legume farming in crop rotation programs is an important point in recent years by focus on agriculture and the future of food supply while the topic will be also important for many years in worldwide. Present paper reviews pest management system, farming legumes and common problems that are need for management by view of sustainability principles.

Sustainable Pest Management in Agriculture

Agricultural production systems are changed from conventional techniques to intensive applications that are including pesticides and fertilizers in the world. Discovery of chemical fertilizers was realized during the 18th century by super-phosphates and ammonia including types, respectively. Similarly, pesticides were invented almost end of 18th century (Horne and Page, 2008). According to archives (Faostat, 2015), using of nitrogen, phosphorus and potassium is realized around 69.3 kg ha^{-1}, 25.8 kg ha^{-1} and 14.8 kg ha^{-1}, respectively. On global scale, amount of pesticide using is approximately; for herbicides; US $35 billion, for insecticides; US $15 billion, for fungicides; US $5 billion and for other products; US $5

billion in 1999 (Agrios, 2005). Additionally, almost 3 billion tons of pesticides are applied every year over the world (Pimentel, 2009a). It is reported that; benefit/cost ratio 2/1 by many risks for pest resistance, limitations on specificity and many harmful side effects (Pimentel et al., 1980; Pimentel, 2009b; McDougal, 2010). Although many efforts to use less chemicals for the agriculture, it is fair that usage of chemicals are too much and cause to serious problems on health of public and environment (Pimentel et al., 2005). The potential risks may be shown in the long term by compaction and erosion of soil and decline in fertility, health problems and extinction of biodiversity (Stinner, 2007).
For the mentioned problems on pest management, one of the feasible solutions is; integrated pest management that depends on a well following system from soil tillage for cultivation to eat on the table. This solution may be realized by integration of; science, policy and business (Hokkanen, 2015). Crop rotation has a great importance on pest management and, legumes in a crop rotation program are an important source for nitrogen and availability of nitrogen for plant to management of pest management. Legume cover crops are also provides to breaking of pest or weed cycles welded by fixation mechanism (Daniel et al., 2018).

Legumes and Pesticide Using

Common problems on legumes are several weeds, diseases (*Phytophthora capsici*, *Xanthomonas axonopodis pv. Phaseoli*, *Colletotrichum lindemuthianum*, *Pseudomonas savastoni pv. Phaseolicola*, *Fusarium spp.*, *Rhizoctonia solani*, *Macrophomina phaseoli*, *Uromyces appendiculatus*, *Perenospora viciae*, *Mycospheralla pinodes*, *Ascochyta pinodella*, *Peronospora lentis*, *Fusarium oxysporum*, *Ascochyta rabiei*, *Uromyces ciceris-arietini*), virus (bean common mosaic virus, bean yellow mosaic potyvirus) and insect (*Amicta oberthuri*, *Apion arrogan*, *Bruchus*, *Deliaplatura*, *Epithrix hirtipennis*, *Etiella zinckenella*, *Gryllotalpa gryllotalpa*, *Lampides boeticu*, *Liriomyza bryoniae*, *Liriomyza cicerina*, *Liriomyza huidobrensis*, *Liriomyza trifolii*, *Phytomyza horticol*, *Phyllotreta spp.*, *Sitona crinitus*, *Tetranychidae*, leaf flea) welded troubles.
Prices of pesticides to combat with the problems are quite expensive, application is hard on the progressive developing periods, some of the active ingredients are not effective on the level of recommended dose and/or presenting toxic effects to legumes and at the most important point; all the pesticides have potential risk for health by direct or indirect ways. There are many researches on pesticide using on legumes that are supported by breeding studies. In general, main breeding techniques on legumes may summarized as; mass selection, single-seed descent, pure-line selection, bulk, pedigree selection, backcross method in addition to advanced methods likewise; tissue culture, gene transfer, mapping populations, selection by molecular markers etc. methods that are examined under biotechnology. For resistance to pesticides, disease-weed and insect control, there are studies on genetically modified organisms (GMO) as well. Studies on GMOs are; transgenesis, cisgenesis, intragenesis, targeted mutagenesis (zinc finger nuclease technology), oligonucleotide directed mutagenesis, grafting, agro-infiltration, synthetic genomics and other techniques (reverse breeding) may be summarized (Legume Forum, 2018). Similarly, it was highlighted that; as a part of positive effects on pest management, the level of habitat in the field may be improved to other functions of ecosystem like controlling weeds, relaxation of soil erosion, cycling of nutrients that are realized by atmospheric nitrogen fixation by legumes (Simpson et al., 2013).

Conclusions

Demand for increased yield and quality, tolerant and/or resistant for changing climate-disease and insect factors, increased health care statues, high level of competition in the trade of global scale and providing sustainability concepts has been a rising trend for all the agricultural systems. Therefore, protection of biodiversity, better and faster breeding techniques, supporting of farmers, new types of foods and industrial usage, widening of extension besides cooperation and new varieties should be developed for legumes.

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BIODIVERSITY ON PASTURES: HUNTING AND SOCIO-ECONOMIC DIMENSIONS

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Abstract

Pastures are main living area of many living organisms and hunting as well. Environmental pollutions, intensive farming systems, urbanization and excessive usage of natural resources caused to significant negative effects on ecosystem even distinction of many species which gave rise to decrease in biodiversity. Wild species of both animals and plants absolutely depend on pastures while these areas are in danger by corruption of balance which is welded by human eventually while natural disasters and epidemic diseases have just a very few ratio. On the other hand, senseless utilization of the pastures likewise out of purpose, excessively irrigation, fertilization, pesticide application, pasturage, illegal hunting and/or on forbidden period, misidentification, extreme and unconscious collection from nature etc. significant mistakes caused to great negative threats on biodiversity on the pasture areas that are quite important side or direct effects on sustainability of ecosystem. Hunters usually do the activity because of the sporting activity, friendship, food, hobby and habit. Some of the hunters do not have license and have a little knowledge about sustainability components such as; birth season, number of kids, average life time, diet, limitation and techniques. It is fair that most of the interviews with hunters showed that the hunters love the animals very much and their associations realize many activities to increase to animal population. Finally, remediation of destruction on nature is extremely limited and impossible in some cases. Therefore, present paper focused on threats on biodiversity of hunting animals in pastures, its socio-economic perspectives and to make suggestions.

Keywords: *Bioresource, Extinction, Hunter, Sustainable environment, Wild life.*

Introduction

History of hunting is as older as human. Purposes of hunting are; nutrition, controlling of population and balance, sport activity and tourism. Irregular and unconscious hunting caused to some laws to legality and sustainability of life. Additionally, intensive applications on agriculture also gave rise to some concerns about biodiversity of pastures and hunting as well.
Hunting laws are including not only hunting animals but also their environment, other living organisms (Çalışkan, 2003a, b). In many countries that have a higher culture pay attention for hunting animals. Species of these animals, quantity, their environment are defined very well (Çanakçıoğlu and Mol, 1996). According to the official laws, hunting means a verb that; hunting depending on laws that is guiding to exact date, species, places, limit and methods. Hunting animal explains; the animal which is caught by hunting activity. Main purposes of the laws are related with: protection of hunting animals in their natural places. If human focused on protection of only one species, the other organisms would be affected as well. So, the main principle should be focused on sustainability. Because, all of the matters in the environment are component of the ecosystem.
As the changing statues on life and industry over the world, there is an increasing concern about sustainability of life and so all the components of environment. Hunting and other effects of human on ecosystem are directly and/or indirectly effects to sustainability. In the present paper, hunting and socio-economic dimensions are reviewed by biodiversity frame.

From this perspective, main purposes of the review are; importance of biodiversity by view of sustainability, determination of basic factors which have negative impacts on environment and wild life welded by increasing of population – urbanization – industrialization and unconscious hunting activities.

Sustainability of Biodiversity on Pastures

All the living organisms in the ecosystem are under the negative effects and risks of adverse conditions (Aktan, 2006). People who are not related with life sciences and hunter that are not educated are an element of danger/risk for sustainability (Akbaba, 2001). Love for nature, animal and all the living organisms are components for environment consciousness (Türçek, 2006). Industrialization and environmental pollutants are often realized by lack of responsibility and sensitivity (Dinç, 2005).

The term of environmental sustainability includes; long term solutions of environment - that is including the pastures - problems and, examination of conditions for economic development that are suitable for environment, newly planned evaluations for future. In this perspective, biodiversity on pastures is an essential element for the purpose and so, sustainable development should take care about providing of the demands for next generations without risk and also for the present time. Using of the natural sources and also pastures has multiple effects on biodiversity in addition to ecology that are serving to human demands (Ankara, 2001).

Sustainable Hunting

Rapid increasing of human population, modern industry, un-planned urbanization, rashly usage of natural resources etc. welded environment problems are threating on global scale. These factors caused to the term of "sustainable development" including economy, ecology, social sustainability and seeking to find solution on many sectors and sciences (Şermet, 2017). The term of sustainability is explained in "First International Conference On Urban Regeneration and Sustainability" as; persistence of functions of life quality in urban areas that are demanded by public without limitation of present and future choices and as not cause to negative effects (Özden, 2008)

One of the components for protection of nature over the world is wild animals as conscious, providing the balance between environment and wild animals, possibility of access from wild animals in sustainability principles (Ege, 1998). Depending on this definition, sustainable hunting may be summarized as following (Alpat, 1989); protection of wild animals by view of their presence-living environment-nutrition evenly, avoid from destruction of living area including diversity of plant, precautions for extinction, whole time watching to balanced reproduction, precaution for forbidden hunting and disease management, pay attention about agricultural activities-applications and cultural practices as well. In the present day, most of the hunters are aware of; importance of wild life, knowing the rules, and efforts for the topic by authorities are admirable and effective (Çaya, 2010). An excellent project suggests an example of theory for wild meat and food security as it seen on Figure 1 (CBD, 2017).

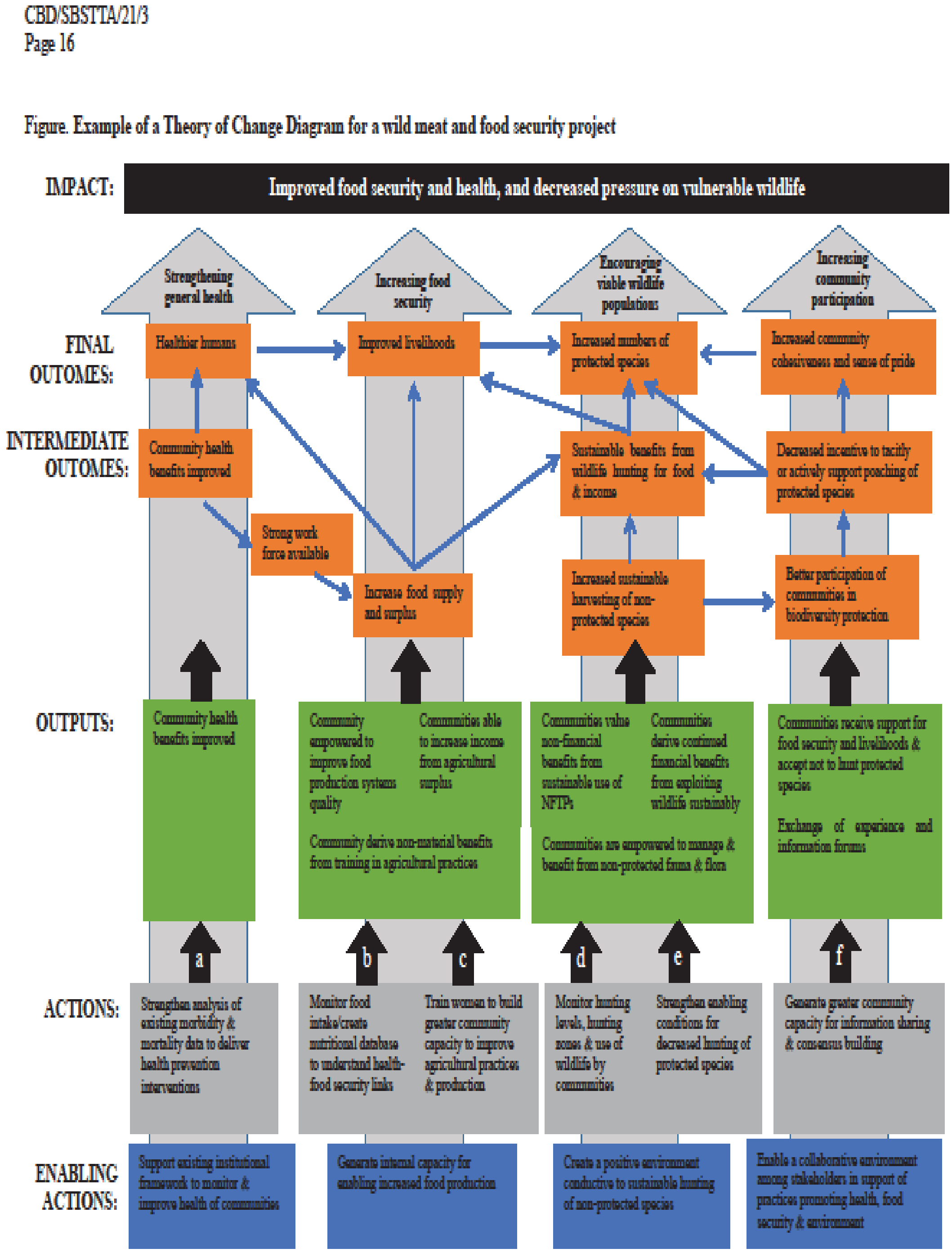

Figure 1. Change diagram for a wild meat and food security (CBD, 2017)

Hunting and Socio-Economic Dimensions

Hunting is an activity which was started by the first human and continues today. All the laws about hunting are focused on sustainability and balance of life. Some of the wrong and/or improper activities in addition to irregular actions give rise to misunderstanding or prejudice by many people who has insufficient knowledge about hunting or hunters. In some cases, wild animals suffers from human activities such as urbanization, mining, fire, industrialization, agricultural activities etc. factors for many times more than hunting and also some of the wild animal species are disappeared already. Therefore, there is need for more education and expansion works on hunters and the people who are strictly objecting to hunting as well. Similarly, all the human activities should be aware of sustainability principles. People has to take into account that; if somebody realizes something wrong, it does not mean all the people on that cite are going to do the same activity.

Conclusions

Some of the points in hunting should be revised such as; sustainability principles, participation, principle about; the person who benefits should contribute, regain, effective protection, honorary inspectorate, sources of income, documents and permission for hunting, hunting tourism, central hunting commission, employment and economy, educational publication and extension, management of ecology and environment, deterrent penalties. It is fair that; biodiversity of pastures, hunting and other human activities and economic development are very important factors for the future. Therefore, all the people who are strictly related with the mentioned topics should decide together.

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EFFECTS OF A SUBSTRATE IMPROVEMENT AGENT ON THE VEGETATIVE GROWTH AND QUALITY OF VEGETABLE SEEDLINGS

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Abstract

Seedling production and the quality of vegetable growing is a determining factor of subsequent yield. Transplant use has further advantages, e.g. earlier yield, improved efficiency of seed germination, increased growth safety, uniformity, etc. Non-profit orientated farmers can also consider using transplants because of these reasons. By applying substrate-improvement agents, farmers have a chance to enhance the seedling productivity in hobby gardens and in commercial production as well. These agents can improve the water capacity and nutrient content of substrates, and it can simplify the use of the media. Multiple type substrates can decrease the amount of chemical fertilizers necessary in production.
This research aimed to study the development of lettuce and tomato seedlings in organic substrate with an improvement agent (BRT® Evergreen – AE), and estimate the efficacy of different amounts of these additives in vegetable transplant growing.
AE is a lightweight substrate additive. It was developed by BRT Ltd., with the purpose of increasing the water holding capacity and nutrient absorption of the growing media.
Differences were found in the development of lettuce and tomato transplants. The additive in different doses increased the nitrogen, phosphorus and potassium uptake of the plants as well. Furthermore, the dry material, nitrogen and SPAD chlorophyll content showed expressive coherences too.
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Keywords: *Tomato, Lettuce, Transplant, Seedling, Additive*

Introduction

Experiences of the past few years proved that seedling production has a particular importance. It can increase earliness, extend the growing period and provide crop safety. Moreover, it allows better utilization of the growing area, and it allows the growers to use the expensive seeds reasonably (used seeds per hectare may significantly reduce) (Pap, 2011). Because of controlled climate conditions, not only the productivity of the plants, but the quality of the yield will also be adequate (Montemurro and Maioranna, 2009). Disadvantages that should be mentioned are the costs of extra work and plant sustainment (Kappel, 2006).
Increase in temperature and decrease in rainfall are the current trends associated with climate change which are perceived to have huge impact in the production of different crops. Considering these conditions and its probable adverse implications, especially in terms of water availability for the growing plants, different interventions and technologies are developed. In addition to the utilization of good quality growing medium that are able to provide optimum growing conditions, different growing mixture additives were used such as superabsorbent (e.g. hydrogels or superabsorbent polymers) which are synthetic substances and water-insoluble polymers that have ability to retain water within its structure. It has been largely utilized during the last decades among different plant species including cotton, oat, onion, watermelon, potato, sage, corns, furthermore *Fagus sylvatica*, *Picea abies*, *Pinus*

sylvestris etc. (Ngoben et al., 2007; Viztiu et al., 2014; Savi et al., 2014; Faried et al., 2014). and was found to be beneficial in moisture conservation and economic reduction of irrigation for agricultural crops (Radó-Takács, 2016). By incorporating this in the growing media, the amount of irrigation needed in the cultivation is expected to be significantly reduced. Similarly, DCM Aquaperla® is a substrate additive developed to improve moisture and nutrient capacity. Aside from the water retention, it is also found to be advantageous in the development of string root, vigorous plant, and greener foliage (WEB 1). In the study conducted by Tilly-Mándy et al. (2016) where a recently developed plant growing media, namely BRT® GreenMoss and Uni-20 professional growing medium had been used in combination with the different levels of substrate improvement agents, specifically BRT® Evergreen and FAIN Bioactivator (FBA) in Tagetes patula "Csemő", it was found that moss can be a suitable growing medium for the plant which promotes significant increase in plant height and width and decline in the stress-enzyme activity of the plant. Another related study that was also done in an ornamental species, particularly Viola x wittrockiana 'Carrera' used BRT® Evergreen and FBA with coconut coir and peat as the growing media. Results indicated that taller and bigger plants have grown from the treatment groups with mix coconut coir, BRT® and FBA as compared to treatment groups with peat as the growing medium (Radó-Takács, 2016).
In this study the effect of additive agent was examined on some vegetable transplants, and tried to define the optimal use of additives with mixtures of black peat.

Material and Methods

The experiment was conducted in a forcing system, in a 50 m^2 type greenhouse at the Department of Vegetable and Mushroom Growing (Szent Istvan University, Faculty of Horticulture, Hungary, Budapest). 40 cell type plastic trays were used for tomato, and 66 cells type trays were used for lettuce seedling growing. The used tomato variety was 'Vilma', which is characteristically a dwarf type cocktail tomato. Sotalis F1 butter head lettuce variety was applied in our experiment. Lettuce transplants were grown from 27th of March 2018 to 24th of April 2018, while tomato transplants were grown from 12th of April 2018 until 24th of May 2018. All treatments had 6 replications, in case of tomato we used 20 transplants in one repeat, and in case of lettuce 30 plants. During the growing we used only clear tap-water for irrigation. The temperature and air humidity were measured by Voltrcraft DL 181THP data-logger. Table 1 shows the applied treatments with signs and important components.

Measurements

The following parameters were determined:

- Plant length to the tip of the longest leaf in case of lettuces
- Plant height to the tip of the plant in case of tomato
- Dry matter content of foliage (calculated from fresh and dry weights)
- Nitrogen and phosphorus in leaves and roots
  - ✓ Nitrogen – Kjeldahl method (Sáez-Plaza et al, 2013)
  - ✓ Phosphorus – spectrophotometric evaluation method (Thamné et al, 1968)
- Chlorophyll content from measured SPAD values with use of Konica-Minolta SPAD 502 Chlorophyll meter

Statistical analysis was evaluated with Microsoft Office Excel 2016 Analysis ToolPak.

Table 1: Marks of the treatments and type of substrate and additives.

Treatment	Marks	Notes
Control Florasca	**F**	55% Black peat 25% white peat and 20% Hungarian grey cattle manure
Florasca + 10% of BRT® Evergreen (WEB 2)	**F 10**	BRT (Biomass Refine Technologies) → Carbamide formaldehyde polymer, Formaldehyde, Carbamide, Clarified phosphorus acid, Alkylbenzole sulfur acid, Surfactant, Water
Florasca + 20% of BRT® Evergreen	**F 20**	
Florasca + 30% of BRT® Evergreen	**F 30**	
Florasca + Aqua Perla® additive (WEB 3)	**F AP**	Aqua perla → Potassium-poliacrilate

Table2: Physical evaluation of applied substrate (F).

Mineral content	Values
pH (KCl)	6,07
Total Salt (TS - m/m%)	0,05
Na (mg/kg)	835
Nitrogen (NO_2+NO_3-N mg/kg)	1040
Phosphorus (P_2O_5 - mg/kg)	6180
Potassium (K_2O - mg/kg)	7500
Mg (mg/kg)	1910
Cu (mg/kg)	13,4

Results and Discussion

According to physical evaluation of transplants a remarkable difference was observable in case of diameters of tomato seedlings (Table 3). In as much as F value is higher than F crit., and p-value does not reach 0,05, the H1 is acceptable. In this case significance is observable between treatments, where F 30 and F AP is presenting relevant difference from other groups.

Table 3. One-way ANOVA - Diameter of stem of tomatoes

SUMMARY

Groups	*Count*	*Sum*	*Average (mm)*	*Variance*	*St. Deviation*
F	30	100,74	3,36	0,11	0,33
F 10	30	99,24	3,31	0,07	0,26
F 20	30	103,62	3,45	0,08	0,28
F 30	30	114,79	3,83	0,20	0,45
F AP	30	106,69	3,56	0,11	0,33

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit.*
Between groups	5,06	4	1,27	11,26	5,5332E-08	2,43
Within groups	16,30	145	0,11			
Total	21,369	149				

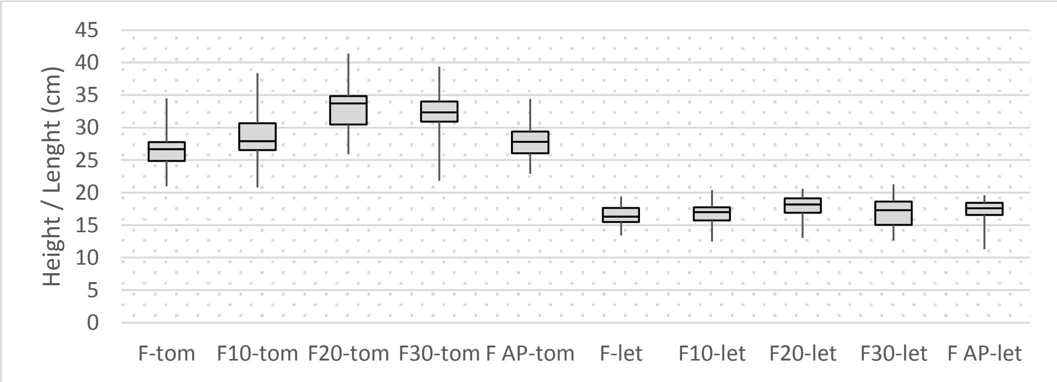

Figure 1. Plant length to the top of the longest leaf in case of lettuces and plant height to the top of the plant in case of tomato

Plant height of tomato and length of lettuce gave the similar results in Figure 1., because in case of F20-tom, F30-tom and F20-let the plants were higher or with longer leaves. In case of tomato they had thicker stems as well. They were well-developed, however transplants of F AP in case of tomato were stocky with thicker and shorter stem. Stem and height data can supplement each other correctly, however it was suitable only for tomato, because in the case of lettuce measurement of stem wasn't feasible.

Also, dry matter content in leaves can supplement the results of physical properties of transplants (Figure 2.). However, in case of lettuce the differences weren't significant, F30-let gave higher dry matter contents. Parallel in case of tomato, treatment of F30-tom gave a robust difference from two kind of plants, however because of high standard deviances we couldn't find significant variance.

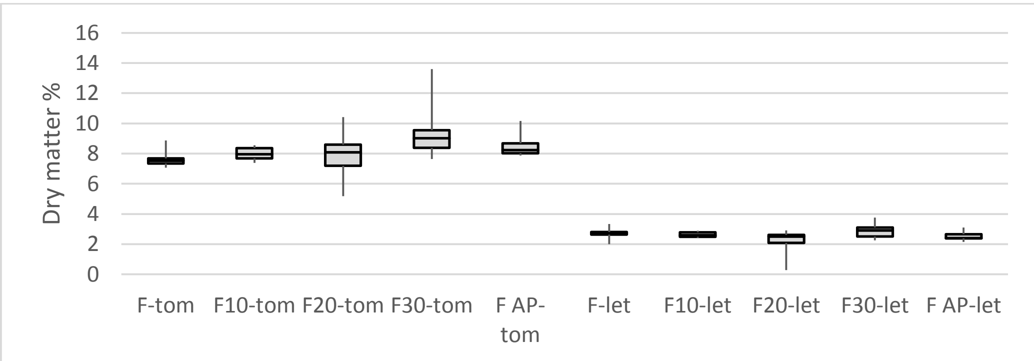

Figure 2. Dry matter content of foliage in case of tomato and lettuce transplants

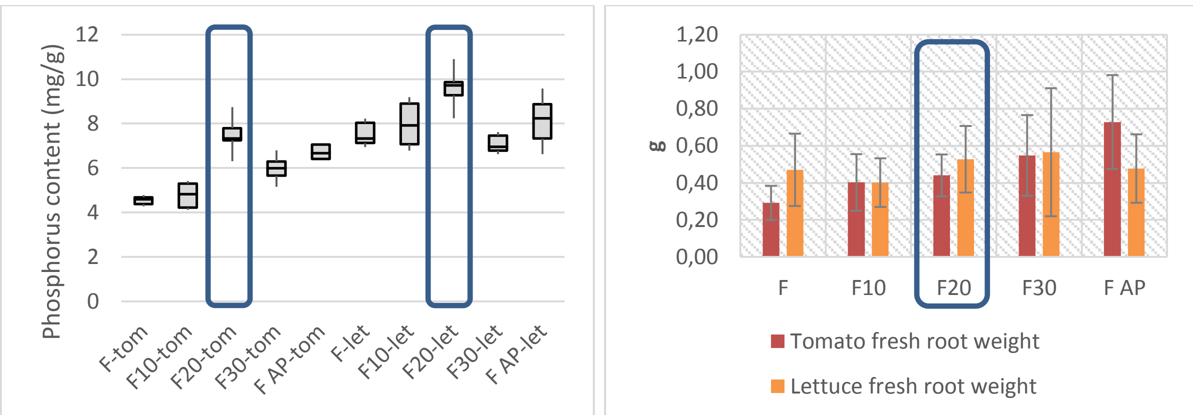

Figure 3. Phosphorus content of roots (left) and fresh root weight (right) in case of examined plants

Phosphorus is an important factor for stronger and powerful root development. Moreover, root system is determines the quality of transplants, and mostly in field growing the better survival and latter stress tolerance (Hopkins and Hüner, 2009). In the case of both plant the treatment F 20 affected higher phosphorus uptake and content. However, in Figure 3. the fresh root weight was not observed to follow this trend.
Also, another relationship was important in our investigation, to prove the earlier results about connection between nitrogen content and SPAD Chlorophyll value. Nevertheless, in our experiment the SPAD values did not follow the changing of data of Nitrogen (Figure 4.).

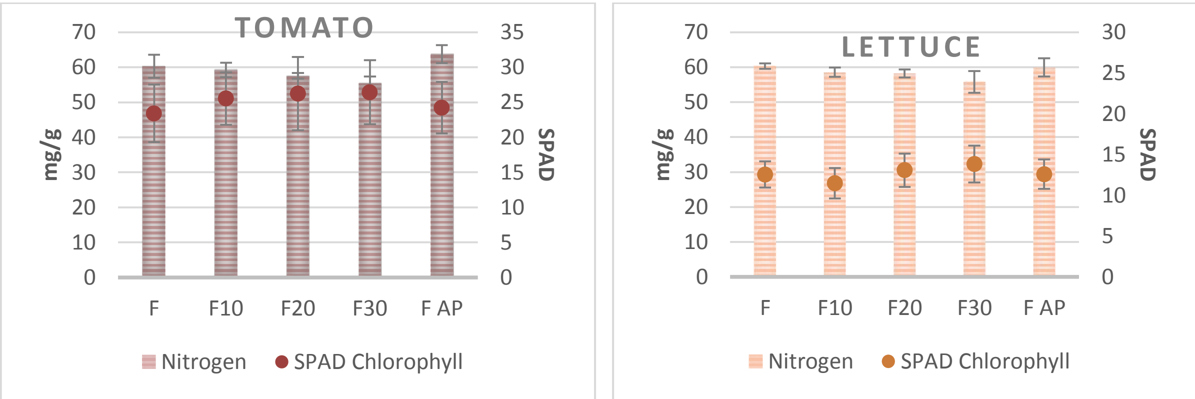

Figure 4. Correlation of Nitrogen content and SPAD in case of tomato (left side) and lettuce (right side) transplants.

Conclusion

During this experiment the development of plants was dependent on two factors in different substrate mixes: nutrient content of substrate and nutrient content of additive. How we increased the amount of additive, we gave more nutrients, however this quantity was moderated. All our additives were advantageous from side of increasing the water capacity of substrate, however in the first stage of our experiment we were curious for the effect of the additives for plant development and behavior. Growth of plants followed the opportunities of higher nutrient values of substrates. In this case, the higher amount of BRT has provoked higher height of tomato seedlings and longer leaves for lettuce. This result could be visible in leaf dry material content as well. At the same time fresh weight of root followed the results above, because of higher volume of nutrients, but the phosphorus value increased extremely higher in treatment of F 20 (in case of both plant). This result did not follow the changes of fresh weight of roots, however according to Hopkins and Hüner, (2009) Phosphorus is important for strong root development. Higher P uptake of treatment of F 20 can give chance for other questions that is recommendable for a latter experiment.
According to Sharaf et al (2015) the SPAD values follow the nitrogen content in leaves, because nitrogen has relevant effect to Chlorophyll synthesis. In our experiment we could not prove this, however amount of nitrogen was similar in different treatments and the differences between SPAD values were not high as well.

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EFFICACY OF MULTILAYER CROPPING ON FIG PRODUCTION IN ARID CONDITIONS OF KUWAIT

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Abstract

This study examines the influence of intercropping on the growth and performance of figs in a multilayer system of planting was carried out under the arid climatic conditions of Kuwait. The experiment was laid out in Randomized Block Design comprising of two treatments with six replications in two sites. The two treatments were fig plants intercropped with or without vegetables. The main tree date palm was planted with a spacing of 7 X 7 m and the vegetables were planted in between the lines of fig trees. The results of the study revealed that date palm +fig +vegetables multitier system exhibited better performance, which has been reflected in the significant ($p<0.05$) increase of the soil nutrients, growth parameters and fruit quantity and quality than the fig plants without vegetables. Even though the trees were in initial orchard years and still young, fruit yield in terms of number of fruiting shoots, number of fruits, weight per fruits, and average yield per tree, the response to inter planting with vegetables was significant. Based on these results, it could be concluded that intercropping technique in date palm plantations along with figs and vegetables resulted in maximizing the use of unit land. Nevertheless, fruit quality of fig was influenced by the intercropping systems, which was considered to be a good sign for farmers in adapting and promoting inter-planting practices.

Keywords: *Inter-planting, date palm, microclimate, vegetables*

Introduction

Fig belongs to the *Moraceae* family (Vallejo *et al.*, 2012) and is among the earliest cultivated fruit trees in the world (Solomon *et al.*, 2006). Today, it is an imperative world crop and has been cultivated in Turkey, Egypt, Morocco, Spain, Greece, California, Italy, Brazil, and other places with typically mild winters and hot dry summers (Zohary and Hopf, 2000).
Kuwait's climatic conditions are harsh and are typical of an arid environment characterized by high temperature during summer, very low rainfalls, frequent winds, sand storms, and soil erosion. This limitation coupled with progressive buildup of salts in the soil hinders the growth of a wide range of plants. Hence, the State of Kuwait is making efforts to strengthen agricultural production and achieve partial food security in selected commodities. To achieve these goals, many types of figs has planted in home gardens and in designated areas in Kuwait. The suitability of land for fig production, combined with positive farmer perception, indicates that there is a potential for the development of this plant on a commercial basis.
The importance of the fig tree lies in the fact that it can withstand severe climatic conditions of arid regions such as low rainfall, high temperature and high soil calcium carbonate content. Due to its easy management properties, the crop is more acceptable in dry lands either as a monocrop or in mixed cropping. Its inclusion in agricultural land use system certainly helps achieve crop diversification

Materials and methods

The intercropping experiment was conducted during April to September of 2018 in an existing 10-year-old bearing date palm plantation site at Public Authority for Agriculture and

Fish Resources (PAAFR), located at Wafra. The experimental sites with enough plant-to-plant spacing were selected to assure a good shade. Out of the two areas, one area planted with Medjool date palms was selected as "Site 1" and the other with Barhi palms as "Site 2". The experiment was arranged in a randomized complete block design (RCBD) with two treatments and 6 replications in each sites. In both of the sites, the grafted seedlings of seven selected cultivars (Black Mission, Brown Turkey, Calimyrna Red, Calimyrna White, Sequoia, Sierra, and Tina) were planted in between the date palms at a spacing of 4 m, and the vegetables were planted in between the lines of fig trees. In short, there were two treatments, such as the interspacing area between date palms and figs planted with leafy vegetables and without vegetables. Similarly, there were two sites, and the vegetables were planted in two seasons.

In the first season, two leafy vegetables, parsley or garden parsley (*Petroselinum crispum*) and arugula or rocket (*Eruca sativa*), were planted on the inter rows of date palms as single strips, and each stripe were started 10 cm away from the basins of the fig plants. In intercropping, each row of fig was inserted between every rows of the parsley or arugula. In the second season, common purslane (*Portulaca oleracea*) and basil (*Ocimum basilicum*) were planted as interplant with fig plants. For the cultivation of these vegetables, the seedbed was prepared by ploughing the land twice at 30 cm depth. The ploughing was carried out perpendicular to the space in between the fig plants to prevent the runoff and improve water retention; basins were prepared around this prepared land. The seeds of all the four vegetables were broadcasted in the prepared areas and irrigated using adjustable drippers. However, no organic or inorganic fertilizers were given to the fig plants until the end of the experiment.

The plant height of fig trees was estimated by measuring the length of the main stem at the beginning and at the termination of season. Stem thickness of each plant was measured using a caliper at the 10-cm height of the plant above the ground surface. Leaf area was calculated on selected four leaves from each plant and expressed as centimeter squared. In the first week of July, the number of fruiting shoots were counted and recorded. The fruits were harvested at the peak of the color development (second week of August), and recorded number of fruits, fruit weight (g) and yield/plant (g). The growth rate of each plant was calculated using the equation as follows:

$$\text{Growth rate} = [(\text{Final total growth} - \text{Initial total growth}) / \text{Initial total growth}] \times 100 \quad (1)$$

The soil samples were analyzed for soil physical and chemical variables at the PAAFR laboratories, Kuwait. The percentages of N, P, K and Mg in plant leaves were determined according to the procedures that outlined by USDA, 2004. Total phenolic compounds of each sample were extracted by the Folin–Ciocalteu's method as described by Fu *et al*. (2011), while total flavonoid content was measured by aluminum trichloride assay (Koolen *et al*., 2013).

Results and discussion

Nutrient Status of Fig Plants

From the data in Table 1, it is clear that the intercropping practices resulted in the improvement of physical conditions and nutrient status of the soil. Intercropping fig plants with vegetables caused a substantial reduction of EC from 5.21 to 3.80 mS/cm, thereby making the major nutrient contents available to the fig plants, which reflects in the vegetative growth of plants under intercropping than the other treatment. Similarly, the lowest values of pH were noticed under intercropped areas, which enables smooth retention and availability of the plant essential nutrients.

Soils of fig plants intercropped with vegetables had a high concentration of major nutrients compared to pure stand of figs. Even though there was an uptake of nutrients by the main and sub crops, a high content of nitrogen was observed in rhizosphere of intercropped plants. The increase in the available nitrogen content of soil might be due to the greater recycling of bio-mass in the inter space with higher percentage of nitrogen in the vegetable based inter cropping system as compared to sole cropping systems (Manna and Singh, 2001). According to Thorup-Kristensen and Sorensen (1999), the planting of shallow rooted crops as intercrop with deep rooted crops where available N is present in deeper soil layers, nitrogen availability can be increased. This increase in available nitrogen content of the soil can be because of the in situ incorporation of intercrops biomass and increased the enzymatic activity of the effective microorganisms, which leads to the release of nutrients in the soil. Swain and Patro (2007) also reported an increased available nitrogen content of the soil through intercropping in mango intercropping systems. The available phosphorus content of soil under intercropping systems might be due to increase in the phosphorus solubulizers due to the incorporation of vegetable biomass. Also, Swain and Patro (2007) observed similar findings on beneficial effect of intercropping in increasing the availability of phosphorus in the soil. The intercropping of vegetables also increased the K levels in soil, which in turn provided resistance to plants to thrive prevailing drought conditions. The results also indicated that the intercropping system were advantageous in increasing the available potassium contents of soil, which corroborates with the findings of Swain and Patro (2007). This recycling of biomass had increased the content of humus in the soil and there by increased the available potassium content. Previous studies of Maheswarappa *et al.* (1998) also supported these findings by their results of improvement in major nutrient status of soil due to intercropping.

Table 1. Soil chemical properties as affected by intercropping of fig plants with vegetables

Treatments	pH	ECe (mS/cm)	Water Soluble Cations (Meq/L)				Water Soluble Anions (Meq/L)			SO_4	PO_4	NO_3
			Ca	Mg	Na	K	CO_3	HCO_3	Cl		(ppm)	
Fig + vegetables	7.1	3.80	10	4.5	9.25	5.5	1.9	4.6	16.4	24.0	37.6	42.6
Fig - vegetables	7.9	5.21	12	1.3	14.4	2.8	0.7	4.2	21.5	21.4	12.4	28.2

Plant Growth of Fig

Results of intercropping of fig plants with vegetables and sole croppingof figs on some vegetative parameters displayed that there was statistical difference between the intercropping and sole cropping ($p<0.05$) on relative growth rate and leaf area, while there was no difference on plant height, stem thickness in both the sites. Nevertheless no significant variation was found between the two sites in any of the vegetative parameters studied. The highest plant height, stem thickness, relative growth rate and leaf area were obtained from intercropped areas. While the highest relative growth (27.9) was obtained from fig plants interplanted with vegetables in Site 2, sole cropping of fig plants (26.6) from Site 1 followed it. The fig plants with vegetables intercropped surpasses the sole cropped plants in the leaf area in both sites. The sites and intercropping interactions had a statistically significant effect ($p<0.05$) on the leaf area only. The maximum leaf area was obtained from the leaves of fig plants planted in intercropped areas in both the sites. In general, the intercropping of fig plants with vegetables significantly higher than the sole cropping of figs in some of the primary growth characteristics. These results are in accordance with the findings of Aksoy *et al.*(1987).

The results of the studies showed that the vegetative growth characteristics of fig plants were positively influenced by intercropping. The reason for increase in growth parameters of fig plants might be due to this input utilization. According to Panda *et al.* (2003), adoption of suitable intercropping systems in fruit crops aids in efficient utilization of available natural resources and improves the input use efficiency in the system. Intercropping with nitrophilous crops particularly with purslane was helpful in the positive effective increase in nitrogen that results in higher vegetative growth in fig plants. More research findings supporting the observations on increase in tree height, girth, and canopy area of fruit crops due to intercropping were reported by Singh *et al.* (1996).

Table 2. Figs growth and yield parameters as affected by interplanting of fig trees with vegetables.

	Plant Growth Characteristics.							
	Site 1				*Site 2*			
Intercropping Treatments	*Plant Height (cm)*	*Stem Thickness (cm)*	*Relative Growth Rate (%)*	*Leaf Area (cm^2)*	*Plant Height (cm)*	*Stem Thickness (cm)*	*Relative Growth Rate (%)*	*Leaf Area (cm^2)*
Fig +vegetables	174.2	4.6	26.6	95.7	174.1	4.7	27.9	94.6
Fig - vegetables	172.5	4.6	25.3	82.4	174.0	4.5	25.5	86.9
Site 1	173.4	4.5	25.9	89.1				
Site 2	174.2	4.6	26.7	89.9				
Site (S).	NS	NS	NS	NS				
Treatments (T).	NS	NS	*	*				
S*T.	NS	NS	NS	*				

	Yield Attributes							
	No. of Fruiting Shoots	*No. of Fruits per Plant*	*Fruit Weight (g)*	*Yield/ Tree (g)*	*No. of Fruiting Shoots*	*No. of Fruits per Plant*	*Fruit Weight(g)*	*Yield/ Tree(g)*
Fig + vegetables	5.93	12.82	39.63	484.2	5.36	12.10	37.27	450.40
Fig - vegetables	5.13	11.98	37.71	449.2	4.66	11.19	36.91	435.34
Site 1	5.53	12.40	38.67	466.7				
Site 2	5.01	11.65	37.09	442.8				
Site (S).	NS	*	NS	*				
Treatments (T).	*	*	*	*				
S*T.	NS	*	NS	*				

	Fruit Quality.			
	Total Phenolics (mg GAE/g DE)	*Total Flavonoids (mg QE/g DE)*	*Total Phenolics (mg GAE/g DE)*	*Total Flavonoids (mg QE/g DE)*
Fig +vegetables	46.270	16.393	43.194	15.932
Fig -vegetables	44.675	15.976	41.543	14.895
Site 1	45.472	16.185		
Site 2	42.369	15.413		
Site (S)	NS	NS		
Treatments (T).	*	NS		
S*T.	NS	NS		

	Leaf Macronutrient Contents (% Dry Weights)									
	N	*P*	*K*	*Ca*	*Mg*	*N*	*P*	*K*	*Ca*	*Mg*
Fig +vegetables	1.77	0.14	1.07	3.59	0.85	1.69	0.15	1.10	3.60	0.88
Fig -vegetables	1.68	0.10	1.06	3.53	0.83	1.63	0.11	1.08	3.56	0.83
Site 1	1.72	0.12	1.07	3.56	0.84					
Site 2	1.65	0.13	1.09	3.59	0.85					
Site (S).	*	NS	NS	NS	NS					
Treatments (T).	*	NS	*	*	NS					
S*T.	NS	NS	NS	NS	*					

* denotes the significant difference at $p<0.05$; NS- Non-significant.

Fruit Weight and Yield of Fig Plants

Even though the fig trees were in initial orchard years and still yielded young fruit in terms of number of fruiting shoots, number of fruits, weight per fruits, and average yield per tree in response to interplanting with vegetables was significant. Plants represented the intercropped area produced more fruiting shoots, fruits, fruit weight, and yield per tree than in areas where vegetables were not planted as intercrops in both the sites. However, statistical variation among the sites and the interaction of sites and treatments were observed in the fruit characteristics of number of fruits per plant and yield per tree. Intercropping practices with vegetables in Site 1 had a significantly higher number of fruits per plant (12.4) and yield per tree (466.7 g) compared to those in Site 2. Regarding the interaction effect of sites and treatments, intercropping vegetables with fig plants in site 1 produced significantly more number of fruits per plant (12.8) and yield per tree (484.2 g) than the other studied interaction treatments under study.

The data showed that the intercropping of date palm, figs, and vegetables significantly increased all the fruit characteristics under study. This result may be because the palm tree provided enough space for the filler crop (figs) and the interplants (vegetables) to get favorable conditions for growth. Akyurt *et al.*(2002) found that date palm supply enough space for intercropping even if they are fully grown as they do not cover much area being a very tall tree. Aksoy (1998) reported that competition for resources between component crops and efficient utilization of natural resources such as solar radiation, soil moisture, and nutrients may be the reasons for higher yield parameters particularly fruit weight and fruit yield under intercropping systems. Moreover, cultural practices followed for the intercropped areas contributed to the higher production of fruits (Ghosh, 2001; Rath and Swain, 2006). According to Ali *et al*. (1998), the date palms could be intercropped with citrus and are possible to be grown as a mixed fruit orchard. Previous studies by different researchers concluded that intercropping those trees that are cultivated with various intercrops had no considerable adverse effects on the yield and fruit quality of main crops (Ashour *et al.,* 1994; Abouziena *et al.,* 2010).

Fig Fruit Quality

Results of total phenolic and flavanoid contents are shown in Table 2. The methanolic extracts of fig leaves from vegetable intercropped areas contained significantly the highest amounts of total phenolic and flavanoid compounds than the other treatments. A higher amounts of phenolic compounds with a value of 46.270 (mg GAE/g DE) were obtained from the intercropped with vegetable areas in site 1 and 43.194 (mg GAE/g DE) from the intercropped areas in Site 2. Similarly in this study, the highest amounts of flavonoids were noted in leaves of fig and vegetables intercropping areas with 16.393 and 15.932 mg QE/g DE in Site 1 and Site 2, respectively.

The analysis of quality parameters of fig plants in Table 2 revealed that there was no appreciable influence on fruit quality by intercropping practices. The fruit quality aspects such as total phenolic acids showed significant differences while there was no difference in total flavonoids between the treatments. Previous studies in fig showed that fruit quality parameters were unaffected by planting distances (Kumar *et al.,* 2014; Hosomi *et al.,* 2013). Similar results were obtained from the researches of Kanwar *et al.* (1993), and Ghosh (2001), where the quality of fruits were not affected due to growing of intercrops, in mango, citrus, and guava orchards.

Leaf Macronutrient Contents

Results clearly revealed that the different nutrients in the fig leaves were significantly varied in the main effects of nitrogen, potassium, and calcium contents. Regarding the interaction effect of sites and treatments significant variation was found in the content of Magnesium. Intercropping of fig plants with vegetables significantly increased the contents of four macronutrients compared to figs without intercropping. A gradual and significant increase in nitrogen, potassium, calcium and magnesium contents were recorded with growing figs with vegetables. These trends of nutrient increment were true in both sites.

The leaf analysis results indicated that the N, K, Mg and Cacontents of fig plant leaves were maximum under fig and vegetable intercropping system. The availability of nutrients in the soil is increased due to the *in situ* incorporation of vegetable residues might be the reason for the increase in the major plant nutrient status of fig leaves when compared to fig plants without intercropping. Maheswarappa *et al.* (1998) concluded that the incorporation of intercrops residues might be helpful in improving the soil physical, chemical, and biological environments, which in turn favors the higher uptake from the nutrient pool in the soil.

Conclusions

Multilayer cropping is becoming a sustainable crop production technique by utilizing a number of environmental beneficial effects and effective utilization of available land for diversifying agricultural outcome. High, medium, and low plants were integrated in a most sustainable manner in this production model. From the results of the study, it was revealed that intercropping was the ideal practice in increasing plant growth and fruit yield of fig plants. The plant growth, fruit weight and fruit yield of fig were found significantly higher in intercropping with vegetables under the canopy of date palms. Nevertheless, fruit quality of fig was influenced by the intercropping systems, which was considered a good sign for farmers in adapting and promoting interplanting practices. The introduction of intercropping technique in date palm plantations along with figs and vegetables resulted in maximizing the use of unit land. The results of the study also conclude that intercropping of fig plants and vegetables with date palm indicated that these techniques of intercropping could be used for combating desertification in the arable sandy soils of Kuwait.

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ASSESSMENT OF SAP FLOW TECHNIQUES AS AN AUTOMATED INDICATOR OF *VITIS VINIFERA* WATER STATUS IN THE PORTUGAL'S DOURO VALLEY

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Abstract

The Douro Demarcated Region, NE of Portugal is characterized climatically by low precipitation and high water losses due to evapotranspiration during the summer. In this sense, experiments were carried out to evaluate the effect of different water availability on the water status of grapevines and soils. In commercial vineyards, the 'Granier' thermal dissipation technique ('Moscatel-Galego-Branco' variety over 2008-09 growing seasons) and the 'compensated heat-pulse' method ('Boal' variety in 2012) were used to continuously monitor sap flow within the xylem of mature vines. Weather variables, soil moisture and leaf water potentials were also measured. Clear relationships between sap flow and increased water availability, after rainfall and irrigation events, were shown. Additionally, relationships between stress indicators, such as predawn leaf water potential, were examined. Furthermore, variations of trunk and root sap flow (using 'compensated heat-pulse' method) were observed during nocturnal periods, and were associated with high vapour pressure deficits. Relative transpiration (calculated as the relationship between the sap flows of water stressed plants and irrigated vines) showed a closer relationship with predawn water potential compared to soil moisture, suggesting being a potential indicator of automated water availability. However, the requirement for a (well-watered) control plot limits its practical applicability.

Keywords: *Grapevine, Survival strategies, Water dynamics, Predawn leaf water potential.*

Introduction

In northeast of Portugal, winegrapes are a major crop constituting an important source of income for local farmers. Located in deep valleys, protected by mountains, the climate in the Douro Demarcated Region is characterised by scarce rainfall and large evapotranspiration losses during the summer season. Thus, a deep root system is crucial for the survival of the vine during the driest period. Thus water redistribution within the plant, particularly between the roots, has been identified as a crucial survival strategy (Malheiro *et al.*, 2016). However, the mechanism generally considered most important for plant survival in a hot and dry environment is stomatal control to reduce transpiration (Dinis *et al.*, 2014). Plant transpiration can be determined indirectly by measuring evapotranspiration minus soil evaporation and/or directly by measuring sap flow, provided an adequate calibration is performed (Ferreira *et al.*, 2012). Sap flow determinations can be performed by methods such as the thermal dissipation technique (Granier, 1985), which stands out for its simplicity and low cost. However, the literature has shown that among the sap flow techniques, the compensation heat-pulse method is the most sensitive in detecting small flows. On the other hand, to evaluate the crop water status, the relative transpiration can be used, when the plants under water stress and in water comfort are available (Patakas *et al.*, 2005). Thus, the objectives of the present study were (i) to evaluate the water dynamics of mature vines using two sap flow methods and (ii) to evaluate the relationship between relative transpiration as an automated indicator and others water stress indicators.

Material and methods

The study was carried out in a commercial vineyard (41º15'N, 7º28'W, 600 m), located in the Douro Demarcated Region in Portugal, in two periods/experiments: 1) 2008 and 2009 with the Moscatel-Galego-Branco variety (syn. Muscat Blanc à Petits Grains) and 2) in 2012 with the Boal variety, in both cases grafted onto 196-17 Cl and planted in 1998 with spacing of 2.2x1.0 m. The climate of the region is of the Mediterranean type characterized by a sinusoidal pattern of precipitation with dry summer and wet winter. In the experimental area, the annual average long-term precipitation is about 630 mm. The average minimum and maximum annual temperatures are of 7.7ºC and 19.4ºC, respectively (www.ipma.pt). The soil, affected by human activity, is essentially of schist origin with a loam texture. Soil moisture was determined from the surface to a depth of 1.5 m by a portable TDR (Trime-FM, Imko). Predawn leaf water potential (Ψ_P) measurements were performed on 6 leaves per replicate of each treatment, with a pressure chamber (ELE International) on selected days during the summer. The data of an automatic weather station placed near the experimental plot were used to determine the reference evapotranspiration (ETo).

Experiment 1

The experimental design, with the Moscatel-Galego-Branco variety, consisted of four replications of irrigated plots and an equal number of irrigated plots. Water in the irrigated treatment was applied through a drip system from August to early September, with a total amount of 150 mm in 2008 and 190 mm in 2009. A Ψ_P threshold of -0.3 MPa was defined to start irrigation, in order to keep the plants in water comfort throughout the experimental period. Subsequently, irrigation was calculated through a simple water balance, so that the available water content varied around 60-70% in the 1.5 m of the soil profile. The thermal dissipation technique (Granier, 1985) was used to evaluate the sap flow of six vines in each treatment from July to September. Relative transpiration (RT) was calculated as the ratio between transpiration of plants under water stress (rainfed) and well-watered.

Experiment 2

The vines of the Boal variety were kept in a rainfed regime. Additional information on soil moisture conditions, physiological performance and yield components can be found in Dinis *et al.* (2014). The compensation heat-pulse method (Green *et al.*, 2003) was used to continuously measure sap flow in trunks and roots of eight vines. Thus, one set of probes (Tranzflo NZ Ltd, New Zealand) was placed on the vine trunk and another set on one of the larger diameter roots. The flow of sap ($L.h^{-1}$) was calculated using the methodology described by Green *et al.* (2003).

Results and discussion

Experiment 1

A decrease (that is, more negative) of Ψ_P was generally found in rainfed vines (-0.3 to -0.5 MPa) as soil moisture (SWC) decreased over the growing seasons (not shown). A close relationship was found between RT and Ψ_P. A similar approach was used for SWC, but with a weaker relationship (Figure 1). The higher dispersion pattern between RT and SWC for lower SWC values may be related to increasing deep-rooted activity, possibly beyond 1.5 m, towards the end of the vegetative cycle. In fact, together with the deep root system of mature grapevines, the typical stony soil characteristics are a major difficulty in monitoring soil water profile in the region. These results confirm a better performance of Ψ_P as a stress indicator in this crop. However, SWC is an estimation of water availability in upper soil layers and can be used to validate the amount of irrigation depth to apply, which can be of value when implementing an irrigation programme for this crop in the region. Close relationships between RT and Ψ_P were also reported in another wine region (Patakas *et al.*, 2005). These results indicate that RT is a potential indicator of water shortage. However, the requirement for a (well-watered) control plot and the need for careful data analysis (*e.g.* correction for natural thermal gradients) limit its practical applicability.

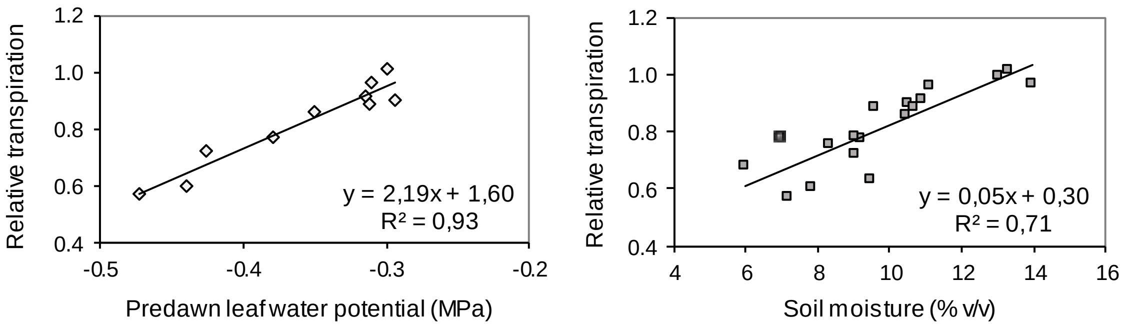

Figure 1. Relation between relative transpiration with predawn leaf water potential (left panel) and soil moisture (right panel): includes data from 2008 and 2009.

Experiment 2

The seasonal evolution of the (normalized) sap flow in the trunk (FSt/ETo) and roots (FSr/ETo) in the Boal variety was relatively stable throughout the growing season (Figure 2), suggesting an equally stable crop water status, despite the occurrence of "peaks" associated with the occurrence of precipitation and slight water recovery. This pattern is consistent with the moderate values of water stress from July to September, based on measurements of predawn leaf water potential (Ψ_P, -0.3/-0.5 MPa, Dinis *et al.*, 2014). The values observed for FSt/ETo (Figure 2) correspond to the basal crop coefficient (Kcb) multiplied by the stress coefficient (Ks). After significant precipitation events in September-October (when we can assume a Ks = 1), FSt/ETo reached values close to 0.4 corresponding to expected Kcb (Ferreira *et al.*, 2012). These results and the above comparison point out that the vines responded to moderate water stress with a reduction in sap flow (transpiration) compared to that at the beginning of the growing season. On the other hand, the fraction of available water in the upper 0.3 m of soil decreased to about 5% during summer (not shown). Such findings show that these grapevines are able to cope with a dry upper soil. The sap flow data showed that one of the plant survival strategies in such dry soil was to exhibit a midday stomatal depression, which tended to disappear after summer rains (not shown). In addition, there was a close relationship between ETo and FSr (Figure 2). Thus, it is possible that a significant amount of sap flow at the root has been converted to transpiration, rather than supporting the flow for rehydration of other roots. In fact, grapevine stomata are likely to remain slightly open during nights and, in conditions of elevated vapour pressure deficit, the resulting atmospheric demand being the main driver of water uptake. However, that response can be significantly reduced with increasing water stress conditions (Fuentes *et al.*, 2014).

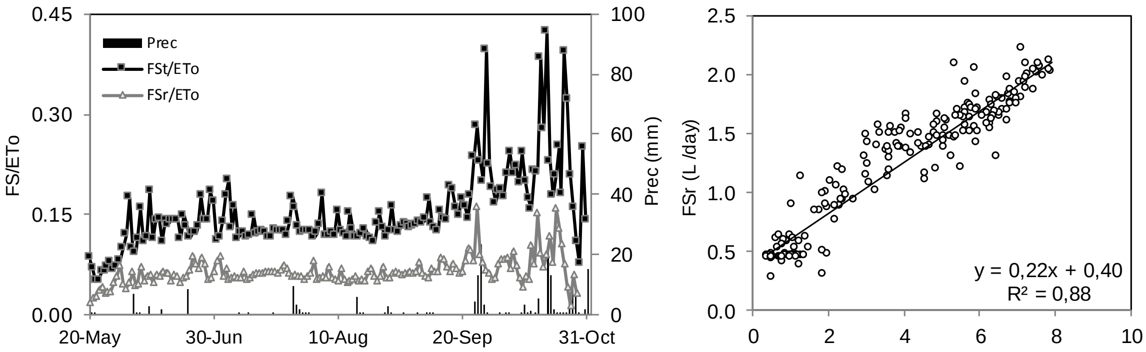

Figure 2. Seasonal evolution of precipitation (Prec) and sap flow in the trunk, FSt/ETo and roots, FSr/ETo (left panel) and the relation between ETo and FSr (right panel): 2012.

Conclusions

The "thermal heat dissipation" and "heat pulse" methods were used successfully in the measurement of sap flow in mature vines. The values found revealed the sensitivity to precipitation (or irrigation) events and conditions of significant atmospheric evaporative demand. A close relationship between relative transpiration and predawn water potential was found. The results suggest that relative transpiration is a potential automated indicator of water stress. However, the need of a well-watered plot limits its practical applicability.

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THE EFFECT OF DIFFERENT FOLIAR FERTILIZERS AND DOSAGES ON THE YIELD AND SEED QUALITY CHARACTERISTICS OF DURUM WHEAT

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Abstract

Durum wheat is one of the most common cereals which is widely grown and constitutes the cereal with the highest monetary yield. There are many studies where the different varieties are compared in case to find the better one with increased harvested yield and better seed quality. Therefore, it appears that the main objective of the investigation is to increase seed quality and seed yield. Furthermore, many chemical substances are sold stating that they can increase the final yield and the seed protein content. For the purposes of the study, a field experiment was established at the Experimental Farm of the Technological Educational Institute of Thessaly in Greece (TEI; Larissa plain) in 2018 (November), where the effects of three different foliar fertilizers in different dosages and their combination (10 different treatments in 3 replications) on final yield and seed quality characteristics were investigated. Few initial observations on the field resulted in more vivid plants with more intense colouring, which could lead to the expectation of higher harvested yield. There was not found any statistically significant difference on seed yield, where control reached almost the 4500 kg ha^{-1}and 6750 kg ha^{-1} for the 3rd treatment (highest). On the other hand, there was found statistically significant differences for the protein content with control reaching the 13.3% and the higher 17.37 reached at the 9th treatment. Due to the fact that the above results were found through the first year of experimentation, safer conclusions expected to arise after the repetition of the experiments in the same place for a second year.

Keywords: *Wheat, seed yield, protein, foliar fertilizers, cereals.*

Introduction

Durum wheat (*Triticum durum*) is one of the most important winter cereals, occupying more than 25 million hectares worldwide. The Mediterranean basin produces about 60% of the world production, and has the ideal growing conditions for producing quality grains (Nachit, 1998), while the Mediterranean countries (Italy, Greece and Spain) together account for 82% of total EU production, but grain yields are lower than those obtained in Northern European countries (European Commission, 2002). The rest is mainly produced in the USA and Canada (Belaid, 2000).

The Mediterranean agricultural region comprises the lands surrounding the Mediterranean Sea: the southern strip of Europe, northern lands of Africa and west Asia. This region is characterized by relatively cold and wet winters, dry and hot spring-summers (Aschmann, 1973) while the soils have typically low fertility (Ryan et al., 2009). Rainfall seasonality restricts agriculture to winter-spring crops and the amount and variation of rainfall drives relatively low and highly variable yield.

Quality of durum wheat is generally dependent on the protein content of the grain, at least in the range of protein percentages encountered in commercial wheat cultivars (Grant and Flaten, 1998; Anderson, 2000; Clarke, 2001). By carefully managing N fertilization, less N

may be needed while grain wheat yields and protein may be maintained or increased (Chaney, 1990; Alcoz et al., 1993; Gate, 1995; Grant and Flaten, 1998).
Matching N supply with crop demand is important to optimize fertilizer uptake, and utilization and retention in the cropping system. Adequate N fertilization is a prerequisite to produce high yields of wheat and to increase grain quality, though depending on the background fertility level. High levels of protein are important for superior durum wheat flour (Feil, 1997).
This study was conducted in the main agricultural plain (Thessaly) to evaluate the effects of three different foliar fertilizers in different dosages and their combination on final yield and seed quality characteristics in Greece.

Material and Methods

For the purpose of the study, a field experiment was established in a typical soil-climatic environment of Thessaly plain, in central Greece. The experimental site is located at the Experimental Farm of the General Department of the University of Thessaly, (ex Technological Educational Institute of Thessaly; Larissa plain, coordinates: latitude 39°62′69″ N, longitude 22°38′14″ E). The selected durum wheat variety was the "Meridiano", in order to assess the effect of different foliar fertilization scenarios in its performance, due to the fact that wheat is the most prevalent winter cereal in Greece.

Field management and experimental design.

Sowing was occurred using a modern cereal seeding machine (on November 2018), applying 225 kg ha^{-1} of seeds in a row-distance of 12.5 cm.
Basic fertilization applied one-two days before sowing using a dispenser and then the fertilizer was incorporated using a rotary cultivator. There was performed post-emergence herbicide application to control weeds.
The experimental design was a completely randomized design with ten foliar fertilization treatments and three replications (blocks), presented in Figure 1. Specifically:
Treatment 1: control, only basic fertilization (250 kg ha^{-1} of 20-10-0).
Treatment 2: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Tidal (2000cc/ha, in total) applied with one dose on 1st of April.
Treatment 3: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Zinctip plus (2000 cc/ha, in total) applied with one dose on 1st of April.
Treatment 4: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Miconic (2000 cc/ha, in total) applied with one dose on 1st of April.
Treatment 5: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Tidal (3000 cc/ha, in total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.
Treatment 6: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Zinctip plus (3000 cc/ha, in total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.
Treatment 7: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Miconic (3000 cc/ha, in total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.
Treatment 8: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Miconic (2000 cc/ha, in total) + Bumper (1000 cc/ha, in total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.
Treatment 9: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Zinctip plus (2000 cc/ha, in Total) + Bumper (1000 cc/ha, in total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.

Treatment 10: basic fertilization (250 kg ha^{-1} of 22-10-0), and foliar fertilization with Zinctip plus (2000 cc/ha, in total) + Miconic (2000 cc/ha, in Total) applied with two doses, half dose at the first on April 1st and the other half at the second on April 16th.

Figure 1. Field experimental design.

Laboratory measurements and data analyses
Soil analysis

Soil samples were analyzed using the following methods which are referred by Page et al. 1982.

Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

Inorganic forms of nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) were extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy. Exchangeable forms of potassium were extracted with 1 mol L^{-1} $CH3COONH_4$ and measured by flame Photometer (Essex, UK).Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylenetriaminepentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. In the case of the determination of total metals Mn, Cu and Zn, 1 g of wet material, was digested at 350 ^{o}C + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by (Allen et al., 1974 and Varian, 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture.

Plant Measures

Total biomass and seed yield measured by a final sampling (final harvest; according to Zadocks scale: ripening stage 91-99). To avoid any border effect, 1 m^2 in the inner plot was harvested in each sampling above the ground. The samples were weighed at the field and then a sub-sample was taken for further laboratory measurements.

Protein, starch and gluten content was determined in dry seeds of each sample by near-infrared reflectance (NIR) spectroscopy technique using the DA 7250 NIR analyzer (Perten Instruments, Hägersten, Sweden).

The statistical package GenStat (7^{th} Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982).

Results and Discussion

Soil characteristics

The soil of the experimental site is characterized as a, calcareous (pH = 7.8), withlow organic matter content (0.9) and low salinity (0.11) at a depth of 30cm. Analytically, the soil properties/characteristics of the experimental field are shown in Table 1.

Table 1. Chemical properties of the field experiment.

Property	Soil depth (0-25) cm
Texture	Loam
pH (1part soil:5parts H_2O)	7.81 ± 0.16
Electrical conductivity, extract(dSm^{-1}) (1part soil:5parts H_2O)	0.11 ± 0.01
Organic matter (%)	0.93 ± 0.05
N-inorganic (mg kg^{-1})	44.8 ± 4.07
K-exchangeable (mg kg^{-1})	373.3 ± 7.45
P –Olsen (mg kg^{-1})	13.1 ± 1.87
$CaCO_3$ (%)	0.63 ± 0.07

Data represent average means and SE deviation. (n)=4

Yield

The results have shown none statistical significant differences between the tested foliar fertilization practices but only numerically superiority of few treatments (Table 2), especially in seed yield case which is the product of economic value.

Analytically, the treatments that treatments with the higher seed yield production were treatment 3 and 6 with seed yield of 4744 and 6048 kg ha^{-1}, respectively. This yield is 1500 – 2000 kg ha^{-1} higher in comparison with the control (treatment 1). The highlight of this is that both are Zinc tip plus treatments using different dosages. Therefore, even if there is not statistically significant difference the treatment 3 with the lower dosage and the use of only one spraying shows higher productivity.

Table 2. Biomass, hay and seed yield of *Triticum durum* under different foliar fertilization practices and its combinations.

Treatments\Characteristics	Biomass	Hay	Seed Yield
	kgha^{-1}	kg ha^{-1}	kg ha^{-1}
Treatment 1	11400	6920	4480
Treatment 2	8480	3885	4595
Treatment 3	12533	5789	6744
Treatment 4	11200	6487	4713
Treatment 5	11247	6837	4410
Treatment 6	10753	4705	6048
Treatment 7	11640	6328	5312
Treatment 8	9100	4808	4292
Treatment 9	10033	5622	4411
Treatment 10	11360	6392	4968
$LSD_{0.05}$	ns	ns	ns
CV (%)	13.2	19.4	23.8

Seed quality characteristics

Unlike the quantitative characteristics of wheat where there was no statistically significant difference, the quality characteristics are statistically significant different. Particularly there was found differences in protein and starch content, while in gluten content (dry and wet) there was not observed any statistically difference.

The results are presented in Table 3, where it is shown that the higher protein content was measured in treatment 9 reaching the 17.3 %. Other treatments with high protein content (> 15 %) were the 6th, 8th and 10th. The particular feature of these treatments is the fact of using double spraying with duration between the two sprays about fifteen days. Once again, it is noticed that the use of the commercial formulation Zinc tip plus (in this case, in combination with other commercial formulation) gives better results, where then protein content is higher compared with the control (which has the lower protein content) more than 2 %.

On the other hand, in case of starch content, the control is the treatment with the higher content indicating once again that seeds of higher protein content have lower starch content. It has been mentioned that proteins can have some obvious effects on enzymatic degradation of starch, such as by physically hindering access of degradation enzymes to starch (Zou et al. 2015). In a study for the barley by Wenwen et al. (2017), it was concluded that the protein content significantly and negatively correlated with starch molecular structures and therefore barley with lower protein content would have higher starch content and larger grain size, which is in agreement with our findings.

Finally, in gluten case, there was found none statistical significant differences between the tested foliar fertilization practices but only numerically superiority of few treatments (Table 3).

Analytically, the treatment with the higher gluten content was the treatment 9, which is the treatment that also gave seeds with higher protein content. In this case there was found that the wet and dry gluten content was 33.15 and 12.91 %, respectively. All treatments of lower protein content had also lower gluten content, indicating once again the effect of the commercial formulation Zinc tip plus to the quality seed characteristics (Table 3).

Table 3. Protein, starch and gluten % content of *Triticum durum* seeds under different foliar fertilization practices and its combinations.

Treatments\Characteristics	**Protein As is**	**Protein Dry basis**	**Starch Dry basis**	**Dry Gluten**	**Wet Gluten**
	%	**%**	**%**	**%**	**%**
Treatment 1	11.947	13.300	73.267	10.80	27.72
Treatment 2	12.553	13.907	72.433	10.51	26.41
Treatment 3	13.147	14.407	70.700	13.09	33.32
Treatment 4	12.213	13.507	70.633	11.32	28.64
Treatment 5	15.040	16.733	69.633	11.71	30.43
Treatment 6	14.263	15.800	70.867	12.41	31.67
Treatment 7	12.287	13.700	72.800	10.45	26.37
Treatment 8	13.783	15.367	71.433	12.71	32.37
Treatment 9	15.637	17.367	70.167	12.91	33.15
Treatment 10	14.167	15.767	71.333	12.41	31.73
$LSD_{0.05}$	0.1157	0.1972	0.3355	ns	ns
CV (%)	0.5	0.8	0.3	12.3	12.8

Conclusions

In all characteristics there was found an effect of the treatments using of the commercial formulation Zinc tip plus. In the quantity characteristics there were not found statistically significant differences but only numerically superiority. In case of the quality characteristics there found statistically significant differences with protein content achieving high values. Due to the fact that the above results were found through the first year of experimentation, safer conclusions expected to arise after the repetition of the experiments in the same place for a second and a third year. In case that the results of the third year will confirm the previous results then foliar fertilization practice with the commercial formulation Zinc tip plus should be proposed in future fertilization schemes, while an economic appraisal of interventions should be made to conduct safer conclusions.

Acknowledgments

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THE EFFECT OF LIMING ON THE ALUMINIUM CONTENT IN A WHEAT ROOT

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Abstract

Numerous chemical factors limit normal growth of plants on acid soils. On soils with a pH ≤ 5.5, Al toxicity is a main stress factor for plants, and it is reflected through the inhibition of root growth, a numerous of damages above-ground plant organs, as well as on decreasing of solubility of important biogenic elements. Liming is one of the key measures that can maintain or increase the productivity of acidic soils. Research in the field trials, were conducted on soil type Dystric cambisol. The objective was to determine the reasonable amount of lime material required, in order to mobile Al content in the soil type Dystric cambisol to bring below the hazardous level. The aim was to determine the Al content in the roots of wheat plants, as well as plant's response to the reduction of content of this element in the soil. The three doses of hydrated lime (CaO x H_2O) have been applied in order to reduce acidity: the two were on the level of partial liming (1/3 Y_1 CaO and 1/2 Y_1 CaO), and the one as full liming (Y_1 CaO). Even in the partial liming, response of soil and wheat plants has been very strong. Compared to variants where no lime applied, by partial liming content of aluminum in the soil has been halved, and in the roots of wheat during the whole vegetation it has been multiply decreased.

Keywords: *liming, aluminium, Dystric cambisol, wheat, root*

Introduction

At acid soils with pH ≤ 5.5 Al-toxicity is the main stress factor for plants (Merino-Gergichevich et al., 2010), and in such conditions it is linked to the prevailing pressure for adaptation of cultivated plants (Ryan and Delhaize, 2010). First, its indirect impact by reduction of solubility is being noticed, as well as by availability of nutrients. Namely, aluminum ions, among other things, block the adsorption of phosphorus and potassium, and cause disturbing crop growth and development (Zheng, 2010). In addition there are indirect and direct effects, when Al ions act toxic to the plant. The acidic environment increases presence of trivalent aluminum cations - Al^{3+} (Lidon and Barreiro, 2002; Kochian et al., 2005), which is the most toxic of all types Al. The best recognized Al-toxicity effects, have been noticed and well described, in the roots (Barceló and Poschenrider, 2002, Ma, 2007; Panda and Matsumoto, 2007). A serious problem that appears at high concentrations of aluminum is the inhibition of root growth. It has been noticed that aluminum influences on the formation of immature and poor root systems, which have limited ability to adopt mineral nutrients and also increase the risk of stress caused by drought (Marschner, 1991). More precise, toxic effect is being caused by the inhibition of lateral roots and root hairs, interruption of feeding with P and Ca and inhibition of growth of shoots (Fageria et al., 1988). However, the upper parts of the plants may be damaged (Merino-Gergichevich et al., 2010), especially leaves, and about which is rarely known. Today, there are more evidences of the negative effects of aluminum on the light absorption, photosynthetic electron transport, gas exchange (Chen et al., 2005a, Chen et al., 2005b; Chen, 2006), photoprotective systems (Chen et al., 2005a; Ali et al., 2008), pigments (Chen et al., 2005a; Mihailovic et al., 2008;

Milivojević et al., 2000), as well as other elements associated with the structure or function of the photosynthetic apparatus. Aluminium toxicity in acid soils negatively impacts the production of staple food crops, particulary grain crops (Pineros et al., 2005). Calcination of acid soils is one of the key factors which can keep or even improve their productivity (Mao et al., 2008; Repšiene and Skuodiene, 2010). Therefore, this research aim is to investigate use of different levels of calcification in an acid soils, such as the Distric Cambisols, defines its effect on the degree of change on acidity and content of mobile Al in the soil and wheat roots.

Material and Methods

Studies were conducted on the soil type Dystric Cambisol near Leposavić municipality (43^0 16' N; 20^0 36' E) during two consecutive years (2010 and 2011). Leposavić belonging to the southern and central part of the Ibar-Kopaonik region, and located in southwestern Serbia and is located at an altitude of 545 m, and is characterized by a temperate continental climate.

Test crop was wheat cultivar Pobeda. For calcification has been used CaO with high degree of purity. The material is applied in both years in September, precisely before primary treatment, so it has been properly distributed over the surface and entered into the soil by plowing.

The quantities of CaO which have been applied, were calculated according to the value Y_1 in the soil and the size of the experimental plots (50 m^2). Three variants of calcification have been determined and applied: 1/3 Y_1 CaO (V-3), 1/2 Y_1 CaO (V-4) and Y_1 CaO (V-5), and two variants without calcification: variant with NPK (V-2) and the variant without liming and fertilization - control (V-1). Fertilizers were applied with the variant performed with calcification. In all cases, the dose of active nutrients: nitrogen, phosphorus and potassium were the 120 kg N/ha for P_2O_5 and K_2O, 90 kg/ha ($N_{120}P_{90}K_{90}$).

The experiment has been performed as a randomized complete block design (RCBD) with four replications. In both years, during the stage of wheat tillering (TL), 5 months after liming and after the harvest (AH), 10 months after liming, the pH was determined in 1M KCl using a pH meter, and exchangeable Al or cell by the method of Sokolov in soil extract with 1M KCl, so it has been firstly determined by the total substitutional acidity, and then precipitation of Al with NaF and influence of Al-ions in forming of substitutional acidity. The content of Aluminum has been determinate in the average samples of wheat roots during the phenophasis of tillering (TL) as well as the full maturity (FM) on the atomic absorption spectrophotometer (AAS). Data were analyzed using standard statistical methods of analysis of variance (ANOVA) using Microsoft Excel 2007 and Statistical Program 5.0. Data analysis has been used to interpret the results and draw conclusions.

Results and Discussion

The effect of three levels of liming on active, substitutional, and hydrolytic acidity has been in accordance with the applied dose of CaO (Table 1), the highest in the variants with complete liming (V-5), and the lowest in partial liming (V-3). The soil quickly reacted to entered CaO, and changes were obvious and complete already at the first check, 5 months after liming, i.e. in the stage of wheat tillering. The differences between the treatments where CaO has not been used (V-1 and V-2) and the treatments in which CaO has been used (V-3, V-4, and V-5) are highly significant. The differences between the variants in which liming has been performed can clearly be observed. All the differences are highly significant ($p < 0.01$), except for the changes in active acidity in the tillering stage (T) in 2010, when a difference in the level of statistical significance ($p < 0.05$) has been found between the first (V-3) and the second level (V-4) of partial liming.

Table 1. The change in pH (H_2O and KCl) and Y1 after liming

Variants	pH H_2O				pH KCl				Y1	
	2010		2011		2010		2011		2010	2011
	T	AH	T	AH	T	AH	T	AH		
V-1	5.45	5.43	5.42	5.50	4.83	4.83	4.84	4,83	16.32	14.12
V-2	5.50	5.51	5.41	5.47	4.75	4.79	4.79	4.82	14.32	14.56
V-3	5.92	5.89	5.90	5.84	5.23	5.30	5.29	5.24	7.76	7.66
V-4	6.17	6.15	6.18	6.14	5.57	5.56	5.60	5.58	5.21	5.38
V-5	6.79	6.77	6.87	6.73	6.24	6.20	6.24	6.19	3.00	3.45
Lsd 0.05	0.24	0.08	0.10	0.10	0.12	0.10	0.14	0.09	1.69	1.56
Lsd 0.01	0.35	0.12	0.14	0.14	0.17	0.14	0.20	0.12	2.43	2.25

The resulting changes, both in active and substitutional acidity, are in favor of the claims of the need for liming acid soils (Busari et al., 2008; Jelić et al., 2011; Mao et al., 2008), in order to perform the neutralization and create favorable conditions for smooth growth and development of plants. It particularly refers to a group of plants that are insufficiently tolerant to soil acidity.

The content of mobile Al^{3+}, in both years of research, was strongly changed in all the variants where CaO was applied (Table 2). Thus, trace aluminum content was found in the first measuring, in the stage of wheat tillering, in the complete liming variant. According to the results of other authors, liming had the same effect on other soil types, such as pseudogley (Jelić et al., 2011; Pivić et al., 2011).

Table 2. The changes in mobile Al content (mg · 100 g^{-1}) after liming

Variant	2010.		2011.	
	T	AH	T	AH
V-1	12.28	12.21	13.62	13.80
V-2	12.32	12.54	13.92	13.87
V-3	5.26	5.56	6.25	6.39
V-4	2.17	2.37	2.44	2.62
V-5	0.48	0.40	0.47	0.42
Lsd 0.05	0.35	0.28	0.35	0.07
Lsd 0,01	0.50	0.40	0.50	0.10

At the same time, also in the variants of partial (V-3) and particularly half-liming, the content of mobile aluminum was reduced to a level at which the risk of its toxic effect on the crops was significantly reduced. In V-3 and V-4 variants, a slight increase in the content of mobile Al was found during the growing season.

The reaction of the plant versus to lower content of Al in the soil in which liming has been applied, fully veryfy this measure. Namely, starting from partial and endin with full liming, it has been recorded constant decrease of Al content, in teh wheat roots (Table 3.).

Table 3. Changes of aluminium content in wheat roots (mg · 100 g^{-1}) after liming

Variant	2010.		2011.	
	T	AH	T	AH
V-1	54.00	64.50	55.00	66.75
V-2	55.50	64.00	55.00	65.00
V-3	43.50	42.00	44.50	44.50
V-4	26.00	25.75	24.00	25.25
V-5	21.25	21.25	20.75	21.25
Lsd 0.05	4.52	4.71	3.56	3.32
Lsd 0,01	6.25	6.51	4.92	4.60

By full liming, content of aluminum has been reduced at the normal level. The similar effect has been achieved at the variant with half-liming, what can completely recommend this level of the applied lime materials. A positive influence of liming materials on the Al content at other grains has been noted by other authors as well, like in the rotts and lieves of oats. (Djuric et al., 2011) and above land wheat mass (Pivić, et al, 2011).

Since the problem of acidity cannot be definitively resolved, it is completely acceptable regular application of lime material that would soil acidity maintain at an acceptable level (Garscho and Parker, 2001). In this way benefits would be multilateral. Smaller quantities calcification materials would be on one side, economically viable, and on the other side can ensure better availability of nutrients, as well as a more favorable environment for the growth and development of crops.

Conclusions

The degree of reduction in content of mobile Al with partial (1/3 Y_1) and in half calcification (1/2 Y_1) justify and affirm these levels in calcification of acid soil. Main benefits of lower levels of calcification were lower investments in calcification material, and reducing the content of mobile Al Tha plants response was in the reduction of Al content in the values which are usual in the lower level of calcification. By this it is appearing the imperative that further work should be targeted toward the defining of more precise, and economic reasonable quantities of lime materials lower than complete quantities for neutralization of acid soils, which can significantly improve soil conditions for unpended plant growth.

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DETERMINATION OF VITAMIN C CONTENT IN DIFFERENT EXTRACTS OF THE *ALCHEMILLA VULGARIS* L.

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Abstract

In recent years, researchers are interested and focused on the identification of bioactive components in plants and food that affects the health, and may also reduce the risk of some diseases. The research of bioactive components, includes very extensive studies both in conventional breeding and biotechnological researches, with special reference to the possibility to increase their content. *Alchemilla vulgaris* L. is a plant from the *Rosaceae* family. Recent scientific research has shown that the source prevents the growth of many types of bacteria including staphylococci - a bacterium that has become resistant to many antibiotics. Within the experimental part of this final work, the following analyzes were performed: preparation of macerates, extraction of samples in the Soxlet apparatus, ultrasonic extraction and determining the content of vitamin C in the extracts obtained. In determining the content of vitamin C, we noticed that the highest content of this vitamin, determined in the extract obtained by maceration (9.75 mg / 100 g), was slightly lower in ultrasonic extraction (7.50 mg / 100 g), and the smallest content in Soxhlet- of this extract (3.45 mg / 100 g).

Keywords: *Alchemilla vulgaris* L, *maceration, ultrasonic extraction, extraction, vitamin C.*

Introduction

Vitamin C (ascorbic acid) is, by chemical structure,Figure 1., lactone close to L-glucose. It is technically obtained starting from D-glucose, which reduces to D-sorbitol by reduction, and by the action of enzymes of special microorganisms it passes into the L-Sorbose, which with HNO_3 produces 2-keto-L-gluconic acid, the methylester is treated with $NaOC_2H_5$ and then hydrolysed in Vitamin C is formed in the acidic environment. (Duh *et all.*, 1999).

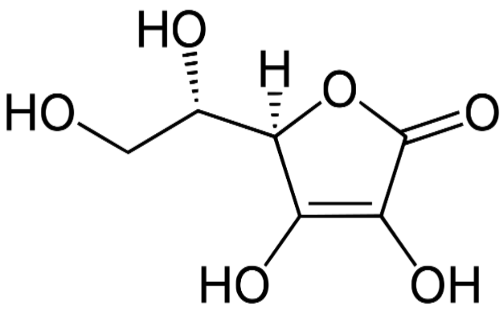

Figure 1. Vitamin C structure

Vitamin C is involved in the construction of cyclic amino acids, steroid hydroxylation, folic acid hydration in tetrahydrofuran, increases breathing intensity, activates many enzymes and improves the acquisition of microelements. Oxidation of L-ascorbic acid first produces monohydroascorbic acid, followed by dehydroascorbic acid. Both have vitamin activity, because they can be converted to ascorbic acid by reduction. L-ascorbic acid is of utmost importance in the metabolism of humans and animals (Tandon *et.*, 1995).

In vitamin C plants is 95% in reduced form, and only 5% is in the oxidized form as dehydroascorbic acid. It is important for the synthesis of collagen and carnitine. Also, the role of vitamin S in the creation of collagen is important for the regeneration of tissue, blood vessels, bones and teeth. The most important group of herbal preparations are extracts, which are obtained by applying different extraction methods, ranging from simpler technologies to advanced techniques. Extraction is the separation and concentration of certain constituents of plant and animal tissues by selective solvents using standard procedures. Depending on consistency, the extracts are divided into liquid, semi-solid and solid. Herbal extracts are obtained by crushing, mainly dry, parts of the plant into contact with the extraction solvent in the appropriate device, the extractor (Daker *et al*.,2008). Extraction material can be: plant, animal and mineral origin. The rate of extraction is influenced by: the size of the contact surface of the solvent and the particulate matter, the thickness of the boundary layer around the particle, and the temperature of the system. In organic laboratories, organic compounds are usually extracted from aqueous solutions using an organic solvent, which is not mixed with water or mixed in part, and two layers are formed. This technique is known as liquid-liquid extraction (Yan *et al.*, 2006).

Material and Methods

As a material in this final work, a virak plant (*Alchemilla vulgaris* L.) was used. The plant material originates from the locality of Cacak, Moravica District, acquired in November 2017. Extracts were obtained from dry plants. In Figure 4, the plant material used for the analysis is shown.All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). *Alchemilla vulgaris* L. is a plant from the Rosaceae family. This is a perennial herbaceous plant up to 50 cm high. Blooms from June to September. Ground leaves in the rosette from which the tree grows, more or less covered with hair. The rounded-kernel leaves, starched on the 7-11 lobes, along the entire periphery, are bent or chopped. The stem is upright, developing laterally on the rosette. The leaves on the stem are shaped like rosette leaves, but they are smaller. The flowers are small, yellow-green, naked, without crocheted leaves. The fruit of virka is nuts. The bloom is a wide broom. Three measurements of the fragmented sample were performed. Samples were dried in a drying oven at the prescribed temperature (at 105 ºC) under atmospheric pressure to constant mass. The height of the applied temperature depends on the type of sample whose water content is determined (Hsu *et al*.,2008). Before the samples are put on drying, it is necessary to dry the veggel with a lid in the dryer to a constant mass (at least 1 hour) at the prescribed temperature (105 ºC). The veggle is placed on the test sample and measured. Then it is put on drying in the dryer. During the drying of the vegeglas with the pattern, it must be opened, i.e. The lid is located next to it during drying. After drying, the vegeglas is cooled in a desiccator, and then measured.

Extraction by maceration

The extraction is the uniform separation of one or more constituents of a solid or liquid mixture (starting material) with another solvent which is not mixed or mixed with the solvent of the initial mixture, and the other ingredients are not soluble or less soluble in it.

The milled and homogenized sample (5 g), was poured with a solvent (200 mL 96% ethanol), then left in a closed, protected enamel mist. Maceration is done for five days, with shaking every day twice a day. After five days, the plant material from the maceration was cut through the gauze, and after and through the filter paper, black strip. The solvent is removed by evaporation on an aqueous bath, and the resulting extract is dried to a constant weight at a temperature of 50 ° C (Merken H. M. and Beecher G. R., 2000).

Extraction by Soxlet

The best-known apparatus for the continuous extraction of solids is the descendant of Soklet. Socklet extraction takes place by placing the starting material in the cauldron. The pattern with the sample is then inserted into the middle part of the extractor which is connected to the refrigerator and the balloon. The balloon was pre-dried for 1 hour at 105 ºC and measured on an analytical scale. Using a small funnel on the upper side of the condenser, so much solvent was poured into the apparatus that the extractor was filled and poured into the balloon. Further solvents (96% ethanol) are then added, ensuring that the total amount of solvent does not exceed more than ¾ of the volume of the balloon.

Ultrasonic extraction

Ultrasonic extraction is performed in an ultrasonic water bath (EUP540A, Euinstruments, France). The sample (5 g) was placed in a balloon and poured with 200 mL of 96% ethanol. The mixture was extracted for 30 minutes at a frequency of 40 kHz and ultrasound effect 90% (216 W), (Macheix, J.-J. and Fleuriet, A., 1998). For the quantitative determination of vitamin C, the Tillmans method (Tillmans), based on oxidimetric titration, is used in which L-ascorbic acid is oxidized to dehydroascorbic, while simultaneously reducing the reagent used. Titration with 2,6-dichlorophenolinophenol, i.e. The Tilmans Reagent (TR) is carried out in an acidic medium at pH 4-6. The oxidized form of the Tilmans reagent solution (which also has the role of the indicator) has a dark blue color (at pH 5,2), while in the presence of ascorbic acid, TR passes into its reduced, leuky form.

Calculation

The content of ascorbic acid (in mg / 10 g of extract) = ((V - Vsp) * s * 100) / Val,

V - mean value of the volume of TR solution used for the titration of the test probe (mL),

Vsp - the mean value of the volume of the TR solution consumed for the titration of the blank (mL),

c - titre of TR solution (mg $C_6H_8O_6$ / 1 mL TR solution)

Val - the volume of the aliquot part of the sample (mL).

Results and Discussion

Content of total extracted matter

After completed extractions, the extraction of the obtained herbal extracts to dryness was carried out, and then the measurement of the obtained residues was obtained. The yield of the extraction is calculated. From 5 g of plant material of virka (*Alchemilla vulgaris* L.)
The results obtained in percentages are shown in Table 1.

Table 1. Percentage yield extractions

Sample	Maceration	Soxhlet extraction	Ultrasonic extraction
herbal drug *Alchemilla vulgaris* L.	9.45%	4.72%	32.54%

Based on the results obtained, we can conclude that the smallest yield was obtained by Soxlet extraction, followed by maceration, and the highest yield was obtained by ultrasonic extraction. The method of ultrasonic extraction proved to be the most optimal method for this plant species, because it shortly lasts for maceration and takes place at a lower temperature, which is not the case with the Soxlet method, which occurs at high temperature, and it is assumed that there has been degradation of vitamins and other thermolable compounds.

Table 2. Vitamin C content

Type of extraction	Vitamin C mg / 100 g
Maceration	9.75
Soxhlet extraction	3.45
Ultrasonic extraction	7.50

In determining the content of vitamin C, Table 2, we concluded that the highest content of this vitamin is determined by maceration (9.75 mg / 100 g), followed by ultrasound (7.5 mg / 100 g) and the smallest in Soxhlet's extract (3.45 mg / 100g). We assume that maceration as an extraction method has proved to be the most optimal, with the highest yield of vitamin C, because the isolation was carried out at room temperature, and we know that vitamin C is thermolabile.

Conclusions

The smallest extraction yield was obtained by Soxlet extraction, slightly higher maceration, and the highest yield was obtained by ultrasonic extraction. In determining the content of vitamin C, we noticed that the highest content of this vitamin, determined in the extract obtained by maceration (9.75 mg / 100 g), is slightly lower in ultrasonic extraction (7.50 mg / 100 g), and the smallest content in Soxhlet extract (3.45 mg / 100 g). The maceration extraction method proved to be the most optimal and with the highest yield of vitamin C, since the isolation was carried out at room temperature, bearing in mind the fact that vitamin C is thermolabile.

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GERMINATION OF CAPPARIS SPINOSA L. SEEDS UNDER DIFFERENT DORMANCY BREAKING TREATMENTS

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Abstract

Capparis spinosa is a spiny, evergreen shrub, that grows to 1 m tall, spreading by semi-prostrate branching to 2 - 3 m wide. It is native mostly to Mediterranean coastal regions, growing on sandy or rocky soils, stone walls and rock crevices. Its unopened flower buds are edible and used as a flavouring in sauces or salads. Also, caper bush is an ornamental and drough tolerant species that is suitable for landscape use. Generative propagation of this species is difficult due to its seed coat dormancy, followed by embryo dormancy that develops after drying. The aim of this study was to investigate the effect of different seed treatments on dormancy breaking. The seeds collected in Perast, Montenegro at the end of summer, were stored for six months before setting the experiment. The treatments included: soaking seeds in sulfuric acid at 96 % for 15 or 30 minutes, mechanical scarification, hot water (40ºC) treatment, 3 months of cold stratification at 3-5^{o}C in perlite, treatment with 0.2% KNO_3. The obtained results showed that KNO_3 has negative impact on caper bush germination, and in all combined treatments, the germination rate was lower with the addition of KNO_3. The mechanical scarification showed the best germination rate (36%), but it is necessary to conduct the additional research in order to improve germination of dried caper bush seeds.

Key words: *Caper bush, stratification, sulfuric acid, embryo dormancy, scarification.*

Introduction

Capparis spinosa L. (caper bush) is a xerophilous, evergreen shrub that typically grows to 1 m tall. Its branches are semi-prostrate and the width of a shrub is 2 - 3 m. The native range is wide, including Mediterranean, southwestern Asia, Black Sea coast (Crimea and Armenia), east side of the Caspian Sea, Iran, Afghanistan, extending eastward to the Himalayas, Pacific Islands and northern Australia (Miras-Avalos and Baveye, 2018; Lansky et al. 2013; Chedraoui et al. 2017). The stems are spiny, with small, round, evergreen leaves, 5 cm long. The flowers develop from the leaf axils, with white petals and pale purple stamens. Diameter of flowers is 5 - 8 cm. *C. spinosa* blooms from spring to early autumn, but single flower lasts only one day. The fruits, known as the edible caperberries, are 1 - 2 cm long and they contain numerous and reddish-brown seeds (Lansky et al. 2013; Chedraoui et al. 2017).
Caper bush is grown as an ornamental species due to its decorative and sweet-scented flowers. It can endure drought, sun, and warm climate, and it can be grown on sandy and rocky soils, in a poor soils or even on stone walls and rock crevices (Miras-Avalos and Baveye, 2018). It is also valuable medicinal and edible species. The unopened flower buds are used as a spice, usually salted or pickled in vinegar (Lansky et al. 2013).
C. spinosa is an important plant for growing in the changing climate conditions due to its resistance to environmental stresses and it can be grown even on areas affected by hyper-aridity (Miras-Avalos and Baveye, 2018). Caper bush has an extensive root system which is very effective for water retention during rainfalls, additionally it has a very high root/stem ratio and capability for a high water use efficiency, absorbing water particularly in soil depths (Chedraoui et al. 2017). Also, it is used for the prevention and control of soil erosion in sloppy areas (Khalil et al. 2009). Caper bush tolerates salty or calcareous soils, low amount of

organic matter in soil and it successfully grows in saline and halophytic habitats (Chedraoui et al. 2017). It belongs to climate zones 8 - 10, but in accordance with the recent climate changes, many species belonging to zone 8 now are successfully grown in Serbia, in the warm and protected areas.

Caper bush can be propagated vegetatively, by hardwood, semi-hardwood or softwood cuttings, but rooting percentage is mostly low (Miras-Avalos and Baveye, 2018). Generative propagation of this species is difficult due to its seed coat dormancy, followed by embryo dormancy that develops after storage. Also, seed viability is low, about 2 years. For this reason, different combined seed pretreatments are used in order to overcome its dormancy, including mechanical scarification, the use of concentrated sulfuric acid (H_2SO_4), followed by KNO_3, gibberellins and different cold stratification pretreatments (Bahrani et al. 2008; Khalil et al. 2009; Labbafi et al. 2018; Miras-Avalos and Baveye 2018; Orphanos 1983; Basbag et al. 2009). However, the plants used in published studies of caper bush germination originated from different areas, and the obtained results varied considerably. For this reason, authors decided to evaluate the effect of different seed treatments on dormancy breaking of seeds collected from the elite genotype grown in neighbouring country Montenegro.

Material and Methods

The fruits were collected in Perast, Montenegro, at the end of summer, in 2016. Experiment was conducted in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia). Dimensions and weight of fruits were measured as well as weight of seeds. This data was used for calculation of an extraction factor (the weight of cleaned seeds per given weight of fruits, expressed in percent), number of seeds in 1 kg and absolute weight of seeds (weight of 1000 seeds). The obtained seeds were kept in dry conditions, at room temperature, for 6 months.

The following treatment were used for a seed coat dormancy breaking:

- **chemical scarification treatment**

The seeds were soaked in a concentrated (95%) sulfuric acid (H_2SO_4) for 15 or 30 minutes, then carefully rinsed using a running water and disinfected by 0.8% solution of the fungicide benomyl *(*benlate).

- **mechanical scarification of seed coat**

The seed coat was cut on side opposite from micropyle so the radicle was not damaged, using a sharp scalpel blade.

- **soaking in a warm water**

The seeds were soaked in a warm water (40ºC) for 24 hours, followed by a disinfection using 0.8% solution of the fungicide benomyl.

The following treatment were used for embryo dormancy breaking:

- **treatment with potassium nitrate**

The seeds were treated with 0.2% KNO_3 (potassium nitrate) during germination test.

- **cold stratification**

The seeds were disinfected by 0.8% solution of the fungicide benomyl and stratified in perlite at the temperature 2 - 5ºC, for 3 months.

Each experiment consisted of four replicates with 50 seeds each. The seeds were placed on the top of two layers of filter paper in the petri dishes, humidity was controlled daily by adding the distilled water if necessary. Temperature during germination was 25°C. Germination was carried out in long day conditions (light/dark period 16/8h). The number of germinated seeds was recorded daily during the period of 21 day. After that, the seeds were cut in order to determine the percentage of viable seeds which is necessary to calculate the real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić et al., 2010). The germination energy was recorded on 7th day. Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

Morphometric analysis

The seed was relatively large compared to some available data where number of seeds per gram was 140 - 160 (Gorini, 1981; Bahrani et al. 2008; Miras-Avalos and Baveye, 2018) while in our research the average number of seeds 116.67 per 1g (Table 1). Due to its large native distribution, the morphological differences among different wild populations of *C. spinosa* from different geographical regions are expected which is proved by many research studies that conducted a morphometric analysis of caper bush seeds (Saadaoui et al., 2011; 2013; Chedraoui et al., 2017).

Table 1 Morphometric parameters of collected fruits and seeds

Parameter	Mean ± SE
weight of fruit (g)	9.75 ± 1.16
length of fruit (cm)	4.31 ± 0.17
width of fruit (cm)	2.01 ± 0.08
length of peduncle (cm)	4.35 ± 0.13
number of seeds per fruit	186.67 ± 36.27
the weight of seeds per fruit	1.68 ± 0.41
extraction factor (%)	16.61 ± 2.72
number of seeds per 1 kg	116671.50 ± 6853.51
weight of 1000 seeds (g)	8.71 ± 0.49

Effect of different seed treatments on dormancy breaking

In the control treatment there were no germinated seeds which was expected due to combined seed coat and embryo dormancy of this species (Table 2). Similarly poor results were obtained after 3 months of stratification, following the warm water treatment (40ºC) conducted for 24 hours. Bahrani et al. (2008) used warm water pretreatment for 30 minutes at the temperature of 50ºC, and obtained germination percentage was also very low.

The other treatments for breaking seed coat dormancy proved favorable for germination, and 30 minutes treatment with concentrated H_2SO_4 proved better than 15 minutes treatment (Table 2). Bahrani et al. (2008) use concentrated H_2SO_4 for 15, 30 and 40 minutes, and the best results were obtained after 30 minutes of treatment. Following the H_2SO_4 treatment, Bahrani et al. (2008) treated seeds by different concentration of gibberellic acid (GA_3) and it had favorable effect, increasing germination percentage at all applied concentrations, although the best result was obtained with 200 mg/L GA_3. Labbafi et al. (2018) also reported high germination rate of seeds treated with concentrated H_2SO_4 for 30 minutes followed by GA_3 treatment (75.%). However, Olmez et al. (2004) obtained the highest germination rate (49.7%) of caper bush treating a seed with concentrated H_2SO_4 for 20 minutes, followed by 0.2% KNO_3 application, while the same treatment with concentrated H_2SO_4 followed by GA application gave lower germination (27.4%).

In our research, the negative impact of 0.2% KNO_3 on *C. spinosa* seed germination was recorded (Table 2), and germination is lower after addition of KNO_3 (13% and 16.50%) than without it (17.50%, 22%, respectively). Similarly, Labbafi et al. (2018) also recorded the negative impact of KNO_3 on caper bush germination, obtaining lower results than a control treatment. Khalil et al. (2019) reported the highest germination rate of caper bush with combination of 20 minutes H_2SO_4 treatment, followed by 0.04% GA_3 treatment and then one week of cold stratification at 4ºC

In this research, the highest germination rate (36%) was obtained with mechanical scarification of a seed coat (Table 2), but some other researchers reported low germination rate with mechanical scarification pretreatment (only 20%) compared to treatment with concentrated H_2SO_4 (43% for 30 minutes duration of treatment) (Bahrani et al. 2008).

Table 2 Germination of collected seeds

Treatment	Germination rate	Germination energy	Real germination rate
Control	0.00^{d}	0.00^{c}	0.00^{d}
0.2% KNO_3	0.00^{d}	0.00^{c}	0.00^{d}
24 h warm water + stratification	3.00^{d}	0.00^{c}	3.18^{d}
15 minutes H_2SO_4	17.50^{c}	9.50^{b}	18.18^{c}
30 minutes H_2SO_4	22.00^{bc}	13.50^{b}	23.15^{bc}
15 minutes H_2SO_4 + 0.2% KNO_3	13.00^{c}	7.00^{bc}	14.03^{c}
30 minutes H_2SO_4 + 0.2% KNO_3	16.50^{c}	12.00^{b}	17.14^{c}
mechanical scarification	36.00^{a}	27.00^{a}	36.72^{a}
mechanical scarification + 0.2% KNO_3	28.50^{ab}	23.00^{a}	29.08^{ab}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

There was no considerable difference between real germination (a percentage of viable seeds that germinated) rate and germination rate (Table 2) indicating that large percent of the tested seed was viable, but did not germinated. Miras-Avalos and Baveye (2018) indicated that seed will successfully germinate in 25 -50 days and we conducted germination test for 21 days. Also, caper bush seed viability is about 2 years when kept at 4°C and low relative humidity (Chedraoui et al., 2017) and we kept the seeds at room temperature, for 6 months.

Additionally, Khalil et al. (2009) reported that germination of caper bush seed was considerably higher in soli substrate (64%) compared to testing on filter paper (24%), after the same seed pretreatments.

Conclusion

Caper bush is a multipurpose plant, that can be grown as a medicinal plant, suitable for culinary uses, but it is also the important low-maintenance ornamental plant suitable for arid conditions and very adaptable in environmental changes. Its propagation is dificult due to seed coat and embryo dormancy, and germination percentage is usually low. Our reserach showed that selected elite genotype from Montenegro has large and viable seeds, but the further research is necessary to overcome dormancy. Additional treatments, including the effect of GA_3 treatments should be tested in order to improve germination rate of caper bush collected in Montenegro.

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NITROGEN FERTILIZATION AND SOWING DENSITY INFLUENCE ON WINTER WHEAT YIELD AND YIELD COMPONENTS

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Abstract

Nitrogen management in winter wheat is one of the most studied agricultural practices. Optimization of nitrogen nutrition and sowing density requirements of specific winter wheat cultivar are major objectives for improvement of trade-offs between grain yield, environmental sustainability and maximum profitable production. Therefore, the aim of this study was to assess the effects of interaction between nitrogen fertilization and sowing density on grain yield and yield determinants of modern wheat cultivars. The trial consisted of five winter wheat cultivars, four top-dressing nitrogen doses and four sowing densities was carried out under rain-fed conditions at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The analysis of variance showed statistically significant effects of all three factors on studied traits, while significance of interactions between studied treatments varied among traits. On average, grain yield between cultivars varied from 8.64 to 9.69 t ha^{-1}. Generally, the highest grain yield was achieved under conditions of 100 kg N ha^{-1} treatment. By increasing N fertilization thousand grain weight decreased almost linearly, while maximum grain number per square meter was recorded with 100 and 150 kg N ha^{-1}. The highest yield and grain number per square meter were obtained under increased sowing densities (700 and 900 viable seeds m^{-2}), while the thousand grain weight had lower variation and the highest values were realized with 300 and 900 viable seeds m^{-2}. In conclusion, the presence of significant interaction between cultivars, N fertilization and sowing densities, indicated necessity to adjust different management practices to each cultivar in order to achieve highest grain yield potential.

Keywords: *Triticum aestivum L., nitrogen fertilization, sowing density, yield traits*

Introduction

Wheat is one of the oldest and most important cereal crops, widely cultivated for its grain which is a worldwide staple food. In Serbia, the southern part of the Pannonian plain, wheat is the main winter cereal crop with a harvested area over 550,000 ha. Wheat production needs to continue to grow with increasing demands, and both aspects increasing the yields and sustainability represent major challenges (Hawkesford, 2014). Production systems in the world vary greatly, depending on climatic and soil fertility factors. The agricultural areas of the Pannonian basin are characterized by a relatively short growing season, winter frosts, occasional spring heats and frequent drought stresses at the end of the grain filling period, which influences significant grain yields variations of many cereal crops across different growing seasons (Mirosavljević et al., 2018). For all agricultural systems, especially in areas of higher production there is a constant need for adequate amounts of nutrients, mostly supplied as fertilizers. Efficient nitrogen fertilization is one of the key elements for economical wheat production. Moreover, nitrogen is the most limiting nutrient for wheat production that affects grain yield and biomass production, as well as establishment of high grain weight and grain number per unit area (Lawlor et al., 2001). Adequate nitrogen use is also important in order to protect underground and surface water from pollution caused by the leaching of nitrates due to excessive and inappropriate fertilization (Vuković et al., 2008). Beside balanced nutrition, sowing density also plays an important role for achieving high yields with desirable grain quality. Optimum plant densities greatly vary across areas, diverse climatic and soil conditions as well as cultivar specificity. Due to the presence of different mechanisms of yield determination in wheat cultivars, each cultivar needs to be evaluated over wide range of fertilization and seeding rates to determine optimal combination of agronomic practices (Wiersma, 2002). Considering constant development of new wheat cultivars with specific requirements for appropriate management practices, there is a lack of information about their response to different fertilization and sowing density. Therefore, the objectives of this study were to quantify the variation in grain yield and main agronomic traits of the five new developed wheat cultivars across different N fertilization levels and sowing densities in order to improve wheat production under agroecological conditions of Pannonian Plain.

Materials and methods

This study included five new winter wheat cultivars (NS Pudarka, NS Nafora, NS Petrija, NS Tavita and NS Ilina) released by the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The cultivars were grown under field conditions with combinations of four top-dressed N fertilization levels and four sowing densities. N fertilization treatments included an unfertilized control (0 N) and N fertilization with 50 (50 N), 100 (100 N) and 150 kg N ha^{-1} (150 N), and sowing densities of 300, 500, 700 and 900 viable seeds m^{-2}. Treatments were arranged in a split-split-plot design with three replications. Main plots were assigned to the nitrogen levels, sub-plots to cultivars and sub-sub-plots to sowing densities.
The trial was set up at experimental fields of Institute of Field and Vegetable Crops, Novi Sad under rain fed conditions on carbonate chernozem, with soybean as a preceding crop in 2015/16 growing season. Crops were sown on recommended sowing date for southern Pannonian plain. A fertilizer combination (NPK – 11:52:0) was applied before ploughing to avoid N, P and K deficit, based on previous soil agrochemical analysis. The soil was prepared by ploughing along with two harrowing procedures. Each plot consisted of 10 rows, with row spacing of 0.10 m and length of 5 m. Pests, weeds and diseases were prevented or controlled by applying the recommended insecticides, herbicides and fungicides. No additional irrigation was applied. Grain yield (GY) was determined from combine-harvested plots in each of the three replications. Moisture content was determined using grain analysis computer (Model GAC2100, Dickey-John, Auburn, IL) and GY was corrected to 130 g kg^{-1} moisture. From harvested sample, thousand grain weight (TGW) was determined by three sets of 300 grains per plot and expressed as the weight of 1000 grains. Number of grains per m^2 (GN) was calculated as the ratio of the grain yield and the thousand grain weight. Analysis of variance (ANOVA) and data mean comparison by Duncan multiple range test were performed using Infostat (student version).

Results and Discussion

In combined analysis of variance cultivar (C) contributed the most to the total sum of squares of the studied traits (Tab. 1). Cultivars behaved differently under various N-fertilization (F) and sowing density (SD) treatments. The effect of F × C interaction was significant for all studied traits, with the highest influence of GY. The contribution of F × SD, C × SD and F × C × SD interaction was significant only for TGW.

Tab. 1. Relative contribution to the total sum of squares (%) and the level of significance for grain yield (GY), thousand grain weight (TGW) and grain number (GN) of wheat cultivars (C) under different fertilization (F) and sowing density (SD) treatments

Source of variation	Degrees of freedom	GY	TGW	GN
F	3	5.9**	17.4**	21.5**
C	4	13.4**	71.7**	27.8**
SD	3	8.2**	0.3**	5.5**
F × C	12	8.9*	4.9**	7.6**
F × SD	9	1.6^{ns}	0.4**	1.0^{ns}
C × SD	12	2.3^{ns}	0.6**	1.0^{ns}
F × C × SD	36	3.4^{ns}	1.8**	2.3^{ns}

* significant at 0.05; ** significant at 0.01; ns - not significant

Overall GY average of the analyzed cultivars in the trial was 9.16 t ha^{-1} (Tab. 2). N fertilization significantly altered GY of five winter wheats cultivars, resulting in GY increase in comparison with the unfertilized treatment. On average, the highest GY was observed at 100 N (9.50 t ha^{-1}) and 150 N (9.24 t ha^{-1}). However, absence of N application (control treatment) resulted in the lowest GY average of 8.80 t ha^{-1}. Moreover, cultivars differed significantly in GY, and the average values among cultivars ranged from 8.64 (NS Ilina) to 9.69 t ha^{-1} (NS Pudarka). Also, there was a significant influence of F × C interaction on GY, indicating different cultivar responses to N application, e.g., NS Petrija achieved the highest GY at 50 N, whereas NS Tavita at 150 N treatment. Similarly, various studies showed GY increase with nitrogen application as a result of enhanced tillering, higher biomass production, GN and GW. (Kristensen et al., 2008, Jaćimović et al., 2014; Yang et al., 2019). Although, negative influence of N-fertilizer application on GY (severe lodging) were recorded due to favorable conditions for organic matter mineralization and consequently higher mineral N content in the soil (Aćin et al., 2013; Aćin et al., 2014).

Tab. 2. Grain yield (t ha^{-1}) of wheat cultivars across fertilization and sowing density treatments

Cultivar	**N-fertilization**				**Sowing density**				**Average**
	0 N	**50 N**	**100 N**	**150 N**	**300**	**500**	**700**	**900**	
NS Pudarka	9.52^{a-c}	9.61^{ab}	9.90^{a}	9.73^{ab}	9.52^{a-d}	9.45^{a-e}	10.02^{a}	9.77^{a-c}	**9.69^{a}**
NS Nafora	9.09^{a-d}	9.90^{a}	9.48^{a-c}	9.44^{a-c}	8.77^{a-d}	9.59^{a-d}	9.73^{a-c}	9.82^{ab}	**9.48^{ab}**
NS Petrija	8.69^{cd}	9.33^{a-c}	9.45^{bc}	8.93^{b-d}	8.59^{ef}	8.73^{d-f}	9.48^{a-e}	9.60^{a-d}	**9.10^{bc}**
NS Tavita	8.31^{d}	8.32^{d}	9.14^{a-d}	9.72^{ab}	8.57^{ef}	8.93^{b-f}	8.91^{b-f}	9.07^{b-e}	**8.87^{cd}**
NS Ilina	8.37^{d}	8.26^{d}	9.53^{a-c}	8.40^{d}	8.09^{f}	8.57^{ef}	8.88^{c-f}	9.02^{b-e}	**8.64^{d}**
Average	**8.80^{c}**	**9.08^{bc}**	**9.50^{a}**	**9.24^{ab}**	**8.71^{b}**	**9.05^{b}**	**9.40^{a}**	**9.46^{a}**	**9.16**

Different letters represent significant differences (p<0.05; Duncan multiple range test)

On average for examined cultivars, significantly highest yields were achieved with 700 and 900 viable seeds m^{-2}. In general, GY of each cultivar improved with increasing sowing densities, but due to the absence of C × SD interaction, differences were not significant for most of the cultivars, except for NS Petrija and NS Ilina (Tab. 2). In agroecological conditions of Serbia, optimal SD of winter wheat varieties should vary between 500 and 600 viable seeds m^{-2}, thus producing a sufficient number of good quality spikes. Plants compensate lower population densities by increasing production and survival of tillers and, to a lesser extent, increasing grain numbers per spike (Bokan and Malesevic, 2004). However, although low plant density induces a higher GN and GW per spike, generally this is not sufficient to compensate for the lower spike density per m^{2} generated by a lower tiller density. Therefore, an appropriate increase in plant density to balance yield component factors would appear to be an appropriate agronomic management strategy for enhancing wheat grain yield (Li et al., 2016). The grand mean of TGW was 43.8 g, and it was significantly affected by the change in nitrogen top-dressing doses (Tab. 3). TGW values decreased with higher application of nitrogen, ranged from 46.6 g in 0 N to 41.9 g in 150 N treatments. Moreover, significant differences among cultivars were recorded for TGW, where average values varied from 38.0 g to 49.2 g, for NS Ilina and NS Tavita, respectively.

Tab. 3. Thousand grain weight (g) of wheat cultivars across fertilization and sowing density treatments

Cultivar	N-fertilization				Sowing density				Average
	0 N	50 N	100 N	150 N	300	500	700	900	
NS Pudarka	45.6^{d}	44.1^{ef}	44.2^{ef}	44.6^{e}	45.1^{bc}	44.7^{b-d}	44.1^{d}	44.5^{cd}	**44.6^{c}**
NS Nafora	48.1^{c}	45.5^{d}	43.5^{f}	43.7^{f}	45.4^{b}	45.3^{b}	44.9^{b-d}	45.1^{bc}	**45.2^{b}**
NS Petrija	45.4^{d}	43.4^{f}	41.0^{h}	38.1^{i}	42.4^{e}	40.9^{f}	42.0^{e}	42.6^{e}	**42.0^{d}**
NS Tavita	51.5^{a}	49.9^{b}	47.5^{c}	48.0^{c}	49.7^{a}	49.3^{a}	49.0^{a}	49.0^{a}	**49.2^{a}**
NS Ilina	42.4^{g}	38.4^{i}	36.1^{j}	35.1^{k}	37.9^{h}	37.4^{h}	37.9^{h}	38.7^{g}	**38.0^{e}**
Average	**46.6^{a}**	**44.3^{b}**	**42.4^{c}**	**41.9^{d}**	**44.1^{a}**	**43.5^{b}**	**43.6^{b}**	**44.0^{a}**	**43.8**

Different letters represent significant differences ($p<0.05$; Duncan multiple range test)

Differences in TGW were also observed due to the effects of various SD (Tab. 3). Highest TGW values were recorded with 300 and 900 viable seeds m^{-2}, for all studied cultivars. In comparison with 500 and 700 viable seeds m^{-2}, differences were not have considerable extent, but statistically significant. However, effects of SD on GY were not significant for cultivars NS Nafora and NS Tavita. According to Valerio et al. (2013), TGW did not reveal an effect on the yield variations, due to a change in SD rates. Similar results have been reported, where the TGW appears to be less affected by seeding density but significantly affected by environment and cultivar (Lloveras et al., 2004; Hiltbrunner et al., 2005). However, increase in SD from 500 to 650 viable seeds m^{-2} resulted in increase of TGW, as higher plant density provides a greater number of primary tillers per m^2, which causes the formation of grains with larger size and weight (Zecevic et al., 2014). An increase in N application resulted in an increase in GN, with a trial average of 21109 (Tab. 4). For all examined cultivars, the highest GN were obtained at 100 N and 150 N treatments, respectively, followed by 50 N and 0 N treatment with the lowest value.

Tab. 4. Grain number of wheat cultivars across fertilization and sowing density treatments

Cultivar	N-fertilization				Sowing density				Average
	0 N	50 N	100 N	150 N	300	500	700	900	
NS Pudarka	20859^{d-f}	21803^{c-e}	22462^{b-d}	21809^{c-e}	21112^{c-e}	21135^{c-e}	22721^{a-c}	21965^{a-d}	**21733^{b}**
NS Nafora	18950^{f}	21782^{c-e}	21838^{c-e}	21577^{c-e}	19439^{e-g}	21191^{c-e}	21726^{a-d}	21792^{a-d}	**21037^{b}**
NS Petrija	19112^{f}	21491^{c-e}	23067^{bc}	23436^{bc}	20353^{d-f}	21482^{b-d}	22648^{a-c}	22624^{a-c}	**21776^{b}**
NS Tavita	16147^{g}	16667^{g}	19249^{f}	20229^{ef}	17324^{h}	18164^{gh}	18253^{gh}	18551^{f-h}	**18073^{c}**
NS Ilina	19790^{ef}	21497^{c-e}	26437^{a}	23972^{b}	21570^{a-d}	23125^{a-c}	23610^{a}	23392^{ab}	**22924^{a}**
Average	**18972^{c}**	**20648^{b}**	**22611^{a}**	**22204^{a}**	**19960^{b}**	**21019^{a}**	**21791^{a}**	**21665^{a}**	**21109**

Different letters represent significant differences ($p<0.05$; Duncan multiple range test)

Furthermore, cultivars differed significantly in GN, with an average for all F and SD treatments ranged from 18073 (NS Tavita) to 22924 (NS Ilina). Due to the significant F × C interaction cultivars responded differently to various N treatments. So, cultivars NS Tavita and NS Ilina achieved the highest GN at 150 N and 100 N, respectively, while the increase in N doses resulted in not significant GN variation for NS Pudarka.

In addition, an increase in plant density was followed by increase in the GN, with no significant differences between 500, 700 and 900 viable seeds m^{-2} (Tab. 4). However, interaction of C × SD for GN was merely significant for cultivars NS Nafora and NS Petrija, with lowest values obtained at 300 viable seeds m^{-2}.

Conclusion

Nitrogen fertilization, cultivars, sowing densities and their interactions showed significant influence on GY and grain yield components, whereas cultivar had the highest contribution to the total sum of squares. Nitrogen application resulted in additional yield increase when compared to 0 N, and highest average GY for all examined cultivars and sowing densities was recorded at 100 N treatment. Similarly, N application increased GN up to the 100 N, but conversely, led to significant TGW decrease at higher N doses. In general, highest average GY were achieved with 700 and 900 viable seeds m^{-2}, without significant differences for most of the examined cultivars. Moreover, no significant differences in GN were recorded between 500, 700 and 900 viable seeds m^{-2}. Finally, the significant influence of interactions indicate the importance of constant examination of simultaneous effects of different nitrogen doses and sowing densities on grain yield formation in wheat cultivars in order to adjust management practice for a specific cultivar.

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INFLUENCE OF SUPERAPSORBENT „TVERDAYA VODA" ON MORPHOLOGICAL CHARACTERISTICS OF POTATO

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Abstract

In the experiments that were carried out in 2019 on two localities (East Sarajevo and Bijeljina) in the entity of Republic of Srpska (Bosnia and Herzegovina), the influence of the application of superapsorbent "*Tverdaya Voda*" on the morphological properties of potatoes was examined. The control variant, superapsorbent "*Tverdaya Voda*", superapsorbent "*Tverdaya Voda*" enriched with growth stimulants, superapsorbent "*Tverdaya Voda*" enriched with microorganisms, superapsorbent "*Tverdaya Voda*" enriched with microelements and superapsorbent "*Tverdaya Voda*" enriched with growth stimulants, microorganisms and microelements in the amount of 20 kg ha^{-1}, were applied for this examinations. The morphological traits of potatoes that were monitored are: plant height (cm), number of offshoots, mass of plant (g) mass of leaves (g) mass of stalks (g), leaf area per plant (m^2). In comparison with the multiyear averages, the year 2019 is characterized by higher temperatures and higher precipitation. Temperatures were lower only in May at both localities, which significantly influenced the slower sprouting of potatoes in East Sarajevo. When we used superapsorbent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements, plant height was 88 cm, number of trees was 6.38, plant weight was 896 grams, weight of leaves was 492.9 grams, weight of stem was 403.1 grams and assimilation surface 0.5529 m^2 per plant, while in variant without the use of superapsorbent "*Tverdaya Voda*" obtained results for plant height was 62.62 cm, number of trees was 3.38, weight of plant was 365.5 grams, weight of leaves was 215.3 grams, weight of stem was 150.2 grams and assimilation surface was 0.3401 m^2 per plant.

Keywords: *superapsorbent, potato, morphological properties, locality.*

Introduction

Potatoes grow under different climatic conditions, but it succeeds the best in climates with moderate temperatures, about 20 °C, during the vegetation period. The optimal temperature for the development of the above-ground vegetative part (tree and leaves) of the potato plant is 15-19 °C (Tadesse et al., 2001), and this temperature also represents a biological optimum for the development of underground vegetative organs (stolons and tubers). Potatoes have somewhat higher demands for water at the time of butonization, full blooming and blossom fall, as well as in the initiation phase, early development of tubers and filling of tubers (Poštić et al., 2012a). Dry seasons are often followed by high temperatures, and production is often carried out in conditions of extremely unfavorable agroecological factors. The undesired effects of high temperature on growth and potato yield are numerous and include: reduction of photosynthesis and increased breathing, reduction of root growth, inhibition of initiation and growth of tubers, reduction of dry matter (Struik 2007). Therefore, water scarcity, particularly in arid and semi-arid regions, is viewed as a major threat to long-term food security (Zhang et al., 2014). Good soil-water management is the most important factor of agricultural production in arid and semi-arid areas (Debaeke and Aboudrare, 2004). Applying water absorbing materials to soils may be a viable alternative and practical strategy for solving the problems of limited and intermittent rainfall. These soil amendments can improve soil physical and chemical properties and soil nutrient status, and have a positive impact on soil microorganisms to improve soil productivity (Mann et al., 2011). It was reported that polymer addition to sandy soil increased water and fertilizer use efficiency for plants (Bhardwaj et al., 2007; Islam et al., 2011). Furthermore, polymers potentially influence infiltration rates, density, soil structure, compaction, aggregate stability, crust hardness, and evaporation rates (Sepaskhah and Bazrafshan-Jahromi, 2006). Previous research indicated that application of polymers not only prevents pollution of agro-ecosystem, but also increases farmers' economic return (Islam et al., 2011). By applying some of the soil amendments such as super absorbent polymers, it may be possible to maintain good soil moisture under erratic rainfall and optimize use of water resources for crop production in the arid and semi-arid regions. Effect of synthetic and natural water absorbing soil amendments on soil moisture content, yield and water use efficiency (WUE) of potato production was investigated in a field experiment in a semi-arid region in northern China (Xu et al., 2014). Synthetic polymers had good interaction with natural soil amendment HA; they had positive effect in plant growth, and improved yield and WUE, this was consistent with Huang et al. (2007). The main goal of this research is to determine the influence of the superadsorbents "*Tverdaya Voda*" (created at the Voronezh State Agrarian University named after Emperor Peter the Great) different composition on morphological properties of potato in different cultivation conditions.

Material and methods

Outdoor experiments were set up in 2019 at two localities: locality 1- on the territory of the city of East Sarajevo (Tilava), altitude 550 m (43°49'01 " north latitude and 18°20'57" east longitude) and locality 2 - in the territory of the city of Bijeljina (Trnjaci), elevation of 90 m (44°83'23" north latitude; 19°29'29" east longitude). The agroecological conditions in East Sarajevo are characterized by the strong influence of the continental climate. The average annual temperature is 10.2 ° C, and the average amount of precipitation is about 900 mm. The experiment was set on alluvial soil (fluvisol). The second locality is characterized by a moderate continental climate. The average annual temperature is 12.5 ° C, and the average amount precipitation is 757.2 mm. The experiment was set on a semigley-type soil. The experiments were set by random block system in four reps with a set of 53.333 plants per ha^{-1}. The ground area of basic plot is 15 m^2 (four rows of 5 m length with 20 plants in a row, distance between rows 0.75 m and distance between plants in the row of 0.25 m). For examination of influence of superapsorbent "*Tverdaya Voda*" on morphological properties of potato we used control variant (A_0), superapsorbent "*Tverdaya Voda*" (A_1), superapsorbent "*Tverdaya Voda*" enriched with growth stimulants (A_2), superapsorbent "*Tverdaya Voda*" enriched with microorganisms (A_3), superapsorbent "*Tverdaya Voda*" enriched with microelements (A_4) and "*Tverdaya Voda*" superapsorbent enriched with growth stimulants, microorganisms and microelements (A_5) in the amount of 20 kg ha^{-1}. The experiment in Bijeljina was set on March 24, 2019. Potatoes began to sprout equally on April 13, 2019, and began to flourish on June 2, 2019. Samples for analysis were taken on June 14, 2019. The experiment in East Sarajevo was set on April 25, 2019. Potatoes began to sprout equally on May 15, 2019, and began to flourish on June 29, 2019. Samples for analysis were taken on July 10, 2019. From the morphological properties of potatoes, the following are observed: height of plants (cm) (measurement from the soil surface to the top of the upper flower); number of offsprings; leaf surface per plant (m^2) by contour sheet on paper method (Prokić i Savić, 2012); fresh offspring plant weight (g), weight of leaves (g) and potato stalk weight (g). The obtained results are analyzed by the twofactorial reflection variance analyze (locality, absorbent), and the individual differences tested by the LSD test, using the SAS /

STAT program (SAS Institute, 2000). Soil samples were taken from experimental parcels in East Sarajevo (B_1) and Bijeljina (B_2). After laboratory analysis it was established that alluvial soil from East Sarajevo has pH_{H2O} 6.63, it contains 3.62% humus, 0.23% N, and in 100 g^{-1} soil, it has 14.75 mg of soluble P_2O_5 and 15.59 mg of soluble K_2O, while humophluvisol from Bijeljina had pH_{H2O} 7.16, containing 4.12% humus, 0.27% N and 100 g^{-1} of the soil had a 40 mg soluble P_2O_5 and 36.41 mg of soluble K_2O. The land from both sites is dried to the same constant. In comparison with the multiyear averages, the year 2019 is characterized by higher temperatures and higher precipitation (Table 1). Temperatures lower at both localities only in May, which significantly influenced the slower growth of potatoes in East Sarajevo. Frequent and higher amounts of precipitation with the appearance of high temperatures also affected the occurrence of the causative agent of the disease, that was the reason for repeated treatmant of potatoes with protective agents.

Table 1. Average monthly air temperatures (°C), monthly precipitation (mm) and multiyear averages for Bijeljina and East Sarajevo.

Month			I	II	III	IV	V	VI
Bijeljina	2019.	Temperature (°C)	0.6	4.7	10.2	13.2	14.9	22.4
		Precipitation (mm)	62.8	34.1	33.7	95.4	121	119.5
	Average 1981-2010	Temperature (°C)	0.6	2.3	6.8	11.5	16.5	19.8
		Precipitation (mm)	69.7	59.1	87.5	84.3	89.4	112.4
East Sarajevo	2019.	Temperature (°C)	-6.8	2.7	8.2	12	13	21.8
		Precipitation (mm)	82.5	53,3	53.8	101.4	106.5	100.3
	Average 1961-1990	Temperature (°C)	-0.8	1.7	5.5	10	14.8	17.7
		Precipitation (mm)	74	63	73	76	85	94

Results and Discussion

The decrease in stem height with water deficit during the growing season and in the absence of superapsorbenta use is consistent with the results of research by Motalebifard et al. (2014). Cell growth is the most sensitive stage under the influence of water stress. The size of organs is limited by decreasing cell growth and the first palpable effect due to water deficiency in the crops is shown by decreasing leaf size and crop height (Kirank et al., 2003). Statistically analyzed, significant differences were found for the height of plants, number of shoots, plant mass, leaf mass, stable and leaf surface when using different variants of superapsorbent "*Tverdaya Voda*". A highly significant influence of the locality for plant height, plant mass, leaf mass, tree mass and leaf surface was determined, while the highly significant interaction of the superapsorbent x locality was determined for plant mass, leaf mass, stable and leaf surface. The highest plant height is in the variant where the superapsorbent "*Tverdaya Voda*" is enriched with growth stimulators, microorganisms and microelements (88 cm). The application of various variants of enriched superapsorbent "*Tverdaya Voda*" had a significantly higher potato plant than the control variant (62.62 cm) and a variant where "*Tverdaya Voda"* superapsorbent was applied (75.25 cm) Highly significant differences were determined in plant height between the variant where superapsorbent "*Tverdaya Voda*" and the control variant were applied. The height of plants in Bijeljina is 91.29 cm, while in East Sarajevo it is 68.71 cm. These differences are highly significant. The results of studies by Jahan et al. (2013) and Islam et al. (2011a) showed the positive effect of superapsorbenta on crop height which is consistent with the results of this research. The control variant had the smallest number of offshoots (3.38), and the largest number of offshoots had superapsorbent "*Tverdaya Voda*" enriched with growth stimulators, microorganisms and microelements

(6.38). The determined differences are statistically significant, as well as differences of other variants of the "*Tverdaya Voda*" superapsorbent in comparison with the control variant. Number of offshoots in Bijeljina was 4.96, and in Eastern Sarajevo was 4.42. These differences were not statistically significant.

Table 2. Influence of superapsorbent and locality on the morphological properties of potatoes

		Plant height (cm)	No. of offshoots	Mass of *plant* (g)	Mass of leaves (g)	Mass of stalks (g)	Leaf surface area of 1 plant (m^2)
Superapsorbent (A)	A_0	62.62c	3.38c	365.5f	215.3e	150.2d	0.3401d
	A_1	75.25b	4.12b	517.6e	271.7d	245.9c	0.3938c
	A_2	83.88a	4.75b	568.9d	298.6d	270.3c	0.4688b
	A_3	83.12a	5.12ab	776. 0b	391.4b	384.6a	0.4661b
	A_4	87.12a	4.38bc	662.7c	353.6c	309.0b	0.4602b
	A_5	88.00a	6.38a	896.0a	492.9a	403.1a	0.5529a
Locality	E.Sarajrvo (B_1)	68.71b	4.96	464.3b	248.5b	215.8b	0.3544b
	Bijeljina (B_1)	91.29a	4.42	797.9a	426.0a	371.9a	0.5395a
AxB E.Sarajrvo	A_0	54.25	3.75	398.3h	250.9e	147.4e	0.2860f
	A_1	62.75	4.25	393.0h	220.1f	172.9e	0.3236e
	A_2	69.25	5.25	381.0h	213.4f	167.6e	0.3441e
	A_3	72.25	5.25	528.9f	269.7e	259.1d	0.3607de
	A_4	75.50	4.50	423.2g	209.5f	213.7d	0.3626de
	A_5	78.25	6.75	661.4e	327.3d	334.0c	0.4492c
Bijeljina	A_0	71.00	3.00	332.6	179.6f	153.0e	0.3941d
	A_1	87.75	4.00	642.1e	323.3d	318.8c	0.4639c
	A_2	98.50	4.25	756.7d	383.8c	372.9b	0.5934b
	A_3	94.00	5.00	1023.1b	513.2b	510.0a	0.5714b
	A_4	98.75	4.25	902.1c	497.7c	404.4b	0.5578b
	A_5	97.75	6.00	1130.6a	658.5a	472.2a	0.6566a
ANOVA	A	**	**	**	**	**	**
	B	**	ns	**	**	**	**
	AxB	ns	ns	**	**	**	**

Mean values designated with the same lowercase letter are not significantly different at the 95% level according to the LSD test
*** F-test significant at 0.01; * F-test significant at the 0.05 level; ns non-significant*

The mass of the plants ranged from 365.5 to 896 grams. Using the "*Tverdaya Voda*" superapsorbent enriched with growth stimulators, microorganisms and microelements, potato plants were obtained, which, compared with other variants, had statistically significant weight of plants, while the control variant compared with the use of different variants of superapsorbent "*Tverdaya Voda*" had statistically significant the smallest mass of plants. We determined a significant variation in the mass of plants between the superapsorbent "*Tverdaya Voda*" (A_1), the superapsorbent "*Tverdaya Voda*" enriched with growth stimulants (A_2), the "*Tverdaya Voda*" superapsorbent enriched with microorganisms (A_3) and the "*Tverdaya Voda*" superapsorbent enriched with microelements (A_4).
The mass of plants in Bijeljina was 797.9 g, and in East Sarajevo was 464.3 g. These differences are highly significant. At both localities, the largest plant mass was in the A_5 variant of 1130 g; 661.4 g), and the smallest 332.6 g in the control variant (Bijeljina), ie 381 g in variant A_2 in East Sarajevo.
The weight of leaves ranged from 215.3 grams to 492.9 grams. Using the "*Tverdaya Voda*" superapsorbent enriched with growth stimulators, microorganisms and microelements, potato plants were obtained, which, compared to other variants, had statistically significantly higher leaf mass, while the control variant compared to the use of different variants of the superapsorbent "*Tverdaya Voda*" had statistically significant the smallest leaf mass. High weight variation of the leaves weight was determined between the superapsorbent "*Tverdaya Voda*" (A_1), the superapsorbent "*Tverdaya Voda*" enriched with growth stimulants (A_2), the superapsorbent "*Tverdaya Voda*" enriched with microorganisms (A_3) and the "*Tverdaya Voda*" superapsorbent enriched with microelements (A_4).
The weight of leaves in Bijeljina is 426 g, and in East Sarajevo 248.5 g. These differences are highly significant. At both localities the highest mass of leaves was in the variant A_5 658.5 g; 327.3 g), and the smallest 179.6 g in the control variant (Bijeljina), or 213.4 g in variant A_2 in East Sarajevo.
The mass of the stem ranged from 150.2 grams to 403.1 grams. Using the "*Tverdaya Voda*" superapsorbent enriched with growth stimulants, microorganisms and microelements (403.1 g) and superapsorbent "*Tverdaya Voda*" enriched with microorganisms (384.6 g), potato plants were obtained which, compared to other variants, had a statistically significant increase in the weight of the stem, while the control variant compared with the use of different variants of the "*Tverdaya Voda*" superapsorbent had a statistically significant smallest mass of stem. A significant variation of the mass of the stem between the superapsorbent "*Tverdaya Voda*" (A_1), the superapsorbent "*Tverdaya Voda*" enriched with growth stimulants (A_2) and the "*Tverdaya Voda*" enriched with microelements (A_4) superapsorbent were determined. The mass of the stem in Bijeljina is 371.9 g, and in East Sarajevo 215.8 g. These differences are highly significant. At both localities, the highest mass of the stem was in variant A_5, and the smallest in the control variant.
Awareness of processes linked to the formation and growth of the surface area of leaves helps provide a clearer forecast about the productivity of photosynthesis. the total surface area of leaves defines and differentiates the efficiency of the agrotechnics applied, depending on the specific conditions of growing the plants. the formation of biomass is indirectly linked to the surface area of leaves which in turn participates in photosynthesis (Stoimenov and Kirkova, 2009). The leaf surface ranged from 0.3401 m^2 per plant (A_0) to 0.5529 m^2 per plant (A_5). These differences were statistically significant, as well as the differences between variant A_5 and other combinations of "*Tverdaya Voda*" superapsorbent, as well as the differences between enriched "*Tverdaya Voda*" superapsorbents and "*Tverdaya Voda*" superapsorbent. Potato cultivated in Bijeljina (0.5395 m^2 per plant) had a statistically significantly higher surface area compared to potatoes grown in East Sarajevo (0.3544 m^2 per plant). These differences are highly significant. At both localities, the highest mass of the stem was in

variant A_5, and the smallest in the control variant. At both localities, the largest leaf surface per plant was in variant A_5, and the smallest in the control variant.

Conclusion

The use of different variants of the "*Tverdaya Voda*" superapsorbent at both localities had a positive effect on the morphological properties of potatoes. The use of "*Tverdaya Voda*" superapsorbent, enriched with growth stimulators, microorganisms and microelements, especially stands out, where we had the best results for: plant height, number of stems, plant mass, leaf mass, stem weight and leaf surface per plant. Good results of the examined properties were found in the use of the "*Tverdaya Voda*" superapsorbent enriched with microorganisms (A_3), and the variant in which the superapsorbents were not used (the control variant) had poorer results for the examined properties. In the experiments carried out in Bijeljina, due to better agroecological conditions, better results were obtained for the examined potato properties, compared with those in East Sarajevo.

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NUTRITION VALUES OF HULLED AND NAKED OATS AND BAKING QUALITY OF WHEAT-OAT COMPOSITE FLOURS

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Abstract

Oat (*Avena* spp. L.) belongs to the family *Poaceae* and to the group of grasses (*Gramineae*). In human nutrition, it is mainly used as source of food fiber, β-D-glucan, carbohydrates and proteins. These ingredients are considered health benefits with positive effects for the consumer. The objective of the submitted study was to realize complete characterization of different varieties of oat among the genus *Avena* to find natural sources of beneficial components of the seed (contents of proteins, dietary fibre, β-D-glucan, lipids, starch…) for their next use in the breeding programmes and application in the food industry. Other aim of this work was to determine the effect of oat flour addition at the level of 5, 10, l5, 20, 25 and 30% to wheat flour on baking quality and to determine optimal portion of oat flour to by acceptable by consumers. The study involved determination of protein content, starch content, lipids content, β-D-glucan content and total dietary fibre content in wheat flour and two chosen oat genotypes (Valentin-hulled and Detvan-naked). Effects of oat flour on the flour properties, the rheological properties of dough by farinograph, the quality of final products and their sensory evaluation were determined. Oat is beneficial in nutritional value especially in total dietary fibre, β-D-glucan and lipids content compared to wheat seeds. Addition of oat flour up to 20% to wheat flour is acceptable for technological quality, rheology properties of dough and final products in overall flavour, appearance and texture for consumers.

Keywords: *Oat, nutrition value, β-D-glucan, composite flour, baking quality*

Introduction

Bread is an ideal functional food product, since it is an important part of our daily diet (Flander *et al*., 2007). The nutritional value of bread can be enhanced through the addition of a large number of flours of different origin, as reviewed by Mariotti *et al*. (2006) who also indicated that acceptable bread can be produced with a wheat flour substitution of up to 25–30%. Wheat (*Triticum aestivum* L.) is the most important crop for breadmaking due to its supreme baking performance compared to other cereals (Dewettnick *et al*., 2008). However, the interest in alternative grains is increasing due to the consumer demand for novel and healthy foods (Zhou *et al*., 1998). Oat (*Avena sativa*) is one of the most adventurous cereal grains for human diet since it contains naturally high amounts of valuable nutrients such as soluble fibres, proteins, unsaturated fatty acids, vitamins, minerals and phytochemicals (Flander *et al*., 2008). Integration of wheat flour with oat enhances the β-D-glucan content of bread and may have a significant effect on human health. Potential benefits of soluble dietary fibre include reduction of bowel transit time, reduction in the risk of colorectal cancer, lowering of serum blood cholesterol, regulation of glucose metabolism and promotion of the growth of beneficial gut microflora (Welch & McConnell, 2001; Brennan & Cleary, 2005; Mariotti *et al*., 2006). The effects of oats on dough properties and bread quality have been studied mainly on composite breads made from wheat and oats, with addition levels ranging from 10 to 51 % of oat bran or flour (Flander *et al*., 2007).

The aim of this study was make screening of oats genotypes for analyse nutrition composition and to analyse and compare technological parameters of composite flours (mixture of major component wheat flour and added out flours- minor crops known as donors of nutritional quality), rheological parameters of dough made from composite flours, and quality traits of final bakery products (breads).

Material and Methods

Analyses of oats genotypes. All oat genotypes were obtained from Research and Breeding station in Vígľaš-Pstruša, Slovakia. We made screening of this genotypes for analyse nutrition composition, 63 genotypes were hulled and 13 genotypes were naked. We determined protein content, lipids content, starch content, dietary fibre and β glucan content. Nitrogen content was determined using Dumas method (CNS-2000 Elemental Analyzer, LECO Corp., USA). Protein content was calculated as N x 6.25 for oat. Total dietary fibre content of oat wholemeals was determined using Total dietary fibre assay procedure, AOAC 991.43 (Megazyme, Ireland), β-D-glucan content was determined using Mixed-linkage Beta-glucan assay procedure, AOAC 995.16 (Megazyme, Ireland). The starch content was determined according to Ewers (STN EN ISO 10520, 2002), lipids by Soxhlet extraction (STN 46 1011–28, 1988).

Flour samples and composite flour formulations. Commercial common wheat flour was used and was obtained from PENAM Trnava mill. Oat seeds of two genotypes Valentin (hulled) and Detvan (naked) were obtained from research-breeding station Vígľaš-Pstruša. Oat seeds were grounded with hammer mill, using 1 mm sieve to wholemeal. Composite flours were prepared by mixing wheat flour and oat groats in proportion of 5%, 10%, 15%, 20%, 25% and 30%. Single basic wheat flour was used as control.

Flour analyses and rheological tests. Wet gluten and swelling of gluten were determined according to STN ISO 5531 (1994) (6-places mechanical washing of gluten), total ash content according to STN ISO 2171 (2006), sedimentation index, Zeleny test according to STN EN ISO 5529: 2008 (shaker, Brabender, Germany) and Falling number was determined according STN EN ISO 3093: 2010 (Falling Number 1800, Perten Instruments, Sweden). The rheological properties of doughs were determined using Brabander farinograph by standard procedure ICC No. 115/1 for wheat flour, (ICC 1992).

Baking test. A straight dough breadmaking process was performed. A basic bread formula, based on flour weight, was used: 250 g of flour, 12,5 g yeast, 3,75 g salt, 2,5 g sugar, 2,5 g lard and variable water on the basis of farinograph water absorption. Doughs were optimally mixed, fermented for 20 min near 32 °C ± 1 °C, divided into 2 the same pieces, hand-moulded and sheeted, put into proofer box for 25 min near 32 °C ± 1 °C. Bread was baked in an electric oven for 20 min at 230 °C.

Bread quality evaluation. After 2 h cooling at room temperature, loaves were weighed and loaf volume was measured by rapeseed displacement method. Loaf specific volume (ml/100g) was calculated. Breads were sensory evaluated (5 hedonic rating scale was used, score 1 means extreme dislikes, score 5 means extreme likes). Sensory evaluations of breads were conducted by 5 panellists. We evaluated form of product, colour of crust/crumb, taste of crust/crumb, aroma of crust/crumb, porosity of crumb, pliancy of crumb, firmness of crust/crumb, thickness of crust, tackiness of crumb. The maximal number of points in evaluation was 53.

Statistical analyses were performed by the software package Statgrafics plus for Windows. All determinated parameters were analyzed in two replications.

Results and Discussion

Average, minimum and maximum values of nutrition parameters (proteins, lipids, starch, dietary fibre and β-D-glucan) of oat genetic resources are presented in Table 1 (hulled oat genotypes) and in Table 2 (naked oat genotypes). For next bakery analyses we used genotypes

Valentin and Detvan. The oat flour from both genotypes (Valentin, Detvan) were used in composite flours as a donor of higher content of proteins, starch, lipids, total dietary fiber and β-D-glucan (Table 3). According author Butt *et al*. (2008) oat groat contains significant amounts of β-D-glucan that varies between 2.3% and 8.5%. According to Šterna *et al*. (2018) the concentration of protein in oat grain depending on genotype ranged from 10.4% to 14.9%, and the richest were grains of the variety 'Peppi', the concentration of lipids in oat grain was from 5.4% to 7.9%, β-D-glucan concentration in oat grains varied from 2.9% to 3.6%. In our set of hulled oat genotypes we observed protein content from 10,2% to 14,9% and in set of naked oat genotypes from 15.3% to 18.2%, β-D-glucan content of hulled oats was from 1,73% to 4,40% and of naked oats genotypes from 3,0% to 5.7%

Ash content of basic wheat flour was 0.52%. Ash content increased significantly ($P<0,05$) in wheat-oat flour continually (Table 4). The decreasing ratio of wheat in composite flour significantly ($P<0,05$) reduced the content of wet gluten (Table 4). This is related to the substitution of wheat proteins (gliadins and glutenins) competent to produce viscoelastic gluten by oat proteins which are unable to do this. The wet gluten content was lower than 26%, i.e. the value required by a relevant technical norm for standard bakery quality grade in flours with oat ratio >10%, respectively.

The swelling of gluten significantly ($P<0,05$) decreased at 10% ratio of oat flour, respectively. It may be concluded that each percent of added oat flour reduces swelling of gluten by approximately 1%. Sedimentation index was decreased significantly ($P<0,05$) with increasing ratio of oat flour, but only flour with oat ratio 30% had a lower sedimentation index than is the minimal value of 23 ml required by the relevant technical norm for food wheat (STN 46 1100–2, 2018).

Table 1. Nutrition parameters of set of hulled oat genotypes

	Proteins [%]	**Starch [%]**	**Lipids [%]**	**Total dietary fiber [%]**	**β-D-glucan [%]**
Average	12.0	42.3	4.2	32.6	3.35
Minimum	10.2	40.7	2.7	10.6	1.73
Maximum	14.9	62.8	7.8	35.0	4.40
STD	0.89	1.84	0.86	2.13	0.64
Frequency	63	8	63	6	63

Table 2. Nutrition parameters of set of naked oat genotypes

	Proteins [%]	**Starch [%]**	**Lipids [%]**	**Total dietary fiber [%]**	**β-D-glucan [%]**
Average	16.7	57.7	6.2	13.3	4.28
Minimum	15.3	48.4	3.3	10.6	3.00
Maximum	18.2	62.8	8.5	16.9	5.70
STD	0.92	6.35	1.32	2.50	1.03
Frequency	13	4	13	5	13

Table 3. Nutrition parameters of flours used for development of composite flours

Source of flour	**Proteins [%]**	**Starch [%]**	**Lipids [%]**	**Total dietary fiber [%]**	**β-D-glucan [%]**
Oat flour -Valentin (hulled)	14.9	62.4	4.49	11.00 ± 0.06^{b}	3.55 ± 0.04^{b}
Oat flour - Detvan (naked)	15.0	59.4	5.67	12.94 ± 0.03^{c}	4.03 ± 0.01^{c}
Basic wheat flour	13.8	58.2	1.33	3.53 ± 0.03^{a}	0.61 ± 0.02^{a}
$LSD_{0.05}$	-	-	-	0.093	0.198

SD - standart deviation, superscripts represent statistically significant differences at P<0.05

Table 4. Basic gualitative parameters of composite oat flours (there was not differences between genotypes)

Composite oat flour	**Ash [%]**	**Wet gluten [%]**	**Swelling of gluten [%]**	**Sedimentation test [%]**
Basic wheat flour	0.52 ± 0.00^{a}	31.2 ± 0.02^{g}	15.0 ± 0.00^{g}	29.0 ± 0.00^{f}
Oat flour 5%	0.62 ± 0.02^{b}	29.2 ± 0.09^{f}	14.0 ± 1.00^{fg}	28.0 ± 0.00^{e}
Oat flour 10%	0.71 ± 0.01^{c}	27.5 ± 0.18^{e}	13.5 ± 0.50^{e}	26.5 ± 0.50^{d}
Oat flour 15%	0.79 ± 0.01^{d}	25.6 ± 0.14^{d}	11.5 ± 0.50^{d}	25.5 ± 0.50^{c}
Oat flour 20%	0.86 ± 0.01^{e}	23.6 ± 0.39^{c}	10.5 ± 0.50^{c}	23.0 ± 0.00^{b}
Oat flour 25%	0.96 ± 0.01^{f}	20.2 ± 0.07^{b}	10.0 ± 0.00^{b}	23.0 ± 0.00^{b}
Oat flour 30%	1.04 ± 0.01^{g}	14.9 ± 1.64^{a}	9.0 ± 0.00^{a}	21.5 ± 0.50^{a}
$LSD_{0.05}$	0.023	1.462	1.134	0.706

SD - standart deviation, superscripts represent statistically significant differences at P<0.05

The falling number as the parameter of amylolytic activity of grain enzymatic complex increased significantly (P<0,05) by addition of ≥10% of both oat flours (Figure 1). We determined differences between oat genotypes. The genotype Detvan more increased values of falling number than Valentin.

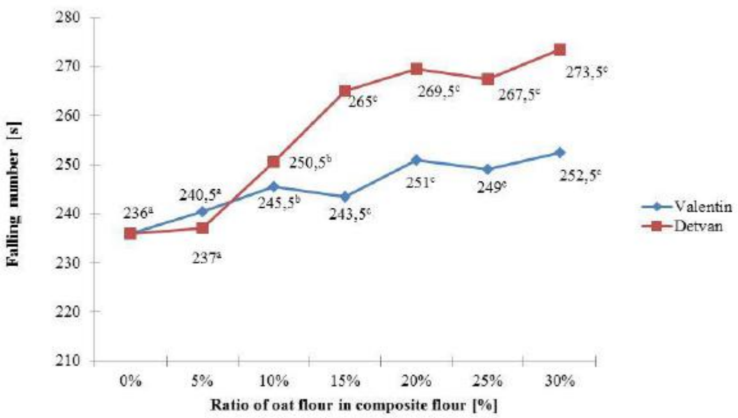

Figure 1. Falling number of composite flours

Farinograph parameters of wheat flour also strongly affect the content and type of proteins (Khatkar *et al*. 1996; Skendi *et al*. 2010). The farinograph parameters of created composite flours determined in our study are given in Table 5. Increased content of oat increased non-significantly (P<0,05) water absorption. With ratio 30 % oat flour was water absorption increased only about 1 or 2 %. Mariotti *et al*. (2006) reported similar effects for the addition 20 %, 30 % and 40 % oat flour. Values of dough development time and dough stability were significantly higher by composite flours than by control. The specific loaf volume in all composite flours decreased this parameter, additions 15 % and more, significantly (P<0,05) (Figure 2). The similar effect reported Mariotti *et al*. (2006).

Table 5. Rheological parameters of dough from composite flours wheat-oat

Composite oat flour	**Water absorption [%]**	**Dough developme nt time [min]**	**Dough stabilit y [min]**	**Dough softeni ng 10 min after test starting [BJ]**	**Dough softening 12 min after maximal consisten cy [BJ]**	**Farinogra ph quality number**
Basic wheat flour	56.2	1.7	2.7	75	82	34
Valentin 5%	56.1	1.9	6.3	49	73	76
Valentin 10%	56.6	5.0	6.2	50	99	80
Valentin 15%	56.9	5.0	4.3	49	96	76
Valentin 20%	57.4	4.5	3.4	51	95	69
Valentin 25%	57.9	4.9	4.8	31	83	88
Valentin 30%	58.4	5.5	7.5	16	81	117
Detvan 5%	56.6	1.7	6.1	45	73	74
Detvan 10%	56.7	6.0	7.4	38	104	89
Detvan 15%	57.3	5.5	6.6	40	111	85
Detvan 20%	56.8	5.8	6.1	38	113	90
Detvan 2 %	57.6	5.5	5.5	34	109	98
Detvan 30%	57.2	6.2	5.8	27	107	110

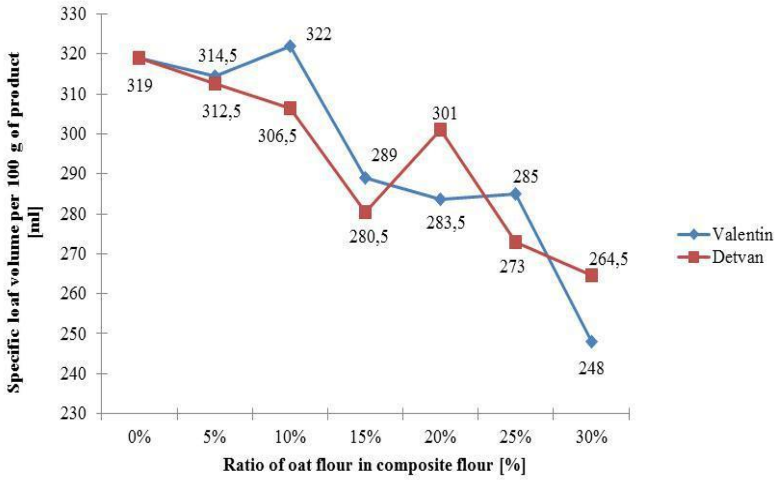

Figure. 2 Specific loaf volumes of berads from composite flours

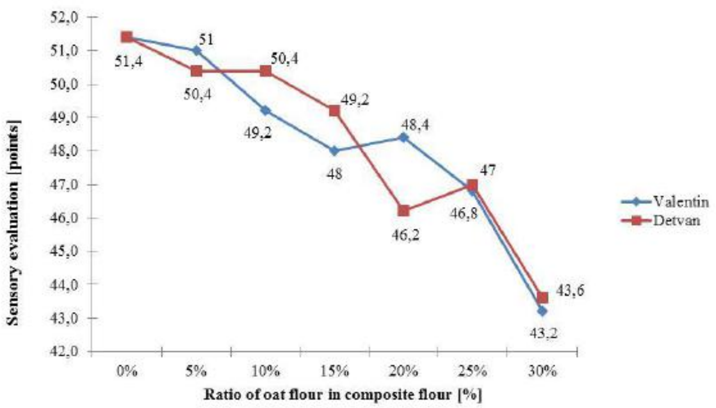

Figure.3 Sensory evaluation of breads from composite flours

Significantly differences in complex sensory parameters of wheat-oat loaves compared to the control were observed only after the ratio of oat was at least 25% when breads were less accepted by referees (Figure 3). The oat addition affected mainly quality of crumb, which was darker by genotype Detvan, with higher elasticity. The taste and aroma of breads changed positively, breads were very tastly, but only to 20%. Optimim addition of both oat flours is maximum 15 %. The optimum amount needed for the brown bread quality, which was improved not only in the crust colour and bread softness but also in the taste itself, was achieved by adding 10 % of oat flour (Gormley, Morrisey, 1999). The addition influences positively not only the sensoric properties and stronger taste but also structure and health benefit of the final product (Flander *et al*. 2007; Škrbić *et al*. 2009).

Conclusions

Flours of oat enhanced composite flours in the amount of starch, lipids, β-D-glucan and total dietary fiber in comparison with wheat flour. Additions of oat into composite flour increased ash content and decreased content and quality of gluten, decreased sedimentation index. The falling number was increased by both oat genotypes. Flours from oat changed parameters of dough by increasing water absorption, dough development time, and increasing dough stability. Specific volume of baked loaves were reduced in composite flours with increased content of oat, respectively. Breads containing oat flour were acceptable in sensory parameters up to the oat ratio of 20%.

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DETERMINATION OF PHYSICAL AND CHEMICAL PARAMETERS OF SELECTED VARIETIES OF BUTTERNUT SQUASH (*CUCURBITA MOSCHATA* DUCH. EX POIR.)

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Abstract

Butternut squash *(Cucurbita moschata* Duch. ex Poir.) is an annual plant of the genus *Cucurbita.* Its fruits are one of the most important crop-plants in traditional agricultural systems in the world and a source of biologically active components. This vegetable has been less grown in Slovakia so far. The aim of the work was to evaluate the influence of genotype on the physical and chemical properties, including carotenoids, vitamin C, and mineral contents of butternut squash in the conventional growing system under the field conditions at Slovak University of Agriculture in Nitra in 2018. Six cultivars of butternut squash (Liscia, Serpentine, Orange, UG 205 F1, Matilda F1, Waltham) were examined in experiment. The physical parameter analysis showed that the average weight of fruit was from 1.59 to 2.25 kg. The edible part of fruit was from 0.52 to 0.75. The fruits of the Orange variety had very unbalanced size, what may have negative impact on the marketing, especially in large commercial chains. The tested varieties are an important source of mineral substances, especially potassium, magnesium and calcium. The total carotenoids content in the pulp of fresh fruits ranged from 3.80 to 8.42 mg.100 g^{-1} FW. Its highest value was determined in case of Orange variety. The vitamin C content ranged in interval from 5.50 to 12.00 mg.100 g^{-1} FW.

Keywords: *butternut squash, variety, minerals, carotenoids, vitamin C*

Introduction

Man has been cultivating important agricultural crops since ancient times. Conventional and also fewer known fruits have represented in various fields of gastronomic, industrial and pharmaceutical spheres (Quintana *et al*., 2018). *Cucurbita moschata* is one of the crops, with crucial role in the agricultural systems of the world. The *Cucurbita* genus is known to be native to the area of central America (Welbaum, 2015). Currently, pumpkins are cultivated as a source of seeds, pumpkin oil (Pinke *et al.*, 2018), edible flowers and nutritionally valuable fruits. However, cultivation of *Cucurbita moschata* in Slovakia is only at development stage being cultivated in small areas of the southern parts of Slovak territory. Butternut squash is a significant crop in terms of the content of bioactive substances with antioxidant properties – vitamin C, vitamin E, carotenoids, polyphenol compounds – as well as B-complex and potassium, vitamins are appreciated by the consumer both raw and heat treated. It is suitable for storage, consumption in the fresh state or heat treatment thanks to its interesting sensory properties (Andrejiová *et al*., 2016). *Cucurbita moschata* is also known as a good source of carotenoids with decent antioxidant properties. The content of these bioactive substances in fresh fruits is variable, they differ from one species to another and can also be influenced by external factors – climate, nutrition, habitat, storage conditions, etc. (Sarah *et al*., 2018). From the nutritional point of view, the key substances in these fruits are mainly pectins, fiber, minerals (Zdunić *et al*., 2016), carotenoids, polyphenol compounds and vitamin C (Grassmann, 2005) are important for antioxidant activity. Gonzales *et al*. (2001) report that α-

carotene and β-carotene, which are precursors of vitamin A have been significantly represented by carotenoids identified in the fruit of pumpkin. From xanthophylls, lutein has been identified. Other carotenoids, such as phytofluene, ζ-carotene, neurosporin, violaxanthin, neoxanthine, flavoxanthine, have been reported to a lesser extent. Other authors also recall the presence of β-cryptoxanthine xanthophyll (Burri *et al*., 2016). The importance of carotenoids for humans is because of the provitamin activity and their antioxidant effect. Carotenoids have been proved to be able to deactivate reactive forms of oxygen, which in excess cause oxidative stress and negatively affecting the body. It is possible that carotenoids have the ability to inhibit the transformation and reproduction of cancer cells and also regulate the expression of genes playing an important role in the formation of certain types of cancer. Furthermore, this squash has been reported with antiinflammation, antibacterial, antidiabetic, antihypertensive and immunomodulation activities (Merhan, 2017; Bogacz-Radomska – Harasym, 2016).
The aim of this research was to evaluate the impact of the genotype on the selected physical and chemical parameters (vitamin C, total carotenoids, refractometric dry matter and the mineral content) of the fresh pulp of *Cucurbita moschata*.

Material and Methods

Experiment site
A field experiment was founded in 2018 in Botanical Garden of Slovak University of Agriculture (below BG SUA) under conventional system of cultivation. Before the experiment was established, a sample of soil was taken from the experimental site in the spring. An agrochemical analysis of the soil was carried out at the Department of Agrochemistry and Plant Nutrition of the Faculty of Agrobiology and Food Resources of the SUA (tab. 1).

Tab. 1 Agrochemical characteristics of the soil before the experiment

humus %	pH/KCl	Nutrients content in mg.kg^{-1} of soil					
		N	P	K	S	Ca	Mg
4,09 H	**6,98** N	**9,6 L**	**177,5 VH**	**615 VH**	**15,0 L**	**7300 VH**	**897,3 VH**

Explanatory notes: Nutrients content: L – low, H – high, VH – very high, pH: N – neutral

Based on the agrochemical analysis of the soil and the recommended normative for the cultivation of pumpkins depending on the production line in the given year, we applied nitrogen in the experimental area two weeks before the planned planting in the form of nitrogen fertilizer LAD (27% N) (60% of the recommended normative) and during vegetation the intense flowering and fruit formation were fertilized with LAD nitrogen fertilizer (27% N) (40% of the recommended normative), which was nitrogen fertilization at 150 kg N.ha $^{-1}$. Other macroelements have not been applied because their content in the soil has met the requirements for growing pumpkins.
A field experiment was founded on 11th of May 2017 from the direct sowing. We used 2.50 x 1.50 m row spacing in frame for every observed variety in 3 repeatings, which represents 2666 plants on 1 ha. Treatment of plants during the vegetation was carried out according to common agricultural techniques. The vegetation was maintained in non-weed condition by manual hoeing 2-3 times up to the stand and treated against the fungal diseases. Fruit harvesting was carried out gradually by picking in botanical ripeness on 10th September, 5th and 19th October.

Characteristics of used varieties

We used 4 varieties and 2 available hybrids of *Cucurbita moschata*: ˈLisciaˈ, ˈOrangeˈ, ˈSerpentineˈ, ˈWalthamˈ, ˈUG 205 F1ˈ, ˈMatilda F1ˈ. The supplier and the origin of the evaluated varieties of *Cucurbita moschata* are listed in tab.2

Tab. 2 Assortment of the evaluated varieties of Cucurbita moschata

Variety	Supplier	Origin
Liscia	**Semo a.s.**	**Czech republic**
Orange	**ZKI Vetőmag KFT**	**Hungary**
Waltham	**Hollar Seeds**	**USA**
Serpentine	**Semo a.s.**	**Czech republic**
UG 205 F1	**Unigen Seeds**	**USA**
Matilda F1	**Enza Zaden**	**Netherlands**

Physical parameters evaluation
We evaluated the physical parameters (fruit dimensions and fruit components weight) after each fruit harvesting period. The length was measured with a tape flexible meter along the curvature of the stalk from end to end (Fig. 1A). The diameter of the bulb was measured by placing the flex meter around the bulb of the squash (Fig. 1B). The diameter of the neck was determined around the thickening section at the end of the neck. Each squash samples and their pulp, shell and seed yield were weighted individually (Fig. 1C).

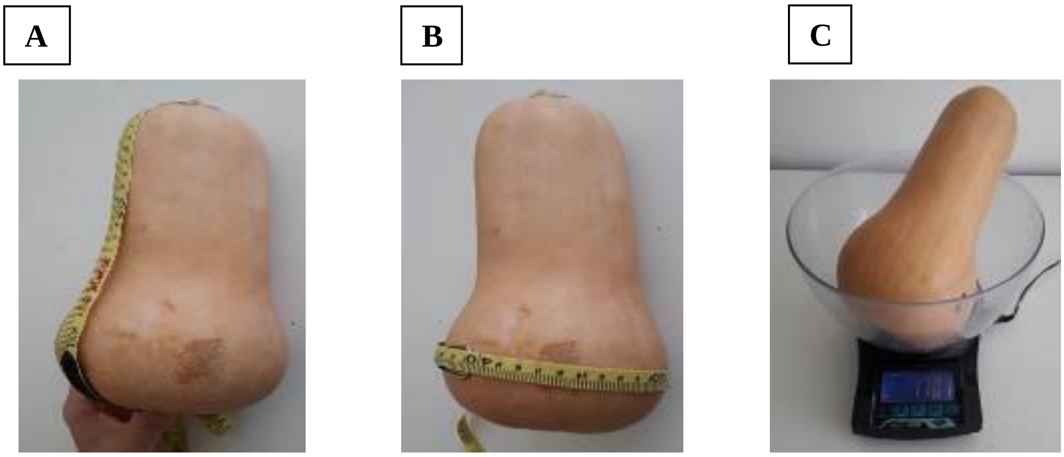

Fig. 1. Physical characterization of *Cucurbita moschata* fruits: external length (Fig. 1A), diameter of the bulb (Fig. 1B), fruit weight (Fig. 1C).

Laboratory analysis
For laboratory analysis were used only fresh fruits in full botanical maturity of all observed varieties of butternut squash. An average sample of 2 000 g was prepared from the harvested fruit yield. We homogenized the opposite two quarters of each sliced fruit within average sample and used it for the determination of total carotenoids and vitamin C content. Fresh fruit analysis was performed within 24 hours after the harvest.

Qualitative parameters

The qualitative parameters were estimated in laboratory of Department of vegetable production, SUA, in Nitra involving:

Total carotenoids estimation - Carotenoids were estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on spectrophotometer PHARO 100 at 450 nm wavelengths.

Ascorbic acid estimation (AA) - HPLC method of vitamin C content estimation was used for its quantity estimation with the help of liquid chromatograph with UV detector (HPLC fy VARIAN).

Mineral substances estimation – optical emission spectrometry with inductively bounded dual-mode plasma ICP-OES apparatus iCAP7600 (Thermo Scentific, USA).

Detectability Limits (LOD) and Quantitation Limits (LOQs) were calculated from BEC value (Background Equivalent Concentration) determined from the condition of the intensity of the analytical signal to the background intensity S/B = 1 (Thompsen, 2012).

Statistical analysis

The analysis of variance (ANOVA), the multifactor analysis of variance and the multiple Range test were done using the Statgraphic Centurion XVII (StatPoint Inc. USA).

Results and Discussion

The evaluation of the physical parameters of selected varieties of *Cucurbita moschata* is shown in Tables 3a, 3b. The largest external length of the fruit was found in the 'Serpentine' variety (80.66 cm), which has a cylindrical shape. Other squash varieties have a pear-shaped fruit and their average length ranged from 30.44 to 36.56 cm. The authors Jaeger de Carvalho *et al*. (2015) found that the average fruit weight was in the range of 1.46 kg to 7.82 kg when evaluating 20 genotypes of *Cucurbita moschata*. Based on the results of our experiments, we can conclude that the average weight of the fruit in the monitored varieties of the squashes at 2,666 plants per ha varied from 1.59 to 2.25 kg. The lowest variability of the fruits weight was found in the 'Waltham' variety. The edible part of the fruit ranged from 0.52 to 0.75. The fruits of the 'Orange' variety have very unbalanced size, which may have a negative impact on marketing, especially in large commercial chains. Jacobo-Valenzuela *et al*. (2011) indicate a higher average weight for the fruit of *Cucurbita moschata* of 3.25 kg.

Tab. 3a Physical parameters of *Cucurbita moschata*

Variety	External length (cm)	Bulb diameter (cm)	Neck diameter (cm)	Fruit weight (kg)
Liscia	32.78 ± 2.70	38.54 ± 2.06	26.28 ± 4.22	1.59 ± 0.35
Orange	36.56 ± 4.04	42.18 ± 2.72	25.12 ± 3.16	1.91 ± 0.46
Waltham	30.44 ± 2.85	37.03 ± 3.27	25.50 ± 3.32	1.32 ± 0.20
Serpentine	80.66 ± 8.08	33.36 ± 3.95	15.48 ± 1.92	1.62 ± 0.40
UG 205 F1	33.08 ± 3.26	38.60 ± 8.47	30.34 ± 3.62	1.87 ± 0.38
Matilda F1	34.52 ± 2.66	44.62 ± 4.24	32.60 ± 3.23	2.25 ± 0.41

Tab. 3b Physical parameters of *Cucurbita moschata* - structural components of fruit (%)

Variety	Pulp	Seed	Placenta	Shell
Liscia	70.74	2.21	4.47	22.58
Orange	70.68	2.54	3.91	19.58
Waltham	67.64	3.56	3.93	24.87
Serpentine	52.19	4.72	11.67	31.42
UG 205 F1	75.26	1.80	3.63	19.31
Matilda F1	70.05	3.11	3.25	23.59

The evaluation of the chemical parameters of the selected varieties of *Cucurbita moschata* is shown in Table 4 and 5.

Tab. 4 Chemical parameters of *Cucurbita moschata*

Variety	Total carotenoids (mg.100 g^{-1} FW)	Vitamin C (mg.100 g^{-1} FW)	Soluble solids (°Brix)
Liscia	6.78 b	8.70 c	8.94 c
Orange	8.42 c	9.70 d	7.76 b
Waltham	6.40 b	7.90 b	10.28 e
Serpentine	3.81 a	5.50 a	5.32 a
UG 205 F1	6.26 b	10.00 e	7.54 b
Matilda F1	5.69 b	12.00 f	9.46 d

Explanatory notes: The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($P<0.05$)

Tab. 5 Mineral content in the pulp of *Cucurbita moschata* (mg.kg^{-1})

Variety	Ca	Na	K	Fe	Mg	Zn	Cu	Mn
Liscia	1654.9	53.6	13263.5	7.44	408.56	3.32	1.73	0.67
Orange	1084.1	52.7	11473.5	6.88	257.24	3.86	1.32	0.57
Waltham	625.1	56.5	14467.6	10.28	459.27	6.90	2.16	1.20
Serpentine	2682.7	58.1	20913.0	8.17	615.51	3.50	1.50	0.50
UG 205 F1	1303.1	52.7	12423.7	9.20	447.15	5.40	1.52	1.52
Matilda F1	1174.6	63.0	9358.9	5.60	466.88	3.45	1.28	0.43

According to several authors, the content of total carotenoids in the pulp of the fruits is very variable and ranges from 2.34 to 25.45 mg.100 g^{-1} FW (Pandey *et al*., 2003; Tamer *et al*., 2010; Jacobo-Valenzuela *et al*., 2011). In our work, the total carotenoids content in the pulp of fresh fruits ranged from 3.80 to 8.42 mg.100 g^{-1} FW. Its highest value was determined in case of 'Orange' variety. Carvalho *et al*. (2015) examined 20 different genotypes of *Cucurbita moschata*, while the total carotenoid content in the fresh matter ranged from 12.46 to 69.9 mg.100 g^{-1}. Jacobo-Valenzuela *et al*. (2011) states that in the pulp of *Cucurbita moschata*

fruit, there is 2.67 mg.100g^{-1} and in the shell 0.33 mg.100g^{-1} of β-carotene. The vitamin C content ranged in interval from 5.50 to 12.00 mg.100 g^{-1} FW. Pandey *et al*. (2003) report that the content of vitamin C in the evaluated assortment of *Cucurbita moschata* was in the range of 1.53 to 6.74 mg.100g^{-1} fresh matter. On the other hand, Andrejiová *et al*. (2016) report the range of interval from 13.88 to 18.69 mg.100g^{-1}.
The tested varieties of *Cucurbita moschata* are an important source of mineral substances, especially potassium, magnesium and calcium as well. Priori *et al*. (2017) indicate in their research, that in the pulp of these fruits are present minerals in following amount: potassium ranged from 33.97 to 94.57 mg.kg$^{-1,}$ magnesium 0.86 to 2.84 g.kg^{-1} and calcium 2.26 to 7.49 g.kg^{-1}.

Conclusion

The butternut squash (*Cucurbita moschata* Duch. ex Poir.) is consumed in small rate in Slovak Republic. In the world, especially in South America, it is one of the crops with a long history of cultivation due to the good climatic conditions of the locations, high biological potential of the crop itself and application in nutrition. The aim of this research was to evaluate the impact of the genotype on the selected physical and chemical parameters of the fresh pulp of *Cucurbita moschata.* In the experiment there was observed 6 butternut squash varieties. The total carotenoids content in the pulp of fresh fruits was varied in the interval from 3.80 to 8.42 mg.100 g^{-1} FW. The vitamin C content ranged in interval from 5.50 to 12.00 mg.100 g^{-1} FW. Based on the evaluation of the average fruit weight and the structural components of the fruit for direct fruit sales, we recommend the following varieties: 'Liscia', 'Orange', 'Waltham' and 'UG 205 F1', for which the average weight of the fruits during the reference period was less than 2 kg. The ´Matilda F1´ variety has large fruits, with average weight 2.4 kg and the fruits of the ´Serpentine´ fruits have very strong curvature (average 80.66 cm external lenght).

Acknowledgement

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IDENTIFICATION OF *S*-ALLELES IN SOME INDIGENOUS SWEET CHERRY GENOTYPES GROWN IN OHRID REGION

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Abstract

Sweet cherry (Prunus avium L.) is one of the major fruit species grown in the Ohrid region. The assortment is primarily based on the indigenous genotype of 'Ohridska Dolga Šiška', which is characterized by very useful biological and agronomic properties that provide high economic value, and which is surrounded with sporadic trees from the other local genotypes for which the growers believe that they are good pollenizers. As the origin of these old genotypes is uncertain, and cases of homonyms or synonyms might occur, a reliable identification is required. Since sweet cherry exhibits gametophytic self-incompatibility, controlled by the multi-allelic S-locus that prevents self-fertilisation, determination of S-alleles of indigenous genotypes is an important step in molecular characterization that is also of enormous significance for growers and breeders to choose appropriate pollenizers in the orchard aiming to the efficient production of fruits and planning crosses in developing new cultivars. The paper presents the results of identification of S-alleles and incompatibility group in eight Macedonian indigenous sweet cherry genotypes [four genotypes of 'Ohridska Dolga Šiška' (ODŠ), two genotypes of 'Ohridska Crvena Krcka' (OCK), 'Ohridska Brza' and 'Ohridska Crna'] collected in the Ohrid region. The use of the polymerase chain reaction (PCR) with consensus primers for the second introns (PaConsII-F and PaConsII-R) of S-RNase and allele-specific primers revealed the following S-allelic constitutions in the assessed genotypes: S2S4 ('OCK-1'), S3S9 ('Ohridska Brza'), S3S12 ('ODŠ-O1', 'ODŠ-O2', 'ODŠ-S1' and 'ODŠ-S2') and S4Sx ('OCK-2' and 'Ohridska Crna'). Based on the obtained S-allelic constitutions, the assessed genotypes have been assigned to incompatibility groups XIII, XVI and XXII.

Keywords: *Prunus avium, autochthonous genotype, S-allelic constitution, incompatibility group.*

Introduction

The Balkan Peninsula is characterized by a large number of indigenous genotypes of many fruit species, which are adapted to difficult and different environments and potentially provided with rich and useful genetic variability. Gjamovski *et al.* (2016a) reported that the genotypes with useful attributes can extend the list of cultivars with a potential to be used as parents in breeding programmes and can also be an important factor for revitalizing major Macedonian fruit growing regions. The Ohrid region is a typical area for sweet cherry growing. In this region, sweet cherries are generally grown in the traditional way, often as isolated trees or in small orchards, and assortment is primarily based on the indigenous genotypes, mainly 'Ohridska Dolga Šiška', which is characterized by high quality fruits (Gjamovski *et al.*, 2016a). Other study of Gjamovski *et al.* (2016b) pointed out that this indigenous genotype showed the highest fruit weight and higher values of fruit-quality parameters among nine commercially important sweet cherry cultivars evaluated in the Ohrid region. A recurrent problem that occurs in the collection and conservation of indigenous

sweet cherry genotypes worldwide is the frequent case of different names for a genotype and different genotypes with the same name.
Apart from very few exceptions, sweet cherry is allogamous and exhibits gametophytic self-incompatibility controlled by the multiallelic *S*-locus with two linked genes, *S-RNase* and *SFB* expressed in the style and the pollen, respectively (Bošković and Tobutt, 1996; Yamane *et al.*, 2003). Therefore, only pollen tubes carrying an *S*-haplotype differing from the two stylar *S*-haplotypes can successfully complete fertilization. The use of the consensus and allele-specific PCR-based methods has enabled the identification of 25 *S*-alleles in sweet cherry (Vaughan et al. 2008). This polymorphism allowed identification of 47 incompatibility groups (IGs), a group of '0' of unique *S*-genotypes and a group of self-compatible cultivars (Schuster, 2012). Due to the high polymorphism, the *S*-locus has also been used as a genetic marker for genotyping and identification of domestic and foreign sweet cherry cultivars at Fruit Research Institute, Čačak (Marić and Radičević, 2014; Radičević *et al.*, 2015).
Since Ohrid region has a distinctive germplasm of sweet cherry that need to be well studied, reliable phenotyping and genotyping are required. Therefore, this study was aimed to identify the *S*-alleles of indigenous sweet cherry genotypes collected in the Ohrid region, as an important step in molecular characterization aiming to assign the genotypes to accurate IGs for planning parental combinations in further breeding programmes and for orchards management by choosing the suitable pollenizer.

Material and Methods

Eight indigenous sweet cherry genotypes, including four genotypes of 'Ohridska Dolga Šiška' ('ODŠ-O1', 'ODŠ-O2', 'ODŠ-S1' and 'ODŠ-S2'), two genotypes of 'Ohridska Crvena Krcka' ('OCK-1' and 'OCK-2'), 'Ohridska Brza' and 'Ohridska Crna', were sampled from the Ohrid region which is located in the south-western part of the Republic of Macedonia at an altitude of 690 m and coordinates 41°7'1"N and 20°48'6"E. Young leaves of the selected genotypes were collected, frozen in liquid nitrogen and stored at –80°C prior to DNA isolation. Frozen leaves were ground in Mixer Mill MM 400 (Retsch GmbH, Haan, Federal Republic of Germany) and extraction of genomic DNA was based on the method reported by Doyle and Doyle (1987), with the addition of β-mercaptoethanol (1%) and polyvinylpyrrolidone (2% PVP 40) in the extraction buffer. DNA samples were dissolved in TE buffer (10 mM Tris pH 8.0 and 1 mM EDTA) and kept at –20°C until using.
Identification of the *S*-alleles in assessed sweet cherry genotypes was performed according to Sonneveld *et al*. (2001, 2003). The PCRs were carried out by using the consensus primer pairs specific for the second introns of the *S-RNase* (PaConsII-F + -R, Sonneveld *et al*., 2003) and the allele-specific primers for S_2, S_3, S_4, S_9 and S_{12} (Sonneveld *et al*., 2001, 2003). For these alleles, the following annealing temperatures were used: 60°C for S_2, 66°C for S_3, 63°C for S_4, 61°C for S_9 and 62°C for S_{12}. Sweet cherry cultivars with known *S*-allelic constitutions were used as standards.
PCR products obtained with the consensus primers specific for the second introns of *S-RNase* were separated by electrophoresis in a 2% agarose gel (70 V/cm for 4 h), whereas products of allele-specific PCRs were separated by electrophoresis in a 1.5% agarose gel (70 V for 2–3 h). Visualization of PCR products was performed by ethidium bromide staining and taking photographs under ultraviolet light in BIO-PRINT-1500/26M imaging system (Vilber Lourmat, Collégien, French Republic). For sizing of DNA fragments, 1 Kb plus DNA ladder (Invitrogen, Groningen, the Netherlands) was used.

Results and Discussion

The identification of *S*-alleles of the assessed indigenous sweet cherry genotypes from the Ohrid region was conducted in two steps, i.e. the second introns of *S-RNase* was amplified using consensus primers in the first step, followed by the next step in which the allele-specific primers were used. The amplification of the second intron of *S-RNase* with consensus primers PaConsII-F + -R revealed two PCR products, which corresponded to *S*-alleles of the assessed sweet cherry genotypes, except for genotypes 'OCK-2' and 'Ohridska Crna'. The size of PCR product for the second intron ranged from ~800 (S_9 or S_{10} allele) to ~2,200 bp (S_2 or S_7 allele) (Figure 1). As reported in earlier studies (Sonneveld *et al*., 2003, Schuster *et al*., 2007, Ipek *et al*., 2011), small size differences were found when using consensus primers for the amplification of the second intron of the S_1 and S_3, as well as S_2 and S_7 alleles. Also due to the similar size of PCR products of the alleles S_9 and S_{10}, an additional analysis with allele-specific primers was required for discrimination of these alleles on an agarose gel, particularly in genotypes in which *S*-allelic constitutions have not been published to date. In order to confirm *S*-allelic constitutions of the assessed sweet cherry genotypes obtained by consensus primers, the genomic fragment of *S-RNase* was amplified using the specific primers for the S_1, S_2, S_3, S_4, S_7, S_9, S_{10} and S_{12} alleles (Table 1). The *S*-allelic constitution of each genotype was determined upon integrating the obtained results with the consensus and the allele-specific primers.

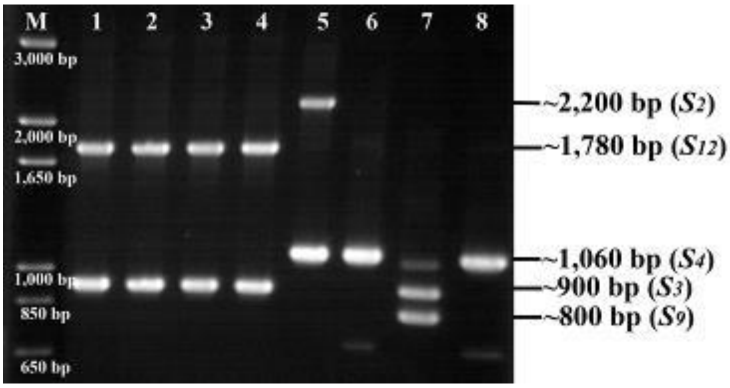

Figure 1. PCR products of the *S-RNase* amplified fragment obtained with consensus primers for the second intron in the indigenous sweet cherry genotypes: 1 – 'ODŠ-O1'; 2 – 'ODŠ-O2'; 3 – 'ODŠ-S1'; 4 – 'ODŠ-S2'; 5 – 'OCK-1'; 6 – 'OCK-2'; 7 – 'Ohridska Brza'; 8 – 'Ohridska Crna'; 1Kb plus DNA ladder (M).

The DNA fragment of ~640 bp corresponding to S_2 allele was identified in 'OCK-1' (Figure 2a). Use of S_3 allele-specific primers enabled amplification of fragment of ~960 bp in 'ODŠ-O1', 'ODŠ-O2', 'ODŠ-S1', 'ODŠ-S2' and 'Ohridska Brza' (Figure 2b). In 'OCK-1', 'OCK-2' and 'Ohridska Crna', the PCR product of ~820 bp corresponding to S_4 allele was obtained (Figure 2c). The PCR product of ~495 bp corresponded to allele S_9 and was identified in 'Ohridska Brza' (Figure 2d). Similarity in product size for S_1 and S_3, S_2 and S_7, S_9 and S_{10} alleles required additional PCRs with primers specific for S_1, S_7 and S_{10} alleles. The absence of amplification with S_1, S_7 and S_{10} allele-specific primers was another confirmation that S_3, S_2 and S_9 are present in abovementioned genotypes. In four genotypes – 'ODŠ-O1', 'ODŠ-O2', 'ODŠ-S1' and 'ODŠ-S2', the PCR product of ~562 bp corresponding to S_{12} allele was obtained (Figure 2e). The size of PCR products for identified *S*-alleles in indigenous genotypes were in agreement with results reported by Sonneveld et al. (2001, 2003).

The S-allelic constitutions for eight indigenous sweet cherry genotypes – 'ODŠ-O1' (S_3S_{12}), 'ODŠ-O2' (S_3S_{12}), 'ODŠ-S1' (S_3S_{12}), 'ODŠ-S2' (S_3S_{12}), 'OCK-1' (S_2S_4), 'OCK-2' (S_4S_x), 'Ohridska Brza' (S_3S_9) and 'Ohridska Crna' (S_4S_x) are published in this paper for the first time. Based on identified *S*-alleles, the genotypes were assigned to their corresponding IGs, previously reported by Schuster (2012). Therefore, the three following IGs (Table 2) were determined: XIII ('OCK-1'), XVI ('Ohridska Brza') and XXII ('ODŠ-O1', 'ODŠ-O2', 'ODŠ-S1' and 'ODŠ-S2').

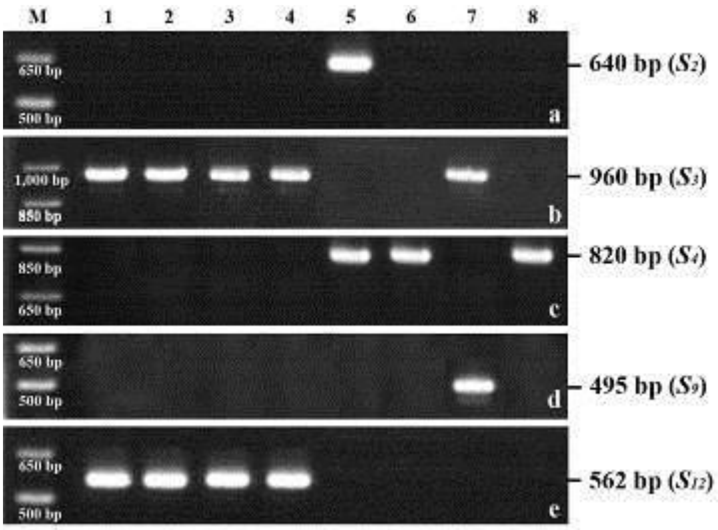

Figure 2. PCR products of the *S-RNase* amplified fragment obtained with primers specific for alleles S_2 (a), S_3 (b), S_4 (c), S_9 (d) and S_{12} (e) in the indigenous sweet cherry genotypes: 1 – 'ODŠ-O1'; 2 – 'ODŠ-O2'; 3 – 'ODŠ-S1'; 4 – 'ODŠ-S2'; 5 – 'OCK-1'; 6 – 'OCK-2'; 7 – 'Ohridska Brza'; 8 – 'Ohridska Crna'; 1Kb plus DNA ladder (M).

Table 1. Identification of *S-RNase* alleles in the indigenous sweet cherry genotypes with consensus and allele-specific primers.

Genotype	Results with consensus primer for the second intron		Amplification with allele-specific primers							
	Allele 1	Allele 2	S_1	S_2	S_3	S_4	S_7	S_9	S_{10}	S_{12}
'ODŠ-O1'	S_1 or S_3	S_{12}	–		+					+
'ODŠ-O2'	S_1 or S_3	S_{12}	–		+					+
'ODŠ-S1'	S_1 or S_3	S_{12}	–		+					+
'ODŠ-S2'	S_1 or S_3	S_{12}	–		+					+
'OCK-1'	S_2 or S_7	S_4		+		+	–			
'OCK-2'	S_4	/				+				
'Ohridska Brza'	S_1 or S_3	S_9	–		+			+	–	
'Ohridska Crna'	S_4	/				+				

The genotypes 'OCK-2' and 'Ohridska Crna', gave a single band in the S_4 position with consensus primers for the second intron of *S-RNase*, while the second allele is still unknown. In general, a single band on the agarose gel could mean either that the two alleles have introns of the same size, or that the second allele is not amplified because the primers do not match the sequence of that allele. Additionally, amplification of *S-RNase* with consensus primers in both genotypes showed weak band of ~650 bp, for which is unknown whether it represents *S* allele or a secondary band. Cloning and sequencing of the DNA fragments will provide their further characterization and possible identification of the second allele in these genotypes.

Table 2. *S*-allelic constitution and incompatibility group of the assessed sweet cherry genotypes.

Genotype	*S*-allelic constitution	Incompatibility group
'ODŠ-O1'	S_3S_{12}	XXII
'ODŠ-O2'	S_3S_{12}	XXII
'ODŠ-S1'	S_3S_{12}	XXII
'ODŠ-S2'	S_3S_{12}	XXII
'OCK-1'	S_2S_4	XIII
'OCK-2'	S_4S_x	/
'Ohridska Brza'	S_3S_9	XVI
'Ohridska Crna'	S_4S_x	/

Based on our current knowledge, some *S*-alleles are more frequent in some regions and certain alleles are specific to a particular geographic region. Tobutt *et al*. (2004) and Lisek *et al*. (2015) reported a relatively higher frequency of occurrence (> 20%) of alleles S_1 and S_3 in 247 cultivars of different origins, as well as for S_3, S_1 and S_4 in cultivars of Central and Eastern Europe, while the S_9 allele occurred with similar frequency (about 6%). Cachi and Wünsch (2014) showed that alleles S_3 and S_6 are highly frequent all over Europe. In each of four 'Ohridska Dolga Šiška' genotypes, S_3S_{12} allelic constitution was detected and based on it, the genotypes were assigned to IG XXII. Schuster (2012) reported that XXII represents an example of group with known cultivars like 'Schneiders Spate Knorpelkirsche', 'Nasenkirsche', 'Nordwunder', 'Germersdorfi', 'Noire de Meched', 'Ziraat 0900' etc., for which it has not yet been proven if they are different genotypes or only synonyms. Therefore, Macedonian 'Ohridska Dolga Šiška' genotypes also require further genotyping with a set of highly polymorphic microsatellites (SSRs) spanning the whole genome. In order to ensure successful cross-pollination and satisfactory fruit yield in commercial orchards, Radičević *et al*. (2015) reported that cross-compatible cultivars with different *S*-genotypes that flower simultaneously are needed to plant together. From this point of view and on the basis of the results obtained in this work, as well as data stated by Gjamovski *et al*., (2016a) regarding flowering phenophase of this material, some indigenous genotypes are cross-compatible with 'Ohridska Dolga Šiška' genotypes and can be recommended as potentially suitable pollenizers.

Conclusions

This study provides valuable insight into the *S*-allelic constitutions of eight Macedonian indigenous sweet cherry genotypes, as an essential precondition for effective pollination and fertilization. Among the assessed genotypes, 'OCK-1', 'OCK-2' and 'Ohridska Crna' can be potentially suitable pollenizers for 'Ohridska Dolga Šiška' genotypes. Knowledge about the *S*-genotype of indigenous sweet cherries is very important for breeders and growers, as well as for conducting further experiments in order to study other reproductive characteristics of these genotypes.

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RESEARCH ON IMPROVING TOMATO GROWTH ON PERLITE SUBSTRATE BY HORSE URINE AND BENEFITS FOR PLANT RESISTANCE TO PATHOGENS

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Abstract

The purpose of the present research was to improve the growth of tomato plants cultivated on mattresses with perlite in greenhouse conditions using various dilutions of horse urine in nutrient solution and to increase plant resistance to pathogenic fungi mediated by the development of microbial antagonists. In the study it has been used a control variant (non-treated) and two experimental variants treated with concentrations of 4ml/l and 6ml/l horse urine. The most significant effect with the highest plants and early production was registered for the variant with 4ml/l horse urine. Microbial density in perlite estimated by plate dilution method revealed high counts of heterotrophic aerobic bacteria and microscopic fungi. Taxonomic identification carried out by morphologic criteria revealed 7 to 9 bacteria species dominated by the development of *Pseudomonas fluorescens* known as an efficient antagonist for plant potential pathogenic fungi, especially from genus *Fusarium* and 8 to 9 fungal species, generally ubiquitous (from genera *Penicillium, Acremonium, Aspergillus, Fusarium, Cladosporium*) and with high enzymatic abilities for degrading various sources of nutrients (especially cellulose). The variant with 4ml/l horse urine proved to induce the best growth of tomato plants and the most equilibrate bacterial and fungal microflora, able to sustain plant health by the biological control of pathogens mediated by antagonistic fluorescent pseudomonads and actinomycetes.

Keywords: *tomato, horse urine, plant growth, Pseudomonas fluorescens, microbial antagonists*

Introduction

The horse urine contains important quantities of nutrients and because of their high amounts they must be diluted with water or solid matters. Urine boasts a N-P-K ratio of 10:1:4 and few trace elements. When not used properly, it can be a source of pollution for environment, especially for surface waters due to the contents in nitrogen, phosphorus and carbon (organic matter). Recent studies reported horse urine as safe fertilizer used for improving the growth of horticultural plants (cabbage, tomatoes and cucumbers) and microbial activity. Research of metabolites produced by microorganisms revealed the inhibitory role on the plant pathogenic bacteria and fungi. Fluorescent species of *Pseudomonas, Bacillus subtilis* and certain actinomycetes are recognized as antagonists able to produce various metabolites with antimicrobial action (Grosu et al., 2014). The development of species *Fusarium oxysporum* with antagonistic activity against various plant pathogens was stimulated by the fertilization with manure as compared with non-fertilized soil and was positively influenced by root exudates produced by tomato or lettuce plants (Papacostea, 1976). The aim of the present research was to improve the growth of tomato plants cultivated on mattresses with perlite in greenhouse conditions using various dilutions of horse urine in nutrient solution and to increase plant resistance to pathogenic fungi mediated by the development of microbial antagonists.

Materials and methods

Test plant: Tomato plants (*Solanum lycopersicum* L.) cultivar Cindrel, indeterminate type, with excellent cold tolerance, early maturity and fruits with 100-120 g weight were cultivated on mattresses with perlite in experiment under controlled conditions. Research has been conducted in greenhouse of HORTINVEST-Research Center for Studies of Food Quality and Agricultural Products, from University of Agronomic Sciences and Veterinary Medicine – Bucharest, Faculty of Horticulture, during summer 2018.

Experimental variants: a control variant (non-treated) and two experimental variants treated with different concentrations of horse urine, respectively 4ml/l and 6 ml/l horse urine. The horse urine used was collected from animals aged 3 years. Analyzes were made regarding the chemical composition of the urine. It contained 1.23% nitrogen, 0.03% phosphorus and 1.6% potassium.

Plants height (cm) was measured and **early production** (kilograms/plant) accumulated after 120 days from planting was determined by weighing tomato plants from experimental variants with horse urine solutions and from the control.

Microbiological analysis: Substrate samples from the control and variants with 4ml/l and 6ml/l horse urine horse urine were analysed by dilution plate method for the number and composition of heterotrophic bacteria and fungi communities (Dumitru & Manea, 2011). Microorganisms developed on culture media, respectively Nutrient Agar (NA) for bacteria and potato-dextrose-agar (PDA) for fungi, after incubation in the dark at 25°C were counted and identified according to specific determinative manuals for bacteria (Bergey & Holt, 1994) and for fungi (Watanabe, 2002). Microbial density was reported per gram of dry soil (oven-dried soil at 105°C for 24 h). All data represent the average value of triplicate analyses.

Results and discussion

Plants growth parameters from the variants treated with both concentrations of horse urine were positively influenced as compared to non treated control. The highest plants after 120 days from planting grew at the variant treated with 4ml/l horse urine (Figure 1.a). Early production of tomatoes was similar in both variants with horse urine but higher than in non-treated control (Figure 1.b).

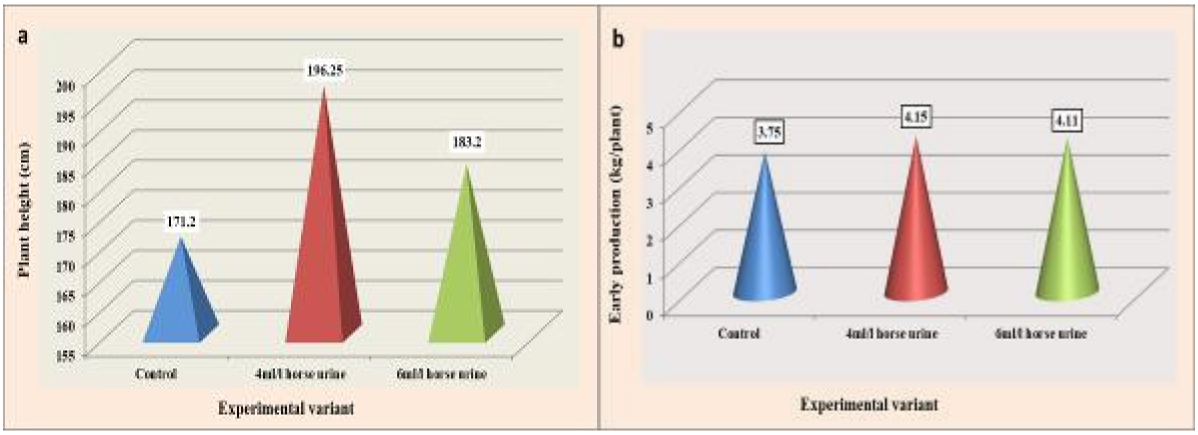

Figure 1. The influence of application of horse urine on the height (a) and early production (b) of tomato plants

The results of microbiological analysis of substrate sampled from mattresses under tomato plants consist of total counts and taxonomic composition of bacterial and fungal communities for control and variants with perlite + 4ml/l horse urine and perlite + 6ml/l horse urine. From the quantitative point of view, the values of bacteria density in analysed samples were considered to be high, especially for Control and the variant with perlite + 6ml/l horse urine and twice lower values (19.466 x 10^6 viable cells x g^{-1} dry soil**)** for the variant with perlite + 4ml/l horse urine (Table 1). Fungal counts from samples presented very high values as compared with values usually registered in soil samples. It was noticed the tendency of equilibration of fungal populations by reducing total counts when horse urine was aded in doses of 4ml/l and 6ml/l as compared with nontreated control.

Table 1. Effect of horse urine solutions on microbial counts

Experimental variant	**Fungal counts** (x 10^3 cfus x g^{-1} dry soil)	**Bacteria counts** (x 10^6 viable cells x g^{-1} dry soil)
Perlite-Non-treated Control	750.879a*	45.230a
Perlite+4ml/l horse urine	495.520b	19.466c
Perlite+6ml/l horse urine	519.325b	41.337b

***** Values in a column followed by the same letter are not significantly different for $P < 0.05$ (Student test)

Microbial communities from soil with perlite and horse urine included 7 to 9 species of heterotrophyc bacteria and actinomycetes identified on Nutrient Agar (NA) medium (Table 2).

Table 2. Taxonomic composition of bacterial microflora

Experimental variant	**No. of species** (S)	**Bacterial Microflora** (Taxonomic Composition)
Perlite-Non-treated Control	11	***Pseudomonas fluorescens, Bacillus sphaericus, Arthrobacter oxydans, Bacillus circulans, Pseudomonas aeruginosa, Flavobacterium sp., Bacillus megaterium, Sarcina ureae, Micrococcus sp.*** Actinomycetes Series Griseus and Albus
Perlite+4ml/l horse urine	**7**	***Pseudomonas fluorescens, Arthrobacter oxydans, Pseudomonas aeruginosa, Arthrobacter citreus, Bacillus circulans, Arthrobacter simplex, Bacillus subtilis,*** Actinomycetes Series Fuscus
Perlite+6ml/l horse urine	8	***Pseudomonas fluorescens, Pseudomonas sp., Micrococcus sp., Bacillus circulans, Bacillus subtilis, Sarcina ureae, Bacillus mesentericus*** Actinomycetes Series Albus

Analysis of species composition of bacterial communities revealed the dominance of fluorescent (*Pseudomonas fluorescens*, producing over 75 metabolites with antimicrobial action) and non fluorescent representatives of the genus *Pseudomonas*, (specific for improving conditions in plant rhizosphere, able to use sugars by oxidative pathway and to reduce nitrates from easily degradable organic materials), accompanied by bacillaceae**,** actinomycetes from Series Albus, Fuscus and Griseus or by species of the genus *Arthrobacter* (Figures 2 - 3). The genus *Flavobacterium*, known to be well developed in substrates with conditions of high humidity, was identified in non treated control, as well as *Sarcina ureae*, more abundant in the substrate from the variant with perlite and 6ml/l horse urine.

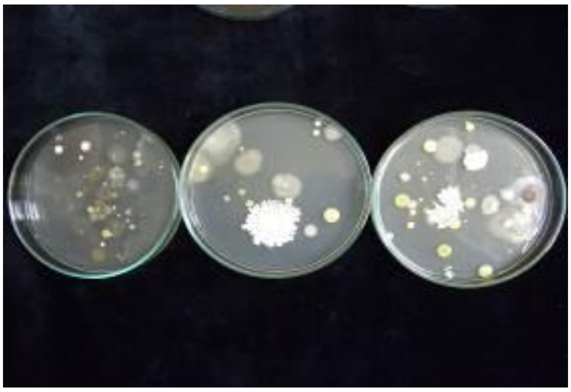

Figure 2. The bacterial microflora in experimental variants
(NA medium, 7 days)

Figure 3. Detail with bacteria in the variant with 6ml/l horse urine (NA medium, 7 days)

The results of taxonomic identification revealed that the fungal coenoses were composed by 8 to 9 species belonging to ubiquitous genera *Penicillium, Acremonium, Aspergillus, Fusarium, Cladosporium* (Figure 4 and Table 3).

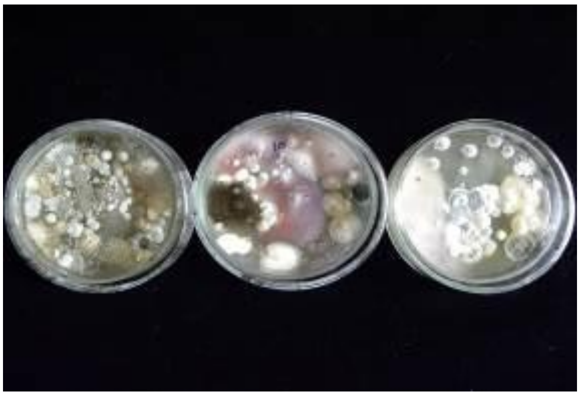

Figure 4. Fungal microflora in experimental variants (PDA medium, 7 days)

Table 3. Taxonomic composition of fungal microflora

Experimental variant	**No. of species** (S)	**Fungal Microflora** (Taxonomic Composition)
Perlite-Non-treated Control	**9**	***Penicillium chrysogenum, Acremonium charticola, Geotrichum candidum, Acremonium strictum, Aspergillus terreus, Cladosporium herbarum, Aspergillus fumigatus, Fusarium verticillioides, Penicillium brevicompactum***
Perlite+4ml/l horse urine	**9**	***Penicillium griseofulvum, Acremonium strictum, Fusarium verticillioides, Geotrichum candidum, Aspergillus fumigatus, Aspergillus terreus, Cladosporium cladosporioides, Aspergillus sydowi, Aspergillus niger***
Perlite+6ml/l horse urine	**8**	***Acremonium strictum, Penicillium aurantiogriseum, Acremonium charticola, Penicillium sp., Cladosporium cladosporioides, Fusarium verticillioides, Aspergillus sydowi, Verticillium sp.***

Many of these species identified are known to possess high abilities for enzymatic degradation of various cellulosic substrates and their utilization as carbon sources in own metabolism. Species *Geotrichum candidum*, with simple morphology, as well as *Aspergillus fumigatus* typical for fungal communities in rhizosphere of cultivated plants were identified in control and in the variant with 4ml/l horse urine but not in the variant with 6ml/l horse urine.The results from the present research showed the presence of microbial species able to produce metabolites with antibiotic and antifungal action, such as: *Pseudomonas fluorescens*, bacillaceae, actinomycetes, *Fusarium oxysporum, Aspergillus terreus, Aspergillus sydowi, Penicillium chrysogenum* in samples from the variants with horse urine and are in concordance with data from experiments demonstrating the beneficial role of various natural sources of nitrogen on the development of species that exert biological control of pathogens by metabolites with antimicrobial properties. Literature cites certain species from bacterial genera *Bacillus*, *Pseudomonas*, especially fluorescent pseudomonads (Attitalla et al., 2001; Redouan et al., 2018), certain lactic acid bacteria (Magnusson et al., 2003; Matei et al., 2015), actinomycetes (Mazzola, 2007) and fungal species of genera *Trichoderma* (Cornea et al., 2009), *Gliocladium*, non-pathogenic *Fusarium* (Weller et al., 2002) as microorganisms acting as efficient agents for the biocontrol of soil-borne plant pathogens. The dominance of these species (able to produce metabolites with antimicrobial action) in microbiomes correlated positively with pathogen suppression and indicated good soil conditions for ensuring plants health (Matei et al., 2017). The mechanism of urine impact consists in stimulating the development of beneficial bacterial populations from rhizosphere, able to use horse urine as easy degradable source of nitrogen and sugars, equilibrating the ratio bacteria:fungi in the substrate, determining the proliferation of antagonistic species able to control pathogens by producing metabolites with antibiotic or antifungal activity and enhancing plant growth and resistance.

Conclusion

Taxonomic identification revealed 7 to 9 bacteria species dominated by the development of *Pseudomonas fluorescens* known as an efficient antagonist for plant potential pathogenic fungi, especially from genus *Fusarium*. 8 to 9 fungal species identified were generally ubiquitous (from genera *Penicillium, Acremonium, Aspergillus, Fusarium, Cladosporium*) and known for enzymatic abilities for degrading various sources of nutrients (especially cellulose). The variant with 4ml/l horse urine proved to induce the best plant growth and early production of tomato and the most equilibrate bacterial and fungal microflora, able to sustain plant health by the biological control of pathogens mediated by antagonistic fluorescent pseudomonads and actinomycetes. Further research are needed to compare the influence of horse urine with manure on plant growth and resistance to pathogens.

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PRELIMINARY RESULTS ON USING CAPILLARY DYNAMOLYSIS IN ASSESSING THE EFFECT OF STRUCTURED WATER ON CUCUMBER PLANTS

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Abstract

Capillary dynamolysis or rising picture is a method widely utilized in EU countries, contributing to quality assessment of fresh foods, medicaments from medicinal plants, certification in biodynamic agriculture or to differentiation between various agricultural practices. A less studied aspect was its application to the assessment of plant quality and vitality under irrigation systems using different types of water. The aim of the present research was to use capillary dynamolysis as picture forming method for acquiring new images reflecting the vitality and biological quality of cucumber plants (*Cucumis sativus* L.) and to assess their usefulness in appreciating the effect of irrigation with structured water when compared with tap water under organic and chemical fertilization in greenhouse experiment. The results from biometric parameters measurements revealed that irrigation with structured water significantly increased plant height and fresh biomass accumulation as compared to tap water, the effect being more visible when associated with organic and especially chemical fertilization. Visual evaluation of the 6 paper images formed by capillary dynamolysis was carried out in the paper. Differences in the aspect of structural elements from both base, bowl and flag zone were observed with evidencing of a higher vital force and quality of plants by stronger, more intensely colored and better-defined images at variants with structured water. A similar pattern of image was recognized for organic fertilizers and for chemical fertilizers, too. Information derived from rising pictures analysis proved its usefulness in comparing the influence of irrigation water and fertilizing systems on quality of cucumber plants.

Keywords: *cucumber, structured water, capillary dynamolysis, biological quality, picture creating methods*

Introduction

Capillary dynamolysis or rising picture is a method widely utilized in EU countries, contributing to quality assessment of fresh foods, medicaments from medicinal plants, certification in biodynamic agriculture or to differentiation between various agricultural practices. As other two image forming methods, namely biocrystallization and circular chromatography utilized in discriminating produce from organic and conventional origin, capillary dynamolysis is based on the evaluation of structures formed by the reaction of product extract with certain inorganic salts. Characteristic qualitative traits result in typical and reproducible image structures (Fritz et al., 2011). A less studied aspect was the application of this method to the assessment of plant quality and vitality under irrigation systems using different types of water. Recent studies evidenced the beneficial effect of irrigation with structured water on plants growth, heat resistance, health, yields quality (Abraham, 2014; Dubey et al., 2018) in basil, tomato, strawberry or cucumbers as compared to plants watered with tap water. The aim of the present research was to use capillary dynamolysis as picture forming method for acquiring new images reflecting the vitality and biological quality of cucumber plants (*Cucumis sativus* L.) and to assess their usefulness in appreciating the effect of irrigation with structured water when compared with tap water under organic and chemical fertilization in greenhouse experiment.

Materials and methods

Test plant: Cucumber plants (*Cucumis sativus* L.) cultivar Siriana, with fruit type cornishon.
Structured water: The water is structured by quantum technology, created exclusively on the basis of natural elements. Tap water was introduced into the apparatus with quantum electromagnetic field (the magnitude of the waves is in the order of 10^{-40}) to obtain structured water. The information transported by this electromagnetic field during the water structuring process remains captive (stored) in the aqueous physical vacuum from the coherence fields of the water and the information is rewritten through the water into the body of the plant.
Experimental variants: cucumber plants were watered with structured water (V1) and using two supplementary variants of fertilization, namely organic product Formulex 5 ml/l (V3) and mineral UNIVERSOL 2 g/l (V5) in experiment under controlled conditions. We calculated so that the dose of nitrogen, phosphorus and potassium was the same for all variants. Similar pots were prepared and in these variants the plants were watered with tap water (V2) or supplemented with identic quantities of organic (V4) and mineral fertilizers (V6). The experiment was carried out in in controlled conditions regarding the humidity and temperatures (24^{o}C days and 22^{o}C night) in Hortinvest greenhouse, which belongs to the Research Center for Horticultural Products Quality, Faculty of horticulture, UASVM Bucharest, during March-June 2019.
Plants height (cm) was measured and **total biomass** (grams) **accumulated** was determined by weighing cucumber plants in experimental variants.
Capillary dynamolysis: Quality differences between cucumber plants under the influence of experimental conditions of irrigation and fertilizing were evaluated by capillary rising picture method also called capillary dynamolysis, according to the method described by Kolisko (1953) and refined by Zalecka *et al*. (2010). A measured standard quantity of aqueous filtered cucumber leaf extract was migrated on a vertical tube of filter paper in a Kaehlin glass dish, followed by intermediate drying, by metal salt (silver nitrate) and iron sulphate migration and development of specific images. Images obtained after the development of colors were scanned. For the analysis of structures developed in images of capillary dynamolysis were utilized the criteria described by Zalecka *et al*. (2010), Unluturk *et al*. (2011) and Böttgenbach (2018).

Results and discussion

The experimental results confirmed the initial hypothesis and evidenced that structured water exhibited plant growth promoting properties and supplementary added fertilizers (organic and mineral) were also beneficial for the development of cucumber plantlets as compared to those watered with tap water or non-fertilized. The results from biometric parameters measurements revealed that irrigation with structured water significantly increased plant height and fresh biomass accumulation as compared to tap water, the effect being more visible when structured water was associated with organic and chemical fertilization (Figure 1).

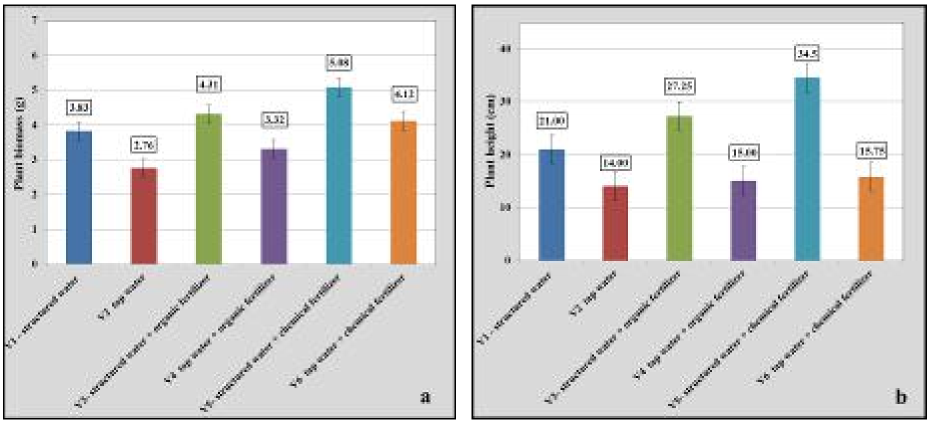

Figure 1. The effect of irrigation water and fertilizers on cucumber plants
a) total plant biomass accumulated b) plant height

The results from the present research confirmed the beneficial effect of structured water on cucumber plants that accumulated significantly more biomass (39%) and were with 50% higher and more vigorous than those watered with tap water. They are in concordance with results from literature reporting improvements in quality, quantity and health of cucumber and other three horticultural plants in Texas, when watered with structured water produced by NAT Structured Water Units from Natural Action Technologies Inc. (Nolte, 2019). Other research brought data on the antioxidant properties of structured water (Higgins *et al*., 2006), improvement of normal cells bioactivities and suppression of malignant cells development (Hwang *et al*., 2017). Sharma *et al*. (2017) listed the benefits of using structured water, including: up to 100% increase fruit/grain/vegetables; up to 100% reduction of chemicals consumption; up to 60% reduction in water usage; improves pests and molds control; increases plant resistance to extreme temperatures; healthier crops and animals; improves soil conditions; enhances taste, texture and shelf life of fruits and vegetables. Visual evaluation of the 6 paper images formed by capillary dynamolysis was carried out. Differences in the aspect of structural elements from both base, bowl and flag zone were observed with evidencing of a higher vital force and quality of plants by stronger, more intensely colored and better-defined images at variants with structured water (Figure 2). A similar pattern of image pairs was recognized for organic fertilizers (Figure 3) and for chemical fertilizers, too (Figure 4). In Figure 2, the colors of the three layers of the basal zone are more pronounced in the image from the sample watered with structured water (left) than in image from the sample watered with tap water (right). Corona is in the first case more irregular, with white beards and interrupted by the base of a long pipe and more regular (uniform parallel layers), without beard in the second image. Bowl zone is more or less diffuse in forms and contrast in both images, the structures from the left are larger, more colored and those from the right are present in compact thin form only on 2/3 of the image. The flag zone from the sample watered with structured water is higher with rare large parallel pipes (flags) narrowed towards the drop zone, open ended, some started from the base, continued in a diffuse grey zone and others formed on the apical third, few being in contact with long brown reduction spots that pass the white zone coming generally in pairs from the thin superior irregular layer. The same zone in image from the sample watered with tap water is shorter than the former, presents the small compact parallel and thin pipes continued with diffuse lighter grey zone without pipes and large, open, irregular flags formed towards a larger white zone ended with a thicker superior layer, with regular contour and reduction spots not in pairs. The pair of images from the samples with organic fertilizer added (Figure 3) revealed the presence of three colored layers in image from the sample watered with structured water, the relative compactness between bowls, the parallel, very large apical openness of the long continuous pipes and narrow end of those formed towards the border of flag zone and only two light colored layers in image from the sample watered with tap water, non-compact structures in bowl zone, with grey middle flags concave ended and large flags formed in apical region. Reduction spots are similar in form with those from the precedent pair of samples non-fertilized, but more dark-colored in the variant with structured water and longer in the variant with tap water. In the pair of images from the variants with mineral fertilizers added (Figure 4), the colors of both basal, bowl and the flag zone are more pronounced in the case of variant watered with structured water than in that with tap water but the differences in form, compactness of bowl structures and flags (pipes) broadness are less expressed than in the case of samples with organic fertilization. Even though a general pattern can be recognized for the fertilization type, the differences induced by irrigation water utilized were: a better zonation of basal zone, generally longer pipes in the flag zone, more pronounced colors and contours with a brown reddish tint and typical rounded reduction spots formed frequently in pairs, characteristic for the structured water and lighter colors, the grey tint of layers and structures from the flag zone and more regular superior margin with pointed reduction spots, not in pairs, characteristic for tap water.

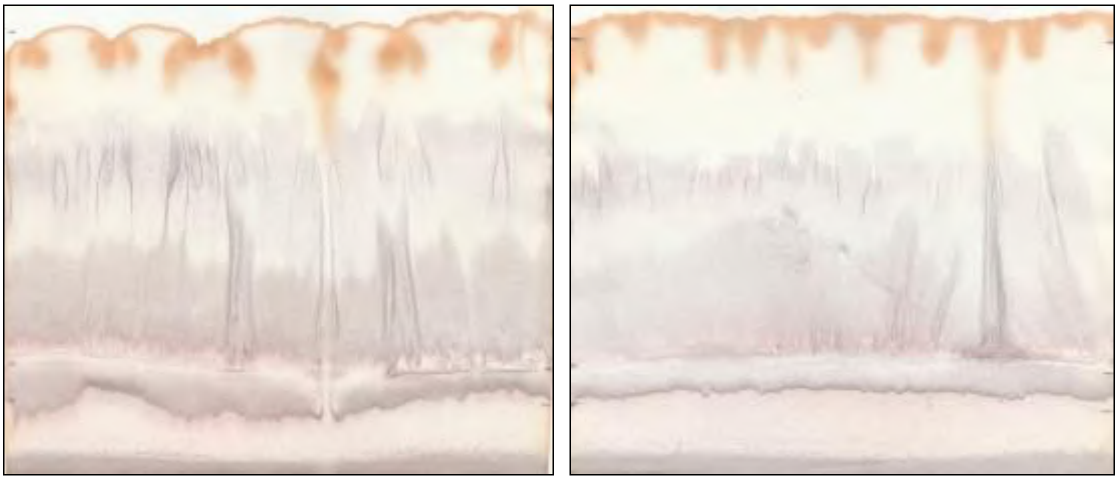

Figure 2. The capillary dynamolysis images from V1 (left) and V2 (right)

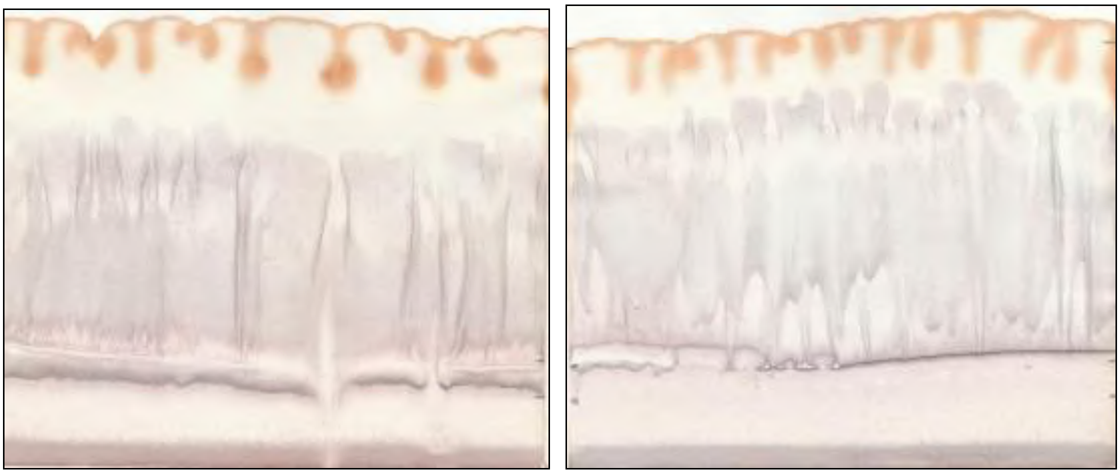

Figure 3. The capillary dynamolysis images from V3 (left) and V4 (right)

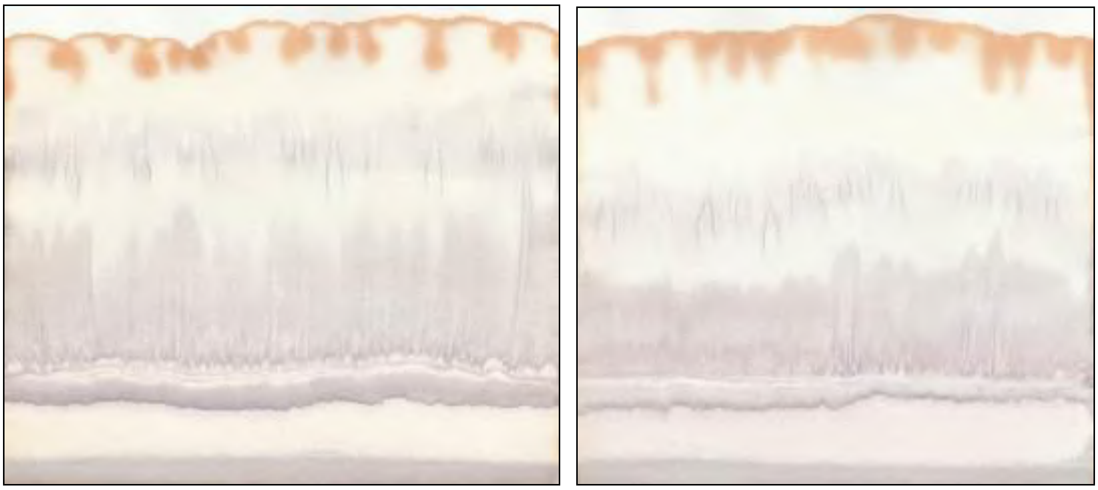

Figure 4. The capillary dynamolysis images from V5 (left) and V6 (right)

Information derived from rising pictures analysis, coupled with biometric data proved its usefulness in comparing the influence of irrigation water and fertilizing systems on cucumber plants. Similarly, Fritz *et al*. (2017) assessed the effects of conversion of vineyard plots from integrated to organic and biodynamic management versus integrated management on grape juice quality using three image forming methods, respectively circular chromatography, capillary dynamolysis and biocrystallization. The parallel application of three methods proved to be useful for successful differentiation and characterization of products from integrated vs. organic and biodynamic production plots and recommend image forming approach as a valuable tool complementary to established methods (chemical analysis) utilized for food quality evaluation. The analysis of images generated with capillary dynamolysis evidenced that for certain samples, images were different in their color in the lower part and by bigger and darker bowl-like forms. Similar to images obtained by circular chromatography, the images produced using the capillary dynamolysis promoted quality characterization with respect to strength of form expression for four samples. As in our interpretation of images, the authors considered that samples yielding images characterized as "strong form expression" were ranked higher than those characterized as "weak form expression" in the qualitative assessment of juice. The image forming methods evidenced the high quality of products from biodynamic plots, followed by organic plots as compared to integrated ones and for the first time, the different application schemes of horn silica were differentiated. The results support other research that proved the use of image forming methods to differentiate the quality of agricultural products from different farming systems or plant production measures (Fritz *et al*., 2011). Authors concluded that the different fertilization systems influence image structures in a reproducible and typical manner and permit the grouping and classification of wheat samples from organic and conventional production plots. Literature cites other trained authors (Skjerbaek *et al*., 2004) capable of visual interpreting and judging the images of capillary dynamolysis and to connect pictures with different farming systems but others used the Gram-Charlier Neural Network (GCNN) methodology for the discrimination of capillary dynamolysis images and successfully applied it to quality assessment of organic and conventional tomatoes (Unluturk *et al*., 2011).

Conclusion

Irrigation with structured water significantly increased plant height and fresh biomass accumulation as compared to tap water, the effect being more visible when associated with organic and chemical fertilization, too. Differences in the aspect of structural elements from both base, bowl and flag zone were observed with evidencing of a higher vital force and quality of plants by stronger, more intensely colored and better-defined images at variants with structured water. A similar pattern of image was recognized for organic fertilizers and for chemical fertilizers, too. Information derived from rising pictures analysis proved its usefulness in comparing the influence of irrigation water and fertilizing systems on quality of cucumber plants. Further investigations are necessary to link capillary dynamolysis results with other image forming methods and chemical analysis for better elucidating the nature of quality differences obtained.

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HARVEST TIME IMPACT ON SHATTERING HABIT AND MILLING QUALITY OF RICE CULTIVARS

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Abstract

During harvest season, grain shattering is an important trait which affects both yield and milling quality of rice crop. A two-year study was carried out in order to investigate the variation of grain shattering habit and head rice yield at different harvest times. The experiments were laid out in a factorial based on completely randomized design with five replications in order to examine the effects of rice varieties (Hashemi, Khazar, and Ghohar), harvest time (26, 28, 30, 32, and 34 days after 50% flowering), and grain position on the panicle (upper, middle, and lower portions) on rice grain pedicel breaking tensile strength (BTS) and head rice yield (HRY). The results revealed that the highest and lowest BTS were associated with Hashemi and Ghohar with an average of 0.60 and 0.35 N, respectively. With delaying in harvesting, grain detaching force from the pedicel decreased by approximately 50% among the earliest and the latest harvest time. Because of the non-uniformity in grain maturity, there was a nearly 30% variation in BTS of the grain pedicel for upper and lower portions of a single panicle. For selected intervals, harvesting time had no significant effect on HRY by itself but interacted significantly with other parameters. Rice variety affected HRY ($p<0.01$), whereupon other variables could intensify its effect.

Keywords: *Rice, shattering habit, harvest time, breaking tensile strength, milling*.

Introduction

Grain shattering is defined as detachment of a grain from its pedicel at relative ease during maturity. Shattering habit as an impressive trait affects harvest efficiency. Easy shattering grains cause remarkable yield loss within harvest period. Also, hard shattering varieties lead to higher threshing force and machine power requirement in consequence. Mainly, rice grain shattering is influenced by its genetic characteristics but such parameters as climate conditions, nourishing, pests and diseases, harvesting implements and machines, and harvest management can affect it. Therefore, in breeding, scientists should select a cultivar which has an appropriate shattering threshold. Before releasing a cultivar, conducting experiments regarding its engineering properties is necessary in order to recognize it thoroughly. Having such information, it would be possible to manage harvesting so that crops encounter the minimum damage and losses.

Studies have been accomplished by researchers to characterize engineering aspects of rice shattering habit. Ichikawa *et al.* (1990) introduced two testing devices and examined shattering habit of 42 varieties. Kawamura *et al.* (2002) used a tensile tester to measure detaching force of a single grain from stem. In a research, shattering habit was evaluated by using the grain detachment force in two forms of parallel and perpendicular pull (Inoue *et al.*, 2003). In an attempt, Ji *et al.* (2006) employed breaking tensile strength (BTS) to measure the degree of shattering at different intervals after heading. Oduori *et al.* (2008) developed a model to evaluate shattered rice grain loss in a combine header. Their model consisted of crop properties and interactions of reel and crop. Lamo *et al.* (2011) developed a hand-held, single grain shattering tester suitable for field assessment of shattering habit. In an investigation, a

universal testing machine was employed to determine the BTS of the rice grain pedicel instead of conventional shattering tester devices (Matsushita *et al.*, 2012).
Another issue is the role of various factors on rice quality properties. Among them, appropriate harvest time has a great importance in preventing crop loss. In a research by Lu *et al.* (1995), it was revealed that rice harvested at moisture contents lower than 15% or higher than 22% encountered significant gross income reduction. Sürek and Beşer (1998) announced that the maximum grain yield and white rice, the minimum breakage during milling, the least unfilled and partially filled grains, acceptable moisture content, and the minimum chalkiness in white rice obtained at 49 days after flowering (DAF) in Turkey. Thompson and Mutters (2006) reported that average rice moisture content could not be an adequate predicting measure for head rice yield (HRY). Siebenmorgen *et al.* (2007) found out that the optimum harvest moisture contents in which head rice yield had the highest value varied from 18.7 to 23.5% and 21.5 to 24% for long and medium grains, respectively. Hossain *et al.* (2009) concluded that aromatic rice should be harvested at 30 to 35 DAF in order to have higher HRY, elongation, volume expansion ratio, and amylose.
This study aimed to investigate rice shattering habit through measuring the BTS of the grain pedicel at different harvest times, grain shedding trend for a single panicle, and the effect of various harvest time on head rice yield.

Materials and Methods

This study was conducted at Rice Research Institute of Iran (RRII), Rasht, Iran over two cropping season of 2016 and 2017. Rice varieties were Hashemi (a local cultivar), Khazar and Ghohar (two improved cultivars). Samples were collected from fields with proper uniformity. The BTS of the grain pedicel was measured by a force gauge (Lutron, FG-5000A, Taiwan) mounted on a specially designed platform. The same procedure described by Alizadeh and Allameh (2011) was followed.

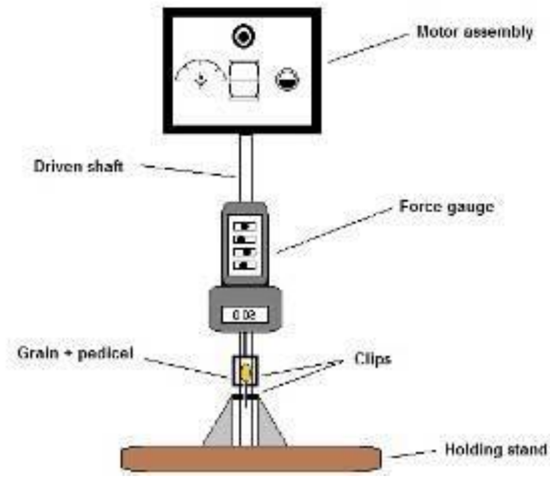

Figure 1. Schematic diagram of the BTS testing device

After finishing the BTS experiments, milling test was commenced. The milling tests were performed as the method mentioned by Allameh and Alizadeh (2013). The experiments were conducted in the form of factorial based on complete randomized design (CRD) in five replications. Independent variables were as rice variety (at three levels of Hashemi, Khazar, and Gohar), harvest time (at five levels of 26, 28, 30, 32, 34 days after 50% flowering), and the grain position on the panicle (at three levels of upper, middle, and lower portions). Dependent variables were defined as the breaking tensile strength (BTS) of the grain pedicel and HRY. Data analysis was performed by the SAS 9 software (2004, SAS Institute, USA).

Results and Discussion

Data analysis indicates that the effect of year, variety, harvest time, and grain position on the panicle is significant on BTS of the grain pedicel. Table 1 displays mean values of BTS for two years of study. The highest force (1.14 N) required for separating a grain from its pedicel belonged to Hashemi cultivar at the first harvest time of the second year of the study. In case, the lowest one related to Khazar cultivar harvested at 32 DAF of the first year of the study. The downswing of the required force for detaching a grain from its pedicel is clearly viewed in the second year of the study in particular.

Table 1 Mean value of BTS at different harvest times (N)

year	Harvest time (DAF)	variety		
		Hashemi	Khazar	Ghohar
1	26	0.33^{jk}	0.15^{p}	0.33^{jk}
	28	0.33^{jk}	0.2^{no}	0.3^{kl}
	30	0.34^{j}	0.16^{p}	0.27^{lm}
	32	0.23^{mn}	0.14^{p}	0.23^{mn}
	34	0.25^{lm}	0.16^{p}	0.17^{op}
2	26	1.14^{a}	0.81^{d}	0.75^{e}
	28	1.05^{b}	0.58^{f}	0.53^{g}
	30	0.94^{c}	0.61^{f}	0.44^{hi}
	32	0.82^{d}	0.47^{h}	0.32^{jk}
	34	0.59^{f}	0.43^{i}	0.2^{no}

Common letters represent no significant difference at 1% level.

There was a significant difference between the two years of study. The reason was that in the first year, due to an unintentional incident, it became inevitable to use samples of a late cultivated crop. Chosen varieties cultivated with a delay of roughly 30 days compared to normal. Therefore, harvest time faced to seasonal rainfalls that resulted in damage to rice plants. Cultivars have behaved differently. Hashemi, a locally grown cultivar, has medium shattering characteristic genetically, while Khazar and Ghohar, high yielding cultivars, are easy shattering. In other words, by applying a lower force, grain will detach from its pedicel and shed. Scientists suggest that genetic rules on shattering habit and other factors rank next to it (Ji *et al.*, 2006; Lin *et al.*, 2006). Investigations demonstrate that the development of abscission layer is in association with shattering habit and in easy shattering varieties, it is completely formed (Fukutae *et al.*, 1994; Ji *et al.*, 2010; Okubo, 2014). Ichikawa *et al.* (1990) expressed that easy shattering varieties needed force less than 1 N to separate grain from its pedicel. In Figure 2 the variations of the BTS of the grain pedicel has been illustrated based on grain position on the panicle. It could be clearly seen that for every single cultivar, the grain position on the panicle had a significant effect on the required force for detaching the grain from its pedicel. From top to down, there was an increasing trend for detaching force.

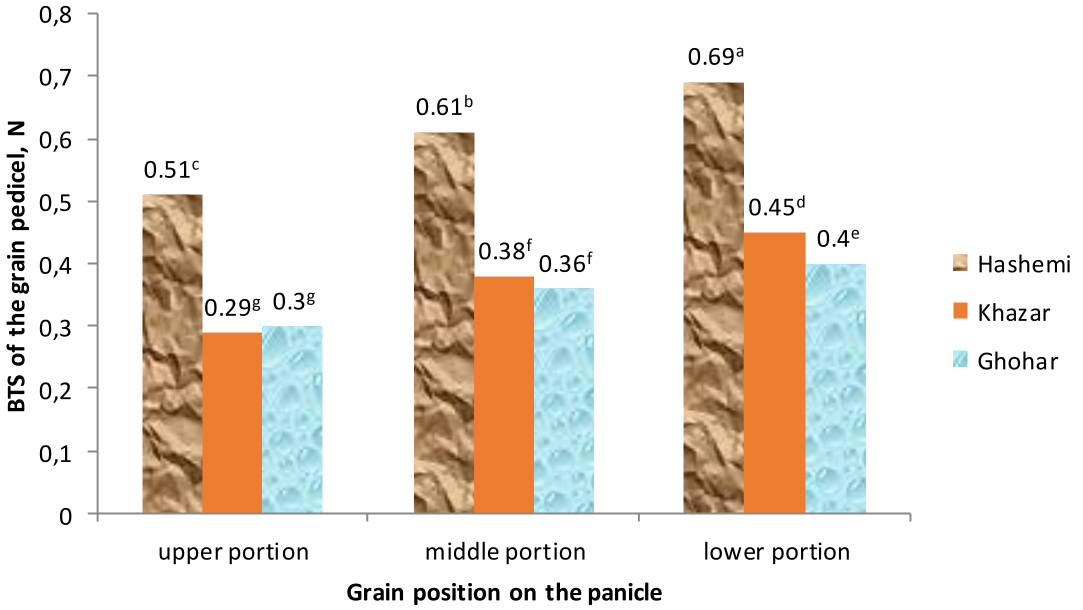

Figure 2. Variations of BTS of the grain pedicel across a panicle

On a panicle, the grain maturity starts from upper portions to lower ones. Upper grains mature soon, lose their moisture content and become yellowish. Hence, they would separate from the pedicel by applying less force. Whereas, lower parts of a panicle have immature or ripening grains which are greenish. Some researchers have pointed out this in their studies (Suastawa *et al.*, 1996; Szot *et al.*, 1998; Kawamura *et al.*, 2002; Ji *et al.*, 2006; Choe *et al.*, 2013; Tang *et al.*, 2015). Head rice yield variations of tested cultivars are illustrated in Figure 3. The maximum variation is related to Hashemi cultivar (nearly 10%) due to late cultivation in the first year. Late cultivation made rice crop encounter seasonal rainfalls which in turn caused lodging and damaging the grains. This has been reported by others (Ghosh *et al.*, 2004; Sha and Linscombe, 2007). Many studies have also referred to the effect of variety on HRY (Zhao and Fitzgerald, 2013; Zhou *et al.*, 2015).

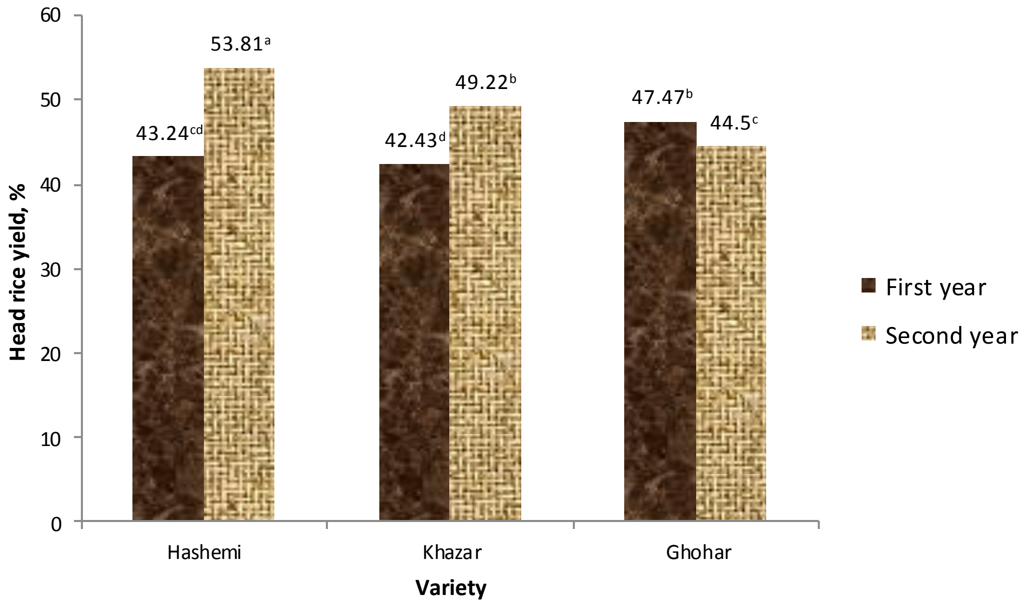

Figure 3. Interaction of year and variety on HRY

In Figure 4, HRY variations have been shown in terms of different harvest time. Over the time interval assigned for this study, with the exception of Hashemi cultivar that its growth period completes nearly 30 DAF and whole the panicle becomes uniformly yellowish, the two other cultivars do not have such a situation. Khazar and Ghohar are among late matured cultivars and it takes 5-10 days longer in comparison with Hashemi. This usually coincides with seasonal rainfalls which causes grain damage and crop loss. For Ghohar cultivar, while upper parts of the panicle turned totally yellow, lower parts were still green or greenish. This conformed to other investigators (Siebenmorgen *et al.*, 2007; Hossain *et al.*, 2009).

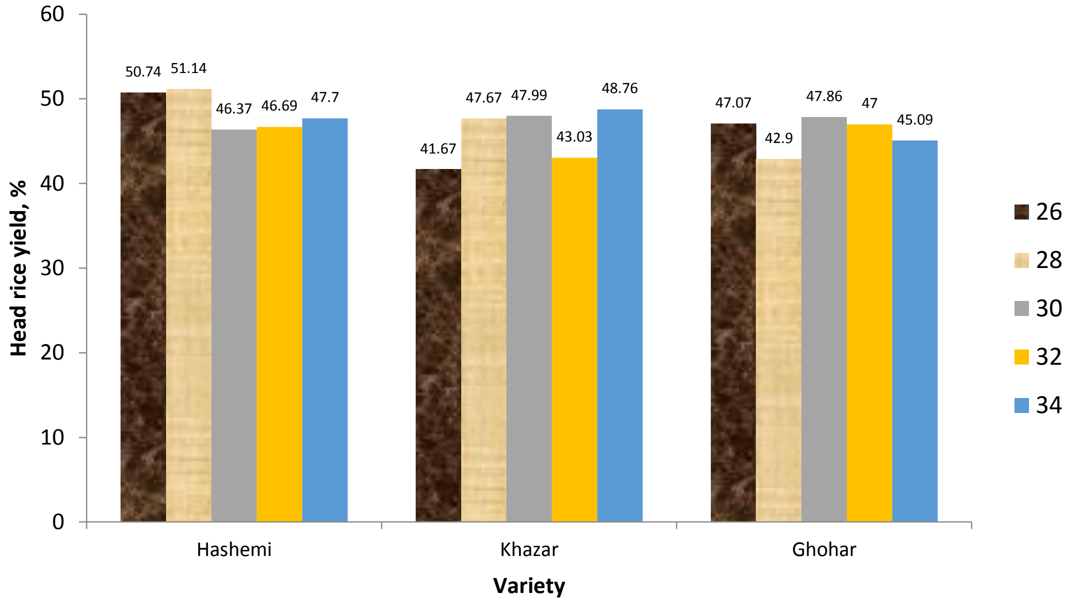

Figure 4. HRY variations at different harvest times

Conclusions

According to the results obtained, the followings could be summarized:

- Among the cultivars studied in this research, Hashemi had the highest mean BTS (0.6 N) but the lowest belonged to Ghohar (0.35 N).
- The more delay in harvest, the lesser would be BTS and the more shedding in consequence. Within the time interval in this study, a reduction of nearly 50% in BTS of grain pedicel occurred from the first to the last harvest time.
- Grain maturity will not proceed across an individual panicle uniformly. On the average, there was a rough difference of 30% in BTS of grain pedicel between the upper and lower portions of a single panicle.
- In each cropping season, planting date had significant effects on BTS and HRY. Late cultivation, through creating specific physiological circumstances, led to decrease in BTS of grain pedicel and easier shattering of the grain subsequently. In addition, coinciding harvest time with seasonal rainfalls lowered milling quality. For instance, Hashemi experienced an approximate reduction of 10 percent in HRY because of late cultivation.

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ANALYSIS AND CHARACTERISTICS OF THE NEW VIRGINIA HYBRID LINES IN CMS FORM

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Abstract

V-88/09 CMS F1, V-120/15 CMS F1 and V-79/09 CMS F1 are male-sterile hybrid lines of Virginia tobacco created during 2009-2017 at the Scientific Tobacco Institute-Prilep by inter-varietal hybridization. Following the previously obtained data on their characteristics, they were included in the comparative trials carried out in the experimental field of Tobacco Institute –Prilepin 2012-2016. Foreign tobaccos were included in the trial as check varieties. Throughout this period, the lines V-88/09 CMS F_1, V-120/15CMS F_1 and V-79/09 CMS F_1showed better bio-morphological and production traits in comparison with the check varieties. It allowed them to overcame all other lines and varieties included in the trials. The time of flowering, the length of the growing period and the stalk height in these lines are characters typical of Virginia tobacco. Excellent results were obtained with regard to yield and quality (average price USA $ / kg) and to the gross income (USA $ / ha). In most of the traits, these lines exceeded the control varieties and achieved statistical significance level of 1% and 5%. The purpose of this paper is to inform the domestic and general public with the achievements in this area, in hope to raise their attention and interest. Data presented here shall recommend these lines to be enrolled in the National list of varieties.

Key words: *tobacco, Virginia, line, traits.*

Introduction

Virfinia tobacco is the most common type of tobacco in the word. Raw material of this tobacco participates with 60-70 % in the composition of modern cigarette blends. Which require precisely determined agro-ecological conditions and cultural practices for their growth and development. The Virginia tobacco raw material is an inevitable component of modern cigarette brands, participating with varying percentage in their composition. The conditions for obtaining a good yield and quality of this tobacco are: good variety, strictly controlled production and proper curing, typical of the type. According to Beljo J. (1996) and Uzunoski M. (1985), the Virginia tobacco belongs to the group of high tobaccos (about 200 cm), which require precisely determined agro-ecological conditions and cultural practices for their growth and development, including the specific method of drying (flue-curing). In R. Macedonia the production of Virginia tobacco was ceased in 2002 and this discontinuance has been unjustifiably extended until now. In the production chain of this tobacco, variety is highly important segment which determines the yeld and quality of the raw material. Tobacco Institute – Prilep has created a number of hybrid lines in CMS form. The solution for this problem is only in the restart of production. In the production chain of this tobacco, variety is highly important segment which determines the yield and quality of the raw material. Tobacco Institute – Prilep has created a number of hybrid lines in CMS form. The results of comparative studies with other varieties and lines show that they can be of interest to potential primary producers.

Material and methods

Cultivation and testing of some newly created lines of Virginia tobacco was carried out at the experimental field of Tobacco Institute - Prilep in the period 2009/2017. Prior to hybridization and creation of a new line, the goals were set up and parental pairs were determined. Breeding lines in male-sterile form (minimum F7 generation) were used as mothers, and fertile varieties and lines (also of min. F7 generation) were used as fathers. The newly obtained progeny (line) is also male-sterile (unisexual), indicating that this trait is inherited by the mother line, in which the heterotic effect is strongly expressed. Hawks S.N. (1978) and Gornik R. (1973) reported that F1 hybrids in male sterile form are characterized by higher yields, higher resistance to some diseases, early maturation etc. The results presented in the tables are summarized average values from the comparative trials in which these lines were included. Statistical data analysis was made and the above lines showed significant differences at a level of 5% and 1% compared to the check varieties, but they also dominated over the other investigated varieties and lines. Conversion in US$ was calculated by middle exchange rate of National Bank of Republic of Macedonia on 22.08.2019 55.38 den. For 1 US$.

Results and discussion

Some more important traits of the newly created Virginia tobacco lines included in the investigation are presented in Table 1.

Table 1. Flowering and length of the growing season

Line	Beginning of flowering, in days	50% flowering	End of flowering, in days	Length of the growing season, in days
V-79/09 CMS F_1	72	77	82	125
V-120/15 CMSF_1	68	73	77	118
V-88/09 CMS F_1	67	73	79	120

- Beginning of flowering – Flowering and formation of seed to prolong the species is a natural phenomenon also present in tobacco. The hybrid lines that are subject of this research are blooming but they do not form a seed. Rubin B. A. (1971) concluded that the first flower that blooms is the central (top) flower of the inflorescence. Naumoski K. et al. (1977) reported that tobacco plant has already built 90% of its plant mass by the end of flowering. Data in Table 1 show that line V-88/09 CMS F_1 ends with flowering in 67 days, line 120/15 CMS F_1 in 68 days and line V-79/09 CMS F_1 in 72 days.
- 50% flowering – After the beginning stage, the process of flowering is intensified and in just a few days (5-7) 50% of flowering is reached. Lines V-120/15 CMS F_1 and V-88/09 CMS F_1 reach this stage in 73 days and line V-79/09 CMS F1 in 77 days. According to Beljo (1996), all investigated lines belong to the group of tobaccos with moderate time for 50% flowering.
- End of flowering - It is varietal and type characteristic strongly affected by the soil and climate conditions, applied agricultural practices, day length, etc. Risteski and Kocoska (2014) in investigation of 7 domestic and foreign tobacco varieties and lines in the region of Prilep in 2010/2011 reported that the end of flowering occurs after 71.5 - 79 days. S.N. Hawks (1978) reported that in some Virginia varieties with so-called mammoth characteristics the beginning and end of flowering stage occurs later, as a result of the day length. The data in

Table 1 show that the line V-120/15 is the first to finish the flowering (77 days) and the line V-79/09 is the last (82 days).

- Length of the growing season – This trait is measured in days from the day of planting until the day when the last leaves are harvested. Data in Table 1 show that the length of the growing season ranged from 118 days in line V-120/15 CMS F_1, 120 days in line V-88/09 CMS F_1 and 125 days in line V-79/09 CMS F_1. According to Beljo (1996), all these lines belong to the group with early to moderate growing season.
- Morphological features

Table 2. Morphological traits

Line	Leaf size Length/Width, cm			Leaf number	Stalk height, cm
	5th leaf	10th leaf	15th leaf		
V-79/09 CMS F_1	52×37	69×40	65×35	34	213
V-120/15 CMS F1	54×39	64×42	61×33	31	207
V-88/09 CMS F_1	55×34	66×41	60×30	33	186

- Leaf size – it is very important that the length of the middle belt leaves (5th-15th) in Virginia tobacco exceeds 35 cm, because it is the first condition for them to be ranked as a first class tobacco. Dražić S. et al. studied 13 genotypes of Virginia tobacco in Nova Pazova (Serbia) in 2011 and found that the average size of the middle-belt leaves was 64 cm length and 30 cm width. Kalamanda O. (2009) in Republika Srpska performed 3-year investigations with two Virginia tobacco varieties and reported that the middle belt leaf had 48.7 cm average length and 22.7 cm average width. From the data presented in Table 2 it can be seen that the leaf length ranged from 52 cm in the 5th leaf of the line V-79/09 CMS F_1 to 69 cm in the 10th leaf of the same line. In other lines, this trait ranges from 54 cm in V-120/15 CMS F_1 to 66 cm in V-88/09 CMS F_1. The largest width of 42 cm (10th leaf) was measured in line V-120/15 CMS F1 and the smallest width of 30 cm (15th leaf) was found in line V-88/09 CMS F_1. According to the established Purchasing rules and due to the adequate length and width of their leaves, all the analyzed lines are ranked in the Class I.
- Leaf number is genetically controlled character which is also affected by the length of the day, applied cultural practices, soil and climate conditions, etc. Devcic K. and Triplat J. (1982) in their trials with 3 varieties of Virginia tobacco (H-30, H-31 and H-32) in three locations in Croatia, reported that the leaf number ranged from 22 in H-31 and H-32 to 23 in H-30. Dražić S. et al. (2012) investigated 13 genotypes of Virginia tobacco in Nova Pazova during 2011 and found that the number of leaves ranged from 22 to 26. According to the data in Table 2, the largest leaf number (34) was counted in line V-79/09 CMS F_1 and the smallest (31) in V-120/15 CMS F_1. In line V-88/09 CMS F_1 the number of leaves was 33. According to Beljo J. (1996), all analyzed lines belong to the group of multifoliate tobaccos.
- Stalk height - Virginia tobacco belongs to the group of high tobaccos. Risteski I. (1999) in his investigations of Virginia variety MV-1 in Prilep found that, depending on the size of nutrient area, the height of the stalks averaged between 193 cm and 213 cm. Devčić K. et al. (1982) reported that the height of the Croatian varieties H-10, H-31 and H-32 was 170 cm. Data presented in Table 2 reveal that the largest average height (213 cm) was measured in line V-79/09 CMS F_1 and the smallest (186 cm) in line V-88/09 CMS F_1. The height of the stalk in line V-120/15 CMS F1 was 207 cm. In all the analyzed lines this trait is within the range cited in literature and is typical of the Virginia tobacco.
- Yield and quality

Data on this characteristic are presented in Table 3.

Table 3. Yield and gross income

Line	Yield kg/ha	Average price, USA $ kg	Gross income, USA $ ha
V-79/09 ЦМС F_1	3450	1.28	226 158
V-120/15 ЦМС F_1	3180	1.23	251 788
V-88/09 ЦМС F_1	3591	1.22	242 825

-Yield per hectare - In addition to the genetic properties of the type and variety, the yield is closely related to soil and climate conditions where tobacco is grown, applied agrotechnics, etc. Risteski I. and Kocoska K., (2012) reported that in 1987 the yield of the first recognized Macedonian Virginia variety MV-1 was about 2,500 kg. In the process of selection, the yield in all further lines increased gradually and presently it is about 3590 kg in line V-88/09 CMS F_1. Dražić S. et al. (2012) studied 13 varieties and lines in the location of Starchevo, Serbia, in which the yield varied between 1710 kg/ha and 4030 kg/ha. S.N. Hawks (1978) presented the movement of yields in the United States by periods. Thus, in the period 1934-1938 the average yield was only 959 kg/ha, and in the period 1964-1967 it raised up to 2224 kg/ha. The data in Table 2 show that the yield per hectare in the analyzed lines ranged from 3693 kg/ha in line V-120/15 CMS F_1 to 3591 kg/ha in line V-88/09 CMS F_1, while in V-79/09 CMS F_1 it was 3450 kg/ha. The above data indicate that the yield obtained in the analyzed lines is quite satisfactory and is typical of the hybrid varieties and lines.

- Average price - The quality of tobacco raw material determines the average price per 1 kg. It is highly affected by the variety, proper and timely applied agrotechnics, yellowing, curing and other factors. The data presented in Table 3 show that the average price ranged from 1.22 USA $ /kg in line V-88/09 CMS F_1 to 1.23 USA $ /kg in line V-120/15 CMS F_1 and 1.28 USA $ /kg in line V-79/09 CMS F_1. According to the above data, it can be concluded that there are no major differences in terms of price or quality between lines. In the period of investigation (2012 – 2016), the lines included in the trials achieved higher average price compared to other varieties and lines.

- Gross income - economic effect - This trait is a summarized expression of the average price per 1 kg of raw tobacco and the yield per hectare achieved by the lines represented in the trial. The data in Table - 3 reveal that line V-120/15 CMS F_1 had the highest gross income (251 788 USD $ ha), primarily due to the high yield per hectare. This parameter in line V-88/09 CMS F_1 was 242 825 den/ha and in line V-79/09 CMS F_1 it was 226 165 den / ha.

Conclusions

Based on the results of the research, the following statements can be drawn:
According to the time required for completion of the flowering stage, the analyzed lines can be included in the group of tobaccos with moderately long time of flowering.
With regard to the length of the growing season, all analyzed lines belong to the group of tobaccos with early to moderate length of growing season.
According to the leaf size (length and width) and in compliance with the Purchasing rules, the investigated lines can be ranked in Class. Regarding the number of leaves (31 to 34), the studied lines belong to the group of multifoliate tobaccos.

According to the stalk height (186 cm - 213 cm), all three lines belong to the group of high tobaccos.
The yield per hectare is relatively high (3450 kg/ha - 3693 kg/ha), which is considerably good result, typical of hybrid varieties and lines.
The amounts of the average price and gross income guarantee a stimulating production of these lines.

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EVALUATION OF ANDROGENIC COMPETENCE OF DIFFERENT PEPPER, TOMATO AND EGGPLANT GENOTYPES

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Abstract
The methods of biotechnology such as androgenesis introduce new possibilities for faster creation of new varieties or at least faster development of improved genotypes with desirable traits that can give an answer to new abiotic and biotic challenges in agricultural production. Androgenesis is a method that opens possibilities for development of haploids and spontaneous dihaploids plants via anther culture. *In vitro* anther culture utilized for gaining haploid/dihaploid plants serves as a tool for improvement of some solanaceous crops such as tomato, eggplant and pepper, but it always faces obstacles for high productivity of regenerants in those crops. In the present study, androgenic competence of 3 pepper genotypes (Edita, Bela Duga and Homera), 3 tomato genotypes (Bellfort, Rally and Policarpo) and 1 eggplant genotype (Domaci srednje dugi) were evaluated. The pepper anthers were cultured according to the method developed by Dumas de Valux *et al.* (1981), tomato anthers were cultivated according to Corral-Martínez *et al.* (2011), while eggplant anthers were cultivated according to Dumas de Valux and Chambonnet (1982). The experiment showed that androgenesis was successfully implemented only in pepper genotype Edita, whereas the eggplant genotype did not show any response to anther induction media. Cultivation of anthers from all tomato genotypes resulted only in callus formation. Our results are one more confirmation that androgenesis applied on pepper, tomato and eggplant has its limitations and the successfulness of androgenesis depends on many factors as growing conditions and donor plant age, donor plant genotype, microspore developmental stage, culture media and cultivation conditions.

Keywords: *Androgenesis, Anther culture, Capsicum annuum, Lycopersicon esculentum, Solanum melongena*

Introduction
Androgenesis is one of the successful biotechnological methods that is included in the group of novel techniques for improving agricultural crops and it can be combined with other biotechnological methods for achieving new selection goals (Koleva Gudeva *et al.*, 2008; Irikova *et al.*, 2016). It is considered as the fastest way to achieve homozygosity and to get homozygotic lines, where dihaploids have many advantages for their involvement in fundamental and breeding research (Koleva Gudeva *et al.*, 2007a; Seguí-Simarro, 2016). Nevertheless, obtaining regenerated and fertile androgenetic plants in the Solanaceae horticulture crops is still low (Seguí-Simarro, 2016).
Generally, there are a small number of scientific publications related to the full regeneration of the androgenetic solanaceous plants, their characterization and evaluation and direct participation in selection programs for scientific and commercial purposes. According to Seguí-Simarro *et al.* (2011), pepper (*Capsicum* sp.) is characterized as a third-class crop by fam. Solanaceae which is "resistant" to methods of androgenesis, after tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melangena* L.). The aim of our research was to test and establish effective androgenesis protocols for pepper, tomato and eggplant.

Material and Methods

Pepper anther culture

Three pepper (*Capsicum annuum* L.) varieties were used as anther donors in the experiment: Edita (sweet, long type), Bela Duga (sweet, long type), Homera (long, hot type). The flower buds were harvested when the corolla was of the same length as the calyx or slightly longer. Flower buds were surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water. After the removal of the filaments, anthers from three flower buds were placed in Petri dish, with the concave face down, touching the culture medium. The media employed for anther culture was: Cp (Dumas de Valux *et al.*, 1981) supplemented with kinetin (0.01 $mg \cdot l^{-1}$) and 2.4-D (0.01 $mg \cdot l^{-1}$). The anthers cultivated on Cp medium with the supplementary hormones were incubated for 8 days at 35±2 ^{o}C in the dark, and then transferred to 25 ± 2 ^{o}C with 12 h photoperiod. After 12 days of induction on Cp medium, the anthers were transferred each month onto fresh R_1 medium supplemented with 0.01 $mg \cdot l^{-1}$ kinetin and simultaneously the deteriorated or infected anthers were removed. The cultures were observed regularly, and the data were recorded every week. The frequency of callus formation and the number of emerged embryoids were recorded. Young shoots emerging from the anthers were transferred onto hormone free V3 media in order roots to be formed.

Tomato anther culture

Three tomato (*Lycopersicon esculentum* Mill.) varieties were used as anther donors in the experiment: Bellfort (rounded shape), Rally (rounded shape) and Policarpo (plum-shaped tomato). Buds varying in length were collected from flowering plants and they were surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water. The anthers were dissected and plated on the MS induction medium prepared according to Corral-Martínez *et al.* (2011). It consisted of MS basal medium + vitamins (Murashige and Skoog 1962), supplemented with 2.5 $g \cdot l^{-1}$ Phytagel, 20 g/l sucrose, 1 $mg \cdot l^{-1}$2ip and 2 $mg \cdot l^{-1}$ IAA, pH 5.7. Petri dishes were kept in a growth cabinet at 25 ^{o}C, in darkness for 1 month, and then under a 16/8 photoperiod. Anthers and developing calli were transferred to fresh medium on a monthly basis. Green or partially green, proliferating calli were transferred to regeneration medium composed of 4.4 g/l MS medium plus vitamins, 2.5 $g \cdot l^{-1}$ Phytagel, 20 $g \cdot l^{-1}$ sucrose and 0.25 $mg \cdot l^{-1}$ zeatin, pH 5.7.

Eggplant anther culture

One eggplant (*Solanum melanogena* L.) genotype, Domaci srednje dugi, was used as anther donor genotype. Flower buds of the certain size were harvested from anther donor plants and surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water and immediately dissected. Anthers were cultured according to Dumas de Vaulx and Chambonnet (1982) on Ct inductive medium. The Ct medium was supplemented with 0.01 $mg \cdot l^{-1}$ 2,4-D and 0.01 $mg \cdot l^{-1}$ kinetin. Anthers were inoculated in petri dishes on Ct inductive medium and cultured for 8 days in darkness at 35^{o}C. Then, they were transferred to light (12 h light/12 h darkness photoperiod) and 25 ^{o}C for 4 more days. At day 12, anthers were transferred to R_1 medium supplemented with 0.01 mg/l kinetin, where they were cultured indefinitely at 25 ^{o}C, with medium refreshing every 20 days.

Results and Discussion

The results of androgenic response of different pepper genotypes are presented in Table 1 and Figure 1. On the inductive Cp medium were incubated 555 anthers from Edita, 619 anthers from Bela Duga and 640 from Homera with mean anther length of 3.3; 3.3 and 3.2 mm, respectively. The cultivated anthers of three varieties responded with callus formation with different percentage as Edita (12.7%), Bela Duga (11.6%) and Homera (17.2%). The percentage of embryogenic anthers varied from 0.22% for Bela Duga and 2.3% for Edita. Only for the variety Edita there was successful regeneration of 5 embryo into plants. As expected, the anthers of the hot variety Homera did not responded with embryo formation.

Table 1. Androgenic response of different pepper genotypes (*Capsicum annuum* L.) incubated on Cp medium + 0.01 mg·l^{-1} kinetin and 0.01 mg·l^{-1} 2.4-D (+35 ^{o}C, 8 days in darkness).

Species / Variety	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Edita	555	3.3	12.7	2.3	5
Bela Duga	619	3.3	11.6	0.22	/
Homera	640	3.2	17.2	/	/

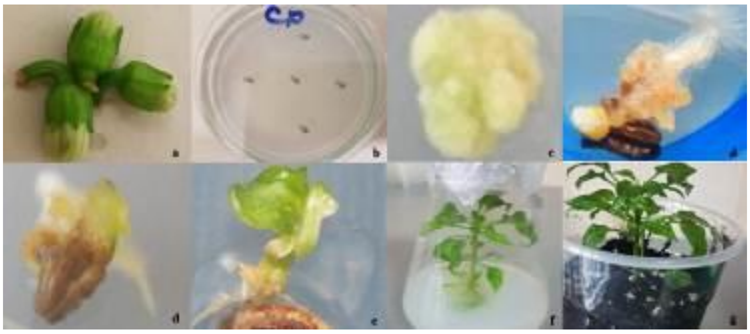

Figure 1. a) pepper buds collected for anther isolation b) anthers inoculated on R_1 medium developing c) callus developed from anther d) emerging androgenic embryo e) fully developed androgenic embryo f) regenerated androgenic plant on V_3 medium g) acclimatized androgenic plant.

According to Koleva-Gudeva *et al.* (2007a,b), the process of embryo formation on different media under different thermal conditions showed that the formation of haploid embryos occurred only in the CP medium exposed to heat thermal stress (+35 ^{o}C) and hot pepper genotypes are low in androgenic response which is in agreement with our results findings (Koleva Gudeva *et al.*, 2007a). Even with difficulties and low efficiency of pepper, there are several laboratories that successfully applied pepper androgenesis and fully regenerated androgenic plants which are characterized either phenological or/and molecular as the Hungarian group (Mitykó and Gémes Juhász, 2006); the Macedonian group (Trajkova, 2013; Trajkova and Koleva Gudeva, 2017); the Bulgarian group (Todorova *et al.*, 2013); the Korean group (Luitel *et al.*, 2012) and the Polish group (Olszewska *et al.*, 2015; Nowaczyk *et al.*, 2015).
The results of androgenic response of different tomato genotypes are presented in Table 2 and Figure 2. On inductive MS medium were incubated 80 anthers from Bellfort, 49 anthers from Rally and 64 from Policarpo with mean anther length of 2.4; 3.0 and 3.5 mm, respectively. The anthers of these three varieties responded with callus formation with different percentage as Bellfort (63.8%), Rally (28.6%) and Policarpo (28.1%). There was no embryo formation from tomato anthers from all three varieties, consequently no regenerated androgenic plants.

Table 2. Androgenic response of different tomato genotypes (*Lycopersion esculentum* Mill.) incubated on MS medium + 1 $mg \cdot l^{-1}$ 2ip and 2 $mg \cdot l^{-1}$ IAA (+25 °C, 1 month in darkness).

Variety	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Bellfort	80	2.4	63.8	/	/
Rally	49	3.0	28.6	/	/
Policarpo	64	3.5	28.1	/	/

Figure 2. a) tomato buds collected for anther isolation b) inoculated anthers on MS medium c-d) calli developing from anthers.

In tomato, anther culture has also been demonstrated possible, but for very few genotypes. Seguí-Simarro (2016) highlight that mostly two key bottlenecks impose strong limitations to the efficiency of double haploids technique in recalcitrant solanaceous crops as induction efficiency and embryo development, where it is widely known that the genotype is the most influential factor. Seguí-Simarro (2016) discussed that only two laboratories have published the complete regeneration of entire tomato plants with a demonstrated haploid or DH origin (Shtereva *et al.*, 1998; Zagorska *et al.*, 2004; Seguí-Simarro and Nuez, 2005, 2007; Corral-Martínez *et al.*, 2011).

The results of androgenic response of one eggplant variety are presented in Table 3. On inductive Ct medium were incubated 144 anthers with mean anther length of 2.4 from eggplant genotype Domaci srednje dugi. The utilized medium and incubation conditions did not induce any response of cultivated anthers.

Salas *et al.* (2011) published successful protocol for eggplant androgenesis where responding anthers produced both calli derived from anther tissue and embryos derived from microspores, suggesting that the protocol established by Dumas de Vaulx and Chambonnet (1982) it's not always a source of androgenic competence among different eggplant genotypes.

Table 3. Androgenic response of eggplant (*Solanum melongena* L.) incubated on Ct medium + 0,01 $mg \cdot l^{-1}$ 2,4-D and 0,01 $mg \cdot l^{-1}$ kinetin (+35 °C, 8 days in darkness).

Genotype	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Domaci srednje dugi	144	2.4	No response	No response	/

However, our results showed that the same protocol did not worked for the eggplant genotype Domaci srednje dugi and no anthers response was reached. On the other hand, Rivas-Sendra *et al.* (2017) evaluated androgenic capacity through microspore culture of the eggplant commercial F1 hybrid Bandera and its first and second generation of DHs and obtained a population of 80 DH individuals via microspore cultures. These findings show that beside the genotype, cultivation medium has the main role in positive androgenic response in eggplant, as well as thickness of eggplant anther walls and heterostyly, which might delay the access of inductive factors to the anther locule, thus reducing their effect over inducible microspores (Salas *et al.*, 2012).

Conclusions

The low androgenic rate in pepper and no androgenic response in tomato and eggplant, as shown in our experimental results, are one more confirmation that these solanaceous crops are still far from an efficient and reliable technology to be applied on a routine basis in breeding programs. Even with the incredible importance of this family for agriculture, double haploid technology is not yet competently applied in these crops and they appear to respond to androgenic induction very differently.

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SOIL PROPERTIES AND QUALITY GRASSLANDS OF THE MUNICIPALITY OF IVANJICA

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Abstract

In the total agricultural area of the Municipality of Ivanjica, natural grasslands predominate and they are present at about 64.3% of these surfaces. Depending on the way and intensity of the use in the soil, dynamic processes are taking place that lead to changes in structure, fertility, and microbiological activity. Microorganisms are key players in the cycling of organic matter and nutrients, their activity contributes to a wide variety of soil ecosystem functions, including and the emergence of soil structure. They are the most numerous group of organisms in overall metabolic activity of soil and represent good indicators of soil health, since they respond quickly to changes in soil ecosystem. The total number of microorganisms represents one of the indicators of the biogenicity of soil. Chemical composition of soil and the total microflora, as well as the floristic composition of 15 natural grasslands were determined. Examined soil samples had humus contents ranging from 1.43 to 4.85 % and acidic chemical reaction, except for one sample which pH was KCl 6.55. The total number of microorganisms ranged from 5.602 to 6.322 (log of number) of absolutely dry soil. The examined grasslands have different percentage of presence of grasses, leguminous and plants from other families.

Keywords: *total microflora, floristic composition, grasses, legumes*

Introduction

The territory of the municipality of Ivanjica lies in the south- west of Serbia, covering a part of the Moravički distict also known as. Stari Vlah and Raška plateau. A large part of Ivanjica municipality, excluding the valleys of the river, occupy grasslands that are very important primarily, which represent an important source of food for domestic and wild animals, and therefore a prerequisite for the development of livestock production is necessary. In addition, they are also important from the aspect of environment preservation, represent the habitat of many plant and animal species, the source of medicinal substances used in pharmacy, and they prevent soil erosion, etc. (Babić *et al.,* 2016). Their production potentials are different and under the influence of numerous factors. Dominant influences are the soil, precipitation level and management (Lazarević *et al.*, 2003).

Interactions between plants and soil microbes play an important role in structuring terrestrial ecosystems by influencing plant growth and competitive ability. Soil microbial communities can respond rapidly to changes in the environment (Meisner *et al*.,2013) and changes in the composition of the soil microbial community influence plant species, it can lead to changes in plant community composition (Hawkins and Crawford, 2018). Plant and microorganisms are indivisible whole. Microorganisms in their metabolic processes excrete a variety of biotic substances into their environment affecting plants, their growth and development, the process of photosynthesis, resistance to diseases and pests, and therefore, the yield and quality of cultivated plants (Šarčević - Todosijević *et al.,* 2018). On the other hand, plants, over root system, excrete different substances that stimulate the development and activity of rhizospheric microorganisms. They are the source of carbon and energy for rhizospheric microorganisms and their chemical composition and amount depend on the plant species. The

chemical compositions of the compounds differ from species to species of both plants as well as microbes. Also, the physiological status of plants affects the chemical composition (Huang *et al.*, 2014; Mitra *et al.*, 2019). Soil microorganisms are the main players in the nutrient cycling in soil systems, and soil microbial community structure has been shown to be influenced by both abiotic and biotic factors. Among the deterministic processes governing the composition of microbial communities, environmental factors such as soil pH, temperature and moisture are considered to be main drivers of microbial community assembly (Castro *et al.*, 2010; Kaiser *et al.*, 2016).
The aim of this investigation was to examine floristic composition and the total microflora in the soil of natural and sown grasslands in the Ivanjica municipality area.

Material and Methods

In August of 2018 soil samples from 15 natural and sown grasslands at altitudes between 703 m and 1239 m were taken for microbiological analyses from a depth of 0-25 cm. Thirteen samples were taken from natural grasslands and marked with N1 to N13, while two were from sown grasslands and had codes A1 and A2.
These grasslands located on the territory of the Municipality of Ivanjica. Soil sampling was carried out in three sites from different parts of one location, they are mixed and present as one sample. The chemical properties of the soil were determined by standard methods in the chemical laboratory of the Institute for Forage Crops Kruševac. From each grassland samples of green fodder were collected and the percentage of grasses, legumes and other species were analysed according to the Flora of Serbia (Josifović, 1970-1977).
Biological activity of the soil of grasslands was monitored on the basis of presence the total number of microorganisms. In the lab, each of the samples was analyzed in three repetitions. Total number of microorganisms was established with standard microbiological methods of introducing a certain specific quantity of soil suspension diluted 10^{-5}, method of agar panels (Pochon and Tardieux, 1962). The incubation lasted for five days on 28°C. The number of grown colonies was calculated per 1 g of absolutely dry soil (Jarak and Djurić, 2006).
The results were processed by means of STATISTICS 8.0 computer program, using Fisher's LSD test.

Results and Discussion

Based on the pH value, one could say that tested soils are acidic, between 3.79 and 4.92 and only one of the analyzed soils from one artificial grassland (A1) showed a neutral reaction (Tab. 1). The chemical reaction of the soil has a strong influence on the growth of plants, the regime and biological availability of nutrients and the way that the ions from nutrients reach the root of the plant. Soil pH is one of the most important soil properties related to the composition of microbial communities (Baah and Anderson, 2003). In a large sampling study including 150 forest and 150 grassland soils Kaiser *et al*. (2016) found soil pH as the best predictor for bacterial community structure, diversity and function.
According to the content of humus, nitrogen, potassium and phosphorus soil samples from different grasslands were under different among themselves (Tab. 1). The largest number of samples were characterized by the average content of humus, which is favorable from the aspect of the number and activity of the soil microflora. Microbial metabolism in soil is limited by the availability and types organic substrates, and the quantitative and qualitative differences in substrates between grasslands are responsible for the variation in microbial community (Moussa *et al.,* 2007). The sampled soils were relatively well provided by nitrogen and easily soluble potassium. The content of easily available phosphorus in the grassland soils, covered by our research, was generally low, which is directly related to the acidity of the soil. The application of adequate agro-technical measures creates conditions for

maintaining microbial population and increasing the capacity of these microorganisms for the mobilization of phosphorus (Jarak and Čolo, 2007). Phosphorus dynamics in soil is closely related to the dynamics of the biological cycle in which microorganisms play a central role (Wakelin *et al.*, 2004).

Table 1. The chemical composition and the total microflora (log of number) of studied soils

The samples	pH		N	P_2O_5	K_2O	Humus %	Total number of microorganisms
	H_20	KCl	%	mg/100g	mg/100g		Log of the number
N1	5.61	4.01	0.193	4.20	4.37	3.35	5.602 ^{n}
A1	7.17	6.55	0.359	15.30	225.2	4.87	6.322 ^{a}
N2	5.75	4.63	0.219	7.7	13.08	2.79	6.114 ^{d}
N3	5.68	4.53	0.211	4.2	17.93	3.40	6.000 ^{g}
N4	6.37	4,.97	0.265	4.10	25.82	3.96	6,041 ^{f}
N6	6.06	4,.92	0.164	3.90	37.10	1.43	6.230 ^{b}
N6	5.20	4.03	0.193	4.00	3.43	2.69	5.954 ^{h}
N7	5.85	4.30	0.164	3.90	2.71	2.01	5.903 ^{i}
N8	5.51	4.32	0.365	5.5	15.9	1.96	5.845 ^{k}
N9	5.72	4.77	0.428	7.15	13.18	2.16	6.146 ^{c}
N10	5.77	4.79	0.237	4.00	27.79	3.06	6.079 ^{e}
N11	4.51	3.79	0.205	7.7	16.78	2.70	5.778 ^{l}
A2	5.22	4.12	0.223	12.9	17.09	3.32	5.845 ^{j}
N12	5.60	4.13	0.338	4.10	46.49	3.62	6.000 ^{g}
N13	4.96	3.96	0.136	4.20	7.94	4.85	5.699 ^{m}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p < 0.05$)

In our study the total microflora in soil samples from varied from 5.602 to 6.322 (log of number) per one gram of absolutely dry soil (Tab. 1). According the results of Fisher's LSD test, samples of soil among themselves shows statistically significant differences presence the total number of microorganisms. The vast differences in the composition of soils, together with differences in their physical characteristics and the agricultural practices by which they are cultivated, result in corresponding large differences in the microbial population both in total number microorganisms. many of the world's natural grasslands are in poor condition and showing signs of degradation (O'Mara, 2012). According to Grayston *et al.* (2001, 2004) comparing microbial community structure under different vegetation communities has established that microbial diversity is greater in unimproved than improved grasslands.

Distribution, quality and botanical composition of pastures and meadows largely depend on the management, whereas excessive utilization, such as over-grazing and fertilization at one point, and abandonment from the other, cause dramatic effects on floristic and vegetation diversity (Dajić - Stevanovic *et al.*, 2010).

Distribution (in %) of grasses, legumes and other plant species in studied grasslands

The samples	Family	Percentage	The most common species
N1	Grasses	18.49	*Agrostis capillaris, Anthoxantum odorattum, Festuca ovina*
	Legumes	16.41	*Trifolium pannonicum, Trifolium campestre, Trifolium pratense, Genista sagittalis*
	Other	65.10	*Stachys officinalis, Plantago lanceolata, Hypericum perforatum, Euphrasia stricta, Moenchia mantica*
A1	Grasses	70.0	*Festuca rubra, Agrostis capillaris, Holcus lanatus*
	Legumes	2.0	*Trifolium repens*
	Other	28.0	*Hieracium bauchini, Hieracium pilosella, Achillea millefolium*
N2	Grasses	83.0	*Arrhenatherum elatius, Festuca arundinacea, Holcus lanatus, Agrostis capillaris*
	Legumes	2.0	*Trifolium pratense, Trifolium repens*
	Other	15.0	*Lactuca serriola, Crepis conyzifolia*
N3	Grasses	57.18	*Agrostis capillaris,Festuca rubra,Phleum pratense, Arrhenatherum elatius*
	Legumes	11.44	*Trifolium ochroleucon, Lotus corniculatus, Lathyrus pratensis, Genista sagittalis*
	Other	31.38	*Prunella vulgaris, Centaurea jacea, lišće Populus tremula*
N4	Grasses	43.31	*Danthonia calycina, Festuca rubra, Agrostis capillaris, Cynosurus cristatus, Arrhenatherum elatius*
	Legumes	5.12	*Trifolium campestre, Lathyrus pratensis*
	Other	51.57	*Centaurea jacea, Thymus pulegioides, Filipendula hexapetala*
N5	Grasses	70.49	*Agrostis capillaris, Festuca rubra,Phleum pratense*
	Legumes	2.81	*Trifolium campestre, Lotus corniculatus*
	Other	26.70	*Centaurea jacea, Viola tricolor, Silene sendtneri, Prunella vulgaris, Filipendula hexapetala, Achillea millefolium*
N6	Grasses	57.18	*Agrostis capillaris,Festuca rubra,Phleum pratense, Arrhenatherum elatius*
	Legumes	11.44	*Trifolium ochroleucon, Lotus corniculatus, Lathyrus pratensis, Genista sagittalis*
	Other	31.38	*Prunella vulgaris, Centaurea jacea, lišće Populus tremula*
N7	Grasses	63.18	*Agrostis capillaris, Festuca rubra, Arrhenatherum elatius*
	Legumes	11.44	*Trifolium pannonicum, Vicia villosa*
	Other	26.38	*Achillea millefolium, Rumex acetossa, Centaurea jacea, Galium verum*
N8	Grasses	58.64	*Agrostis capillaris, Cynosurus cristatus, Holcus lanatus,Phleum pratense, Agropyron repens*
	Legumes	10.17	*Trifolium pratense,Trifolium repens, Lotus corniculatus*
	Other	31.19	*Centaurea jacea, Plantago lanceolata, Achillea*

			millefolium
N9	Grasses	65.53	*Festuca rubra, Agrostis capillaris, Cynosurus cristatus, Phleum pratense,*
	Legumes	10.19	*Lathyrus latifolius, Lathyrus pratensis*
	Other	24.27	*Centaurea jacea,Achillea millefolium*
N10	Grasses	35.94	*Festuca rubra, Agrostis capillaris, Cynosurus cristatus*
	Legumes	10.14	*Trifolium pannonicum, Trifolium campestre, Lathyrus pratensis, Genista sagittalis*
	Other	53.92	*Centaurea jacea,Achillea millefolium, Hieracium pillosela*
N11	Grasses	61.92	*Agrostis capillaris, Festuca rubra, Cynosurus cristatus, Dactylis glomerata*
	Legumes	8.22	*Lathyrus pratensis, Trifolium alpestra*
	Other	29.86	*Centaurea jacea, Hieracium bauchini, Prunella vulgaris, lišće Populus tremula*
A2	Grasses	95.0	*Dactylis glomerata, Phleum pratense, Agrostis capillaris*
	Legumes		
	Other	5,0	*Rumex acetossa*
N12	Grasses	41.8	*Agrostis capillarisDactylis glomerata, Cynosurus cristatus, Festuca rubra, Chrisopogon grillus*
	Legumes	17.21	*Lotus corniculatus, Trifolium pratense, Trifolium campestre*
	Other	40.98	*Centaurea jacea, Achillea millefolium, Fillipendula hexapetala, Hieracium pilosella, Euphrasia stricta*
N13	Grasses	53.54	*Agrostis capillaris*
	Legumes		-
	Other	46.46	*Polypodium vulgare*

For agronomic purposes, all species that participate in the floristic composition are classified into grasses and legumes and a group of other species (Stošić and Lazarević, 2007). Localities of the examined area differ in floristical composition and thus in capabillity in animal nutrition (Tab.2). The percentage of legumes and other plant species in the green matter yield indicated the state of the grassland, primarily the degree of degradation of these areas. Grasslands in this area have leguminous species from 2 % to 17.2 I% and presence of plants of this family at two locations was not recorded. Đurić *et al.* (2007) was recorded the distribution of legumes ranged from 6.73% to 34.12% on grasslands of the Moravicki District. The percentage of species from the Poaceae family anges from 18.49 to 83, while the group of other species present with 5 % to 65.10 %. Acoording to Grynia and Krysza (1998) environmental factors as well (precipitations, temperature, soil fertility) can affect the floristic composition of grasslands and their yield and quality.

Conclusions

Results of this study shown that the presence of microorganisms in the tested soil samples is influenced by numerous factors and that each soil provides different conditions for the life of the microbes. Grasslands in this area have a basic role in the production of fodder and provide important ecosystem services, including erosion control. In order to ensure adequate conditions for the development of the root system of plants and the activity of soil microorganisms, phosphatization and calcification measures should be applied.

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YIELD AND CONTENT OF STARCH AND PROTEIN IN THE SEED OF THE QUINOA GENOTYPES PUNO AND TITICACA

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is considered as a very important agricultural crop due to its nutritional value and tolerance to different stress factors. The aim of this study was to investigate the differences in yield, the content of starch and protein in the seed of two introduced genotypes of quinoa (Puno and Titicaca) and the possibility of their cultivation in Serbian agroecological conditions. The experiment was carried out during the 2017 growing season in the rain-fed condition in Subotica, Republic of Serbia. The seeds were sowed in the first part of April. Sowing was done at a depth of 2 centimeters, the distance between the rows was 50 cm and between the plants in the row 5 cm. The crops were harvested in the first half of August when quinoa seeds were mature and the amount of moisture in seed was 12%. The harvest of Puno and Titicaca seeds was made by hand. The content of crude proteins was determined according to the Kjeldahl method, while for starch measurement the Ewers polarimetric method was used. Obtained results showed that the yield of plants Titicaca genotype (24.4 g/plant) was higher compared to the yield of plants Puno genotype (21.3 g/plant). Our results did not show significantly different values in protein content between the seeds of Puno (14.1%) and Titicaca (14.0%). Also, the seeds of Titicaca and Puno contained similar starch content (54.1 and 55.6%, respectively). These results indicate that both investigation genotypes can be grown in Serbian agroecological conditions.

Keywords: *Quinoa, yield, seed, starch, protein*

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal plant, belonging to the family Amaranthaceae, native to the Andean regions of South America. Quinoa being adaptable to different types of soil and climatic conditions and to adapt to different abiotic stress conditions including frost, drought, salinity (Jacobsen and Muica, 2002; Jacobsen, 2017). Furthermore, the crop has a remarkable adaptability to different agro-ecological regions. It can grow at relative humidity from 40% to 88%, and withstands temperatures from -4 °C to 38 °C. It is a highly water efficient plant, is tolerant and resistant to lack of soil moisture, and produces acceptable yields with rainfall of 100 to 200 mm (Valencia-Chamorro, 2003). In comparison to most cereals, quinoa seeds have a higher nutritional value (Matiacevich *et al*., 2006). The nutritional values of quinoa are the result of high content of minerals, vitamins, proteins and essential amino acids, high quality fatty acids, antioxidants and other important multiple bioactive compounds (Vilcacundo and Hernández-Ledesma, 2017). The nutritional value of quinoa seeds is reported to meet, and even surpass, that recommended by the World Health Organization (Hirose *et al*., 2010). It is known that quinoa has considerably positive effects on metabolic, cardiovascular, and gastrointestinal health in humans. The protein content of quinoa seeds varies from 8% to 22%, which is higher on average than that in common cereals such as rice, wheat, and barley. The storage reserves of proteins in the seeds are mainly located in the reduced endosperm and the cotyledons (Valencia-Chamorro, 2003), while carbohydrate reserves are found in the perisperm, nominated as seed storage tissues

(Prego *et al*., 1998). Agronomic research, including the plant density, potential ultivation, phenology, morphology, physiological maturity, yield, and weeds control, should be performed. Production of quinoa has been prevalently conducted in Bolivia and Peru and still is with small productions in other Andean countries like Ecuador, Chile, Argentina, and Colombia. Jacobsen (2003) and Pulvento *et al*. (2010) has been investigated quinoa adaptability to both northern and southern European conditions. Many other countries are performing tests on quinoa with very promising results. Growing period of quinoa varied between 70 to 200 days (Kenya 65-98 days, Denmark and Sweden 120-160 days, Greece 110-160 days) (Ramesh *et al*., 2017). The highest yield of 7.500 tons/ha was recorded in Lebanon closely followed by Egypt 3.872 tons/ha, while lowest yield was recorded in Mauritania 0.230 tons/ha.
The aim of this paper is present the possibility of cultivating quinoa and achieving satisfactory yields and quality (protein and starch content in the grain) in the agroecological conditions of Serbia.

Material and Methods

The experiment was carried out during the 2017 growing season in rain-fed conditions, using two introduced genotypes of quinoa adapted to the European climate, Puno and Titicaca. Used genotypes selected at the University of Life Sciences in Copenhagen, Denmark (Jacobsen and Muica, 2002). The quinoa was grown on Serbian farm near Subotica, located in Vojvodina, northern Serbia. The soil type was chernozem, medium rich in nitrogen (0.24%) and hummus (3,19%), highly rich in phosphorus (34.68 mg P_2O_5 per 100 g of soil) and rich in potassium (29.42 mg K_2O per 100 g of soil), slightly alkaline (pH 7.6). The analysis showed that the nutrient content of the soil was satisfactory and fertilizer wasn't applied during the vegetative season. The seeds were sowed in the first part of April. The experiment was laid out in a split-split plot system, with four replications. The size of the main plot was 12 m^2. The distance between the rows was 50 cm and between the plants in the row 5 cm (approximately 400 000 seeds per hectare). The seeds were sown at a depth of 2 centimeters. The crops were harvested in the second half of August when quinoa seeds were ripeness, the moisture content was 12%. The height of plants, plant fresh weight, number of flower branches and yield was measured. All investigated parameters were calculated on the basis of 120 plants. The seeds were ground by using a laboratory mill (model Cemotek Sample Mill Foss, Sweden) and then the contents of proteins and starch were analyzed. The content of crude proteins was determined according to Kjeldahl method (Stikić *et al*., 2012), while for starch measurement the Ewers polarimetric method was used (ISO 10520: 1997). The temperature data was obtained from the automatic meteorological station located in the center of Subotica (4-km from the experimental field). The measurements were collected using "Nexus" instruments and "Weather Display" software (http://www.sumeteo.info). The amount of precipitation was measured on site, at the experimental field.
Monthly reviews of average air temperature and total precipitation are represented in Table 1.

Table 1. Climate conditions during the 2017 growing season.

	Average temperature (°C)	Total precipitation (mm)
April	11.2	35
May	17.1	38
June	22.1	35
July	22.8	44
August	23.3	33

Results and Discussion

In the territory of Serbia, there is a tendency to increase the air temperature as well as the number of tropical days (Ruml *et al*., 2017). Growing period of quinoa in Serbia is from beginning of April to the second half of August (about 140 days). The growing season 2017 in the Palić (Subotica) area features a temperatura mean of 18.9 ºC and a total rainfall of 245 mm. A comparison with the perennial average (1971-2000) shows that the growing seasons of 2017 were warmer, which was most pronounced during July and August (Table 1). Regarding the amount of precipitation, the growing season 2017 has been very dry. August was the warmest (23.3ºC), with the least rainfall (33 mm). Growing season 2017 is characterized by a pronounced deficit, higher than the average in the area of Serbia, which is 286 mm (Matović *et al*, 2013). The quinoa's need was as much as 534 mm higher than the incoming rainfall.
Results of plant height, plant fresh weight, number of flover branches and yield two genotypes (Titicaca and Puno) are presented in Table 2.

Table 2. Plant height, plant FW, number of flover brances and yield of two investigation genotypes (Titicaca and Puno)

Genotype	Plant height (cm)	Plant fresh weight (g)	Number of flower branches	Yield (g/plant)
Titicaca	139.43	134	13	24.35
Puno	122.78	96.6	16	21.33

Our results did not showed the big variation between Titicaca and Puno plants in investigation parameters: plant hight, plant FW, number of flower branches and yield (as the most important parameter). Average yield of Titicaca plants was 24.35 g/plant (4.9 tons/ha), while the average yield of Puno plants was 21.33 g/plant (4.3 tons/ha) (table 2). If we compare the yield obtained in our agro-ecological conditions to other countries (Kenya 4 tons/ha, Greece 2 tons/ha) (Ramesh *et al*., 2017), it can be considered a high yield. Compared to the conventional cultures involved, quinoa production could be more cost-effective in existing agro-agroecological conditions. For example, corn and quinoa record a similar yield per ha. Higher economic cost-effectiveness of quinoa production lies in the cost of cost. In Serbia, in 2017, the same price of corn was recorded, and in 2010 it was 0.13 eur/kg, which is approximately 35 times lower than the quinoa price. Between 2006 and early 2013, quinoa crop prices have tripled. In 2011, the average of quinoa crop price was 3,115 USD per ton with some varieties selling as high as 8,000 USD per ton (Ruiz *et al*., 2014).
We have compared Puno and Titicaca cultivars and found similar percentages of proteins (14.1 and 14.0%, respectively) (Czekus *et al*., 2019). Similar protein content in grain Puno and Titicaca quinoa genotypes (14.7 and 14.4%, respectively) was found Aluwi *et al*. (2017) The main carbohydrate component of quinoa is a starch and in our research the seeds of Titicaca and Puno contained similar starch content (54.1 and 55.6%, respectively) (Czekus *et al*., 2019). According to Aluwi *et al*. (2017), a higher percentage of quinoa seed starches was detected in Puno (62.6%) than in Titicaca (56.4%). In general, as for the Titicaca cultivar, the total carbohydrate represents the main seed component at approximately 54-57%, according to the study of Pulvento *et al*. (2012).

Conclusions

In conclusion, our study demonstrated that quinoa cultivars Puno and Titicaca has grown in Serbia (Southeastern European agro-ecological conditions), gave a very satisfactory yield, as well as the starch and protein content in the seed, in the year with a high precipitation deficit. With their introduction in production, given the current prices of quinoa seed, high profits could be achieved. Quinoa is a plant species extremely tolerant to drought and its cultivation could contribute to overcoming the increasingly negative effects of drought on agricultural production in Serbia.

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BOTANICAL COMPOSITION AND FORAGE QUALITY OF NATURAL GRASSLANDS OF PEŠTER HIGHLANDS

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Abstract

Improvement of animal feed production and animal husbandry, particularly beef and sheep production, is interrelated with natural and sown meadows and pastures in hilly - mountainous region. High quality of forage has positive effect on profitability of milk and meat production and it is prerequisite for improvement and reduction of costs in livestock production. Because of that, the main role of grassland is to ensure a supply of livestock production as a source of healthy and safe animal food and that way they have strong contribution to rural agricultural and economic development. Grasslands in this area are preserved from the use of large amounts of mineral fertilizers and chemicals for plant protection, because of what production of healthy and safely animal food, with minimal investments, is still possible. The presence of useful and high quality species in grasslands enables the production of high quality biomass. One of the possibilities for improving the quality of biomass is to increase the percentage of quality plant species in grasslands. For this reason, research was carried out on the territory of the Pešter highlands were is collected 61 samples from natural grasslands on altitude of 1200 to 1300 m and determined botanical composition and dry matter quality. Natural grasslands analyzed in these research had an unsatisfactory botanical composition and low quality of dry matter. From analyzed samples, 54.1% showed low dry matter quality with less than 8% of the crude protein content, 37.7% samples contained 8-11%, and 8.2% samples contained 11-14% of crude protein.

Key words: *natural grasslands, botanical composition, quality, crude protein content.*

Introduction

Grasslands are the largest ecosystems in the world (Suttie et al., 2005) and representing more than 40% of the terrestrial areas, excluding Greenland and Antarctica. In Serbia, natural grasslands are the most important source of voluminous livestock feed in hilly mountainous regions. Also, they are important component of the environment, particularly in terms of conservation of biodiversity (Dajić Stevanović et al., 2010) and protection of soil from degradation and erosion. Natural grasslands are the most widespread in hilly mountainous region in Serbia. With altitude increase, the share of grasslands in the total agricultural area increases. The participation of grasslands in hilly mountainous regions is from 30 to 60% of the total agricultural land. In this area natural grasslands present main or only source of livestock feed. Intensive researches of methods and possibilities for improvement of livestock feed production on natural grasslands in Serbia have been initiated during fifties and sixties of the twentieth century. In previous period most researches were focused on the effect of fertilization on yield, botanical composition, dry matter quality, conservation on biomass from natural grasslands, etc. (Lazarević et al., 2005; Stošić et al., 2005; Tomić et al., 2005; Tomić et al., 2009). Natural grasslands are not fully exploited (average yield on natural grasslands is below 2 t ha^{-1} on meadows and about 0.5 t ha^{-1} on pastures), which is confirmed by numerous results obtained in previous period which have shown that production on the grasslands can be considerably increased in short time without big investments, with preserving the natural balance and without disturbing the environment. On natural grasslands is possible to produce over 4 t ha^{-1} dry matter yield (Vučković et al., 2005), and 7-10 t ha^{-1} on sown grass-leguminous mixtures (Lazarević et al., 2005). However, some natural grasslands are in most cases neglected, with low level of utilization. On Pešter highlands, share of natural grasslands in total agricultural lend is more than 60%, which indicates their great importance in this region. This area is characterized by typical climatic conditions with long and cold winters, low average temperature and short vegetation period.

Aims of this investigations was to determine to botanical composition (share of species of two the most important families, grasses and legumes, and group of other species) and dry matter quality of biomass collected on grasslands on Pešter highlands. Presence of legumes on grasslands is indicator of their quality and specific soil traits. Based on this, it is possible to determine appropriate agro-technical measures, especially mineral fertilizers, that can be used for improvement of forage yield and quality. Productivity on grasslands is possible to increase by application of fertilizers, especially nitrogen (Stošić et al., 2005), but this can change botanical composition and decrease presence of legume component in grasslands, which will lead to a decrease of forage quality (Salis and Vargiu, 2008). Only usage of balanced fertilizers will resulted in full contribution and will have the greatest influence on productivity and quality of grasslands on Pešter highlands. In this way, the direct effect on increase of milk and meat production is achieved, as well as final livestock products.

Material and methods

In August 2018 samples from 61 natural grasslands were taken for analyses. All sites are located in the territory on the Pešter highlands (southwestern Serbia) at altitudes between 1 200 and 1 300 m. Botanical composition was determined from sample of 1 m^2 from which plants were cutted and aboveground biomass is collected. In each sample the plants from the same botanical family are separated from total biomass and weighed and percentages calculated. Plants were separated per categories: quality grasses, quality leguminous and useful and conditionally useful species from other families, according to Kojić, 1990 and 2001. Dry matter quality (crude protein, fat, ash, ADF and NDF) were determined in the laboratory of the Institute for Forage Crops, by the Weende system.

Results and discussion

Grassland management has a decisive influence on the production capacity and floristic composition of grasslands. The grasslands from which the samples were taken are managed in a traditional way, but with a reduced number of domestic animals compared to the earlier period. In these grasslands, a small amounts of mineral fertilizers are used or this measure does not apply at all. The cutting is done usually very late, and after that the areas are left to the grazing of cattle or sheep. Considering the fact that the number of domestic animals on grasslands is significantly lower than in the previous period, in the biggest part of the grasslands no one of agrotechnical measures was applied.

According to results presented in Table 1, the largest number of analyzed samples had the highest percentage of species from *Poaceae* family (on average the share of grasses was more than 77%), while the share of legumes, as a carrier of dry matter quality, was lowest, only 12%. Species from other families are represented on average by 17% on natural grasslands on Pešter highlands.

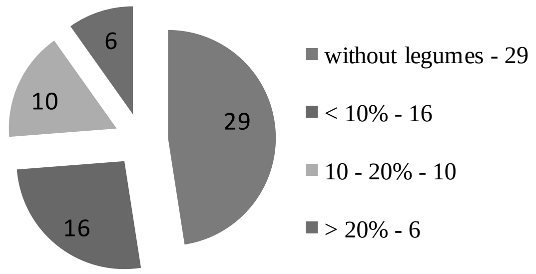
Table 1: Botanical composition of analized natural grasslands

Samples from KP*	Share of family (%)			Samples from KP*	Share of family (%)		
	Poaceae	*Fabaceae*	Other		*Poaceae*	*Fabaceae*	Other
1055	91.2	/	8.8	**2834**	66.7	10	23.3
1324	96	/	4	**211**	46.1	13.6	40.3
831	98	/	2	**212**	63	20.6	16.4
1855-1	84.7	/	15.3	**1109**	67.9	14.2	17.9
1855-2	98	/	2	**1240**	100	/	/
1844	97	/	3	**601**	40	40	20
1191	84.9	10.7	4.4	**619**	38.6	4.6	56.8
423	62.9	6.5	30.6	**1428**	95.8	1.4	2.8
1229	45.5	51.5	3	**428**	32.7	17.8	49.5
1237	100	/	/	**566**	91.6	/	8.4
409	38.5	1.9	59.6	**656**	94.6	/	5.4
2029	47.4	3.2	49.4	**561**	50.3	/	49.7
2027	82.6	9.2	8.2	**832**	67.5	/	32.5
524	95.5	2	2.5	**847**	97	3	/
570	94	1	5	**602**	91.5	/	8.5
588	57.4	/	42.6	**400**	52.8	18.9	28.3
1403	71.4	4.8	23.8	**518**	40	10	50
1318	57.5	22.5	20	**1057**	82.4	/	17.6
2079-1	87.4	5.7	6.9	**1059**	90.7	/	9.3
2079-2	80.8	16.8	2.4	**1099**	88.5	/	11.5
353	72.5	26.5	1	**1104**	97	0.8	2.2
1444	85	/	15	**1122**	95	/	5
2150-2	68.2	/	31.8	**1124**	88	/	12
2151	95	/	5	**1601**	88.3	7.1	4.6
1070	83.4	/	16.6	**1737**	96	/	4
1077	90.8	3.5	5.7	**1789-2**	64.4	34.7	0.9
3534	67.9	/	32.1	**1798**	98	/	2
3524-1	81.3	/	18.7	**1779**	80	/	20
3524-2	95.2	/	4.8	**233**	49.3	17.9	32.8
2214	63.1	10.8	26.1	**31**	90	2	8
2216	90.4	3.9	5.7	**/**	/	/	/
Average *Poaceae* (%)					**77.2**		
Average *Fabaceae* (%)					**12.4**		
Average Other families (%)					**17.2**		

*KP – kadastar plots

In this study, on average, the share of legumes in analyzed samples from a natural grasslands from Pešter highlands, as a carrier of quality is very low (Chart 1). Of the 61 samples, 29 were without legumes, 16 contain less than 10% of plants from this family, 10 contain 10-20%, while over 20% of legumes are found in only 6 samples.

The share of plant species from other families is relatively satisfactory considering the fact that in these natural grasslands agrotechnical measures were applied at the minimum or did not applied. The greatest number of samples (29) had less than 10% plant species from other families, 12 samples had 10 to 20% of these species, 17 samples were with over 20% and only 3 samples did not contain unwanted plant species.



Graf. 1. Share of legumes

Botanical composition of analyzed grasslands in the best way illustrates how the way in the management and use of grasslands affects their productivity and quality. On grasslands with organic and mineral fertilizers application, plants are more productive, they contain a higher percentage of legumes and less species from other families. On poorly maintained grasslands dominated rough grass species, weeds and other undesirable species.
The largest number of biomass samples had less than 8% crude protein content (33 samples or 54,1%), 8-11% crude protein content had 23 samples, respectively 37,7%, and 11-14% was found in only 5 samples of biomass (8,2%), Table 2. High levels of precipitation during June and July 2018 did not allow timely cutting, which affected low quality of biomass in the analyzed areas.

Table 2. Quality of biomass from natural grasslands (% in absolutely dry matter)

Samples from KP	Crude protein	ADF	NDF	Ash	Fat
1055	4.7	40.8	84.0	10.4	1.3
1324	3.1	64.3	91.7	10.0	0.5
831	9.2	39.8	77.1	6.2	0.4
1855-1	9.7	46.5	78.5	8.7	1.4
1855-2	5.7	46.7	77.6	5.5	1.7
1844	7.7	43.8	81.7	9.4	1.4
1191	6.1	48.8	79.0	5.6	1.4
423	9.4	48.0	79.1	7.3	1.2
1229	7.8	36.7	73.8	10.6	1.8
1237	9.3	46.7	83.0	9.7	1.2
409	8.8	40.2	71.6	9.1	1.5
2029	8.6	39.7	73.8	8.4	1.4
2027	8.2	39.3	76.5	9.9	1.3
524	6.6	46.9	77.0	5.1	1.5
570	5.1	47.0	76.0	3.5	1.7
588	8.6	43.2	69.4	6.4	1.9
1403	8.9	40.7	73.6	8.0	1.7
1318	9.1	40.6	71.2	9.3	1.9
2079-1	8.1	47.8	75.9	7.1	1.6
2079-2	6.5	49.7	77.4	4.5	1.5
353	7.6	50.8	76.9	5.3	1.5
1444	9.1	43.8	75.9	7.4	1.6
2150-2	11.5	41.3	78.8	10.0	1.1

2151	8.7	33.6	85.2	10.1	0.9
1070	3.3	42.0	81.5	11.3	1.0
1077	8.9	39.3	83.5	9.6	0.5
3534	3.1	48.0	93.1	8.9	0.5
3524-1	7.8	40.0	84.3	10.5	1.0
3524-2	7.3	35.0	89.6	10.8	1.1
2214	11.8	40.9	76.5	11.1	1.4
2216	8.7	36.7	77.6	9.3	1.5
2834	13.3	39.5	64.3	11.8	2.3
211	3.8	35.9	88.9	8.9	0.5
212	2.9	38.9	80.1	10.4	0.5
1109	7.0	42.3	75.7	10.5	0.2
1240	8.7	43.2	77.2	7.0	1.5
601	3.0	52.3	96.7	10.5	0.2
619	3.0	42.3	84.5	10.5	0.4
1428	3.0	46.8	87.1	11.6	0.3
428	3.7	34.0	77.8	11..4	1.1
566	5.2	45.8	77.0	6.5	1.9
656	3.8	44.1	71.9	9.0	2.0
561	6.6	44.1	67.4	3.8	2.2
832	6.3	44.5	76.3	6.2	1.7
847	2.6	39.6	98.2	10.2	0.1
602	9.2	43.6	77.0	8.8	1.5
400	11.8	41.0	66.3	7.1	1.9
518	8.0	39.7	76.8	8.5	1.6
1057	7.9	40.4	76.1	8.1	1.8
1059	8.7	42.4	72.1	8.0	1.8
1099	8.2	41.4	83.7	10.7	1.2
1104	3.1	43.4	95.7	9.1	0.3
1122	2.4	32.3	97.1	10.8	0.4
1124	2.7	37.8	92.8	9.1	0.1
1601	12.8	42.7	72.9	10.3	0.6
1737	4.2	35.5	79.4	9.1	1.3
1789-2	9.9	38.1	79.6	8.0	0.8
1798	10.0	40.7	73.5	8.1	1.8
1779	8.9	45.2	77.8	6.7	1.6
233	3.0	39.3	73.1	8.6	0.5
31	6.9	41.8	69.3	5.0	2.0

Forage quality from the analyzed natural grasslands is not at the satisfactory level. The main reason for the poor quality of biomass is late cutting and a large amount of precipitation during the vegetation season. With late cutting, the share of structural carbohydrates (ADF and NDF) increases, which reduces the digestibility and nutritional value of forage. According to fact that first cut, in hilly mountainous regions, participates with two thirds to 90% in total production, it is necessary to make compromise between yield and quality (Lazarević et al., 2010). The main problem in forage production is the low level of crude protein which is usually compensated by concentrated nutrients or is not compensated at all, so the production of milk and meat is at a very low level. Addition of protein in the form of concentrates makes production more expensive and increases the cost unit of end products in total costs.

Conclusion

In order to revitalize livestock production it is necessary to implement the agrotechnical measures to improve natural grasslands as the most important source of voluminous livestock feed in hilly mountainous regions in Serbia. An easy way to improve the forage quality is earlier cutting, approximately, at the beginning of heading the dominant grass species on the grassland and increasing the share of legumes in grasslands. Low level of legumes in grasslands is possible to increase by application agrotechnical measures like in-sowing, balanced fertilization, the way of utilization, adequate melioration measures, etc. In this way is possible to improve current quality on natural grasslands.

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EFFECT OF GROWTH STAGE ON MINERAL CONCENTRATION IN THE TOP ALFALFA AND DRY MATTER YIELD

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Abstract

Mineral concentrations in forages vary greatly, and are affected by soil mineral level, soil pH, plant species, stage of maturity, and application of fertilizers or waste materials. Soil tests are not useful in determining some mineral levels and are only good at predicting growth responses from applied P and K when test levels are very low. Plant tissue test is very reliable for minerals regardless of growth stage, but is not as reliable in predicting P and K responses with alfalfa sampled from early bud to 1/10 bloom. The aim of this study was evaluating the nutrition status of the alfalfa regarding to the best results of alfalfa dry matter yield. The samples were collected at three stages of alfalfa growth: early bud, full bud and at the beginning of flowering - 10% of bloom. 40-60 stems including leaves were collected from at least 30 plants. The top third samples were discarded and dried. Top third samples were analyzed for nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, zinc, mangan, boron and sulphur regarding to DMY (dry matter yield) of alfalfa at different stages of growth. Results of investigation showed that alfalfa top had sufficient amount of nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, zinc, mangan, boron and sulphur in all stages of growth, but it was deficient in potassium at full bud stage and zinc at the begining of flowering.

Keywords: *alfalfa top, mineral concentration, dry matter yield*

Introduction

Mineral concentrations in forages vary greatly, and are affected by soil mineral level, soil pH, plant species, stage of forage maturity, and application of fertilizers or waste materials. Forage mineral concentrations are of limited value in assessing mineral status of ruminants, because little is known regarding availability and factors affecting availability of minerals in ruminants fed forage diets (Spears, 2003). The relatively high solubilization of forage minerals in the rumen suggests that, in most instances, the release of minerals in a soluble form is not a major factor limiting absorption. Interactions of minerals with other minerals, other plant constituents, and microbial cells following solubilization appear to be more important as regards mineral absorption (Khan et al., 2007).

Due to the economic and agronomic importance of alfalfa, a considerable amount of inorganic fertilizer is applied to this crop (Gardner et al., 2000; Koenig et al., 1999). Determining when applications of phosphorus, potassium, sulfur and boron are needed is important to insure adequate alfalfa yield and quality. Therefore, some form of soil and tissue testing program must be used to identify deficiencies and monitor the nutrient status of alfalfa over time (Koenig et al., 2001; 2002; Mortvedt et al., 1996). Improvements in forage production have the potential to increase income and significantly reduce livestock production costs. Rotating forages with annual grain crops can increase grain yields, reduce weeds, improve soil quality, and reduce system energy requirements (Entz et al., 2002). Soil fertility is important for forage production, stand health/longevity, and forage quality (Smith et al., 2007).

Adequate amounts of P, K, S and B are necessary for healthy root growth, withstanding drought and winter stres, high quality forage, and N fixation in legumes. Soil testing helps fine-tune nutrients needed for early growth and identify possible limitations for establishment. Because P and K are relatively immobile, they should be placed in the root zone. If soil P and K are below critical levels, soil P and K levels should be build up prior to planting by incorporating broadcast fertilizer. Unfortunately, soil tests for sulfate-S are not a reliable indicator of plant available S. Therefore, plant tissue analysis is the best tool to determine S status and a valuable tool for in-season management of other nutrients. The critical tissue nutrient concentration is the level at which approximately 90-95 percent of maximum yield is obtained and varies with growing conditions. Because tissue concentrations change with plant maturity, it is important to sample the correct tissue at the correct time. Plant and soil samples taken from an affected area can be compared to healthy samples to help identify a limiting nutrient (Meyer et al., 1997).

The aim of this investigation was to determine the level of some nutrients such as macrominerals and microminerals in the soil and in the same time to investigate the levels of those nutrients in the top alfalfa samples taken at the different growth stage. These data should be used as tool for fertilizer application if it is necessary for achieve high yield and forage quality.

Materials and methods

The experiment was designed with three replications according to a randomized complete block. Alfalfa was grown at the experimental field of Institute for forage crops, Kruševac – R Serbia (21° 19' 35" E, 43° 34' 58" N). The study area was situated at altitude of 166 m above sea level in Central Serbia.

Soil and plant material were sampled in May 2015, which was the second production year of alfalfa. In pre-arranged field preparation, NPK 15:15:15 fertilizer was broken up in an amount of 300 kg ha^{-1}. Soil samples were collected in disturbed state using agricultural probe from 0-30 cm depth. One composite soil sample consisted of 15-20 individual samples. The collected samples were air-dried and grinded to particle size < 2 mm according to SRPS / ISO 11464:2004.

Alfalfa (*Medicago sativa* L.) – *cv* K 28 selected at Institute for forage crops, Kruševac was sampled at three stages of maturity I – full bud (harvested on the 04^{th} May – 20 days of vegetation), II - early bloom – 10-15% of flowering (harvested on the 21^{st} May – 32 days of vegetation) and III - mid bloom – 50-60% of flowering (harvested on the 29^{th} May – 42 days of vegetation). 40 to 60 stems from at least 30 plants in each of the parcels were collected. The top alfalfa – 15 cm was analyzed for macro and micronutrients.

Soil pH was determined by potentiometric method according to ISO 10390:2005. Total nitrogen in soil was determined by Khejldal method. Extraction of available phosphorus and potassium from the soil was carried out according to the AL method. Potassium was determined by flame emission on AAS PERKIN ELMER 1100 B, and phosphorus was measured on a HALO RB.10 spectrophotometer at a wavelength of 580 nm. Humus content in soil was determined according to Tjurin method. Calcium carbonate content in soil was determined by volumetric method according to ISO 10693:1995. The soil samples for boron determination were extracted with hot water. The suspension was filtered. The aliquot part of the filtrate was evaporated on an aqueous bath or heated plate with the addition of a sodium hydroxide solution and burnt in a furnance at a temperature of 450° C to destroy the nitrates and the organic substance. The residue was dissolved in 0.5N hydrochloric acid. In the aliquot part of this solution, a colored complex of boron with carmine was developed. The carmine solution in concentrated sulfuric acid is red and, in the presence of boron, turns into a purple-blue color. The color intensity is measured on a HALO RB-10 spectrophotometer at a wavelength of 585 nm. Sulphur was determined gravimetrically according method by

Jakovljević et al. (1985). The concentrations of Na, Ca, Mg, Fe, Zn and Mn were measured by atomic absorbance spectrophotometry - AAS PERKIN ELMER 1100B.
The amount of total nitrogen was measured by the Kjeldahl method on the TECATOR KJELTEC AUTO ANALYZER 1030. The total phosphorus in the plant was determined according to the standard method ISO 6491 with a molybdenum-vanadate reagent, spectrophotometrically. Potassium was determined from the solution by direct measurement of the intensity of the emission at a wavelength of 766 nm, using AAS PERKIN ELMER 1100B. Sulphur in the plant was determined gravimetrically according to the method by Sarić et al. (1967). The determination of boron in plant samples with carmine was based on the formation of a complex boric acid ester with carmine which was purple blue color. The color intensity was measured on a HALO RB-10 spectrophotometer at a wavelength of 585 nm. The concentrations of Na, Ca, Mg, Fe, Zn and Mn were measured by atomic absorbance spectrophotometry. Samples for Ca and Mg analysis were prepared with $1gL^{-1}$ lanthanum.
Study data were processed by the methods of descriptive statistics. Significance of differences between treatments was tested by analysis of variance (ANOVA). The significance of differences between arithmetic means was tested by Fisher's LSD test. Effects were considered different based on significant ($p< 0.05$) F ratio.

Results and discussion

The content of available nutrients in the experimental soil site is presented in the Table 1.

Table 1. The content of available nutrients in the experimental soil site, 0-30 cm depth

Parameter	Unit	Values
pH (H_2O)		6.98
pH (KCl)		5.96
Total Nitrogen	%	0.170
P_2O_5	mg 100 g^{-1}	4.40
K_2O	mg 100 g^{-1}	18.58
Humus	%	2.86
Ca-carbonate	%	0.80
Boron	mg 100 g^{-1}	2.75
Sulphur	mg kg^{-1}	35.66
Calcium	mg 100 g^{-1}	12.08
Magnesium	mg 100 g^{-1}	5.49
Sodium	mg 100 g^{-1}	19.03
Iron	mg kg^{-1}	6.96
Zinc	mg kg^{-1}	5.73
Manganese	mg kg^{-1}	226.0

The results of investigation showed that soil was mild acidic in reaction (pH in suspension of 1N KCl: 5.96); with middle level of total nitrogen (0.170%); low in available phosphorus (4.40 mg 100 g^{-1}); medium in available potassium (18.58 mg 100 g^{-1}) and low in humus content. The soil was high in available S (35.66 mg kg^{-1}) and B (2.75 mg 100 g^{-1}). In general, the fertility status of the experimental site was optimum with few limitations.
Mean alfalfa DMY and mineral concentration in top alfalfa are presented in the Table 2. The harvest time at a certain stage of development significantly influenced the alfalfa DMY. The highest DMY was obtained when the plants had 50-60% of the flowers (5.74 t ha^{-1}), and the lowest when they were in the bud stage (3.78 t ha^{-1}). A large number of investigations indicated that domestic varieties have excellent adaptability to existing in certain environmental conditions which influenced high DMY throughout the period of explaitation

(Lukić et al., 2001; Mladenović et al., 2001; Popović et al., 2003). The results of this investigation showed that concentrations of phosphorus and iron did not differ significantly between stages of growth, whereas concentration of phosphorus increased from 3.93 g kg^{-1} to 4.06 g kg^{-1} and concentration of iron increased from 126.41 to 127.76 mg kg^{-1} from the full bud to mid bloom stage. Although, concentrations of both those elements were above critical levels in top alfalfa (Table 3).

Table 2. Effect of alfalfa growth stage on DMY, macro and microminerals content in top alfalfa (15 cm)

	I stage of growth	II stage of growth	III stage of growth
DMY, t ha^{-1}	3.78^{c}	5.03^{b}	5.74^{a}
Ash, g kg^{-1}	9.92^{a}	8.39^{b}	$9{,}80^{a}$
Nitrogen, g kg^{-1}	49.90^{a}	41.90^{b}	49.65^{a}
Phosphorus, g kg^{-1}	3.93^{ns}	4.00^{ns}	4.06^{ns}
Potassium, g kg^{-1}	23.82^{a}	19.65^{c}	21.39^{b}
Calcium, g kg^{-1}	24.20^{b}	14.46^{c}	27.06^{a}
Magnesium, g kg^{-1}	2.81^{c}	5.85^{a}	3.93^{b}
Sodium, g kg^{-1}	0.75^{a}	0.55^{b}	0.50^{b}
Iron, mg kg^{-1}	126.41^{ns}	126.69^{ns}	127.76^{ns}
Zinc, mg kg^{-1}	42.11^{b}	40.23^{c}	44.94^{a}
Manganese, mg kg^{-1}	41.91^{b}	38.55^{c}	48.71^{a}
Boron, mg kg^{-1}	51.80^{a}	50.55^{a}	44.38^{b}
Sulphur, mg kg^{-1}	4.33^{a}	3.92^{b}	3.64^{b}

Different letters denote significantly different means ($P< 0.05$)

Concentration of nitrogen, potassium and calcium decreased from the first to the second stage of plant growth, and with plant development content of those elements increased. The highest content of nitrogen and potassium were in the first stage of growth, whereas the highest content of calcium was in the third stage of growth (Table 2). Mean top alfalfa nitrogen and calcium content were above the critical level in all samples taken from the different growth stage, but content of potasssium was below the critical level in the second and third stage of growth. Concentration of zinc and manganese also decresed from the first to the second stage of growth and after that increased with plant growth (Table 2), but the concentrations of those elements were above critical level at all samples taken from different stage of growth (Table 3). On the other hand, concentration of magnesium increased from 2.81 g kg^{-1} at the first stage of growth to 5.85 g kg^{-1} at the second stage of growth, and after that decreased to 3.93 g kg^{-1} (Table 2), but the concentrations were above critical level (Table 3). Concentrations of sodium, boron and sulphur decreased with alfalfa growth and development (Table 2), and the results of this investigation showed that the concentration of those elements were above critical levels of nutrients (Table 3).

Table 3. Sufficiency levels of nutrients, top alfalfa – 15 cm, (Meyer et al., 1997).

Nutrients	Low	Sufficient	High
Nitrogen, g kg^{-1}	< 25.00	25.00-40.00	> 40.00
Phosphorus, g kg^{-1}	< 2.50	2.50-4.50	> 4.50
Potassium, g kg^{-1}	< 22.50	22.50-34.00	> 34.00
Calcium, g kg^{-1}	< 7.00	7.00-25.00	> 25.00
Magnesium, g kg^{-1}	< 2.50	2.50-7.00	> 7.00
Sodium, g kg^{-1}	-	-	-
Iron, mg kg^{-1}	< 30.00	30.00-250.00	> 250.00

Zinc, mg kg^{-1}	< 20.00	20.00-60.00	> 60.00
Manganese, mg kg^{-1}	< 20.00	20.00-100.00	> 100.00
Boron, mg kg^{-1}	< 25.00	25.00-60.00	> 60.00
Sulphur, mg kg^{-1}	< 2.50	2.50-5.00	> 5.00

Soil and plant tissue tests have been developed to assess the nutrient content of both the soils and plants. By analyzing this information, plant scientists can determine the nutrient need of a given plant in a given soil. In addition to the levels of plant-available nutrients in soils, the soil pH plays an important role in nutrient availability and elemental toxicity (Uchida, 2000). Plant analysis consist of testing nutrient concentrations in specific plant parts during specific growth stage (Jacobsen and Jasper, 1991). If nutrient concentrations in a sample are below or above on established sufficiency range, then the plant is defficient or in excess for that element. Plant analysis can be performed relatively quickly in the field using semi-quantitative test kits or more extensively in a laboratory (Havlin et al., 1999). As a diagnostic tool, plant analysis improves the chances of making a correct diagnosis and can be particularly useful in identifying hidden hunger or pseudo deficiencies.

Conclusions

Nutrient deficiencies and toxicities cause crop health and productivity to decrease and may result in the appearance of unusual visual symptoms. Understanding each essential nutrient`s role and mobility in the plant can help determine which nutrient is responsible for a deficiency or toxicity symptom. When in excess, many nutrients will inhibit the uptake of other nutrients, thus potentially causing deficiency symptoms to occur as well. According to the results obtained in this investigation we can conclud that soil contained sufficient amount of available nutrients, except the potassium content which was deficient in the second and third stage of growth. So, higher amount of potassium fertilizer could be applied.

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EFFICIENCY OF EQUIPMENT FOR CLEANING OF THE NATURAL RED CLOVER (*Trifolium pratense* L.) SEEDS

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Abstract

The paper presents results of examination of various red clover seed lots of the process of cleaning on the seed processing equipment. The experiment was carried out at the seed processing center of the Institute for Forage Crops Kruševac. In the Republic of Serbia, among the perennial fodder leguminous plants, red clover (*Trifolium pratense* L.) occupies the second place immediately after the alfalfa. The seed for planting red clover crop must have high purity, germination and genetic value. These properties of red clover seeds are accomplished by processing, i.e. by removing all impurities and seeds of poorer quality. In order to achieve optimum results during seed processing, each seed lot needs to be carefully analyzed and the machines should be appropriately adjusted. The aim of the study was to show the effectiveness indicators of seed processing in the red clover seeds. The basic indicators of the efficiency of machines in seed processing were the quality and quantity of the seed obtained at the end of the seed processing. The relevant parameters that define the characteristics of seed processing machines were: pure seed (%), weed seed (%), seed of other crops (%), inert matter (%), amount of processed seed (kg), seed losses (%) and processing output (%). On the basis of the obtained results it is possible to select the appropriate equipment, as well as their correct adjustment in the seed processing, depending on the quantity and type of weeds and other ingredients found in the natural red clover seeds. Machines and devices used for seed processing were: intake pit with belt conveyor, belt conveyors, bucket elevators and the fine-cleaning machine. For the separation of weeds used a magnetic separator with rollers.

Keywords: *Relevant parameters, Machines, Red clover, Processing, Impurities.*

Introduction

In the Republic of Serbia, red clover (*Trifolium pratense* L.) occupies the second place in the areas where it is grown, right after alfalfa (*Medicago sativa* L.). Red clover (*Trifolium pratense* L.) is most grown in the area of Sumadija and Western Serbia where it is harvested on an area of 33.883 ha with an average hay yield of 3.0 t ha^{-1} (Statistical yearbook of the Republic of Serbia, 2018). For animal, red clover is used as green fodder plants; as a single crop or as a mixture; for grazing or conservation as hay, silage, or dehydrated as flour, in the form of pellets and briquettes (Vučković, 1999). Red clover is characterized by high yield of biomass, high feed quality and rapid regeneration after mowing. In particular, the leaves are rich in proteins (about 25% in the phase of butonization). It has a high content of provitamin A, vitamin C, D, E, K, B1, B2, B3, as well as microelements - molybdenum, cobalt, boron, copper and manganese (Marković et al., 2007). In comparison with lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) is better tolerated by acidic soil. It is recommended to cultivate on neutral to slightly acidic soils with low pH (5.5-7), and the soils with worse structure. It can be grown on different types of soils: medium heavy, wet, with medium fertility – brown earth, alluvial and smonitza (Lugić et al., 2000).

The natural seed for seed processing is a very complex mechanical mixture in which there are large and small weeds, parts of organic and inorganic matter and broken seeds. Seed processing, as a part of seeds production, is a very hard work with high energy consumption (Orobinskij at al., 2017). For the seed processing and storage, the physical characteristics of the seed are very important. The most important physical characteristics of the seeds are: humidity, shape, dimensions, sphericality, mass of 1000 seeds, seed volume, porosity of the seed, volume-hectolitic mass, density, static and dynamic angle of internal friction - free fall angle, static coefficient of friction on known surface (Copeland et al., 2004; Black et al., 2006, Đokić and Stanisavljević, 2012; Đokić et al., 2012; Baskakov et al., 2018). The right combination of seed processing equipment is necessary to achieve the best quality of processed seed in the shortest possible time, where the quality of the seed corresponds to the standards for seed (Đokić et al., 2017; 2018; 2019). In red clover, weed species contaminate seeds and can make difficult processes of harvest and seed processing. In particular, there is a detrimental presence of quarantine weed, Dodder (*Cuscuta* sp). This weed belongs to the most dangerous and economically most damaging quarantine weeds in the red clover crop. If it can not be prevented, it causes enormous crop damage (Karagić et al., 2007). Seeds must be of high purity, germination and moisture. The Law of Seeds and Planting Material prescribes all conditions related to the production, processing, use, trade, import and testing of seeds of agricultural plants (Gazette of the Republic of Serbia, No. 45, 2005). The quality of the red clover seeds should correspond to the Rule on the Quality of Seeds of Agricultural Crop Seeds (Official Gazette of SFRY, No. 47/87). The red clover seeds must have a minimum purity of 95%, 2% of other seeds, less than 0.5% of weeds (without *Cuscuta* sp and *Rumex* sp), up to 2.5% inert matter, minimum germination of 70% and maximum moisture of 13%.
The aim of this study was to show the effectiveness indicators of seed processing in the red clover seeds. The basic indicators of the efficiency of machines in seed processing are the quality and quantity of the seed obtained at the end of the seed processing. In the process of seed processing, it is necessary to get as much quality seeds for the shortest possible time, and with the least losses.

Materials and methods

The test was carried out at the seed processing center of the Institute for Forage Crops Kruševac. In three replications, the natural seed of the red clover of seven seed lots with different purity was processed. The content of the weed species was different for each seed lot. The seed processing equipment was manufactured by Danish manufacturers Kongskilde and Damas. Machines and devices used for seed processing were: intake pit with belt conveyor, belt conveyors, bucket elevators, and the fine-cleaning machine type Alfa - 4. In the upper and lower shaker shoe on the fine-seeding machine Alfa-4 there were six sieves arranged in two levels. In the upper shaker shoe were sieves with round openings of diameter: 2.75 mm; 2.5 mm; 2.25 mm; 2.2 mm; 1.9 mm and 1.9 mm. In the lower shaker shoe were sieves with longitudinal-cut openings of width: 1.3 mm; 1.2 mm; 1.1 mm; 0.6 mm; 0.5 mm and 0.5 mm. For the separation of weeds we used a magnetic separator of the German manufacturer Emcek Gompper-type 4. During the test, the steel powder of Nutra Fine was used.
An analysis of the content of basic seed and other ingredients in the red clover seeds was done in a Laboratory for the sample analysis of the Institute's seed processing center. For the analysis of the content of the other matters in the seed samples, a magnifying glass and a precision electronic scale was used. Seed samples for analysis were weighing 5 g (working) and 50 g (average). An electronic scale of measuring range up to 300 kg was used to measure the mass of the natural and processed seeds. During the process of seed processing, the following parameters were measured: quantity of pure seed (%), seed of other species (%),

inert matter (%), weed (%), and amount of processed seed (kg). After seed processing, the processing output (%) and seed losses (%) were calculated. The obtained results were processed by statistical analysis of variance (ANOVA), and the significance of the difference of the mean was tested with the Tukey test. The statistical program Minitab 16.1.0 was used for data processing (statitics software package).

Results and discussion

The purity of the red clover natural seed of seven different lots is shown in Table 1. The smallest seed purity (52.0 %) was for seed lot II, and the highest (88.0 %) was for seed lot V. The purity of seed lot I was 81.0 %. For seed lots III and VI, the seed purity was 78.0 %. Seed lots IV and VII were seed purity of 86.0 %.

Table 1. The average purity of the natural red clover seed

Lot	I	II	III	IV	V	VI	VII	*F test*
Seed structure	%	%	%	%	%	%	%	
Pure seed	81.0 ab	52.0 c	78.b	86.0 a	88.0 a	78.0 b	86.0 a	***
Other species	-	-	-	-	grass		0.6 alfalfa	
Inert matter	19.0 bc	48.0 a	22.0 b	14.0 c harvest residues, empty seed	12.0 c harvest residues, soil	22.0 b harvest residues	13.4 c harvest residues	***
Weed	in 5 g 5 *Cuscuta* sp	in 5 g 4 *Cuscuta* sp	-	Barnyard grass in 5 g 2 *Rumex* sp	in 5 g 2 *Cuscuta* sp, in 5 g 2 *Rumex* sp	in 5 g 40 *Cuscuta* sp, in 5 g 5 *Rumex* sp	barnyard grass in 5 g 40 *Cuscuta* sp	
Total	100	100	100	100	100	100	100	-

F test, statistical significance levels: *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ns – not significant (p ≥0.05)
Tukey test statistical significance levels: p≤ 0.05, differences in row marked in small letters a, b, c...

Inert materials in the form of harvest residues (stems, leaves, pods), sickly, damaged seeds and soil were from 12.0 % for seed lot V; to the highest values of 48.0 % for seed lot II. In seed lot VII seed of other plant species (grass and seed of alfalfa) was discovered. In the working sample (5 g), the highest presence of the quarantine weed Dodder (*Cuscuta* sp) was founded in seed lots VI and VII (a total of 40 seeds of *Cuscuta* sp). In the seed IV, V and VI there were also seeds of curly dock (*Rumex* sp). In the samples of seed lots IV and VII, there were also Barnyard grass (*Echinochloa crus-galli*).

The purity of the red clover seeds after seed processing is shown in Table 2. During the cleaning, the seed passes through a system of machines that separate impurities such as dry stems, weeds and broken seeds (Uhlarik et al., 2018; Baskakov et al., 2018). After seed processing on the fine- cleaning machine, the seed is processed on a magnetic machine to remove weeds. Seed samples for quality analysis are taken after processing the seed on a magnetic machine (Đokić et al., 2019). The purity of the red clover after passing through the magnetic separator was high and ranged from 98.4% for seed lot I, to 99.0% for seed lots II, V and VI. The content of other plant species, inert materials in the form of sickly seeds and weeds, were within the legally prescribed limits. In the seed lot III three seeds of curly dock (*Rumex* sp) were found in the average sample of 50 g, which is less than the legally prescribed amount (maximum 4 seeds in a sample of 50 g).

Table 2. The average purity of processed red clover seeds

Lot	I	II	III	IV	V	VI	VII	*F test*
Seed structure	%	%	%	%	%	%	%	
Pure seed	98.4 a	99.0 a	98.5 a	98.6 a	99.0 a	99.0 a	98.6 a	ns
Other species	in 10 g 10 seeds alfalfa, 2 seeds grasses	0.6 chamo mile	0.2 (grasses) 0.2 (common vetch)	0.3 alfalfa	in 10 g 3 seeds grasses	-	0.6 alfalfa	
Inert matter	1.6 a	0.4 c	1.1 b	0.9 b	1.0 b	1.0 b	0.8 bc	**
Weed	8 seeds Barnyad grass, daisy	-	in 50 g 3 curly dock 3 chamo mile	0.2	-	in 5 g 1 Barnyad grass	-	
Total	100	100	100	100	100	100	100	-

F test, statistical significance levels: *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ns – not significant (p ≥0.05)
Tukey test statistical significance levels: p≤ 0.05, differences in row marked in small letters a, b, c...

The quantities of natural red clover seeds at the beginning of the seed processing, as well as the amount of processed seeds at the end of the processing are shown in Table 3. Processing output and seed losses on the processing machines, also calculated and displayed (%). The highest utilization of seed yield (82.59%) was found in the seed lot VII (with initial purity of 86%) with losses on the processing machines of 3.8%. Đokić et al. (2018) obtained the highest red clover seed yield of 80.56%, with at least losses of 7.4% on processing machines (purity 87%). In the examined material, the minimum seed utilization of 46.64% was found for seed lot II. This seed lot, also, had the lowest initial purity of natural seed (52.0%) with the highest content of inert substances (48.0%). The highest seed losses during seed processing (17.34%) were found in seed lot I.

Table 3. Amounts of proceseed red clover seeds on the processing machines

Seed structure	Lot (kg)							*F test*
	I	II	III	IV	V	VI	VII	
Natural seed	1150.0 a	268.0 c	1027.0 a	663.0 b	593.0 b	896.0 b	293.0 c	***
Processed seed	770.0 a	125.0 c	700.0 a	520.0 b	473.0 b	655.0 a	242.0 c	**
Processing output (%)	66.96 b	46.64 c	68.16 b	78.43 a	79.76 a	73.1 ab	82.59 a	**
Losses **(%)**	17.34 a	10.3 bc	12.62 b	8.8 c	9.36 bc	6.28 c	3.8 d	***

F test, statistical significance levels: *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ns – not significant (p ≥0.05)
Tukey test statistical significance levels: p≤ 0.05, differences in row marked in small letters a, b, c...

Quantities of natural seed (p≤ 0.05) were significantly higher in the seed lots I and III. For processed seed significantly higher quantity were in lots I, III, and VI. The processing output were statisticaly signifficant in part IV, V and VII, while seed losses were significantly higher the in seed lot I (Table 3).

Conclusions

Based on the obtained research results, it can be concluded that the effectiveness of the seed processing machines depends on the purity of the natural seed, the content and the type of quarantine weeds, as well as the correct adjustment of the finishing machines in the processing of red clover seeds. By increasing the content of weeds and impurities in the natural seed, the technological process of processing is longer, the consumption of energy is higher, and therefore the cost of the harvested seeds are high. The purity of natural red clover seeds ranged from 52% to 88%. The content of inert materials ranged from a minimum of 12% for seeds lot V to a maximum of 48% for seeds lot II. The most seeds of quarantine weeds were found in the seeds of the VI and VII. There was a total of 40 seeds of *Cuscuta* sp in each seed lot. Any defects and failures in the technological process can lead to large losses of seeds, or large economic losses. The quality of the processed seed must comply with the legislation on seed material.

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GRAIN YIELD, YIELD COMPONENTS, MACRO AND MICROELEMENTS VARIATION ANALYSIS IN WHEAT GROWN ON SOLONETZ SOIL

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Abstract

There are more than 7.7 billion of humans at the moment. It seems unavoidable that, if it weren't any calamity, our population would be about to reaching 10 billion mouths by the mid-century. The crop production increment in developing countries relies on agricultural production intensification per area unit by 80% and the remainder on arable land expansion. Hence, the part of strategy is to seize the soil degradation and to enhance or restore productivity to degraded soils. Solonetz is a less productive alkaline soil, with high sodium content, covering about 2.1% of agricultural land in Serbia. Bread wheat, one of the main sources of plant generated energy for humans, is moderately tolerant to alkaline soil. Long term trials for studying the wheat reaction to solonetz growth conditions, has been established. The results of 11 wheat varieties and 1 triticale are given. Analyses of grain yield, its components, as well as macro and microelements storage in plants has been conducted. Varieties showed considerable variation in grain yield, plant height, spike length and weight, grain weight and number per spike. The content of selected elements (Ca, Cd, Co, Cr, Cu, K, Mg, Mn, Na, Ni, Pb, and Zn) varied in harvested plant material, as well. Pearson correlation coefficients were used to study interrelationship between observed sources of variation. Low positive correlation coefficients between grain yield and plant height were in consequence of the genetic variation generated in plant breeding program under similar selection criteria. The mutual influence within the yield components, the spike traits, was considerably higher. Away from K and Na, the variation of elements had weak to negligible influence on grain yield variation. Further analysis using ridge regression and cluster analysis showed more complex relationship..

Keywords: *wheat, yield, components, elements, solonetz.*

Introduction

Though gradually slowing in rate, the human population is still exponentially growing (UN DESA PD, 2019). Hence, using up resources is closely related to the demand for food increment, particularly targeting those energy sources like key cultivars for global food security, that wheat and cereal, as a whole, surely are (Mondal *et al*., 2016). The crop production increment in developing countries relies on agricultural production intensification per area unit by 80% and the remainder on arable land expansion (Alexandratos and Bruinsma, 2012). The part of this strategy is to seize the soil degradation and to enhance or restore degraded soils productivity. An inventory of the soils of Serbia and their degradation revealed that solonetz which is a class of a less productive alkaline soil, with high sodium content, covers 2.1 - 2.6% of 3.4 million hectares of agricultural land in Serbia (Ličina *et al.*, 2011; Belić *et al*., 2012). In Vojvodina, there is approximately 80,000 hectares of this type of soil, mostly in the Banat (Belić *et al.*, 2006). This soil type belongs to alkaline class with high sodium content, occurring as a result of extensive irrigation, or could be spontaneously formed in a lowland steppe arid climate. Solonetz covers about 150 million hectares in the World, about 0.5% i.e. 20 million hectares of Europe is solonetz covered soil, mainly in Russia, Ukraine, Hungary, Bulgaria, Romania, and Kazakhstan (ESBN, 2005; Spaargaren, 2008). This type of soil is known as alkaline, clay and sodium reach land, deficient for nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), cuprum (Cu), manganese (Mn) and iron (Fe), after Blum (2018). There are 118 elements that have been discovered (Karola *et al.*, 2016). However, 98 of them occurred in nature, 90 among them could be found in appreciable amount, and 6 of them, namely, carbon (C), hydrogen (H), N, oxygen (O), P and sulphur (S) are essential for life entering the protein and nucleic acid structure. Other 12 are very important for organism activities, chlorine (Cl) and iodine (I) from halogen family of elements (non-metals) and bio-metals sodium (Na), K, magnesium (Mg), calcium (Ca), Fe, cobalt (Co), Cu, Zn, Mn and molybdenum (Mo). Elements that play an essential role in the living world could be divided into 4 groups: 1. *The main or bulk elements* (C, H, N, O, P, S); 2. *Macrominerals and ions* Na, K, Mg, Ca, Cl, phosphate ion (PO_4^{3-}), sulphate ion (SO_4^{2-}); 3. *Trace elements* (Fe, Zn, Cu) and 4. *Ultratrace elements* comprises of: a. *Non-metals* fluorine (F), I, selenium (Se), silicon (Si), astatine (As), boron (B) and b. *Metals* Mn, Mo, cobalt (Co), chromium (Cr), vanadium (V), nickel (Ni), cadmium (Cd), tin (Sn), lead (Pb), lithium (Li) (Giribabu and Narayanan, 2015). Essential mineral elements for plants could be classified in four groups: *Macronutrients - major* (N, P, K), *Macronutrients - secondary* (Ca, Mg, S), *Micronutrients - essential* (Fe, Mn, Zn, Cu, B, Mo, Cl, Ni), and *Micronutrients - beneficial* (Na, Si, Co, Se), Ronan (2007). The increasing demand for food pushes primary agricultural production toward the increment of cultivation intensity, not only in a sense of getting a higher yield, but also in obtaining higher quality. To reach this goal, a better understanding of plant nutrient acquisition, complex interaction between genotypes, environment and chemical elements is required (Etienne *et al.*, 2018). Solonetz is commonly utilized for extensive pasture, but some crops and remedial measures can raise the productive value of this land. Salt tolerance is an inherent trait existing in plants in order to adapt to various environments. Small grains including bread wheat are generally regarded as moderately tolerant to alkaline soil (Dimitrijević *et al*., 2012; Zafar *et al*., 2015). Phenotypic variability of yield studied in trials established *in situ* in abiotic stress conditions of solonetz soil agro-ecological environment could provide valuable informations in creating desirable wheat variability in order to put less productive land in more intensive use for food production (Petrović *et al*., 2016). However, in stressful production conditions, some Green Revolution ways do not apply in full. Firstly, that means that individual plant contributes more to overall grain yield than in normal intensive production environment, where the main contributor is population per area unit. Consequently, individual plant trait variation, the yield components like plant height, and generative part traits influencing individual plant productivity (spike length and weight, grain number and weight per spike), gain in importance in final yield formation.

Hence, the aim of this work is to examine and analyse varietal variation of grain yield and the yield components in wheat grown on solonetz soil, as well as, to get an insight in complex interrelations in these variations.

Material and Methods

There were 12 genotypes included in the experiment, where 11 were 6X bread wheat varieties (*Triticum aestivum ssp. vulgare* L), all created within a Cereal Breedeing Program of the Institute of Field and Vegetable Crops in Novi Sad, namely Renesansa, Pobeda, Evropa 90, NSR-5, Dragana, Rapsodija, Simonida, Cipovka, Banatka, Bankut 1205 (winter types), Nevesinjka (facultative) and winter type triticale variety Odisej. The trial is a part of broader research experiment conducted for about two decades parallel in normal, chernozem i.e. black soil, (pH = 6.86) experimental conditions at the test field of the Institute of Field and Vegetable Crops - near Novi Sad (latitude: 45.3249360N, longitude: 19.8428830 E), and in solonetz soil (pH = 9.86) abiotic stress conditions at the experimental field in Kumane village (latitude: 45.5219940N, longitude: 20.1949190 E). The experimental plant sample was chosen according to previously accumulated experience of varietal behaviour in harsh environmental conditions that solonetz soil is. Varieties Renesansa, Rapsodija, Pobeda, NSR-5 and Evropa 90 are selected as particularly adaptable genotypes. Varieties Nevesinjka and NSR-5 are particularly tolerant to less productive soil conditions. All the genotypes express tolerance to low temperatures. Varieties Renesansa and Rapsodija are particularly resistant to drought conditions. Varieties in study are grown not only in Serbia, but also at the Balkans. Variety Rapsodija is grown in the EU and Ukraine, as well. Triticale Odisej was included as a control genotype, because triticale as a synthetic species has been created to withstand abiotic stressful growth environments. Moreover, two varieties Pobeda, and Renesansa were followed parallel on chernozem and solonetz as control genotypes, too. The raw data were collected in one vegetation period, to diminish environmental variance in total phenotype variation. The variation observed in the experiment was analyzed using descriptive statistics, as well, as Analysis of variance (ANOVA) and direct and indirect correlations. Additional analysis of trait interrelations and variation was conducted using Cluster analysis as non-parametric method. The content of macro and micro chemical elements was followed by atomic absorption spectrophotometry in dry plant material. The data were standardized in order to be comparable in statistical models. All the statistical calculations were done according to Microsoft Excel procedures. The experiment was set up by Randomized Complete Block Design in three replications. Each variety was sawn in eight 12.5cm spaced rows, 155m of length. Fifty kilograms of NPK 15:15:15 fertilizer was applied with sowing.

Results and Discussion

The analysis of grain yield variation shows that abiotic stress caused mainly by sodium rich solonetz soil influenced grain yield decrement by about 40%, comparing yield to control varieties Pobeda and Renesansa grown in normal conditions of chernozem at the locality of Rimski Sancevi. Local population Banatka, and old variety Bankut 1205 were expectedly the tallest genotypes, however modern varieties did not exhibit no significant loss in plant height (PH) on solonetz comparing to cernozem, including varieties Pobeda and Renesansa. Hence, the immediate cause of the grain yield decrement is to be searched in mass property parameter variation, including grain weight and grain number per spike (GWS, and GNS, respectively), which were affected by solonetz stressful growth conditions by lowering its average values about 30% comparing to control averages. Triticale Odisej exhibited the best performance on solonetz soil (tab. 1). According to visual screening from the field, the other reason of the grain yield decrement on solonetz was canopy that suffered more in a harsh conditions of Kumane part of the trial. Concerning chemical elements that were found in plant dry material by atomic-absorption spectrophotometry, varietal variation in content has been denoted. Wheat varieties in trial absorbed chemical elements differently, exhibiting variation that could be meaningful in wheat breeding program for special purposes i.e. higher salinity tolerance, or remediation of soil burdened with toxic elements. There is a significant difference in absorbed cobalt (Co), chromium (Cr), sodium (Na), nickel (Ni), and lead (Pb) comparing plant material samples gathered on solonetz, and chernozem soil. Sodium richer solonetz soil caused higher absorption of this element. The higher absorption of Co, Cr, Ni, and Pb, on solonetz soil, could be the result of prolonged effect of phosphogypsum application as an ameliorative measure, in the past (tab. 2). Pearson's correlation coefficients varied between none to very high, graded after Akoglu (2018). The relation between the grain yield and the yield components varied from weak ($r = 0.21$ to 0.28) to moderate ($r = 0.31$ to 0.33). In a whole, yield components under study were quite evenly related to grain yield, and no one stood out as an early phenotypic marker of higher yield (tab.3)

Table 1. Grain yield, and the yield components (plant height – PH, spike length – SL, spike weight – SW, grain weight per spike – GWS, and grain number per spike – GNS) variation in wheat varieties grown on solonetz and control varieties grown on chernozem at R. Sancevi.

GENOTYPES	TRAITS					
	YIELD	PH	SL	SW	GWS	GNS
	t/ha	cm	cm	g	g	
Pobeda-R. Sancevi	6,3	83,7	9,0	2,2	1,7	42,8
Renesansa-R. Sancevi	6,3	87,7	10,0	2,5	1,9	48,8
Renesansa	3,5	77,6	8,5	2,0	1,5	36,9
Pobeda	4,1	80,0	7,8	1,8	1,5	36,1
Evropa 90	4,1	87,3	8,5	1,9	1,5	38,5
NSR-5	3,6	71,2	7,7	2,0	1,5	34,3
Dragana	4,2	83,0	7,9	1,6	1,2	26,9
Rapsodija	3,7	73,9	7,3	1,8	1,5	39,6
Simonida	4,0	75,6	7,9	1,7	1,3	33,6
Cipovka	3,9	79,5	8,0	1,6	1,2	35,7
Banatka	3,4	118,7	7,1	1,1	0,9	21,2
Bankut 1205	3,8	114,6	8,0	1,3	0,9	23,0
Nevesinjka	4,0	78,9	9,7	1,8	1,3	38,5
Odisej	5,2	107,5	10,8	3,3	2,6	51,6
Confidence Level(95.0%)	*0,55*	*8,80*	*0,62*	*0,31*	*0,24*	*4,95*
Confidence Level(91.0%)	*0,46*	*7,46*	*0,53*	*0,26*	*0,21*	*4,20*

Table 2. The content of chemical elements found in dry plant material using atomic-absorption spectrophotometry.

GENOTYPES	ELEMENTS [mg/kg]										
	Ca	Co	Cr	Cu	K	Mg	Mn	Na	Ni	Pb	Zn
Pobeda-RS	6152,0	0,0	3,9	20,9	9366,0	2721,0	138,8	588,5	5,5	1,6	52,4
Renesansa- RS	5656,0	0,0	2,5	18,7	9074,0	1954,7	90,2	434,2	6,2	0,0	47,8
Renesansa	5613,7	3,5	12,2	28,6	7123,7	3032,7	307,7	2008,0	10,6	7,1	73,0
Pobeda	5683,3	1,8	6,1	20,3	6964,7	2782,0	219,3	1057,0	6,6	4,2	58,1
Evropa 90	5133,0	3,6	10,0	20,9	7056,3	3008,7	329,8	1548,9	10,7	8,0	57,6
NSR-5	6346,0	4,5	13,1	35,5	8163,3	3366,0	382,1	2123,8	12,2	10,0	67,1
Dragana	7435,0	6,1	15,4	26,1	6856,7	4007,0	480,2	1412,7	17,1	13,4	70,3
Rapsodija	7070,7	4,7	12,0	23,3	6561,7	3435,3	395,7	1106,7	14,1	10,0	71,1
Simonida	5465,3	2,3	7,3	21,1	6399,0	2819,3	264,4	1170,2	8,3	5,7	47,9
Cipovka	6146,0	6,6	17,0	25,0	8233,3	3686,3	534,9	1474,2	17,0	13,5	69,1
Banatka	5474,0	2,8	8,5	19,7	5545,5	2780,5	245,7	1640,7	8,0	5,9	69,7
Bankut 1205	6003,5	3,5	11,3	21,9	7630,0	3262,0	292,0	1310,9	12,5	7,9	75,9
Nevesinjka	6663,0	4,6	13,4	24,8	9099,0	3456,0	425,7	1534,6	12,2	10,5	71,7
Odisej	6013,3	4,2	13,7	24,9	10801,0	3251,7	364,0	1393,6	12,1	8,7	95,6
CL (95.0%)	*373,48*	*1,13*	*2,47*	*2,54*	*821,04*	*289,75*	*71,61*	*268,34*	*2,14*	*2,28*	*7,34*
CL (91.0%)	*316,66*	*0,96*	*2,10*	*2,15*	*696,13*	*245,67*	*60,71*	*227,51*	*1,81*	*1,93*	*6,22*

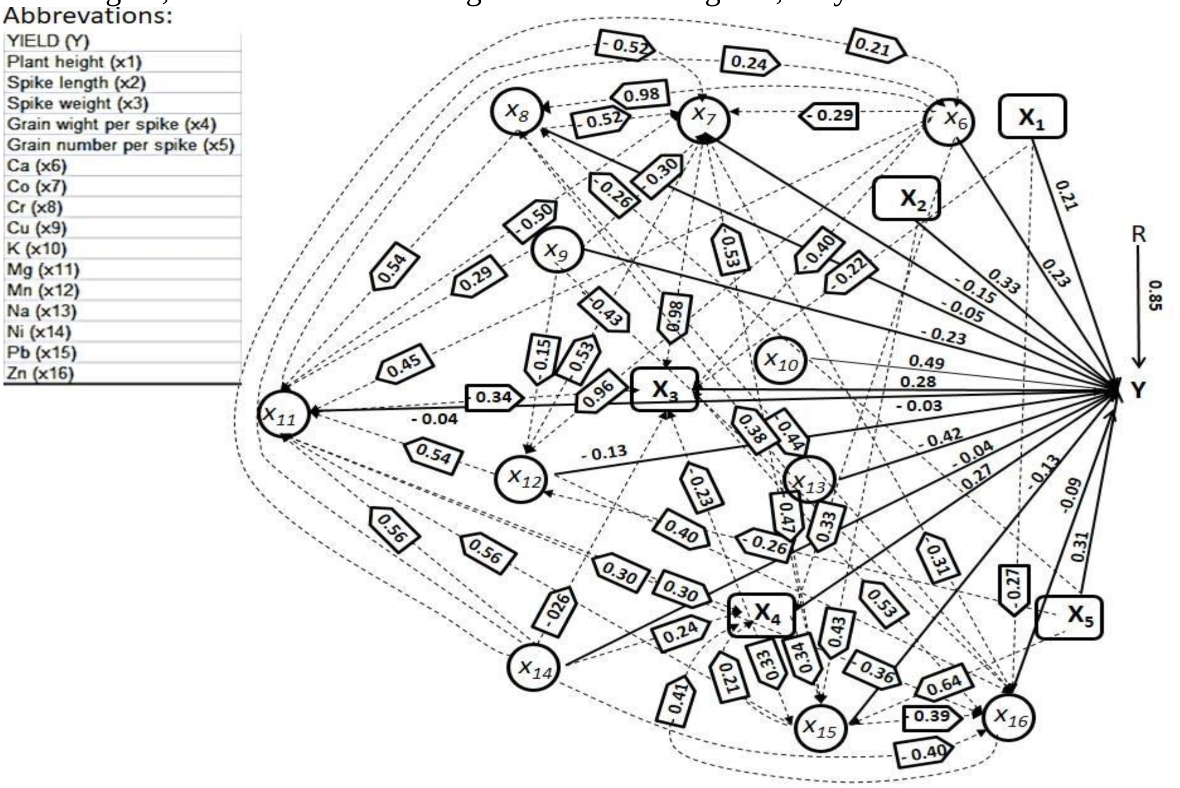
The mutual relation between the grain yield and chemical elements content, predominantly varied from none to weak at r = -0.03 to 0.23. However, higher sodium content of solonetz caused higher content in plants as well, resulting in moderate to strong (r = -0.42) negative correlation between Na content and grain yield. The effect of regular and usual application of fertilizers reflected in moderate to strong positive correlation between K and yield (tab. 3).

Table 3. Pearson’s correlation coefficients giving interrelationship of examined biological traits, and chemical elements content in wheat sample of 11 wheat and one triticale varieties.

	TRAITS						ELEMENTS [mg/kg]										
PEARSONS CORR.	YIELD	PH	SL	SW	GWS	GNS	Ca	Co	Cr	Cu	K	Mg	Mn	Na	Ni	Pb	Zn
	[t/ha]	[cm]	[cm]	[g]	[g]												
	Y	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	x13	x14	x15	x16
YIELD (Y)	1,00																
Plant height (x1)	0,21	1,00															
Spike length (x2)	0,33	0,23	1,00														
Spike weight (x3)	0,28	0,09	0,74	1,00													
Grain wight per spike (x4)	0,27	0,07	0,71	0,99	1,00												
Grain number per spike (x5)	0,31	-0,06	0,75	0,88	0,88	1,00											
Ca (x6)	0,23	-0,14	-0,24	-0,17	-0,16	-0,23	1,00										
Co (x7)	-0,15	-0,02	-0,13	-0,09	-0,09	-0,19	0,55	1,00									
Cr (x8)	-0,05	0,03	-0,04	-0,02	-0,03	-0,14	0,53	0,97	1,00								
Cu (x9)	-0,23	-0,16	-0,06	0,05	0,01	-0,05	0,28	0,55	0,60	1,00							
K (x10)	0,49	0,14	0,74	0,56	0,53	0,60	0,06	-0,06	0,08	0,04	1,00						
Mg (x11)	-0,03	-0,07	-0,17	-0,15	-0,15	-0,26	0,76	0,92	0,91	0,50	0,02	1,00					
Mn (x12)	-0,13	-0,07	-0,11	-0,07	-0,07	-0,14	0,53	0,99	0,95	0,54	-0,04	0,90	1,00				
Na (x13)	-0,42	-0,16	0,06	0,07	0,06	-0,09	-0,09	0,16	0,21	0,25	0,07	0,17	0,11	1,00			
Ni (x14)	-0,04	0,01	-0,15	-0,11	-0,12	-0,21	0,64	0,97	0,94	0,53	-0,05	0,94	0,95	0,06	1,00		
Pb (x15)	-0,13	-0,04	-0,13	-0,10	-0,10	-0,19	0,56	0,99	0,97	0,55	-0,05	0,93	0,99	0,14	0,96	1,00	
Zn (x16)	-0,09	0,38	0,15	0,23	0,20	0,08	0,28	0,58	0,63	0,61	0,24	0,51	0,56	0,05	0,57	0,55	1,00

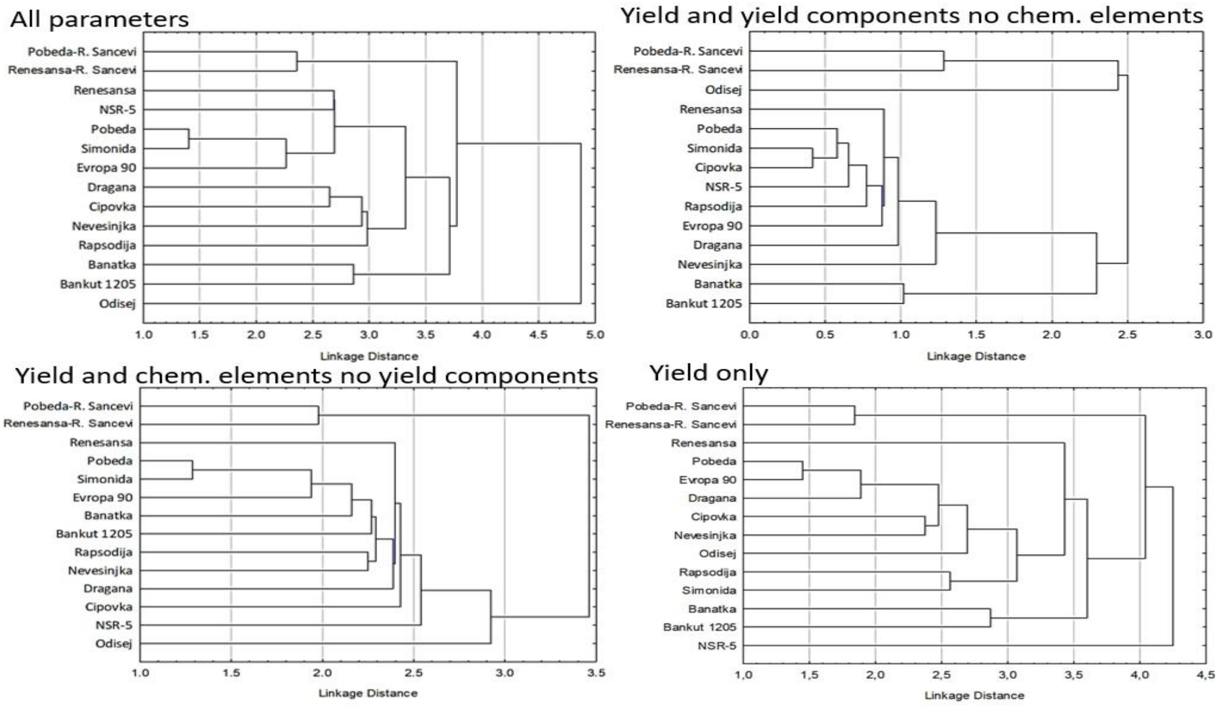
Path coefficient analysis was conducted on normalized data by subtracting varietal averages from the grand mean, and dividing by standard deviation, in order to weight values. Since, there is no room for detailed analysis, the sample figure given illustrates very complex indirect relations to grain yield, that were observed (fig. 1)

Figure 1. Path coefficient analysis giving closer analysis of direct effect of yield components and chemical elements on grain yield, through indirect effects screening. Due to complexity of the figure, indirect coefficients higher than 0.20 are given, only.



Clusterization of genotypes in trial was conducted as a non-parametric evidence of the influence of the yield components and chemical elements content as a whole in plants on grain yield formation. By drawing a comparison between genotype array in cluster grouped using all parameters – yield, yield components, and chemical elements content in plant material, and other three clusters it is evident that all the examined biological traits, as well as, chemical element content had an influence on the yield. The effect of soil stress absence on chernozem led to the grouping of control wheat varieties, Pobeda and Renesansa at the locality of Rimski Sancevi, in all the clusters. Triticale Odisej was genotype for itself, as expected. All the other varieties more or less differed in grouping depending on clusterization criteria. Although the grain yield had its influence on genotype grouping, the variation of the yield components, as well as, chemical element content variation exhibited its strength to rearrange the genotype grouping to some extent (fig. 2).

Figure 2. Cluster analysis for 11 wheat and 1 triticale varieties grown on solonetz soil and two wheat varieties grown on chernozem as control.



Conclusions

This is a pilot report of the results obtained on two localities for 12 genotypes in one year, so there are indications and trends in order, rather than hard conclusions. The inferiority of sodium reach solonetz soil in wheat production is well illustrated, once more. However, the existing genetic variability created for intensive agricultural practice in favourable growth conditions, could withstand abiotic salinity stress giving up to 60% of grain yield comparing to chernozem environment, without any additional investment. Moreover, analysing some tiny influences of chemical element content in plants, we could find genetic variability not only for grain yield increment, but also for phytoremediation of soil with higher content of adverse chemical elements. The higher zinc content in varieties grown on solonetz soil comparing to control varieties grown on chernozem, as well as, other macro, and micro nutrients varietal variation observed in this investigation, points out the existence of variability that could be utilized in fighting the "hidden hunger" of malnutrition. Anyway, the further research is required for analysing deeper a complex structure of phenotype variation in order to cultivate genetic variability adequate for drastic climate changes, and higher, and higher demands in food production the humanity is facing.

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MINERAL ELEMENTS TRANSLOCATION AND RELATIVE YIELD AND YIELD COMPONENTS TOLERANCE IN WHEAT GROWN ON SOLONETZ SOIL

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Abstract

The Green Revolution had great, but ambiguous impact on food production. Though it has increased agricultural output dramatically, the loss of a third portion of arable land due to erosion or pollution has been going on, as well. Moreover, the similar selection preasure, as it seems, has led to nutritionally inferior crops, engendering malnutrition, and even a stand that we live in a toxic food culture. The narrowing of genetic variability, and industrialized, unsustainable food system, made a modern diet as a leading risk factor for diseaes development. Increased demand for nutrients and health-safe food in the situation of the exponential growth of human population represents a some of heavy challenges to agriculture. The utilization of less productive soil in wheat production, as well as, the studies of nutritional and toxic elements dynamics in vegetative and generative wheat plant parts is given in the article. The trial has been conducted on solonetz soil. The phenotypic varietal variation of the yield and yiels components (plant height, spike length and weight, grain weight and number per spike) in 11 bread wheat and the one triticale genotypes was followed, as well as, the source to sink dynamics of 13 selected elements (B, Ca, Cd, Co, Cr, Cu, K, Mg, Mn, Na, Ni, Pb, and Zn). The varietal ability to endure abiotic stress growth conditions of solonetz was estimated through the relative tolerance index obtained by comparing a phenotypic variability of particular trait for each genotype in sample with corresponding phenotype variation of two standards, wheat varieties Renesansa and Pobeda, grown in normal, intensive production conditions of the experimental fields of the Institute of Field and Vegetable Crops in Rimski Šančevi. Notable variation has been found and commented in the article.

Keywords: *wheat, solonetz, yield components, tolerance index, chemical elements.*

Introduction

Starting in the middle of previous century, two phenomena have label our past, present and future – The Green Agricultural Revolution and Human Population Geometric Growth. World's food production per-capita increased about 25%, and the yield of cereals, substantial for securing human energy supply, has grown 3-5 times for the four decades since 1960s. In the same period, the human population has more than doubled (Roser and Ritchie, 2019). Parallel processes, however, have been the loss of a third portion of arable land due to erosion or pollution has been going on, biodiversity and dietary diversity narrowing, as well as, ongoing overall erosion of the environment. The soil, as well as total environmental pollution, largely depends on micro and macro elements content and flow. High concentrations of heavy metals, as a special group of elements, for example, could exhibit a toxic effect on plants. However, from 1970s there has been a rising perception of the preventable influence of dietary micronutrient deficiency on human health and mortality (Dubock, 2017). In the same time, the growing level of environmental awareness has started. In order to find adequate pathways toward food production sustainability, stability and increment, complex and multidisciplinary scientific programs are needed. Within this strategy the research of chemical elements in biosphere and food is of relevance. Chemical elements influence environmental pollution, food nutritive value, healthcare, and is fundamental for cell and genetic and epigenetic processes. There are 11 heavy metals - lead (Pb), cadmium (Cd), mercury (Hg), cuprum (Cu), tin (Sn), vanadium (V), chromium (Cr), molybdenum (Mo), cobalt (Co), and nickel (Ni) and three metalloids - antimony (Sb), arsenic (As), and selenium (Se) listed as the most hazardous elements. (Vodyanitskii, 2016). Because of water and soil pollution, metals in the environment should be considered as environmental contaminants that represent a permanent threat to mankind. Hence, there are some serious concerns over their potential health effects on humans (Jan *et al.*, 2015). In fact, there are 118 elements discovered up to 2016 (Karola *et al*., 2016). In fact, there are 118 elements discovered up to 2016 (Karola *et al.*, 2016). Six of them - carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P) and sulphur (S) are essential for life building the protein and nucleic acid structure, while twelve are of remarkable importance for organism activities, chlorine (Cl), iodine (I) as non-metals and sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), cobalt (Co), cuprum (Cu), zinc (Zn), manganese (Mn) and molybdenum (Mo), as bio-metals. According to Ronan (2007), there are four groups of elements having an essential role in organisms: I. the main or bulk elements (C, H, N, O, P, S); II. Macrominerals and ions Na, K, Mg, Ca, Cl, phosphate ion (PO_4^{3-}), sulphate ion (SO_4^{2-}); III. Trace elements (Fe, Zn, Cu) and IV. Ultratrace elements comprises of: IVa. Non-metals fluorine (F), I, selenium (Se), silicon (Si), astatine (As), boron (B) and IVb. Metals Mn, Mo, cobalt (Co), chromium (Cr), vanadium (V), nickel (Ni), cadmium (Cd), tin (Sn), lead (Pb), lithium (Li) (Giribabu and Narayanan, 2015). Essential mineral elements for plants could be classified in four groups: Macronutrients - major (N, P, K), Macronutrients - secondary (Ca, Mg, S), Micronutrients - essential (Fe, Mn, Zn, Cu, B, Mo, Cl, Ni), and Micronutrients - beneficial (Na, Si, Co, Se). As it could be seen from above classification, the effect of particular element depends of its nature i.e. chemical properties and its concentration. Consequently the role of plants could be double-natured. Plants, through food, bring these elements to our diet and plants through elements mobilization could be utilized in phytoremediation i.e. cleaning up contaminated soil using the ability of plants to uptake pollutants from the environment (Ali *et al*., 2013). Solonetz is sodium reach soil, of higher pH value, being considered as a stressful environment for cultivar growing, and is commonly used as extensive pasture. The genetic group at the Faculty of Agriculture, University of Novi Sad has been running long term parallel trials on solonetz and chernozem soil, in order to study usable variation among the existing genetic variability in wheat, wheat adaptation to abiotic stress, and the possibility of novel superior variability selection (Petrović *et al.*, 2016). Through these studies the experience and knowledge is to be gathered to respond to more and harsher food production conditions brought by climatic changes. The aim of this article is to follow genetic variability of salinity stress tolerance, as well as, varietal variation in chemical elements translocation from source to sink in wheat.

Material and Methods

A sample of 11 bread wheat varieties (*Triticum aestivum ssp. vulgare* L), - Renesansa, Pobeda, Evropa 90, NSR-5, Dragana, Rapsodija, Simonida, Cipovka, Banatka, Bankut 1205 (winter types), Nevesinjka (facultative) and a winter type triticale variety Odisej, was pulled out from the larger multiyear experiment. All 12 genotypes where creations of Breedeing Program of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The parallel trial was established in normal, chernozem soil, (pH = 6.86) at the test field of the Institute of Field and Vegetable Crops - near Novi Sad (latitude: 45.3249360N, longitude: 19.8428830 E), and in solonetz soil (pH = 9.86) at the experimental field in Kumane village (latitude: 45.5219940N, longitude: 20.1949190 E). Varieties Renesansa, Rapsodija, Pobeda, NSR-5 and Evropa 90 were choosen as very adaptable genotypes. Varieties Nevesinjka and NSR-5 are more tolerant to less productive production conditions. All the genotypes were tolerant to low temperatures. Varieties Renesansa and Rapsodija are more tolerant to water deficiancy. Varieties Pobeda, and Renesansa were analyzed both on chernozem and solonetz as control genotypes. Data were gathered in one year, to eliminate the influence of multiyear environmental variance in total observed variability, and to underline genotype differences. The data were analysed using statistical measures of central tendency and dispersion (Hadživuković, 1973). The content of macro and micro chemical elements was followed by atomic absorption spectrophotometry in dry plant material in the National Institute of Field and Vegetable Crops (NIFVC), Novi Sad, Serbia. Relative tolerance to solonetz abiotic stress production condition was calculated as follows: T = Ti /T1 x 100 [%], where Ti represents the mean value of a trait at solonetz soil, and T1 is mean value of average performance of control varieties Pobeda and Renesansa on chernozem soil. All the statistical calculations were done according to Microsoft Excel procedures. The experiment was set up by Randomized Complete Block Design in three replications using standard spacing (eight 12.5cm spaced rows, 155m of length), and plant feeding (50kg of NPK 15:15:15 fertilizer) was applied during sowing.

Results and Discussion

The varieties exhibited statistically significant variation in reaction to abiotic stress growth conditions of solonetz soil. The grain yield was on average 27% lower than in control part of the trial, except 17% decrement denoted for triticale variety Odisej, and the highest RTI of 0,82. The relative tolerance index for grain yield in wheat varieties varied from 0.55-0.58, for varieties Renesansa, Banatka, and NSR-5, respectively, to 0.65, 0.66 and 0.67 for varieties Evropa 90, Pobeda, and Dragana. Variety Banatka is and old local wheat population, instinctively bred, with good dough quality, but lower grain genetic potential in general. Variety NSR-5 is an early genotype. Abbreviation NSR stands for "Novi Sad's Early". Early varieties their "earliness" commonly pay with lower yield potential. Moreover, the clay fraction in the Bt horizon of solonetz, that Belić *et al.* (2012) reported, could cause the accumulation of excessive amount of water, that commonly greatly affect the yield, and plant development as a whole, beside exchangeable sodium and a high alkaline reaction. A higher tolerance index of varieties Evropa 90, Pobeda and Dragana was in accordance to previously reported results (Dimitrijević *et al.*, 2011; Dimitrijević *et al.*, 2012; Petrović *et al.*, 2016). The RTI for the yield components revealed significant genotype variation, as well. Plant height showed RTI nearly to 1, meaning high tolerance of genotypes for that trait in this experiment. Weight spike parameters exhibited RTI of about 0.80, in general, whereas local population Banatka had lower RTI, while triticale Odisej was with the highest RTI in trial. Triticale Odisej was compared with bread wheat control, so triticale as a synthetic plant species exhibited superior variation of tolerance to abiotic stress of solonetz growth environment comparing to 6x wheat varieties Pobeda and Renesansa as control. According to coefficient of variation as a relative parameter of phenotypic variability, mass traits SW and GWS exhibited the highest varietal variability. Hence, CV was the highest for RTI of these two traits, as well. Bread wheat varieties Evropa 90, Renesansa, Nevesinjka, Simonida, Pobeda, Rapsodija and Pobeda with overall RTI of 0.80, and higher. Variety Nevesinjka is facultative wheat variety, especially bred for harsh growing conditions in special purposes wheat breeding program established in the IFVCNS in cooperation with former Yugoslav People's Army in S.F.R. Yugoslavia, proving its value in this trials, as well, almost half of century later (tab. 1).

Table 1. Mean values of 11 wheat, and 1 triticale (Odisej) varieties for PH - plant height [cm], SL – spike length [cm], SW – spike weight [g], GWS – grain weight per spike [g], GNS – grain number per spike, and YIELD – grain yield [t/ha]. All the varieties were grown on solonetz soil. Varieties Pobeda and Renesansa were parallel grown on chernozem soil at the

Experimetal Field of NIFVC in Rimski Sancevi (R.S.) as control varieties. Relative tolerance index (RTI) was calculated, as well, for each variety in trial. Basic statistic was calculated – Se (standard error of the average), STD (standard deviation), CV (coefficient of variation), and LSD – Least Significant Differences at the 5% and 1% levels.

Genotip	PH	RTI	SL	RTI	SW	RTI	GWS	RTI	GNS	RTI	YIELD	RTI
Pobeda-R.S.	83,7		9,0		2,2		1,7		42,8		6,3	
Renesansa-R.S.	87,7		10,0		2,5		1,9		48,8		6,3	
Mean	**85,7**		**9,5**		**2,4**		**1,8**		**45,8**		**6,3**	
Renesansa	77,6	0,91	8,5	0,89	2,0	0,84	1,5	0,86	36,9	0,80	3,5	0,55
Pobeda	80,0	0,93	7,8	0,82	1,8	0,77	1,5	0,81	36,1	0,79	4,1	0,66
Evropa 90	87,3	1,02	8,5	0,90	1,9	0,79	1,5	0,82	38,5	0,84	4,1	0,65
NSR-5	71,2	0,83	7,7	0,82	2,0	0,84	1,5	0,83	34,3	0,75	3,6	0,58
Dragana	83,0	0,97	7,9	0,83	1,6	0,68	1,2	0,66	26,9	0,59	4,2	0,67
Rapsodija	73,9	0,97	7,3	0,77	1,8	0,78	1,5	0,81	39,6	0,86	3,7	0,60
Simonida	76,5	0,89	7,9	0,84	1,7	0,72	1,3	0,73	33,6	0,73	4,0	0,63
Cipovka	79,5	0,93	8,0	0,84	1,6	0,68	1,2	0,68	35,7	0,78	3,9	0,62
Banatka	118,7	1,38	7,1	0,74	1,1	0,47	0,9	0,47	21,2	0,46	3,4	0,55
Bankut 1205	114,6	1,34	8,0	0,84	1,3	0,54	0,9	0,49	23,0	0,50	3,8	0,60
Nevesinjka	78,9	0,92	9,7	1,02	1,8	0,75	1,3	0,75	38,5	0,84	4,0	0,63
Odisej	107,5	1,25	10,8	1,14	3,3	1,40	2,6	1,42	51,6	1,13	5,2	0,83
Mean	**87,4**	**1,00**	**8,3**	**0,87**	**1,8**	**0,79**	**1,4**	**0,79**	**34,7**	**0,77**	**4,0**	**0,63**
Se	4,8	0,03	0,3	0,02	0,2	0,04	0,1	0,05	2,3	0,03	0,1	0,02
STD	16,5	0,19	1,0	0,13	0,5	0,25	0,4	0,26	8,1	0,20	0,5	0,14
CV [%]	18,9	18,61	12,6	14,76	29,5	31,95	30,7	33,29	23,4	25,41	11,8	21,78
LSD (95.0%)	8,80	0,065	0,62	0,05	0,31	0,09	0,24	0,09	4,95	0,07	0,55	0,05
LSD (91.0%)	7,46	0,056	0,53	0,04	0,26	0,08	0,21	0,08	4,20	0,06	0,46	0,04

Trace microelements showed different dynamics among themselves and genotype variation, as well. Cobalt was not detected in control varieties grown on chernozem, while on solonetz translocation from vegetative to generative plant parts was not observed. Mean content indicate accumulation of Zn in generative parts of varieties Renesansa, Pobeda, older Banatka, Bankut 1205, and facultative Nevesinjka (tab. 2). The higher Na content in plants grown on solonetz than in control varieties was expected due to solonetz chemical properties. However, the Na translocation from vegetative to generative parts was the same on solonetz and on chernozem, according to source to sink content variation. Genotype variation was exhibited for Mg content variation. Magnesium is an important element in plant physiological processes, photosynthesis in particular, important for enzyme activities, and plant tolerance to stressful environmental conditions. The content of Mg was higher in varieties grown in stressful solonetz conditions, than in control varieties grown on chernozem (tab. 3).

Table 2. Trace microelements, (Cu and Zn are "trace elements", as well as, heavy metals, and Co and Mn are "ultratrace elements"), content in vegetative (straw), and generative (grain) parts.

	Co [mg/kg]		Cu [mg/kg]		Mn [mg/kg]		Zn [mg/kg]	
Genotip	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
Pobeda-R. Sancevi	nd	nd	11,7	9,2	93,4	45,5	17,6	34,8
Renesansa-R. Sancevi	nd	nd	8,3	10,4	49,2	41,0	16,9	30,9
Mean			**10,0**	**9,8**	**71,3**	**43,3**	**17,3**	**32,9**
Renesansa	3,5	nd	18,3	10,3	267,9	39,8	32,8	40,2
Pobeda	1,8	nd	12,2	8,0	181,8	37,5	20,9	37,2
Evropa 90	3,6	nd	11,7	9,2	294,7	35,1	28,9	28,7

NSR-5	4,5	nd	25,2	10,3	343,5	38,6	42,2	24,8
Dragana	6,1	nd	17,9	8,2	445,7	34,5	41,5	28,7
Rapsodija	4,7	nd	14,9	8,4	361,4	34,3	36,1	35,0
Simonida	2,3	nd	12,7	8,4	226,0	38,4	23,4	24,6
Cipovka	6,6	nd	14,9	10,1	491,6	43,3	39,0	30,1
Banatka	2,8	nd	10,5	9,2	218,7	27,0	26,9	42,8
Bankut 1205	3,5	nd	13,2	8,7	260,0	32,0	29,3	46,6
Nevesinjka	4,6	nd	16,0	8,8	385,6	40,1	32,9	38,8
Odisej	4,2	nd	13,4	11,5	321,9	42,2	45,6	50,1
Mean	**4,02**		**15,08**	**9,26**	**316,55**	**36,90**	**33,29**	**35,64**
Se	0,41		1,15	0,30	27,09	1,32	2,25	2,43
STD	1,41		3,98	1,05	93,84	4,58	7,78	8,41
CV [%]	35,18		26,38	11,39	29,64	12,40	23,37	23,59
Confidence Level(95.0%)	0,898		2,397	0,589	71,792	2,818	5,328	4,524
Confidence Level(91.0%)	0,758		2,032	0,500	60,870	2,389	4,518	3,835

Table 3. Macroelements i.e. macromineral content in source (straw) and sink (grain) plant parts

	Ca [mg/kg]		K [mg/kg]		Mg [mg/kg]		Na [mg/kg]	
Genotip	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
Pobeda-R. Sancevi	4189,0	1963,0	6860,0	2506,0	1298,0	1423,0	402,7	185,8
Renesansa-R. Sancevi	3793,0	1863,0	6790,0	2284,0	766,7	1188,0	258,2	176,0
Mean	**3991,0**	**1913,0**	**6825,0**	**2395,0**	**1032,4**	**1305,5**	**330,5**	**180,9**
Renesansa	3645,7	1968,0	4851,3	2272,3	1780,1	1252,7	1820,0	188,0
Pobeda	3816,3	1867,0	4564,7	2400,0	1476,7	1305,3	879,6	177,4
Evropa 90	3301,3	1831,7	4682,0	2374,3	1669,7	1339,0	1364,7	184,2
NSR-5	4519,0	1827,0	5625,0	2538,3	2050,7	1315,3	1935,7	188,1
Dragana	5578,7	1856,3	4510,7	2346,0	2713,7	1293,3	1229,7	183,0
Rapsodija	5087,0	1983,7	4014,3	2547,3	2139,0	1296,3	922,5	184,3
Simonida	3622,7	1842,7	3871,7	2527,3	1557,7	1261,7	988,3	181,9
Cipovka	4154,7	1991,3	5771,7	2461,7	2333,7	1352,7	1278,7	195,4
Banatka	3492,0	1982,0	2887,0	2658,5	1369,0	1411,5	1438,0	202,7
Bankut 1205	4174,0	1829,5	5355,5	2274,5	1745,5	1516,5	1132,9	178,0
Nevesinjka	4698,7	1964,3	6418,3	2680,7	2126,7	1329,3	1351,6	183,1
Odisej	3983,3	2030,0	7589,7	3211,3	1818,3	1433,3	1200,1	193,5
Mean	**4172,78**	**1914,46**	**5011,82**	**2524,36**	**1898,38**	**1342,25**	**1295,14**	**186,63**
Se	198,01	22,44	359,82	73,59	112,06	22,16	93,74	2,14
STD	685,92	77,74	1246,47	254,91	388,19	76,78	324,71	7,42
CV [%]	16,44	4,06	24,87	10,10	20,45	5,72	25,07	3,98
Confidence Level(95.0%)	369,026	42,812	763,451	140,345	281,245	49,299	266,310	4,266
Confidence Level(91.0%)	312,884	36,299	647,303	118,993	238,457	41,799	225,795	3,617

Heavy metals are widespread and dramatically increased environmental pollutants. They require particular attention because being non-degradable they could persist in the environment as constant environmental and health threat. The results obtained, are encouraging for phytoremediation of heavy metals in soil. No translocation of Cr, and particularly of Pb, from vegetative to generative wheat parts, has been denoted. Moreover, the Ni content in grain has been considerably lower than in vegetative part. Genetic variability in heavy metal presence is observed, as well (tab. 4).

Table 4. Heavy metals (trace microelements, "ultratrace elements") content variation in wheat

	Cr [mg/kg]		Ni [mg/kg]		Pb [mg/kg]	
Genotip	Straw	Grain	Straw	Grain	Straw	Grain
Pobeda-R. Sancevi	3,9	nd	4,0	1,5	1,6	nd
Renesansa-R. Sancevi	1,1	1,3	2,6	3,5	nd	nd
Mean	**2,5**	**1,3**	**3,3**	**2,5**	**1,6**	
Renesansa	12,2	nd	9,9	0,7	7,1	nd
Pobeda	6,1	nd	6,1	0,5	4,2	nd
Evropa 90	10,0	nd	10,0	0,7	8,0	nd
NSR-5	13,1	nd	11,6	0,6	10,0	nd
Dragana	15,4	nd	16,7	0,4	13,4	nd
Rapsodija	12,0	nd	13,5	0,6	10,0	nd
Simonida	7,3	nd	7,8	0,5	5,7	nd
Cipovka	17,0	nd	16,5	0,5	13,5	nd
Banatka	8,5	nd	11,1	0,4	10,6	nd
Bankut 1205	11,3	nd	11,2	0,5	7,5	nd
Nevesinjka	13,4	nd	10,3	0,5	7,6	nd
Odisej	13,7	nd	8,1	0,5	6,5	nd
Mean	**11,66**		**11,06**	**0,54**	**8,67**	
Se	0,93		0,93	0,03	0,84	
STD	3,23		3,22	0,10	2,90	
CV [%]	27,66		29,15	19,14	33,48	
Confidence Level (95.0%)	2,590		2,512	0,536	2,394	
Confidence Level (91.0%)	2,196		2,130	0,454	2,030	

Conclusions

The analysis could be considered as a pilot study of the variation denoted in larger multiyear research of wheat genetic variation in abiotic stress caused by chemical and physical properties of solonetz soil, as well as, harsh environmental conditions of western Banat area. Observed genetic variability in bread wheat, reflected through phenotypic variation followed using RTI, relative tolerance index for grain yield, and its components, provides basis for further wheat improvement for withstanding abiotic stressful growing conditions, salinity stress in particular. Triticale variety Odisej was superior to wheat varieties in durability under abiotic stress. The variation in chemical elements variability has been denoted not only through genotype variability, but also depending on localities in the experiment, predominantly due to solil properties and agricultural practice. Further investigation in this direction is required because of phytoremediation, the enhancement of nutritional properties in wheat, searching for special purpose food production on less favorable soil. Moreover, epigenetic pathways are very important in plant reaction to the environmental stress (Pikaard & Mittelsten, 2014). The plant responsiveness is highly depending on balance between enzyme activities, small RNA gene expression regulation, long non-coding RNAs regulation etc. All these processes have in their core the dependency on chemical elements dynamics in plant. The study of micro- and macro-elements variability in source, and particularly in generative plant parts is of rising importance in epigenetic pathways comprehension.

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THE EFFECTIVENESS OF SOIL TILLAGE SYSTEMS IN MAIZE CULTIVATION UNDER VARIABLE METEOROLOGICAL CONDITIONS OF CENTRAL SERBIA

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Abstract

Soil tillage system is a basic in maize crop production. Under variable climatic conditions and frequent occurrences of dry years, soil tillage adjusted to the soil type, agro-ecological conditions and the crop, can contribute to the achievement of the maximum yield potential. Systems of reduced tillage, particularly direct sowing, have been widely used in Europe. Under agro-ecological conditions of central Serbia, the highest maize yields have been achieved by using conventional soil tillage with 20-25-cm deep autumn ploughing and a single pass land preparation in spring as 10-12-cm shallow tillage. In the Maize Research Institute, Zemun Polje, the long-term experiment was conducted (2005-2016) in order to test the efficacy of zero-, reduced and conventional tillage together with the application of different rates of mineral fertilisers in maize cultivation. The experiment was performed under rainfed and irrigation conditions. The data were processed by four factorials ANOVA and differences were analysed by the LSD-test. Based on the precipitation sum in the investigated period, three years were extremely dry, four were moderately dry and five were favourable for maize production. Under irrigation conditions, the 12-year maize grain yield average was higher by 2.1 t ha^{-1} than under rainfed conditions. Under rainfed conditions, differences between conventional and reduced or zero-tillage were 1.7, and 1.3 t ha^{-1}, respectively, while under irrigated conditions conventional tillage was more productive by 1.4 t ha^{-1} than reduced and zero-tillage, respectively. In some dry years, such 2012, the maize grain yield was higher under conditions of reduced tillage than conventional tillage.

Key words: *Maize, Tillage system, Meteorological conditions, Yield.*

Introduction

Tillage contributes to the formation of a loose soil layer and the accumulation and moisture preservation, which leads to the improvement of the air-temperature regime of the soil (Kovačević, 2010). Appropriate soil tillage provides more efficient water use, prevents erosion, changes CO_2 concentration, reduces soil compaction and enables better soil structure. It ensures the introduction of organic residues of the preceding crops and fertilisers, increases crop competitiveness, influences weed presence and biomass of rhizomes, provides better efficacy of the applied herbicides, etc. (Stefanović et al., 2011). Under variable climatic conditions and frequent occurrences of dry years, soil tillage adjusted to the agro-ecological conditions and the crop, can contribute to the achievement of maximum yield (Bodner et al., 2015). Yield potential of maize hybrids developed in Serbia is much higher than an actual yield (Videnović et al., 2013) and, except soil tillage, irrigation and the fertiliser application are very important for maize yield increase (Simić et al., 2018).

The highest yields of maize are recorded on loose and well-permeable soils with high water capacity. Soils containing great moisture reserves, easy available nutrients and at the same time if well-aerated, are favourable for the maize cultivation. Such properties are characteristic for soils with a medium texture/mechanical composition (medium and light clay) and soils rich in organic matter (chernozem and brown forest soil and similar). Maize grown on well-permeable soils with good physical properties can utilise moisture from the depth of 1.5 to 2 or more meters because maize has great demands for water, which is a decisive factor for its production in Serbia (Pejić et al., 2009). A necessary precipitation sum during the maize growing season amounts to approximately 570 l/m^2. It is also important that maize plants are well supplied with moisture in the second part of their growing season (July and August) during the ear formation, flowering and until the end of grain filling (Pejić et al., 2009). In the regions with insufficient precipitation sums and irregular distribution of precipitation, water should be provided by irrigation (Kresović et al., 2013). The maize root system was better developed under irrigated (76.7 g) than under conditions of soil water deficit (46.5 g). The water intake from the 80-cm soil layer was higher, as well as, the maize grain yield under irrigation (442 mm and 13.7 t ha^{-1}, respectively) than under rainfed conditions (304 mm and 8.4 t ha^{-1}, respectively), (Pejić, 2000). Maize plants can use the stored water during periods of drought. It is well known that accumulated water increases maize yield. In the majority production areas, the different measures should be taken to collect and store moisture in the soil (proper tillage and fertilisation, autumn ploughing, summer ploughing, snow retention).

Systems of reduced tillage, particularly direct sowing, are entered in widespread use in North America (Fulton, 2010), Australia (Llewellyn et al., 2012), South America and in Europe (Morris et al., 2010). Direct sowing also means saving energy, labour and machinery and helps prevent erosion and increases the efficiency of water and fertiliser utilisation. However, the use of these soil cultivation systems requires the implementation of measures for intensive weed control (herbicides). Under irrigated conditions, soil tillage greatly increases maize yield, because it enhances positive effects of beneficial soil micro-organisms and fertilisers. Under conditions of a higher water supply in the soil, 80– 85% of field water capacity, the maize yield was the highest (15.08 t ha^{-1}), and also mobility of soil N and accumulation of nitrates in the upper soil layers were increased (Kresović et al., 2012).

Conventional tillage mainly includes autumn ploughing (20-25 cm) and a single pass land preparation in spring - shallow tillage (10-12 cm). In such a way a good soil structure is provided, moisture is maintained, the soil is not trampled, weed seedlings are destroyed, which is all of a particular importance for the efficient herbicide actions (Stefanović et al., 2011). Conventional soil tillage should be conducted in autumn to the depth of 20-30 cm, depending on the soil type, the amount of harvest residues, etc. At the same time, organic and mineral fertilisers containing phosphorus (P) and potassium (K) should be incorporated. The soil over winter remains with open furrows to be exposed to the frosts, which contribute to a better soil structure and reduction in the number of pests. Immediately after harvest of small grains, crop residues should be ploughed to the depth of 10-15 cm. In such a way the water regime of soil is improved, while a portion of elements removed by harvest is returned.

The effectiveness of conventional, reduced and zero-tillage system of chernozem soil in the central part of Serbia (Zemun Polje) together with the application of mineral fertilisers in three rates, on maize grain yield were investigated under rainfed and irrigated conditions in twelve meteorologically different years, 2005-2016.

Material and Methods

The study was conducted in the experimental field of the Maize Research Institute, Zemun Polje, Belgrade, Serbia, during the 2005-2016 period. The soil was slightly calcareous chernozem with 47 % clay and silt, and 53 % sand. The 0–30-cm layer contained 3.3 % organic matter, 0.21 % total N, 1.9 % organic C, 14 and 31 mg per 100 g of soil available P and extractable K, respectively and 9.7 % total $CaCO_3$, and pH of 7.8.

The experiment was performed under rainfed and irrigation conditions as a split-plot design with four replications. The main plots were soil tillage systems: zero soil tillage or direct

sowing - ZT, reduced soil tillage system - RT and conventional soil tillage system- CT and subplots were the levels of N fertiliser applied in the following rates: F0 – without fertilisation; F1 - 180 kg N ha^{-1} and F2 - 240 kg N ha^{-1}. The elementary plot size was 21 m^2. Fertilisers in the amount of 50 kg P ha^{-1} and 50 kg K ha^{-1} were applied in the autumn, while N fertiliser was incorporated in the spring, prior to sowing. Winter wheat was the preceding crop to maize in each year. The irrigation was applied to maintain 75% of field water capacity (FWC).
Maize grain yield was measured from two inner rows within the elementary plot and calculated to 14% moisture at the end of a growing cycle. The data were processed by two factorial ANOVA and differences were analysed by the LSD-test.
Meteorological conditions. Based on the precipitation sum in the investigated period, four years were very dry – 2008, 2012, 2013 and 2015, while the extremely dry with a highest average temperature was 2012; four were moderately dry – 2007, 2009, 2011 and 2016 and four years were favourable for maize production – 2005, 2006, 2010 and 2014 (Table 1). The highest average temperature, i.e. precipitation sum during the growing season (April-September) was recorded in 2012, i.e. 2014, respectively (the year with extreme floods).

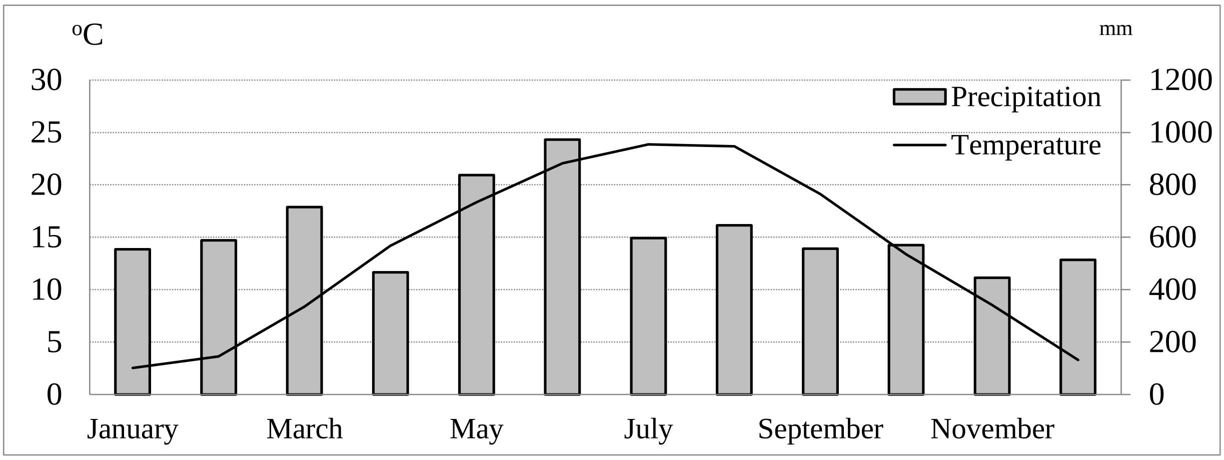

Figure 1. The average monthly temperatures and precipitation sum (2005-2016)

Table 1. Meteorological data for the growing season of maize (April-September) in the years of investigation, 2005-2016

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
°C	18.8	18.0	20.7	20.3	21.1	19.8	20.8	22.1	20.2	19.3	21.1	20.3
mm	387.4	417.1	290.1	224.6	321.0	453.9	301.6	174.8	245.4	652.5	285.4	320.1

In average for all years within a period of investigation, 2005-2016, April was a month with the insufficient amount of precipitation as well as July, August and September (Figure 1).

Results and Discussion

The long-term experiment results showed considerable differences in maize grain yield in dependence on the applied soil tillage system, as well as, on the fertilisation level and their interactions. Maize grain yield was higher under irrigated conditions especially in some years - 2007, 2012 and 2013 (Table 2). In 2012, the average maize yield was 5.5 t ha^{-1} (63.2%). It was higher under irrigated conditions (8.7 t ha^{-1}) than in the rainfed treatment (3.5 t ha^{-1})

because of extremely unfavourable meteorological conditions. In total, maize yield was, on average, higher by more than 2 t ha^{-1} in the irrigation treatment. The highest yield was obtained in 2005, under irrigation and CT (14.5 t ha^{-1}). In both water regimes, the conventional soil tillage system significantly contributed to the achievement of higher maize yield (10.0 and 11.9 t ha^{-1}) than reduced tillage system (8.3 and 10.5 t ha^{-1}) and direct sowing (7.0 and 9.1 t ha^{-1}). In some dry years, such 2012, the reduced tillage system was advantageous over the conventional system regarding to maize grain yield, even though differences were not significant. Bescansa et al. (2006) and Fernández-Ugalde et al. (2009) reported the same trend for a clay loam and a silt loam soil, respectively, in a semi-arid climate in Spain, leading to better crop performance in the reduced tillage systems during dry years.

Table 2. The effects of soil tillage on maize yield under rainfed and irrigated conditions,

Year	Rainfed				Irrigation			
	NT	RT	CT	Average	NT	RT	CT	Average
2005	9.2	12.4	13.6	11.7	10.6	11.8	14.5	12.3
2006	8.0	10.8	12.1	10.3	11.6	12.5	13.0	12.4
2007	6.6	7.8	7.8	7.4	10.3	11.9	11.9	11.4
2008	3.9	6.3	9.6	6.6	6.2	9.6	12.0	9.3
2009	12.0	11.1	12.1	11.7	11.1	12.3	13.1	12.2
2010	8.5	8.9	10.1	9.1	9.7	10.4	10.8	10.3
2011	6.1	8.1	10.4	8.2	6.7	9.3	11.6	9.2
2012	2.8	3.5	3.3	3.2	7.7	8.7	9.6	8.7
2013	5.5	7.6	8.7	7.3	8.7	11.5	12.7	11.0
2014	8.7	10.3	13.6	10.9	10.2	10.8	13.0	11.3
2015	3.6	3.8	6.4	4.6	6.6	7.0	9.1	7.6
2016	9.1	8.8	11.8	9.9	9.6	9.7	11.3	10.2
Average	7.0a	8.3b	10.0bc	8.4	9.1c	10.5c	11.9d	10.5
$LSD_{0.05}$ soil tillage x water regime = 2.821**								

Tillage system is important soil and plant management practice which has both, short- and long-term impacts on a yield limiting processes (Bodner et al., 2015). In the long term, soil water and air performances and plant physiological limitations may be overcome by altering tillage systems and cultivating new varieties that are a more resistant to stresses. Interactions between plant and soil, particularly in the rhizosphere, are a way towards better crop water supply. Kell (2011) pointed to the unexploited potential of breeding for deeper rooted crops to substantially improve resource uptake efficiency in deep rooted cropping systems. Deeper root systems can be achieved either by cultivar selection (Wasson et al., 2012), tillage system (Pietola, 2005) or crop stand establishment (Sharratt and McWilliams, 2005).

Another important measure, which can significantly contribute to higher yield of maize is fertilisation. Results show that the application of mineral fertilisers (F1 and F2) increased maize grain yield especially under rainfed conditions (Table 3). In average for 12 years, maize yield was higher by 1.1 t ha^{-1} in F1 and by 1.8 t ha^{-1} in F2 than in F0 in rainfed variant, while differences were 2.2 and 2.8 t ha^{-1} in irrigated conditions. The highest maize yield was achieved in CT-F1 under irrigation (12.7 t ha^{-1}) and it was higher by 2.3 t ha^{-1} than yield in CT-F1 and rainfed conditions (10.4 t ha^{-1}). Higher amounts of fertilisers, F2, did not increase maize yield significantly in comparison to F1, neither in rainfed nor irrigated conditions.

Turner (2004) reviewed measures for sustainable crop production under drought in Mediterranean climates and attributed half of yield improvement over the last decades to agronomic measures, mainly advanced sowing time and fertilisation both leading to higher early vigour and better match of water availability with crop demand.

Table 3. The effect of tillage systems and fertiliser application on maize grain yield, long-term average 2005-2016, Zemun Polje

Tillage systems	Rainfed			Irrigation			Average
	F0	F1	F2	F0	F1	F2	
NT	5.2	7.2	8.5	6.9	9.7	10.6	8.0a
RT	6.7	8.8	9.4	8.9	10.9	11.6	9.4b
CT	9.3	10.4	10.1	10.5	12.4	12.7	10.9c
Average	7.1a	8.8b	9.3b	8.8b	11.0c	11.6c	$LSD_{0.05}$ tillage=3.001**
$LSD_{0.05}$ fertilization x water regime = 2.856**							

Conclusion

The conventional tillage system including the application of fertilisers is the most suitable maize cropping system for achieving high yields on high-quality soil, such as chernozem, under agro-ecological conditions of central Serbia. In dry years, reduced tillage system could be more appropriate, while the fertilisation level should be adjusted to crop demands. In order to maintain the optimum plant water supply, irrigation is the most important factor, which together with conventional soil tillage system and fertilisation may increase maize yield by more than 2 t ha^{-1}.

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THE INFLUENCE OF PLANT CUTTING ON GRAIN YIELD TRAITS IN MAIZE

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Abstract

In this research an influence of the strong source restriction meaning cutting off the whole plants at the first internodes 5 (5DAPt), 10 (10DAPt) and 15 (15DAPt) days after pollination on grain yield and its corresponding traits in maize were tested. Control represented plants harvested at full maturity. Four inbred lines were used, two historical ones (Mo17 and B73) and two commercial ZP inbreds (ZPL and ZPB). The experiment was conducted at Zemun Polje, Serbia, in 2014 and 2015. The trait of particular importance was the number of kernels per ear and its average values were 37.73 at 5DAPt, 115.14 at 10DAPt and 175.20 at 15DAPt, being sufficient for planting next generation of breeding. According to the results obtained, ZPL represented an improved Lancaster line over Mo17 regarding drought tolerance, that could not be stated for ZPB over B73. Hybrid among these two lines is drought tolerant due to heterosis (epistatic effects) or dominant origin of ZPLs good response to drought stress. Values for seed set and eventually for grain yield per plant were 0.00 for line B73 at 5DAPt in 2015. An improved breeding scheme for increased drought tolerance could be proposed, namely self-pollination of border plants on high density sown selfing progenies or dihaploid (DH) lines, cutting off selfed plants at 15 DAP and evaluating their kernel properties. Open-pollinated progeny would serve to estimate other important traits for selection. From the chosen progenies kernels of selfed and cut-off plants should be used for the next generation of breeding.

Keywords: *drought, grain filling, Zea mays L.*

Introduction

Drought is one of the most important limiting factors in maize production, and new methods for increasing drought tolerance in maize are searched for. Maize plants are most susceptible to drought at the flowering time, resulting in low kernel number and grain yield per plant (Grant *et al.*, 1989). Following in sensitivity is grain filling phase, while the vegetative phase is the least sensitive (Fereres and Villalobos, 2016). Some researchers have suggested that the early grain filling phase is very suitable for breeding for drought tolerance (Grant *et al.*, 1989; Nesmith and Ritchie, 1992). However, kernel number is highly correlated with grain yield under drought stress (O'Neill *et al.*, 2004).

The source-sink interaction in plants represents the connectivity between the source of assimilated material and the pathway of this material to the sink, a region where carbon-based products are metabolized and energy (in the form of ATP) is synthesized. Source-to-sink ratio is very important in all cereals in determining grain yield. The supply of assimilates from mother plant (source) or the capability of the reproductive ear (sink) to accumulate assimilates limits growth of maize kernels (Gambín *et al.*, 2006). Assimilate supply can be increased by the environment (nitrogen N supply) and genotype. Under normal growing conditions maize yield is mostly sink limited (Borrás *et al.*, 2004). Drought and/or deficiencies in N supply decrease grain yield by reducing kernel number per plant (poor synchronization between silking and pollination) and greater kernel abortion (poor grain filling). The same authors denoted maize as source-limited crop under stress conditions. Maize plants set sink potential early during grain filling, indicating importance of studying first stages of this phenophase.

Yield responses of major crops to different source-sink manipulations were often studied. Usually, this was done in five ways: 1) stand density manipulations (Hernández *et al.*, 2014; Solomon *et al.*, 2017); 2) induced drought stress at flowering time (Oveysi *et al.*, 2010; Siahkouhian *et al.*, 2013); 3) pollination treatments during seed set (Borrás *et al.*, 2003a, 2003b), 4) seed removal (Jones and Brenner, 1987) or truncation of the ear (Seebauer *et al.*, 2010), and 5) manipulation of N supply in the soil (D'Andrea *et al.*, 2008).
The objective of our research was to estimate the effects of cutting off the whole maize plants at the first internodes at different days after pollination (DAP) on grain yield traits. This procedure would provide a high source restriction, and make an impact on grain filling and other kernel traits. Finally, it could serve as a tool for estimating drought tolerance in maize.

Materials and Methods

The four maize inbred lines were used: two historical ones (Mo17 of Lancaster and B73 of BSSS origin) and two commercial ZP inbred lines (ZPL of Lancaster and ZPB of BSSS origin) which are components of the commercial late maturity drought tolerant ZP hybrid. The trial was conducted at Zemun Polje, Serbia, in 2014 and 2015 according to the Split-Plot Randomized Complete Block Design (RCBD) in two replications. Three treatments (sets) were applied: cutting off plants at first internodes at 5 (5DAPt), 10 (10DAPt) and 15 (15DAPt) days after pollination. Plants harvested at full maturity were used as control set. Each genotype was sawn in two rows 0.75 m apart, five plants per row and 0.3 m between plants. Rows were overplanted (three kernels per hill) and thinned to one plant per hill at 5-7 leaf stage. Only border plants were cut, providing the same environmental conditions to the all plants measured, and also only border control plants were measured. Cut-off plants were put into the shed house, away from direct sunlight.
A total of 15 traits were tested on both cut-off and control plants. Morphological traits were: anthesis-silking interval (ASI) in days, ear (EH, cm) and plant height (PH, cm), total number of leaves (NL), number of leaves above the uppermost ear (LAE), length (LL, cm) and thickness (LT, cm) of the ear leaf. Average chlorophyll content (CL, SPAD units) was measured by a SPAD (Minolta) chlorophyll meter, and duration of chlorophyll content (CD) in days from silking till SPAD values reached units beneath 10. Ear and kernel traits were: number of ears per plant (EP), seed set (SS, %) - visual estimate of the percent of pollinated kernels on the ear, grain filling (GF, %) - visual estimate of the percent of filled kernels from the total of pollinated kernels, number of kernels per ear (KE), grain yield per plant (GP, g) and 100 kernel weight (KW, g).
Statistical analysis was done by threre-way analysis of variance (ANOVA) for genotypes combined over sets and years for each trait. Significances of differences among years, genotypes and treatments were determined by LSD test at 0.05 probability level. Pearson's correlation coefficients were calculated for CL, CD, EP, SS, GF, KE, GP and KW for three cut-off treatments and control in order to estimate prediction ability of the treatments on the final values of control plants.

Results and Discussion

Highly significant differences between years ($p<0.001$) were found for ASI, EP, ES, EH, PH, NL, LL, LT, CD, SS, KE and GP, and significant ($p<0.01$) for CL and KW (data not shown). These was expected, since climate conditions in 2014 and 2015 were drastically different at Zemun Polje. Namely, 2014 was highly favorable in comparison with 2015, as sum of precipitation was higher for 56.3%. The largest difference was observed in July, during maize pollination, with precipitation of 187.4 mm in 2014 and only 7.2 mm in 2015. On the other hand, more rainfall occurred in August (time of grain filling) in 2015 than in 2014 (56 and 41 mm, respectively). In south-east Europe the first part of maize vegetation is most often sufficient in rainfall, but about two to three weeks before or at the flowering time drought frequently occurs. The consequence is poor development of root system in the first part of vegetation that remains inefficient to compensate drought stress for rest of vegetation. The second peak of drought occurs during grain filling, when uptake of soil assimilates by maize plants is poor (Anders *et al.*, 2014). Our experiment was designed to simulate this common situation - by cutting off the plants at the first internodes assimilates from the soil became unavailable, thus imitating poor development of the root system.

Genotypic differences were highly significant ($p<0.001$) for all traits except KW (non-significant), which is in accordance with review given by Borrás and Vitantnonio-Mazzini (2017). Year × genotype interaction was significant for ASI, EH, CD, EP, SS, GF and KE. Differences among sets were non-significant for all the morphological traits, indicating they were fully developed before time of stress (Abrecht, 1999). Induced stress provoked significant differences in all other traits ($p<0.001$ and for CL $p<0.01$). Minimal, maximal and average values for these traits are given in Tab. 1. The trait of particular importance is KE and the average values were 37.73 at 5DAPt, 115.14 at 10DAPt and 175.20 at 15DAPt. From breeding standpoint these KE are sufficient for planting the next generation. In the previous pilot experiment with gene bank material, kernels obtained in this way were viable and had good emergency (data not published). The minimum values for SS, GF, KE, GP and KW were 0.00 for B73 at 5DAPt in 2015. This line has passed ear formation (V10) successfully, but the severe drought stress during pollination caused the absence of seed set.

Table 1. Minimal, maximal and average absolute values of the analyzed traits

Trait	Treatment (DAP)					Checks
	Range	5	10	15	Trend	
Chlorophyll content	Min	18.20	21.96	19.20	ʌ	19.34
CL (SPAD units)	Max	33.98	37.35	35.94	ʌ	39.32
	Aver.	27.19	28.63	26.41	ʌ	28.77
Chlorophyll duration	Min	29.50	34.00	31.00	ʌ	35.58
CD (days)	Max	101.50	101.25	99.75	↓	96.59
	Aver.	71.68	68.92	63.20	↓	58.43
Ears per plant	Min	0.50	1.00	0.75	ʌ	0.81
EP (number)	Max	1.33	2.00	2.00	↑	1.81
	Aver.	1.04	1.29	1.28	ʌ	1.36
Seed set	Min	0.00	10.00	8.50	ʌ	11.28
SS (%)	Max	87.50	88.75	94.00	↑	95.08
	Aver.	39.42	59.85	64.41	↑	63.39
Grain filling	Min	0.00	10.00	30.00	↑	43.44
GF (%)	Max	78.75	88.75	93.75	↑	97.58
	Aver.	26.84	59.03	74.24	↑	82.42
No.of kernels per ear	Min	0.00	10.00	12.00	↑	22.13
KE (number)	Max	124.25	250.50	494.50	↑	484.67
	Aver.	37.75	115.18	175.20	↑	240.99
Grain yield per plant	Min	0.00	2.43	2.25	ʌ	5.83
GP (g)	Max	13.92	24.44	58.10	↑	164.13
	Aver.	3.52	13.00	21.03	↑	71.15
Kernel weight	Min	0.00	3.63	7.33	↑	16.89
KW (g)	Max	13.33	68.60	25.36	ʌ	36.92
	Aver.	6.31	14.27	14.49	↑	27.07

ʌ - maximum value of the particular parameter is at 10 DAP; ↓ - decreasing value trend of the trait from 5 towards 15 DAP; ↑ - increasing value trend of the trait from 5 towards 15 DAP

All three values (min, max and av.) for CL were highest at 10DAPt, although still smaller than for control plants. This was unexpected, since plants cut-off at 10DAPt had five days less for photosynthesis than those at 15DAPt. Probably photosynthesis continued for some time on the cut-off plants of this treatment. These results are in accordance with Borrás *et al.* (2004), who found that maize kernel growth is highly source-dependent when tested in drastic reduction of assimilates during grain feeling. Minimum CD values showed the same trend, but maximum and average values decreased from 5DAPt to 15DAPt, and for all three treatments values were higher than for checks. As a response to stress, leaf tissue probably shrunk more than in checks, so the relative chlorophyll concentration in the leaves was higher. For EP erratic values and trends were obtained, presumably due to differences between the genotypes tested. For all other traits mostly increasing trends were obtained and in almost all instances values of the treatments were smaller than those of the checks.

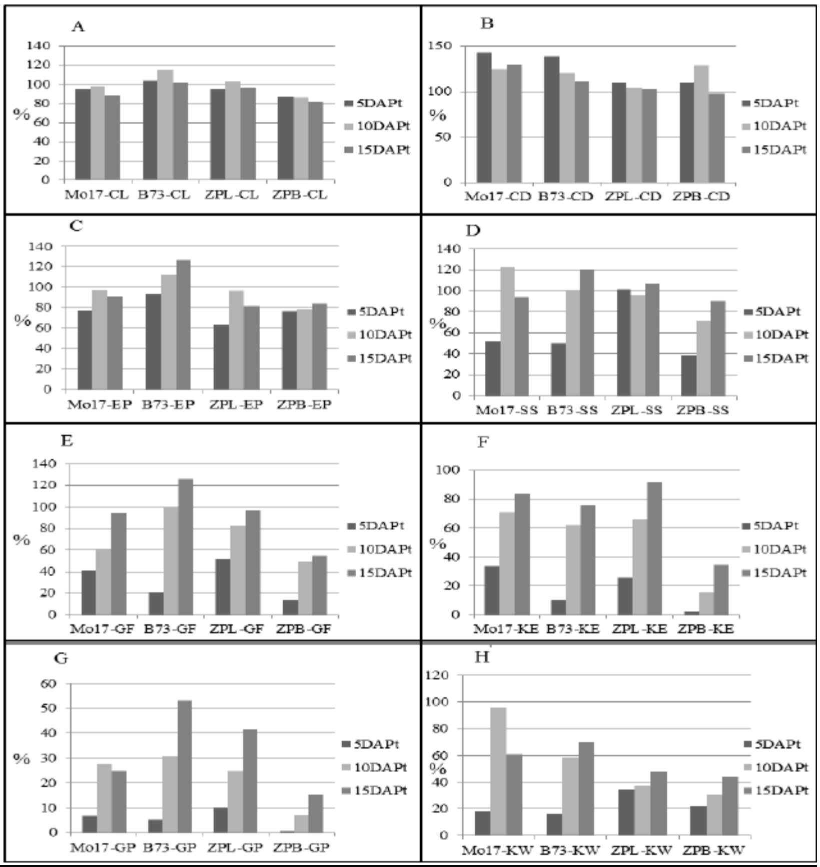

Figure 1. Values for CL (A), CD (B), EP (C), SS (D), GF (E), KE (F), GP (G) and KW (H) are presented in percent of the control for four lines and three cut-off treatments.

LSD values at 0.05 level for genotypes over years were calculated (data not shown). For all analyzed traits, ZPL was significantly better or at the level of Mo17, indicating that it is an improved Lancaster line regarding drought tolerance. On the other hand, B73 and ZPB had, in most cases, non-significant differences between trait averages, except for GF at 15DAPt and KE at 10DAPt where B73 was superior, but only overall both seasons. Therefore, ZPB showed better stability of drought tolerance over B73, but it is not significantly improved for this trait considering both years of research.

If pollination occurs 3-8 days after first silk exposure maximal seed set is expected (Carcova *et al.*, 2000; Anderson *et al.*, 2004). According to this, plants would be adequately pollinated already at the first treatment in our experiment (5DAPt). The results for SS, however, showed that pollination was not sufficient. Namely, SS was less than 33.00% for three lines and 76.25% for ZPL. SS in our experiment raised with number of DAP. This trait, however, remained constant in the review presented by Borrás and Vitantnonio-Mazzini (2017). Regarding GF the experiment was efficient, since there was a growing trend from 5DAPt to 15DAPt. The least values were obtained for ZPB. In maize and other cereals there are three phases of grain filling: a lag phase, a linear phase and a phase of slowing down approaching the physiological maturity (Johnson and Tanner, 1972). The first phase lasts 15-18 DAP and during this time very little dry weight is accumulated. In our experiment normal grain filling was stopped at 5DAPt and 10DAPt since the lag phase was not finished. KE was particularly low at 5DAPt for all lines and again ZPB was the poorest one. The most jeopardized trait was GP with maximum value of 53.2% in comparison with the control for B73 at 15DAPt. KW showed increasing trend from 5DAPt to 15DAPt, except for Mo17 at 10DAPt, and smaller values for all treatments were obtained for ZPL and ZPB. Middle leaves have essential role in photosynthesis and deriving grain yield (Siahkouhian *et al.*, 2013). Defoliation in mentioned research produced many immature and small kernels on ear tips. Similar phenomenon occurred in our experiment. Premature death of leaves induced by cutting off the stalks resulted in yield losses and plants most probably remobilized stored carbohydrates from the leaves and/or stalks to the developing ears, but yield potential was already lost. Death of all plant tissues, occurring about 15 days after cutting off the plants, stopped any further remobilization of stored carbohydrates into the ears.

Pearson's correlation coefficients between treatments and checks were significant only for 15DAPt and checks for KE (0.681; $p<0.01$) and GP (0.543; $p<0.05$), and we could assume that at 15DAPt our experiment became feasible for predicting final grain yield under uncontrolled environmental conditions.

Conclusions

In the literature we could not find cutting off plants as a way of source-sink manipulation in plants. High plant density is often used during inbreeding or double haploid breeding for obtaining new, drought tolerant maize inbred lines. An improved breeding scheme for increased drought tolerance could be proposed. The new approach would include self-pollination of border plants on high density progenies, cutting off selfed plants at 15 DAP and evaluating their kernel properties. Besides for SS and GF, open-pollinated progeny could be used for estimating other important traits (ASI, stay green, tolerance to plant and stalk lodging, diseases and pests). From the chosen progenies kernels from selfed and cut-off plants should be used for next generation of breeding. One of the advantages of such approach might be shortening of vegetative period of late materials, thus allowing more generations per year.

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TROPHIC STRUCTURE OF THE NEMATODE COMMUNITY IN MONOCULTURES OF CARROTS (*DAUCUS CAROTA SUBSP. SATIVUS*)

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Abstract

The carrot (*Daucus carota* L.) has been used as food since ancient times. Despite of the nutritional importance of carrots, their production in small farms is stagnant or declining. The main reason is the monoculture of the carrots, where the risk of attack and damage from pests is high. The carrots change their shape and appearance under the influence of a number of biotic stress factors such as bacteria, fungi and plant-parasitic nematodes which affect their quality. Plant-parasitic nematodes are long known to cause significant damage to carrots. The results of the present work have been obtained on the basis of conducted field and laboratory studies, as for the research were used agricultural areas in the region of Targovishte. The aim of the present work was to assess the phytosanitary status of the areas in the monocultures of carrots on the basis of the trophic structure of the nematode communities. A total of 11 genera of plant parasitic nematodes were found in the analyzed samples. The genus *Pratylenchus* was found in 92% of the samples tested. The genus *Meloidogyne* was found in 53% of the samples. In addition to plant parasitic nematodes, entomopathogenic nematodes of the genus *Steinernema*, omnivorous nematodes of the genus *Dorylaimus* and bacterial-feeding nematodes of the genus *Cephalobus* were also found. The knowledge of the trophic structure of nematode communities in monoculture is a key to deciding on a crop rotation that will effectively reduce plant-parasitic populations.

Key words: *carrots, nematodes, monoculture.*

Introduction

The carrots (*Daucus carota* L.) are widespread in all countries of Europe, Asia, Africa and America because of their high plasticity, adaptability to environmental conditions, keeping edible and good taste quality. However, irrespective of the nutritional importance of carrots, their production on small farms is stagnant or declining. Some of the major problems are technological, related to the quality of the seed, germination and germination energy, which leads to low yields (Lada et al., 2004). Despite the application of chemical and organic fertilizers that guarantee the successful production of carrots, yields are often low on these farms. The main reason is undoubtedly the monoculture cultivation of carrots, where the risk of attack and damage by pests is greatest. The carrots change their shape and appearance under the influence of a number of stress biotic factors such as bacteria, fungi and plant parasitic nematodes. The plant-parasitic nematodes have long been known to cause significant damage to carrots (Fitzgerald 1950). They feed on the root system (with both root, lateral roots and root hairs) as a result, the nutritional value decreases and the root deformations are observed (Pretorius & Engelbrecht 2009, Tangvik 2017). Deformed carrots do not meet the market criteria

Many of the farmers' questions refer to clarifying the causes that lead to distortions and poor quality of production. All this is leading to finding ways of reducing the negative effects while ensuring plant health and the potential of the crop.

The purpose of the present work is to evaluate the phytosanitary status of the areas in monoculture cultivation of carrots on the basis of the trophic structure of nematode communities.

Materials and Methods

Characteristics of the region, experimental area and plant material

The results of this work were obtained on the basis of field and laboratory studies. For the purposes of the study, agricultural land was used in the area of Targovishte (Vasil Levski village, GPS: 43°15'48.4"N 26°37'36.9"E). In the region, the soil in mechanical composition can be classified as heavily sandy-clayey. It is are characterized by good water-physical properties, with medium to low water permeability, good water holding capacity and relatively favorable air regime.

Carrots (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens, variety Nantes 2 were used for the experiments. The investigations were conducted during the growing season in 2015-2018. The experiments were carried out in the first half of March, on an area where carrots have been grown for 10 years ,with an area of 180 m^2, with a total number of plants 1200, with planting scheme: 0.06 m in-row and 0.25 m in-row distance, rows oriented east-west. The soil tillage was done manually. During the growing season, plant protection products were not used.

Sampling methods

The plant health condition of the fields has been satisfactory; there were zones of reduced plant growth and plant damage. Soil samples were taken to evaluate the quantitative and qualitative composition of the migrating root nematodes.

According to the meteorological data, abiotic factors, which could cause these symptoms, are excluded. The period of sampling of plant and soil samples was consistent with that recommended by Knuth et al. (2003). In order to determine the increase or decrease of density of the populations, the samples were taken depending on the season, before sowing and repeatedly during the growing season. The soil samples were taken randomly from 15 – 25 cm depths and then transferred in plastic bags to the laboratory. After mixing the samples, the average samples of 100 cm^3 were determined by means of a measuring cylinder. The resulting quantitative and qualitative data was related to this volume.

Samples from the root system of healthy and diseased plants were also taken for analysis. The root systems and roots were harvested at the same time as the soil and placed in the same bag. A simple amount of 500 g of root was sufficient for the pooled sample.

Extraction of the nematodes

The methods for the extraction of the nematodes from the soil and roots and their subsequent mounting on permanent slides for identification are according to the Baermann pan method described by Townshend (1963). In addition, the samples were examined for the presence of cyst nematodes by flotation methods.

Quantitative and qualitative assessment of nematodes

The liquid nematode suspension was filled to 100 ml with water to include the number of extracted individuals. The suspension was transferred (5 times per 1 ml) into Bogorov Modified Counting Chamber. Initially, all nematodes were counted under stereomicroscope and the plant parasitic nematodes were separated at recount. The average number of live nematodes in the sample (100 cm^3) in the starting suspension of 100 ml was determined by Peters (2013). Species characterization and identification were based on morphology of various life stages [1. Andrassy 1998, Holovachov et al., 2017). Due to the complexity of the process of identification, the some extracted nematodes were determined up to genus level.

The dominant genera were described by using Engelmann's method (Engelmann, 1978). All individuals were subdivided into trophic groups based on the classification of Yeates et al. (1993). The number of nematodes in the various trophic groups was calculated by the relative density of taxa (in 100 cm^3) included in the group.

Results and Discussion

Trophic structure and analysis of nematode communities

A total of 11genera of plant parasitic nematodes were detected in the analysed samples. In the table 1, lists the identified genera and species of plant-parasitic nematodes. The number of samples tested is also indicated.

Table 1. Plant-parasitic nematodes in soil and plant samples

Genus	Speies	number of individuals [n=67]
Aphelenchoides	*Aphelenchoides saprophilus*	2
Ditylenchus	*Ditylenchus dipsaci*	1
Geocenamus		17
Helicotylenchus	*Helicotylenchus sp.*	11
Meloidogyne	*Meloidogyne hapla*	36
	Meloidogyne naasi	8
Paratrichodorus	*Paratrichodorus sp.*	2
Paratylenchus		41
Pratylenchus	*Pratylenchus neglectus*	61
	Pratylenchus penetrans	13
	Pratylenchus crenatus	81
Rotylenchus	*Rotylenchus sp.*	5
Trichodorus	*Trichodorus similis*	21
Tylenchorhynchus		142

The genus *Pratylenchus* with the species *P. crenatus*, *P. neglectus* and P. *penetrans* was detected in 92% of the tested samples. The genus *Meloidogyne* with *M. hapla* and *M. naasi* species was found in 53% of the samples.

Other plant-parasitic genera were *Paratylenchus*– 56% and *Trichodorus/ Paratrichodorus,* which were represented in 41% of the samples. *Ditylenchus* was observed in only 5% of the samples. The nematodes of genus *Ditylenchus* were difficult to detect in soil. A significant number of plant analyses were required to accurately identify them. The Pratylenchus species during sampling may be in the root rather than in the soil and thus have lower values. Also, values in the Meloidogyne genus could be higher, as Meloidogyne nematodes can hardly be covered by the sampling. Therefore, nematodes of this genus may not be found in soil samples from areas with typical *Meloidogyne* damage (root knotting). The widespread genus *Tylenchorhynchus* is irrelevant to carrot production. The relative frequency of genera is shown in Fig. 1.

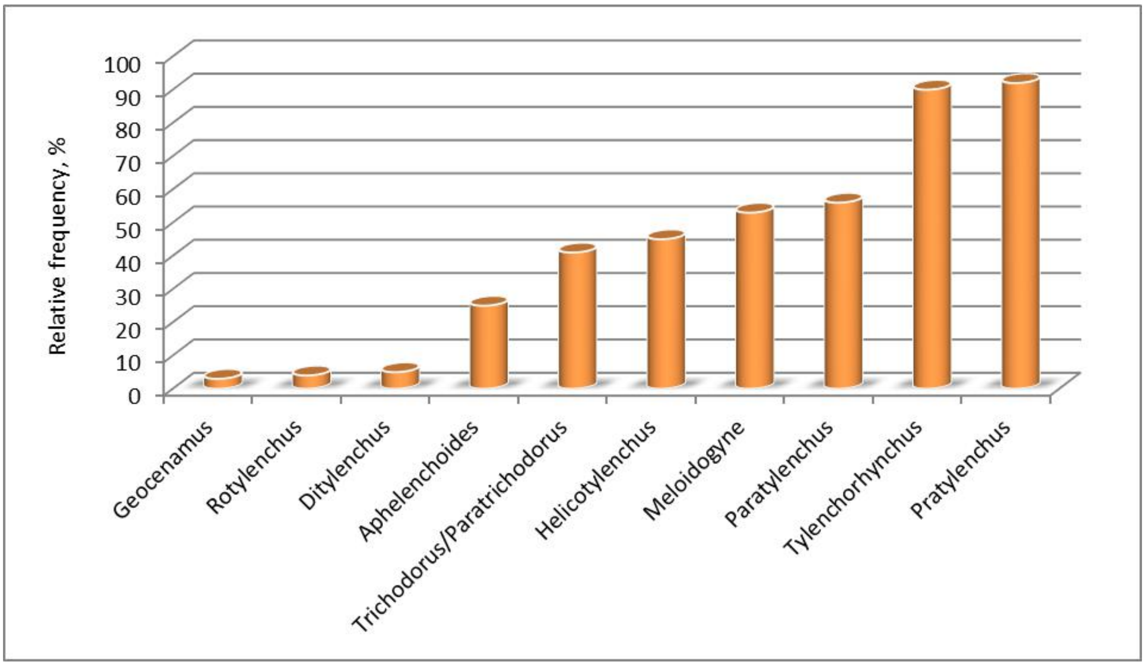

Figure 1. Relative frequency of plant parasitic nematodes in the tested samples (n = 67)

In addition to plant-parasitic nematodes, entomopathogenic nematodes of the genus Steinernema were detected in 14% of the tested samples. Saprophagic nematodes of the genus Dorylaimus were detected in 19% and in 3% of the samples found the genus Cephalobus, which feeds on bacteria. Predatory nematodes of genus Mononchus were found in only 1 sample (Fig. 2).

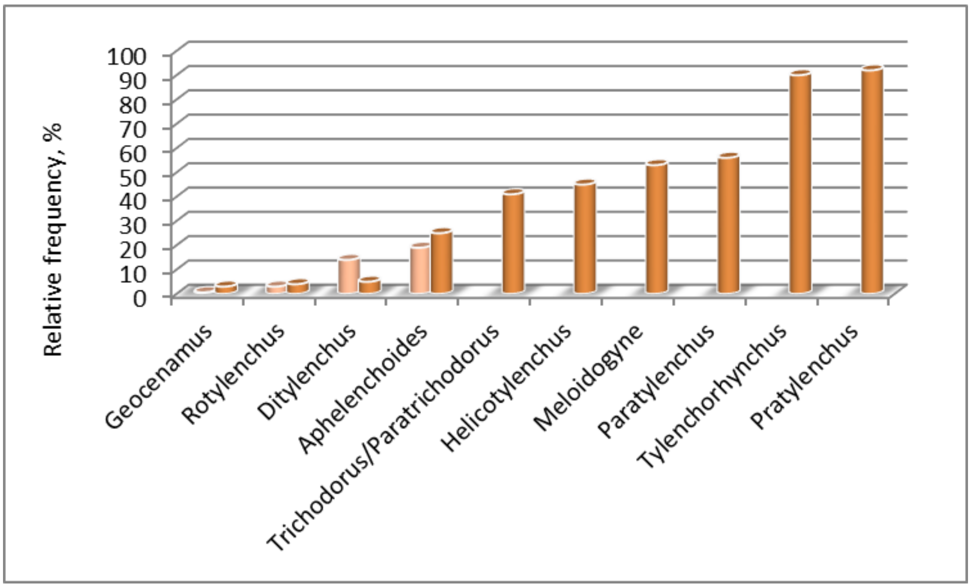

Figure 2. Relative frequency of beneficial and indifferent nematode species in the samples tested (n = 67**)**

Depending on the relative share of individuals in the nematode community, the genera *Pratylenchus*, *Meloidogyne*, *Paratylenchus* were dominant; eudominant - *Tylenchorhynchus*, and subdominants *Helicotylenchus*/*Rotylenchus* (Engelmann 1978) (Fig. 3).

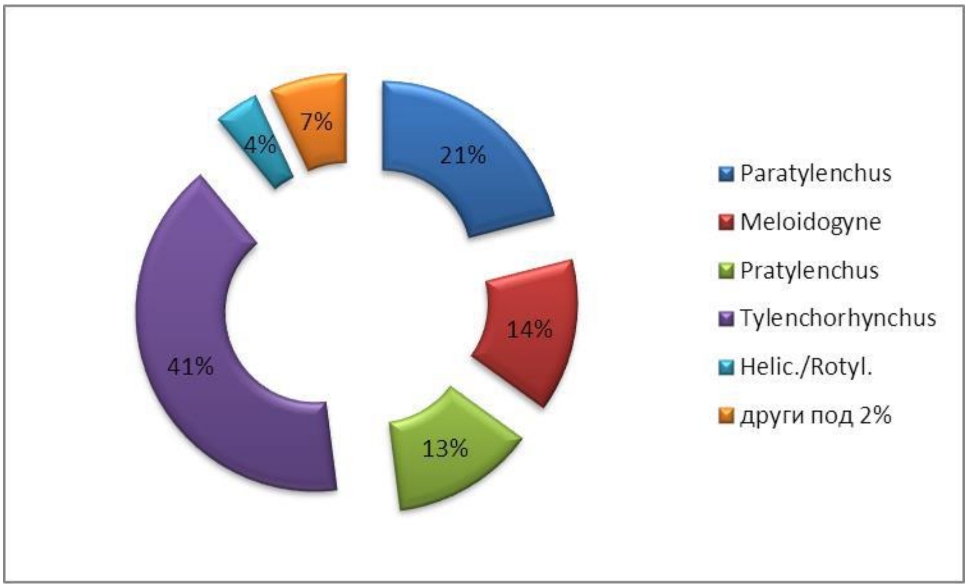

Figure 3. Dominant structure of plant-parasite genera in the tested samples (n = 67)

Figure 4 shows that more than 3 genera/sample were detected in most samples.

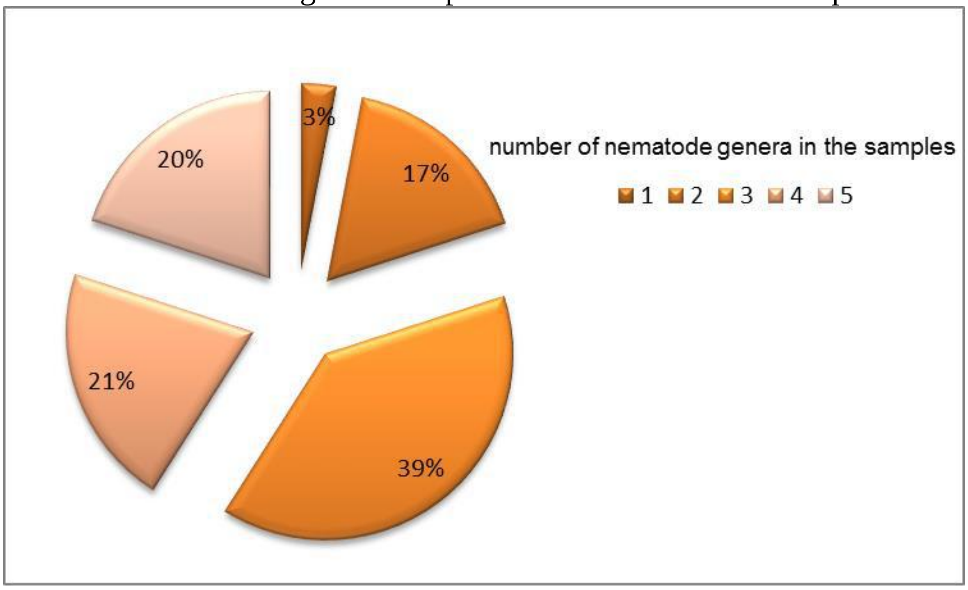

Figure 4. Relative share of the jointly founded plant-parasite genera in the tested samples (n = 67)

In more than 28% of all samples, the 3 genera Meloidogyne, Pratylenchus and Paratylenchus were found together (Tab. 2). Joint populations of the genera *Pratylenchus* and *Paratylenchus* as well as *Meloidogyne* and *Pratylenchus* have been proven. *Meloidogyne* spp. alone were detected in only 4 soil samples.

Table 2. Occurrence of genera (n = 195)

Genus	Number of individuals in the samples	Relative share,%
Meloidogyne	4	2
Pratylenchus	39	19
Paratylenchus	3	2
Meloidogyne Pratylenchus	40	20
Meloidogyne	5	3

Paratylenchus		
Pratylenchus Paratylenchus	50	26
Meloidogyne, Pratylenchus Paratylenchus	54	28
total	195	100
number of samples	67	93

Table 2 shows that nematode genera that cause significant damage are often found together. Therefore, their regulation through crop rotation becomes more difficult as each species has a different range of hosts. The nematodes in the soil were of low numbers (between 1 and 20 nemotodes in 100 cm3 of soil) most often. The genus Pratylenchus shows proportionally higher levels of infection than the genus Meloidogyne (Fig. 5). There are various factors affecting the number of the nematodes, such as the sampling time and phenophase of the carrots during sampling.

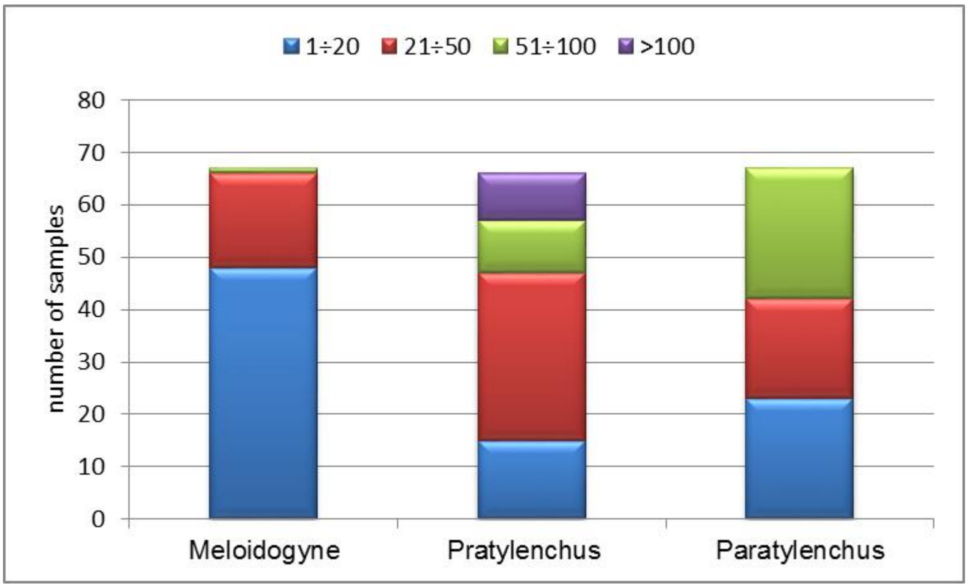

Figure 5: Number of dominant genera in nematode communities)(nematodes / 100 cm3 soil) divided into 4 different classes (n = 67)

During all time points in the study, an average of 5-6 species of plant-parasitic nematodes were identified. The main plant- prasitic nematodes in our research were *Meloidogyne hapla* and various species of *Pratylenchus*, mostly *P. crenatus*, *P. neglectus* and *P. penetrans*. Since several Pratylenchus species always appeared simultaneously in the samples, they can be assembled in *Pratylenchus* spp. Other authors also point out that main plant-parasitic nematodes on carrots are species of the genera *Meloidogyne*, *Pratylenchus*, *Paratylenchus* and (*Para*-) *Trichodoru*s (Hallmann & Meressa 2018; Sikora et al. 2018). Significant yield losses can be observed even at low levels of nematode infection on carrots of (Hay et al. 2004).
As the application of nematocides in small farms is economically unjustified, the control of plant-parasitic nematodes is based mainly on non-chemical methods. Knowing about the trophic structure of nematode communities in monoculture cultivation is a key factor leading to decisions of applying crop rotation, which will effectively reduce plant-parasitic nematode populations. Further studies are needed to determine causes of species damage, to improve the prognosis, and to develop easily a practically applicable control measures**.**

Conclusions

In the analysed samples were detected a total of 11 genera plant-parasitic nematodes. Eudominant was the genus Tylenchorhynchus; dominant - genera Pratylenchus, Meloidogyne, Paratylenchus, and subdominants Helicotylenchus and Rotylenchus. The genus Pratylenchus with the species *P. crenatus*, *P. neglectus* and *P. penetrans* was detected in 92% of the samples tested. The genus *Meloidogyne* with the species *M. hapla* and *M. naasi* was found in 53% of the samples. Nematode genera, which cause significant damage to the carrots, were common together. Joint populations between the genera *Pratylenchus* and *Paratylenchus* as well as *Meloidogyne* and *Pratylenchus* have been proven. *Meloidogyne* spp. In more than 28% of all samples, the three genera *Meloidogyne*, *Pratylenchus* and *Paratylenchus* were found together. In addition to plant parasitic nematodes, entomopathogenic nematodes of the genus Steinernema were detected in 14% of the tested samples. Saprophagic nematodes of the genus Dorylaimus were found in 19% and in 3% of the samples the genus Cephalobus was found to feed on bacteria. Mononchus predatory nematodes were only found in 1 sample.

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MICROMORPHOLOGICAL AND BIOCHEMICAL ASPECTS IN *MENTHA x PIPERITA* L. FOR INDUSTRIAL APPLICATIONS

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Abstract

Aromatic and medicinal plants are a natural source of volatile oils studied for the antimicrobial, insecticidal or antioxidant activity. The composition of volatile oils of the same species may vary depending on the geographical origin. The purpose of the present research was to acquire new Scanning Electron Microscopy (SEM) images on secreting tissues of *Mentha x piperita* L. plants (Romanian ecotype originated in Ulmeni, district Teleorman) and to identify the main organic compounds from biochemical composition of volatile oil, underlining their importance for utilization in pharmaceutical, alimentary or cosmetics industry. The study of secretory tissues was performed using electronic microscopy at the Research Center HORTINVEST, U.A.S.M.V. Bucharest. Separation and identification of the compounds in volatile oil was carried out by gas chromatography. NIST spectral library was utilized to identify the essential compounds and verified using Kovat's indices. Micromorphological and anatomic analysis of SEM images of leaf tissues revealed secretory structures represented by hairs with unicellular and multicellular glands. Chemical composition of volatile oil of the Romanian ecotype of *Mentha x piperita* L. included 23 compounds, most important proportion being recorded for the next compounds: alpha-terpinolene (29.80%), terpinen-4-ol (23.33%), gamma-terpinene (9.84%), alpha-terpinene (6.40%), sabinene (4.26%), cis-p-terpineol (3.97%). These volatile compounds, generally recognized as safe (GRAS), are known to confer the character of medicinal and aromatic plant and recommend its utilization in pharmaceutical industry, plant protection, food industry and perfumery.

Keywords: *Mentha, volatile oil, scanning electron microscopy, secreting tissue, chemical composition.*

Introduction

Aromatic and medicinal plants are a natural source of volatile oils studied for the antimicrobial, insecticidal or antioxidant activity (Pandey and Tripathi, 2011). The composition of volatile oils of the same species may vary depending on the geographical origin (Al-Bayati, 2009). Originating in the regions around the Mediterranean Sea, cultivated peppermint has spread worldwide in regions with favorable thermic climate, including countries from Europe, Asia and America. Taxonomy studies based on phylogenetic analysis, morphology, the number of chromosomes and the main chemical constituents of volatile oil (Cantino *et al*., 1992) redefined the genus *Mentha* (from Family *Lamiaceae*, order *Lamiales*). In present, 18 species and 11 hybrids grouped in 4 Sections are included in this genus (Tucker and Naczi, 2007). Of these, 6 species of *Mentha* (with varieties) and 6 hybrids have been described in Romanian flora (Beldie,1979; Ciocârlan, 2009). *Mentha x piperita* L. requires cultivation on well aerated, permeable, fertile and humid soils (humidity around 80-90%).

Plants cultivated on sunny fields produce volatile oils rich in menthol and poor in menthone. These properties warranty a superior quality of mint volatile oil (Păun,1975). The hybrid *Menta x piperita L.* is frequently met in spontaneous flora but varieties of the taxa are cultivated for their special properties, presenting economic importance (Tucker and Naczi, 2006). It is cultivated on large areas in USA, Russia, Bulgaria, Italy, France. In Romania, this hybrid can be found especially in districts Bistriţa-Năsăud, Sibiu, Argeş and Ilfov (Oprea, 2005). Plants of this hybrid are resistant to -10 ºC unprotected or even until -25 ºC – 30 ºC under the snow layer or protected with various materials. They can grow in shadow but a high light intensity is more favorable for obtaining higher yields. The plants content in volatile oil and its quality are also influenced by soil characteristics, such as good aeration, permeability, drainage and fertility. The purpose of the present research was to acquire new Scanning Electron Microscopy (SEM) images on secreting tissues of *Mentha x piperita* L. plants (Romanian ecotype originated in Ulmeni, district Teleorman) and to identify the main organic compounds from biochemical composition of volatile oil, underlining their importance for utilization in pharmaceutical, alimentary or cosmetics industry.

Materials and Methods

***Mentha x piperita* L. plants** (Romanian ecotype originated in Ulmeni, district Teleorman) were sampled in flowering stage.

Micromorphological analysis of leaf and stem tissues (the study of secretory tissues) was performed using electronic microscope Inspect S50 (FEI) at the Research Center HORTINVEST, U.A.S.M.V. Bucharest.

Chemical composition of volatile oil: Volatile oil was extracted from plants by hydrodistillation using a Clevenger-type apparatus. Heating was carried out with Bunsen burner, with precaution to avoid overheating of distillation recipient. Generally, the vegetal mass and water ratio was 1:3 (w/v). Separation and identification of the compounds was carried out by gas chromatography (Agilent 7890B-MSD 5977A apparatus with sample introduction system GC 80, HP-5 MS Ultra inert column of 30 m, Ø = 0,250 mm and fill of 0,25µm). Chromatographic conditions were: injecting temperature: 250 °C; detector temperature: 150 ºC; initial temperature of the oven: 50 °C, programmed to grow gradually until 150 °C. Helium was utilized as carrier gas to a linear flow rate of approximately 0,6 ml min. Retention time and peaks were processed using Agilent 7890B-MSD 5977A software. NIST spectral library was utilized to identify the essential compounds and was verified using Kovat's indices.

Results and Discussion

Micromorphological analysis of SEM images of leaf and stem tissues of *Mentha x piperita L.* revealed secretory structures represented by hairs with unicellular and multicellular glands (Figures 1, 2, 3).

For most species in this family there are two types of secretory hairs: peltate and capitate (Fahn, 1988). The results obtained are similar to those obtained by Svoboda et al. (2001) and Andro (2012) in *Mentha* spp. or in *Thymus pannonicus* (Boz *et al*., 2009).

Chemical composition of volatile oil of the Romanian ecotype of *Mentha x piperita* L. determined by GC-MS analysis (Figure 3) included 23 compounds, the most important proportion being recorded for the next compounds: alpha-terpinolene (29.80%), terpinen-4-ol (23.33%), gamma-terpinene (9.84%), alpha-terpinene (6.40%), sabinene (4.26%), cis-p-terpineol (3.97%).

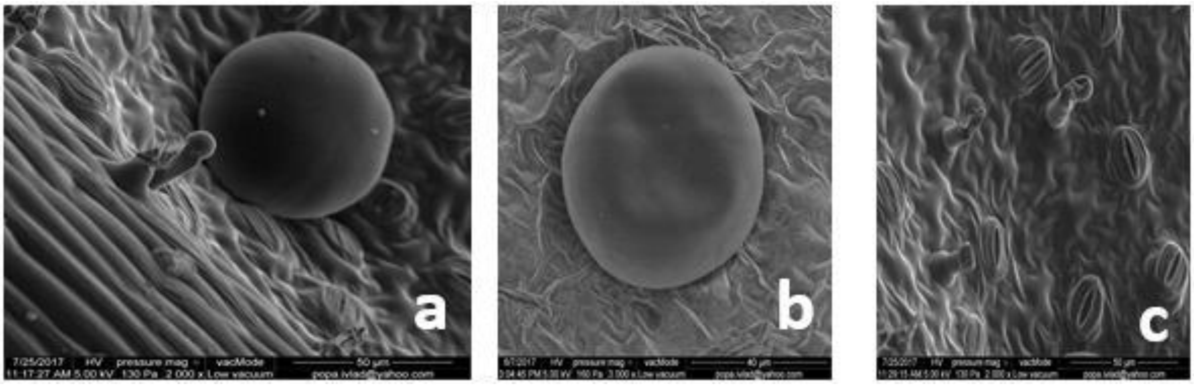

Figure 1. The scanning electron - microscopy image of lower (abaxial) epidermis of *Mentha x piperita L.* leaf. Secretory hair with unicellular (a,c) and multicellular glands (a, b).

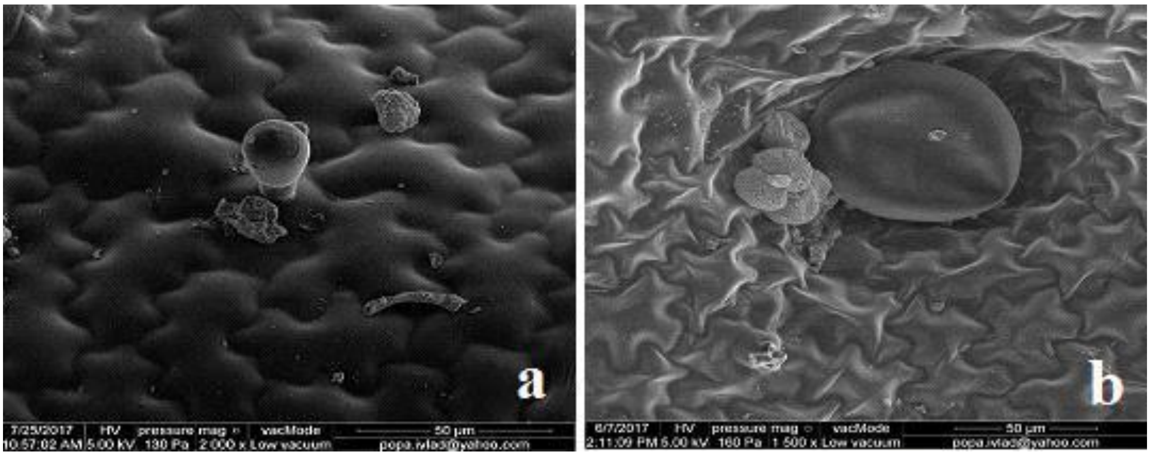

Figure 2. The scanning electron - microscopy image of upper (adaxial) epidermis of *Mentha x piperita L.* leaf (a) and (b). Secretory hair with unicellular (a) and multicellular glands (b).

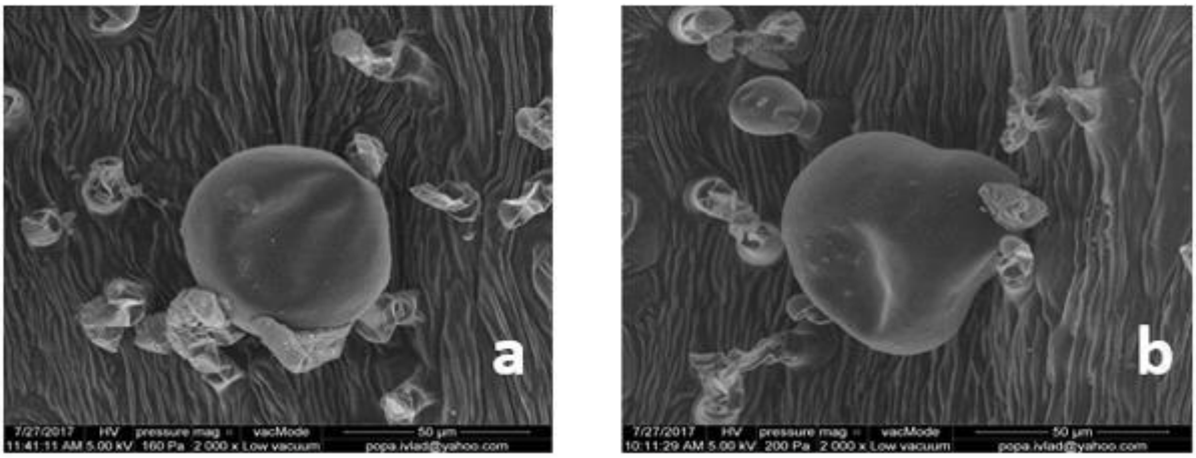

Figure 3. The scanning electron - microscopy image of stem - Secretory hair with unicellular and multicellular glands of *Mentha x piperita* L.

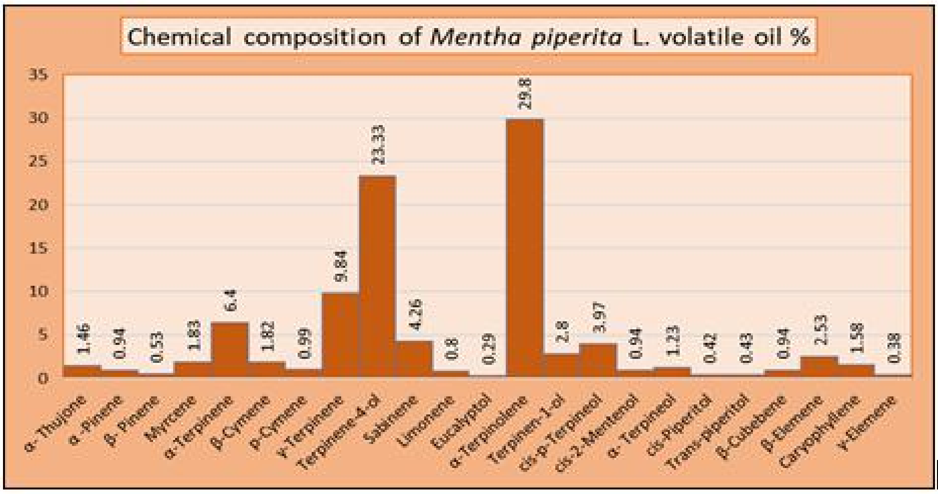

Figure 3. Chemical composition of *Mentha x piperita* volatile oil (%)

These volatile compounds, generally recognized as safe (GRAS), are known to confer the character of medicinal and aromatic plant and recommend its utilization in pharmaceutical industry, plant protection, food industry and perfumery. α -terpinolene (29.80%), belongs to the group of terpenoids, also known as terpenes, a category of phytochemical products which provide a wide range of therapeutic benefits. These substances can present sedative and antioxidant properties as well as antimicrobial activity. Terpinolene is commonly used to improve the odor of various industrial and household products, like mixing paints and varnishes. Terpinolene is found in citrus, allspice, peppermint, juniper, and parsnip essential oils. Terpinolene is used as an input in soap, detergent, creams, lotions, perfume, fragrances, flavoring ingredients, and laundry and dishwashing products. In certain cannabis varieties, terpinolene can be the predominate terpene (https://praxis-laboratory.com/terpene-analysis). α – terpinene (6.4%) is a flavoring and fragrance chemical used in the personal care and cosmetic and food industries, also used in the pharmaceutical and electronics semi-conductor manufacturing industries (https://foreverest.cn/products/turpentine-derivatives/99-alpha-terpinene.html). Like other terpenes, α -terpinene is commonly used in essential oils and in the production of perfumes. α-Terpinene is often used to synthesize other products and reagents (https://praxis-laboratory.com/terpene-analysis/). Terpinen-4-ol (23.33%) is utilized in perfumery for its flavor (smells like lemon). It has strong anti-allergenic, anti-asthmatic, antiseptic, antibacterial, diuretic and fungicidal effect (http://www.alegesanatos.ro/cautare/).
γ -terpinene (9.84%) is a terpene with acaricide, insect repellent and antioxidant properties. γ-Terpinene is colorless, oily terpene with an odor that resembles turpentine. γ -Terpinene exudes a tropical, lemon-lime, woody scent and has a bitter taste. γ-Terpinene is an insectifuge, a compound that repels insects non-lethally, and can also be an irritant. γ -Terpinene is also an antifeedant, a naturally occurring substance that adversely affects creatures that feed upon plants that produce it. γ-Terpinene is found in a variety of foods, including allspice, carrots, cinnamon, oregano, sage, and thyme. Commercially, γ -terpinene is used as a flavor and a production input in perfumery (https://praxis-laboratory.com/terpene-analysis/). Sabinene (4.26%) presents antimicrobial, antiseptic, bactericide properties (www.ars-grin.gov) and is utilized in perfumery (Burzo, 2015). Andro (2012) found: α -pinene, sabinene, β -pinene, β -myrcene, 3-octanol, β -ocimene, limonene, eucalyptol, cis- β -ocimene, menthone, iso -menthone, menthol, terpinen-4-ol, α -terpineol, pulegone, piperitone, menthyl -acetate, β -burbonene, β -caryophyllene, β -cubebene, mint furanone, germacrene D, caryophyllene oxide, ledole as main chemical compounds of essential oil from stems, leaves, flowers of *Mentha x piperita*. According to Burzo (2015), the volatile oil extracted from peppermint during flowering period had the following composition: 61.42% piperitone oxide,

7.56 % eucalyptol, 3.57 % beta-cubebene, 2.26 % beta-caryophyllene, 2.24 % piperitone oxide, 2.08 % beta-pinene, 2.01 % alpha-caryophyllene, 1.68 % trans-beta-ocimene, 1.44 % mint-furanone, 1.16 % 3-octanol, 1.15 % myrcene, 1.08 % alpha-pinene, 1.16 % sabinene, 0.79 % octil acetate, 0.76 % cis-beta-terpineol, 0.59 % carvacrol, 0.47 % hydro-piperitone, 0.40 % burbonene, 0.34 % cubenol, 0.29 % alpha-cadinol, 0.29 % germacrene D and 0.27 % calamenen. Research carried out on the composition of essential oils from *Mentha x piperita* L. reported menthol (53.28%), methyl-acetate (15.1%), menthofuran (11.18%) as main constituents of the total 17 compounds identified and significant antimicrobial activity against human pathogenic species of *Candida* (Saharkhiz et al., 2012). Other studies found menthone, neomenthol, menthol and carvacrol as compounds of peppermint essential oil from Brazil responsible for antifungal activities in postharvest deteriorating species of *Aspergillus*, *Colletotrichum* and *Fusarium* (Freire et al., 2012). In peppermint essential oil from Romanian ecotype, menthol was not detected, as also reported for essential oil from Portugal (Martins et al., 2004). Strong antimicrobial, insecticidal and antioxidant activities in essential oils from different species of the genus *Mentha* offer the prospective of their use as natural, environmentally friendly products with commercial value ((Rahnama et al., 2017; Singh and Pandey, 2018).

Conclusion

Micromorphological analysis of SEM images of peppermint leaf tissues revealed open stomata and secretory structures represented by hairs with unicellular and multicellular glands. Chemical composition of volatile oil of the Romanian ecotype of *Mentha x piperita* L. from Ulmeni, district Teleorman, included 23 compounds, most important proportion being recorded for the next compounds: alpha-terpinolene (29.80%), terpinen-4-ol (23.33%), gamma-terpinene (9.84%), alpha-terpinene (6.40%), sabinene (4.26%), cis-p-terpineol (3.97%). These volatile compounds, generally recognized as safe, are known to confer the character of medicinal and aromatic plant and recommend its utilization in pharmaceutical industry, plant protection, food industry and perfumery.

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STUDYING THE ACCLIMATIZATION AND AGRICULTURAL PECULIARITIES OF EARLY SPECIES OF TANGERINE INTRODUCED FROM JAPAN IN CONDITIONS OF ADJARA

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Abstract

In the Black Sea subtropical zone, the reservs to assimilate the new areas for the citriculture has been exhausted and further development of the sector is mainly possible with the introduction of modern technologies, while increasing the cost of the plant should be carried out by replacing biologically amortized plants by arranging sedimentation nurseries, raising soil fertility, timely conduct of agro-techniques and plant pest control measures and correctly organizational-practical issues. Since our region belongs to the extremely northern subtropical zone, it is necessary to select varieties that are suitable for our zones, which are distinguished by early ripening and high yield. In 2011, with the initiative of the Ministry of Agriculture of Adjara for solution of this problem, 11 varieties of tangerine were imported from Japan: Nichinani, Iura-Vase, Taguchi - Vase, Miagava - Vase, Kavada, Nankani-20, Ohotsu-Vase, Ueno-Vase, Aoshima, Okitsu-Vase, Mukaiama, which was planted in the Kobuleti municipality, the plants are planted with nutritional air of 4 × 2.5 meters, at 35-40 meters above sea level, the soil is red. We had ten plants in each variant that we have studied: - The formation of buds, the beginning and end of vegetation, the massive flowering, setting, the beginning of the ripening of the fruit and the full ripening, high yielding (piece, kg), the average weight of one fruit (g), the color of the fruit, the nature of the skin removal from the flesh, number of seals in flesh and organoleptic characteristics. We received fruit from different expositions of the plant and explored biochemical indicators at the University Laboratory. The novelty of the research is that we have examined the first phonological, agro-ecological and agro-technological characteristics of eleven varieties of introductive tangerins, from which only three varieties: Nichinani, Iura-Vase, Taguchi - Vase identified the best characteristics for our zone and were recommended to be a super early sorts, which are 40-45 days prior to the local in breeding. We recommend the cultivation of such plants by growing citrus areas and therefore productivity. The novelty of the research is also that the intensive technological complex also includes organizational and economic measures and economic issues that will be directed to improve organizational management and management of the sector.

Keywords: *Citrus, fruit, subtropical, varieties, phenology, vegetative growth*.

Introduction

The problems of development of citriculture in Georgia can not be considered only in terms of the characteristics of the market economy. It needs special approaches and support from the state, and the manufactured products will meet the requirements and standards of the world market. After the collapse of the Soviet Union, due to the breakdown of old economic ties and market relations, export was significantly reduced and import increased. Currently, harvesting of citrus cultivars is 10 times lower, but the quality of harvest is reduced and the export is

difficult. For example, in 1995 citrus exports comprised 7 million US dollars, and in 2000 the export volume decreased to 2.3 million, and in 2018 only $ 1.1 million (Jabnidze, 2018).
Plants in our citrus garden in the age of eighty years and more. Theoretically and practically it is proven that citrus fruits are highly productive until the age of 50-55, then the harvest is gradually decreasing, and any agronomic activities do not give the desired result.
One of the effective measures for solving this issue is the rejuvenation of plants in the citrus plantations, namely, the biologically aged plants should be replaced by the abundant and stablely fruit-bearing, early ripening varieties and forms of species, which are better adapted to the local conditions. The replacement of amortized trees with young plants will increase yield and decrease the timing of harvesting. This will increase economic efficiency and in a few years we will get completely rejuvenated plantations (Lamparadze, 2014).
In order to solve this problem, it is necessary to arrange new nurseries and gardens from district and collective gardens. At the same time, it will be essential to establish such ratio of the varieties of breeds, which provide the prolonged period of harvest and relatively early adoption of harvest, which is unimaginable without early ripening breeds at different times, which have already been tested and approved in many foreign countries, which produce citrus. From the financial point of view, the event will be economically advantageous, our calculation confirms it. In the amortized gardens averaging 10-11 tons of fruit per hectare, younger (25 years) up to 30 tons, which can bring extra income for farmers and private owners annually (Beridz, 2010).

Material and Methods

The plant material included 11 varieties of tangerine (Nichinani, Iura-Vase, Taguchi-Vase, Miagava-Vase, Kavada, Nankani-20, Ohotsu- Vase, Ueno-Vase, Aoshima, Okitsu-Vase, Mukaiama) which were imported from Japan in 2011, with the initiative of the Ministry of Agriculture of Adjara, Our experiment was conducted on the south-eastern slopes of the 25-35 degrees in the Chakvi citrus collection of the Kobuleti municipality, where the plants are planted at 4 × 2.5 meters, at an altitude of 35-40 meters above sea level. Under the plants, the soil is red, we had ten plants in each variant, and we had tested and examined about 110 plants. Studies were conducted according to the preliminary draft and methodology. All trial plants were numbered, labeled, obtained data was written in a special journal where all the data of each plant was envisaged for research.
On each varieties we did the phenological monitoring: the formation of buds, the beginning and end of vegetation, the massive flowering, setting, the beginning of the ripening of the fruit and the full ripening, yield (piece, kg.), the average weight of one fruit (g.). In 2011-2018 we also studied biometric indicators on tangerine varieties, thus determining the height of the plant in cm, the base and bot diameter in mm for grafting (table 3).

Results and Discussion

Observations have shown that the following varieties of tangerine are distinguished with the early ripening, quality and productivity: Nichinani, Iura-Vase and Taguchi – Vase.
As shown in the 1-2 table, the blossoms, vegetation and massive blossoming on the above-mentioned plants almost coincide with each other. The fruit setting was begun in the third decade, the second vegetation in the second decade of August.
Nichinani - The massive ripening of the fruit began in the second decade of October. On average one tree has produced 415 pieces of fruit, resulting in an average of 24.3 kg. The average weight of one fruit is 57.7 g. The number sections in the flesh is 12 (Table 1-2). The leaves are oval, the average size is 12 × 6 cm, the length of stem is 2 cm. Attached with the sharp angle. The size of the plants in the range was 223 cm, the diameter of the base and the bot for grafting is 62-52 mm (Table 3).

Iura-Vase - The massive ripening of the fruit began in the first decade of September, and the mass of ripening phase in the third decade of September, on average, 458 pieces of fruit was gathered per tree, which reached averagely of 20.1 kg. The average weight of one fruit is 52.3 grams. In comparison with breed Nichinani, the fruit is smaller in size, the number of sections in the flesh is 11 (Table 1-2). The leaves are rhombic-oval, the average size is 13X7 cm, attached with the sharp angle. The length of the stem is 2,5 cm. The size of the plants in the range was 171 cm, the diameter of the base and the bot for grafting is 60-58 mm (Table 3).

Taguchi – Vase - The second vegetation beginning was at the first decade of August. The massive ripening of fruit began at the second decade of September, averagely, 425 pieces of fruit was gathered per tree, which reached averagely of 28,5 kg. The average weight of one fruit is 62,4 grams. The number sections in the flesh is 10 (Table 1-2). The plant is well-leafy, the leaf is attached with the sharp angle and is of the dark-green color, the length of the stem is 2.0 cm. The average size of the leaf was 13.5X5 cm. The size of the plants in the range was 198 cm, the diameter of the base and the bot for grafting is 56-48 mm (Table 3).

Table 1. Basic phenological pbservation pata on pifferent species of tangerine (2011-2018 average)

	Variety	Waking buds	The first vegetation	Massive flowering	The second vegetation
1	Nichinani	II decade of March	III decade of April	I decade of May	II decade of August
2	Iura-Vase	II decade of March	III decade of April	I decade of May	II decade of August
3	Taguchi-Vase	II decade of March	III decade of April	III decade of May	II decade of August
4	Miagave-Vase	III decade of March	III decade of April	II decade of May	II decade of August
5	Kavada	III decade of March	III decade of April	III decade of May	III decade of August
6	Nankani-20	III decade of March	III decade of April	III decade of May	III decade of August
7	Ohotsu-Vase	I decade of April	I decade of May	III decade of May	III decade of August
8	Ueno-Vase	III decade of March	III decade of April	II decade of May	II decade of August
9	Aoshima	I decade of April	I decade of May	III decade of May	III decade of August
10	Okitsu-Vase	III decade of March	II decade of April	I decade of May	II decade of August
11	Mukaiama	III decade of March	III decade of April	I decade of June	II decade of August

Table 2. Basic phenological, productivity and fruit weight observation data on different species of tangerine (2011-2018 average)

N	Species	End of 2nd growth	Beginning of ripening fruit	Massive ripening of fruit	Productivity		Average weight of one fruit (gr)	Number of particles in the pulp (units)
					Pieces	In Kg		
1	Nichinani	II decade of August	I decade of September	III decade of September	415	24.3	57.7	12
2	Iura-Vase	I decade of August	I decade of September	III decade of September	458	20.1	52.3	11
3	Taguchi-Vase	I decade of August	II decade of September	I decade of October	425	28.5	62.4	10
4	Miagave-Vase	II decade of August	I decade of October	III decade of October	355	23.5	54.2	11
5	Kavada	II decade of August	III decade of September	II decade of October	252	21.5	49.2	10
6	Nankani-20	I decade of August	III decade of September	II decade of October	265	21.8	63.2	11
7	Ohotsu-Vase	III decade of August	II decade of October	I decade of November	231	22.4	62.1	11
8	Ueno-Vase	II decade of August	II decade of September	I decade of October	371	24.2	51.3	10
9	Aoshima	III decade of August	I decade of October	I decade of November	81	8.4	84.2	10
10	Okitsu-Vase	I decade of August	III decade of September	I decade of October	347	25.2	68.4	12
11	Mukaiama	II decade of August	III decade of September	III decade of October	297	21.3	65.2	13

Table 3. Results of biometric observations on tangerine varieties based on 2018 data on 3 plants in the variant

N	Variety	2018		
		Height cm	Rootstock dm/mm	Grafted dm/mm
1	Nichinani	223	62	52
2	Iura-Vase	171	60	59
3	Taguchi-Vase	198	56	48

Conclusions

Following our experiment and analyzing the results of scientific research data we have made following conclusions:

Among the 11 varieties of tangerine, which was imported from Japan, three varieties of tangerine; Nichinani, Iura-Vase and Taguchi – Vase are distinguished with the early ripening, high yielding, productivity.

These plants are well adapted to soil-climatic conditions of humid subtropical zones and we can recommend them for planting on agriculture farming.

With the cultivation of early ripening, high-yielding breeds, the citrus areas will intensively be increased and therefore, the productivity as well, which will help to improve the economic condition of the population.

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GENERAL COMBINING ABILITIES OF ELITE MAIZE INBRED LINES FOR YIELD AND IMPORTANT TRAITS

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Abstract

General combining abilities (GCA) is an important biometric parameter for maize that can be used to predict the contribution that the investigated genotype can give to its progeny. The purpose of this study was to determine GCA values in six elite maize inbred lines, components of commercial hybrids of Maize research institute „Zemun Polje“ and belonging to different heterotic groups. Field trials were set on three locations (Zemun Polje, Becej and Pancevo) during two years (2011 and 2012). GCA was calculated for eight different trait the most important being grain yield. Significant values of GCA for examined traits with positive and negative sign, were evaluated in both years of trials and differences were recorded both between different years and genotypes. GCA estimates for grain yield ranged from -0.870 t ha-1 for ZPL3 line in 2011, to 0.909 t ha-1 for ZPL6 inbred line. Significant values of GCA for plant height (cm) were evaluated for each inbred line except for ZPL1 in 2012 with ZPL 5 being the best general combiner for that trait in both years: 19.4 cm in 2011 and 18.55 cm in 2012. Traits that were in correlation exhibited GCA values of similar significance. Inbred ZPL6 had significant GCA values for most of the wanted traits making this inbred line a valuble source of desirable alleles for maize breeding programs. This GCA analysis provided a better insight and understanding of the inbred lines chosen in this study as breeding material.

Key words: *maize, general combinig ability, grain yield, morphological traits*

Introduction

Maize as an important crop was subject of intensive breeding practices resulting in the increase of areas under cultivation and constant increase in yield (Troyer i Hendrickson, 2007) with the discovery and explotation of heterosis has had a great impact on modern agriculture and was a milestone in maize breeding (Lamkey and Edwards, 1998). The performance of a hybrid and expressed heterosis is related to the general (GCA) and specific (SCA) combining abilities of the inbred lines involved in the cross with SCA being more important in elite breeding material due to a greater importance of dominance and epistasis (Hallaur and Miranda, 1988; Gama et al., 1995; Nass et al 2000). Therefore general combining abilities (GCA) is an important biometric parameter for maize that can be used to predict the contribution that the investigated genotype can give to its hybrid progeny and the expressed heterosis for the investigated traits (Sharareh et al, 2018).

The concepts of general combining ability (GCA) and specific combining ability (SCA) was first explained by Sprague & Tatum (1942), defining GCA as an average performance of a line in a hybrid combination. They interpreted GCA largely as a indication of genes having mostly additive effects. Its predominance can be useful to a breeder in increasing selection efficacy in segregating populations (Bocanski et al., 2009). The value of any cultivar in breeding programes depends on its potential *per se* and its combining ability in crosses (Vacaro *et al*., 2002).

Different mathematical modeling were proposed to estimate the GCA and SCA values for specific traits: diallel crosses (Griffing ,1956), topcross (Jenkins i Brunson, 1932), line x testers analysis (Kempthorne ,1957; Singh and Chaudhary, 1976), principal component biplot technique for diallel analysis (Yan and Hunt, 2002)... Most commonly used is the Griffing's method (1956), which partitions the total variance to GCA variance of parents and SCA variance of crosses. The diallel is simple to manipulate in maize and supplies important information about the studied populations, as general and specific combining ability, genetic variances, heritability and maternal effects, among others. This analysis also permits an evaluation of the populations *per se* (Vacaro *et al*., 2002).

Material and methods

For this study, six maize inbred lines belonging to the elite core of „Zemun Polje" institute gene bank were chosen. Inbred ZPL1 is of unknown heterotic origin, lines ZPL2, ZPL3 and ZPL4 are sharing a common BSSS origin, while ZPL5 and ZPL6 belong to a Lancaster heterotic group. Crossings according to a incomplete diallel design (Griffing model II) were performed among the inbred lines in order to obtain 15 of their F_1 progenies. Inbreds and their crosses were tested in field trials set at 3 locations (Zemun Polje, Becej and Pancevo) during two years (2011 and 2012). While the 2011 had average temperatures and precipitation, 2012 is considered arid with significantly lower precipitations especially during critical phases of maize growth. The trials were sown according to a RCB experimetal design in 4 repetitions. The size of the elementary plot was $7.5m^2$ and the sowing was done mechanicly with a 0.75m spacing between rows. Traits that were evaluated in this trial are grain yield (t/ha), plant height (cm), ear height (cm), total number of leaves, number of leaves above main ear, ear lenght (cm), number of kernel row and number of kernel in a row.

Estimates of general combining abilities (GCA) for the investigated traits were evaluated according the method 2 by Griffing (1956). The following formula was used for the calculation of general combining abilities (GCA):

$$Sij = \frac{1}{p+2}\left[(Ti+ii) - \frac{2}{p}GT\right]$$

Tj + ii = total i- row + average value of parent i

Results and Discussion

Estimation of GCA for investigated traits were calculated for each year of the trials (2011 and 2012) and they're presented in table 1 and table 2.

Grain yield is the most important and most complex agronomic trait of maize, which represents the ultimate, although not the only goal of most selection programs (Duvick, 2005). Therefore, the determination of the inbreds with high positive values of GCA for grain yield and their inclusion in the breeding programs is very important. GCA estimates for grain yield ranged from -0.870 t ha^{-1} for ZPL3 line in 2011, to 0.909 t ha^{-1} for ZPL6 inbred line. In 2011, four lines showed statistically significant values of the GCA for grain yield. Inbred lines ZPL2 and ZPL3 had negative values (-0.393 t ha^{-1} and -0.870 t ha^{-1}) while ZPL5 and ZPL6 had positive GCA values of for this trait. In the more unfavorable year of 2012, significant GCA for grain yield were evaluated for all tested lines with only inbreds ZPL2 and ZPL4 having a negative value. Lines ZPL6 and ZPL5 emerged as best combiners for grain yield in both years, while ZPL1 had highest GCA value in arid 2012 but insignificant in the rainy 2011, indicating that this line is a potential source for drought tolerance.

Table 1. Estimates of GCA for grain yield (YI), plant height (PH), ear height (EH), total number of leaves (TNL), number of leaves above main ear (NLE), ear lenght (EL), number of rows of kernel (NRK) and number of kernel in a row (NKR) in 2011.

	YI	PH	EH	TNL	NLE	EL	NRK	NKR
ZPL 1	0.256	-1.791*	-1.714*	-0.607**	-0.240**	0.334*	-0.703**	0.373
ZPL 2	-0.393**	-6.922**	0.911	0.063	-0.129**	-0.635**	0.555**	-1.401**
ZPL 3	-0.870**	-10.697**	-6.128**	-0.243**	-0.184**	-0.796**	0.944**	-2.057**
ZPL 4	-0.203	-5.376**	-3.629**	0.199**	0.103*	-1.626**	1.094**	-4.557**
ZPL 5	0.302*	19.408**	9.557**	0.489**	0.277**	1.239**	-0.416**	2.202**
ZPL 6	0.909**	5.378**	1.004	0.100	0.172**	1.486**	-1.476**	5.440**
0.01	0.352	2.215	2.155	0.147	0.112	0.368	0.170	0.760
0.05	0.265	1.666	1.62	0.110	0.084	0.276	0.128	0.571

*,** Significant at the 0.05 and 0.01 probability levels, respectively

In maize breeding , plant height and in particular the height of the upper ear are considered undesirable agronomic traits in which the tendence is to obtain lower values (Duvick et al., 2004, 2005). All investigated lines had statistically significant values of GCA for plant height in both years of testing, except for the ZPL1 line in 2012. It can be noticed that all BSSS lines (ZPL2, ZPL3, and ZPL4) had negative values of GCA for plant height in both years, while the Lancaster lines ZPL5 and ZPL6 had significant positive values for plant height GCA. ZPL 5 is the best general combiner for that trait in both years with GCA estimates of 19.4 cm in 2011 and 18.55 cm in 2012. Inbred lines that were recognised as best general combiners for grain yields have also significant positive GCA values for plant height which is in accordance with results by Čamdzija (2014).

Table 2. Estimates of GCA for grain yield (YI), plant height (PH), ear height (EH), total number of leaves (TNL), number of leaves above main ear (NLE), ear lenght (EL), number of rows of kernel (NRK) and number of kernel in a row (NKR) in 2012.

	YI	PH	EH	TNL	NLE	EL	NRK	NKR
ZPL 1	0.481**	0.861	1.006	-0.712**	-0.376**	0.865**	-0.184	1.730**
ZPL 2	-0.742**	-4.850**	-1.181	0.189**	-0.056	-0.618**	0.362**	-2.104**
ZPL 3	0.152*	-11.369**	-4.599**	-0.167**	-0.106**	-0.889**	0.672**	-1.849**
ZPL 4	-0.576**	-2.998**	-3.866**	0.216**	0.122**	-1.701**	0.776**	-4.108**
ZPL 5	0.289**	15.855**	9.195**	0.472**	0.296**	0.829**	-0.481**	1.392**
ZPL 6	0.395**	2.502**	-0.556	0.001	0.012	1.514**	-1.146**	4.940**
0.01	0.167	2.303	1.708	0.165	0.083	0.394	0.248	0.903
0.05	0.125	1.732	1.284	0.124	0.062	0.296	0.186	0.679

*,** Significant at the 0.05 and 0.01 probability levels, respectively

The estimates and direction of GCA for ear height show a compatibility with GCA estimates for the plant height, which is in agreement with numerous authors who examined the correlation between these two traits (Bello et al., 2010; Pavlov et al., 2012). Four inbred lines in 2011 (ZPL1, ZPL3, ZPL4 and ZPL5), and three lines in 2012 (ZPL3, ZPL4 and ZPL5) had statistically significant values of GCA for ear height raging from -6.128 cm (ZPL3) to 9.557 cm (ZPL5).

The total number of leaves, and especially the number of leaves above the upper ear is in a positive correlation with the yield and maize yield components (Jalilian and Delkhosh, 2014) making these traits highly desirable in maize breeding. For the total number of leaves, significant GCA values in both years were estimated in four lines: ZPL1 and ZPL3 with negative GCA values, versus ZPL4 and ZPL5 lines with positive.

Ear lenght is an important yield component trait correlated to grain yield in maize (Rafiq et al, 2010; Pavlov et al, 2012). The results of the study in both years pointed to statistically significant values of GCA for this trait for all inbred lines. Inbred ZPL6 had greatest positive GCA values for ear lenght in both year 1.486 cm in 2011 and 1.514 cm in 2012, which is consistent with the said line being the best general combiner for grain yield.

Greatest positive GCA estimates for number of rows of kernel were found in ZPL4 inbred (1.094 in 2011, 0.776 in 2012), while inbred ZPL 6 had highest negative values of GCA for this trait in both years. The fact that the ZPL6 line is the best general combinator for yield, despite being the worst combiner for the number of rows of grains, is in agreement with the results of the research that came from Zarei et al (2012) and Camdzija (2014), which found poor correlation of grain yield and number of grain rows in hybrids.

As expected, the values of the GCA for the number of kernel in a row are have the same sign and high correlation with the values of GCA for ear lenght. The ZPL5 and ZPL6 lines are particularly distinguished as positive general combiners for this trait.

Conclusions

In this study inbred lines with significant GCA values for each of the analysed trait have been idetified. Inbred ZPL6 had significant GCA values for most of the wanted traits and can be considered as such as a source of desirable alleles for maize breeding programs. Interestingly, inbred line ZPL1 has emerged as the best general combiner for grain yield in the arid 2012, although its values for the same trait in 2011 were without significance. In this regard, it can be estimated that inbred ZPL1 is a carrier of the drought tolerance genes and should be included as a source material in breeding programs having for purpose the creation of drought tolerant maize hybrids. Inbred lines ZPL2 and ZPL4 having significant negative GCA values for grain yield would be of limited usability and therefore not recomended in breeding programs with a purpose of creating high yielding maize lines and hybrids. Although been great general combiner for grain yield, inbred lines ZPL5 and ZPL6 had significant positive GCA values for undesirable traits of plant and upper ear height so it would be advisable to cross them with inbred lines with significant negative GCA values for these unwanted traits. This GCA analysis provided a better insight and understanding of the inbred lines chosen in this study as breeding material, allowing a greater precision and efficiency of its use in breeding programs.

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ESTIMATION OF YIELD STABILITY OF ZP MAIZE HYBRIDS USING DIFFERENT STATISTICAL MODELS

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Abstract

Eight ZP maize hybrids, belonging to FAO 300-400 maturity groups, were tested during the 2018 year at six different environments in Serbia. All examined hybrids have been recently registered in Serbia. The aim of the study was to identify hybrids with the highest grain yield, as well as with high yield stability over different environments. The experiment was conducted according to Randomized Complete Block Design in three replications. For the estimation of yield stability, different statistical analyses were done: regression coefficient (bi), deviation from regression (S^2di), Shukla stability variance (ri^2), and cultivar superiority measure (Pi). Maize hybrids ZP 4540 and ZP 4077 showed the highest yield potential, but they also had high moisture content in harvest. Hybrid ZP 4567 had above average yield and also high stability, while hybrid ZP 3536 had average yield, good stability and the lowest moisture content in the harvest. Although hybrid ZP 4540 obtained the highest grain yield in trial, it performed unstable according to the results of stability analysis. Based on the results obtained in the experiment, hybrids ZP 4567 and ZP 3536 are recommended for further testing in 2019 and initial commercialization.

Key words: *maize hybrids, grain yield, yield stability*

Introduction

Maize is the most important field crop in Serbia. Traditionally, late maturity hybrids were dominant in maize production, but in the last decade medium-early hybrids are becoming more popular, due to better stability and lower harvest moisture content compared to late maturity hybrids. The main challenge for maize breeders is to create hybrids with high yield potential, but also with stable yield under different seasons and environments. The presence of genotype by environment interaction is of major concern for breeders, because large interaction can reduce yields and complicate identification of superior cultivars (Rasul et al., 2005). There are genotypes that have similar performance regardless of the productivity level of the environment, while there are others whose performance is directly related to the productivity potential of the environment, clearly indicating the importance of stability analysis (Babu et al., 2017, Kaya et al., 2014). Stability of expected grain yield is one of the most desirable properties, in order to recommend hybrid for use (Čvarković et al., 2009).

Multi environments trails play an important role in selecting the best cultivars to be used in future years at different locations and in assessing cultivars stability across environment before its commercial release (M.A.A. Hassan, 2015, Vargas et al., 1999).

Gauch and Zobel (1996) reported that if the number of new genotypes to be evaluated were large, five different environments would be adequate to determine different stability parameters and/or optimize the amount of GE interaction. The objective of this research was to identify hybrids with high yield potential, low moisture content in harvest and good stability.

Material and methods

Eight maize hybrids, belonging to FAO 300-400 maturity groups were tested during the 2018. year at six different locations in Serbia. Hybrids included in this trial have recently been registered in Serbia and also three check hybrids were included in trial. Trials were set up in three repetitions and each hybrid was sown in eight rows. In order to avoid the effect of border rows, only grain yield from four middle rows was used for statistical analysis. Sowing and harvesting were done mechanically. The experiment was conducted according to Randomized Complete Block Design in three replications. For the estimation of yield stability, different statistical analysis was done: Eberhart and Russell (1966) regression analysis, Shukla (1972) stability variance and cultivar superiority measure, proposed by Lin and Binns (1988). According to joint regression model developed by Eberhart and Russell (1966), a stable variety is one with a high main yield, regression coefficient close to one (bi = 1) and deviation from regression close to zero (S^2di = 0).

Shukla method (1972) is based on variance components. A genotype is stable if its stability variance (ri^2) is equal to environmental variance ri^2, which mean that ri^2= 0. A relatively larger value of ri^2 indicates higher instability, while stable genotypes are those having minimum stability variance ri^2. Lin and Binns (1988) proposed cultivar performance measure (Pi) as the mean squares of distance between genotypes i and 'i' where 'i' is the genotype with maximum response over all locations. The smaller the value of Pi, the smaller the distance to the genotype with maximum yield, the better the genotype. Pi values were measured on overall location means and it represents superiority in the sense of general adaptability (wide adaptation).

Results and discussion

Average grain yield obtained at six different locations, as well as average moisture content in harvest are shown in Table 1.

Table1: Grain yield (t/ha) and average moisture content (%) obtained at six locations in Serbia

Grain yield (t/ha) per locations								Moisture %
Hybrid	Z.Polje	Bečej	Bajša	S.Mitrovica	Pančevo	Požarevac	Average	
ZP 3536	12.81	13.97	10.89	11.72	14.28	14.54	13.04	14.4
ZP 366	12.71	13.76	9.85	10.87	14.43	14.45	12.68	18.4
ZP 4007	11.62	14.09	10.09	10.94	12.43	13.09	12.04	16.8
ZP 4073	13.12	14.15	10.36	12.22	14.24	15.22	13.22	17.6
ZP 4077	14.42	14.51	11.20	12.38	15.25	14.59	13.73	17.4
ZP 427	13.87	14.95	10.31	11.53	14.47	12.45	12.93	15.7
ZP 4540	11.42	15.42	12.22	11.91	16.26	15.23	13.74	17.3
ZP 4567	13.69	14.65	11.37	11.69	14.82	15.22	13.57	15.5
Average	12.96	14.44	10.79	11.66	14.52	14.35	13.12	16.6

Grain yield of hybrids included in this trial was in the range from 12.05 to 13.75 t/ha. The highest grain yield was observed for hybrids ZP 4540 (13.75 t/ha) and ZP 4077 (13.73 t/ha), but both hybrids had higher moisture content and they could be proposed for further testing in FAO 500 maturity group. Hybrid ZP 4567 had above average grain yield and moisture content was lower than the average, which is desirable, while ZP 3563 achieved grain yield which is slightly lower than the average of the trial, but at the same time it had the lowest moisture content in harvest. Stability parameters are presented in Table 2. In present study, the regression coefficient values (bi) ranged from 0.84 (ZP 4007) to 1.19 (ZP 366). According

to joint regression model developed by Eberhart and Russell, a stable variety is one with a high main yield and regression coefficient equals to one (bi = 1) Hybrids with the bi value close to 1 were ZP 4567 (bi = 1.02) and ZP 4073 (bi = 1,03) and those two hybrids could be considered as a most stable, according to the bi values. According to the deviation from regression values, the most stable genotypes are those with the S^2di values close to zero. In our experiment hybrids ZP 4077 and ZP 4073 could be considered as a most stable with the S^2di values 0,02 and 0,07 respectively. The most stable genotypes based on the values of Shukla stability variance were ZP 3536 (ri^2 = 0) and ZP 4567 (ri^2 = 0.06), while the most unstable were ZP 427 (ri^2 = 1.18) and ZP 4540 (ri^2 = 1.76).
Finally, values of superiority index (Pi), proposed by Lin and Binn's ranged from 0.27 (ZP 4077) to 2.95 (ZP 4007). Beside ZP 4077, which had the lowest Pi value, hybrids ZP 4567 and ZP 4540 had also low values of Pi (0.37 and 0.77 respectively).
Hybrid ZP 4567 had above average grain yield, low moisture content and performed very stable, according to the majority of used stability parameters. Those results are in accordance with the results published by Crevar et al. (2011) and Kaya et al. (2014), who also identified hybrids with high yield and good stability. On the other side, hybrid ZP 4540 had the highest grain yield, but performed very unstable. Similar results were reported by Stevanović et al. (2018).

Table 2: Values of stability parameters for investigated hybrids

Hybrid	bi	Rank	S^2di	Rank	ri^2	Rank	Pi	Rank
ZP 3536	0.92	5	-0.22	6	0.00	1	0.94	5
ZP 366	1.19	8	-0.18	5	0.15	3	1.46	7
ZP 4007	0.84	7	0.12	3	0.43	6	2.95	8
ZP 4073	1.03	2	0.07	2	0.28	5	0.90	4
ZP 4077	0.93	4	0.02	1	0.24	4	0.27	1
ZP 427	0.96	3	0.91	7	1.18	7	1.32	6
ZP 4540	1.10	6	1.42	8	1.76	8	0.77	3
ZP 4567	1.02	1	-0.15	4	0.06	2	0.37	2

Conclusions

Hybrids ZP 4540 and ZP 4077 had the highest grain yield in trial, but at the same time both of them had higher moisture content in harvest, so they could be proposed for further testing in FAO 500 maturity group. Although hybrid ZP 4540 obtained the highest grain yield in trial, it performed unstable according to the results of stability analysis.
Taking into account obtained grain yield as well as harvest moisture content, hybrid ZP 4567 is the most desirable genotype for further testing in FAO 400 maturity group. It also showed very good stability estimated by different stability parameters.
Hybrid ZP 3536 had average grain yield, but the lowest harvest moisture content and good stability. Based on obtained results, it can be proposed for further testing in FAO 300 maturity group.

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EFFECT OF DIFFERENT SIZES OF (PRE BASIC SEEDS) MINITUBER ON PLANT GROWTH AND SEED YIELD OF POTATO

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Abstract

Potato (*Solanum tuberosum*) is one of the major stable foods in the world. Worldwide, potato minitubers are used at initial stage of seed multiplication. Minitubers are the progeny tubers produced from *in-vitro* derived plantlets. Field performance of different size minituber can vary and it is important to plant optimum size minituber in order to get higher seed yield. Hence, a study was conducted at Agriculture Research and Development Centre, Sita-Eliya to investigate the effect of different sizes of potato minituber on yield and growth performance. Potato variety granola was used and four grades of potato minituber (<10 mm, 10-15 mm, 15-20 mm, >20 mm) were taken for the study. Plant emergence percentage, number of main stems and plant height were measured as growth parameters and there was no significant effect ($p<0.05$) observed in plant emergence with different sizes of mintubers. But, a significant influence on number of main stems due to different minituber size was recorded. Plant height was also, significantly affected by minituber size. Total yield was also, significantly affected by planted minituber size. But, seed tuber yield (28 - 55mm) was not significantly affected by different minituber sizes. It was found that mini tuber size >10 mm was most suitable for the field planting in order to obtain high seed yield.

Keywords: *Different size, Minituber, Potato.*

Introduction

Potato (*Solanum tuberosum*) plays significant role in developing countries with its high yield potential and high nutritional productivity compared to rice, wheat and maize. Potato is introduced to Sri Lanka in 1850's by the British rulers and at present successfully cultivated in Nuwara-Eliya, Badulla and Jaffna Districts. Average potato productivity in 2017 is 16.46 t/ha in Sri Lanka (Agstat, 2018). Non availability of good quality disease free seeds of desirable varieties at the correct time for planting is considered as the main constraint for increasing productivity of potatoes in Sri Lanka (Nugaliyadde *et al*., 2005). In 2017, 151,438 tons of potatoes were imported for consumption at a cost of Rs million 5,440,129 (Agstat, 2018). This emphasizes the importance of increasing local potato production and productivity. Potato cultivation still depends on imported seed potatoes which amount to about 1611 t in 2017. The total annual seed potato requirement in Sri Lanka is about 16,000 tons and out of which 750-1000 t/year seed is produced formally. Rest of the seed potato requirement of about 80% is met by farmers own seed production. Since the quality of farmer produced seed is not assured, productivity is also low. Thus there is a high potential for increasing productivity of potato with high quality seeds. It was realized that the quality of farmer produced seed should be increased to improve the productivity. *In-vitro* mother plant production, rapid multiplication and production of pre basic seeds (mini tubers / G_0) through aeroponic, hydroponic and geoponic are the main consecutive steps of potato seed production techniques. Minitubers are the progeny tuber produced by *in-vitro* derived plantlets (Struik, 2007). Repeated harvesting method is practiced under these three technologies, in order to maximize the G_0 production. During harvesting different sizes of minitubers are harvested.

The size of minitubers may range from 5-25 mm and sometime it grows even bigger. Larger size minituber also demanded by farmers.
Even though sufficient information is available on the performance of minitubers under protected houses, a little information exists on the field performance of minitubers of potato. A massive programe is launched in Nuwara-Eliya and Baddulla districts to improve the seed quality produced by the farmers in order to achieve higher productivity and intern lower the cost of production. It has been realized that suitable size of minitubers should be identified to streamline the field multiplication of G_0 tubers. This study is therefore intended to evaluate the effect of different sizes minituber on growth and yield of potato under field condition and identify the best size of minituber for increasing the seed tuber yield of potato.

Materials and methods

This study was conducted at the Agriculture Research and Development Centre Sita-Eliya, Sri Lanka in the up country wet zone during 2015 *yala* and 2016 *yala*. Potato minituber seeds were divided in to four different diameter classes and tested in this experiment as T_1 seed diameter below 10 mm, T_2:10 -15mm, T_3:15-20 mm and T_4:20-25 mm. Treatments were arranged in a randomized complete block design and replicated three times during two seasons.
Before planting pre basic seeds of potato variety Granola was harvested and stored for 3 and 1/2 months for sprouting. Uniformly sprouted seeds with 3-4 sprouts were selected and planted in plots (2.25 m^2) contained 50 plants at the spacing of 30‹15 cm. All crop management practices were performed as per the DOA recommendations. Number of plant emerged at 20 days after planting, number of main stems and plant height at 30 days after planting was recorded. Harvesting was done at physiological maturity i.e. 105 days after planting and number of tubers/m^2 and tuber yield/m^2 were recorded. Harvested yield were graded according to seed diameter (i.e. <28 mm, 28-55 mm and >55 mm) and seed weight and seed number were recorded.
The analysis of variance and mean separation were done for the experimental data using SAS statistical package.

Results and discussion

Plant emerge percentage

There was no significant difference ($p<0.05$) in plant emerging percentage among mini tuber sizes at 20 days after planting (DAP) (Table 01). Dimante and Gaile (2015) also reported that minituber size had no effect on emergence. In contrary, Hossain *et al.*, (2015) and Kawakami and Iwama (2012) reported that minituber size had significant effect on plant emergence.

Number of main stems

There was a clear effect of minituber size on number of main stems. With increasing of minituber size, number of main stems was also increased (Table 1). This is due to the presence of more eyes and reserve food in large size tuber and it help for rapid growth of stems. Dimante and Gaile (2015) also reported that main stems depend on both cultivar and mini tuber size.

Plant height

Plant height also significantly higher in bigger tubers than that of small tubers except in 2015 *yala* (Table 01).This finding also is in agreement with the findings of Hossain *et al*. (2015). This also could be attributed to presences of more reserve food in tubers, which support rapid growth of plant during initial stages.

Tuber yield (Kg /m^2) and tuber number (m^2)

The maximum tuber yield was recorded from the 15 -20 mm size minituber during 2015 *yala*. But, during 2016 *yala* maximum yield was obtained from the >20 mm size minituber. During two seasons, the lowest yield was recorded in < 10 mm seed size (Table 2). However there was no significant difference observed between seed size of 15-20 mm and of >20 mm (Table 04).

Table 1: Percentage of plant emergence 20 DAP, number of main stems at 30 DAP and plant height at 30 DAP in potato plants grown in different size minitubers.

Treatments	**2015 *Yala***			**2016 *Yala***		
	Plant emerge %	**No of main stem**	**Plant Height (cm)**	**Plant emerge %**	**No of main stem**	**Plant Height (cm)**
<10 mm	82 a	1.2 c	15.3 a	97.3 a	1.5 b	11.0 b
10-15 mm	86.6 a	1.4 b	20.0 a	98.6 a	1.4 b	12.7 b
15-20 mm	84.6 a	1.5 b	20.6 a	92.6 a	1.5 b	12.3 b
>20 mm	86.6 a	1.9 a	18.8 a	94.0 a	2.2 a	15.9 a
CV	5.5	5.2	17.7	3.3	11.3	8.7

Table 2: Total yield of different sizes minituber in 2015 *yala* and 2016 *yala*

Treatments	**Total G_1 Seed Yield (Kg/m^2)**	
	2015 *Yala*	**2016 *Yala***
<10 mm	3.26 b	5.23 c
10-15 mm	3.56 ab	5.73 bc
15-20 mm	4.13 a	6.6 ab
>20 mm	3.76 ab	7.4 a
CV	10.8	10.4

Table 3: Total tuber number of different sizes minituber in 2015 *yala* and 2016 *yala*

Treatments	**Total Tuber Numbers/m^2**	
	2015 *Yala*	**2016 *Yala***
<10 mm	88 a	112 a
10-15 mm	95 a	119 a
15-20 mm	86 a	126 a
>20 mm	84 a	122 a
CV	7.8	19.3

Table 4: Graded yield according to the recommended tuber size of different sizes minituber in 2015 *yala* and 2016 *yala*

Treatments	**2015 *Yala* (Kg/m^2)**			**2016 *Yala* (Kg/m^2)**		
	<28 mm	**28-55 mm**	**>55 mm**	**<28 mm**	**28-55 mm**	**>55 mm**
<10 mm	0.17 ab	2.76 a	0.36 a	0.08 a	5.01 a	0.15 b
10-15 mm	0.19 a	3.13 a	0.23 a	0.14 a	5.03 a	0.55 b
15-20 mm	0.11 b	3.16 a	0.88 a	0.08 a	5.19 a	1.31 a
>20 mm	0.11 b	2.86 a	0.78 a	0.09 a	5.64 a	1.68 a
CV	21.7	10.1	57.6	47.6	9.7	29.1

Table 5: Graded tuber number according to the recommended tuber size of different sizes minituber in 2015 *yala* and 2016 *yala*

Treatments	**2015 *Yala* (Tuber No/m^2)**			**2016 *Yala* (Tuber No/m^2)**		
	<28 mm	**28-55 mm**	**>55 mm**	**<28 mm**	**28-55 mm**	**>55 mm**
<10 mm	24 ab	61 a	3 ab	16 a	96 a	1 b
10-15 mm	27 a	67 a	2 b	16 a	99 a	4 b
15-20 mm	17 b	63 a	6 a	11 a	106 a	9 a
>20 mm	19 ab	61 a	5 ab	13 a	98 a	11 a
CV	19.2	6.7	47.5	50.5	18.6	35.3

The result shows, that there was no significant difference on tuber numbers during 2015 *yala* and 2016 *yala*. With increasing size of minituber, number of tubers wasn't shown clear increasement during 2015 *yala* and 2016 *yala* (Table 3).
Mahmoudpour (2014) reported that the size of minituber has an impact on tuber yield, but not in the tuber number. However, Hossain *et al*. (2015) observed that minituber size and planting distance interacted significantly on tuber weight and tuber number.
Highest seed size category (28-55mm) was recorded from minituber of 10-15 mm size planted during 2015 *yala* seasons, whereas during 2016 *yala* season more seed size tubers were harvested from mini tubers diameter 15-20 mm and there was no significant differences (Table 5). Dimante and Gaile, (2015) also found tuber yield at >25 mm was significantly affected only by the cultivars, but not by seed size.

Conclusions

It was found that mini tuber size had a positive effect on tuber yield, tuber number, number of main stem and plant height. However, mini tuber size did not influence the amount of seed size tubers produced in the next progeny. Considering the growth parameters and seed yield G_0 minitubers of size >10 mm are most suitable for open field multiplication.

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INFLUENCE OF SOIL MOISTURE REGIME ON ALLUVIAL FOREST HABITAT

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Abstract

At the mouth of the Tamis into the Danube there is a complex of alluvial forests that represents a separate ecological unit within the Managment unit „Donje Potamišje". In unchanged natural conditions, there were favorable ecological conditions for the development of forest vegetation, defined by the periodically high water levels of the Danube and Tamis, as well as moistening of groundwater. The construction of Hydropower plant "Djerdap" changes the regime of the Danube and Tamis waters, which implies the process of degradation of land and vegetation, that is, the formation of ponds and wetlands on the entire surface of the forest management unit. By raising the embankment towards the Danube and Tamis, as well as by building ameliorative canals and evacuating excess water, more favorable ecological conditions for the development of forest vegetation were created. In addition, the area, called "Gradska Suma", is used as a source for the city's water supply, which affects groundwater levels, whose impact on forest vegetation should not be neglected.

Keywords: *hygrophilic forests, habitat, ecological unit, water regime, forest and swamp ecosystems, forest management class, levees, melioration*

Introduction

Spacious alluvium at the mouth of the Tamis into the Danube, edged with earth embankments, which protect it from the large waters of these rivers and it represents a special ecological unit on the territory of the municipality of Pancevo (Serbia). This area, designated as "City Forest" - GJ "Donje Potamišje" under the unchanged conditions of the humidification regime, which was occasionally flooded by large waters of the Tamis and the Danube, represented, under the existing climate and soil conditions, the natural habitat of hygrophilic species. The construction of HPP "Djerdap" in 1972 created a slowdown in the Danube and even the Tamis, which floods the alluvial plain for most of the year and causes the existing habitat of forest species to decay, turning it into a swampy and grazed area. This area is inhabited by hydrophilic species, that is, represents the habitat of numerous species of swamp birds and other aquatic organisms, which excludes it from forest production by decaying of hygrophilous forests. In addition, one part of the city of Pancevo has the need for drinking water from the aquifer from the site. Protective embankments, built since 1998, create special environmental conditions with controlled humidity. In the conditions of the so-called melioration section, floods are excluded, and the precipitation water is collected and transferred to the Tamis by constructed pumping system and a system of reclamation canals. The aim of this paper is to analyze the changes of habitat characteristics, conditioned by changing the basic ecological factor and humidification regime to establish the possibilities of biomass production in accordance with the use of water resources of the source "City Forest".

Material and methods

A number of different methods were used in the preparation of this paper, based on the available database of which:

- Descriptive method, used to familiarize with the basic characteristics of the ''Gradske Sume'' area, the potentials and limiting factors of the natural environment for the use of water and forest resources, as well as the mutual influence of water and forest vegetation;
- analysis of data collected using available literature, planning documents, scientific and professional papers, made it possible to explore and identify key elements of the use of available resources of the ''Gradske Sume''.
- The synthesis method was used to draw an appropriate conclusion based on the descriptions and analyzespreviouslyused.

The analysis will use the data on the forest management units for characteristic periods of changes in the habitat conditions of the study area: natural conditions (1953), the period of influence of the Danube slowdown (1983) and controlled wetting regime (2015), which is achieved by protective embankments and drainage network channels. The area of research of the Donje Potamišje Unit is mainly located on the left bank of the Danube and is a homogeneous spatial unit that extends along a river flow, with various widths, from several hundred meters to 2.5 km. Part of the management unit ''Gradske Sume'' has been separated into a separate ecological unit, due to the erection of a protective embankment near Tamis, as this area is no longer flooded.

Figure 1. View of the ecological unit "Gradska Suma", GJ "Donje Potamišje", Forestry Holding "Banat" Pancevo

The ecological unit "Gradska Suma" covers an area of 348.55 ha, bordered on the north by an old protective embankment, from the bridge over Tamis to the embankment, which connects the old road with the new E-94, on the south side by the embankment on which the E-94 road lies and from the east side with the right coast of Tamis, Figure 1. The terrain of the ecological whole, "Gradske Sume", is mostly flat with characteristic elevations, beams, along the bed of old Tamis, Sibnica and Tamis itself. The highest elevation in the area of the City Forest is 74.5 m (Lugarnica), the lowest is 69.0 m, the bed of old Tamis and the pits along the old Pancevo - Belgrade road. Analyzing the relief by altitude zones, it can be seen that the largest surface area between the 70.0 and 70.5 m elevation is about 142 ha, the smallest area of only 2.0 ha is in the height zone above 73.5 m. Altitude zones that still stand out are: zone with an altitude below 70.0 m, with an area of 87 ha and an altitude zone from 70.5 m to 71.0 m, with an area of 49 ha. The hydrographic network of the study area is primarily represented by the Danube and Tamis Rivers, which, as typically plain rivers, have a large number of branches, meanders, sections of old streams and still waters. In addition to these two rivers, smaller tributaries of the Danube such as Sibnica, Galovica and Nadela, as well as a number of melioration canals should be noted.

Table 1. Average monthly and annual values of the Danube water level H (cm) Water meter station: Pancevo elevation "0" 67.33 (according to RHMZ, Belgrade)

year	I	I	III	IV	V	VI	VII	VII	IX	X	XI	XII	mid
2006	391	321	497	710	577	535	346	318	309	254	276	267	400.1
2007	317	377	419	337	299	295	276	251	333	298	381	402	332.1
2008	331	313	403	439	388	359	320	327	259	261	275	399	339.5
2009	325	407	455	492	373	344	426	302	273	265	321	334	359.7
2010	492	370	488	443	441	615	444	389	366	352	372	522	441.2
2011	485	376	349	331	296	300	293	306	246	260	241	262	312.1
2012	293	314	354	378	381	373	293	261	266	270	335	328	320.5
2013	386	426	499	578	495	487	332	246	276	287	342	309	388.6
2014	303	358	367	340	514	369	315	378	455	382	410	390	381.7
2015	396	414	427	394	320	332	272	251	243	299	273	277	324.8
mid	**372**	**368**	**426**	**444**	**408**	**401**	**332**	**303**	**303**	**293**	**323**	**349**	**360.0**
Max	**492**	**426**	**499**	**710**	**577**	**615**	**444**	**389**	**455**	**382**	**410**	**522**	**441.2**
Min	**293**	**313**	**349**	**331**	**296**	**295**	**272**	**246**	**243**	**254**	**241**	**262**	**312.1**

An analysis of the land profiles in the "Gradska Suma" area revealed the remains of the Chernozem that are typical of this area. The presence of sand and alluvium indicates the same origin of these lands with the formations of the large belt of the Danube coast. The fact that the Danube and Tamis flooded this terrain at high water levels and at low water drained both surface and groundwater, which was very suitable for growing soft and hard deciduous trees. The application of fertile sludge brought about by each flood wave had very positive effects on the processes of pedogenesis and forestry.

Table 2. Distribution of land types in the area of "Gradska Suma" before the rise of the Danube ("Energoprojekt", Belgrade, 1983)

Types-subtypes of soil	**Area**	
	ha	**%**
Chernozem	48.99	14.09
Swamp-gley soil-epigley	11.50	3.3
Swamp-gley soil on chernozem	117.35	33.76
Hydromorphic (gley) alluvium	4.25	1.2
Hydromorphic (gley) alluvium on chernozem	157.02	45.17
The unexplored surface of the Tamish meander	8.52	2.45

According to the European classification (FAO-UNESCO), Škorić et al., (1985), the following types of land have been identified in the surveyed area of the Gradska Suma according to the European classification (Table 2).

Results and discussion

According to the available data on the isolated forest management classes for characteristic periods of time, the area of the ecosystem under study can be observed, "Gradske Sume", their very uneven representation, table 3, diagram 1. According to these data the following forest management classes (gk) are separated: AB-Euro-American Poplar, Black and White Poplar and American Ash; C-white willow, black and white poplar and American ash; D-American ash and oak; E-gullies (1983 only) and G-forest openings and paths.

During the period of natural humidity conditions (1953), the area under study is dominated by forest management class C, which comprises 60.18% (from Salicetum albae to Populeto-Fraxinetum angustifoliae), and forest clearings and paths are the least represented, forest management class G with 6.40% of total area, Table 3, Diagram 1, Figure 2.

The period of the impact of the Danube slowdown (1983) on the surveyed areas of the Gradske Sume is characterized by a drastic decrease in the areas under forest and an increase in forest clearings, forest management class G at 53.77% (with predominant aquatic and swamp vegetation) and isolation of special forest management class E, thinning out of old forest, which increased the woodless level by an additional 3.51%, Figure 3.

Table 3. Share of forest management classes in ecological unit "Gradska Suma" of GJ "Donje Potamišje" (Matijević, 2016)

FOREST MANAGEMENTCLASS	PERIOD OF TIME					
	1953		1983		2015	
	ha	%	ha	%	ha	%
AB	52,43	15,50	36,56	10,81	188,01	55,59
C	203,52	60,18	34,33	10,15	14,17	4,19
D	60,60	17,92	73,57	21,75	99,17	29,32
E	0,00	0,00	11,87	3,51	0,00	0,00
G	21,63	6,40	181,85	53,77	36,83	10,89
Σ	338,18	100	338,18	100	338,18	100

Institute of Topology of Novi Sad, (1976), in the newly created habitats of "Gradske Sume", along with the channel network, vegetation units of Salicetum triandrae and Salicetum albae will survive; on the Chernozem and swamp gley areas, the sets of pedunculate and field ash will be expanded; deep sandy soils will be occupied by white poplar and European oak, and the sets of peduncle and hornbeam will replace the sets of black poplar and oak with fluvisols and humofluvisols, Figure 4.

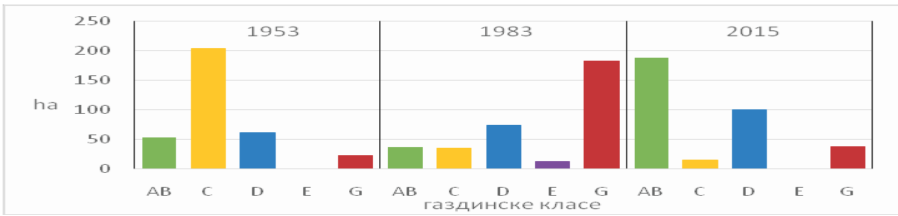

Diagram 1. Separate forest management classes in the ecological unit "Gradska Suma" GJ "Donje Potamišje"

The controlled wetland period (2015) of the eco-unit under study (protected from external and inland waters) is characterized by an increase in areas with forest management class AB at 55.59%, as well as a significant decrease in forest management class G to only 10.89% with the absence of forest management class E. According to the available data (Table 3, Diagram 1), there is a significant increase in the area of forest management class D, non-coniferous wood species, which ranges from 17.92% in natural conditions to 29.32% in controlled humidity conditions, is especially emphasized, which is confirmed by the above forecasts of the Institute in Novi Sad. The ecological conditions of the ''Gradske Sume'' were dictated by the water regimes of the Danube and Tamis, as the area is influenced by these rivers and was exposed to flooding and wetting of groundwater. Depending on the relief, or hydrographic position, soil moisture in this area was different and directly depended on the water level, its duration and frequency. According to the available data, before the construction of HPP "Djerdap", the Danube level ranged between 66.5 m and 73.9 m above sea level, while groundwater oscillation amplitude ranged from 2.0 to 3.5 m. The existence of favorable ecological conditions (Herpka, I., 1979) is confirmed by isolated forest management classes where the indigenous vegetation of the field, as well as artificially raised plantations occupy about 94% of the area of the ''Gradske Sume''.

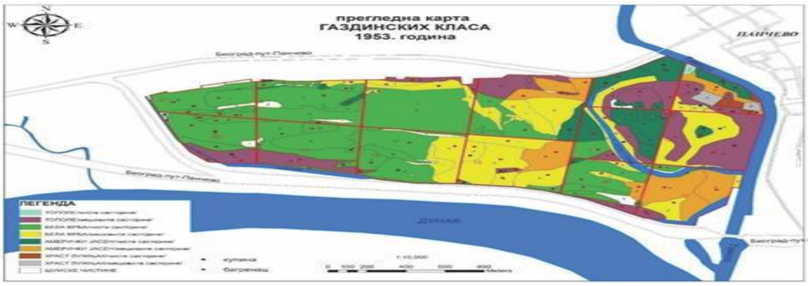

Figure 2. Overview map of the forest management classes of the "Gradska Suma" in 1953 - Natural Conditions ("Energoprojekt", Belgrade, 1984)

Figure 3. Overview map of the forest management classes of "Gradska Suma" in 1983. - Period of influence of slowdown ("Energoprojekt", Belgrade, 1984)

Changing the regime of the Danube waters, after the construction of HPP "Djerdap", in this area, the regime of soil moisture as well as the intensity of wetting was changed, which led to permanent appearance of ponds and swamps. As a result of the pronounced ecological changes (the impact of the basic ecological factor changed), land degradation occurred and forests were prevented from restoration as well as any kind of forest economic activity. Separate forest management classes indicate numerically the previously exposed ones, so that over 50% of the area is covered with clearings and paths.

Hydrological indicators of the flow regime of the Danube in slow conditions indicate significant changes, the most important of which are in small waters and the amplitudes of their oscillations. In the 68/63 m low water regime, they increased by an average of 2.0 m, especially in the period July - September, did not go below 68.5 m. In the 69.5 / 63 m regime, small waters were 3-3.5 m higher than in natural conditions (Energoprojekt, 1984). Median waters also increased, more in the vegetation than in the non-vegetation period, with the regimes for both periods approaching each other. The mean waters for the regime of 69.5 / 63 m were at an elevation of 71.1 m above sea level. All regimes have acquired characteristics of lake - river regimes (Nikolić, V. et al., 2012).

Figure 4. Overview map of the "Gradske Sume" forest management classes in 2015

The large waters of the Danube and Tamis previously flooded the study area, until defense embankments were built along these rivers and a canal system in the ''Gradske Sume''. The construction of the canal system drained many ponds and wetlands. The water regime in them depends on the height of the atmospheric precipitate as well as the water level of the surface currents. Rainfall is particularly dominant in June and July. During such periods, these channels accept excess water and from the water to the pumping station, which transfers to Tamis. The controlled soil moisture regime provides more favorable ecological conditions for cultivation of both the softwood species and the extension of the surface of forest management class AB and hardwood ones, as indicated by the increase in forest management class D, (Matijevic, 2016).

Conclusions

The natural conditions of the alluvial surfaces at the mouth of the Tamis into the Danube are established by the wetting regime, characterized by the appearance of flood-meadows in the first half of the vegetation period and their withdrawal in the second part of the period. Such a regime of wetting, depending on the position and type of soil, establishes an adequate vegetation of hygrophilic species, soft and hard deciduous forest, as indicated by the layout and participation of the separated forest management classes with dominant C class.

The impact of anthropogenic factors is hampered by the natural regime of surface and groundwater (slowdown by HPP "Djerdap" since 1983, "Nera" regime 69.5 m and more), which leads to degradation and decay of forest habitats and the creation of ponds and wetlands. Deforestation and degradation of forest habitats defines the relationship of forest management class G, especially singled out E, whose participation exceeds the other forest management classes;

Improved ecological conditions for the cultivation of forest species are achieved by protection against external and internal waters, that is, by controlled soil moisture in the analyzed area of the ''Gradska Suma''. Larger participation, over half the surface, separated by forest management class AB, as well as a significant increase in forest management class D, refers to the process of raising the production capabilities of the habitat of the analyzed area;

Excessive pumping of water for the purposes of water supply has negative consequences, reducing groundwater level below the reference level, which in specific conditions is 1.5 to 2.0 m;

The prognosis of cultivation of hygroviline species, softwoods and hardwoods in the area of such ecological units, is achieved by providing optimal conditions for their cultivation. Under current conditions, these are mainly habitats: A-white willows, B-white and black poplars and C-field ash and European oaks on different types of land.

In general, the land of the ''Gradske Sume'', despite its degradation forms, is a solid basis for the cultivation of characteristic forests of inundation. Although the pedogenetic processes in such land melioration areas differ from the flood-meadow zones of the alluvial planes, where the precipitated sludge allows optimal biomass production in short time cycles;

It can be generally stated that forest vegetation in the "Gradska Suma" area has a production function (production of biomass) and a protective function of forests (special purposes), which is for the time being rare to use water and wood from the same area as a resource.

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EFFECT OF NITRIC OXIDE (NO) APPLICATION ON THE DEVELOPMENT OF PEPPER PLANT UNDER DROUGHT STRESS

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Abstract
The study was carried out in the Physiology Laboratory, Faculty of Agriculture, Van Yuzuncu Yıl University, in a climate room where normal atmosphere was provided. The main purpose of the normal atmosphere in the experiment was to ensure that salt stress effects occur under normal conditions. The study was carried out under controlled conditions in a light / dark photoperiod of 16/8 hours, climate room of 25^{o}C and 70% humidity. The aim of the study was to determine the possible role of NO (nitric oxide) as a precursor molecule in some metabolic changes occuring under the effect of drought stress in plants and to try to determine its effects on plant growth. Demre pepper variety was used as a test material. Tested plants in the controlled climate were cultured in cups containing Hoagland nutrient solution. For drought stress application, 10% polyethylene glycol (PEG 6000), equivalent to the osmotic potential of -0,40 MPa, was added to the nutrient solution. Different doses of sodium nitroprusside (SNP) and potassium salt (carboxy-PTIO) (SNP 0,01, SNP 1, SNP 100 and SNP 0,01 + cPTIO, SNP 1 + cPTIO, SNP 100+ cPTIO) were applied to the pepper seedling before drought stress was applied. On the 10th day of drought application, the root, stem and leaf weights, leaf counts and inter-node distances of the plants were examined as growth parameters. In terms of plant growth parameters, it was determined that the growth and growth of plants pretreated with 0.01 and 1 doses of SNP were better.

Keywords: *Capsicum annum, Carboxy-PTIO, Drought stress, Nitric oxide, SNP*

Introduction
One of the abiotic stress conditions affecting the plant growth and development is drought (Farooq *et al.,* 2009; Yasar *et al.,* 2013; Yasar *et al.,* 2014). This change in growth depends on the duration and severity of the drought. In the early stages of arid conditions, the plant slows the body's elongation and triggers root development to reach more water. On the contrary, if arid conditions last long enough to cause damage to the plant, both stem and root growth will be halted, leaf area and number of leaves will decrease and even some leaves will be poured out with yellowing (Ozpay, 2008; Kose, 2010; Yasar *et al.,* 2013; Yasar *et al.,* 2014). The decline in plant growth is due to the division of cells in the shoot and root meristems and the arrest of the expansion of the cells. The disruption of cell division or enlargement is directly related to the decrease in the rate of photosynthesis due to water insufficiency (Anjum *et al.,* 2011).
In plants, nitric oxide (NO) is a messenger molecule in another of the metabolic changes that take place under the effect of drought stress. NO is a colorless inorganic molecule, consisting of a nitrogen and an oxygen atom, which is lipophilic, gaseous, easily diffusible without a receptor-dependent, very short half-life, containing an unpaired electron, also called free radical (Olson, 2011).
NO is synthesized and released in small amounts in plant cells under normal conditions. In plants, NO is synthesized by two different enzymatic and non-enzymatic metabolic pathways. The NO synthesis varies depending on the plant species, the tissue, and the growing conditions. The NO production site in plant cells is the cytosol, nucleus, peroxisome matrix

and chloroplasts (Barroso *et al.,* 1999 and Pedroso *et al.,* 2000). At the same time, non-enzymatic processes also play a role in NO formation in plants.
In acidic or light media, NO2 can be converted to NO (Cooney *et al.,* 1994). Nitric oxide is an important signaling molecule with a variety of physiological functions in plants. It is thought that the plants play an important role in the growth and development of the fruits from the seed to the flowering stage. In addition, NO can be produced in different plant species and organs in case of environmental stress caused by abiotic and biotic factors. Nitric oxide is a very active molecule that has been proven to protect plants against a variety of biological pathways to the damage caused by oxidative stress conditions (Carlos and Lorenzo 2001).

The main objective of this work was to identify possible complementary roles in plant development of NO with the messenger molecule property in some metabolic changes that occur under the influence of drought stress in plants.

Material and Methods

Material

Plant material

Demre's pepper varieties with strong root structure and resistant to diseases are used in the study.

Method

Pepper (*Capsicum annuum*) Seedlings Growing:
The study was carried out in the Physiology Laboratory, Faculty of Agriculture, Van Yuzuncu Yıl University, in a climate room where normal atmosphere was provided. Pepper seeds were germinated in pumice-filled plastic germination containers in a climate-controlled chamber (split conditioning system, 25 ± 1° C, and 70% humidity). The seedlings that began to show their first true leaves began to be made with Hoagland nutrient solution (Hoagland and Arnon, 1938). In the ponza environment, the seedlings pretreatment process, which consists of the second true leaves, was carried out. This was done with sodium nitroprusside (SNP) and nitric oxide (NO) capture 1 µM cPTIO in Nitric oxide (NO) buffer at concentrations of 0.01, 1, 100 µM in ½ Hoagland solution for 2 days in brown bottles. Later, pre-treated and untreated seedlings were placed in aquaculture environment. Sprouted seedlings were transferred at the second true leaf stage to a water culture system consisting of 25 × 25 × 18 cm plastic baths containing Hoagland nutrient solution. The pepper seedlings were wrapped with small foam pieces and placed on specially made plastic trays, fitted with holes to accommodate each seedling. The trays were placed in the baths, with the plant roots immersed in the nutrient solution. The nutrient solution was aerated by thin plastic hoses attached to an aquarium pump. Drought stress was applied by adding 10% Poly Ethylene Glycol (PEG 6000). The implemented applications are shown in Table 1.

Table 1. Experimental practices

1-Application: ½ Hoagland (Control group)
2-Application: ½ Hoagland + 10% Poly Ethylene Glycol (PEG 6000)
3-Application: ½ Hoagland + 0.01 µM SNP + 10% Poly Ethylene Glycol (PEG 6000)
4-Application: ½ Hoagland + 1 µM SNP + 10% Poly Ethylene Glycol (PEG 6000)
5-Application: ½ Hoagland +100 µM SNP + 10% Poly Ethylene Glycol (PEG 6000)
6-Application: ½ Hoagland + 1 µM C-PTIO + 0.01 µM SNP +10% Poly Ethylene Glycol (PEG 6000)
7-Application: ½ Hoagland + 1 µM C-PTIO + 1 µM SNP + 10% Poly Ethylene Glycol (PEG 6000)
8-Application: ½ Hoagland + 1 µM C-PTIO + 100 µM SNP + 10% Poly Ethylene Glycol (PEG 6000)

Samples were taken from the plants harvested on the tenth day after the application of the drought. Root, stem and leaf weights, leaf counts and inter-node distances were looked at as growth parameters of the plants.

The experiment was designed as a completely ran-domized plot with three replicates. Data were analyzed statistically, and the means of each treatment were analyzed by Duncan's multiple range test using SAS software (Sas-Instıtue,1985).

Results and Discussion

Responses to drought stress in pest plants subjected to drought stress with PEG 6000, and in plants pre-applied with SNP and c PTIO were also investigated (Table 2).

Table 2. Root weight (g), stem weight (g), leaf weight (g), number of leaves (number), plant height (cm), distance between plant nodes (cm).

Application	Rot. W.	Stem.W.	Leaf.W.	Leaf.Num.	P. Leng.	D.B.P.N.
CONTROL	4,43 B	4,34 BC	15,11 A	14,66 B	22,00 A	2,16 BC
PEG	4,29BC	3,09 DE	9,60 C	10,66 C	18,33BC	2,67 AB
SNP 0,01+ PEG	4,44 B	4,84 AB	14,51 A	16,33 A	19,83 B	2,83 A
SNP 1+ PEG	5,54 A	5,07 A	12,48 B	13,67 B	18,67BC	2,16 BC
SNP 100+ PEG	3,34 D	2,66 E	9,98 C	10,33 C	15,67 E	2,16 BC
C.PTIO+SNP 0,01+ PEG	3,81CD	3,75 CD	11,35 B	11,00 C	18,00CD	2,33 BC
C.PTIO+SNP 1+ PEG	3,33 D	2,51 E	9,64 C	10,66 C	16,67DE	2,00 C
C.PTIO+SNP 100+ PEG	2,65 E	1,60 F	5,52 D	8,33 D	15,67 E	1,83 C

The difference between the averages with the same capital letter in the same column is insignificant compared to $P \leq 0.05$.

When we examined root weights of plants as a result of application of PEG 6000 to pepper plants, it was seen that there were differences between applications. In the case of SNP 1 application, root weight increased compared to control, while C. PTIO + SNP100, C. PTIO + SNP1 and SNP 100 decreased compared to control. PEG and SNP 0,01 were found in the same statistical group interval as the control. In terms of body weights, it is seen that there were differences between applications. In the case of SNP 1 and SNP 0,01 application, body weight was increased compared to the control, while C.PTIO + SNP100, C.PTIO + SNP1 and SNP 100 decreased compared to the control. If SNP is 0,01, the same statistic was found in control group. When we examined leaf weights of plants, it was observed that there were differences between applications and there was no application which shows an increase compared to leaf weight control. C. PTIO + SNP100, C. PTIO + SNP1 and PEG decreased with respect to the control. If the SNP is 0.01, the same statistic is found in the group interval (Table 2).

When we examined leaf counts of plants as a result of applying PEG 6000 to pepper plants, it was seen that there were differences between applications. In the application of SNP 0.01, leaf numbers increased with respect to the control, while C. PTIO + SNP100, SNP 100, C. PTIO + SNP1 and PEG decreased with respect to the control. SNP 1 was found in the same statistical group interval as the control. When we examined the size of the plants, it was seen that there were differences between the applications. There has not been any application in terms of plant height, which is higher than the control. SNP100, C. PTIO + SNP100 and C. PTIO + SNP1 were decreased compared to the control. SNP 0.01, SNP1 and PEG were found in the same statistical group interval. It is seen that there are differences between applications at the intersection points of plants. SNP 0.01 and PEG, SNP100 and C.PTIO + SNP1 were decreased compared to controls. SNP1, SNP 100 and C.PTIO + SNP0.01 were found in the same statistical group interval as the control (Table 2).

Doses of 0.01 and 1 μM of SNP and c.PTIO + SNP pre-treatments of root, stem and leaf weight of the growth and development parameters of the pepper plants pretreated with SNP and c-PTIO at different doses were in the same range as the control plants without drought It has become. Much better than PEG treatment without pretreatment with SNP and c.PTIO +

SNP. As the SNP and cPT + SNP did not have a positive effect of 100 μM, it was seen that the plants got more stressed compared to the PEG without pretreatment. The same cases were also observed in terms of number of leaves, plant height and inter-node spacing. Sekmen *et al.,* (2005) found that plants subjected to pretreatment by salt stress on tomato plant showed increased root and stem weights and lengths on day 28 compared to plants treated with salt stress without pretreatment. On the 43rd day of stress, however, pretreatment lost its effect and plants gave the same response as plants without pretreatment. Likewise, Tuna and Eroglu (2017) applied NO pretreatment to pepper plants under salt stress and looked at stem root and leaf weights of plants under stress. While the root, stem and leaf growth of the plants were decreased compared to the control, they were found to be better than the salt treatment without pretreatment. Many researchers who have done similar work in our study have achieved similar results. Erkılıç, (2005) also found that exogenous salicylic acid applied to salt stressed pepper plants had a positive effect on some growth parameters such as wet and dry weight. Kausar *et al.,* (2013) reported that nitric oxide pretreatment of wheat (Triticum aestivum) plants subjected to salt stress affects the growth and yield of plants in a positive way.

In a different study by Tian and Lei (2006) the effects of SNP, a NO donor, on drought stress induced by 15% PEG in wheatgrass were investigated. Whereas 0.2 mM SNP administration increased the growth of shoots and provided high water content. In addition, investigators have reported that oxidative damage was reduced by the addition of 0.2 mM SNP.

Conclusion

In this study aimed to determine the possible role of NO (nitric oxide) as a precursor molecule in some metabolic changes occuring under the effect of drought stress in plants and to try to determine its effects on plant growth; it was concluded, that of 0,2 mM SNP application increases the plant growth.

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OLIVE PRODUCTION AND TRAITS OF MAIN CULTIVARS GROWN SOUTHEAST ANATOLIAN PART OF TURKEY

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Abstract

Olive (*Olea europea* L.) is grown in Mediterranean countries in the world. Turkey is the fourth largest producing country. It is grown for table consuming or oil production. The main olive producing country in the world is Spain. Generally the oil needs of the world population equates to 186 billion tons per year. World olive oil production is 3 billion tons approximately. Olive oil consuming is important for cardiovascular system for human health. The highest olive oil consumption in the world is 12.8 kg/person in Greece.

Olive production in Turkey is 1,300,000 tons, with 190,000 tons for olive oil. Table olive is also divided into two types; one of them black, another is green olive which pickled. The yield is not enough due to lack of some technical applications such as irrigation, fertilization etc. The intensive producing provinces are Eagean, Mediterranean, Marmara, Southeast part of Turkey. General common growing cultivars are 'Gemlik', 'Halhali', 'Kilis Yaglik', 'Kan Celebi', 'Kalembezi', 'Nizip Yaglik' etc. Main black olive is 'Gemlik'. It is used as table consumption. However, each cultivar has different traits.

Keywords: *Olive cultivar, Southeast Anatolian Region, Turkey.*

Introduction

The total average olive production in the world, between 2010 and 2017, was 20,007,854 tons. Turkey between the years 2010-2017 the average olive production is 1,744,875 tons. Olive production in the world ranking, Spain is first country, second is Italy, the others are Greece and Turkey.

The European olive (*Olea europaea* L.) is a major source of edible oil and processed edible olives. Today, the olive is cultivated over a total world surface area of almost 10 million hectares, on 60% of which it represents the main crop. The traditional area of olive cultivation is the Mediterranean basin, which includes 95% of the olive orchards of the world, and where more than the 95% of the olive oil and the 75% of the table olives are produced. A rough estimate of the global number of olive trees is over 800 million. The annual yield of olives is estimated at 10 million tonnes, most of which is used for oil production and less than 10% consumed as table olives (Fabbri et al., 2004).

The presence of olive trees in the world of table olives and grain producing countries significantly parallel to the olive production in Spain, Turkey, Italy, Greece, USA, Morocco, Syria and Egypt. Turkey ranks second after Spain in the production of table olives. Turkey ranks first in the world production of black table olives for table olive production. Spain ranks first in the production of green table olives. Turkey produced 85% of fruits black and green color, 70% of the table olives produced in Spain are green, 26% are color-oriented and 4% are black; 73% of table olives produced in Italy are black, 22% are green and 5% are color-oriented; 65% of table olives produced in Greece are processed and evaluated in black, 27% in green, and 8% in color (Tunalioglu, 1995).

In Turkey, 20% of the Marmara region of olive production is almost all kinds of Gemlik table production, 65% of the Aegean Region, Mediterranean and Southeast Anatolia Regions have a production of 15%. Overall Turkey produced 85% of black and 15% are covered in green

and color-oriented. According to these data in Turkey the Gemlik variety of black table olives is the most produced (Tunalioglu, 2003).
Olive oil yield, along with fruit production, is the main factor determining productivity in olive oil cultivars, thus explaining why it is one of the main breeding selecting criteria for the species (Rallo, 1994). Varieties to be planted for fruit production in a region has great importance. Soil structure is of great importance as well as the conditions that should be taken into consideration in terms of climatic conditions for the region where the variety will be grown. It is very important that climatic conditions are resistant to winter cold, they meet the cooling requirements required for flowering and fruit set. Tthe amount of rainfall in winter and the sum of summer temperatures necessary for the ripening of fruits. Soil structure in terms of pH, salinity rate, organic matter amount, water holding capacity and so on. The suitability of the properties should also be considered (Ak and Parlakci, 2007).

Classification of Olive Cultivars in Turkey

When it is desired to grow olives economically in a region, the answer to this question should be sought. The product to be obtained will be used for table consumption or for oil production. In other words, according to consumption or evaluation methods, olive is divided into two groups as table and oil. Varieties to be evaluated as table; Grain Weight (g) is defined as 9-12 g good, 5-6 g Medium and 1-2 g Small (Bad). In the varieties considered as oily; Fat Ratio (%) 25-30 good, 20-25 Medium and 15 and below (bad) is called. Another way of determining the variety of characteristics is the number of fruits per kilogram. If there are 175 fruits per/kg, it is classified as Jumbo size, 176-250: very big size, 251-335: Moderate, 336-421: Small,> 421: Very Small (Canozer, 1991).
All kinds of olives can be processed for table consumption. However, because of differences in quality, black, green and color-oriented table olives are prepared for consumption using different processing techniques. Generally, the proportion of flesh is small, the kernel is small, can be separated from the flesh, thin and flexible peel, high sugar content and preferably low fat varieties are processed as table. Table olives are presented to consumption after various processing techniques in the form of kernel, crushed, peppered, carrot, almond filled and sliced olives (Tunalioglu, 2003).
As it is known, our country has produced 1,022,500 tons of olives according to the averages of the last 10 years. It ranks 4th among the olive countries in the world and 5th with the production of 105.9 thousand tons of olive oil.

Cultivars in Turkey

It is known that there are around 600 varieties of olives in the world (Tsouchtidi, 2007). A significant amount of olive cultivation occurs in these countries (Spain, Italy, Greece, Tunisia, Turkey, Syria, Morocco, etc.) and prominent varieties are available. In this respect, countries that are advanced in production work have done so by concentrating on one or more varieties. In this respect, Arbequina, Blanqueta varieties in Spain, Koroneiki in Greece, Kalamata varieties became widespread.
In Turkey, different varieties have come to the fore depending on different usage purposes. These are; Celebi, Gemlik, Edincik Su, Karamursel Su, Samanli cultivars in Marmara Region, Ayvalik, Cakir, Cekiste, Cilli, Domat, Erkence, Izmir sofralık, Kiraz, Memecik, Memeli, Uslu cultivars in Eagean Region, Büyük topak ulak, Sari hasebi, Sari ulak, Saurani, Tavsan yuregi in Mediterranean Region, Egriburun, Halhali, Kalembezi, Kan Celebi, Kilis Yaglik, Nizip Yaglik, Yag Celebi, in Southeastern Anatolia Region, Marantelli, Otur, Patos etc. in Black Sea region well as different types in Turkey.
For the development and continuity of the export of olive oil in our country, it is the most important factor to increase the quality of the cultivation of oil varieties and the quality of the fruit. For this, both on the basis of production, taking into consideration the periodicity

situation, and on the basis of processing should go to the modernization and protection of both the manufacturer and the exporter (Kaska et al, 2005).

Some Characteristics of Main Varieties are Grown in Southeastern Anatolia

In a research on the pomological characteristics of olive varieties in the Southeastern Anatolia Region, four of the economically produced olive varieties (Kilis oil, Nizip Yaglik, Tatayin and Yag Celebi) were evaluated as oily and the other four (Halhali, Kalembezi, Egriburun and Kan Celebi) were used and will be suitable for processing table (Table, 1). On the other hand, 40% of the olives in the region are Kilis oil, 30% Nizip Yaglik, 10% Halhal, 5% Yag Celebi and 10% Tatayin varieties (Karaca and Ulusarac, 1996). In the study of Karadag et al. (2003), some of the findings obtained from an adaptation study conducted in Kilis. According to these findings, it has been determined that Gemlik variety, which has become widespread in the region, has 247 fruits per 1 kg, weight of 100 fruits is 407 g, flesh ratio is 62.82%, and pit ratio is 37.18%. According to these findings, when compared with the standard features of Gemlik variety, it was seen that fruits were bigger but flesh ratio was low. It was determined that the fat content was 19.76% and it was 29.98% in comparison with the source prepared according to the standards. There are also differences between varieties in terms of showing periodicity. The characteristics of the Gemlik variety, which is widely grown in the Southeastern Anatolia Region and has been cultivated in the region with new facilities in recent years, are also presented below. (Canozer, 1991; Ozkaya, and Kaynas, 2003; Bulbul, 2007).

Nizip Yaglik

Its origin is Nizip district of Gaziantep. It is common in Nizip, Gaziantep and Cizre districts of Şirnak and Kahramanmaras. The tree is of medium vigor and has a large crown formation. It has the characteristic of collective development in youth. It gains a flat and drooping structure when it yields. Branching is good, the branches are well-dressed. Thick branches are beige-green, young branches are green-beige. Old branches are usually narrow, some are right angles, young branches are wide-angle and drooping. Medium strength and beige color of the body, young trees in a smooth structure, the tree gets rough appearance as the bark cracks. The medium long, broad elliptical leaf has a linty, slightly hazy surface with a lighter hue than the typical green color of the olive leaf, usually 000761 (Lavender green), and some with a light colored needle at the tip 000858 (Leek green). Lower surface glabrous, green-grey. Stalk color is yellowish green. The average length 58.48 mm, average width 14.06 mm, length / width ratio is 4.15. Cluster lengths are 14-31 mm, average Cluster length is 24.40 mm, the number of flowers in the Cluster 10-30, the average number of flowers is 17. Cylindrical, close to round, symmetrical in length and transverse, the fruit is large and small mixed, usually very small size. There is no spine formation, the tip is usually flat, some inward collapsed. The handle pocket is large, round, medium depth. Green fruit, typical olive green, ripe fruit dark cherry-black color, medium hardness. The flesh under the skin is cherry in color, but the main flesh is light green-white. The flesh is easily separated from the pit.

The weight of 100 fruits is 217.60 g and the volume is 200.00 cm^3. The number of fruits per kilogram is 460. Fruit length is 20.82 mm, fruit width is 17.17 mm and length / width ratio is 1.21. Flesh ratio of fruit; 81.31%, the oil content is 27.36% and the moisture content is 40.49%. Like fruits, the pits are large and small and can be considered as large according to the size of the fruit. The longitudinal and transverse symmetrical, cylindrical, widest point is in the middle, with a spine protrusion on both sides and a needle at the end. Thin, scattered grooves on the surface of the pit acquire a slightly rough structure and are beige-brown. Pit length 11.76 mm, width 7.07 mm, length / width ratio is 1.66. 18.69% of the fruit is pit.

Kilis Yaglik

Its origin is Kilis district of Gaziantep Province. It is widespread in Gaziantep, Kilis, Oguzeli, Sanliurfa, Turkoglu in Kahramanmaras and Cizre in Sirnak. The tree is of medium vigor and forms a fast-growing, medium-sized, flat and drooping crown. Branching is good, the branches are well-dressed. Two-year-old and older branches are milky brown and young branches are green-beige. Old branches are narrow-angle, young branches are wide-angle and drooping. The medium strength and dark beige body is generally smooth. Medium long, large elliptical leaf, the upper surface of the hairless, slightly hazy 000761 (Lavender green), some 000858 (Leek green) color, with a small needle at the tip. The lower surface is slightly pile grey-green. Stalk color is yellowish green. The average length of 57.46 mm, average width 13.18 mm, length / width ratio is 4.35. Cluster lengths 20-40 mm, average Cluster length 29.7 mm, the number of flowers in the 14-30, the average number of flowers is 20. Round, transverse and longitudinal symmetrical fruit, mixed large and small, usually very small size. The spine is not round, the end is round. The handle pocket is small, of medium depth, usually round, sometimes shuttle-shaped. Green fruit, typical olive green, ripe fruit is bright dark black color, medium hardness. The Flesh is easily separated from the pit.
The weight of 100 fruits is 176.80 g and its volume is 14.91 cm^3. The number of fruits per kilogram is 566. Fruit length is 17.58 mm, fruit width is 14.91 mm and length / width ratio is 1.17. Flesh ratio of fruit; 82.25%, the oil content is 31.82% and the moisture content is 40.79%. The fruit pit is oval, large and has a small needle at the end. The surface is covered with smooth and streaked veins and has a yellowish-beige color with a length of 11.93 mm, a width of 7.81 mm, and a length / width ratio of 1.52. 17.75% of the fruit is pit.

Halhali

Origin is the district of Derik in Mardin Province. It is widespread in Hatay, Gaziantep, Kahramanmaras and Mardin provinces. The tree is of medium vigor and forms a rounded crown. Branching is good, the branches are well-dressed. Old branches are milky brown and right angled, young branches beige and wide angle. The medium-strength and grey-beige body is generally smooth. The long, narrow, elliptical leaf has a lint-free top surface of 000761 (Lavender green), some of which has a color of 000858 (Leek green) and a small needle at the tip. The lower surface color is grey-green. Stalk color is yellowish green. The average length is 63.18 mm, average width 11.82 m, length / width ratio is 5.34. Cluster lengths are 21-35 mm, average Cluster length is 26.6 mm, the number of flowers in the Cluster 9-25, the average number of flowers is 12. The fruit, which is close to the round, symmetrical to the transverse and longitudinal, is of medium size. The spine is not round, the end is round. The handle pocket is small, smooth and round. Green fruit, hazy-light green, ripe fruit dark cherry-black color, medium hardness. The flesh is easily separated from the pit.
The weight of 100 fruits is 383.01 g and the volume is 370.50 cm^3. The number of fruits per kilogram is 261. Fruit length is 19.54 mm, fruit width is 16.09 mm and length / width ratio is 1.21. Flesh ratio of fruit; 82.79%, the oil content 21.11% and moisture content 49.03%. The fruit pit is oval, large and has a needle at the end. The surface is veined, rough and pinkish-beige color, 12.37 mm in length, 6.28 mm in width, length / width ratio is 1.96. 17.21% of the fruit is pit.

Egriburun

Its origin is Nizip district of Gaziantep. It is possible to come across it in Hatay, Iskenderun, Nizip, Gaziantep and Halfeti districts of Şanliurfa. The type of euphorbia has taken its name from the shape of the fruit. Other varieties with the same name can be found. The tree is of medium vigor and forms a medium-sized, rounded crown with a central part. Branching is frequent and well-dressed. Thick branches are beige-green and narrow angled, young branches are green and skirt branches are wide-angle. The medium strength and dark beige-brown body has a rough structure. The upper surface of the leaf is medium-long and wide elliptical and has a color of 000761 (Lavender green), some of which is 000858 (Leek green). The bottom surface is grey-green in color and has a small needle at the tip. Stalk color is yellowish green. The average length is 55.81 mm, the average width is 12.88 m, length / width ratio is 4.33. Cluster lengths 26-33 mm, average Cluster length 20.20 mm, the number of flowers in the wedge is 10-28, the average number of flowers is 20. The fruit, which is oval, transverse and longitudinal symmetrical, is small. There is no spine death and the tip is curved. The handle pocket is medium-sized, deep and slightly grooved on both sides. Green fruit, dark green, ripe fruit is dark purple, the color of the flesh under the skin is purple in color, and the color of the kernel gets pinkish white color. Fruit flesh is hard and easily separated from the flesh pit.

The weight of 100 fruits is 258.00 g and the volume is 250 cm^3. The number of fruits per kilogram is 388. Fruit length is 21.21 mm, fruit width is 15.01 mm and length / width ratio is 1.41. Flesh ratio of fruit; 86.09%, 20.84% and 50.23% of the moisture content. Fruit pit is oval, small, twisted and needle. The surface is slightly rough and dark beige-brown in color with a length of 14.77 mm, a width of 7.80 mm, and a length / width ratio of 1.89. 13.91% of the fruit is pit.

Kalembezi

Its origin is Nizip district of Gaziantep. It is a variety found in some olive groves in Nizip, Kilis districts of Gaziantep Province. The tree is of medium vigor and has a medium-sized, scattered, drooping crown structure. Branching is not very frequent. Old branches are beige and connected with different angles, young branches are beige-green color and skirt branches are wide angle. The body is of medium strength and dark grey and has a corrugated and rough structure. The leaf is medium-long and medium-wide elliptical and its top surface is hazy, lint-free 000858 (Leek green) and in some, 000761 (Levander green). The bottom surface color is grey-green, sometimes with a light yellow-green needle at the tip. Stalk color is light green. The average length is 58.50 mm, the average width 12.86 m, length / width ratio 4.54 species. Cluster lengths 18-27 mm, average Cluster length 24.00 mm, the number of flowers in the 11-23, the average number of flowers is 15. It is oval, round and symmetrical in length and transversely. There is no spine formation and the tip is round. The handle pocket is round and shallow. Green fruit, typical olive green, ripe fruit is dark cherry with hazy, black color. The ripe fruit flesh is greenish yellow and medium hard and easily separated from the pit.

The weight of 100 fruits is 222.00 g and its volume is 225.00 cm^3. The number of fruits per kilogram is 450. Fruit length is 18.28 mm, fruit width is 15.07 mm and length / width ratio is 1.21. Flesh ratio of fruit; 84.29%, the oil content is 31.50% and the moisture content is 46.16%. The fruit pit is oval and medium in size and has two sides of vertebrate which is flattened from the top. The tip of the pit is needle. The surface is veined, slightly rough and brown in color with a length of 13.48 mm, a width of 8.70 mm and a length / width ratio of 1.54. 15.71% of the fruit is pit.

Kan Celebi

Its origin is Nizip district of Gaziantep. It grows in and around Gaziantep and develops well in well-maintained environments. It has a large flat and rounded crown. Branching is normal and branches are common. Old branches are usually narrow-angle, young branches are wide-angle. Two and older branches are light brown, young branches are milky brown. The surface of the body is dark grey and very strong, corrugated, lumpy but smooth. The top surface of the leaf is very long and narrow elliptical, lint-free, 000858 (Leek green) color and the bottom surface is grey-green color with a needle at the end. The average length is 67.62 mm, the average width is 10.96 m, the length / width ratio is 6.16. Cluster lengths 24-45 mm, average Cluster length 38.00 mm, the number of flowers in the Cluster 11-28, the average number of flowers is 19. Round, transverse and longitudinal symmetrical fruit, very large. The spine is not round, the tip is usually round. The handle pocket is smooth, round-shaped, medium depth. Green fruit, typical olive green, ripe fruit is dark cherry-black, the fruit flesh under the skin is cherry color, the color of the kernel turns pinkish cream color. Fruit flesh is medium hard, easy to separate from the pit.

The weight of 100 fruits is 615.10 g and its volume is 620 cm^3. The number of fruits per kilogram is 163. Fruit length is 25.73 mm, fruit width is 21.37 mm and length / width ratio is 1.20. Flesh ratio of fruit; 88.90%, the oil content is 16.90% and the moisture content is 50.03%. The fruit pit is oval, small, with a bent downward tip and marked needles, flattened from the top and vertebrate on both sides. Veins are white lines on the surface and are of beige color. Pit length 16.52 mm, width 9.15 mm, length / width ratio is 1.80. 11.10% of the fruit is pit.

Yag Celebi

Its origin is Gaziantep. It is a variety grown in Kilis and Nizip districts of Gaziantep Province. The tree is of medium vigor and forms a medium-sized drooping crown. Branching is good and the leaf density of the branches is good. Main branches are grey-beige, young branches are green-grey. Old branches are steep or close to the narrow angle of the dike, young branches are wide-angle. Medium strength, dark grey color, corrugated and lumpy body, smooth surface. The upper surface of the leaf is medium long and wide elliptical in grey-green color. Stalk color is greenish yellow. The average length is 52.84 mm, the average width is 12.10 mm, the length / width ratio is 4.36. Cluster lengths 24-36 mm, average Cluster length 31.50 mm, the number of flowers in the 6-6, the average number of flowers is 18. Long oval, downward slightly bent tip, asymmetrical fruit is large. There is no spine formation, the tip is bent down and there is a nipple formation at the tip. The handle pocket is deep, with protrusions at the edges and grooved in 2 or 4 separate directions. Green fruit, typical olive green, ripe fruit is bright dark cherry-black color, medium hardness. The flesh is easily separated from the pit.

The weight of 100 fruits is 442.15 g and the volume is 430.00 cm^3. The number of fruits per kilogram is 226. Fruit length is 26.32 mm, fruit width is 17.44 mm and length / width ratio is 1.50. Flesh ratio of fruit; 84.57%, the oil content is 21.10% and the moisture content is 53.40%. Fruit pit is long cylindrical, slightly asymmetrical, medium-sized, with a small needle at the tip. Due to the deep grooves, the surface is rough and dark beige-brown with a length of 19.46 mm, a width of 8.91 mm, and a length / width ratio of 2.18. 15.43% of the fruit is pit.

Gemlik

This variety is not actually of Southeast Anatolian Region. However, intensive planting has been done in the region in recent years. Its origin is Gemlik district of Bursa province. It is grown in Bursa, Tekirdag, Kocaeli, Bilecik, Kastamonu, Zonguldak, Sinop, Samsun, Trabzon, Balikesir, Izmir, Manisa, Aydin, Icel, Adana, Antalya and Adiyaman. Medium-vigor tree, large, smooth, round forms an angle. Branching is good, the branches are well-dressed. Branches turn green-grey color and the knuckle is short. The average length is 50.68 mm, average width 11.84 m, length / width ratio 4.28. Cluster lengths 15-26 mm, average Cluster length 20.30 mm, the number of flowers in the wedge is 10-23, the average number of flowers is 14. Close to the round, cylindrical, symmetrical transverse and longitudinal fruit, medium-sized. It does not have a spine formation and has a rounded v-tip. The handle pocket is small, smooth, round-shaped and medium-sized. Green fruit, hazy typical olive green color, ripe fruit bright, dark black color, medium hardness. The fruit flesh is easily separated from the pit.

The weight of 100 fruits is 372.80 g and the volume is 370.00 cm^3. The number of fruits per kilogram is 268. Fruit length is 22.33 mm, fruit width is 17.91 mm and length / width ratio is 1.24. Flesh ratio of fruit; 85.86%, the oil content is 29.98% and the moisture content is

45.05%. Fruit pit is oval, medium-sized and has a needle at the end. The surface is deep, frequently corrugated, rough and brown-beige in color with a length of 13.81 mm, a width of 7.98 mm, and a length / width ratio of 1.73. 14.14% of the fruit is pit.

The yield of this variety which develops to medium strength in a good maintenance environment is good. It gives fruit regularly every year. May 12-June 9 flowering varieties, between May 16-June 13 between the fruit vineyards. The fruit reaches the green ripening period between August 20 and September 10 and reaches black ripenin on 1-15 November. Its production is produced with thick or green branch cuttings. This variety, which is partially resistant to cold, is the most important variety considered for black table according to the Gemlik method. Since the fruits are rich in oil, non-table quality products can be processed as oil. This cultivar has 80% of the wood being in Marmara, Turkey. The presence of Gemlik trees in general constitutes 11%..

Conclusions

Over the last 30 years, the production and the consumption of olive oil have increased together. It is unlikely that this trend will change in the near future, considering the recent introduction or increase of olive cultivation and olive-oil consumption in countries such as Japan, Australia, China and South Africa. Remarkable increases (up to 10-fold) have been observed in several countries, including Australia, where olive-oil consumption has passed the level of 1 kg/person/year. Hence, the volume of olive oil consumed worldwide during the next 10 years is expected to exceed three million tonnes annually (Fabbri et al., 2004)

Olive cultivation is carried out economically especially in Gaziantep, Kilis, Mardin, Adiyaman and Şanliurfa provinces in Southeast Anatolia Region. However, certain varieties have gained weight in the region. Lack of irrigation, high alkaline soils, nutritional problems with low yield per tree, small fruit, all contribute to the production of mainly oil varieties. Olive is called an immortal plant because of its continuous regeneration feature. Therefore, the life of an olive tree can be up to 500 years depending on the climate and maintenance conditions. In fact, such a monument tree occurs in Sanliurfa (Ak and Korkmaz, 2018).

In recent years, with the support of the Ministry of Agriculture, olive plantation areas have increased rapidly in Şanliurfa and Adiyaman. It is known that especially Gemlik variety has become widespread in the region. This variety is grown as a black table variety under irrigated conditions in the western regions of our country. It is not possible to predict how it will work in both irrigated and non-irrigated conditions in the Southeastern Anatolia Region. The reason why this variety is widespread is that they can be easily produced by cuttings. On the other hand, under irrigated conditions, it was observed that this variety yielded in a short time and yielded fruit regularly without showing periodicity. It is also a kind of table and oil olive, and is partially resistant to cold. When the physiological water requirements of the trees are not met, grains are crumpled and spilled. It was observed that the fruits of the Gemlik variety produced in the region, with low air humidity and high temperatures, lower the table quality and the outer skin is thicker.

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THE TRAITS OF PISTACHIO ROOTSTOCKS AND THEIR IMPORTANCE

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Abstract

Rootstock is very important for fruit production. They effect some traits such as vigour, quality of fruits, yield, diseases, resistance for different soil types etc. Rootstocks are very important for pistachio nut production as well. Rootstocks may give resistance to biotic and abiotic stresses such as insect pests and diseases, low and high temperatures, drought, low soil moisture, salinity and its effects on the size of the tree size, alternate bearing, yield, quality, shell splitting and blank nut production, etc. There are 11 Pistacia species and hybrids in general. But for P. lentiscus, almost all of the Pistacia species can be used as rootstock for P. vera. Most common Pistacia spp. are P. vera, P. khinjuk, P. atlantica, P. terebinthus, P. palestina etc. in Turkey. In Greece and Italy (Sicily) the use of P. terebinthus is also common. In North Africa P. atlantica is used. In California, although both P. atlantica and P. terebinthus were used in the early days of the pistachio industry, now P. atlantica is the preferred rootstock because of its greater success in budding and faster growth than P. terebinthus. However, since both species are very susceptible to Verticillium wilt, a new rootstock, P. integerrima, or UCB=1 (hybrid) are now recommended to growers because of its vigour and resistance to the disease.

Keywords: *Pistacia, Rootstock, pistachio, fruit*

Introduction

Almost all commercially available fruit trees have been budded or grafted; that is, the top portion, or scion, of the desired fruit variety is attached to the root system, or rootstock, of a different variety. Trees are grown this way because some popular varieties grow and crop better on rootstocks other than their own. In some cases, the rootstock is more resistant to certain troublesome diseases. The choice of rootstock is very important for some fruits, such as apples, but not of much consequence for others. Apple trees are grown on a wide variety of rootstocks. These are called size-controlling rootstocks because they control the size of the tree; however fruit size is not reduced. In general, the smaller the tree, the sooner it will bear fruit after planting (Choudhary and Mehta, 2010).

Rootstocks are propagated by seed or vegatatively i.e. cuttings, layering. *Pistacia* spp. Are generally propagated by seed. Rootstock is very important for pistachio nut production. Choice of rootstock depends on resistance to insect pests and diseases, low and high temperatures, drought, low soil moisture, salinity and its effects on the size of the crown, alternate bearing, yield, quality, shell splitting, blank nut production, etc. (Ferguson et al., 2005; Ak, 2002)

The pistachio nut belongs to the genus *Pistacia* of the family *Anacardiacea.* The *Pistacia* genus has 11 species. The edible pistachio of commerce is the species *Pistacia vera* L. In addition to many named cultivars, significant populations of wild germplasm exist, primarily in central Asia from Turkey to Afghanistan. The species has been long propagated for nuts throughout the Mediterranean and Middle East. Several reports (Hormaza et al., 1994) suggest that the Romans were responsible for the spread of *Pistacia vera* within the Mediterranean basin. According to Zohary (1952), the distribution range of *Pistacia* species comprises five different centres and our literature survey supports this idea: (1) Northwestern America region), (2) Mediterranean region, (3) Irano-Turanian region, (4) Sino-Japanese region and (5)

Northeastern African region. Zohary (1952) also indicated several isolated areas such as Saudi Arabian desert (*P. palaestina*), Egypt (*P. khinjuk*) and southern Crimea (*P. atlantica*).
Apart from P. lentiscus almost all of the Pistacia species can be used as rootstock for P. vera. (Ak and Parlakcı, 2009). Although in Iran and Turkey P. vera seedlings have been used as common rootstock for centuries, seedlings of P. terebinthus in Turkey and P. mutica in Iran have also been used during the last few decades. In Greece and Italy (Sicily) the use of P. terebinthus is also common (Table 1). In North Africa P. atlantica is being used such as in Morocco. In California although both P. atlantica and P. terebinthus were used at the beginning of the pistachio industry, now P. atlantica is the preferred rootstock because of its greater success in budding and faster growth than P. terebinthus. However, since both species are very susceptible to Verticillium wilt, a new rootstock, P. integerrima is recommended to the growers because of its vigour and resistance to the disease (Needs and Alexander, 1982; Crane and Maranto,1988; Kaska, 1990).

Table 1. The Countries and common *Pistacia* species (Ak, 2013)

Afghanistan	*P. vera (from seed), P. khinjuk, P. atlantica, P. mutica*
Algerie,Morocco	*P. lentiscus, P. terebinthus, P. atlantica, P. vera*
Armenia, Georgia	*P. mutica*
Azerbaijan	*P. mutica, P. atlantica*
Greece	*P. lentiscus, P. terebinthus, P. palaestine*
Iran	*P. vera, P. atlantica, P. khinjuk*
Israel	*P. lentiscus, P. atlantica, P. palaestina, Hybrid P. saportae*
Italy	*P. lentiscus, P. terebinthus, P. vera*
Lebanon	*P. palaestina, P. lentiscus, Hybrids*
Mexico	*P. mexicana, P. texana*
Syria	*P. atlantica, P. palaestina, P. khinjuk, P. vera*
Spain	*P. atlantica, P. lentiscus, P. terebinthus*
Tunisia	*P. atlantica, P. lentiscus, P. terebinthus*
Turkey	*P. terebinthus, P. atlantica, P. khinjuk, P. palaestina, P. vera*
USA	*P. integerrima, P. atlantica, P. terebinthus, PG II and UCB=1 (Hybrid)*

Pistacia vera and *Pistacia mutica* species are cultivated in Iran. Generally Ghazvin and Badami pistachio cultivars seeds are used as rootstocks because of their resistance for salinity and *Phytophthora* (Hokmabadi et al., 2008). In Syria *P. atlantica* and *P. vera* cultivar Ashoury seeds are used as rootstock.
In Turkey, there are four common *Pistacia* species (*P. vera, P. atlantica, P. khinjuk, P. terebinthus*) used as rootstocks. Except for *P. vera*, the others are not commonly used as seedlings because of their low percentage of seed germination. According to observation, Siirt cultivar seeds have better growth and development than the other cultivars.
In U.S.A, *P. atlantica, P. terebinthus* and *P. integerrima* were used in last decade. Very recently the hybrid of *P. integerrima* and *P. atlantica* have been used for Kerman pistachio cultivar. The main hybrid rootstocks used is UCB=1 (*P. atlantica x P. integerrima*) and PG-II (*P. integerrima x P. atlantica*) for their different features (Ferguson et al., 2005). In Spain commonly *P. atlantica* is used as rootstock besides *P. terebinthus*.
Some important characteristics of the main rootstocks are summarized below (Kaska and Bilgen, 1988; Kaska, 1990; Ak, 2015; Ak, 2019).

Pistacia vera, L.

The trees of P. vera are big in size and around 10 meters tall. They have wider crowns than the other pistachios. The compound leaves consist of 1-3 pairs of leaflets and one terminal leaflet which is of the same size as the lateral leaflets. The petioles are short. The upper surfaces of the leaves are quite dark and shiny green but the lower surfaces are dull-green (Bilgen, 1968). The male flower clusters are bigger and more compact than those of the other pistachios.

P. vera seedlings are stronger and more homogeneous than the other rootstocks. Budded trees on this rootstock grow slowly during the first year but growth becomes faster in the following years. There is no incompatibility problems in budding with the varieties of pistachio (Ozbek, 1978, Ozbek and Ayfer, 1959). In the budded trees the juvenility period lasts long. Therefore, the trees bear fruit quite late. In arid zones the trees reach the bearing stage 15 to 20 years after planting. They can withstand drought, high lime content and salinity in the soil but are sensitive to high soil moisture, therefore, drainage is necessary. P. vera seedlings are sensitive to Melodogyne nematodes and some soil borne diseases (Kaska, 1990). In Turkey it is used because of rapid growing traits. But in the last years it is affected by excessive irrigation water or rainy weather conditions. In such conditions P. khinjuk will be better as rootsotock for pistachio cultivars.

Pistacia khinjuk, Stocks.

The trees of P. khinjuk look like P. vera. Hovever, they are taller in height and compact in shape. The compound leaf consists of five pairs of leaflets and one terminal leaflet which is quite large. The petioles of leaflets are short. In comparison to P. vera the ovaries are small but the styles are more pronounced and the stigmas are yellow-green (Özbek, 1978). During the ripening process the exocarp first becomes red and then turns olive-green. The endocarp is rather hard. The female flowers are similar to most of P. vera whereas the anthers in the male flower clusters are smaller and redder. The fruit is small (7 to 9 mm in length and 8 to 10 mm in width) but rich in oil. The exocarp is green-blue and the kernel is darkish pistachio green. Because of this attractive colour the nuts of P. khinjuk are used in the pastry and ice cream industry (Kaska 1990).

The fruit which is grown in Turkey did indicate differences in terms of size. There are generally two types of P. khinjuk called Buttum. These are divided into A and B type. Buttum A type fruits are bigger than B type and it is called Cultured Buttum in Siirt region. The fruit in this Buttum (A type); is 10.42 mm long and 7.31 mm wide on average. 100 fruit weighs are 38.69 g, as well as the number of fruits per 100 g was determined to be 258.6. The thickness of the hard shell is 1.00 mm. On the other hand, the outer green shell of the Buttum A is more firmly attached to the darker green and harder shell (endocarp) than type B. Buttum B; is about half the size of type A and in Siirt province called "Wild Buttum". This type is 8.3 mm long and 5.4 mm wide. The weight of 100 fruit is 19.98 g. This classification and its characteristics were determined by Bekir Erol Ak (Ak, et al. 2012).

This rootstock is hardier and more resistant to drought than P. terebinthus. Therefore it is grown at much higher altitudes (2,500 to 3,000 m) and in arid areas. In the eastern part of South East Anatolia the trees of P. khinjuk are grown as woods and form big trees. P. khinjuk occurs naturally in South East Anatolia, Sinai Desert, Syrian Desert, North of Iraq, Iran, Baluchistan and Kashmir (Yaltirik, 1967; Ayfer, 1959). The value of this rootstock is not known well. Hovever, when the giant trees of P. khinjuk are top-budded, they bear fruit within 5 to 6 years. Detailed studies are needed on the value of this rootstock vhen it is grown from seed. Moreover, the behaviour of different pistachio varieties budded on this rootstock needs to be investigated. In the last decades P. khinjuk has showed better performance according to rootstock x scion trials.

There is little swelling on the rootstock and scion of the cultivated pistachio grafted on the *P. khinjuk* rootstock. *P. khinjuk* was found to be the best utulizing from nitrogen in soil among *Pistacia* species used as rootstock ((Ak, et al. 2012).

Pistacia atlantica, Desf.

P. atlantica is one of the important rootstocks for P. vera. The nuts are small in size; therefore, their germination creates problems. Since they are open pollinated and compatible with other pistachios, the seedlings may not be homogeneous. However, by discarding the non-homogeneous materials this problem can be overcome. The seedlings grow slowly and come to the budding stage within two to three years on dry sites. Tap roots of P. atlantica are not as strong as P. vera, therefore they are not as difficult to transplant as P. vera. This rootstock can be recommended for irrigated plantations. They are also successful in somewhat heavier soils. Thus in such soils they give better results than P. vera seedling roots which are sensitive to high moisture in soils (Özbek, 1978, Ayfer, 1959, Spiegel-Roy et al. 1972; Needs and Alexander, 1982; Crane and Maranto, 1988). P. atlantica is sensitive to temperatures below -10°C (Maranto and Crane, 1982). This is the reason why it is widespread along the Mediterranean coastline but is not grown in continental areas. One of the most important facts about P. atlantica is its resistance to Melodogyne nematodes and some soil borne diseases such as Phytophtora parasitica. Hovever, the seedllngs are sensitive to Verticillium wilt. Therefore they are not recommended for planting in Verticillium infested soils where cotton was previously grown.

Pistacia atlantica Desf.'s trees grow up to 20 meters, forming trees with a width of 10-15 meters. P. atlantica is a strong rootstock and well-compatible with pistachio cultivars. There is no swelling or in-compatibility signs at the grafting point. The tree which is grafted on P. atlantica has big size and is a high yielding tree. It is a decidious tree and the seedling grows slowly for the first years.

P. atlantica can be used as rootstock for growing pistachios under irrigated conditions. They grow well in non-heavy base soils. P. atlantica is sensitive to temperatures below –9.4 oC. therefore, it is spread all over the Mediterranean coastline. On the other hand, this rootstock is resistant to nemetodes and some soil-borne diseases such as Phytophthora parasitica. Although it reaches the thickness for budding but sometimes the bark is not easily sparated from wood part. This means it may create problems in terms of removing the bark.

Fruits (P.atlantica)are oval-shaped, 5-7 mm long, 4-5 mm wide, dark green or bluish if filled, and one side reddish light yellow if non-filled. The number of fruits is 1033 per 100 g and it is determined that 100 fruits are 9.70 g. (Ak, et al. 2012). The trees of P. atlantica are rather tall. Mature trees may reach up to 7 to 20 m. The compound leaves consist of one terminal and 2 to 5 pairs of pointed leaflets. This species and some of its subspecies are distributed in Turkey, Greece, Aegean Islands, Cyprus, North Africa, Syrian Desert, Crimea (Russia), Caucasia, Iran, Afganistan, and Baluchistan (Davis, 1967).

Pistacia terebinthus, L.

The trees of this species are grown generally as bushes. The plant has a strong and deep root system (Ayfer, 1964). Therefore it can be grown in poor, rocky and stony soils. It is a kind of dwarfing rootstock for P. vera so the trees budded on this rootstock can be easily recognized. The male flower clusters of this species are distinguished by their red colour in comparison to pale or yellow colour of the other species. The leaflets are pointed. In each leaf there are generally 4 to 6 pairs of leaflets. The nuts are very small (5 to 6 mm in length, 3 to 5 mm in width) (Ozbek, 1978).

P. terebinthus is as hardy as P. vera but hardier then P. atlantica. Therefore, it is preferred to P. atlantica in cold areas. In Turkey, it can be grown successfully at elevations up to 1200 m

(Ak, 1992). It is also resistant to Melodogyne nematodes but very susceptible to Verticillium wilt. P. terebinthus is suitable for planting in calcareous soils along the Mediterranean coast. It grows very well in places where the annual precipitation is about 400 to 600 mm. As in the case of P. atlantica it can also grow in somewhat heavy soils. According to Joley (Woodroof, 1982) pistachio varieties budded on P. terebinthus and P. atlantica grow faster than the ones on P. vera.

P. terebinthus, grows more slowly than pistachio seedlings, but they are more resistant to nematodes than are also resistant to Phytopthora. They can be sized 3-5 m depending on maintenance and soil conditions,. They are usually found in the mountains in the form of bushes in forests. Each bushes have an average of 20 plantlets.

Fruit; It is 3-5 mm wide and 5-6.5 mm long and similar to lentil grains. When the outer shell is mollusc and ripe, it is bluish, olive-green and fleshy. Fruits are eaten freshly or roasted with this outer crust or squeezed out of the oil. On the other hand, fruits are crushed and roasted and ground like coffee. Bekir Erol Ak determined that the number of fruits per 100 g is 1455 and the amount of 100 fruits is 6.88 g. (Ak, et al. 2012).

P. terebinthus has a very strong root system and the roots have a very good growth ability in rocky soils. It is a good rootstock for rocky barren and especially non-fertile soils. They also grow naturally in both chalky and clay and deep soils and can withstand long summer droughts. Among all *Pistacia* species, the most powerful root system is considered for *P. terebinthus*. Some researchers have reported that pistachio grafted on *P. terebinthus* have been alive for 200 years.

Pistacia integerrima

P. integerrima is considered to be a variety of P. chinensis (Joley, 1979) but Monastra et al. (1988) stated that it is a synonym of P. khinjuk. In recent years it has been used in California as rootstock for pistachio as it grows vigourously and is resistant to Verticillium wilt (Needs and Alexander, 1982). It is recommended for replanting and new plantations in the cotton areas where the soils are infested with the wilt-causing fungus. According to Maranto and Crane (1982) P. integerrima is less tolerant of cold temperatures than either P. atlantica or P. terebinthus (Kaska 1990; Ferguson et al., 2005)

Hybrid Rootstocks

There are two common and main hybrid rootstock developed in Californa. They are respectively Pioneer Gold II (PG II) and UCB I. PG II is the result of open pollination in an isolated situation with multiple *P. atlantica* trees as the female parent and multiple *P. integerrima* trees as the pollen parents. UCB I is the result of a closed pollination with a single tree of the same parents. (*P. integerrima* pollen is introduced into a closed greenhouse in which the *P. atlantica* tree is grown.) Thus, both are the same interspecific hybrid but are produced from different seedling parents and therefore both exhibit seedling variability. Both of the hybrid rootstocks have increased vigor compared to *P. atlantica*, and generally equal or greater vigor compared to *P. integerrima*. (Ferguson et al., 2005).

After *P. integerrima* was discovered to be tolerant of Verticillium wilt, it became the most widely planted rootstock in California. It is vigorous, buds easily and results in a uniform tree stand. It is the least cold tolerant of the commonly used rootstocks and can be damaged by winter freezes and early frosts, particularly if they occur when the tree is young. The researches indicated that its growth rates and early production is superior to *P. atlantica* and 19.1% less than UCB-I, equal to or slightly less than the two interspecific hybrids. Nutrient studies have shown *P. integerrima* to be less efficient than *P. atlantica* for boron, zinc and copper uptake (Ferguson et al., 2005).

Conclusions

Rootstock is very important for pistachio as it is other fruit trees. The relationship between rootstocks and cultivars are very important. It can be affected by soil structure, salinity, mineral nutritions, resistance to soil borne diseases etc. Some researches are determined that Pistacia khinjuk is seems to be prefferable rootstock for pistachio. Rootstocks also effects the scion if there is facilities of irrigation or not. Excessive irrrigation occurs diseases problems.

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EFFECTS OF DIFFERENT SOIL TYPES ON SOME FRUIT PHYSICAL TRAITS OF 'KETEN GOMLEGI' PISTACHIO CULTIVAR

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Abstract

Pistachio is mainly grown at Southeast Anatolian part of Turkey. It is grown in a very bad soil conditions and non-irrigated areas. It is often called "rich plants for poor soils". In this research the plants were grown on three different soil types. Soil samples were taken from 3 different regions of these three different types of soil (A Parcel: Deep Red Soil (Kepir), B Parcel: Konglomera type (Pur), C Parcel: Boz soil (Lime content high) in fall. According to obtained results, pH was determined between 7.68 to 7.86 and lime content was 18.46 % to 35.10 %. Organic matter was very low because of high temperature. The highest shell splitting was obtained from the trees grown in A type soil conditions. Shell splitting rate varied between 38.65% to 50.39%. The empty fruit rate also varied according to soil types. Fully filled fruit rate was affected by soil type as well.

Keywords: *Pistachio, soil types, splitting, empty fruits.*

Introduction

Pistachio can be grown in only several parts of the world due to special climatic requirements. The most important pistachio producer countries in the world are Iran, U.S.A., Turkey and Syria, respectively. Growing and processing techniques after harvest in Iran and U.S.A differ from those in other countries. Modern growing and processing techniques are performed in USA only (Ak, 1992).

Pistacia vera is grown at 30-40 m altitude and suited in microclimate areas in the world. The biggest (420,000 ha) production areas belong to Iran. Second country is Turkey. But its yield is very low when the production areas are compared (Arpaci et al., 2005). This situation makes the scientist to think about the problems and solutions. Pistachio is growing in unfertile, rocky and high lime soil and rainfed conditions. It has grown in non-irrigated areas so far. But now, there is a dam named Ataturk, it provides facility of irrigation in Southeast Anatolian region where pistachio have been growing for hundreds of years (Kaska, 1995).

There are major cultivars in each main producing country. According to Sheibani (1995) there are 60 named cultivars in Iran. But main production cultivars are Ohady and Kaleh Ghochi. In Turkey, Kirmizi, Uzun and Siirt are the major cultivars. Keten Gomlegi is a domestic regional cultivar. Turkish cultivars are preferred in many European and U.S.A. markets due to their good taste and uniformly green kernels. In U.S.A. the main cultivar is Kerman. In Syria, different cultivars are common such as Ashoury, Oleimy, White Batoury etc. But the main cultivar is Ashoury (Ak, 2014).

The aim of the this research is to determine the effects of different soil types on splitting, blank, and shelling percentage of pistachio cultivar " Keten Gomlegi".

Material and Methods

Material

This research was carried out during period 2016-2017, in Bozova town in Sanliurfa in Turkey. The orchards were established in three different parcels with three different soil types such as: A Parcel: Deep Red Soil (Kepir), B Parcel: Konglomera type (Pur), C Parcel: Boz soil (Lime content is high). Some climatic conditions are given in Table 1.

Pistachio trees were "Keten Gomlegi" cultivar of pistachio (Figure 1). The trees are 30-40 years old and grown in rainfed conditions. In the orchard, five trees were selected which are similar to each other for yield and appearances. Each tree was accepted as one replication. **Keten Gomlegi cultivar description:** This cultivar is mainly grown as domestic cultivar in Sanliurfa and Gaziantep province in Turkey. Tree grows weak. Habit is semi-erect. Flowering time mid-late. Density of branches are very high and length of branch is small. The colour of florescence is yellowish-green. Leaf pedicel is short and color is deep green and thick. Oil content is approximately 59 % and protein content is 26 % around. (Gokce and Akcay, 1993; Ak, 2014).

Figure 1. "Keten Gomlegi" Pistachio cultivar grown in Soil Type A.

Table 1. Some climatic conditions of Sanliurfa province.

Months	Minimum Temp. (^oC)	Maximum Temp. (^oC)	Average Temp. (^oC)	Relative Humidity (%)	Rainfall (mm)
January	-3.1	17.2	6.2	68.8	82.5
February	-0.6	18.2	7.6	74.3	100.8
March	2.5	24.8	11.7	58.9	79
April	4.7	29.9	15.7	49.7	24.3
May	11.8	36.9	22.8	38.0	10.3
June	16.7	38.4	27.7	35.3	0.7
July	21.4	42.8	33.2	26.5	0.2
August	22.1	43.1	31.5	37.4	-
September	18.7	40.4	29.8	30.5	-
October	12.7	33.0	21.6	50.5	58.8
November	5.6	24.3	14	48.1	7.9
December	0.5	20.0	8.6	50.8	25.2

*Source: Sanliurfa province General Directorate Meteorology.

Methods

The fruit samples were taken from five marked trees during the harvesting time. Samples are taken from four sides of trees, each side two clusters, totally four clusters for each tree and after drying some physical analysis were done such as splitting, blanking, unsplitted rate or totally filled nut rates.

The soil samples were taken in Fall after the first rain. Soil samples were taken from three soil depths (0-20 cm, 20-40 and 40-60 cm) and three different places. These samples were analyzed in the laboratory and results are given Table 2 and Table 3. Statistical analysis, (LSD: Least Significant Differences) were done on either soils or fruits.

Results and Discussion

In this research, the effect of different soil types on Keten Gomlegi pistachio cultivar was investigated. During the harvesting time analyses were done on each of five trees. The results were given in tables.

Soil Analysis

Soil samples were taken from different soil types and parcels. The soil analysis results were given Table 2 and Table 3.

Table 2. The contents of pH, salt, lime, structure and organic matter of different soil types

SOIL TYPES	Soil Depth	Some Physical Traits of Soil				
		pH (SC)	**Salt (%)**	**Lime (%)**	**Structure (%)**	**Organic Matter (%)**
A TYPE	0-20	7.92*	0.032	18.16	54.63	0.69
	20-40	7.82	0.037	19.83	57.56	0.53
	40-60	7.83	0,037	17.39	60.13	0.60
	AVERAGE	**7.86**	**0.035**	**18.46**	**57.44**	**0.60**
B TYPE	**0-20**	7.76*	0.027	35.40	47.30	0.87
	20-40	7.68	0.030	35.47	48.03	0,78
	40-60	7.62	0.023	30.60	47.30	0.69
	AVERAGE	**7.69**	**0.026**	**33.82**	**47.54**	**0.78**
C TYPE	**0-20**	7.66*	0.017	35.30	41.80	0.61
	20-40	7.68	0.018	34.48	42.90	0.56
	40-60	7.72	0.017	35.54	44	0.67
	AVERAGE	**7.68**	**0.017**	**35.10**	**42.90**	**0.61**

*: This is average of different three samples.

Different soil types contain different traits. But the deepness is also differing by means of pH, salt, lime etc. The contents of pH is higher (7.86) in soil Type A. Besides pH, salt content is also higher while lime content was lower than others. Of course, it can be seen as contrast to soil types. This is may be due to different part of orchards and different soil types. Organic matter found was very low in the all soil types (Table 3). This value is normal because summer temperature is very high (Table 1). Organic matter is getting lower because of temperature. Tekin et al. (1985) conducted trials in Sanliurfa, Gaziantep, Ceylanpinar where pistachio is densely grown and similar results were determined. Bozova city area where this research has been performed is located in the same part of Turkey.

Table 3. Comparison of traits of different soil types.

SOIL TYPES	Some Physical Traits of Soil				
	pH (SC)	**Salt (%)**	**Lime (%)**	**Structure (%)**	**Organic Matter (%)**
A TYPE	7.86* a	0.035 a	18.46 b	57.44 a	0.60 b
B TYPE	7.69 b	0.026 b	33.82 a	47.54 b	0.78 a
C TYPE	7.68 b	0.017 c	35.10 a	42.90 c	0.61 b
AVERAGE	**7.74**	**0.026**	**29.12**	**49.29**	**0.66**
LSD (%5)	**0.109**	**0.005**	**3.587**	**3.455**	**0.153**

*: This is average of different three samples.

Statistical analysis also shows that all soil types are different to each other. pH is high, salt is normal and lime content is high while organic matter is very low. (Table 3). Generally, soil Type A is the better than others. It is named "Kepir" deep red, fertile soil conditions.

Splitting Rate (%)

The effect of different soil types on pistachio nut splitting rates is shown in Table 4 and Table 5. As seen in Table 4, there are some differences between the trees. According to Table 5, the highest splitting rate (50.39 %) was obtained in Type A soils while the lower is determined as 36.64 % in Soil type C.

Totally Filled Nut Rate (%)

Filled nut rates varied depending on soil types. According to obtained results, total nut rate (100 %) is obtained from the trees grown in Soil Types C. The lowest (87.85 %) totally filled nut rate was obtained from Soil Type A.

Blank Nut Rate (%)

Blank nut rate can be changed due to irrigation, fertilization, pollination etc. In these orchards the mentioned factors were eliminated and all conditions were similar. According to obtained results the blank nut rate was higher in Soil Type A then in other soil types. The reason can be due to mineral nutrition of soils. Fruits are remaining on the branches after June dropping time. The other soil types' fruits are dropping because of the fruit load or other reason.

Table 4. Effects of different soil types on some physical traits of pistachio nut.

TYPE A	**Split Nut (%)**	**Unsplit Nut (%)**	**Full nut rate among unsplit nut (%)**	**Blank Nut (%)**	**Totally Filled Nut (%)**
A1	59.18	40.81	26.53	14.28	85.71
A2	42.85	57.14	36.73	20.41	79.58
A3	46.66	53.33	37.77	15.56	84.43
A4	60.41	39.58	29.16	10.42	89.57
A5	42.85	57.14	57.14	0	100
MEAN	**50.39**	**49.6**	**37.46**	**12.13**	**87.85**
TYPE B					
B1	39.58	60.41	58.33	2.08	97.91
B2	66.67	33.33	33.33	0	100
B3	8.11	91.89	91.89	0	100
B4	64.29	35.71	35.71	0	100

B5	31.71	68.29	68.29	0	100
MEAN	**42.07**	**57.92**	**57.51**	**0.41**	**99.58**
TYPE C					
C1	38.10	61.90	61.90	0	100
C2	18.43	81.57	81.57	0	100
C3	30.44	69.56	69.56	0	100
C4	51.17	48.83	48.83	0	100
C5	55.11	44.89	44.89	0	100
MEAN	**38.65**	**61.35**	**61.35**	**0**	**100**

Table 5. Average of pistachio trees physical nut traits grown in different soil types

SOIL TYPES	Split Nut (%)	Unsplit Nut (%)	Full nut rate among unsplit nut (%)	Blank Nut (%)	Totally Filled Nut (%)
A TYPE	50.39	49.61	37.46	12.13	87.85 b
B TYPE	42.07	57.93	57.51	0.41	99.58 a
C TYPE	38.65	61.35	61.35	0	100 a
AVERAGE	**43.70**	**56.30**	**52.10**	**4.18**	**95.81**

Generally speaking, unsplit nut rate is higher (56.30 %) than split nuts rate. This is mainly due to lack of water. This situation is caused by non-effective fertilization or feedings as some elements are effective when soil is irrigated.

Shelling Percentage (%)

The obtained results regarding shelling percentages of Kernel are given in Table 6, which is related to growth on different soil types. According to results, the highest value at 52.40 % in Full and split fruits were in Soil Type A. The lowest (50.51 %) value is determined in Soil Type C. The results between the unsplit fruits were similar to splitted fruits. This means that Soil Type A had better conditions for shelling percentage.

Table 6. Comparison of shelling percentages of Pistacio cultivar “Keten Gomlegi” grown on different soil types.

SOIL TYPES	Splitted nut (%)	Non-Splitted nut (%)	Shelling Average (%)
A TYPE	52.40 a	50.63	51.51 a
B TYPE	52.26 a	49.32	50.77 ab
C TYPE	50.51 b	48.55	49.53 b
AVERAGE	**51.72**	**49.50**	**50.60**
LSD (%5)	**1.608**	**N.S.**	**1.425**

Shelling percentages among the unsplitted fruits is not statistically significant. The best one was in Soil type A and split fruits (Table 6).

Conclusions

Pistachio grows well in different soil conditions. But it is more fruitful if it is grown under fertile and physically good soil conditions, otherwise, it can be grown as a plant in all kinds of soil types. Soil type A is very well conditioned which provides positive factors for the kernel or fruits.

Although blank nut formation is a varietal character, it is extensively affected by climate, efficiency of pollination, fertilization, irrigation and rootstocks. Blank nuts found in the Turkish cultivars are due to inefficient pollination and lack of irrigation. Insufficient pollination conditions of the orchards are mainly responsible for high blank nut percentages (Ak et al., 1998). Besides this, vegetative or stimulative parthenocarpy and embryo abortion have another impact on blank nut formation. The tendency to parthenocarpy and percentage of abortive embryos can be different from one cultivar to another (Caglar et al., 1997). Splitting of pistachio nut depends on cultural practices such as irrigation, fertilization and soil cultivation (Ak and Parlakci, 2006). But it is a genetic character. Generally it depends on kernel development. In this experiment we showed that when pistachio is grown in fertile soil the quality will be higher.

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ECO-FRIENDLY WATER-RETAINING AGENTS AS THE BASIS FOR POTATO AGROTECHNOLOGY (Black-Earth Region, Russia)

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Abstract

The sorbent, or water-retaining agent, we produced is based on the introduction of biodegradable units into the macrochain at the stage of synthesis of polymers. The sorbent granules, containing biodegradable fragments in the mesh structure, were obtained by radical polymerization in the redox environment. Bacillus sp/ microorganisms were introduced into the sorbent matrix. It also seems highly effective to use a whole complex of products for processing vegetable and animal raw materials containing starch, pectin, chitosan and other polymeric materials. They are both available and can be decomposed as the result of microbial activity into water, carbon dioxide and nitrogen derivatives. The purpose of this work was to study the effect of moisture-retaining materials on the yield of potato. The sorbent demonstrated the ability to absorb and retain moisture in the plant root area, which allowed an increase in the potato crop production. The data showed that due to the moisture retained inside the sorbent and the moisture released to the plants, it was possible to form an additional potato crop. If the sorbent is enriched with microbes Bacillus sp. and the dose of the sorbent is 20 kg / ha, it increases the yield production by 1.7. The difference of yield output in the pilot plots caused the extra water retention in the soil. The biological activity of the soil varied considerably at the experimental plots, and the figures for the catalase activity were by 1.1-1.5 higher than the control option. Thus, we could observe a tendency of the increase in its activity by the end of the vegetation season.

Keywords: *sorbent/water-retaining agent, humidity sorption, Bacillus sp, microbiological activity of the soil, barley production capacity.*

Introduction

The issue of water retention in the soil has always been extremely important for the regions lacking in rains, as it inevitably affects the yield production capacity (Rode, 2008). The black-earth region in Russia is no exception. Farming in the risky climate faces a lot of problems, for example, when the lack of rains coincides with a particular vegetation phase of the plants (Kadyrov et al., 2005).

Thus, solutions to such problems should aim at developing efficient water-saving technology and thus effectively provide plants with moisture. The research shows that using microorganisms as elements in the water-retaining agents can contribute a lot to the eco-safety of the latter. Providing plants with necessary moisture during a particular vegetation period as well as increasing the microbiological activity in the root area will definitely decrease the stress effect from bad weather conditions and enhance the yield production capacity.

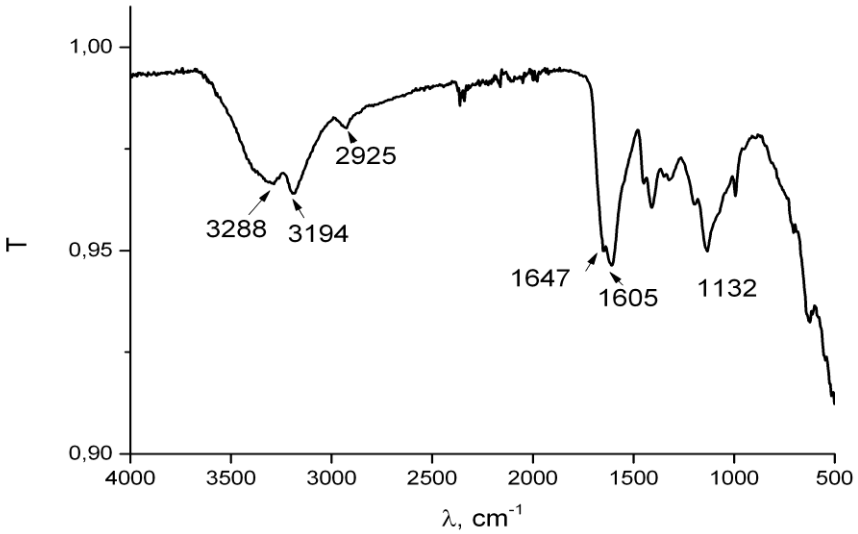
Materials and Methods

The polymer we used in our research, or the agent with qualities of super absorbing agent (SA), was synthesized in accord with traditonal methods (Bellamy et al., 1963). We added non-organic and organic matter to the reaction mass (concentration ratio 4:1) during the last stage of polymerization and as a result developed the modified polymers. IR spectra of the SA

samples were developed on the basis of Fourier transformer *Bruker Vertex 70*. In order to study the effect of the SA on potato production capacity, we launched a field experiment in 2017-2018 (Vernekhavsky District of the Voronezh Region, Russia, the site location data is 51.800372, 40.216045). The methods used were developed by B. Dospekhov (Dospekhov, 1985).
The research was based on the Dutch potato sort Picasso. It is a medium-late variety species (cooking type), with a high yield capacity. It was developed in the Netherlands by "Agrico" company, and later, in 1995, included into the Russian State Plant Registry.
The experiment had three variants: 1. Control – potato; 2. Control + SA 10 кg/ha; 3. Control + SA 20 кg/ha; 4. Control + SA 20 кg/ha + microorganisms;
The microbial agents used in the research are produced in Russia. The concentration used was 10 g/кg, which was added to the SA at the last stage of its development.
We used traditional methods (Khaziev., 1990) for estimation of the amount of ammonifying bactreia and microbiota participating in the decomposition of the non-nitrogenous compounds in the soil, and also the ferment activity of the soil. The soil in the experimental plots is qualified as typical black-earth with the average amount of organic matter of % - 6.16, $pH_{кс1}$ 5.9, the amount of labile soil nutrients (мg/кg) P_20_5 108, K_2O 163. The amount of nitrate nitrogen and nitrogen exchange ammonium in absolutely dry soil was 17.7 мg/кg and 4.59 мg/кg, respectively.

Results and discussions

The SA (i.e. the water-retaining agent used in the research) is based on the principle that at the stage of polymer synthesis the macro chain is joined by biodegradable links. It also seems highly effective to use a whole complex of products for processing vegetable and animal raw materials containing starch, pectin, chitosan and other polymeric materials. Such materials are widely available and biodegradable as they decompose into water, carbon dioxide and nitrogen derivatives.
FTIR spectra data were used to confirm the structure and composition of the obtained sorbents (picture 1).



Picture 1. FTIR spectrum of the original polymer

The FTIR spectrum of the polymer without added microelements and humic acids contain absorptions bands for values of 1647 и 1605 cm^1, corresponding to stretching modes of the

C=O-group (amide I) and NH_2-group (amide II) of amide parts (Bellamy et al., 1963). Broad absorption bands with the value of 3180-3300 cm^{-1} signal that there are OH и NH_2-groups of a biodegradable component (Kuznetsov et al., 2016, Kuznetsov et al., 2017). The FTIR spectra of the SA samples containing additives show the shift of typical absorption bands. It means there is an interaction and formation of associates by functional macromolecule groups of the polymer with its additives that are embedded in the polymerization mass. In this regard, the obtained SA can be used not only as «water reservoirs» but also as fertilizers, which are able to carry functional additives. In regard to the above-mentioned, bacteria *B. Subtilis sp* seem to be of interest with a view to developing a water-retaining agent with efficient functions:

1. They can function as antagonists for pathogenic and opportunistic pathogenic microorganisms due to their production of antibiotics and their ability to acidify soil;
2. They can produce ferments, which remove the rotten matter in fibre;
3. They synthesize a number of aminoacids, vitamins and biologically immune-activating factors;
4. The activity of *B. subtilis* in rhizosphere contributes to the plant resistance to the toxic effect of heavy metals.

At present, farmers are using an innovative microbiological agent called «Phytosporin M», which contains *B. Subtilis* 26 D. It is highly effective for fighting fungi- and bacteria-based diseases found in any crops (Selyavkin et al., 2015). *B. Subtilis, being aerobiotic sporogenic bacteria, are quite common in our environment. They have a unique ability to survive in plants in the highly competitive environment and act as antagonists to phyto pathogenic organisms. Due to this quality, such bacteria can be effectively used in biological plant protection.* It is especially important that they are able to carry endophytic mircoorganisms to the plant root area and thus not only to protect the plant from pathogenic factors but also to improve its physiology. During the experiment, we tested and compared the original soil samples and those enriched with the bacteria-based polymer (Popov, 2004).Phytotoxity of soils is the result of pollutants and toxic substances getting into soil and inhibiting the growth and development of higher plants. It is always necessary to identify the degree of phytotoxity when monitoring chemically contaminated soils or when assessing the possibility to use some wastes as fertilizers. It could be, for example, the sewage sediment, composte or hydrolyzed lignin.

The degree of soil toxity can be assessed through analyzing some plants development. It is a well-known fact that plant resistance to environmental stresses depends on its age, or rather a particular phase of development. In this regard, the highest degree of vulnerability is found in seeds during the germination phase. In this period plants are most susceptible to the environmental impact. Hence, plants at this stage are most convenient objects of ecological analysis and testing. It is necessary to analyze and assess different parametres during their germination and make conclusions.

The main parametres studied during biotesting plants for phyto toxity is germinating capacity and seed vigor. Testing is usually based on wild plants, which have a good germinating capacity and are locally growing. For example, to test soils in central Russia (sod-podzol type), they usually use peas and oats, for steppe and forest-steppe areas – wheat, beans and medick (lucerne). According to the National State Standard system, we can also use seeds of oil radish and oats. Soil contamination with heavy metals can be detected by using seeds of garden-cress pepperweed, which are quite sensitive to ions of heavy metals. Therefore, if the soil is contaminated, the seeds sprouting and germinating capacity become low. In addition, in this case the plant shoots and roots demonstrate vivid morphological changes, such as delayed growth and shoot distortion as well as the decrease in the length and weight of the roots.

In the next 3-4 days after the incubation of seeds, we counted the number of the shoots in the control group and experimental one, and then estimated the seeds germinating power (i.e. the percentage of sprouts within a given period) according to the following formula (1):

$$B = а/в \times 100\ (\%) \qquad (1)$$

where ***a*** is the number of the sprouts; ***в*** is the total number of the seeds under the experiment. The experiment can be extended for another several days to verify the obtained results. For this, it is necessary to provide lighting, estimate the germinating capacity of the seeds, measure the average length of the plant herb and roots, and the total weight of the plants in the control and experimental groups. To obtain the comparable results, testing data are used to estimate the toxity index of a particular evaluated factor: germinating power, germinating capacity, the length of the shoots and roots, the weight of the sprouts in each group. It is done according to the following formula (2):

TIF (toxity index of the factor) = TFo / TFk (2)

Where TFo – average value of a particular characteristic/factor in the experiment;
TFк – average value of the same characteristic in the control group. To estimate the toxity index of the soil, usually the following scale is used (Table 1).

Table 1. The scale of the soil toxity

Value of TIF	Toxity class
>1.10	VI (stimulation)
0.91 – 1.10	V - norm
0.71 – 0.90	IV – low toxity
0.50 – 0.70	III – average toxity
< 0.50	II – high toxity
Unsuitable habitat for the object	I – superhigh toxity causing death of the tested object

As a rule, the result can be considered to be true if an average length of the plant shoots and roots differs from that of the control group, and the difference is over 20%. If the difference is lower, it means the decrease in the plant growth and hence the possible high toxity degree of the plant. The lower the figures are, the more degraded the soil is and the less fertile it is. It eventually leads to the soil's inability for self-purification.
Table 2, as given below, shows the results of biotesting of the radish seeds. The data include values for the control soil and experimental sample enriched with the polymer.

Table 2. Biotesting of seeds

Results of biotesting							
Variant	Germinating power 3 days (%)	Weight of 100 sprouted seeds, grams	Toxity index of the factor (TIF)	Length of the shoot, cm	Toxity index of the factor (TIF)	Length of the root, cm	Toxity index of the factor (TIF)
1	100	10.6	-	3.6	-	5.7	-
2	100	3.9	-	0.4	-	0.6	-
4	100	4.3	1.1/0.4	0.7	1.75/0.19	1.4	2.3/0.25
5	100	11.9	3.05/1.1	4.4	11/1.2	5.3	8.8/0.92

1. control – water;
2. control – soil; 3. Initial/ original polymer . 4. The plymer enriched with microorganisms.

In Table 2 the values given with a slash (/) indicate the toxity index in relation to soil and distilled water.

The data in Table 2 show that all the TIF values for the soil control samples demonstrate a stimulating effect, and for the distilled water control sample the polymer affected the length of the shoots. Biological activity of soil has a number of indicators (Emtsev et al., 2006, Zvyagintsev, 1987). One of the signs of the soil fertility, for example, is the amount of microorganisms and, as a result, their effect on the mineralization of the organic matter in the soil. Also, one of the experiment objectives was the assessment of catalase activity, which shows the degree of soil biological capacity. The results demonstrate that the amount of catalase slightly varied across the board and reached its maximum by the end of harvesting, which was confirmed by some earlier data (Selyavkin et al., 2015). The variants enriched with the bacteria-based polymer showed the increase in ammonifying bacteria and those which use mineral forms of nitrogen. The data given below demonstrate some of biological characteristics of soil and figures for potato production in 2017- 2018.

Table 2. Characteristics of biological activity of soil and potato production in 2017-2018.

Variants	Phase pf potato growth												Yield tones/ha
	Sprouts				Blossoming				Top necrosis				
	beef peptone agar (BPA)	starch-and-ammonia agar (SAA)	BPA / SAA	Catalase	BPA	SAA	BPA / SAA	Catalase	BPA	SAA	BPA / SAA	Catalase	
1	9.1	10.9	1.2	1.5	7.3	5.1	0.7	3.3	7.0	8.4	1.2	3.3	7.2
2	11.0	14.3	1.3	1.7	11.4	21.6	1.9	3.6	14.0	16.8	1.2	3.6	11.8
3	22.0	30.8	1.4	1.8	26.0	49.4	1.9	3.6	15.0	19.5	1.3	3.8	12.2
													LSD 0.05= 0.64 tonnes/ha

Catalase - ml 0.1M $KMnO_4$ per1gr of soil within 20 minutes

During the phase of potato vine necrosis, the biggest amount of both groups of microorganisms was found in Variant 3. The soil there was enriched with the SA and Bacillus sp. It enhanced the biological soil activity and contributed to rehabilitation of the root area and plant protection capacity against phyto pathogens. As a result, the potato production was significantly improved (Popov, 2004).

The decrease in soil moisture capacity below 60-70 % from the minimal value can cause a negative impact on plant development. The maximum production of particular potato sorts in the Black-earth region of Russia largely depends of water: for example, 1 tonne of potatos needs up to 10 tonnes of water, which normally comes from underground water or rainfall. During the research period, the amount of moisture at the stage of planting the potato seeds and during the vegetation period reached about 2900 m^3 /ha. In our opinion, it was a limiting factor for potato production capacity. Despite favourable agrophysical conditions of black earth and the opportunity to control the moisture level in the soil, the process of water retention and its influence on the production capacity varied.

The field experiments in 2017 - 2018 were based on standard agrotechnology and involved the introduction of a fertilizer complex containing $N_{20}P_{11}K_{11}$ at the planting stage (250 кg/ha). The total amount of the rainfall during the vegetation period was about 70% of the long-term annual average, which allowed providing the effective moisture capacity and, as a result, obtain only 7.2 tonnes/ha of the potato in the control field. Apparently, it was because of limited moisture due to its high evaporation. After adding the bacteria-based SA in the near-seed area, the biological soil activity in the root area increased and gave boost to production capacity of the potato. The potato production figures for soil with the SA and the bacteria-based SA were 11.8 и 12.2 tonnes/ha, respectively.

Conclusions

The obtained results clearly demonstrate a positive effect of the ammonifying bacteria-based absorbent. At all stages of the potato development the amount of this type of bacteria (Variant 8) was 4 times as high as their amount in the control variant.

The structure of the polymers enriched with bacteria enables them to retain moisture effectively and therefore makes them functionally efficient. The experiment also showed that both the original, or innitial, polymer and the bacteria-based one do not have a toxic effect on plants and enhance its biometric characteristcs. The stimulating effect reaches values of 3 to 8.8 according to the toxity scale and positively affects the size of the formed roots and the total weight of sprouts. Biological soil activity varied across the board: for example, the catalase activity became 1.2 times as high in comparison with the control.

According to the results of the research, the SA dose of 20 кg/ha contributed to the potato production capacity increasing it 1.6 as high. The potato yield was over 1.7 times as high compared to the control variant, which is due to the difference in providing soil with moisture and its retaining as well as involving bacteria.

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CHEMICAL COMPOSITION AND YIELD OF DILL AS AFFECTED BY METALLURGICAL SLAG AND ORGANIC FERTILIZER APPLIANCE TO MARGINAL SOIL

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Abstract

Anethum graveolens L., commonly called dill, is frequently grown in herb, vegetable and flower gardens not only for harvest of its aromatic leaves and seeds, but also for ornamental display of its attractive foliage and flowers. It prefers well-drained sandy and medium loamy soils with pH in the range from 5.3 to 7.8 and highly acidic conditions could stunt its growth. Thus, a necessary lime should be applied. The aim of this study was to investigate the influence of an appliance of Ca - containing metallurgical slag from Steel factory from Smederevo, Republic of Serbia, on chemical composition of an aerial biomass of dill, through vegetative experiments performed in semi-controlled greenhouse conditions. The effects of metallurgical slag were compared to those of commercial lime material ($CaCO_3$) in combination without and with standard mineral and organic (NPK nutrient of animal origin) fertilizers. Lime material, along with metallurgical slag, particularly in combination with organic fertilizer, showed positive effects on elemental composition of dill and its yield. The contents of toxic heavy metals in herb comparing to non-fertilized treatment were not significantly increased and were within the permissible levels in plants in all the treatments in spite of their higher content in metallurgical slag. Based on the results obtained in present study, the studied metallurgical slag of the standardized chemical composition can be recommended for the wider practice toward improving the productivity of highly acid soils such as Stagnosol.

Keywords: *Metallurgical slag, Commercial lime material, Organic and mineral fertilizers, Stagnosol, Dill.*

Introduction

High soil acidity is a property of the majority of Serbian soils and application of only organic and mineral fertilizers is not enough to sustain their productivity. On these soils, along with regular fertilization, it is necessary to apply calcium containing fertilizers - calcifiers, for improving their biological and physico-chemical properties and obtaining higher yields (Pivić *et al.*, 2011).

The use of traditional commercial alkaline liming materials such as limestone, dolomite and burnt lime to acid soils for the amelioration of acidity consequently improving crop production is a common practice (Huang *et al.*, 2012). Along with these materials present in Serbia and regarding its alkaline nature, metallurgical slag from the steel factory, located in Smederevo, Serbia, can be of great importance.

Crops vary greatly in their soil affinity and ability to tolerate low pH. Dill (*Anethum graveolens L.)* is a commonly cultivated herb which grows mainly for its edible leaves and seeds, though it is also used medicinally. It requires moderately rich loose, well-drained, sandy and medium loamy soils. In general, it is an easily grown plant, although it belongs to those herbs that tolerate a soil pH in the range 5.3 to 7.8 and extremely acidic conditions could stunt its growth (Dressendorfer, 2010). Thus, a necessary lime should be applied.

Although the significant quantities of metallurgical slag are generated as waste material every day from steel industries, its physicochemical property offers a high potential for its utilization in agriculture. As metallurgical slag contains fertilizer components such as CaO, SiO_2 and MgO, its alkaline property remedies soil acidity. The liming materials in metallurgical slag comprise water-soluble and less water-soluble Ca and Mg compounds. Free Ca in slag reacts rapidly with water to form $Ca(OH)_2$. The $Ca(OH)_2$ will react rapidly with soil acidity (NSA, 2011). In addition to these three components, it also contains components such as FeO, MnO and P_2O_5, so it has been used for a broad range of agricultural purposes. Certain authors reported on field trials in Pennsylvania that crop yields of corn, wheat, oats, buckwheat and soybeans with metallurgical slag application were as good or better than an equivalent amount of limestone (White *et al.*, 1937). In developed countries such as Germany, USA, France and Japan, converter slag is used to produce siliceous and phosphorus fertilizer, as well as micronutrient fertilizer (Huang *et al.*, 2012).

Some slags may contain elevated concentrations of trace metals such as Fe, Cd, Cr, Cu, Pb, Mo, Ni and Zn. They occur naturally in soil and some of them are essential plant nutrients. If concentrations in the slag are similar to soil concentrations, they present no problem. If they are present at substantially higher concentrations in the slag than in the soil, repeated application of the slag could significantly increase soil metal concentrations, leading to plant toxicity. Nevertheless, the bioavailability of these metals in slags is very low (NSA, 2001).

The aim of this research was to investigate the effect of Ca-containing metallurgical slag, a by-product from steel factory, on yield and chemical composition of the aerial parts of dill (*Anethum graveolens L.)*. The effects of metallurgical slag were compared to those of other lime material (calcite, $CaCO_3$) in combination with and without standard mineral and organic (NPK nutrient of animal origin) fertilizers.

Material and Methods

The study was carried out under semi-controlled condition in the greenhouse of the Institute of Soil Science, Belgrade (Serbia), in experimental cassettes of diameter 1.0x1.0x0.5 m, from the fourth decade of March to the fourth decade of June, during 2017. The trial was undertaken with Stagnosol (WRB, 2014), a type of soil from Western Serbia region, generally characterized by very acid reaction and poor physical and biological properties (Pivić *et al.*, 2011). Each cassette was filled with 650 kg of the studied soil, brought from an experimental field of Institute of Soil Science - Varna, near Šabac town. In the experiments the comparison of the effect of metallurgical slag (MS) with other lime materials (calcite, $CaCO_3$, containing 60% of carbonate) in combination with and without mineral NPK [composite NPK (15:15:15)] and commercial organic (solid NPK 4:3:4 nutrient of animal origin - Nervosol Complex, NC) fertilizers were studied.

The following seven designed treatments were carried out in three replications: control (untreated soil) - T1; $CaCO_3$ - T2; NPK mineral fertilizer + $CaCO_3$ - T3; NC + $CaCO_3$ - T4; MS - T5; NPK mineral fertilizer + MS - T6; NC fertilizer + MS - T7.

Before sewing the dill, the amount of fertilizers, lime and slag was measured according to the experimental design and mixed with soil (calculated as for 1 ha): NPK fertilizer (15:15:15) = 500 kg ha^{-1}; NC fertilizer = 170 kg ha^{-1}; $CaCO_3$ = 4 t ha^{-1}; $Ca(OH)_2$ = 2,8 t ha^{-1}; MS = 4 t ha^{-1} (same as the amount of $CaCO_3$, in spite of lower amount of slag). Both MS and calcite with granulation of 0.2 mm were used in the experiment.

Chemical composition of MS applied (Table 1) was determined in our previous study (Pivić *et al.*, 2011). Accordingly, this material has very alkaline reaction, with the content of calcium in oxide forms (CaO) from 33-45%, of which about 50% is easily soluble in 1 M ammonium acetate; content of the total magnesium was mainly in forms of MgO, while nearly all the amount of phosphorus(P) is in available forms for plants; contents of the total iron (Fe) and manganese (Mn) are high, but with lower amounts of their soluble forms; zinc (Zn) is contained in lower amounts, while the content of copper (Cu) is a little higher.

Table 1. Chemical composition of MS (Pivić *et al.*, 2011).

Parameter	Average value	Parameter	Average value
pH in H_2O	12.48	Total P_2O_5 (%)	0.61
Total Ca (%)	26.20	Total Fe (%)	15.34
Total CaO (%)	36.60	Available Fe (mg kg^{-1})	3.38
Total $CaCO_3$ (%)	65.80	Total Mn (%)	1.80
Available Ca (%)	17.18	Available Mn (mg kg^{-1})	3.12
Total Mg (%)	0.41	Total Zn (%)	14.60
Available Mg (%)	0.70	Total Cu (%)	228.8

Organic fertilizer used is a solid NPK 4:3:4 nutrient of animal origin, commercially called Nervosol Complex. According to its main chemical composition, it consists of 4% of total nitrogen (N), 4% of organic N, 3% of phosphorus in the form of P_2O_5, 4% of potassium in the form of K_2O, and 30% of organic carbon (C) (Đurić et al., 2015).

The soil samples for analysis were air-dried, crushed, passed through a sieve (≤ 2 mm) and crushed into dust by hand. The preliminary observation of the soil studied included the analysis of the following chemical parameters: soil acidity (pH in 1M KCl) was analyzed potentiometrically with glass electrode (SRPS ISO 10390:2007, 2007); total nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III (SRPS ISO 13878:2005, 2005); available phosphorus (P_2O_5) and potassium (K_2O) were analyzed by Al-method (Đurđević, 2014); the total contents of investigated trace biogenic elements - iron (Fe), zinc (Zn) and copper (Cu), as well as cadmium (Cd) as the toxic heavy metal, in soil samples were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) after the digestion of the samples with aqua regia (ISO 11466:1995, 1995; ISO 22036:2008, 2008).

The aerial parts of dill plants were taken and after drying at 105^{o}C the plant biomass was weighed. For all the plant samples from all the treatments the chemical composition of the aerial parts was analyzed. The content of N was determined on elemental CNS analyzer Vario EL III (Nelson and Sommers, 1996). Phosphorus (P) was determined by spectrophotometer with molybdate (Đurđević, 2014a), and potassium (K) - by flame emission photometry (Đurđević, 2014b). In the determination of investigated trace biogenic elements - Fe, Zn and Cu, as well as cadmium (Cd) as the toxic heavy metal, atomic absorption spectrometry - AAS was used (Wright and Stuczynski, 1996).

The obtained data on soil properties represent the arithmetic means of three replicates and standard deviation values. The effects of T1-T7 treatments on the studied chemical parameters and yield of the plants were evaluated using the analysis of variance (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at P = 0.05.

Results and Discussion

In Table 2 the data on main chemical characteristics of plowed layer of the studied Stagnosol are displayed. The soil is characterized by very acid soil reaction, low content of available P, then, it is well supplied with available K, medium provided with total N, Cu and Zn, and very highly provided with Fe (Table 2). Content of toxic metal Cd was low and far from the maximum limiting value of 0.8 mg kg^{-1} (OGRS, 2018).

Table 2. Chemical properties of the studied soil (mean ± standard deviation value*)

Property	Value*	Property	Value*
pH in 1M KCl	4.12±0.14	Total Fe (mg kg^{-1})	133.5±1.05
Total N (%)	0.12±0.02	Total Zn (mg kg^{-1})	0.61±0.03
Available P_2O_5 (mg 100g $^{-1}$)	9.42±1.39	Total Cu (mg kg^{-1})	1.68±0.04
Available K_2O (mg $100g^{-1}$)	17.00±2.58	Total Cd (mg kg^{-1})	0.08±0.00

Certain types of soil, including stagnosols, require the periodic application of soil conditioners such as commercial liming materials to provide aeration, increase moisture retention, and promote root permeation and growth. In several experiments in European countries it was determined the ability of metallurgical slag to raise the pH of acid soils (Rodriguez *et al.*, 1994; NSA, 2011).

The optimum pH range in soil for growth of most crops in soil is between 5.5 and 7.0, within which most plant nutritives are available (Prasad and Power, 1997). Data on the content of macroelements in dill aerial parts (Table 3) show the statistically significant differences (*P* *) between the treatments at P<0.05 for P and K, and no significant difference (NSD) for N. Nevertheless, there is a noticeably tendency of an increase in the content of N, P and K in tested plant material in the treatments that included NC fertilizer and NPK mineral fertilizer, respectively, in combination with MS (T6 and T7), in relation to other treatments. Improved organic and mineral nutrition in combination with MS would explain the promotion of dill biomass growth which led to the promotion of its yield. The data on yield of dill were in accordance with chemical ones, meaning that the yield was highly significantly higher (*P* ***) at P<0.05 in treatments T6 and T7 (Table 3).

Table 3. Macroelements content in dill and its yield depending on the treatment used

Treatments	Macroelements (mg kg^{-1} of dry biomass*)			Biomass yield (kg per cassette*)
	N	P	K	
T1 - control (untreated soil)	2.84±0.08[ab]	0.29±0.02[cd]	2.90±0.51[c]	3.08±0.10[b]
T2 - $CaCO_3$	3.07±0.10[ab]	0.43±0.08[ab]	3.27±0.37[abc]	3.14±0.13[b]
T3 - NPK mineral fertilizer + $CaCO_3$	3.21±0.31[a]	0.39±0.03[abcd]	3.64±0.24[ab]	3.27±0.13[b]
T4 - NC + $CaCO_3$	3.19±0.34[a]	0.36±0.07[bcd]	3.61±0.32[ab]	3.11±0.12[b]
T5 - MS	2.96±0.10[b]	0.30±0.06[d]	2.94±0.29[c]	3.09±0.09[b]
T6 - NPK mineral fertilizer + MS	3.24±0.16[a]	0.43±0.04[abc]	3.69±0.41[ab]	3.77±0.20[a]
T7 - NC fertilizer + MS	3.25±0.16[a]	0.46±0.02[a]	3.82±0.30[a]	3.82±0.18[a]
P value	NSD	*	*	***
LSD (0.05)	0.446	0.108	0.547	0.227

*means ± standard deviation; LSD - least significant difference; NSD - no significant difference at P=0.05; *, **, *** - statistical significant differences at P<0.05, P<0.01 and P<0.001, respectively; values followed by the same letter in a column are not significantly different at P<0.05.

The nature of applied treatments and their combinations have an impact on trace elements accumulation, their mobility and storing capacity in plant tissues (Riesen and Feller, 2005). Some of them may pose a toxicity threat if present at elevated levels as their availability and mobility increases under acidic conditions (Pawlowski, 1997). The concentration of trace elements in dill aerial parts shows that there are statistically highly significant differences (*P* ***) between different treatments at P<0.05 for Zn and Cu, and no significant differences (NSD) for Fe and Cd (Table 5). It should be noted that there was not found higher accumulation of Fe in tested plants in the treatments where metallurgical slag was applied in spite of its significant content in this raw material. According to the reference values of Kloke *et al.* (1984), the content of Cd was within the safety limits and permissible levels in all the treatments, which is a highly desirable outcome (Table 4).

Table 4. Effect of applied treatments on the content of trace elements in dill biomass

Treatments	Trace elements (% of dry biomass)			
	Fe	Zn	Cu	Cd
T1 - control (untreated soil)	98.10±2.01^{a}	48.58±2.02^{de}	6.88±0.13^{d}	0.28±0.09^{b}
T2 - $CaCO_3$	108.61±7.38^{a}	60.22±8.63^{c}	7.67±0.45^{c}	0.39±0.09^{ab}
T3 - NPK mineral fertilizer + $CaCO_3$	115.07±25.92^{a}	63.56±3.70^{abc}	8.30±0.26^{abc}	0.37±0.07^{ab}
T4 - NC + $CaCO_3$	100.49±0.46^{a}	46.68±1.81^{e}	8.25±0.15^{abc}	0.35±0.09^{ac}
T5 - MS	107.12±3.56^{a}	69.77±3.03^{a}	8.45±0.08^{a}	0.44±0.06^{a}
T6 - NPK mineral fertilizer + MS	107.32±13.60^{a}	60.93±4.64^{bc}	8.24±0.12^{abc}	0.40±0.08^{ab}
T7 - NC fertilizer + MS	102.63±8.19^{a}	56.43±4.91^{cd}	8.33±0.23^{ab}	0.30±0.05^{ab}
P value	NSD	***	***	NSD
LSD (0.05)	18.105	7.509	0.577	0.131
Reference value				
Normal	50[1]	15[3]	3[3]	<0.1-1[3]
Critical	250[1]	150[2]	15[2]	5[2]
MPL	600[2]	200[2]	20[2]	10[2]

*means ± standard deviation; LSD - least significant difference; NSD - no significant difference at P=0.05; *, **, *** - statistical significant differences at P<0.05, P<0.01 and P<0.001, respectively; values followed by the same letter in a column are not significantly different at P<0.05; MPL - maximum permissible levels; literature source: [1]Schulze *et al.* (2005), [2]Kastori *et al.* (1997), [3]Kloke *et al.* (1984)

Conclusions

The results of the paper indicate that the treatments with combination of metallurgical slag and organic and mineral fertilizer, respectively, showed positive effects on the content of main and beneficial biogenic macroelements in aerial biomass of dill, particularly in relation to control. Improved organic and mineral nutrition in combination with metallurgical slag would explain the promotion of dill biomass growth which led to the promotion of its yield, as the data on yield of dill were in accordance with chemical ones. The contents of trace elements in herb, including toxic Cd, comparing to non-fertilized treatment were not significantly increased and were within the permissible levels in plants in all the treatments in spite of their higher content in metallurgical slag. Generally, the studied metallurgical slag of the standardized chemical composition can be recommended for the wider practice toward improving the productivity of highly acid soils such as Stagnosol.

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ASSESMENT OF THE POSSIBILITIES FOR ALTERNATIVE WEED CONTROL IN SOYBEAN

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Abstract

The aim of the present study is to assess the possibilities for an alternative weed control in soybeans by using cover crops with proven allelopathic potential as oats and rye. The study was conducted in the period 2015-2017 on the Experimental Field of the Soybean Experimental Station in Pavlikeni (Bulgaria). Weed communities in the studied soybean agrophytocenosis ranged between 5 to 8 species and practically does not change under the influence of meteorological factors. Weeding was of a mixed type with a prevalence of one-year late spring weeds (58% - 92%), which were also identified as dominant weed species. The values of the diversity indices and distribution uniformity of the weed species are negligibly influenced by the dynamics of the weather conditions. It was found that under the specific conditions of the experiment, the survival capacity of the soybean plants to the technical maturation phase was in the range of 0.76 to 0.96. The ability to self-restore of soybean was relatively good (from 1.16 to 5.09) and a slight increase in the soybean population (from 0.15 to 1.63) was observed. The use of oats as an allelopathic active cover crop in soybean reduces the weed infestation rate from 33.6% to 67%, as well as the amount of fresh and dry biomass accumulated in the late spring weed group by 12% to 68%. The capping ability is the result of limiting the density of some annual weeds (*Amaranthus* spp., *Abutilion theophrasti* Medik., *Chenopodium album* L.), although compensatory processes in the population density of other weed species. The use of rye as an allelopathic active cover crop in soybean reduces the weed infestation from 49% to 63% with the increasing of the rye sowing rate when compared to the control ($p<0.05$).

Keywords: *soybean, oats, rye, allelopathy, weeds*

Introduction

Weed management takes an important place in soybean cultivation, where herbicides represent the biggest part of the applied pesticides. In last decades, the chemical method for weed control is a main element into the management strategies for weed density regulation due to its rapid action and easy application (Georgiev *et al.*, 2008; Koger *et al*., 2002). Significant problems are a rich variety of weed species, high biological and ecological plasticity, resistance to herbicides, leading to their fast expansion (Nakova, 2003; Heap, 2007). Some significant changes in the weed associations under the influence of many factors revealed to the search for new alternative methods and resources for weed control which are non-destructive to the environment (Hwu *et al*., 1999; Duke *et al*., 2000; Fujinaria and Yoshida, 2000). Many scientific publications recommend the development of non-chemical approaches for weed management, including allelopathic active cover crops or intercropping. Main findings are related to the significant weed-suppressing ability of *Hordeum spp., Avena spp., Secale spp*. and their use as an effective method for biocontrol against weeds in many important forage crops, and also to the economic benefits of such usage (Stancheva, 2000; Bhowmik and Inderjit, 2003; Dimitrova, 2008). Insufficient and extremely limited are studies on the effect of allelopathic and / or cover crops on the growth and reproductive forms of various grain legumes, including soybean (Camal-Maldonado *et al*., 2001; Vasilev and Kertikov, 2003; Adler and Chase, 2007). Aim of the present study is to assess the possibilities for alternative weed control in soybean (*Glycine max* (L.) Merill) by using cover crops with proven allelopathic potential as oats (*Avena sativa* L.) and rye (*Secale cereale* L.).

Material and Methods

The study was conducted in the period 2015-2017 on the Experimental Field of the Soybean Experimental Station in Pavlikeni (Bulgaria). Each experimental variant was set in four replications with an area of the harvested plot of 5 m^2, respectively. Object of research was soybean (*Glycine max* (L.) Merill), Srebrina variety) and the sowing was mechanized at a seed density of 280 000-300 000 germinating seeds/hectare and a distance of 70 cm between rows (Georgiev *et al*., 2008). Evaluation of natural background of weed infestation was based on the dynamics of weed density in the soybean agrophytocenosis. The level of weeding and the species composition of weeds have been quantified at the end of the vegetation of the crop and were presented in number individuals on m^2. The values of the diversity indices (H) and distribution uniformity (J) of the weed species were calculated on the basis of the experimental data following the method of Begon *et al*. (1986). Aggregation and intra-population distribution of weed species ($S\sqrt{x}$) was assessed according to Odum (1975). The degree of aggregation of weeds was determined by comparing the groups of different sizes with the theoretical distribution of Poisson, which shows the frequency of groups of different sizes for each unit of investigation (Sendecor, 1961).

Assessment of the survival capacity of the soybean plants under the natural background of weed infestation have been made at the end of each vegetation period. Structural elements of yield (number of beans per plant; number of seeds per plant; mass of seed per one plant) were determined according to the methodology of Stoimenova (1990). The ability to self-restore of soybean agrophytocenosis under the natural background of weed infestation was evaluated using the methodology of Begon *et al*. (1986). Assessment of the possibilities for alternative weed control in soybean using oats (*Avena sativa* L.) and rye (*Secale cereale* L.) as an allelopathic active cover crop was made in mixed soybean-oats and soybean-rye agrophytocenosis, respectively. Sowing of the cover crop was made in parallel with sowing of the soybean, but in perpendicular direction and a distance of 10 cm between rows. Three experimental sowing rates of oats/rye were applied – 12.5%, 25% and 50% of the standard sowing rate (180 kg/ha). Weed composition, weed density and other indices in mixed agrophytocenosis were determined as described above. For the determination of the relative index of competition (RCI) between weed and soybean plants, the formula of Goldberg *et al*. (1999) was used. Raw experimental data of the study were processed (descriptive statistics, t-test, $p<0,05$) by the statistical package Statistica 7.0 (StatSoft Inc., 2004).

Results and Discussion

Evaluation of natural background of weed infestation

Identification of the main patterns in weed associations dynamics in soybean was a key element of the theoretical basis for the development of integrated weed control systems defined by the specific conditions. The level of total weed infestation varied considerably over the years - from 62 to 486 individuals per m^2, depending on the quantity and distribution of rainfall during the growing season. In years with relatively unfavourable conditions, total weed infestation was in the diapason 62 to 363 individuals per m^2, and in years with higher precipitation the weed density ranges from 249 to 486 individuals per m^2 (Table 1). The increase in total weed infestation of soybean plots was mainly due to the increased density of *Sorghum halepense* (L.) Pers. (number of stems per unit area) and was not so expressed to the annual and dicotyledonous weed species (*Amaranthus retroflexus* L., *Chenopodium album* L., *Setaria* spp.). The species composition of weed communities (S) varies in relatively narrow ranges of 5 to 8 weed species and practically does not change under the influence of meteorological factors. The weed diversity index (H) in the studied agrophytocenosis also varied in relatively narrow ranges and was independent of the amount and distribution of rainfall during the study period with an average value of 0.920, assuming values in the range of 0.835 to 1.035. Weeding was of a mixed type with a prevalence of one-year late spring weeds (58% - 92%), which were also identified as dominant weed species. The values of distribution uniformity (J) of the weed species was negligibly influenced by the dynamics of the weather conditions with an average of 0.508, ranging in the diapason from 0.428 to 0.578. Dominant weed species (over 15%) in the studied soybean agrophytocenosis were *Solanum nigrum*, *A. retroflexus* and *S. halepense*, while subdominant weed species (from 5% to 15%) was *Setharia* spp. We found that was the degree of weed aggregation ($S\sqrt{x}$) was in accordance with the quantitative ratio (P_i) of weed species. Dominant (over 15%) and subdominant (5-15%) weed species, forming the main infestation in the studied agrophytocenosis, have group distribution ($S\sqrt{x}$<1), while the secondary (0.1% to 5%) and the tertiary (0.1%) weed species were evenly distributed (($S\sqrt{x}$>1) during the whole period of our study ($p<0.05$). Analysis of the results obtained showed that species composition (S), diversity index (H) and distribution uniformity (J) in weed communities changed negligible during the three-year study period, despite the dynamics of meteorological factors. This fact could be very useful in practice as allow the weeds to be experimentally determined in a shorter period of time and more efficient weed control systems to be developed and applied.

Table 1. Dinamics of weed density in studied soybean agrophytocenosys

Year	Weed species	Number/m^2	P_i	$S\sqrt{x}$	S	H	J
2015	*Abutilon theophrasti* Medic.	0,2	0,003	0,03	6	-1,035	-0,578
	Amaranthus retroflexus L.	12,7	0,206	106,9			
	Chenopodium album L.	0,5	0,008	0,03			
	Hibiscus trionum L.	0,1	0,005	0,05			
	Solanum nigrum L.	36,4	0,589	12,6			
	**Setaria spp.*	11,6	0,189	6,5			
2016	*Amaranthus retroflexus* L.	67,6	0,552	76,4	5	-0,835	-0,519
	Chenopodium album L.	4,0	0,033	0,41			
	Hibiscus trionum L.	0,1	0,001	0,05			
	Solanum nigrum L.	50,4	0,411	73,3			
	**Sorghum halepense* (L.) Pers.	0,5	0,004	0,04			
2017	*Abutilon theophrasti* Medic.	1,0	0,004	0,21	8	-0,889	-0,428
	Amaranthus retroflexus L.	9,3	0,038	70,9			
	Chenopodium album L.	1,3	0,001	4,1			
	Datura stramonium L.	1,7	0,007	0,37			
	Hibiscus trionum L.	1,0	0,004	0,21			
	Solanum nigrum L.	9,4	0,039	26,5			
	**Setaria spp.*	47,0	0,188	53,0			
	**Sorghum halepense* (L.) Pers.	178,7	0,717	142,2			

Assessment of the survival capacity of the soybean plants and the ability to self-restore of soybean agrophytocenosis under the natural background of weed infestation

Many authors have shown that the comparatively low competitiveness of soybean against weeds was predetermined by the slow growth in the first stages of its development (Lyubenov, 1988; Stoimenova, 1989). Studies by Stoimenova (1987), Alexieva and

Stoimenova, (2004) and Bensch *et al.* (2003) have shown that when the soybean stand was infested with *A. retroflexus* and other late-spring weed species by the end of crop growth, than the structural elements of the soybean yield were decreased by 29.3% to 73.2%.
It was found that under the specific conditions of the experiment, the survival capacity (l_x) of the soybean plants to the technical maturation phase was in the range of 0.86 to 0.96 in the years with favourable meteorological conditions, decreasing to 0.76 in the years with unfavorable ones. The intensity of soybean dying (K_x) ranged from 0.02 to 0.14, with dependence on both the degree of weed infestation and the agrometeorological conditions. In the years with rainfall, it took values from 0.02 to 0.14, and in the case of drought - 0.12. The higher intensity of soybean dying in the more favorable year (2015) may be explained by the higher degree of weed infestation of the crop combined with an increase in weed density in the studied agrophytocenosis. In the studied period, the degree of weed infestation in soybean stands from phenophase sprouting to phenophase technical maturity had a negative impact on the structural elements of soybean yield, with statistical reliability at $p <0.05$ for all tested parameters. The correlations between yield and precipitation ($r = 0.758$-0.998) and between yield and weed density ($r = 0.836$-0.999) were very high ($p<0.05$). The ability to self-restore of soybean was relatively good (from 1.16 to 5.09) and a slight increase in the soybean population (from 0.15 to 1.63) was observed. Regardless of the relatively high reproduction rates (R) of soybean plants grown under the natural background of weed infestation, a significant reduction of the structural elements of the yield was found as follows: number of beans per plant - 78.7%; number of seeds per plant - 83.3%; mass of seed per one plant - 67.3% ($p <0.05$). So, it could be concluded that weeding and agrometeorological conditions were the most significant limiting factors for the development of the soybean crop, as well as the most important factors for the quantity of the yield.

Assessment of the possibilities for alternative weed control in soybean using oats as an allelopathic active cover crop

The use of oats as a cover crop affected the dynamics and extent of weed infestation in the studied soybean agrophytocenosis. From the soybean germination to phenophase R2, the weed suppressing effect of oats was in the range of 33.6% to 38.4%, disproportionate to its increased sowing rate, at a relative competition index (RCI) of 0.62 to 0.66 (Table 2). By prolonging the vegetation period to phenophase R4, the weed suppressing effect of oats increased significantly and ranged from 64.7% to 67.0% while the RCI was in the range of 0.33 to 0.35 ($p<0.05$). An exception was found in the soybean phenophase R8 at the lower sowing ratios of oats (12.5% and 25%) where the differences reliably exceed the same compared to the control variant ($p = 0.05$). Reducing the degree of weed infestation was found to be a result of the elimination of *S. halepense* (L.) Pers., *Echinochloa crus-galli* (L.) Beauv., *Setaria spp*. and the inhibition of late-spring weeds density (*Amaranthus spp., Abutilon theophrasti* Medik*., Ch. album* L.), although some compensatory processes at the population's density of *Convolvulus arvensis* L. have been established. The species composition (S) of weed communities in the mixed soybean-oats agrophytocenosis varied from 4 to 8, while the weed diversity index (H) varied from 8.4 to -21.2 with a distribution uniformity (J) of -4.2 to -10.2. The experimental data obtained were consistent with the published results of Stancheva (2000) and Dimitrova and Serafimov (2007a, b), according to which the compaction of crops is an agro-technical approach to biological control of weeds. The weed suppressing capacity of the cover crops is due to their phytotoxic compounds that act inhibitory on the growth of weeds (Fujinaria *et al*., 2000; Dimitrova and Serafimov, 2009).

Table 2. Weed parameters in mixed soybean-oats agrophytocenosys

Development from emergence (VE) to	Experimental variants	Parameters											
		P	$\%K_{nv}$	RCI	cm^{-1}	$\% K_{nv}$	RCI	g_{fb}^{-1}	$\% K_{nv}$	RCI	g_{db}^{-1}	$\% K_{nv}$	RCI
Flowering R2	K_{nv}	117,4d	100,0	0	33,0c	100	0	19,8c	100	0	4,6c	100	0
	12,5%	40,9c	34,8	0,65	41,7d	126,4	-0,26	25,0d	126,3	-0,26	4,9d	196,5	-0,07
	25%	45,1b	38,4	0,62	28,7b	87,0	0,13	13,4b	67,7	0,32	2,9b	63,0	0,37
	50%	39,5a	33,6	0,66	24,6a	74,5	0,25	10,9a	55,1	0,45	2,4a	52,2	0,48
Pod formation R4	K_{nv}	135,6d	100,0	0	67,7c	100	0	22,9d	100	0	2,6a	100	0
	12,5%	90,8c	67,0	0,33	36,9b	54,5	0,45	12,8c	55,9	0,44	3,0a	115,4	-0,15
	25%	89,8b	66,2	0,34	36,1a	53,3	0,47	7,4a	32,3	0,68	1,9a	73,1	0,27
	50%	87,7a	64,7	0,35	36,1a	53,3	0,47	8,1b	35,4	0,65	2,3a	88,5	0,12
Technical ripeness R8	K_{nv}	116,2b	100,0	0	82,8d	100	0	36,0d	100	0	5,6b	100	0
	12,5%	139,8b	120,3	-0,2	79,1c	95,5	0,05	24,8c	68,9	0,31	4,6ab	82,1	0,18
	25%	121,3c	104,4	-0,04	47,3b	57,1	0,43	31,5c	87,5	0,12	3,3ab	58,9	0,41
	50%	87,8a	75,6	0,24	30,4a	36,7	0,63	24,7a	68,6	0,31	2,9a	51,8	0,48

Assessment of the possibilities for alternative weed control in soybean using rye as an allelopathic active cover crop

The analysis of the experimental results from 2015 (high amount of rainfall) showed that in the period from sprouting to phenophase R2 the level of the weed infestation in mixed soybean-rye stand significantly decreased from 49% to 63% with the increasing of the rye sowing rate when compared to the control ($p<0.05$). By prolonging the vegetation period to the phenophase R4 and phenophase R8 of soybean, the weed suppression capacity of rye was maintained and the relative index of competition (RCI) was in the range of 0.21 to 0.78 ($p<0.05$). An exception was found in the soybean phenophase R8 at the lowest rye sowing rate where the weed density of experimental mixed stand statistically exceeds that of the control variant and the RCI has a negative value of -0.04 (Table 3).

Table 3. Weed parameters in mixed soybean-rye agrophytocenosys

Development from emergence (VE) to	Experimental variants	Parameters											
		cm^{-1}	$\%K_a$	RCI	LER	g_{fb}^{-1}	$\% K_a$	RCI	LER	g_{db}^{-1}	$\% K_a$	RCI	LER
Flowering R2	K_a	46,0b	100	0	-	50,4b	100	0	-	10,3b	100	0	-
	K_{nv}	37,0ab	80,4	0,2	-	35,2ab	69,8	0,3	-	7,9ab	76,7	0,23	-
	12,5%	34,5a	75	0,25	1,55	28,3a	56,2	0,44	1,26	4,5a	43,7	0,56	1,2
	25%	32,1a	69,8	0,3	1,5	24,5a	48,6	0,51	1,18	5,0a	48,5	0,51	1,25
	50%	31,8a	69,1	0,31	1,5	21,5a	42,7	0,57	1,13	3,5a	34	0,66	1,11
Pod formation R4	K_a	67,7b	100	0	-	104,4b	100	0	-	27,2b	100	0	-
	K_{nv}	54,9ab	81,1	0,19	-	32,5a	31,1	0,69	-	8,3ab	30,5	0,69	-
	12,5%	60,2ab	88,9	0,11	1,7	48,7a	46,6	0,53	0,78	13,1a	48,2	0,52	0,79
	25%	57,7ab	85,2	0,15	1,66	40,5a	38,8	0,61	0,7	10,8a	39,7	0,6	0,7
	50%	48,3a	71,3	0,29	1,52	26,6a	25,5	0,75	0,57	7,4a	27,2	0,73	0,58
Technical ripeness R8	K_a	75,2b	100	0	-	29,3b	100	0	-	26,9b	100	0	-
	K_{nv}	56,5ab	75,1	0,25	-	4,4a	15	0,85	-	4,0a	14,9	0,85	-
	12,5%	57,6ab	76,6	0,23	1,52	7,8a	25,9	0,74	0,41	6,9a	25,7	0,74	0,41
	25%	52,5ab	69,8	0,3	1,45	5,0a	17,1	0,83	0,32	4,6a	17,1	0,83	0,32
	50%	49,1a	65,3	0,35	1,4	5,4a	18,4	0,82	0,33	4,9a	18,2	0,82	0,33

Weed suppressing capacity of the rye in 2016 at the sum of the rainfall close to the mean annual value (I = 20.9) was strongest to the phenophase R2 ($p<0.05$). By prolonging the vegetation period to the phenophase R4, the allelopathic inhibitory effect of the rye has decreased and the differences were significant only at the higher sowing rates of rye (25% and 50%) at a relative index of competition (RCI) of 0.16 to 0.26 ($p<0.05$). In phenophase R8 of soybean the weed infestation level of mixed soybean-rye agrophytocenosis significantly exceeded that of the control variant ($p<0.05$) and the RCI had a negative value ranging from -0.34 to -0.48 with the exception of the highest rye sowing rate of 50%, where RCI = 0.29.

Reducing the degree of weed infestation during the early stages of the development and formation of mixed soybean-rye agrophytocenosis could be explained by the presence of some residual amounts of the glycoalkaloid 2,4-dihydroxy-1,4(H)-benzoazin-3-one (DIOBA) separated from the roots of the rye and/or the presence of 2,3(H) –benzoxazinilone, derived from DIOBA degradation. Many authors have shown that these glycoalkaloids act as strong inhibitors of the germination and initial development of some mono- and dicotyledonous weed species (Yenish *et al*., 1995, Creamer *et al*., 1996, Weston, 1996; Reddy, 2001).

Conclusions

Use of oats as an allelopathic active cover crop in soybean reduces the weed infestation rate from 33.6% to 67%, as well as the amount of fresh and dry biomass accumulated in the late spring weed group by 12% to 68%. Capping ability is the result of limiting the density of some annual weeds (*Amaranthus* spp., *Abutilion theophrasti*, *Chenopodium album*), although compensatory processes in the population density of other weed species. The use of rye as an allelopathic active cover crop in soybean reduces the weed infestation from 49% to 63% with the increasing of the rye sowing rate when compared to the control ($p<0.05$).

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ALLELOPATIC EFFECT OF FIVE WEED SPECIES ON SEED GERMINATION OF SORGHUM CROPS

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Abstract

Aim of this study was to evaluate the allelopathic effect of five invasive weeds (*Sorghum halepense* L. Pers., *Sonchus arvensis* L., *Cirsium arvense* L. Scop., *Xanthium strumarium* L.and *Aristolochia clematitis* L.) in forage crops on two *Sorghum* crops (*Sorghum sudanense* (Piper) Stapf and *Sorghum vulgare* var. *technicum* (Körn.)). An ex-situ experiment was carried out as follows: 10 seeds of each test variety were placed in Petri dishes between filter paper and the dried weed biomass extract was added at concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2% w/v. Petri dishes were placed in a thermostat-operated device at a temperature of 22 ± 2°C for a seven-days period. Distilled water was used as a control. Number of germinated seeds, % of seed germination against the control, and weed inhibition rate (IR) were measured. Our study revealed that the alellopathic effect of the tested five invasive weeds on seed germination of *S. sudanense* and *S. vulgare* var. *technicum* varied according to the plant species (both weed and cultural) and the concentration applied. Most pronounced negative effect on the germination process of *S. sudanense* seeds have expressed the cold aqueous extracts from *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop. – IR varied from 4.67-5.0% up to 70.3% at the highest test concentrations ($p<0.05$). Maximal inhibition of seed germination of *S. vulgare* var. *technicum* in comparison with the control was found at 3.2% w/v extract of *Sorghum halepense* (L.) Pers. (IR=58.4%), followed by the two highest concentrations of *Xanthium strumarium* L. extract (IR=41.6-45.9%).

Keywords: *interaction, allelopathy, seed germination, inhibition rate*

Introduction

Sorghum, originated in East Africa, is one of the first crops, which was taken into cultivation (House, 1985). At present, *Sorghum sudanense* hybrids are preferred as a fodder due to higher yields and tillering ratios and also their thin stems and higher leaf ratios (Uzun *et al.*, 2009). *Sorghum vulgare* var. *technicum* is mainly utilized for producing brooms, washing brushes, knittings, paper, wallboard, fences, biodegradable materials for packaging due to their peculiar resistance (Popescu and Condei, 2014). Sorghum cultivar plants are more tolerance to drought and high temperatures, diseases, pests, various soil types and show higher water use efficiency and, higher production capacity per unit area (Undersander and Lane, 2003; Uzun and Cigdem, 2005). Main disadvantage of Sorghum crops is their high sensitivity against weed infestation in the first 30-40 days after sowing (Marinov-Serafimov and Golubinova, 2015). Drastic effects of weeds on germination and growth on *Sorghum* and other crops are due to their competiveness with cultivated crops for resources and allelopathic potentials. Through allelopathy, weeds may cause significant effects on the growth and germination capacity of other crops. Weeds may possess diverse allelochemicals, which can interfere with other plants in a number of ways by either retarding or enhancing the germination and growth of receiving plants (Oerke, 2006; Marinov-Serafimov and Golubinova, 2015).

The summarized results of the experimental work of Cheema *et al*. (2002; 2008), Alsaadawi and Dayan (2009), Jesudas e*t al*. (2014), Głąb *et al*. (2017), Jabran (2017) show that the skrining to determine the allelopathic effect of species of the genus Sorghum on germination and initial development of a number of weed species has been well performed. Allelopathic interference in the system "weed - Sorghum crop" has been poorly studied (Shahrokhi *et al*., 2011; Asghar *et al*., 2013; Dafaallah and El-Twom, 2017), necessitating the establishment of allelopathic tolerance of Sorghum crops towards invasive weed species.
Aim of this study was to evaluate the allelopathic effect of five invasive annual and perennial dicotyledonous weed on germination and initial development at *Sorghum sudanense* (Piper) Stapf and *Sorghum vulgare* var. *technicum* (Körn.) in order to determine species sensitivity which could serve as a means of increasing efficiency in the early stages of the selection process.

Material and Methods

Collection of weed material and preparation of cold aqueous extracts

Aboveground biomass from five invasive weed species (*Sorghum halepense* (L.) Pers., *Sonchus arvensis* L., *Cirsium arvense* (L.) Scop., *Xanthium strumarium* L., *Aristolochia clematitis* L.) in forage crops was collected in a natural environment of weed infestation in the Institute of Forage Crops, Pleven at BBCH 51-55 (Hess *et al*., 1997). No separated aboveground biomass of available weed species was chopped together to the length of 0.5-3.0 cm, drying to a constant dry weight at 50 ± 5°C and was grind in a grinder Retsch SM – 1 at a sieve size of 1.0 mm. Cold aqueous extracts of dry weed aboveground biomass were prepared as follows: 100 g of dry weed biomass were soaked in 1 l distilled water and leaved at a temperature of 24 ± 20C for 24 h in a shuttle apparatus at 240/60 c^{-1}. After that they were decanted, filtered through filter paper and centrifuged in K24 centrifuge at 5000/60 s^{-1}. From these stock solutions were prepared test extracts of each weed plant with concentrations of 0.1%, 0.2%, 0.4%, 0.8%, 1.6% and 3.2% w/v, respectively. The experimental data were evaluated using analysis of variance with means separation based on Fisher's least significant difference test at $p<0.05$ with the software Statgraphics Plus for Windows Ver. 2.1 and Statistica Ver. 10.

Experimental design

Ex-situ experiment was carried out as follows: 10 seeds of *S. sudanense* (300/43 mutant form) or *S. vulgare* var. *technicum* (local population), respectively, were placed into Petri dishes between paper and 10 ml weed test extract and one of the above mentioned concentrations were added. Seeds of the tested *Sorghum* genotypes were provided from the Selection Collection of the Institute of Forage Crops in Pleven, Bulgaria. Distilled water was used as a control. Each treatment consisted of three replicates including the control treatment. Samples were placed in a thermostat-operated device at 22 ± 2°C for 7 days and the number of germinated seeds, % of seed germination against the control, and weed inhibition rate, were measured. Percentage of seed germination was calculated after preliminary arcsin-transformation following the formula $Y=\arcsin\sqrt{(x\%/100)}$, forwarded by Hinkelman and Kempthorne (1994), and to induce half-maximal inhibition of growth (LC50) and P=0.05 confidence intervals were calculated according to Hamilton *et al.* (1977). Inhibition rate (IR) of tested weeds was calculated by the formula of Ahn and Chung (2000): $IR= 100 \times (n_{control} – n_{experiment})/n_{control}$, where n is the number of the germinated seeds. All collected data were analyzed using the software Statgraphics Plus for Windows Ver. 2.1 and Statistica Ver. 10.

Results and Discussion

Seed germination and seedling development are increasingly used in various bioassays as they allow the biological activity of various substances (herbicides, metabolites, allelochemicals

and even radionuclides) to be established with high reliability and low cost (Piotrowicz-Cieślak *et al.*, 2010). Our study revealed that the alellopathic effect of the tested five invasive weeds on seed germination of *S. sudanense* and *S. vulgare* var. *technicum* varied according to the plant species (both weed and cultural) and the concentration applied (Table 1).
When regarding the experiments with seeds of *S. sudanense*, it was obvious that the most pronounced negative effect on the germination process expressed the cold aqueous extracts from *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop. – IR varied from 4.67-5.0% up to 70.3% at the highest test concentrations ($p<0.05$). Lowest inhibition was found under the extract from *Xanthium strumarium* L., where the % germination against the control was in the range between 63.0% - 92.56% and the IR had values from 7.4% to 37.0%. The extracts of the other two weed species - *Sorghum halepense* (L.) Pers. and *Aristolochia clematitis* L., also have medium expressed negative effect on the seed germination of *S. sudanense*, with the maximal values of IR=44.4% at 1.6% w/v concentration (Table 1).

Table 1. Allelopathic effect of five studied weed species on the seed germination of *S. sudanense* and *S. vulgare* var. *technicum*

Weed species	Concentration (w/v)	*Sorghum sudanense* (Piper) Stapf		*Sorghum vulgare* var. *technicum* Körn.	
		% germination against the control	Inhibition rate (IR)	% germination against the control	Inhibition rate (IR)
Sorghum halepense (L.) Pers.	0.1%	85.22cd	14.8	66.63c	33.4
	0.2%	81.44c	18.6	95.88e	4.1
	0.4%	81.44c	18.6	87.50d	12.5
	0.8%	92.56d	7.4	62.50bc	37.5
	1.6%	55.56a	44.4	58.38b	41.6
	3.2%	70.33b	29.7	41.63a	58.4
Sonchus arvensis L.	0.1%	85.22e	14.8	70.88bc	29.1
	0.2%	85.22e	14.8	62.50a	37.5
	0.4%	74.11d	25.9	62.50a	37.5
	0.8%	48.11c	51.9	87.50d	12.5
	1.6%	33.33b	66.7	66.63b	33.4
	3.2%	29.67a	70.3	62.50a	37.5
Cirsium arvense (L.) Scop.	0.1%	85.22e	14.8	83.38d	16.6
	0.2%	85.22e	14.8	87.50d	12.5
	0.4%	74.11d	25.9	58.38a	41.6
	0.8%	48.11c	51.9	66.63b	33.4
	1.6%	33.33ab	66.7	66.63b	33.4
	3.2%	29.67a	70.3	70.88c	29.1
Aristolochia clematitis L.	0.1%	85.22cd	14.8	79.13b	20.9
	0.2%	81.44c	18.6	100.00d	0.0
	0.4%	81.44c	18.6	79.13b	20.9
	0.8%	92.56d	7.4	75.00b	25.0
	1.6%	70.33b	29.7	91.63c	8.4
	3.2%	55.56a	44.4	58.38a	41.6
Xanthium	0.1%	85.22d	14.8	66.63c	33.4

strumarium L.	0.2%	92.56e	7.4	79.13d	20.9
	0.4%	74.11b	25.9	83.38e	16.6
	0.8%	77.78c	22.2	87.50f	12.5
	1.6%	63.00a	37.0	54.13a	45.9
	3.2%	77.78c	22.2	58.38b	41.6
Means followed by the same letter within column are not significantly different at ($p<0.05$)					

Considering the results with seeds of *S. vulgare* var. *technicum,* it was obvious that the negative effect on the germination process as a whole was not so strong in comparison with the *S. sudanense* seeds ($p<0.05$). Maximal inhibition of seed germination in comparison with the control was found at 3.2% extract of *Sorghum halepense* (L.) Pers., followed by the two highest concentrations of *Xanthium strumarium* L. extract. Least pronounced was the negative effect of the extract from *Aristolochia clematitis* L., where in all studied concentrations the germination was close to 80% and above. The extract of *Cirsium arvense* (L.) Scop. had the IR values in the range from 12.5% up to 41.6% and was quite similar to the extract of *Xanthium strumarium* L. with IR values in the range 12.5% - 45.9%. only in the experiment with *Sonchus arvensis* L. extract we could not observe some significant dynamics of the inhibitory effect related to the concentration variations. Germination % against the control varied from 62.50% to 87.50% and the IR was close to 37.5% in almost all cases (Table 1).

Seed germination is a critical phase of plant development (Ernst, 1998). This process begins with the uptake of water from the dry seed and its swelling and ends with the germination of the germ root through all the seed coatings (Bewley, 1997). Germination inhibition is one of the most studied effects of toxic exposure to heavy metals (Ernst, 1998; Sfaxi-Bousbih *et al.*, 2010), herbicides and biologically active substances, including allelochemicals (Kalinova *et al.*, 2012; Marinov-Serafimov *et al.,* 2017).

On the basis of their inhibition rate on the *S. sudanense* seed germination the five tested weed species could be ranked as follows:

1 group – IR<29.99%: *Xanthium strumarium* L. (IR average=21.59%), *Sorghum halepense* (L.) Pers. (IR average=22.24%) and *Aristolochia clematitis* L. (IR average=22.24%)

2 group – 30%<IR<49.99%: *Sonchus arvensis* L. (IR average=40.72%) and *Cirsium arvense* (L.) Scop. (IR average=40.72%)

On the basis of their inhibition rate on the *S. vulgare* var. *technicum* seed germination the five tested weed species could be ranked as follows:

1 group – IR<29.99%: *Aristolochia clematitis* L. (IR average=19.46%), *Cirsium arvense* (L.) Scop. (IR average=27.77%) and *Xanthium strumarium* L. (IR average=28.48%)

2 group – 30%<IR<49.99%: *Sorghum halepense* (L.) Pers. (IR average=31.25%) and *Sonchus arvensis* L. (IR average=31.25%).

This could be explained by the presence of glycoalkaloids, various phenolic acids (vanilla, syringe, ferulic, N-coumaric, etc.), condensed tannins, cyanoglycosides and hydrophobic p-benzoquinone, which are concentrated in the aboveground biomass of the studied weed species. Glycoalkaloids are known to have a highly toxic effect, with higher concentrations causing a lethal effect on germination of wheat seeds, while lower ones inhibit germination to varying degrees (Agarwal *et al.*, 2002). The differences found between reaction of two studied cultural Sorghum genotypes may also be due to differences in their allelopathic tolerance, since comparisons between them are made under the same conditions (Marinov-Serafimov *et al.*, 2017).

Conclusions

The results of this study are in good agreement with the previous findings that the alellopathic effect of weeds on seed germination varied according to the plant species (both weed and cultural) and the concentration applied. Both studied genotypes showed significantly high allelopathic tolerance to the invasive weed species – 68.1% for *Sorghum sudanense* (Piper) Stapf and 78.3% for *Sorghum vulgare* var. *technicum* Körn. This fact demonstrated that they are suitable for incorporation into various breeding programs as allelopathic tolerance donors. Reading of the allelopathic reaction is proved as fast and effective method for determining plant tolerance at different stages of the selection process.

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IN-DEPTH BRAIN PHOSPHOPROTEOME STUDY REVEALS NEUROBIOLOGICAL UNDERPINNINGS FOR FORAGER HONEYBEE WORKERS (*APIS MELLIFERA LIGUSTICA*)

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Abstract

The forager bees perform the foraging tasks outside the hive in order to enhance the honeybee coloney survival. There is a lack of knowledge about how the neurobiological activities via protein phosphorylation in the brains of forager honeybee workers align with their tasks performances. The phosphoproteome in the brains of forager honeybee workers was characterized using Ti^{4+}-IMAC phosphopeptide enrichment, shotgun proteome, label-free quantitation, and bioinformatics. The identified 916 phosphoproteins in the nurse bee brain were involved in a wide spectrum of biological functions, metabolic pathways and kinase activities, indicating their pivotal roles to drive the brain maintenance, the neurobiological activities, learning, memory, and the cognition of the forager bee brain during navigation autside the hive. The more strongly represented phosphoproteins in the forager brains were strongly involved in the biological pathways of phosphatidylinositol signaling system, inositol phosphate metabolism, phototransduction, glycerophospholipid metabolism, and Wnt signaling. The most enriched kinases in the nurse bee brain were CDK2_CDK3, p38, JNK, ACTR2_ACTR2B_TGFbR2, CK2, CLK, PKC, and PKA, suggesting their vital roles in brain maintenance, signal transduction, and the olfactory learning processes to enhance the foraging activities performance. This study is the first in-depth and comprehensive phosphoproteome report on forager honeybee worker brains and provides novel insights into the molecular details of phosphoproteins that employ protein function to the needs of forager honeybee workers. These data provide basis information for future research to better understand the neurobiological roles of targeted proteins in the forager bee brain.

Key words: *Brain, Forager bees, Honeybee, Phosphoproteome.*

Introduction

The honeybee workers participate in all scenarios of routine activities that the colony needs. The worker's numerous tasks are very precisely organized depend on the division of labor which is largely dependent on age (Page and Peng 2001). Once the transition to foraging is made, bees no longer engage in within-hive tasks (Seeley 2009). Instead, they focus on foraging for the four resources colonies need: propolis, water, pollen, and nectar (Johnson 2010). Of the four, pollen and nectar make up most of the foraging activity, except in periods of heat stress in which water collection can be as labor intensive (Seeley 2009). The interaction within the brain three distinct regions; the mushroom bodies, the optic lobes and the antennal lobes (Menzel and Giurfa 2001) in the response of the environmental signals, generates and drives the forager bees' neurobiological activities during the foraging performance such as cognition, learning and memory. Therefore, to perform the navigation, cognition, learning, memory, and decisions during the foraging activities (Kappeler 2010) , honeybee brain involves varieties of activities in terms of changing the brain chemistry, brain structure, endocrine activity, and temporal patterns of gene and protein expressions (Robinson and Ben- Shahar 2002). Recently, Proteomics studies have become an important to gain in-depth insights and information about the neurobiological mechanisms in the neuro-cell level. To this end, however there is a gap of knowledge about phosphoproteins roles in the neuro-activities in forager bees' brains, the honeybee forager brain phosphoproteome will rather enhance our understanding about the phosphoprotein's roles in the forager brain neurobiological activities during foraging tasks performance.

Materials and methods

Chemical Regents

Unless otherwise specified, all chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). All reagents were analytical grade or better. Modified sequencing grade trypsin was bought from Promega Corporation (Madison, USA). Ti4+-IMAC material was kindly offered by Prof. Zhou H. F. (Dalian Institute of Chemical Physics, Chinese Academy of Sciences). ZipTip C18 column was from Millipore (MA O1821 USA). Protease Inhibitor Cocktail Tablets were from Roche (Mannheim, Germany). Other chemicals not mentioned here are sourced in the text.

Sampling Forager Honeybee Brains and Protein Preparation

We sampled the brains from five colonies of honeybee (*Apis mellifera ligustica*) maintained at the Institute of Apicultural Research, Chinese Academy of Agricultural Science, Beijing, China. 150 foragers were sampled at the entrance of the hives. The worker bees for foragers collecting didn't mark as the neurobiological activities are in link with the task performance. Only those bees returning with a pollen load were sampled to ensure they were really participating in foraging activity. Three biological replicates were produced. The collected bees were immediately anesthetized on ice, and the dissected brains were placed into tubes containing a cocktail solution of protease inhibitors and then stored at -80°C until analyzed.

Protein extraction of honeybee brains was carried out according to our previously described method (Zhang *et al.*, 2012) with minor modifications. Briefly, the sample was mixed with a lysis buffer containing 8M urea, 2M thiourea, 4% CHAPS, 20 mM tris-base, 30 mM DTT (1mg of brain/10μl of buffer). The mixture was homogenized for 30min on ice and then sonicated for 3min and centrifuged at 13,000g at 4°C for 15min. Ice-cold acetone was added to the recovered supernatant at a final concentration of 80% (V/V) and the mixture was kept on ice for 30min for protein precipitation. Subsequently, the mixture was then centrifuged at 13,000g at 4°C for 15min. The supernatant was discarded, and the pellets were used as protein samples for the following analysis. The final protein concentration was determined using Bradford assay.

Trypsin Digestion and Phosphopeptides Enrichment using Ti4+-IMAC

The protein sample was dissolved in 40 mM of (NH4)HCO3 followed by reduced with 100 mM of DTT [protein solution/DTT (V/V, 10:1)] for 1h to prevent reformation of disulfide bonds (Zhang et al., 2012). Then the mixture was alkalized with 100 mM of iodoacetamide (IAA) [DTT/iodoacetamide (V/V, 1:5)] for 1h in the dark. The protein mixture was digested by trypsin (20 ng/μl) in the ratio of 1:62.5 [enzyme/protein (W/W)] at 37°C for 14 h. The digested proteins were centrifuged at 13,500g at 4°C for 10min. The supernatant was recovered for phosphopeptide enrichment.

Phosphopeptide enrichment was performed using Ti4+-IMAC material according to the previously described method with minor modifications (Yu et al., 2009). Specifically, 10 μl of the prepared Ti4+-IMAC were dissolved in 500 μl of binding solution (80% ACN/6% TFA). Then, a 500 μl sample of digested peptide was added to the above mixture and incubated at room temperature for 60min. The mixture was centrifuged at 13,500 g at 4°C for 5 min. The supernatant was discarded and the precipitate was washed with 1ml of washing buffer I (50% ACN/ 6% TFA, NaCl 200 Mm) and washing buffer II (50% ACN /0.1% TFA). Finally, the

phosphopeptides were eluted twice with 200 µl of elution solution (0.5M K2HPO4) and the two fractions were combined.
The enriched phosphopeptides were manually loaded onto Reversed-Phase ZipTip C18 columns (desalting column) for concentrating and desalting prior to MS analysis. The desalted peptides were extracted in a Speed-vac system (RVC 2–18, Marin Christ, Germany) and dissolved in 0.1%formic acid (FA) thereafter.
NanoLC-MS analysis
An 8 µl sample of phosphopeptides was loaded onto a Q Exactive mass spectrometer (Thermo Fisher Scientific, Germany) coupled to EASY-nLC 1000 system using a nanoelectrospray ion source (Thermo Fisher Scientific, Germany) according to our previously established protocol with minor modification (Han et al., 2013). Specifically, reverse phase chromatography was performed using the Thermo EASY-nLC 1000 with a binary buffer system consisting of 0.5% acetic acid (buffer A) and 80% ACN in 0.5% acetic acid (buffer B). The peptides were separated by a linear gradient of buffer B up to 90% in 180min with a flow rate of 350 nL/min in the EASY-nLC 1000 system. The following gradient program was used: from 5 to 8% B in 5min, from 8 to 20% B in 115min, from 20 to 30% B in 40min, from 30 to 90% B in 10min and 90% B for 10min. The LC was coupled to a Q Exactive mass spectrometer via the nanoelectrospray source (Thermo Fisher Scientific, Germany). The Q Exactive was operated in the data dependent mode with survey scans acquired at a resolution of 70 000 atm/z300. Up to the top 10 most abundant isotope patterns with charge ⩾2 from the survey scan were selected with an isolation window of 1.6 Th and fragmented by HCD with normalized collision energies of 25. The maximum ion injection times for the survey scan and the MS/MS scans were 20 and 60 ms, respectively, and the ion target value for both scan modes was set to 1 ×106. Repeat sequencing of peptides was kept to aminimum by dynamic exclusion of the sequenced peptides for 30s.
Database Searching and Mapping Phosphorylation Sites
The raw files were searched against the in-house database generated from protein sequences of Apis mellifera (downloaded April, 2012) and augmented with sequences from Saccharomyces cerevisiae (downloaded April, 2012), totaling 61,380 entries using PEAKS studio software (version 6.0, Bioinformatics Solutions Inc.Canada). The search parameters were: Cysteine carbamidomethylation (C, +57.02) as a fixed modification; and methionine oxidation (M, +15.99), protein phosphorylation (+79.9663 Da) (STY) as variable modifications; enzyme, trypsin; missed cleavages, 2; peptide tolerance, ± 30 ppm; and MS/MS tolerance, ± 0.05 Da. A fusion-decoy strategy was employed to control FDR of protein and peptide identification used the cutoff score of >20 (−10logP) and FDR <1.0%. Scaffold PTM Version 2.0 (Proteome Software, Oregon, USA) was used to estimate the phosphosites localization probability by assigning Ascores (Beausoleil et al., 2006) to each PTM call. All MS/MS queries with an Ascore for every protein having a 95% or better probability were considered.
Label-free Quantitation of Protein Abundance
To evaluate the altered expression level of phosphorylated proteins between the brains of nurse and forager bees, a label-free quantitation was employed using Progenesis LC-MS software (Version 4.1; Nonlinear Dynamics, UK). The raw MS data was imported and processed with the software using a quantify-then-qualify strategy. The following MS feature alignment, editing MS1 spectra, peak modeling algorithm, quality control, peak detection, and statistical analysis of differentially expressed phosphoproteins between the nurse and forager bees' brains followed our recent published protocol (Fang et al., 2014). Then a file of a merged peak list containing differentially expressed phosphoproteins was generated by the software and it was searched against the above search engine, and a protein identification database using the same parameters thereafter. The search results of quantified proteins were

imported into software again to match each signal feature with the best peptide assignment. Similar proteins were grouped and only non-conflicting features were used for quantitation.

Bioinformatics Analysis

Functional annotation of the identified phosphoproteins having at least one site with a 95% localization confidence was performed using Blast2GO Pro v2.6.4 (http: //www.blast2go.com/ b2ghome, BioBam, Valencia, Spain).

Biological pathways were mapped using on-line iPath2.0 software (http://pathways.embl.de/) (Yamada et al., 2011) to navigate and explore the KEGG metabolic pathways of the forager bee brain phosphoproteins on a global and metabolic pathway-centric level.

Phosphorylation is catalyzed by protein kinases (Manning et al., 2002) and these enzymes can be recognized by specific sequence motifs in their substrates (Miller et al., 2008). The significantly enriched phosphorylation motif sets were extracted from all phosphopeptides with confident localized phosphosites (probability ⩾ 95%) using motif-X algorithm (http://motif-x.med.harvard.edu/motif-x.html) (Schwartz and Gygi 2005). The background was the uploaded Apis mellifera proteome (<10M of database size that randomly generated from A. mellifera proteome), the motif width was 13, occurrences were 20, significance was1x10-6, and motifs were extracted separately by pS, pT and pY sites at position 7. The motifs were categorized as basic, acidic, proline-directed, tyrosine, or "other" using a decision tree algorithm described previously (Huttlin et al., 2010). To assign the motifs to known kinases, two programs (http://www.hprd.org/PhosphoMotif_finder (Amanchy et al., 2007) and http://phosida.de/ (Gnad et al., 2007)) were used. All phosphorylation sites were subjected to kinase prediction using NetworKIN 3.0 algorithm (http://networkin.info/version_2_0/newPrediction.php) (Linding et al., 2008) to predict possible upstream kinases. A score threshold of NetworKIN score ⩾ 1.0 and String score ⩾ 0.6 were regarded as significant, and all kinases predictions were used. Since A. mellifera is not included in the NetworKIN database, Drosophila was selected as an alternative.

Results and discussion

Mapping the Phosphoproteome of Forager Brains of the Honeybee worker (*A. m. ligustica*)

In an effort to map the phosphoproteome in the brains of forager honeybee workers, IMAC phosphopeptide enrichment and shotgun proteome strategies were employed. A total of 13,253 phosphopeptides (3,091 nonredundant) from 916 phosphoproteins were detected in foragers with an FDR <1.0% both at the peptide and protein level.

The identified phosphoproteins in forager bees' brains were assigned to the GO categories and the phosphoproteins were classified to 21 functional categories including; regulation of biological processes (32.5%), metabolic processes (27.8%), transport (25.0%), response to stimulus & stresses (24.5%), development (23.9%), cellular component organization or biogenesis (23.3%), cell communication (22.6%), protein metabolic processes (22.6%), localization (22.2%), signaling (21.7%), nucleobase-containing compound metabolic processes (19.3%), cell differentiation (16.6%), signal transduction (16.5%), anatomical structure morphogenesis & development (15.6%), protein modification processes (11.0%), catabolic processes (9.7%), cytoskeleton organization (9.0%), biosynthetic processes (8.8%), cell-cell signaling (8.3%), behavior (7.2%), and unknown (6.9%) (fig. 1).

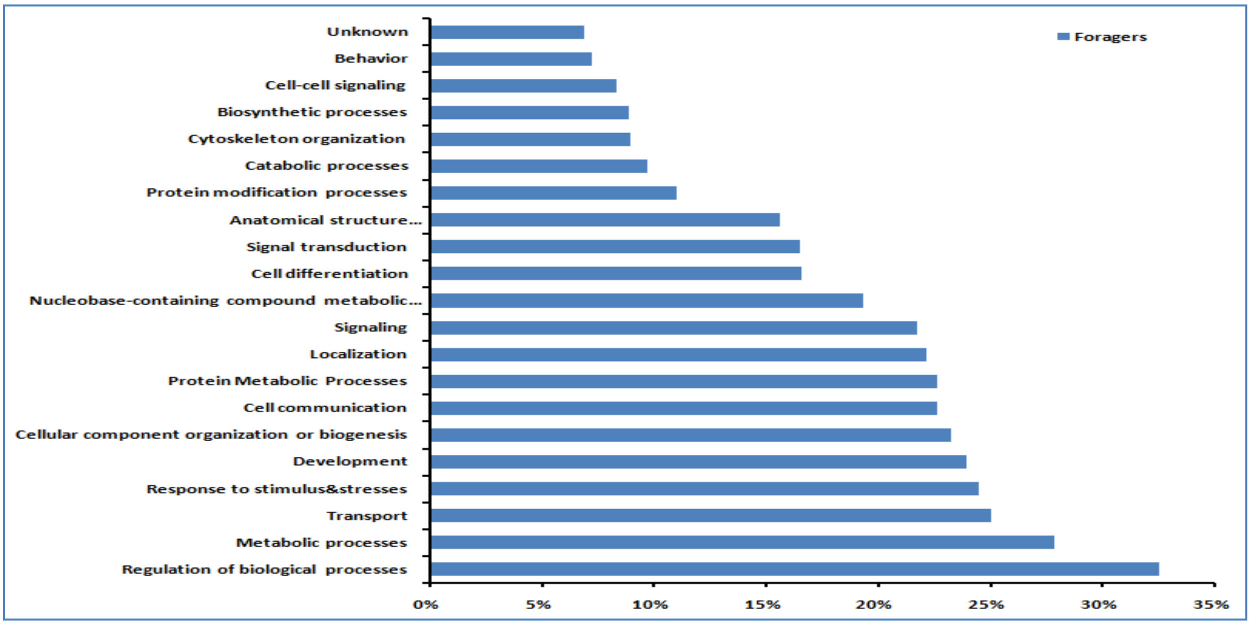

Fig. 1. The functional category of all the identified phosphoproteins in forager brains of the honeybee worker (*A. m. ligustica*). The percentage of each functional group is obtained on the basis of the number of phosphoproteins under each of the functional groups divided by the total number of the identified phosphoproteins in foragers individually.

Protein−Protein Interaction PPI Analysis

Of the 916 identified phosphoproteins in the forager’s brains, 208 phosphoproteins ($\geqslant$ 10 interactions of each protein) were identified as key node proteins in the PPI networks with a total of 11,621 interactions. These key node phosphoproteins in forager bees’ brains were implicated in the regulation of biological processes, protein metabolic processes, development, response to stimulus & stresses, cellular component organization or biogenesis, metabolic processes, cell communication, transport, signaling, localization, nucleobase-containing compound metabolic processes, anatomical structure morphogenesis & development, cell differentiation, signal transduction, protein modification processes, biosynthetic processes, catabolic processes, cytoskeleton organization, behavior, and cell-cell signaling funcional categories (fig. 2).

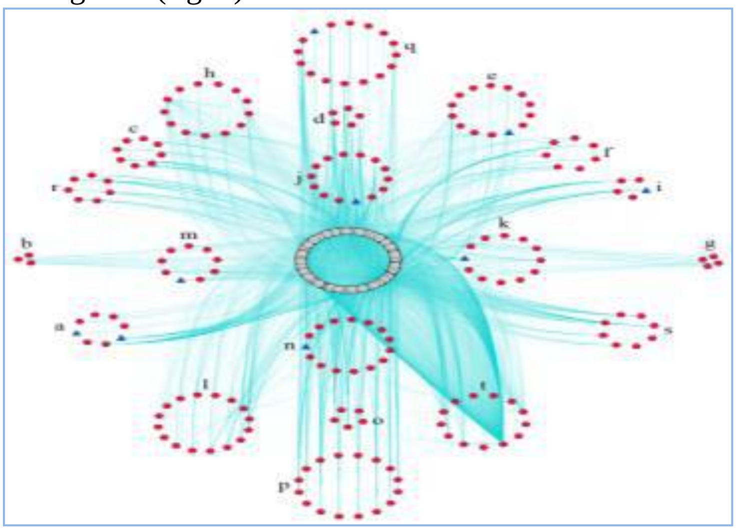

Fig. 2. Protein-protein interaction PPI network of identified phosphoproteins in forager brain of the honeybee worker (*A. m. ligustica*). PPI is predicted using the Interologous Interaction Database (I2D) and visualized by NAViGaTOR software (Brown and Jurisica 2005; Brown and Jurisica 2007b). The color symbols represent identified proteins connected in the PPI

network with at least 10 interaction degrees. The regular triangles stand for up-regulated phosphoproteins in forager brain of the honeybee worker. Letters from "a" to "t" represent the categories of anatomical structure morphogenesis & development, behavior, biosynthetic processes, catabolic processes, cell communication, cell differentiation, cell-cell signaling, cellular component organization or biogenesis, cytoskeleton organization, development, localization, metabolic processes, nucleobase-containing compound metabolic processes, protein metabolic processes, protein modification processes, regulation of biological processes, response to stimulus & stresses, signal transduction, signaling, and transport.

The top 10 key node proteins with high degrees of interaction in forager bees' brains were: polyubiquitin-A-like (610 interactions), actin related protein 1 (286 interactions), eukaryotic initiation factor 4A-like (256 interactions), fasciclin-2 isoform 1 (255 interactions), autophagy 1 (213 interactions), cyclin-dependent kinase 7 (213 interactions), histone deacetylase 4 (203 interactions), tubulin beta-1 chain (189 interactions), tubulin alpha-1 chain-like (169 interactions), and voltage-dependent anion-selective channel (166 interactions).

Pathway Mapping and Enrichment Analysis

To better explore and navigate the key functionality of the phosphoproteins in forager's brains, all identified 916 phosphoproteins in forager bees' brains were mapped to KEGG-derived metabolic pathways using iPath2.0. These phosphoproteins were matched to 81 metabolic map elements (redundant entries included). Pathways involved in carbohydrate metabolism such as fructose and mannose metabolism, amino acids metabolism, amino acids biosynthesis, and the chlorocyclohexane and chlorobenzene degradation were highly represented. Of which, pathways of the phosphatidylinositol signaling system, inositol phosphate metabolism, phototransduction, glycerophospholipid metabolism, and Wnt signaling were significantly enriched in the KEGG pathway database.

Motif Analysis and Kinase Prediction

Of all the confidently localized phosphosites in the forager bees' brains, 1,390 kinase-substrates were obtained from the phosphosites in foragers. These kinase-substrates mainly involved in 28 kinase families group in foragers brain phosphoproteins including; CDK2_CDK3, p38, JNK, ACTR2_ACTR2B_TGFbR2, CK2, CLK, PKC, PKA, GSK3, p70S6K, PKB, PAKA, PAKB, CK1, ATM_ATR, CaMKIIalpha_CaMKIIdelta, InsR, MAP2K6_MAP2K3_MAP2K4_MAP2K7, MAPK3_MAPK1_MAPK7_NLK, CDK1, MSN, AuroraA, CDK5, ROCK, DMPK, MST, NEK1_NEK5_NEK3_NEK4_NEK11_NEK2, and PKGcGK.

All the phosphosites harbored in the phosphoproteins identified in forager bees' brains were subjected for motif analysis by Motif-X algorithm. In total, [15] Ser motifs were extracted from the serine phosphorylation sites, i.e., [SP××××R], [SP××S], [SPS××S×××E], [KS×SPS], [S××××SP], [KS×SP], [SP], [SPS], [DS], [S×××S], [S××S], [S×E], [S××S×××SP], [S××××S] and [S××××P]. There were [12] Thr motifs were enriched from the threonine phosphorylation sites, i.e., [SVT××××E], [S××××T×E], [SPT], [SP××T], [SP××××T], [TP], [T×××SP], [S××T], [S×××T], [P××T], [T×××××P], and [S×T]. In addition, one motif [S×××Y] was identified from the tyrosine phosphorylation sites. In forager bees' brains, 4 acidic, 5 proline-directed, 1 tyrosine, and 18 "other" motifs were identified, of which 16 were novel, and the remaining 12 were matched to known kinases of ERK1, ERK2, CKII, CK1, and b-Adrenergic Receptor kinase (fig. 3).

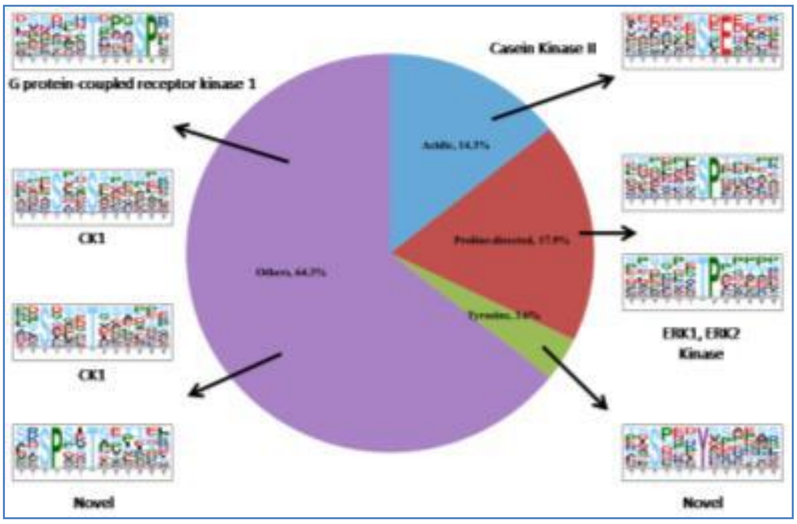

Fig. 3. Representative extracted motifs from the phosphorylated peptides identified in forager brain of the honeybee worker (A. m. ligustica). Motif extraction was done using the Motif-x algorithm (http://motifx.med.harvard.edu/motif-x.html). Phospho-motifs were classified according to ref (Lasonder et al., 2012). Known motifs were searched for the kinases using the resources at http://www.hprd.org/PhosphoMotif_finder (Amanchy et al., 2007) and http://phosida.de/.

As well known, honeybee (*Apis mellifera*) is the typical model for the molecular studies of social behavior. In addition, the division of labor behavior in the social insects brings to the light how much these social insects organized and advanced. Honey bee worker performs a sequence of tasks throughout her adult life as she shifts from a young nurse bee living inside the hive to a forager bee that navigates the outdoors. The foraging activities are the key factor of the honeybee colony survival, whereas outdoor worker bees collect the materials for the colony nutrition and maintenance such as; pollen, nectar, propolis, and water. To perform the different tasks, honeybees efficiently regulate highly advanced social behaviors and intelligent decisions (Tereshko and Loengarov 2005) by the functionality of brain cell chemistry, structure, endocrine activity, and changes in temporal patterns of gene and protein expression (Robinson and Ben - Shahar 2002; Robinson 2002). With age development, the brains of honeybee workers have already physiological matured and engaged in the neural activities associated with the field foraging tasks. To this end, the involovement of the identified 916 individual forager bee brain phosphoproteins in regulation of biological processes, metabolic processes, transport, response to stimulus & stresses, development, cellular component organization or biogenesis, cell communication, protein metabolic processes, localization, and signaling is supposed to help the forager to achieve their task performance by supplement of biological molecules for brain maintenance and energy depletion to support the intense cerebral activity during the foraging activities.

The top detected key node phosphoproteins in the PPI network of the forager bee brain phosphoproteome imply their importance in further supporting the regular maintenance and demands of the forager brain via synthesis of proteins and energy supplies during foraging. The actin related protein 1 is assumed to be involved in transportation of mitochondrion (Haghnia *et al.,* 2007) and vesicle-mediated transport, which is a vital process in signal transduction regulation. The polyubiquitin-A-like proteins involved in the cell phagocytosis (Stroschein-Stevenson *et al.,* 2005) and activating the autophagy-related protein 7-like which regulates the neurodegeneration and aging in *Drosophila* (Chen *et al.,* 2012). Eukaryotic initiation factor is important for resolving mRNA secondary structures (Fraser and Doudna 2006), which in turn, support forager brain maintenance during the forage activities, while Fasciclin-2 isoform 1 protein has been reported in relation to neuron recognition (Grenningloh

et al., 1991), synapse organization (Ashley *et al.,* 2005), olfactory learning, and short-term memory (Waddell and Quinn 2001) suggest that this protein is important for enhancement the honeybee workers cognition and decision. Moreover, tubulin proteins involved in centrosome duplication in drosophila (Müller *et al.,* 2010) suggests its pivotal role in cell cycle progression and forager brain maintenance. The histone deacetylase protein plays pivotal roles in neurogenesis and neuron generation within the nervous system (Neumüller *et al.*, 2011). As vision is a key factor for foraging and navigating, voltage-dependent anion-selective channel proteins participate in ion transport (Komarov *et al.*, 2004) and maintaining the photoreceptor response as a key factor in phototransduction (Lee *et al.,* 2007a). The key driver proteins for these networks highlight potential novel targets for further mechanistic studies of honeybee neurobiology.
The forager brain phosphoproteins molecular network covered the major central metabolism categories including carbohydrate metabolism such as fructose and mannose metabolism, amino acids metabolism, amino acids biosynthesis, and the chlorocyclohexane and chlorobenzene degradation were highly represented. These metabolic pathways imply the phosphoproteins importance in further supporting the regular maintenance and demands of the forager brain via synthesis of proteins and energy supplies during foraging. Foraging activity requires the development of a wide repertoire of navigational skills due to the spatial complexity and the acquisition of novel sensory information from the environment outside the nest (Tereshko and Loengarov 2005). As forager bees, information acquisition from the environment is the first step in the process of learning, memory, and navigation. The eyes of foragers are vital for the cognition of flower colors and patterns and to learn the new route to a food source (Pahl *et al.,* 2010). Toward this goal, the more strongly represented phosphoproteins which are significant in the phototransduction pathway reveal the paramount importance of visual perception (Srinivasan 2010) via the efficient information processing. In addition, the other significant enriched biological pathways in the forager brain coherent with the brain functions such as; the phosphatidylinositol signaling system and inositol phosphate metabolism pathways play central role in cellular responses (Dickman *et al.*, 2006), synaptic vesicle endocytosis and neurotransmitter secretion (Morrison *et al.*, 2000). The Wnt signaling pathway has a pivotal role in the signal transduction to support the brain interaction with the environment events (Nusse and Varmus 1992).
The enhanced enzymatic activities of kinase in forager brains such as CDK2_CDK3, p38, JNK, ACTR2_ACTR2B_TGFbR2, CK2, CLK, PKC, and PKA may play vital roles in the nerve cell differentiation (Morgan 2007), and cell proliferation regulation (Pearson *et al.*, 2001). This suggests their importance in the enhancement of the signal transduction function in the forager's brain. On the other hand, the enriched motifs from the phosphopeptides in the forager bee brain are matched with the extracellular-signal regulated kinases (ERK1/2), CKII, CK1, and b-Adrenergic Receptor kinase. of which, the ERKs are widely involved in the regulation of cellular proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis functions, and they are also important to many different stimuli such as heat shock via activating the ERK pathway (Pearson *et al.*, 2001). Therefore, ERKs are important both in response to environmental stimuli and brain maintenance in forager bees. Casein kinase 2 (CKII) has been reported to be activated Wnt signaling pathway (Gao and Wang 2006), while the casein kinase 1 (CK1) family are selective enzymes that function as regulators of signal transduction pathways in most eukaryotic cell types and involved in Wnt signaling, , DNA repair, and DNA transcription (Eide and Virshup 2001). Therefore, the forager brain phosphosites motifs provide specific binding sites for kinase recognition that is potentially useful for a mechanistic understanding of how kinases mediate phosphorylation events in the forager bee's nervous systems.

Conclusion

This study provides the first comprehensive and large scale in vivo dynamic phosphoproteome profile into the forager honeybee worker brain up to date. The identified 916 phosphoproteins in the nurse bee brain are mainly associated with regulation of biological processes, metabolic processes, transport, response to stimulus & stresses, development, cellular component organization or biogenesis, cell communication, protein metabolic processes, localization, and signaling. The forager brains' phosphoproteins expressions in the common biological pathways and kinase activities are vital to sustain central neural activity during the foraging activities. The more strongly represented phosphoproteins in the forager brains that are implicated in pathways phosphatidylinositol signaling system, inositol phosphate metabolism, phototransduction, glycerophospholipid metabolism, and Wnt signaling, and activated kinases such as CDK2_CDK3, p38, JNK, ACTR2_ACTR2B_TGFbR2, CK2, CLK, PKC, and PKA are required for brain maintenance, signal transduction, and the olfactory learning processes to enhance the foraging activities performance. Overall, the neural associated phosphoproteins networks identified in this study require further mechanistic studies of honeybee neurology. The knowledge gained from the further studies will lead to major advances in the honeybee brain neurobiology and other social insects.

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BEHAVIOR OF YOUNG CONSUMERS REGARDING FLAVORED WINES - CASE STUDY: RETSINA AND VERMOUTH

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Abstract

Flavored wines consist of a neutral wine/grape juice base, with the addition of alcohol and a mixture of dry ingredients, such as aromatic herbs. One of the most popular Greek flavored wines is Retsina, which results by addition of Aleppo Pine resin in grape juice during alcoholic fermentation. "Retsina" is a wine of traditional appellation produced only in Greece, while some Retsina wines produced in the Greek regions of Attiki, Viotia and Evia have additionally become entitled to bear a geographical indication of origin. Vermouths, also designated into the flavored wines category, originate from the addition of herbs and spices in a base wine. Both Retsina and vermouths are consumed by young people. Specifically, Retsina is also named "drink of students" because of its low cost and easy combination to a variety of foods of Mediterranean cuisine. Vermouths, on the other hand, is a very popular choice among young people, for an after-office drink or aperitif. The purpose of this survey was to assess the knowledge and preferences of young consumers regarding flavored wines, in particular Retsina and vermouth. The study was conducted from January to May 2019 in the region of Thessaly (central Greece). Four hundred questionnaires were filled in by randomly selected individuals, who were asked to answer key questions about flavored wines. The results of the survey showed that 79.5% of the respondents consumed flavored wines without being aware of the fact (45.5%). Respondents' consumption was limited to less than one bottle per month. They preferred Retsina over any other flavored wine (64.4%). They preferred domestically produced wines (53%), originating mainly from the Greek region of Thessaly (21.6%), because of their desire to support local businesses. The present study provides detailed statistical information on the behavior of young consumers regarding flavored wines in the Thessaly region.

Key words: *flavored wines, vermouth, retsina, young consumers.*

Introduction

The original purpose of aromatization, like fortification, was to preserve the wine from microbiological or oxidative spoilage. Nowadays, due to vastly improved winemaking techniques, materials and equipment, the original purpose of this practice has been lost, however the taste for aromatized wines remains strong in many parts of the world (Buglass, 2011).

Alcoholic beverage industry is one of the most dynamic sectors in Greece, with a high degree of competitiveness and big concentration of enterprises. Retsina is a traditional Greek white wine with the added spicy pine resin flavor. It has a history of thousands of years. It is one of best known Greek products in international markets, labeled "Traditional Wine" which means that only Greece can produce it (Kourakou – Dragona, 2015). It is a powerful "weapon" in the hands of capable wine-makers who have realized that a small country like Greece on the international markets can only stand if it invests in absolute quality. There are several producers currently working in this direction, making wonderful Retsinas and winning praise

from well-known wine-critics. The product's image inside the country is not so good, being labelled as a student /cheap wine and getting a negative vote by wine drinkers.
According Panesar et al. (2009), vermouth, which is officially classified as an "aromatized fortified wine", is produced by the fortification with alcohol of a white wine base and subsequent infusion with aromatic plants, seeds, fruits and other botanicals. Depending the recipe, its characteristic taste should be occur by the utilization of *Artemisia* species, according to Regulation (EU) No 251/2014 (Annex II). These types of wines are quite popular in European countries and in the USA (Amerine et al, 1980). Italy and France are famous producers of vermouths, however, a few Greek companies also have begun producing vermouths recently.
The aim of the project was to investigate the behavior of young people in terms of their knowledge and preferences in flavored wines and especially in Retsina and vermouth. Consumers are influenced by a variety of external factors (demographic, social, economic, technological, etc.) when making purchasing decisions, and age according to Garcia et al. (2013) is an important variable in differentiating consumer preferences. For this reason, the sample of respondents was selected on the basis of age, and especially younger consumers, who are the potential consumer audience of a society that can highlight a trend and reflect its needs. This study provides information to the wine industry in order to take steps to improve the knowledge of new consumers about these products and in particular about Retsina, which is a popular Greek wine.

Materials and Methods

The research was based on the collection of data on the behavior of young consumers towards flavored wines. The focus group selected based on legal drinking age in Greece (18 years old), the demographic cohort named Gen Z (18-23 years of age), along with Gen Y.1 (age range 24-29 years old), which represent the main age groups of university students in Greece (graduate or post-graduate levels) (Kamenidou et al. 2019). Millennials or Gen. Y cohort identify as wine drinkers, and correspond to a fast growing wine segment, according to Atkin & Thach (2012).
A total of 400 randomly selected young people, currently studying or owning a university diploma, with a level of income of € 13,000 to € 24,000, answered face to face questions about their knowledge and preferences regarding flavored wines. Participants answered 34 questions, grouped in 3 parts. The first part of questions negotiated consumer attitudes towards the beverage market and consumption of wine, the second part consisted of questions regarding wine selection (imported - domestic and organic), while the third part was composed by questions referring to Standardization - Packaging - Price - Label - Advertising. The survey was conducted from January to May 2019, in the region of Thessaly (central Greece). Data were processed using SPSS ver 17 statistical package. Limitations of the survey is the geographically restricted sample, which does not represent neither the general population, nor the whole country.

Results and Discussion

Considering that age is a variable that influences consumer preferences and is based on the principle that each generation has its own specific values, which in turn lead to different behaviors (Garcia et al, 2012), the research was conducted on young people 18 - 24 years (93.5%) to the majority of students (92.1%), since Retsina is also called 'student drink', as it is not lacking in any student hangout and is the first drink in their preferences.
Although largely young people are unaware of the particulars regarding aromatized/flavored wines (composition, alcohol, etc.), Retsina has got 64.4% of their preference among flavored

wines, vermouth 11%, while 25% of respondents prefer another flavored wine (e.g. aperitifs, sangria) (Figure 1).

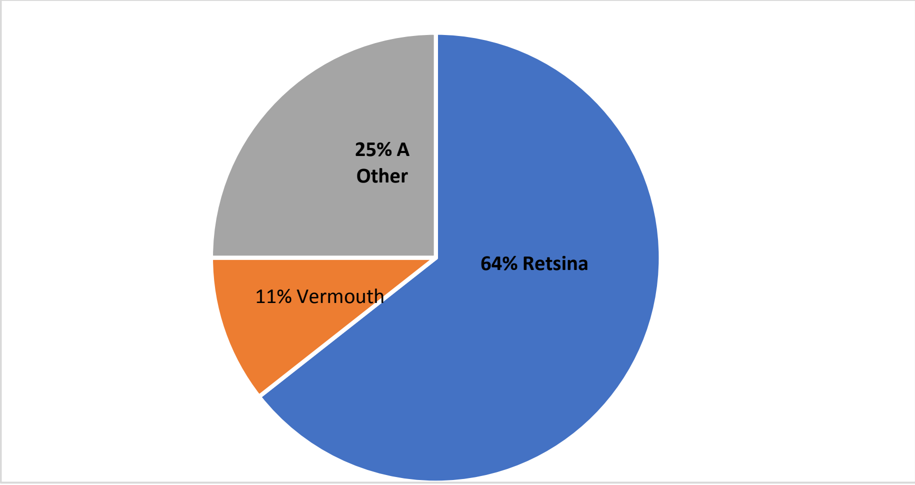

Figure 1. Consumption of flavored wines by young people

The largest percentage of respondents (44.9%) say that they spend less than 10 euro / month on the purchase of flavored wine (Figure 2), and they probably represent the consumers of Retsina, since the cost of a Retsina bottle (0.5L) range from 1.75€ to 10€. One out of four young consumers spends more than 20 euro per month for the purchase of aromatized wines (vermouth drinkers probably are included, since these products cost over 20 euro per bottle. Finally, 3.4% of the respondents spends 50-60 euro / month, thus enhancing the growth power of the alcoholic beverage industry and the entire wine production and marketing chain in general.

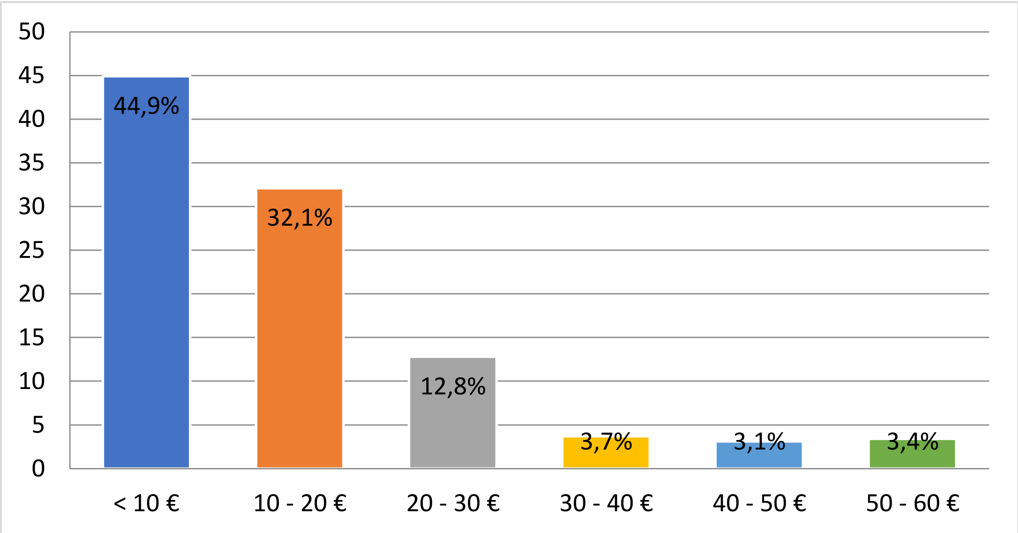

Figure 2. Money spent for the purchase of flavored wines in a monthly basis

It is important to note that over 50% of the research participants prefers to consume domestically produced aromatized wine over imported, due to quality, uniqueness, but also to support Greek companies. In addition, over one third of those preferring domestic aromatized wines express a strong preference for locally produced aromatized wine, originating from the region of Thessaly, which indicates that the criterion "local" plays a significant role in their buying behavior. Regarding the respondents' opinion on the criteria for the selection of flavored wines, the highest percentage of agreement is related to the parameters of taste (Table 1) and aroma, followed by the production company (agricultural cooperative), the price and quality (quality certification).

Table 1. Criteria to be considered for the selection of flavored wines

Parameters	Average value
Region of origin	3.1
Aroma	3.8
Clarity	3.4
Good taste	4.4
Affordable	3.7
Easily found (available in several selling points)	3.3
Packaging	2.6
Variety	3.4
Offers	3.0
Advertising	2.4
Denomination of origin	2.8
Winery	2.9
Cooperative winery	3.8
Informative and nice label	2.8
Brand	2.8
Product /company certification	3.6

*1-not at all, 2-little, 3-moderate, 4-a lot, 5-too much

It is impressive, however, is that more than 66% of the respondents do not buy organic flavored wines, despite recognizing their superior quality (data not shown).
As far as prices are concerned, the majority of respondents think that they are good enough and in the event of a price increase consumers would not stop buying the products, just maybe reducing quantities.
Research participants usually prefer to consume flavored wines accompanied by food and especially at celebrations/gatherings. This is the case mainly for Retsina, which is served in restaurants by rarely in bars, while vermouths and other flavored wines are served almost exclusively in bars.
Finally, it is worth mentioning that the purchase of a flavored wine is highly influenced by the taste and personal experience (previous tasting), price and quality, while not letting the brand and advertising direct it. These research findings are also confirmed by the research of Garcia et al., (2013), with the exception of young people's preference for organic wines which in this study do not seem to have the same impact, which may be due to the lack of information about organic flavored wines.
The results indicate a conscious consumer audience regarding the consumption of flavored wines (Table 2)

Table 2. Criteria affecting the consumers for the selection of flavored wines

Criteria	Average value
Good taste	4.3
Reasonable price	3.7
Advertising	2.4
Brand	2.8
Packaging	2.6
Country of origin	3.2
Variety	3.3
Company of production and packaging	3.0
Quality characterization (eg. PGI)	3.2
Opinion of friends and family	3.3
Personal experience (previous tasting)	4.2

*1-not at all, 2-little, 3-moderate, 4-a lot, 5-too much

Conclusion

In this study it was revealed that young people consume flavored wines and prefer retsina over vermouth because it is a Greek product, is quite affordable and they believe that they contribute to the enhancement of local market. Characteristics that appear to affect young consumers of flavored wines in the region of Thessaly are the taste, personal experience (previous tasting) and price of the product. In addition, the characteristics that least influence the choices of the research participants appear to be advertisement, packaging and brand, indicating a conscious consumer audience interested in taste and good quality. This research could be used by wine companies as a reference to their marketing strategies targeting young consumers. Finally, conducting a more extensive country-wide research could confirm the interpretation of the existing findings.

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ASSESSING STABILITY IN MAIZE

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Abstract

Commercial maize hybrids should incorporate high yield potential and stability across environments in order to be profitable for the farmers. A stable genotype must show almost no interactions with the environments where it is cultivated. The only effects acceptable in the field, are the favorable conditions in some environments that may increase yield above the level of expected performance. The purpose of the present research was to analyze yielding stability in maize, based on data from Randomized Complete Block designs conducted in two contrast environments: in Florina and Thessaloniki (Northern Greece). Ten commercial F1 maize hybrids were used: ZS-680, ZS-8720, ZS-650, ZS-600, ZS-500, ZS-700, Cresus, Jetta, Funo and ZP-704. Plots consisted of four rows 5m long, 0.75m apart, 20cm plant-to-plant spacing and four replications. Mean yield was estimated for each location and hybrid and stability index (a specific ratio of mean and standard deviation) was used to depict which commercial hybrids were more stable across the environments. Generally, there were differences in stability across environments and some hybrids showed stability in both Greek environments. ZS-700 generally showed the greatest values (83.43) followed by Cresus (54.29). In Florina, stability index values were generally greater than Thessaloniki, indicating a more stable performance in this specific environment. The more stable hybrids were ZS-700 and Cresus for Florina, and ZS-600 for Thessaloniki. Jetta showed satisfactory values in both environments, indicating a more balanced and stable performance across the two environments. These commercial hybrids could be recommended for cultivation in certain Greek regions. The stability index is a useful criterion for cultivar recommendation. From our dataset, Florina region contributed positively to stability performance.

Key words: *stability index, environment, performance*

Introduction

Commercial maize hybrids in order to be profitable for the farmers must incorporate high yield potential and stability across environments (Stratilakis and Goulas, 2003). Evans (1980) stated that breeding contributed to yielding performance by a) increased adaptability of genotypes, b) increased resistance to insect attacks and diseases, and c) increased responsiveness to inputs and new cultivation techniques. Fasoulas (1981; 1993) reported the main principles for efficient breeding, such as absence of competition during selection, special designs to reduce soil heterogeneity and exploitation of individual plant yielding capacity, in order to have high and stable yielding performance. Stability of genotypes across environments is considered of great importance for a commercial cultivar (Fasoulas, 1988) and a stable genotype must show almost no interactions with the environments where it is cultivated. The only effects acceptable in the field, are the favourable conditions in some environments that may increase yield above the level of expected performance. Stability estimations were previously conducted on the basis of stability index

$(\bar{x}/s)^2$, where $\bar{x}$ and s are the entry mean yield and standard deviation (Fasoula, 2013). Fasoulas (2004) stated that this index reveals genes that control stability and tolerance to various biotic and abiotic stresses. Initially, Fasoulas (1988) proposed the ratio VC or 1/CV (the coefficient of variation) between mean and standard deviation for stability estimations and later Fasoula (2013) used the squared form as a stability criterion considering this approach as the most appropriate to analyse stability. The concept of the use of coefficient of variation (CV) is not a new one. Edmeades and Daynard (1979) used it for stability estimations and Tollenaar and Wu (1999) as a means of efficacy in breeding. Edmeades and Deutch (1994) used many parameters for estimating stability and also they proposed evaluations in many different environments.
The purpose of the present research was to analyse yielding stability of ten maize commercial F1 hybrids, based on data from Randomized Complete Block designs conducted in two contrasted environments in the Greek regions of Florina and Thessaloniki.

Material and methods

Environment evaluation (Fasoulas, 1981; Ipsilandis, 1996; Greveniotis *et al.* 2019) was based on maize data from Randomized Complete Block designs conducted in Florina (Technological Educational Institute of Western Macedonia in Florina, Greece) and Thessaloniki. For this purpose, in the above mentioned locations, 10 maize hybrids were sown at the same date in 20 April: ZS-680, ZS-8720, ZS-650, ZS-600, ZS-500, ZS-700, Cresus, Jetta, Funo and ZP-704. Plots consisted of four rows 5 m long, 0.75 m apart, 20 cm plant-to-plant spacing and four replications. Only one of the four rows was harvested by a single-row harvester, the left of the two inner rows. Mean yield was estimated for each location for the 10 commercial F1 maize hybrids. Stability estimations were based on stability index $(\bar{x}/s)^2$, where $\bar{x}$ and s are the entry mean yield and standard deviation (Fasoula, 2013).

Results and discussion

In the quest of stability breeders mainly and then farmers, spent a lot of time testing genetic materials to prove their stable performance across environments and then in specific environments. Breeders especially choose their way to stability through different paths (Bernardo, 2002).
In our dataset the different genetic materials showed significant differences between their stability index. Differences were also found across environments. ZS-700 generally showed the greatest values (maximum 83.43) followed by Cresus (maximum 54.29). In Florina stability index values (mean= 25,64) were generally greater than Thessaloniki (mean= 6,88), indicating a more stable performance in this specific environment probably because of the local conditions that proved to be more favourable (Fasoulas, 1988). Thus, the more stable hybrids were ZS-700 and Cresus for Florina, and ZS-600 for Thessaloniki (16,19). Jetta showed satisfactory values in both environments (Florina and Thessaloniki), indicating a more balanced and stable performance across the two environments.
According to Fasoulas (1988) and Ipsilandis (1996) stability of performance is essential for the establishment of a commercial cultivar/hybrid. Thus, the stability index is a useful criterion for cultivar recommendation. Modern maize hybrids are considered to be more productive than older ones, because of their stability of performance even under difficult environmental conditions. They also correspond better to various inputs according to previous reports (Castleberry *et al.* 1984; Carlone and Russell, 1987; Duvick, 1992; 1997).
According to Fasoulas (1988), stability estimations may also lead to clarification of the type of a trait inheritance, meaning that stability index may also indicate if a specific trait exhibits

quantitative or qualitative behavior. Comparisons between traits may depict if a trait is controlled by a few, one or many genes.

Table 1. Stability index for all hybrids within and across environments

	Florina (Env A)		**Thessaloniki (Env B)**	
HYBRIDS	$(\bar{x}/s)^2$	$\bar{x}/s$	$(\bar{x}/s)^2$	$\bar{x}/s$
ZS-680	4.47	2.11	3.83	1.96
ZS-8720	13.18	3.63	3.00	1.73
ZS-650	10.14	3.18	2.92	1.71
ZS-600	22.90	4.79	16.19	4.02
ZS-500	5.43	2.33	6.51	2.55
ZS-700	83.43	9.13	6.86	2.62
CRESUS	54.29	7.37	6.63	2.57
JETTA	19.97	4.47	9.40	3.07
FUNO	4.70	2.17	9.47	3.08
ZP-704	37.85	6.15	3.97	1.99

Conclusions

Maize hybrids ZS-700 and Cresus for Florina, ZS-600 for Thessaloniki and Jetta in both regions, showed high stability according to stability index in the two Greek environments used. The differences between genotypes were also very significant, depicting the more stable ones. These hybrids could be recommended for cultivation in Greece, since the stability index proved to be a useful criterion for cultivar recommendation. Stability in extreme environments like Florina may prove to be very important for maximizing field yield. From our dataset Florina contributed positively to stability performance.

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DEVELOPMENT OF CHANGES OF LACTIC ACID AND COLOR CONTENT IN HARVEST SEASON FROM ELDERBERRY (SAMBUCUS NIGRA L.)

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Abstract

Elderberry (*Sambucus nigra L.*) can be used in many ways in the food industry due to its nutritional characteristics its organoleptic properties and its rich color content. Currently this plant is used as a natural coloring or coloring food (concentrate, powder) in the food industry. In recent years, the rise in lactic acid content has been a problem for both Hungarian producers and the industry, which has a negative impact on the quality of products. Lactic acid bacteria often appear in plantations, due to weather conditions, especially temperature and precipitation. However, this also affects the anthocyanin content. Many items are rejected on receipt of raw material due to excess lactic acid limit, which makes it difficult to process of elderberry for both producers and acquiring companies. In our research, we monitored the development of lactic acid and color content in the elderberry samples from the plantations of BOTÉSZ (Bodzatermesztők értékesítő Szövetkezete – Hungarian elderberry association) under the elderberry harvest season. In our investigation, we used a variety of color content and lactic acid measurements to select a method that can be routinely applied in industrial environments. Based on our results, we would like to develop recommendations for good harvest practices that are planned to be presented at BOTÉSZ members' meetings and conferences.

Keywords: *Elderberry, lactic acid, color content, BOTÉSZ*

Introduction

Elderberry (*Sambucus nigra* L.) is variably usable fruit in food industry because of its taste, coloring properties and positive health benefits. It is a source of protein, fatty acids, vitamins and high biologycal activity compounds, like polyphenols. They have numerous positive medical effects, for example immune system stimulation, blood pressure stabilization, decreasing of uric acid levels and anti tumor activity. (Andrzej Sidor, October, 2015)
Due to these properties, elderberry is used in many different food products in Europe, such as pies, jellies, yogurts, syrups, jams, food colorants and alcoholic drinks. (Pedro Silva, January, 2017)

Measurements and methods

Samples: The harvesting season consists of mainly the next five days: 24 August – 30 August (on 25 and 26 August it was raining). The mass of the elderberry samples were each 700 – 800g in plastic bags, the next table shows the distribution of total 25 samples.

Table 1. Distribution of elderberry samples

2018. 08. 24.	2018. 08. 27.	2018. 08. 28.	2018. 08. 29.	2018. 08. 30.
8 samples	8 samples	3 samples	3 samples	3 samples

Preparation of samples: We took off the samples from the freezer, put them in a kitchen blender and made a homogene puree.
After this, we made an extraction with a solution consisting of 60% destilled water, 39% methanol and 1% formic acid. We added 30 ml of this solution to 1 g sample in a centrifuge

tube, put it in ultrasonic bath for 15 minutes and centrifugated for 10 minutes on 4000 rpm. This extraction is needed to perform some further measurements.

Table 2. Used measuring methods for each parameters

Measured parameter	**Method**
Anthocyanin content	pH differential method (J. Lee, 2005)
Lactic acid content	Food Lab Analysis Sytem (by Agrana Juice Ltd.)
Colour parameter (L*)	CIE Lab Color Measuring System with a Konica Minolta CR 410 manual digital color meter
Dry matter (refraction %)	Codex Alimentarius 558/93/EEC

Results and Discussion

As shown in the abstract, we examined the lactic acid and anthocyanin content during the harvest of 2018. As we see on Figure 1, the temperature decreased to 15°C from a light summer-end weather because of two rainy days, but after it started to rise again.

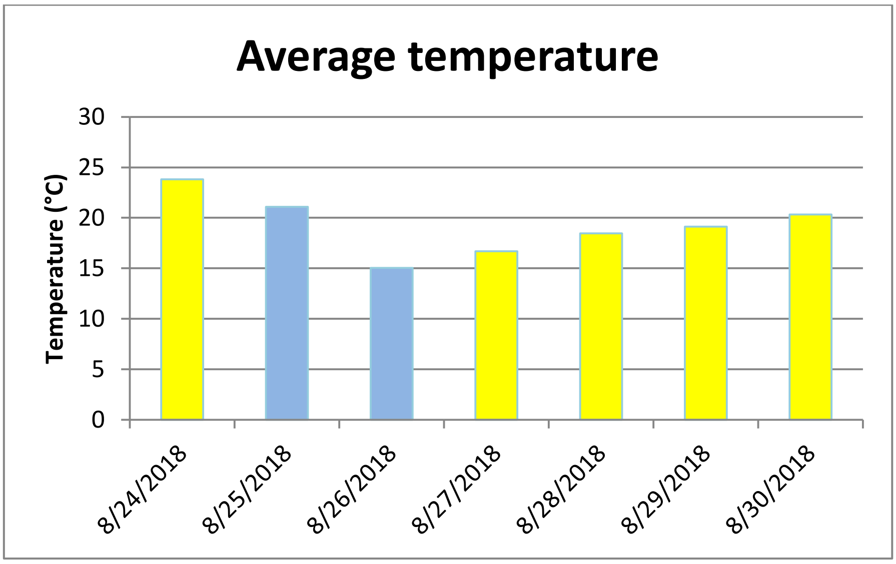

Figure 1. Average temperature changing during harvest

The problematic compound, the lactic acid had a level of about 40 mg/l in the samples of the first day. Three days later, after raining and temperature decreasing, this level dropped to the half, 20 mg/l. As the weather got warmer again, the lactic acid content grew back fastly, moreover to 80 mg/l, twice higher than the original level.

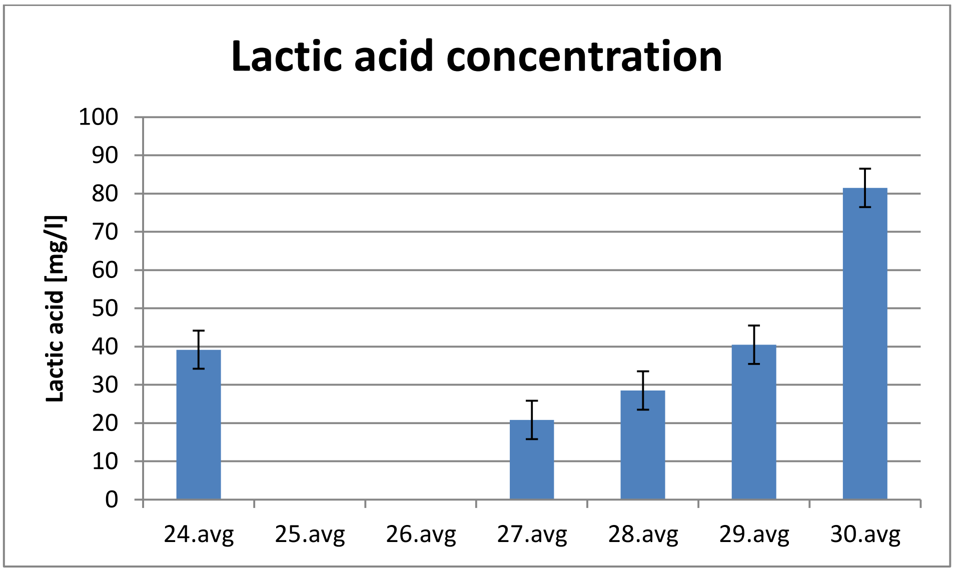

Figure 2. Lactic acid growing of elderberry samples

According to Figure 3., dry mass content was around 13% on the first day, which also decreased after raining to 11%, and started to grow as the effect of warming. Comparing to lactic acid content, this parameter rised only to the level before raining, not significantly higher.

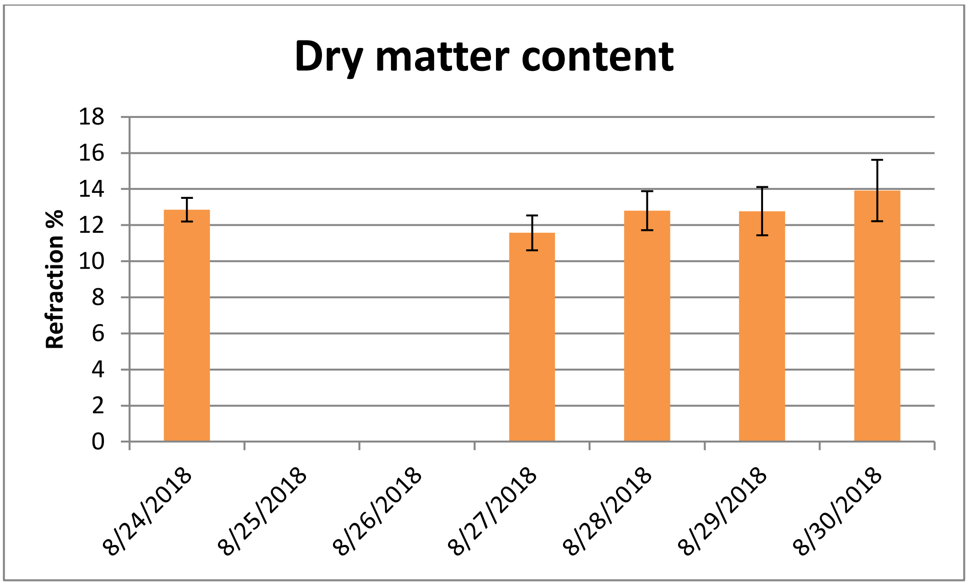

Figure 3. Refraction values during the mentioned days

The other main subject of our project is the coloring compound, the anthocyanin content. During maturation, many biochemical reactions occur in fruits, like aromatic compunds, sugars, ethylen and anthocyanins are synthesized. Some fruits and vegetables are bought according to the colouring content.
As shown on Figure 4., the anthocyanin concentration was the lowest on the first day of harvest: around 3900 mg/l. After raining, it got higher and higher (from 4000 mg/l to 4300 mg/l), but the levels do not follow each other in order.
Accordingly, there is an inverse proportion between anthocyanin concentration and lightness value: when the anthocyanin increased a lot on a harvesting day, the lightness value

decreased, as well. This tendency is the most visible on the pair of 2018. 08. 28. samples, where the anthocyanin concentration is the highest, 4450 mg/l, and the belonging L* is 18,5.

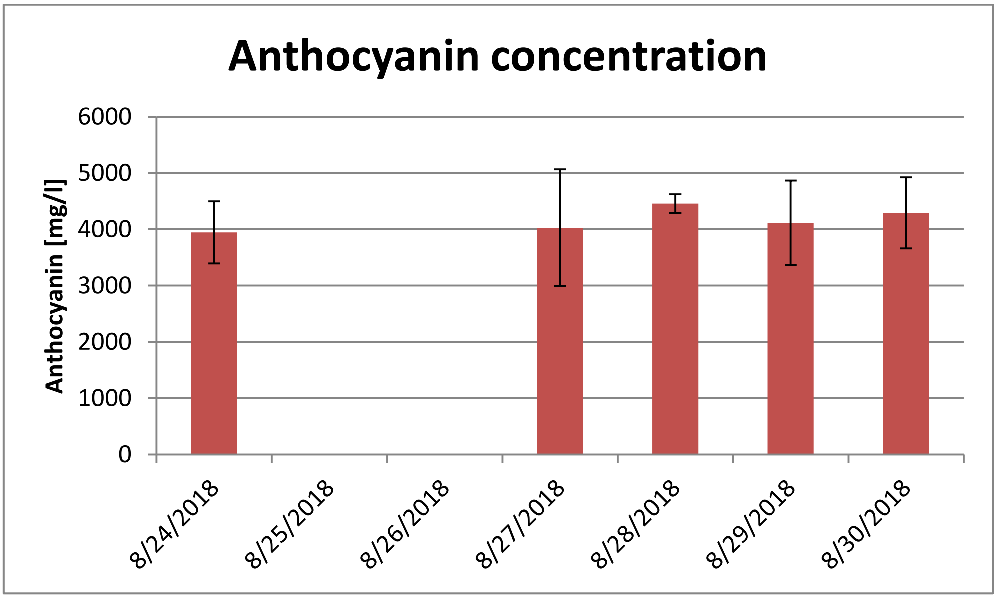

Figure 4. Changing of anthocyanin content in berries

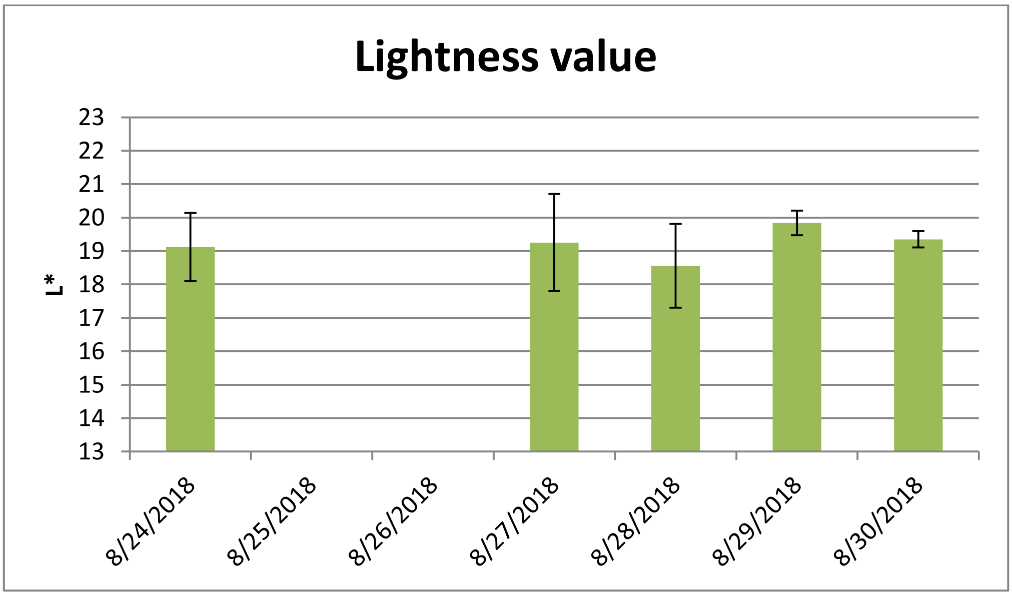

Figure 5. Colour index (L*) changing during the harvest

Conclusion

As the previous figures show, the temperature significantly influences the lactic acid concentration, and also has an effect on dry mass content of elderberry.

The proportion between temperature and lactic acid concentration is straight, which is in connection with the temperature optimum of lactic acid bacteria. They are mesophilic microorganisms, so the temperature lower than 20°C blocks their reproduction and lactic acid production. The refraction % dropping occured because the rain extracted some water soluble components from the berries. The temperature did not have an impact on anthocyanin content. This parameter also increased after raining, but there is not an order in datas, so the time of maturation is the factor that influences coloring content instead of temperature changing.

Acknowledgement

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REVALUATION OF NATIVE CORN TO FACE ENVIRONMENTAL CHANGE

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Abstract

Each sowing with improved maize moves to the native maize (MN). Producers accept them for their good performance under favorable conditions, but this behavior in rainfall agriculture is not certain. It is known that the genetic diversity within the MN ensures a certain yield under droughts in Mexico, so the objective of this work is to analyze the flowering stage in blue MN (MNAz) under the rain variations and point out its advantages under that environmental tension. Three locations (Loc) were planted with MNAz of 14, 16 and 18 rows, in which the dynamics of flowering and yield were recorded. Within each corn, there were cobs of different rows, predominantly those of 16 in the three locations. In 2018, in the eastern region of Tlaxcala, Mexico, agriculture suffered losses due to droughts of 30 and 45 days, causing economic damage to the country. Under these conditions, in Loc1 the yield of MNAz1 was 4,431.4 kg/ha and in MNAz2 2,213.7 kg/ha; in Loc2 with MNR/N it was 3,911.8 kg/ha and in Loc3 with MNAz3 it was 1,076.5 kg/ha. The performance is explained by the dynamics of female flowering under drought, through characters of escape to drought. In Loc1 the prolificity index was 0.92 in MNAz1 and 0.64 in MNAz2; in Loc 2 of 1.2 and 0.54 in Loc3. The improved corn yields between 0.1 and 0.8 ton/ha locally, which stimulates the MN to improve.

Keywords: *Native blue corn, female flowering, rain, prolificity index*

Introduction

The massive planting of improved maize causes its genetic erosion, which endangers native corn. In Mexico, it is estimated that around 25% of the corn planting is improved, reaching up to 70% in regions with technology (Ortega *et al*., 2000). Although there are producers who keep native seeds, at present this is no longer so true, due to the difficulty of preserving them. Therefore, it is also studied how to conserve them in regions where this practice exists, finding that with metallic silos they are conserved with grain moisture below the recommended 13.5%, ensuring germination greater than 86.2% (Manuel *et al.,* 2007). The above is important because in MNAz of the high plateau a variant of the flour starch dominates that makes it specific for tlacoyos, tamales and blue tortillas (Muñoz *et al*., 2009), but very sensitive to the attack of pests in the warehouse.

Yield is determined by the production environment, so, yielding maize families, they were because they flourished in times with good rainfall (PP), while those of low yield female flowers ("jilotes") in times with low PP. With limitations of humidity, the rain that explains the yield is the one that accumulates from two weeks before to two weeks after the "jiloteo". In contrast, without humidity limitations, the temperature around female flowering (FF) is what explains the yield potential (Barrales *et al*., 1984). It is also known that in regions with prolonged drought the production of corn stubble becomes more important than of grain (Muñoz *et al*., 2009).

The nutraceutical importance of colored corn is important today, although it was already known that corn with anthocyanin pigment is what regulates the color of anthocyanins (Salinas *et al.,* 2012), whose safety for consumption human forces to take advantage of its antioxidant, antimutagenic and anticancer properties (López-Martínez, 2009), given the public health problems in Mexico. It was found that the antioxidant activity has a high correlation (r

= 0.72 **) with the anthocyanin content (Ruiz *et al*, 2008), but it is suggested to see its changes when making food.
The quality of native or native corn is not in doubt, and proof of this is the diversity of uses associated with each type of corn. Blue corn has a protein quality with lysine contents between 3.0 and 4.13% and tryptophan between 0.417 and 0.629% (Vidal *et al*., 2008). It is also mentioned that there are maize that contribute more than 50% of the needs of lysine and tryptophan, which makes it possible to suggest, quantify its contents in each pigmented corn and recommend its specific use in infant feeding.
Lately, the genetic improvement in corn is directed to variables of interest for the dough and tortilla industry, such as starch content, oil and protein quality (Vázquez-Carrillo *et al.*, 2018), however, what is needed it is to benefit consumers, which implies recovering the MN with its nutritional, nutraceutical and functional properties. In that sense, given the recurrent presence of droughts during flowering in corn that causes loss of yield, this work is proposed with the objective of analyzing the flowering stage in blue MN (MNAz) under the variations of rainfall at that stage.

Materials and methods

In 2018, during the seasonal agricultural cycle in Cuapiaxtla, Tlax., four batches of evaluation with MNAz with different rows (NH) were sown. In the first batch (Loc1), blue maize was planted with 18-rows (MNAz1) and 20-rows (MNAz2), registering the emergence date (FE) on April 29. In Loc2 16-row mixed red and black corn (MNR/N) was planted with FE on May 8 and Loc3 was planted 16-row corn (MNAz3) with FE on May 7. In each locality four evaluation sub-lots were delimited, five rows of five meters long and 85 cm wide, distributed in the four ends of each lot. Within each sublot 25 plants were marked to record male flowering (FM) or panicle emergence (EmPa) and female flowering (FF) or "jilot" emergency (EmJil) during flowering. Male Flowers and female flower were counted every eight days to have the dynamics of flowering. With simple linear regression (Ostle, 1974), the age of the plant and the number of flowering plants were related, to estimate the daily appearance of "jilotes".
During the biological cycle of the plants, rain was recorded with a Weekly Accumulation Rain Gauge (Barrales, 1980), to analyze the dynamics of FF and the yield in each environment, according to the variation of the rain. The rainfall received by the plants was estimated since two weeks before the FF (PPpreFF), until to two weeks after it (PPposFF). The yield was evaluated in the two central grooves of each sublot, where the number of harvested plants and cobs that were carried at constant weight was recorded and the yield components, grain yield per ha, cob weight and of grain per plant, pot weight, percentage of pot and prolificity index to see the number of ears per plant. Simple linear regression, analysis of variance and test of means were used with the Tukey procedure (Steel and Torrie, 1980), using the SAS package.

Results and Discussion

In all locations there was drought (S) in the flowering stage, a recurring phenomenon in temporary agriculture. In corn, the lack of water during flowering almost does not prevent EmPa, but it does affect EmJil, which in extreme cases does not occur, and with it, although there is little pollen, there are no stigmas that receive it, decreasing the fertility of the ovules and seed formation.
In each variety the typical floral asynchrony of the corn plant was observed, that is, the panicle first appears and then the jilote, as a mechanism to reduce self-fertilization. In heterogeneous populations of heterozygous maize such as MN, not all plants bloom at the same time, so that early plants start with EmPa and then they will be the first to emit jilotes, coinciding with other plants that start their EmPa and that will be the ones that pollinate it.

This mechanism puts the later ones at risk, which emit jilotes when there is no plant with pollen release, causing cobs with few grains. When relating plants with EmPa and EmJil over time, simple linear regression models (Table 1) were found with high coefficients of determination (R^2), greater than 0.9. In the models, according to the regression coefficient, it is observed that every day they appeared between 0.73 panicles in MNR/N and 0.88 in MNAz3. In the case of "jilotes", between 0.61 in MNR/N and 0.71 "jilotes" per day in MNAz1, MNAz2 and MNAz3 were recorded every day. In the latter, 100% of EmJil was never registered, due to the greater intensity of the drought.

Table 1. Linear regression models that quantify the relationship between the number of male or female flowering plants (X) with the age of blue corn plants (Y), planted in three locations under reinfall conditions in Cuapiaxtla, Tlax. 2018.

Locations	Modelo de regresión	R^2
	<u>Male Flowering (panículas)</u>	
Location 1		
MAz1 1	Y= 0.7305X – 47.417	0.9303**
MAz2	Y= 0.7835X – 52.304	0.8659**
Location 2		
MR/N	Y= 0.7722X – 59.889	0.9331**
Location 3		
MAz3	Y= 0.8851X – 63.065	0.9382**
	<u>Female Flowering (jilotes)</u>	
Location 1		
MAz1	Y= 0.6153X – 46.396	0.9569**
MAz2	Y= 0.7126X – 55.594	0.9374**
Location 2		
MR/N	Y= 0.7193X – 60.059	0.9316**
Location 3		
MAz3	Y= 0.6964X – 53.267	0.9504**

In Loc1 with MNAz1 and MNAz2 up to 63 days of age of the plants, 224.35 mm rainfall accumulated, and then have three weeks without rain defining an S of 34 days (between 64 and 98 days dde (days after the emergency)). The average EmPa was day 82 in MNAz1 and one day later in MNAz2, and in both, the average EmJil was at 96 dde. It has been found that the accumulated rainfall since two weeks before until two weeks after the FF (Barrales *et al.*, 1984) influences the yield more; in this case, on average both maize received in this period, 34.3 mm of rain. It was observed that 13.3% of the plants were early and received only 2 mm of rain in pre-flowering; at 90 dde, 48% were blooming and those received 28.1 mm of PPposFF and the rest of the plants received 34.9 mm in the same period. The 12.3% of the plant were the latest and received the greatest amount of rainfall distributed between pre and post-flowering female. In the locality where the MNR/N was evaluated, until day 54 where it rained 235.27 mm, and then there was an S from 55 to 90 dde. In this locality the average EmPa occurred at 83 dde and EmJil at 96 dde, which coincides with a maximum rainfall on day 90, accumulating rain of 148.47 mm until day 131, which allowed the corn to evade effects of drought. During the average flowering period, 34.3 mm of rain were received, especially after FF, although in this case the earliest plants were 5% and received 69.9 mm of rain after EmJil; 22.7% of plants received 73.2 mm of rain after EmJil, and the other 41.3%, 9.3% and 13.3% received 84.1, 111.3 and 91.7 mm of rain before and after EmJil, which allowed them to have good Humidity conditions, although it was not the highest performance. In the lot with MNAz3 the lowest amount of rain was recorded, because until day 70, was

155.02 mm of rain accumulated, then face four weeks without rain. It was until day 91 when it rained, accumulating until day 125, 93.34 mm. The average EmJil occurred at 95 dde, and around flowering 41.4 mm of rain was received, mainly in post FF, from day 96 to two weeks later. Until the 97th day, the plants received rain after EmJil, so that 4% of early plants received 27.1 mm, 36% of plants received 39.9 mm and 24% received 42 mm of rain. Later plants coincided with better rain conditions. Thus, 17.3% of plants received before and after EmJil, 60.6 mm of rain and 5.3% of late plants received 93.3 mm of rain around the FF. Grain yields were 4431.5, 2213.5, 3911.8 and 1076.5 kg/ha in MNAz1, MNAz2, MNR/N and MNAz3 respectively (Table 2). In the locality with MNAz1 and MNAz2 a total of 365.18 mm of rainfall was recorded in the biological cycle, which contrasts with the locality where MNAz3 was with the lowest yield, where it rained 348.80 mm, that is, only 16.38 mm of rain less. It happened that, before the S in both environments, in the first one 224.35 mm had already been received while in the other only 155.02 mm of rain, which caused an effect on the development registering a prolificity index of 0.5 (Table 2).

Table 2. Result of the test of means with the Tukey procedure in variables registered in blue maize evaluated in rainfall agriculture conditions in Cuapiaxtla, Tlax. 2018.

Variable	MAz1	MAz2	MR/R	MAz3
number of plants per hectare	57254.9 a	45882.4 b	33823.5 c	44411.8 b
Number of cob per hectare	52647.1 a	29117.6 c	40294.1 b	24411.8 c
cob weight per hectare (kg)	5041.2 a	2523.5 c	4397.1 ab	1272.1 d
Grain weigth per hectare (kg)	4431.5 a	2213.8 bc	3911.8 b	1076.5 d
Cob weight per plant (g)	88.3 ab	55.4 bc	133.7 a	28.8 c
Grain weight per plant (g)	77.6 ab	48.7 bc	119.0 a	24.4 c
Prolificity index	0.8 ab	0.6 b	1.2 a	0.5 b
"olote" Percentage	12.1 ab	12.3 ab	11.1 b	15.5 a
seed weight (mg)	376	372	343	372

Need to explain what are the letters a, ab and what the different letters mean statistically
Another important element that influenced the performance in Loc1 was that after S, between 99 and 147 days, it rained 136.46 mm, followed by a week without rain and then between 155 and 168 dd, 4.37 mm were received. On the other hand, in the MNAz3 after the S it rained between 91 and 125 dde, 93.34 mm and then returned to present another four weeks without water that directly affected the grain filling. Here, in the end, between 154 and 181 dde were recorded 100.44 mm of rain, which in no way favored the filling of grain, but it affected the quality of it by wetting the cobs and rotting the grain.

In the case of the MNR/N after the four-week S, between 148 and 131 days, 148.47 mm cried, then two weeks without rain, without showing effects of water deficit in the plants, since there was humidity in soil. In the end, between 146 and 159 dde it rained 30.57 mm that could be grained. In this plot it was where he obtained more rain (414.31 mm) and the EmJil had under favorable humidity conditions. Here, plants have no mechanism to evade or resist any adverse condition effects, such as S (Loomis and Connor, 2002).

The current genetic improvement looks for short flowering stages, that is, a short time between EmPa and EmJil, which shows a corn crop blooming almost at the same time. However, when MN does not flowering all at the same time, they can effectively confront the phenomena of S, because they affect when it occurs, it will impact differently to the early, intermediate or late ones within the same MN. Also, we can use varieties of different cycles to face the magnitude of rainfall between corn producing areas (Luna et al, 2005), but when the FF is short, if it coincides with the drought, the yield loss can be total.

Conclusions

The amplitude in the stage of male and female flowering in native maize allows us to confidently face the adverse effects of the drought, as it does not cause equal damage to all the plants within the population, since it will only affect those that affect pre flowering and flowering.

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PRODUCTIVITY OF RED CLOVER-ITALIAN RYEGRASS MIXTURES ON ACIDIC SOIL DEPENDING OF THE LIMING

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Abstract

Grass-legume mixtures are important crops in the production of quality forage. Soil acidity is one of the factors which limit growth of many legumes and grasses. The aim of the study was to analyze the effect of liming (control – without CaO; 3 t ha^{-1} CaO; 6 t ha^{-1} CaO) an acid soil with a pH of 4.8, on the forage yield, hay yield, the proportion of red clover, Italian ryegrass and weed in the total hay yield. The experiment was performed in the period 2014-2016. The trial was set in a randomized block design with three replications and with a plot size of 5 m^2. Sowing was done on 20 cm row spacing of. In the 2014 and 2016 analyzes were carried out on three and in the 2015 on two obtained cuts, due to drought. The total hay yield of grass-clover mixture for all of the three years in control was 36.0 t ha^{-1}, in the treatment with 3 t ha^{-1} of lime, the yield was 39.607 t ha^{-1} and in the 6 t ha^{-1} of lime, the yield was 37.279 t ha^{-1}. Soil liming had an impact on the forage and hay yield in the first cut in the second and third year of production. In drier periods of the year, soil liming influenced the significant increase in the weight ratio of Italian ryegrass. However, in the wet times of the year, the stronger impact of soil liming was reported for the development of red clover.

Keywords: *forage yield, Italian ryegrass, liming, red clover, weeds.*

Introduction

The precondition for intensifying livestock production is to produce sufficient quantity of high quality forage. Average hay yields in meadows in the Republic of Serbia are low and range between 1.5 - 2.0 t ha^{-1}. One of the main reasons for low and unstable yields and poor forage quality is the lack of application of agro-technical measures (Dubljević, 2007).In present days, there are tendencies of reducing energy consumption and environmental pollution, intensifying systems of sustainable agriculture and biodiversity preservation. For this purpose, among the other things, the inclusion of forage legumes into grass mixtures should be carried out. The introduction of legumes in systems of forage crops production reduces the use of nitrogen fertilizers possibility of losing nitrogen from the soil by leaching or emission in form of gasses, is reduced (Ledgard et al., 1999). Symbiotic fixation of nitrogen in legumes is the basic process for maintaining fertility of the soil and productivity of organic systems of cultivation (Vinther and Jensen, 1999). By cultivating legumes and grasses in mixture, a more viable production and better quality of forage is achieved (Nešić et al., 2007). Also, the share of weeds in the total yield of grass-leguminous mixtures is considerably smaller as compared to clean grass or leguminous crop (Sleugh et al., 2000).

Acidity of soil is one of the factors that makes it difficult to cultivate grass and legumes (Wheeler, 1998). Soil reaction affects all of the stages of plant development, resistance to disease, resistance to low temperatures, life span, forage yield and quality. Acidic soils, with addition to the lack of calcium, are distinguished by the high presence of easily mobile forms of Al, Fe, Mn and reduced content of easily accessible P, K and Mo (Su and Evans, 1996). Problems of acidic soils can be successfully solved by the introduction of lime fertilizers

(Grewal and Williams, 2003). Considering that acid soils occupy significant areas in the Republic of Serbia, the aim of the research was to investigate the effect of liming on such the soil on the yield of grass-leguminous mixture of red clover and Italian ryegrass.

Materials and methods

The field trial was set up in 2014 in Čačak (43°54'39.06" N, 20°19'10.21" E, 246 m a.s.l.) on a leached vertisol with acid reaction (pH_{H2O} 4.8), which contains 3.18% organic matter, 0% $CaCO_3$, 22.08 mg P_2O_5, 30.0 mg K_2O 100 g^{-1} of soil. Before the basic cultivation of the soil and after the end of vegetative periods, fertilization was carried out with 300 kg ha^{-1} $N_{15}P_{15}K_{15}$ or how many kg of each element per ha. The trial was set up on a completely random block system in three replications, with the experimental plot size of 3 m^2 (3x1 m). The analyzes were carried out on the grassy leguminous mixture of red clover (variety Kolubara – Institute of Field and Vegetable Crops in Novi Sad) and Italian ryegrass (Tetraflorum – Slovenian tetraploid cultivar). The research covered three levels of soil liming (control – without CaO, treatment with 3 t ha^{-1} CaO and treatment with 6 t ha^{-1} CaO). The introduction of lime material was done by surface application immediately before the pre-seeding preparation of the soil. The sowing was carried out at a distance of 20 cm between rows (Italian ryegrass and red clover were sown in the same rows), with a seed quantity of 12 kg ha^{-1} of red clover, and 12 kg ha^{-1} of the Italian ryegrass. The crop was grown without the use of irrigation. The analysis were carried out on three obtained cuts in 2014, two cuts in 2015 (due to the pronounced drought periods) and on three cuts in 2016. Mean annual air temperature in 2014, 2015 and 2016 was 13.15°C, 13.14°C and 12.4°C respectively, and the annual rainfall was 902 mm, 547 mm and 767 mm respectively (Table 1). The average annual air temperature for many years (1992-2002) is 11.97°C, and the average annual precipitation is 680.3 mm.

Table 1. The amount and distribution of rainfall by month (P) and mean monthly temperatures (T) for 2014, 2015, 2016 and the period 1992-2002.

Month		I	II	III	VI	V	VI	VII	VIII	XI	X	XI	XII	$\bar{x}$ i $\sum$
1992-	P (mm)	0.5	3.1	7.6	11.7	17.9	21.3	22.6	23	16.8	12.2	6.1	0.8	11.97
2002	T (ºC)	30.7	38.9	42.5	51.2	56.4	88.4	82.6	51.6	74.9	57.6	52.8	52.7	680.3
2014	P (mm)	4.0	6.6	10.2	12.5	16.1	21.1	22.7	22.1	17.0	13.5	8.9.0	3.1	13.15
	T (ºC)	21.5	6.0	52.5	104.5	125	103.5	163	56.0	101	60.0	19.0	90.0	902.0
2015	P (mm)	0.9	2.7	9.1	13.4	16.5	22.2	23.0	22.4	18.0	16.5	9.2	3.8	13.14
	T (ºC)	30.0	63.0	74.0	16.0	53.0	87.0	4.0	25.0	42.0	68.0	41.0	44.0	547.0
2016	P (mm)	-0.6	2.4	9.5	12.8	16.8	20.8	22.2	22.1	17.6	15.2	8.8	1.2	12.4
	T (ºC)	49.0	20.0	182	41.5	123.5	74.0	12.5	76.0	44.0	70.0	20.0	55.0	767.0

Mowing was carried out in the stage of budding of red clover. The forage yield was determined by measuring the total biomass from the plot immediately after mowing. From the measured sample (1000 g), after drying at room temperature, the hay yield (t ha^{-1}) was calculated and the weight ratio of the Italian ryegrass, red clover and weeds in the hay was determined. The obtained results were processed using the analysis of variance (ANOVA) in SPSS 4.5 software. The significance of the difference in mean treatment values was tested by the LSD test.

Results and discussion

Soil liming did not have an impact on the forage and hay yield in the first year of growing grass-legume mixture of red clover and Italian ryegrass (Table 2). The average hay yield in the first cut was 10.01 t ha^{-1}, in the second 4.376 t ha^{-1} and in the third 1.41 t ha^{-1}. Simić et al. (2011) was determined lower yields of grass-legume mixture of red clover and Italian ryegrass in the first year of production, which is a result of less precipitation during the vegetation period.

Table 2. Influence of soil liming on the forage yield, hay yield, proportion (%) of Italian ryegrass-red clover and weeds in the total yield in 2014

Cut	Liming	Forage yield ?	Hay yield?	Ryegrass weight ratio (%)	Clover proportion weight ratio (%)	Weed proportion weight ratio (%)
1	ø	27.31	9.83	88.7	8.3 b	3.01
	3 t ha^{-1}	29.88	11.38	84.8	11.9 a	3.31
	6 t ha^{-1}	26.42	8.82	93.3	3.6 c	3.03
2	ø	12.18	3.78	61.0 a	33.2 c	5.8
	3 t ha^{-1}	16.78	4.57	50.1 b	42.1 b	7.8
	6 t ha^{-1}	16.19	4.78	41.3 c	50.9 a	7.8
3	ø	5.18	1.16	32.0 a	61.6 b	6.45 a
	3 t ha^{-1}	6.25	1.35	10.0 b	88.8 ab	1.27 c
	6 t ha^{-1}	7.07	1.72	0.1 c	95.2 a	4.73 b

The values denoted with different small letters within columns are significantly different ($p \leq 0.05$) in accordance with the LSD test.

In the first cut in 2015, on the treatment with 6 t ha^{-1} of lime, a significantly lower forage yield was obtained as compared to the control and treatment with 3 t ha^{-1} of lime, while the hay yield did not differ significantly (Table 3). The reason for this is the impact of liming on the change in the floristic composition of the grassland. Liming had a more positive impact on the red clover, which caused its weight ratio in the mixture to increase, and the weight ratio of the Italian ryegrass reduced get lower?, thus reducing the overall yield. In the second cut there was no effect of soil liming on forage and hay yield. The average hay yield in the first cut in 2015 was 8.01 t ha^{-1}, and in the second cut 4.573 t ha^{-1}.

Tabela 3. Influence of soil liming on the forage yield, hay yield, proportion (%) of Italian ryegrass, red clover and weeds in the total yield in 2015

Cut	Liming	Forage yield	Hay yield	Ryegrass proportion weight ratio (%)	Clover proportion weight ratio (%)	Weed proportion weight ratio (%)
1	ø	34.43 a	8.13	76.0 a	24.0 c	-
	3 t ha^{-1}	30.88 ab	8.35	47.1 b	52.9 b	-
	6 t ha^{-1}	26.42 b	7.54	6.2 c	93.8 a	-
2	ø	13.6	3.89	43.2 a	56.1 b	0.74 a
	3 t ha^{-1}	15.2	4.24	20.7 b	79.2 a	0.10 b
	6 t ha^{-1}	21.2	5.59	17.0 b	82.4 a	0.85 a

The values denoted with different small letters within columns are significantly different ($p \leq 0.05$) in accordance with the LSD test.

In the third year of production, there was no Italian ryegrass, and the weight ratio of weeds was very high, especially in the first cut (Table 4). The forage and hay yield in the first cut was significantly reduced in the treatment with 6 t ha^{-1} as compared to the control, while in the second and the third cut the forage and hay yield did not significantly depend on the liming of the soil. The hay yield in the first cut was 0.646 t ha^{-1}, in the second 5.987 t ha^{-1} and in the third 2.485 t ha^{-1}.

Tabela 4. Influence of soil liming on the forage yield, hay yield, proportion (%) of Italian ryegrass, red clover and weeds in the total yield in 2016

Cut	Liming	Forage yield	Hay yield	Ryegrass proportion weight ratio (%)	Clover proportion weight ratio (%)
1	ø	3.933 a	0.746 a	11.7 ab	88.3 ab
	3 t ha^{-1}	3.544 ab	0.705 ab	7.0 b	93.0 a
	6 t ha^{-1}	2.867 b	0.489 b	19.0 a	81.0 b
2	ø	14.76	5.51	31.5 ab	68.5 ab
	3 t ha^{-1}	16.28	6.36	26.0 b	74.0 a
	6 t ha^{-1}	15.93	6.09	38.6 a	61.4 b
3	ø	9.51	2.552	35.8	64.2 a
	3 t ha^{-1}	9.81	2.652	57.1	4.9 c
	6 t ha^{-1}	9.17	2.250	57.0	43.0 b

The values denoted with different small letters within columns are significantly different ($p\leq0.05$) in accordance with the LSD test.

The total hay yield in 2014 was 15.797 t ha^{-1}, in 2015 12.58 t ha^{-1} and in 2016 9.118 t ha^{-1}. Higher yield in the first year in relation to the second is the consequence of higher precipitation, especially in the second period of vegetation. The total yield for the three years in the control treatment was 36.00 t ha^{-1}, in the treatment with 3 t ha^{-1} of lime 39.61 t ha^{-1}, and on the one with 6 t ha^{-1} 37.28 t ha^{-1}.

In the second and the third cut during the first year of exploitation, the liming of soil affected positively and significantly the weight ratio of red clover in mixture, at the expense of reducing the weight ratio of Italian ryegrass and weeds. However, in the first cut, the weight ratio of red clover was decreased with the application of liming, due to the increase in the weight ratio of the Italian ryegrass. The reason for this inconsistent impact of liming on the change in the weight ratio of clover and ryegrass can be a different amount of precipitation. In drier periods of the year, liming has influenced a significant increase in weight ratio of the Italian ryegrass, as confirmed by the results of Tomić et al. (2013). Thomas (1984) pointed to the similar results, according to which in the conditions of drought, the competitive ability of the Italian ryegrass was more expressed in relation to the red clover. However, in the wet times of the year, the stronger impact of soil liming was noted for the development of red clover, and then the weight ratio have increased.

In 2015, in the first cut, the weight ratio of red clover was increased significantly with the increase in the amount of lime applied, at the expense of reducing the weight ratio of Italian ryegrass. Due to very strong competitive ability and rapid space for growth of red clover and Italian ryegrass, there were no weeds. In the second cut, the weight ratio of red clover was significantly higher and the weight ratio of Italian ryegrass was lower in the treatments as compared to the control, while there was no difference between the treatments. The weight ratio of weeds in the second cut was below 1%. Živanović-Katić et al. (2008) state that the application of liming leads to a change in the floristic composition of plant communities, in way of achieving larger density of crops and reducing the weed weight ratio .

In the third year of production, there was no Italian ryegrass, the weight ratio of red clover was smaller, and the weight ratio of weeds was high. Therefore, the weight ratio of red clover and weeds in all three shots did not differ significantly in treatments, as compared to the control.

Conclusion

Soil liming did not have any effect on forage and hay yields except in the first cut in the second and third year of production, when significantly less forage yield as compared to the control was obtained for the treatment with 6 t ha^{-1} of lime. The hay yield did not change significantly during the second year in the first cut under the influence of liming, while in the third year on the treatment with 6 t ha^{-1} was significantly lower as compared to the control. The total hay yield for all three years was for control 36.0 t ha^{-1}, for treatment with 3 t ha^{-1} of lime the yield was 39.607 t ha^{-1} and for treatment with 6 t ha^{-1} it was 37.279 t ha^{-1}.

The impact of soil liming on the weight ratio of the Italian ryegrass and red clover in the forage was inconsistent. The reason for this is a different amount of precipitation during certain periods of the year. In drier periods of the year, soil liming has influenced a significant increase in the weight ratio of the Italian ryegrass. However, in the wet times of the year, the stronger impact of soil liming was recorded on the development of the red clover, and then its weight ratio increased. In the third year of production there was no Italian ryegrass, the weight ratio of red clover was smaller, and the weight ratio of weeds was high.

Notification

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LENGTH OF THE ONE-YEAR-OLD BRANCHES AND CATKIN POSITION ON THEM AFFECT QUANTITY AND MORPHOLOGICAL CHARACTERISTICS OF STAMINATE FLOWERS IN WALNUT

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Abstract

The influence of the length of the one-year-old branches (under 5 cm, 13-17 cm and above 25 cm) and the catkin position on these branches (basal, middle and terminal) to the number of staminate flowers per catkin, the number of stamens per staminate flower and the number of stamens per catkin was studied. The number of staminate flowers per catkin amounted to 56-123 (average 95.4). From the base to the top of the branch, the number of staminate flowers per catkin increased. The highest average number of stamens per staminate flower was found in catkins on the branches 13-17 cm long (20.8), and the smallest in the branches under 5 cm long (17.8). In the catkins at the base of the branches, the staminate flowers contained the smallest number of the stamens (on average 17.8), while in the catkins in the middle and the terminal zones of the branches the staminate flowers contained an average of 19.4 and 19.9 stamens respectively. The number of stamens per catkin ranged from 1120 to 3075 (average 1825.6). The largest number of stamens per catkin was determined on the branches 13-17 cm long (2001.6), and the branches up to 5 cm in length had the fewest stamens per catkin (1617.4). From the top of the branch to its base, the average number of anthers per catkin decreased from 2084.5 to 1503.3.

Keywords: *Juglans regia, stamen, male inflorescence, plant reproduction*

Introduction

The family Juglandaceae consists of seven genera, comprising about 60 tree species. The genus *Juglans* contains circa 20 species, of which the Persian walnut (*Juglans regia* L.) is the most widely cultivated and economically important species of edible nuts in the temperate regions of the world (McGranahan and Leslie 1990).

Persian walnut is the main nut crop in Serbia. The total production of walnuts in a shell from 2012 to 2017 in Serbia has varied from 12276 tonnes in 2017 to 18336 tonnes in 2013. The total walnut production area in Serbia in 2017 was 3307 ha, and the average yield from 2012 to 2017 was 3712 kg/ha (FAO, 2017).

Walnut is a monoecious, wind-pollinated, and self-compatible fruit tree. Staminate flowers are borne on catkins, usually 10 to 15 cm in length, which emerge from lateral buds on the wood from the previous season's growth (Figure 1).

The individual flowers lack petals and consist primarily of a whorl of green sepals that surrounds up to 40 pollen-bearing stamens on each flower. Each stamen terminates in a pollen-bearing organ, the anther (Polito, 1998).

In the previous studies, the authors have recognized that the main causes of morphological differences in the structure of catkins are the walnut genotypic specificities of the species and cultivars (Molina *et al*., 1996; Mert, 2010; Sütyemez, 2007; Ozcan *et al*., 2017), and that the position of staminate flowers on the axis of catkins is the basic factor determining number of stamens in staminate flowers (Germain *et al*., 1975; Impiumi and Ramina, 1967).

The establishment of staminate catkins in the walnut buds takes place during the spring of the year preceding their flowering, on about the 25th day after the initiation of leaf sprouting (Šebánek *et al*., 1991). The young meristems of walnut buds may develop in three different ways as stamen primordia, as vegetative buds or as terminal buds with pistillate flowers (Sladký, 1972). The origin of three different types of buds on the branches of fruitful walnut trees gives the impression that the course of this differentiation is most probably affected by endogenous regulators (Langrova and Sladky, 1971). According to Atsmon and Galyn (1962), the pattern of primordia development is regulated hormonally with a strong modifying influence exerted by the adjacent leaf.

The aim of this paper is to draw attention to the need to determine the factors expressing the regulatory function in the process of formation of reproductive organs in walnut. One of the unknowns in this process is the way in which the length of one-year-old branches, as well as the position of catkins on these branches, affects the number of staminate flowers in catkins, the number of stamens in flowers, and the number of stamens in catkins.

Materials and methods

This study was carried out in 2014 and 2015 on Serbian walnut cultivar 'Šampion'. Plant material has been collected from walnut trees grown in an orchard situated near the town of Kraljevo (Central Serbia).

Samples of flower branches with catkins were collected from different parts of the crowns and were classified according to length in three groups: 1) up to 5 cm in length, 2) from 13 to 17 cm and 3) over 25 cm. Each group contained 30 branches that were taken from five trees of each variety examined. One catkin from the basal, middle and terminal part of each bearing branch was taken as a sample. The number of staminate flowers per catkin, the number of stamens per staminate flower and the number of stamens per catkin was studied. Since the number of stamens in staminate flowers decreases from the base to the top of catkins, the number of stamens per male flower is determined as an average of three flowers in the middle of the catkin.

Counting of staminate flowers and stamens was performed under a stereoscopic microscope, model STM-9 Pro (Nanjing Microtech Scientific Instrument Co., Jiangsu, China) at a magnification of 10-60 times. Photographing of samples was performed with Canon EOS 700D (Canon Inc., Tokyo, Japan). The number of stamens per catkin was calculated by multiplying the number of staminate flowers per catkin with an average number of stamens in the three flowers in the middle of the catkin.

The data obtained are processed by the statistical method of the analyses of variance. The significance of differences between mean values is determined by Tukey´s test at $P = 0.05$.

Results and discussion

The number of staminate flowers per catkin

The number of staminate flowers per catkin in the two-year research period ranged from 56 to 123, with an average value of 95.4. These results are in agreement with the findings of other authors. Germain *et al*. (1975) stated that catkins of the walnut contain about 100 to 160 staminate flowers, spirally arranged along the axis of the catkin. According to Molina *et al*. (1996), depending on the genotype, walnut catkins contained 95-300 staminate flowers. Sütyemez (2007) examined the production of pollen in 32 genotypes in Turkey and found that the number of staminate flowers in catkins ranged from 109-148. Ozcan *et al*. (2017) state that the catkins of four varieties of walnuts contained 117 to 140 staminate flowers.

The factor year had a significant impact on the number of staminate flowers per catkin. In 2014, the average number of staminate flowers per catkin was 90.2, while in 2015 the average value of this parameter amounted 100.7. Janković (2016) states that the average number of

flowers per catkin over the three-year study period was 120.1. On this parameter, a significant influence had the genotype, year and the interaction between genotype and year.
As the length of branches increased, the number of staminate flowers per catkin increased. The number of staminate flowers per catkin at branches shorter than 5 cm (90.5) was significantly lower than the number of staminate flowers per catkin at branches longer than 25 cm (99.6). The number of staminate flowers per catkin on 13-17 cm long branches did not differ significantly from the value of this parameter to the other two categories of branches. Oparnica and Vulić (2006) concluded that the number of male and female inflorescences in hazelnut, as well as their arrangement at the bearing branches, are in direct dependence on the length of bearing branches.
From the base to the top of the branches, the number of staminate flowers in catkins increased. At the top of the branches, the average number of staminate flowers per catkin was 104.3 and significantly differed from the value of this parameter on the middle and basal part

Table 1. Effect of length of the one-year-old branches and catkin position on the number of staminate flowers and stamens

Factors	Factor levels	Number of staminate flowers per catkin	Number of stamens per flower	Number of stamens per catkin
Length of the branches (A)	< 5 cm	90.5 a*	17.8 a	1617.4 a
	13-17 cm	96.2 ab	20.8 b	2001.6 b
	> 25 cm	99.6 b	18.5 a	1857.7 b
Catkin position on the branch (B)	top	104.3 a	19.9 a	2084.5 a
	middle	97.2 b	19.4 b	1888.8 b
	base	84.7 b	17.8 b	1503.3 b
Year (C)	2014	90.2 a	19.1 a	1728.2 a
	2015	100.7 b	19.0 a	1922.9 b
Average		95.4	19.1	1825.6
ANOVA				
Factors		p		
A		**	***	**
B		***	**	***
C		***	NS	*

*Values followed by the same letter do not differ significantly at P < 0.05
NS – non-significant at P < 0.05
* – the differences are significant at p < 0.05
** – the differences are significant at p < 0.01
*** – the differences are significant at p < 0.001

of the branches, and was 97.2 and 84.7, respectively.

Number of stamens per flower
The number of stamens per flower ranged from 13 to 24, with an average value of 19.1 (Table 1). The number of stamens per flower did not differ significantly over the years. According to Krueger (2000), Californian walnut genotypes contain an average of 40 stamens per flower, while Molina *et al*. (1996) state that in their researches the number of stamens per flower was

9-23. Sütyemez (2007) found that the number of stamens per flower among the 32 walnut genotypes ranged from 16-22. According to Ozcan *et al.* (2017), in the four studied walnut genotypes, the number of stamens per flowers was from 17 to 18.
Significantly more stamens had flowers on branches 13-17 cm long (20.8) than those on branches up to 5 cm (17.8) and over 25 cm (18.5). In the catkins at the base of the branches, the staminate flowers contained the smallest number of the stamens (on average 17.8), while in the catkins in the middle and the terminal zones of the branches the staminate flowers contained an average of 19.4 and 19.9 stamens, respectively. According to Germain *et al.* (1975), staminate flowers, depending on the location of the catkin, can have 2-32 stamens. The number of stamens in the flowers decreases from the base to the top of the catkins. In the studies carried out by Impiumi and Ramina (1967), the male flowers that were at the base of the catkins had an average of 18 stamens, and flowers at the top of the catkins 13 stamens.

Number of stamens per catkin
The number of stamens per catkin ranged from 1120 to 3075 (average 1825.6) (Table 1). In 2015, catkins contained significantly more stamens (1922.9) than in 2014 (1728.2). The highest number of stamens per catkin had the branches 13-17 cm long (2001.6), and the branches up to 5 cm in length had the fewest stamens per catkin (1617.4). The difference in this parameter between the branches belonging the largest and the lowest length category was not significant. From the top of the branch to its base, the average number of anthers per catkin decreased significantly from 2084.5 to 1503.3.

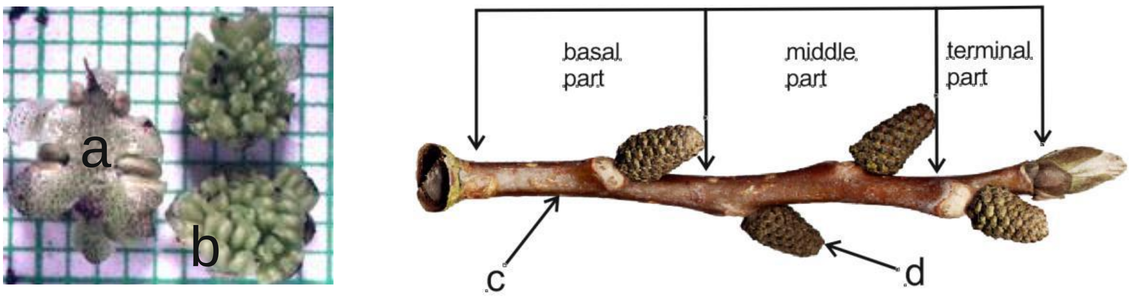

Figure 1. Appearance of staminate flowers, stamens and a bearing branch with catkins in walnut: a - staminate flower; b – stamens; c – bearing branch; d - catkin

In a shoot with multiple flowers, the shoot meristem becomes converted into an inflorescence meristem, which can form one or more floral meristems, each of which develops into a single flower. The floral organ primordia, from which the individual parts of the flower develop, arise in the floral meristem by patterned cell division followed by cell differentiation and enlargement (Wolpert *et al.* 2002). The young meristems of walnut buds may develop in three different ways: as stamen primordia, as vegetative buds or as terminal buds with pistillate flowers (Sladki, 1972). The origin and the development of single buds on the walnut branches is a gradual process. First 2 or 3 basal buds originate from primordia of staminate catkins following 3 to 4 vegetative buds, and only towards the end of the budding period primordia of pistillate flowers are differentiated in terminal buds. This succession in differentiation is analogous, to a certain extent, with the differentiation of staminate and the pistillate inflorescence of maize **(**Langrova and Sladky, 1971). The transition from vegetative to inflorescence shoot meristem is controlled by environmental signals including photoperiod and temperature (Laibach, 1951; Gregory and Hussey, 1953; Napp-Zinn, 1985), by intrinsic growth regulators such as the gibberellins (Napp-Zinn, 1969; Langridge, 1957; Wilson *et al.*, 1992), and by a system of flowering time genes (Martínez-Zapater, 1994).

The establishment of staminate catkins in the buds takes place during the spring of the year preceding their flowering, on about the 25th day after the initiation of leaf sprouting (Šebánek, 1991). The data given by Sladki (1972) show that spraying walnut shoots with 0,2% IAA or 0.1% MH in the period prior to 1 May may reduce the number of staminate buds and increase the number of vegetative buds.

Conclusions

As a monoecious species, in which male and female reproductive organs are separated in different blooms on the same tree, walnut is characterized by a complex sexual process, and thus quite different from most other fruit trees. Since the biology of reproductive organs is not sufficiently studied, many questions about sexual reproduction in walnuts have not yet been answered. Among numerous issues, it is of particular importance to define factors that regulate the process of transferring vegetative meristem into the generative phase of development, that is, to define the conditions that lead to the induction of male and female inflorescences.

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THE EFFECT OF TEMPERATURE ON POLLEN GERMINATION OF TWO WALNUT CULTIVARS

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Abstract

The research was carried out in 2014 and 2015. The influence of temperature (10°C, 15°C, 20°C, 25°C and 30°C) on pollen germination of two walnut cultivars (G139 and Elit) was examined. Cultivar and temperature both showed a significant effect on pollen germination. Conversely, the year did not affect germination. The least germination of pollen in both cultivars was at 10°C. The average pollen germination rates over the two years were 5% and 11.6% for G139 and Elite cultivars, respectively. The highest pollen germination rates were at 25°C, with G139 cultivar having 34.1% and Elite 44.4% germinated pollen grains. The influence of temperature on pollen germination of studied walnut cultivars is presented in the form of graphics of fitted cubic functions, based on the average values over the two years of research. The results highlight the issue of temperature impact on walnut pollen germination, emphasizing that the minimum temperature for walnut pollen germination is not 14°C (as commonly accepted) and suggesting the possibility that there are genotypes of walnut whose pollen can germinate at temperatures lower than 10°C.

Keywords: *Juglans regia, Geisenheim 139, Elit, temperature, cubic function*

Introduction

The Persian walnut (*Juglans regia* L.) is a wind-pollinated, monoecious, herkogamous plant (McGranahan and Leslie,2009). Walnut is self-fertile, but in most genotypes the period of pollen shedding and pistillate flower receptivity does not sufficiently overlap. Therefore, commercial walnut orchards should necessarily be co-planted with pollenizers (Polito, 1998). An important criterion to consider when choosing pollenizers, as well as the male parent for hybridisation in walnut breeding is pollen viability.

Information on pollen viability and germination is important for the study of reproductive biology of walnut and for the development of its genetic improvement program. Various methods can be used for estimation of pollen viability and germinability in horticultural crops. Two different approaches can be taken to estimate pollen viability: staining pollen with dyes and *in vitro* germination assay. Staining techniques aim to determine pollen enzymatic activity and membrane integrity. *In vitro* germination determines the actual germination ability of pollen under suitable conditions (Shivanna *et al*., 1991; Dantas *et al*., 2005; Tuinstra and Wedel, 2000), anditisthemostwidelyusedmethodof testing pollen viability in breeding programs (Marcellán and Camadro, 1996).

In vitro pollen germination is influenced by several factors, such as species, culture medium, temperature and time of incubation, and flower development stage at the time of sampling, in addition to storage conditions (Stanley and Linskens, 1974).

Temperature is one of the most important environmental factors that affect pollen performance during the progamic phase (Hedhly *et al*., 2005). Threshold and optimum temperature requirements for pollen germination are species-specific and, often, cultivar-

specific (Luza *et al*., 1987; Polito *et al*., 1991; Loupassaki *et al*., 1997; Rosell *et al*., 1999; Hedhly *et al*., 2004).
Cardinal temperatures for germination of pollen are usually in high correlation with average temperatures that prevail at the time of flowering, as evidenced by many authors, including: Griggs and Iwakiri (1975), Egea *et al*. (1992), Pirlak (2002), Mellenthin *et al*. (1972), Luza *et al*. (1987).
Polito *et al*. (1991) detected adaptive responses of walnut pollen germination to temperature during pollen development: optimal temperatures for pollen germination whose formation went under regular environmental conditions were lower than for pollen taken from the branches that were held at temperatures of 16-17°C from the beginning of February until the time of catkins shedding.
In spite of several studies conducted in the previous period to determine the minimum, optimal and maximum temperatures for germination of walnut pollen, no accurate answer has been given to this day. This study aimed to determine the cardinal temperatures for *in vitro* germination of the pollen of two walnut cultivars.

Materials and methods

Studies of temperature effects on in *vitro pollen* germination were performed over the two years, from 2014 to 2015.For the experiments, pollen of two walnut cultivars, G139 and Elit was selected. These cultivars are recommended for cultivation in Serbia in areas with the greater risk of late spring frosts due to the later start of their vegetational period (Mišić, 1989). Additionally, cultivar G139 is considered an efficient pollinator for other cultivars with the late start of the vegetational period (Cerović *et al*., 2003).
Pollen was germinated on media comprising: 0.7% agar, 15% sucrose, 300 ppm boric acid and 50 ppm calcium chloride. Five incubation temperatures were studied: 10±1°C, 15±1°C, 20±1°C, 25±1°C and 30±1°C. The temperature was recorded at three-hour interval as a mean value collected from the three thermometers whose accuracy is ±1^{o}C.
Pollen samples were collected between 8 and 10 a.m., at the time the catkins began to shed their pollen. The catkins were sampled from different parts of the crown of each experimental tree at the start of anther dehiscence. Under laboratory conditions, the catkins were laid out on black paper to release their pollen for three to four hours. Six hours after the catkins collection, pollen grains were inoculated onto germination media.
The pollen was germinated in sterile 35-mm-diameter Petri dishes, each containing 3 ml of medium. According to Taylor (1972), Petri dishes with germination media were exposed to a temperature of 4^{o}C for 24h to achieve optimal agar gel solidification before pollen inoculation. This prevents pollen grains from getting immersed too deep into the medium, which could interfere with the germination process or lead to intensive bursting of pollen tubes due to high hydration. The pollen was deposited by a fine brush to ensure even distribution of pollen grains on the surface of the medium. The importance of uniform pollen distribution on media surface was described by Giulivo and Ramina (1974), who discovered that pollen germination rates were higher in the areas with higher pollen grain density. Pollen grains were incubated in the dark for 24 hours at a temperature of 20°C. Upon incubation, the Petri dishes containing the germinated pollen were deep-frozen at -18°C to hold back pollen tube growth and preserve the material until further evaluation. The day before planned pollen observation under the microscope, Petri dishes were transferred from the freezing chamber to refrigerator to 4^{o}C for defrosting, as described by Hedhly *et al*. (2005).
The numbers of germinated and nongerminated pollen grains were counted under a light microscope at 100 x magnification. The pollen grains were considered germinated when the pollen tube length exceeded its diameter. Fifteen fields of view randomly selected from

different parts of the Petri dishes were examined per dish, each containing 20-50 pollen grains. About 400-600 pollen grains were observed per dish.

The association between pollen germination and independent variables was tested using ANOVA. Significant differences among treatments were computed after Tukey's HSD test at $P < 0.05$. Germination rate responses to temperature and maximum seed germination were analysed using linear and nonlinear regression methods for all genotypes in two experimental years.

Results and discussion

Temperature had a significant effect on pollen germination in all cultivars in both experimental years (Table 1), which is in accordance with the results other authors reported for other plant species (Griggs *et al*., 1975; Luza *et al*., 1987; Sukhvibul *et al*., 2000; Pirlak, 2002; Hedhly *et al*., 2005).

Pollen germination rates for both walnut cultivars were lower in 2014 than in 2015, although the differences were not significant. Janković (2016) studied temperature effect on pollen germination of five walnut cultivars and showed the existence of interactions between the walnut cultivar and the year in which measurements were made. In our study, this was not confirmed. The strong influence of ecological conditions in the time of pollen formation and catkins shedding on the functional ability of pollen is described by Beineke *et al*. (1977) in black walnut and Beyhan and Serdar (2008) in chestnut.

Table 1. Effects of temperature, cultivar and year on pollen germination of walnut

Factors	Factor levels	Pollen germination
Tempera-ture (A)	10±1	8.3 a
	15±1	19.0 b
	20±1	35.6 c
	25±1	39.2 c
	30±1	18.6 b
Cultivar (B)	G139	19.6 a
	Elit	28.8 b
Year (C)	2014	21.9 a
	2015	26.4 a
ANOVA		
Factors		p
Cultivar (A)		**
Temperature (B)		***
Year (C)		NS
A × B		NS
A × C		NS
B × C		NS
A × B × C		NS

The lowest pollen germination rates were obtained at 10° C, although germination still occurred in both cultivars. The pollen of G139 (Figure 1 - a) had lower germination rates at 10°C (3,9% in 2014 and 6,1% in 2015) compared to the one from Elit cultivar, which had the germination rates of 11,1% in 2014 and 12,1% in 2015. With further temperature rise, the percentage of germinated pollen grains in both cultivars also increased (Table 1). The highest pollen germination rates for Elit and G139 cultivars were recorded at temperature of 25°C in both studied years (Figure 1 - b). Temperature rise to 30°C had a negative influence on pollen germination. The effect of interactions between cultivar type and temperature on pollen germination was not significant. Conversely, Janković (2016) found a significant influence of genotype on walnut pollen germination at cardinal temperatures. Also, other authors reported the existence of interactions between genotype and temperature in the pollen germination process (Pfahler *et al*., 1997; Mckee and Richards, 1998; Song *et al*., 1999; Sukhvibul *et al*., 2000; Hedhly *et al*., 2004).

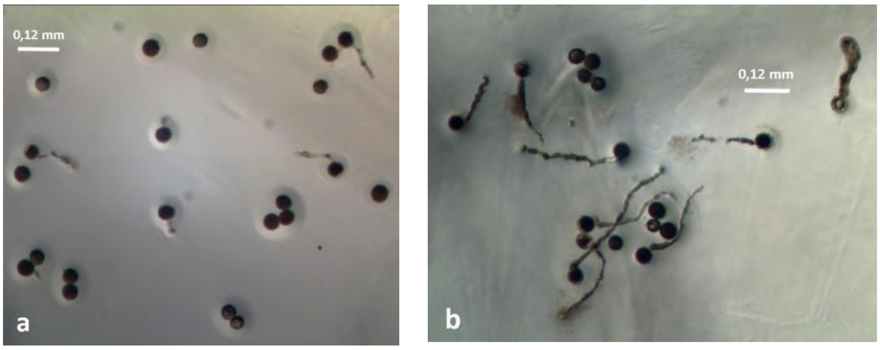

Figure 1. a - pollen of G139 walnut cultivar after germination at a temperature of 10°C; b - pollen of G139 walnut cultivar after germination at a temperature of 25°C

Luza *et al*. (1987) showed that none of the 12 studied Juglans regia and three Juglans nigra cultivars produced pollen that germinated at temperatures below 14°C, and at 14-15°C only pollen from early blooming cultivars germinated.. After their experiments, there were no other studies on cardinal temperatures for walnut pollen germination, so these results are often cited as proof that these cultivars have higher demands regarding heat during pollen germination. Pollen of some almond, sweet cherry and apricot cultivars starts germination already at 5°C (Griggs and Iwakiri, 1975; Egea *et al*., 1992; Pirlak, 2002), while pollen of some pear and mango cultivars can germinate at 10°C (Mellenthin *et al*., 1972; Sukhvibul *et al*., 2000). According to Janković (2016), pollen of all five studied walnut cultivars germinated at 12°C.

Table 2. Equations to describe temperatures' (T) influence on pollen germination rate: T_{opt} - optimal temperature for maximal pollen germination; R2 - regression coefficient

Cultivar	Fitted cubic equations	T_{opt} (°C)	R^2
	2014		
G139	$Y_1 = -0.022T^3 + 1.147T^2 - 16.173T + 73.127$	24.9	0.96
Elit	$Y_2 = -0.019T^3 + 0.949T^2 - 12.660T + 61.740$	24.1	0.92
	2015		
G139	$Y_3 = -0.019T^3 + 0.935T^2 - 11.911T + 51.073$	24.2	0.96
Elit	$Y_4 = -0.019T^3 + 0.919T^2 - 10.901T + 48.527$	24.4	0.92

Results of our study indicate that there are walnut genotypes whose pollen could germinate at temperatures even below 10°C. Among the linear and nonlinear regression models tested, the cubic function best described the response of pollen germination to temperature (Fig. 1) and was used to estimate the optimal temperatures for pollen germination of all genotypes in two experimental years (Table 2).

According to Janković (2016), between pollen germination at a temperature of 12°C and time of cultivar blooming, there were no significant correlations. Mert (2009) studied pollen germination in six walnut cultivars at three temperatures (16°C, 19°C and 27°C) and obtained the highest pollen germination rate at 27°C, and the lowest (1.97-8.92%) at 16°C. Wu *et al*. (2008) reported that the optimal temperature for germination and tube growth of walnut pollen was 25°C. Luza *et al*. (1987) obtained maximum pollen germination at 28°C in *J. regia* and at 32°C in *J. nigra*. In this experiment higher optimum temperatures for pollen germination were associated with later blooming cultivars, that was also observed in our study.

Conclusion

Temperature is an important factor in walnut pollen germination process, especially regarding cardinal values. Opposed to the generally accepted opinion that walnut pollen doesn't germinate at temperatures below 14°C, our results show that germination is possible even at 10°C. During the walnut pollen germination process, a significant effect also display genotypes and environmental factors that affected the process of pollen formation. It can be concluded that under specific ecological conditions, some walnut genotypes can produce pollen with the ability to germination at temperatures even below 10°C, indicating the need for further studies regarding this topic.

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THE EFFECT OF CYTOPLASMATIC MALE STERILITY ON YIELD AND YIELD COMPONENTS OF MAIZE INBRED LINES

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Abstract

Initial studies related to cytoplasmic male sterility (CMS) were performed by Rhoeds in 1931. CMS is used in maize to enhance efficiency of seed production with simultaneous cost redaction. The majority of studies showed positive effects of CMS on maize grain yield. Grain yields recorded male sterile plants were higher by 5-10% than the ones in female fertile plants. Seven maize inbred lines of different origin and growing season were analysed. Each inbred was analysed in five variants: original inbred (N), CMS-C, RfC, CMS-S and RfS. The aim of this study was to compare grain yields and yield components of original inbreds and their CMS and Rf variants. The highest yields of observed inbreds (5.303 and 5.197 t/ha) were recorded in those with C and S cytoplasm, respectively. According to the LSD test, at significance levels of 0.05 and 0.01, the longest ears of 15.57, 15.56 and 15.46 cm were detected in inbreds with original, S and C cytoplasm, respectively. The highest kernel row number (12.98) at both significance levels was established in inbreds with C cytoplasm. The highest number of kernels per row (33.92) at both levels of significance was recorded in inbreds with S cytoplasm. The greatest kernel depth (0.8212 and 0.8196 cm) at both significance levels was established in inbreds with C and S cytoplasm, respectively. The greatest 1000-kernel weight at both levels of significance was detected in inbreds with normal cytoplasm. The highest number of kernels per m^2 (2716 and 2676) was recorded in inbreds with C and S cytoplasm, respectively.

Keywords: *maize, yield, male sterility, inbred lines.*

Introduction

The revolution in maize production began with the discovery of heterosis and the cultivation of hybrids, during 1930s in the United States (Duvick, 2005). Maize is the first plant species in which heterosis is used to produce commercial hybrids. The use of the hybrid F_1 generation leads to an increase in yield by 25-30% compared to free pollinating varieties (Vančetović et al., 2007).

The first research on cytoplasmic male sterility (Cytoplasmatic Male Sterility-CMS) was performed by Rhoeds in 1931. In maize CMS is used to increase seed production efficiency and reduce its cost. The majority of studies showed positive effects of CMS on maize grain yield. Grain yields recorded in male sterile plants were higher by 5-10% than the ones in female fertile plants. Nowadays, production of maize hybrid seed in some parts of the world is increasingly based on using CMS in order to reduce costs of detasseling (Stevanovic *et al.*, 2013). The majority of studies (Stamp *et al.*, 2000; Weingartner *et al.*, 2002; Kaeser *et al.*, 2003) showed a positive effect of CMS on maize grain yield, especially under unfavourable drought conditions (Bruce *et al.*, 1966)

There are three types of CMS known so far in maize (Luo *et al.*, 2002; Shuanggui *et al.*, 2006):

1. CMS-T
2. CMS-C
3. CMS-S

The C and S type are used in commercial production in Serbia. CMS-T type is not used due to its specific susceptibility to the fungus Helminthosporium maydis race T that caused epiphytotic disease in USA during the 1970s (Duvick, 1972).
The aim of this study was to compare grain yields and yield components of original inbreds and their CMS and Rf variants

Material and Methods

Seven maize inbred lines (designated ZPL1, ZPL2, ZPL3, ZPL4, ZPL5, ZPL6 and ZPL7) of different origin and maturity group (FAO 300-500) were used in the trial. All original inbreds have been developed at the Maize Research Institute Zemun Polje. Each inbred was observed in five variants:

1. Original inbred (N)
2. CMS-C
3. Rf-C
4. CMS-S, and
5. Rf-S

Cytoplasmic male sterile and restorer versions have been developed at the Maize Research Institute Zemun Polje by conversion of inbreds with normal cytoplasm.
The trial, set up according to the randomised split-plot design, was carried out in three locations (Zemun Polje and Školsko dobro within the experimental fields of the Maize Research Institute Zemun Polje and in Srbobran) during two years (2010 and 2011). The location of Školsko dobro was rejected in 2011 due to a poor crop stand. The three-replication trail was set up in five sets (blocks). Each block represented one type of the observed inbreds:

I block – N (normal) cytoplasm, i.e. original inbreds
II block – CMS-C inbreds
III block – RfC inbreds
IV block – CMS-S inbreds and
V block – RfS inbreds.

Plots within each replication were composed of four rows. Two border rows represented original inbreds in all five blocks, which was especially important in CMS blocks, since N inbreds were pollinators for their sterile counterparts. Plants from inner rows (avoiding the effect of a border hill) were used for the analysis of grain yield and agronomic traits. Between spacing was 70 cm. The elementary plot size amounted to 7.28 m^2, while the crop density was 71.429 plants ha^{-1}. The yield was calculated in tones per hectare (t/ha) at grain moisture content of 14%.
Least significant difference (LSD) was used to compare original inbreds and converted inbreds at the 0.05 and 0,01 significance levels for yield an yield components. The MSTAT-C software package (MSTAT Development Team, 1989) was used for performing the LSD test.

Results and Discussion

In this study, comparisons were made between original and converted inbreds. The estimation of significance of differences between average values was done by LSD test at the 5% and 1% level.
Table 1 presents results of LSD test for grain yield, kernel rows number and number kernels per row. We detected the highest grain yields across all the seven studied inbreds for both significance levels in variants with C and S cytoplasm (5.303 and 5.197 t/ha, respectievly). In inbreds with the RfS type we detected the lowest yield (4.662 t/ha). LSD-test between inbred lines showed the highest kernel rows number in inbreds with RfC type of cytoplasm (12.98),

and the lowest with N type (12.67) for both investigated levels of significance (0.05 i 0.01). The highest number of kernels per row was observed in S inbreds (33.92), while the lowest number of kernels per row was determined in RfC inbreds (31.45) for both significance levels.

Table 1. Least significance difference test for the grain yield, kernel rows number and number kernels per row

	grain yield (t/ha)				kernel rows number				number kernels per row			
Rang	Tip	Vrednost	0.05	0.01	Tip	Vrednost	0.05	0.01	Tip	Vrednost	0.05	0.01
1	C	**5.303**	A[1]	A[1]	RfC	**12.98**	A[1]	A[1]	S	**33.92**	A[1]	A[1]
2	S	**5.197**	A	A	RfS	**12.87**	AB	AB	C	**33.65**	AB	AB
3	N	**4.878**	B	A	C	**12.79**	BC	AB	N	**33.14**	B	B
4	RfC	**4.850**	B	B	S	**12.76**	BC	B	RfS	**32.19**	C	C
5	RfS	**4.662**	C	B	N	**12.67**	C	B	RfC	**31.45**	D	D
		Lsd	**0.1283**	**0.2230**		**Lsd**	**0.1603**	**0.2112**		**Lsd**	**0.5442**	**0.7170**

1 -valued that have no letter in common are statistically different at the given level of significance

LSD-test (Table 2) between inbred lines showed the highest ear lenght in inbreds with N, S and C type of cytoplasm (15.57, 15.56 and 15.46 cm respectievly). The lowest ear lenght was detected in inbreds with RfS and RfC type of cytoplasm 14.98 and 14.85 cm respectievly. In inbreds with N type of cytoplasm we detected the highest weight of 1000 kernels (261.3g). Lowest value of this parameter was recorded in inbreds with RfS cytoplasm (253.9g). The highest number of kernels per m^2 was observed in C and S inbreds, 2716 and 2676 respectievly, and the lowest with RfS type 2496.

Table 2. Least significance difference test for the ear lenght, weight 1000 kernels and number of kernels per m^2

	ear lenght (cm)				weight 1000-kernel weight (g)				number of kernels per m^2			
Rang	Tip	Vrednost	0.05	0.01	Tip	Vrednost	0.05	0.01	Tip	Vrednost	0.05	0.01
1	N	**15.57**	A[1]	A[1]	N	**261.3**	A[1]	A[1]	C	**2716**	A[1]	A[1]
2	S	**15.56**	A	A	S	**260.5**	AB	A	S	**2676**	AB	A
3	C	**15.46**	A	A	RfC	**258.0**	AB	A	N	**2610**	BC	AB
4	RfS	**14.98**	B	B	C	**257.0**	AB	A	RfC	**2533**	CD	BC
5	RfC	**14.85**	B	B	RfS	**253.9**	B	A	RfS	**2496**	D	C
		Lsd	**0.1800**	**0.2372**		**Lsd**	**0.1603**	**0.2112**		**Lsd**	**0.5442**	**0.7170**

1 -valued that have no letter in common are statistically different at the given level of significance

In our research inbreds with C and S type of CMS achieved a higher grain yield than the inbreds with N type. Our results are in accordance with the results if previous studies (Stamp *et al.*, 2000; Weingartner *et al.*, 2002; Kaeser *et al.*, 2003). Previous studies showed also a positive effect of CMS on maize grain yield, especially under unfavourable drought conditions (Bruce *et al.*, 1966).

The impact of sterile analogs on the yield components should be positive because less nutrients and water are consumed and plants do not form pollen.It can not be concluded that there is a significant influence on the ear length, depending on the type of germplasm. Adding new genes in the cytoplasm leads to an increase in the kernel rows number. For the number kernels per row can be said that it is higher in the lines with the S and C type of cytoplasm. The mass of a thousand kernels did not differ on the level of significance 0.01, while at the level of significance of 0.05 the highest value of this parameter was achieved by genotypes with normal germplasm , which is in agreement with the results of Lim et al. (1974), but these studies were done on hybrids where the normal and T genotypes were compared. Weingartner et al. (2002) observed minimal changes in this indicator between genotypes with normal and T-type cytoplasm, while Munsch et al. (2009) showed that T and C cytoplasm negatively affect these parameter. When the number of grains per square meter is concerned, the highest values are recorded in the lines with S and C type of cytoplasm at both levels of significance. This is very important and positive for seed production on a CMS basis. Results Weingartner et al. (2002) show a significant influence of C cytoplasm on the value of this parameter. Also, Munsch et al. (2009) shows similar results.

Conclusions

It can be concluded that inbreds with C type of cytoplasm have better performances compared with inbreds with N ad S type for following traits: grain yield, kernel rows number and number of kernels per m^2. Sterile analogues were not only better when grain yield was concerned, but also when it comes to almost all other yield components.

Grain yield is the most important trait for the seed production. In this study, inbreds with C type of cytoplasm had best results.

In the period since the seventies of the last century, until now, cytoplasmic male sterility in maize hasn't received much attention from the researchers. The reduced intensity of the research is due to the epiphytosis of the mushroom *Helminthosporium maydis* and for this reason the use of CMS in maize production is considered to be very risky. Just because of all the above research on this topic, it should be continued and found out how new (modern) lines behave if in seed production we use their sterile analogues.

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QUALITATIVE ANALYSIS OF SUNFLOWER SEEDING IN REDUCED SOIL TILLAGE USING PRECISION AGRICULTURE SYSTEM

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Abstract

Reduced tillage has impact on the economical agricultural production. The secondary effect is manifested through the effective implementation of the basic frameworks of sustainable agricultural production. Support to the above concept in the field is represented by applied precision farming systems in accordance with the principles of good agricultural practice. The entire agricultural production technology is supported by agricultural machinery, which exploitation is a pillar of sustainability. Using precision agriculture technologies, it opens up new opportunities in analyzing the quality of sowing of row crops. An analysis of sunflower seeding was performed. The random sample system extracted data from big data records collected from over 18 parcels and an area greater than 380 ha. The average seed population had a coefficient of variation from negative 23% to positive 60% in relation to the given seed rate. The average number of well-planted seeds (single seeded seed, ISO index) ranged from 86.6% to 94.6%. The average number of double seeds (ISO Multiple index) ranged from 1.7% to 8.6%, and the average number of blank places (ISO Miss Index) ranged from 1.4% to 7.1%. Due to the large deviation and the characteristic cyclic repetition of the error in a big data sample, it was concluded that the sowing did not meet the required planting quality standards. The cause of the unacceptable planting quality is caused by poor exploitation of the soil tillage machine. The advantage of using precision farming systems, acquisition and then Big Data analysis is significant from the aspect of profitability of agricultural production. The registered error at the beginning will ensure the quick and effective detection and removal of the cause.

Keywords: *planting, precision agriculture, reduced tillage, sunflower, big data*.

Introduction

The growing need for food, has led to the development of agriculture primarily relying on the use of mechanization and chemicals. As one of the responses to established sowing practices, precise agriculture can offer a variety of techniques and technologies, which can successfully overcome existing problems and shortcomings of the classical production technology (Kutter, T, 2011). Precision agriculture is based on the application of information technologies, satellite navigation, sophisticated control tools and the ability to align agricultural machinery with the desired direction of production management (Pedersen, S.M. 2004). With the help of various sensors, real-time production process parameters can be determined and recorded based on which the reasons for effective / inefficient operation can be determined (McBratney, A, 2005). The collected information is used to create maps showing the variations of the observed parameters such as yield, weeds, soil fertility, seed quality, disease development, and so on. The complete effect of applying precise agriculture is evident through the cost-effectiveness of production, optimizing the costs of engaged inputs in production, and rationalizing the engagement of agricultural mechanization and human resources, timely and effective decision-making, and most importantly, making decisions

based on detected and provable information that has not been available so far (Dillon CR, 2009; Oparnica S, 2018a; Oparnica S, 2018b).
Implementation of precise agriculture has become possible thanks to the development of sensor technology in combination with procedures for mapping variables in the appropriate agro-technical scale in the production area, such as plowing, sowing, fertilization, herbicide and pesticide application, harvesting and livestock breeding (Sarantis, M; Roland B). The key feature comes from a positioning system, primarily global navigation satellite systems (GNSS), which are the main enabler of "precision". It is present in major agricultural producers, especially in large farms and areas of mainly growing regions of Europe, the USA and Australia, and where the business model is the main driver in order to increase profitability (Plant, RE 2000; Daberkow, SG 2003; Fountas, S., 2005). Controlled Traffic Farming (CTF) and auto-pilot systems are the most successful forms of application in arable land that show clear advantages in almost all cases. For the Variable Rate Application (VRA) method, such as the optimization of seeds, fertilizers or pesticide applications (the success varies according to specific application factors which is manifested through the yields by zones (yield sensors - mapping yields by zones). For example, section control implies the exclusion of sections of seeders, spreaders and nozzles outside the boundaries of the plot or on the already treated part of the plot, a double passage (Velandia M, 2013).
Using these systems, some savings are expected. For example, savings were achieved on average 2.24 eur/ha using the aggregate guidance system (Abidine, 2002;). This resulted in savings of 18-48 eur/ha in wheat production. The variable application of the norm can save 10-25 eur/ha depending on the crop and reduce the use of 10-15% nitrogen without affecting the yield reduction. As for the use of sensors in the application of pesticides, usage can be reduced by an average of 13% (Shockley, 2015). If cereals are grown, which have more treatments, it is possible to save over 12 eur/ha for just one pesticide application. Using precision agriculture in irrigation leads to a 25% reduction in the use of water, resulting in a final cost reduction of 44 eur/ha directly. Section control saves between 3% and 5% of the seed.
The application of precise agriculture can be through determining the compaction of the plot and pulling resistance, determining the presence of characteristic parts of the plot and the appropriate selection of the crop production technology. The most important point in production is the quality of sowing (Zarco-Tejada, P 2014; Jensen, H.G. 2012; Hörbe, T.A.N. 2013). The sown represents the crucial point of production because the soil tillage technology is on checking. Although the emergence also depends on the availability of moisture in soil and precipitation, the final plant density and the uniformity of crops depend on land cultivation. After sowing, follow the care measures that will keep the crops at the same level as to prevent the competition of other plants and diseases. Therefore, sowing together with agro technical measures keeps a crucial place in the production of sunflowers and other crops.
Seeding with multiple seeders with the assistance of precision farming systems, automatic management and reduction of human and machine labor costs are the task of this paper. Using the system of precision agriculture does not lead to the necessary emergence of cost-effectiveness of production and its efficiency, it can very easily cause negative effects whose presentation is the goal of this paper.

Material and method

Sowing were done with 4 seeder. All of them are seeder with 12 seeding mettering unit mechanically powered, with vacuum pressure and pneumatically section control. Inter row spacing is 0,7 m. There are some differences between two pairs of them which is presented in figures and text.

Fig. 1. First type of seeding unit

Fig. 2. Second type of seeding unit with more pressure on parallelogram linkage

Seeding units are presented at figure 1 and 2. The heavy-duty parallel linkage (fig. 1-1 and fig. 2-2) with a large travel is mounted on interchangeable bushings. Two springs stabilize the metering unit with additional shock absorber (fig. 2-3). Each metering unit is equipped with an audible safety clutch system (fig. 1-2) with automatic clutching for optimal protection of the metering system. Both of them were powered by electric motor, not with mechanical powered system (fig. 2-1). The adjustment of the clod removers is precise (12 positions) and rapid due to the adjustment system with locking pins. In order to suit different planting conditions, the clod removers are rapidly interchangeable with the trash wheels and this with no tool (fig. 1-3 and fig. 2-5). The planting system is made up of two large diameter discs (380 mm) mounted on sealed ball bearings (fig. 1-4 and fig. 2-6). Together with the inner shoe, this system ensures a constant depth control and planting quality no matter what the planting conditions. Large depth gauge wheels (110 mm) ensure an efficient and uniform depth control (fig. 1-5 and fig. 2-7). Resting on the ground directly in line with the seed drop ensures the unmatched depth consistency (fig. 2-8). The bracket arms of the gauge wheels (fig. 1-6 and fig. 2-9) are assembled on interchangeable bushes. They are helically fluted for a good grease distribution. Scrapers are standard assembly on the gauge wheels. The open rear press wheel unit (fig. 1-7) gives better clearance in sticky conditions. The off-set adjustments and the spacing of the 2 press wheels enable optimal furrow closing no matter what the conditions (stones, trash, hard soil, …), fig. 2-10. The depth adjustment is easily made using a hand wheel (fig. 1-8 and fig. 2-10). This type of adjustment (fig. 1-9 and fig. 2-11) ensures a great control precision of the planting depth even for small seeds at a shallow depth. The hopper in high resistance, transparent plastic has a capacity of 52 liters. The cover locks so will not close during filling, even in windy conditions (fig. 1-10 and fig. 2-12). The front facing of the metering unit integrates the passage of the vacuum hose and the seed monitor and signal kit cables (fig. 1-11).

Sowing quality was checked in accordance with ISO 7256-1: 1984 / Sowing equipment - Precision drills on certain sections of the seeders, and based on this the conclusion on the overall quality of sowing was carried out. By connecting the seeder to the navigation device, the density population is recorded, the percentage of double places, the percentage of empty places on the whole plot area (Figure 3).

In this case, the "point" on the map is the working width of the seeder, 8.4 m and the length of 20 m until the control of the sections is done. Sowing quality parameters are given in common for all seed sections for the given point. On average, 59 points / ha were obtained. When section control turns off/on each section, a new point is created with entered parameters.

Based on the data from the map, a conclusion can be given on the quality of the sowing quality on the entire sown surface, the condition of the seedlings and the conditions that affected the sowing quality.

For each recorded individual layer, the processing and analysis of data in the software provides graphical information, statistics for the entire parcel, as well as a histogram with a partition of surfaces with a certain parameter ranking.

When the answer to the question about the size of the yield is requested after harvest, by overlapping the yield map with the sowing map, it can show the impact of sow quality on the yield.

The research was carried out in the sunflower sowing on 18 plots on a total area of 380 ha. The sowing was done in the last decade of March (south Backa) and the first decade of April (north of Backa).

Results and discussion

Sowing is an operation in which the advantages of the navigation systems are best seen. Sowing along an ideal straight line makes it easy to carry out the next operations and makes them more productive due to the possible higher speed of movement and making less damage to the crop. By using the control of seedling sections, the use of seed can be reduced to up to 5%. The shape of the plot is incorrect, savings are greater. A system of precise agriculture in which the combined seedling and drive aggregate recognize the already sown surface or plot boundary and exclude individual sections. In a wide variety of cultures, the seed that sown and emerges in the corded area is later cut down in between row cultivation. If it remains, in that part there is a larger plant population and one companion for light, nutrition and water.

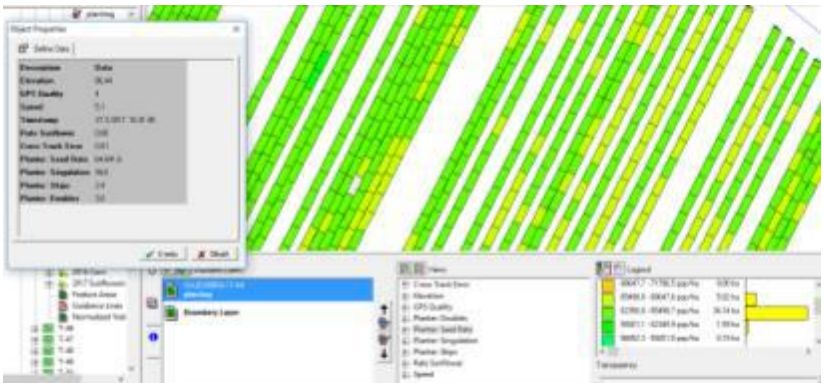

Fig. 3. Sowing map quality

The sowing of multiple seed aggregates on one plot during the day is a challenge, and almost impossible to realize during the night. Simply, operators are not able because of poor visibility to register the sown area. If each sowing unit is on a separate plot, it is often necessary to provide logistics on each plot because they are interchangeably distant, which is required by an additional telehandler, additional worker, a transport unit for seeds and fertilizer for each plot. If one sowing plant sow large plots, an area of more than 200 ha, it takes 2 to 3 days to complete the sowing of that parcel. The consequence of such a method is the uneven emergence. With the use of "Vehicle Sync" technology, up to 6 machines can be connected on a single plot that exchanges information on the finished surface. In this situation, this technology has enabled 4 seeders to work on one plot, with only one telehandler, one assistant worker and one transport unit. In addition, the working time coefficient was about 82.5% with a standard deviation of 2.5%, that is, the seedlings work effectively 19.2 hours - 20.4 hours per day. With a smaller number of seeders and lesser

logistics, larger areas are sown and no more sowing units are needed (Oparnica S, 2017a, Oparnica S, 2017b)
Modern seeders are equipped with sensors that are tasked with tracking the seeded population and to alarm problems with deviation from the norm. Typically, the alarm level is ± 10% to 15% of the current standard. Theoretically there is the possibility that the whole seedling season of the seeder will eject 9% more / less seed, and the alarm will not be activated.

Tab. 2. Seeding analysis results

No	ISO Multiple index)	Area [ha]	Singulation		Double places		Empty places		population			speed [km/h]		AVG Yield [t/ha]
			min 97%		max 2%		max 2%		target 65000 [pop/ha]	max 2%				
	Field		AVG %	STD	AVG %	STD	AVG %	STD	AVG pop/ha	difference	STD	AVG km/h	STD	
1	F-1	30,1	92,1	9,8	3,7	0,9	2,9	1	57961	-11%	6664	5,1	0,7	3,99
2	F-2	4,2	89,9	16	2,9	1,1	3,5	1,1	56673	-13%	10325	5,3	1	3,99
3	F-3	24,6	92,6	5	3,3	1,5	3,7	0,9	58454	-10%	3474	5,8	0,7	3,96
4	F-4	3,6	93,5	11,4	2,1	0,8	2,2	0,7	61466	-5%	1741	5,3	0,8	2,99
5	F-5	43,4	93,6	8,3	2,4	2,7	4	2,9	66655	3%	16027	5,8	0,5	3,25
6	F-6	29,8	91,6	16,1	3	1,1	1,7	1,9	57930	-11%	13838	5,4	1	4,42
7	F-7	12,3	89,7	18,2	2,2	0,9	3,8	1,3	50299	-23%	11581	5,6	0,7	3,99
8	F-8	18,2	88,1	10,4	5,5	3,7	7,6	4	90154	39%	31682	5,6	0,5	4,09
9	F-9	19,8	86,6	7,8	7,1	3	8,6	2,8	54161	-20%	18579	5,6	0,5	3,18
10	F-10	60,5	93,8	13,1	1,4	1,2	2,7	1,2	63253	-3%	13851	5,4	0,5	4,34
11	F-11	13,1	91,3	18,1	1,9	1,2	2,8	1,3	56183	-14%	11336	5,7	0,6	4,32
12	F-12	3,6	86,6	7,8	7,1	3	8,6	2,8	104161	60%	18579	5,6	0,5	4,17
13	F-13	42,5	90,9	17,8	2,7	1,8	2,3	1	60775	-6%	12595	5,1	0,7	2
14	F-14	19,0	87,9	12,8	5,6	3,7	6,9	4,1	66732	3%	20159	5,3	0,4	3,67
15	F-15	7,4	89,9	17,9	2,5	0,8	3,4	1,9	59638	-8%	11134	5,4	0,4	3,79
16	F-16	13,2	89,5	18,4	2,6	0,7	3,7	1	58442	-10%	12165	5,7	0,7	3,85
17	F-17	8,6	91,1	13,9	2,6	2,1	3,5	1,3	60028	-8%	9208	5,4	0,8	4,00
18	F-18	27,9	94,6	9	1,8	1	2,5	2,3	61029	-6%	6575	5,6	0,8	4,1

The random sample system extracted data from big data records collected from over 18 parcels and an area greater than 380 ha. The average seed population had a coefficient of variation from negative 23% to positive 60% in relation to the given seed rate. The average number of well-planted seeds (single seeded seed, ISO index) ranged from 86.6% to 94.6%. The average number of double seeds (ISO Multiple index) ranged from 1.7% to 8.6%, and the average number of blank places (ISO Miss Index) ranged from 1.4% to 7.1%. Due to the large deviation and the characteristic cyclic repetition of the error in a big data sample, it was concluded that the sowing did not meet the required planting quality standards.
Benefits of using the various systems for control in the technology field of precision farming systems obviously require constant control and validation of work. Now when it is clear that the problem exists due to unsatisfactory results of sowing, it was present to find reasons.
Based on the conducted observations when measuring the seedlings in a row, the depth of sowing was very different. The range of change ranged from 15% to over 35% (random sample). In the case of NH seeder (Figure 2), the deviations were smaller, while more extensive dilations were recorded in the NG seeder (Figure 1). The reason for these differences in sow depth can be seen in the following photos. First, Figure 4a shows the direction of soil tillage. The tillage direction was about 30^{o} in a negative mathematical direction from the sowing direction (the angle closing right 1 and 2 in Figure 4a.).

Fig. 4a. Line1 marks direction of sowing and line 2 marks direction of tillage

Fig 4b. Marks A and B for formed different tillage zones

Secondly, two seeders are designed for direct planting of crops, while the other two are for sowing after conventional soil treatment. In Figure 4b two seeding zones are observed. The first zone is marked with A and the other with B. Two seeding zones were observed that differ in the fact that zone A, zone of compacted soil (untreated) and zone B, zone of loose tillage soil. These zones are formed in soil tillage with a combined low-speed soil compacting machine consisting of a short discs (two separate segments) and tins (on elastic supports).
Another factor that affected the poor sowing is caused by the depth of tillage. The tillage depth did not exceed 0.1 m in zone A, and in Zone B, it was usually 0.05 m. The consequence of shallow treating particularly caused problems on plots where there were a lot of harvest residues. Changing the depth of sowing and the appearance of zones of different depths is shown in Figure 5.

Fig. 5. Differences and variance of tillage depth

An analysis of the impact of each seed quality parameter on the average yield was made. In this case it turned out that the population has the greatest impact on yield. Therefore, it is necessary to direct future research and analysis towards the direction of dependence and correlation between plant and yield strata.

Tab. 2. Coefficients of correlations between obtained data and yield

Correlation	**Yield**
Population	-0,23604
Singularity	0,0161
Double	-0,04551
Empty	0,0168

Conclusion

The advantage of using precision farming systems, acquisition and then Big Data analysis is significant from the aspect of profitability of agricultural production. The registered error at the beginning will ensure quick and effective detection and removal of the cause.

Given collected and processed sowing data are obtained:

• Precise information on the quality of the operation performed on the entire surface;

• Mapping gives right information about the proper layout of the seed over the surface, or a trap of an average population without a variation information is avoided;

• There is accurate and precise information about the quality of sowing and business decisions are not made on the basis of assumptions about sowing quality.

In order to achieve the positive effects of the application of precision agriculture, different challenges must be overcome both in terms of both, material and psychological nature. The material challenges are very defined, the size and shape of the parcel, the type of machine to be used, the technology of land processing and ultimately the defined cost of purchasing navigation equipment and sensors. Challenges of psychological nature are the most difficult to overcome. It is difficult to implement training in which operators will change their habits and move from their comfort zone. Although the benefits of applying the new concept are very clear, irrational resistance is created in people who need to apply them in practice. It requires multidisciplinary involvement of agronomists from all areas of agriculture in order to demonstrate all the benefits of using modern technologies of precision agriculture.

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ISO 7256-1:1984 / Sowing equipment -- Test methods -- Part 1: Single seed drills (precision drills)

THE EFFECT OF DIFFERENT FERTILIZER APPLICATIONS ON PLANT DEVELOPMENT AND FLOWERING OF DEMRE PEPPER

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Abstract

In the Study Demre pepper variety was used. The study was carried out in the Physiology Laboratory, Faculty of Agriculture, Van Yuzuncu Yıl University (Van city Turkey), in a climate room where normal atmosphere was provided. The main purpose of the normal atmosphere in the experiment was to ensure that salt stress effects occur under normal conditions. The study was carried out under controlled conditions in a light / dark photoperiod of 16/8 hours, climate room of 25^{o}C and 70% humidity. The study aimed to investigate effect of some commercial fertilizers which have a big market share in the world and Turkey. Nine different commercial fertilizer applications were made to the pepper plants. Base fertilizers were applied to the groups to be applied to the soil before planting seedlings. Other fertilizer applications were applied in 10-15 days intervals according to the instructions of the company. In addition, the group watered with distilled water only (control) and groups with only ½ second Hoagland nutrient solution of irrigation, fertilizer application constituted control of these commercial applications. The fertilization plan was made in accordance with the size of the company said. In this study, plant height, stem diameter, the first flowering date, number of flower, plant internodes length, number of stems; were examined. When the data obtained were evaluated in terms of plant growth; It was remarkable that Bestline fertilizer had the highest values in terms of all the developmental parameters examined except for the plant nodes distance.

Keywords: *Pepper, Number of flowers, Plant growth, Yield.*

Introduction

The pepper plant belong to the Capsicum genus of Solanaceae family. Capsicum genus contains 25 species. Among these species 5 are commercially produced C. annuum L., C. frutescens L., C. baccatum L., C. chinense Jacq., ve C. pubescens Ruiz & Pav. The most widely grown and economically important species of these are Capsicum annuum L. (Pernezny et. al., 2003).

World pepper production in 2016 was 34,497,462 tons. Around 3% of the world's vegetable production is pepper production. 2016 Turkey pepper production was 2,457,822 tons. In 2017, production increased by 6.1% and reached 2,608,172 tons. Turkey produces 7.5% of the world pepper production. (Anonim, 2018a).

Pepper was not as advantageous as tomato and eggplant in terms of exploiting foods due to its hairy root structure and depth. According to them, the root and hairy structure is less deep. If this feature is not taken into account in pepper cultivation, various malfunctions arise due to deficiency of plant nutrients. These also lead to premature deaths, reduced yields and poor quality.

Fertilizer is one of the most important inputs in agricultural production. If it is not applied sufficiently, it causes significant losses in yield and quality, but if it is applied more, it causes pollution of bottom and surface waters, especially with washing of nitrogen and phosphorus fertilizer, and emission of nitrogen oxide (NO, N2O, NO2) and air pollution (Güler, 2004).

The rapid increase in human population necessitates the increase in yield and quality in crop production. Correct fertilization is very important in order to increase yield and quality in crop production. Today, agriculture is still maintained in many places with traditional fertilizer use habits. Unconsciously one-way and large amounts of fertilizer applications without being based on soil analysis bring some yield and quality problems. In addition, this situation has reached the dimensions that threaten human health through nutrition. There are many commercial fertilizers on the market. In this study, the effect of commercial fertilizers on yield and quality criteria in pepper plant which has a large share of the World and Turkey market was investigated.

Material and Methods

The experiment was carried out in a split air-conditioned climate room where normal atmosphere was provided. Demre pepper variety was used in the study.
A mixture of perlite, peat and barn manure (1: 2: 4) was used as growing medium.

Table 1. Some physical and chemical analysis results of the growing environment

Structure	Ph 1.2.4.	Lime %	Organic material %	EC mS/cm	Sand %	Clay %	Silt %
Loamy	8.21	11.34	2.23	2284	49.8	24	26.2

Applied fertilizers

10-30-10, 10-5-40, Potasmag, TSP, Uan, 10-25-20+20(SO_3)+Zn, Bestline, 13-24-12+10(SO_3)+Fe+Zn, 20-20-20 fertilizer, hoagland solution.

Table 2. Hogland nutrient solution content

Macro elements	**g/lt**	**Micro elements**	**g/lt**
$Ca(NO_3)_2.4H_20$	1.180	$C_6H_5FeO_7.5H_2O$	0.02
KNO_3	0.252	$Mn\ Cl_2$	0.00072
KH_2PO_4	0.136	H_3BO_3	0.00116
$MgS0_4$	0.246	$ZnCl_2$	0.000048
		$CuCl_{2.}2H_2O$	0.00004

Pepper seeds were placed in plastic germination containers filled with pumice, after which 100 seeds were planted and watered with tap water. Seeds were planted in the pumice and covered with paper at a temperature of 24-26 0C and left to germinate in the air conditioning room and watered at intervals of 1 day. After germination started, the papers were removed and grown until they had 2 true leaves in a 16/8 hour light-dark photo period. After having 2 real leaves; 1: 2: 4: ratio of perlite, peat and manure growing medium with a size of 9x15 cm 570 ml volume of large six-hole and each cup of 1 seedling in each cup of 12 glasses were confused for each application.
The fertilization plan is based on the dimensions specified by the company. 9 different commercial fertilizer applications were applied to the plants. Base fertilizers applied to the groups were applied to the groups to be applied to the soil before planting seedlings. Other fertilizer applications were applied in 10-15 days intervals according to the instructions of the company. In addition, there are two different groups that constitute the control application of the study. The control group (the group irrigated with pure water only) and the groups made with only 1/2 Hoagland nutrient solution (Hoagland and Arnon, 1938) of irrigation were the control application of these commercial fertilizer applications.
In this study, plant height (cm), stem diameter (mm), length between plant nodes (cm), number of branches (number), first flowering date (day), number of flowering (number) were examined.

For all the properties studied, a randomized trial design (Random Plots) was used to test the differences between the groups. In case of significant differences between the groups ($p < 0.05$), Duncan multiple comparison test was applied to the relevant data to test which group had significant difference from the other. For this purpose, SAS (1985) statistical package program (SAS Institute Inc., Cary, NC) was used.

Results and Discussion

The study lasted 137 days after planting pepper seedlings. The first flowering after planting was observed after 47 days and in the application of 10-25-20 + 20 (SO3) + Zn. This was followed by 20-20-20 applications after 13 days. Among the applications, the latest flowering was observed in TSP application.

Table 3. Plant height (cm), stem diameter (cm), length between nodes (cm), number of branches (pieces), number of flowers (pieces)

Grup	N	Plant height ($\bar{X} \pm S_{\bar{X}}$)	Stem diameter ($\bar{X} \pm S_{\bar{X}}$)	Length between node ($\bar{X} \pm S_{\bar{X}}$)	Number of branches ($\bar{X} \pm S_{\bar{X}}$)	Number of flow. ($\bar{X} \pm S_{\bar{X}}$)
10-25-20+20 (SO_3)+Zn	12	30.750 ± 1.21^{ab}	0.467 ± 0.01^{ab}	$1.333 \pm 0.08^{a/c}$	6.916 ± 0.43^{b}	18.333 ± 1.67^{ab}
(13-24-12)+10 (SO_3)+Fe+Zn	10	28.600 ± 1.41^{b}	0.475 ± 0.01^{ab}	$1.342 \pm 0.15^{a/c}$	7.700 ± 0.50^{ab}	13.600 ± 0.94^{cd}
TSP	12	28.833 ± 1.39^{b}	0.488 ± 0.02^{ab}	$1.137 \pm 0.11^{b/d}$	6.583 ± 0.47^{b}	13.416 ± 1.01^{d}
Potasmag	10	30.300 ± 2.11^{ab}	0.477 ± 0.01^{ab}	1.058 ± 0.08^{cd}	6.500 ± 0.31^{b}	$16.900 \pm 0.77^{a/d}$
20-20-20	10	32.300 ± 1.14^{ab}	0.456 ± 0.02^{ab}	$1.243 \pm 0.09^{a/d}$	6.600 ± 0.50^{b}	$17.700 \pm 1.69^{a/c}$
Bestline	11	33.909 ± 1.88^{a}	0.490 ± 0.01^{ab}	$1.145 \pm 0.09^{b/d}$	8.363 ± 0.64^{a}	19.545 ± 1.52^{a}
UAN	11	33.382 ± 1.30^{ab}	0.447 ± 0.010^{b}	1.426 ± 0.11^{ab}	6.727 ± 0.20^{b}	$15.454 \pm 1.34^{a/d}$
10-5-40	11	32.909 ± 1.38^{ab}	0.449 ± 0.02^{b}	1.500 ± 0.11^{a}	6.636 ± 0.43^{b}	$15.090 \pm 1.02^{b/d}$
10-30-10	11	31.364 ± 1.28^{ab}	0.478 ± 0.02^{ab}	$1.232 \pm 0.07^{a/d}$	7.636 ± 0.54^{ab}	18.636 ± 1.68^{ab}
Hogland	10	29.600 ± 1.70^{ab}	0.494 ± 0.02^{a}	1.060 ± 0.05^{cd}	7.000 ± 0.33^{ab}	$15.700 \pm 0.86^{a/d}$
Kontrol	10	29.500 ± 1.54^{ab}	0.475 ± 0.02^{ab}	0.975 ± 0.07^{d}	7.900 ± 0.35^{ab}	$15.700 \pm 0.76^{a/d}$
P		0,143	0.135	0.002	0.033	0.0082

As a result of measurements in pepper plant, the longest length was obtained from Bestline fertilizer with 33,909 cm. According to the control practices of Bestline fertilizer (Control and Hoagland) it is seen that it creates differences in plant height. The lowest plant height was obtained from 13-24-12 + 10 (SO3) + Fe + Zn fertilizer application (28.600 cm) (Table 3).
As a result of the measurements, it was obtained from plants grown with Hoagland nutrient solution with 0.494 cm of the plants having the highest stem diameter. However, it is noteworthy that UAN and 10.5.40 fertilizer have statistical differences in terms of stem diameter of plants grown with Hoagland nutrient solution. Among the fertilizer applications, the maximum body diameter was measured as Bestline (0.490 mm) (Table 3).
According to the data obtained, the lowest inter-node distance was obtained from Control plants (0.975 cm) while the highest inter-node distance (1.500 cm) was obtained from 10.5.40 fertilizer. The applications in the same statistical group as 10.5.40 application, respectively UAN, 13-24-12 + 10 (SO3) + Fe + Zn, 10-25-20 + 20 (SO3) + Zn, 20-20-20, 10 -30-10 (Table 3).
It was determined that the application with the highest branching was Bestline and there was a statistically significant difference with this fertilizer. The lowest number of branches among fertilizers is Potasmag fertilizer. (Table 3).
The number of flowers varies between 13.416-19.545 pieces. Bestline fertilizer with 19.545 pieces has the highest number of flowers. TSP fertilizers and 13-24-12 + 10 (SO3) + Fe + Zn

fertilizers with 13,600, 13,416 units have low values from control applications (Control, Hoagland) (Table 3).
Koca (2013), potassium sulphate application, plant vegetation growth character (longest plant, the highest number of leaves, fresh dry weight of the whole plant, average weight, average weight, as well as total onion yield per unit, onion diameter) was found to be the highest. In a similar study El Bassiony (2006), the effect of potassium fertilizer on the growth, yield and quality of onion plants in the study of the best growth (plant height, number of leaves / plants) highest yield and onion quality 20 kg / ha by applying potassium sulfate to the soil obtained. Again in Aisha and Taallab (2008), the highest vegetation growth character (longest plant, maximum number of leaves, maximum fresh dry weight of the whole plant, average weight as well as total onion) using chemical forms of K as potassium sulfate. yield and onion diameter) were determined. In the study conducted by Bozkoylu (2008), the effect of chemical fertilizers on plant growth was investigated and it was found that chemical fertilizers were more effective than organic fertilizers.

Conclusion

The first flowering after planting was observed after 47 days and in the application of 10-25-20 + 20 (SO3) + Zn. This was followed by 20-20-20 applications after 13 days. Among the applications, the latest flowering was observed in TSP application. When the data obtained were evaluated in terms of plant growth; It was remarkable that Bestline fertilizer had the highest values in terms of all the developmental parameters examined, except the distance between the plant nodes. Especially in terms of the number of flowers which are among the yield criteria, it was determined that the application of fertilizer which had close values with Bestline was 10-30-10 fertilizer. It can be said that Bestline and 10-30-10 fertilizers yield more positive results on yield compared to other fertilizers.

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BIOCHEMICAL STRESS MARKERS OF PLANTS IN CONDITIONS OF FOREST AND PARK ECOSYSTEMS

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Abstract

The secondary metabolism of plants is very sensitive to the environmental factors. In the processes of physiological adaptations of plants, an important role belongs to the phenolic compounds. The total content and the ratio of phenolic components depend on the nature of stress factors. Thus, the latter can be considered as stress markers and used in the phytoindication of the levels of anthropogenic transformation observed in the forest and park ecosystems. The biochemical markers were studied using the referent species *Acer platanoides* L., *Carpinus betulus* L. and *Sambucus nigra* L., sampled in the National Nature park "Holosiivskyi" (Kyiv, Ukraine). For the studied species, the highest phenolic content was observed in leaves of plants growing on insignificant elevations and slopes. The content of phenolic compounds such as flavonoids and catechins decreased in plants growing on plots with relatively high level of recreation pressure. The content of phenolic compounds and anti-oxidants was comparatively high in *Carpinus betulus*. The plants of *Acer platanoides* were significantly sensitive to the environmental factors. In their leaves, the concentration of phenolic compounds changed almost by half depending on the habitat conditions. In contrast, the leaves of *Sambucus nigra* plants varied more by their qualitative and quantitative composition of flavonoids. The chlorogenic acid, also, was shown to be highly informative, according to the analysis of principal components. That phenolic compound has high anti-oxidative potential. The ratio of chlorogenic acid to the total content of the identified phenolic compounds is a relative index of the plant's physiological state. Hence, the ratio value can be considered a biochemical marker of stress in the indicator plants.

Keywords: *forest ecosystem, marker, phytoindication, phenolic compound, stress*

Introduction

The functions of phenolic compounds in plants are diverse and mostly unstudied (Keech, 2005; Mattner, 2006). These compounds act as components of the electron transport chains of respiration and photosynthesis, and as regulators of growth and development (Cheynier, 2013). They also participate in various redox processes and ensure functions of low molecular weight antioxidants, etc. (Blokhina, 2003).

Phenols are important in the formation of constitutional and induced plant resistance to stress factors (herbivores, pathogens, herbivores, unfavorable temperature and pH, UV radiation, heavy metal stress), in regulation of symbiotic relationships (Nichols S., 2015; Zhu, 2016; Naikoo, 2019; Kidd, 2001). Phenolic compounds in a plant organism are predominantly synthesized by the shikimate pathway, which also involves the formation of aromatic amino acids (Tzin, 2010; Randhir *et al.*, 2004). The initial stage of phenol biosynthesis is the formation of oxycoric acids by the enzymatic conversion of *L*-phenylalanine. The flavonoids are obtained from chalcones with the participation of chalcone synthase (CHS) (Cheynier, 2013). Flavonoids are found in almost all plant tissues and organs, which points to their exceptional role in the regulation of physiological processes and adaptation of plants (Nichols, 2015; Mierziak, 2014). A large group of unreduced flavonoids, such as leukoanthocyanidins and anthocyanins localized in epidermal cells, is known to actively absorb ultraviolet radiation (280-320 nm) (Logemann *et al*., 2000). High selective ability to absorb ultraviolet determines their protective role in the functioning of the photosynthetic apparatus of plants, especially in the early stages of ontogeny (Liu, 1995). In addition, some aglycone flavonoids (quercetin, campferol) significantly affect the transport of auxins, interacting with membrane proteins that are involved in transmembrane transport of growth regulators (Brown, 2001). The structures of many flavonoid groups with antifungal, antiviral and antibacterial activity have now been identified. In addition, several flavonoid groups showed synergistic effects. At the same time, reports of the antibacterial activity of flavonoids are quite contradictory, possibly due to variations in testing conditions and individual sensitivity of study objects. Phyto-antibiotic activity particularly that associated with inhibition of DNA gyrase has now been confirmed for quercetin (Cushnie, 2005). A few mechanisms of action of other flavonoids, including myricetin, apigenin, rutin, etc. are also known (Cheynier, 2013). Taking into account the important role of secondary metabolites in the regulation of plant metabolism, the present work aimed at biochemical profiling and determining the impact of recreational pressure on the quality content of phenolic compounds in leaves of plants, which are widespread in the territories of different biotopes of the National Nature park "Holosiivskyi".

Material and Methods

The research was conducted in 2017–2018 in the hornbeam stands of the largest (1052 ha) green area in southwestern Kyiv (Ukraine), Holosiivskyi mixed deciduous forest. Six sites (S_1–S_6), 2500 m^2 each, were selected for the study. The general condition of trees and shrubs was determined visually. S_1 was located near university building, S_2 was chosen near playgrounds with relatively high recreational pressure, S_3 was chosen in area with average recreational pressure (30 m alongside a city road), S4 was established near Golosijevo ski complex, S_5–S_6 were chosen in Holosiivskyi Forest at areas with low recreational pressure, hardly visited by the city residents.

Acer platanoides L., *Carpinus betulus* L. and *Sambucus nigra* L. plants up to 10 years old were used as indicators of the state of the park ecosystem. The leaves of Norway maple and hornbeam were collected randomly from the lower branches. Elderberry leaves were collected from the middle branches. Samples were stored in a container during field studies. Within one experimental area, leaves were collected from five plants. The extraction was carried out in methanol (1:10 – v/v).

Biochemical profiling of methanol extracts was performed with HPTLC on G60 silica gel plates (Merck). The separation of flavonoids and phenol carboxylic acid conjugates was carried out in a solvent system: ethyl acetate, ethyl methyl ketone, methanol, water – 30:20:5:5 (v/v/v/v) (Reich, 2007). Chromatographic extract volume was 3 μl. Chlorogenic and caffeic acids (1 mg/ml) were used as standards. To obtain individual products and to determine the chemical nature of compounds, the chromatogram after drying was treated with 0.5 % NP reagent in ethyl acetate and 5% polyethylene glycol 400 in dichloromethane. The plate was kept for 5 min at 100 ºC. The chromatogram was visualized at λ=365 nm. R_f indices of the individual compounds were determined photodensitometrically using the *Sorbfil TLC* software.

The biochemical profiles of indicator plants were analyzed using the principal component method in Statistica 7.0.

Results and Discussion

Biochemical profiling of leaf extracts showed that the qualitative composition of phenolic compounds is species-specific and depends on the habitat conditions. The total concentration of phenols, as well as the content of individual components, is a sufficiently informative indicator of the effect on plants of external factors.

The Norway maple was quite sensitive in this aspect among the studied plant species. Under unfavorable conditions the concentration of phenolic compounds, including flavonoids, decreased almost twice in its leaves. On the contrary, both the qualitative and quantitative compositions of flavonoids were variable in the elderberry leaves.

The qualitative composition of phenolic compounds in the hornbeam leaves was quite conservative. Only at the S2 site with high anthropogenic pressure, an additional product was formed in the leaves (Rf~0.43). The product, treated with NP reagent, released a bright blue fluorescence, characteristic of phenolecarboxylic acid conjugates. However, this phenolic compound was not detected in other biotopes.

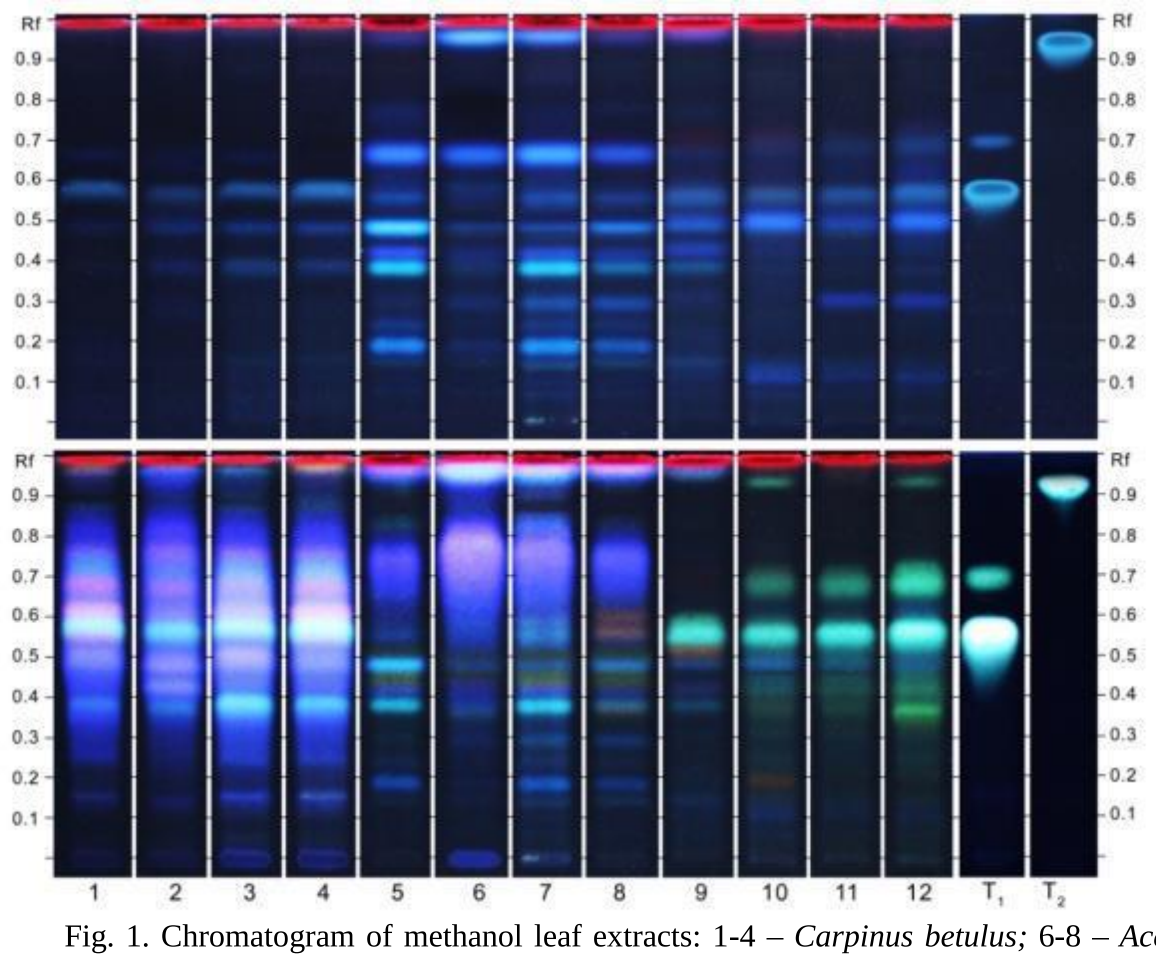

Fig. 1. Chromatogram of methanol leaf extracts: 1-4 – *Carpinus betulus;* 6-8 – *Acer platanoides*; 9-12 – *Sambucus nigra* in different biotopes of National Nature park "Holosiivskyi"; T_1 – chlorogenic acid; T_2 – caffeic acid (lower chromatogram was made after leaf plate was treated with NP reagent (1 % diphenylboryl oxyethylamine) and 5% PEG 400, and then heated to 105 $^{\circ}$C for 3 min).

As a result of photodensitometric analysis of the individual compounds, six phenolic compounds with intense auto-fluorescence (UV excitation 365 nm) were detected in the hornbeam leaves. At the same time, the qualitative composition of this class of substances

was quite variable. The full range of substances was identified in the leaves of trees growing on the S_2 site (Table 1).
According to the fluorescence brightness and Rf~0.58, the hornbeam leaves contain chlorogenic acid or one of its isomers. The highest concentration of chlorogenic acid is found in plants from the S5 site, which is farthest from the major highways, utilities and buildings.
For Norway maple, the range of substances on the chromatograph was wider. The chromatographic profile revealed 14 individual compounds (Table 2). Similarly to the hornbeam leaves, the profile with most of the separated components was seen for plants from the S_3 and S_5 sites. Under these conditions, up to 11 compounds are synthesized in leaf. It should be noted that territorially, these sites are closer to each other. The biochemical profiles of plants from S_1 and S_2 sites were somewhat less wide.

Table 1.
The ratio of individual compounds in the extracts of *Carpinus betulus* leaves according to fluorescence intensity (λ = 365 nm)

Peak	$R_f \cdot 100$	Peak area, S×1000, c.u.			
		S_1*	S_2	S_3	S_5
1	6	0	0	8.16	0
2	15	0	0	9.17	7.38
3	38	0	23.01	42.42	33.05
4	48	9.42	27.31	38.19	43.48
5	58	58.06	38.95	60.95	86.34
6	66	7.08	0	60.01	0
	Σ	74.55	89.26	164.90	170.24

* S_1, S_2, S_3, S_4 – study sites.

Nine compounds were found in the leaves of plants from the first site (S_1), and only seven compounds were determined from leaves of plants from S_2. Lower number of individual phenolic compounds, which are cytoprotectants with high antioxidant potential, in the biochemical profile may indicate a decreased potential ability of leaf tissues to protect macromolecules and membranes from stress-related reactive oxygen species (ROS). Typically, an increase in the total amount of phenolic compounds in plants occurs under the action of external factors that cause a protective reaction. In the hornbeam under considerable recreational pressure, the total number of phenolic compounds has decreased almost twice, according to the results of chromatographic analysis. That response may be explained by the depressed state of the plants, which leads to a decreased metabolic activity, or to the activation of other mechanisms of adaptive responses that require separate studies.
The content of phenolic compounds in the leaves is somewhat different for *Acer platanoides*. Thus, under conditions of recreational pressure (S_1), content of the phenolic compounds increased by 1.4 and 1.7 times, respectively, compared to the not visited areas (S_3-S_4) (Table 2).

Table 2.
The ratio of individual compounds in the extracts of *Acer platanoides* leaves according to fluorescence intensity (λ = 365 nm)

Peak	$R_f \cdot 100$	Peak area, S×1000, c.u.			
		S_1	S_2	S_3	S_5
1	7	0	0	8.57	0
2	14	0	0	9.67	6.49

3	19	110.76	16.36	88.64	52.77
4	24	27.37	0	7.73	4.58
5	30	19.00	24.84	49.26	46.20
6	38	123.78	21.33	96.60	57.83
7	42	47.71	21.34	20.38	14.33
8	48	138.17	30.90	32.00	76.01
9	55	62.30	0	47.25	34.77
10	58	0	24.28	0	0
11	66	130.87	105.07	117.87	88.85
12	76	23.56	0	0	5.07
13	85	0	0	11.36	0
14	96	0	0	0	22.83
	Σ	683.53	244.11	489.32	409.71

At the same time, the qualitative composition of individual products and the total content of phenolic compounds twice decreased in the highly visited recreational area (S_2). Such a feature of metabolic activity coincides with the response of *Carpinus betulus* plants. The content of phenolic compounds increased in *Acer platanoides* leaves in S_1 and decreased in S_2, possibly indicating the relatively greater protective potential of the substances, and confirming that phenylpropanoid synthesis is inhibited under prolonged exposure to stress factors.

Analysis of the individual compounds by the principal component method revealed that the greatest contribution to the overall dispersion is made by substances with Rf~0.66, as well as three substances 0.19; 0.38; 0.48 (Fig. 3).

The compound with Rf~0.66 was found in all leaves. According to fluorescence intensity, its highest concentration is found in the leaves from the study site S1, the lowest concentration in leaves from S_5.

The ratio of this substance to the sum of all identified compounds by the area of peaks on the photodensitogram (K) was an informative indicator of the plant's condition and we consider it a marker of their physiological state, even if the obvious visual signs of damage or inhibition of plants are not observed. The coefficient is calculated by the formula:

$$K = \frac{SRf_{\text{к}}}{\sum_{i=1}^{n} SRf_i},$$

where SRf_{κ} – peak area on photodensitogram of the marker product, $\sum_{i=1}^{n} SRf_i$– sum of peak areas of (*n*) products on a chromatogram of one sample.

The calculated ratios were then put on the map of the study area (Fig. 4). It is shown that the calculated coefficient decreases with the level of anthropogenic pressure by the distance of plant habitat from the main buildings, popular recreational areas and high-traffic highways. For S_2 site, the coefficient was 0.43, for S_3 and S_5 sites, it was 0.24 and 0.22, respectively.

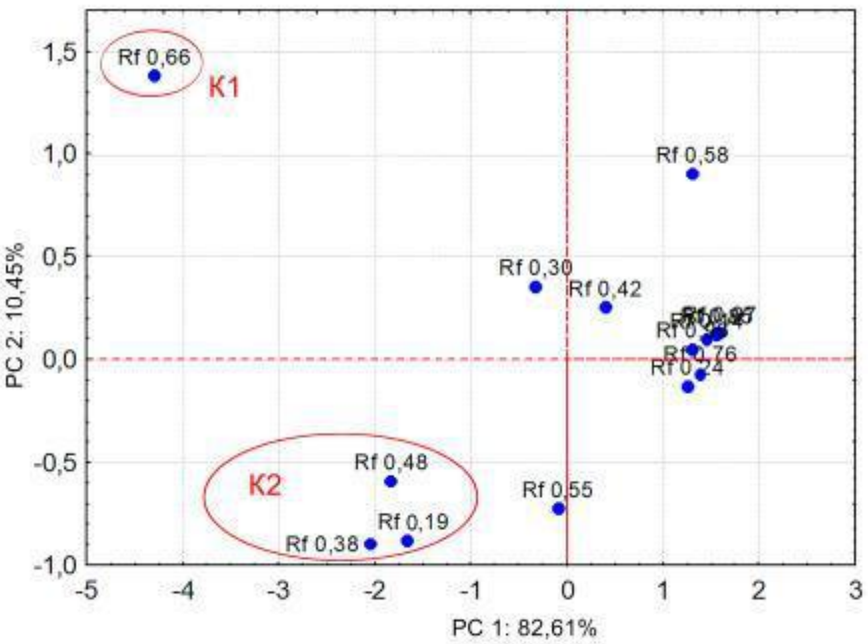

Fig. 3. Principal components analysis of biochemical profiles of *Acer platanoides* leaves

In the studied model, this biochemical marker made the greatest contribution to the overall dispersion and is therefore a highly informative feature. In our opinion, this substance should be considered as one of the key response signs of *Acer platanoides* to the state of the ecosystem.

Fig. 4. Indexes of physiological state of *Acer platanoides* plants by biochemical markers in different habitat of the National Nature park "Holosiivskyi"

Chromatography of *Sambucus nigra* leaves revealed a significant amount of chlorogenic acid (CA) and its derivatives. In this chromatography system, CA has an Rf of 0.56, and a bright blue fluorescence at UV 365 nm.

In addition, 12 phenolic compounds were isolated from elderberry leaves. Several of them are of some interest in terms of the possible chemo-indication of anthropogenic and biotic pressure. CA, as a fairly powerful antioxidant, is one of the key compounds involved in providing systemic protection to the plant organism (Manquian-Cerda, 2018). Photodensitometric analysis made it possible to compare the CA content of plants from different biotopes.

The content of this compound in the leaves was quite variable. At the same time, not only its quantitative content but also its ratio to other phenolic acid conjugates may be important.

Table 3.
The ratio of individual compounds in the extracts of *Sambucus nigra* leaves according to fluorescence intensity (λ = 365 nm)

Peak	$R_f \cdot 100$	Peak area, S×1000, c.u.			
		S_1	S_2	S_3	S_5
1	11	0	19.23	46.07	31.56
2	15	19.96	0	0	0
3	21	7.67	0	0	0
4	31	17.20	42.95	8.82	50.19
5	38	32.73	0	11.05	17.75
6	43	39.35	0	11.26	12.90
7	49	63.03	51.23	91.71	101.9
8	56	61.19	54.81	50.45	79.86
9	63	0	0	6.31	19.52
10	66	16.89	0	0	0
11	68	0	31.97	38.19	66.44
12	77	6.97	0	0	0
Σ		264.98	200.18	263.86	379.92

CA is one of derivatives of the cinnamic acid (cinnamate), which are common in higher plants, mainly in the form of conjugates. After hydrolysis they form phenolic acids such as caffeic (3,4-dihydroxycarboxylic), ferulic (3-methoxy-4-hydroxycarboxylic), mustard (3,5-dimethoxy-4-hydroxycarboxylic), and p-coumaric (4-hydroxycarboxylic). CA reduces the level of malondialdehyde due to its high antioxidant potential. It reduces the prooxidant activity of free radicals in plant tissues under oxidative stress, as evidenced by the decreasing concentrations of lipid peroxidation products and of hydrogen peroxide (H_2O_2). At the same time, CA enhances the enzymatic activity of the antioxidant system (SOD, catalase and glutathione peroxidase).

The most represented plant profiles in the elderberry leaves, were those of the first section (10 compounds), and the second section (5 compounds) was the least represented. This coincides with the data obtained for the hornbeam and Norway maple.

Principal component analysis confirmed the high importance of CA (Rf~0.56). Another potential marker for assessing the plant's physiological state is a prominent substance with Rf~0.49 (Fig. 5).

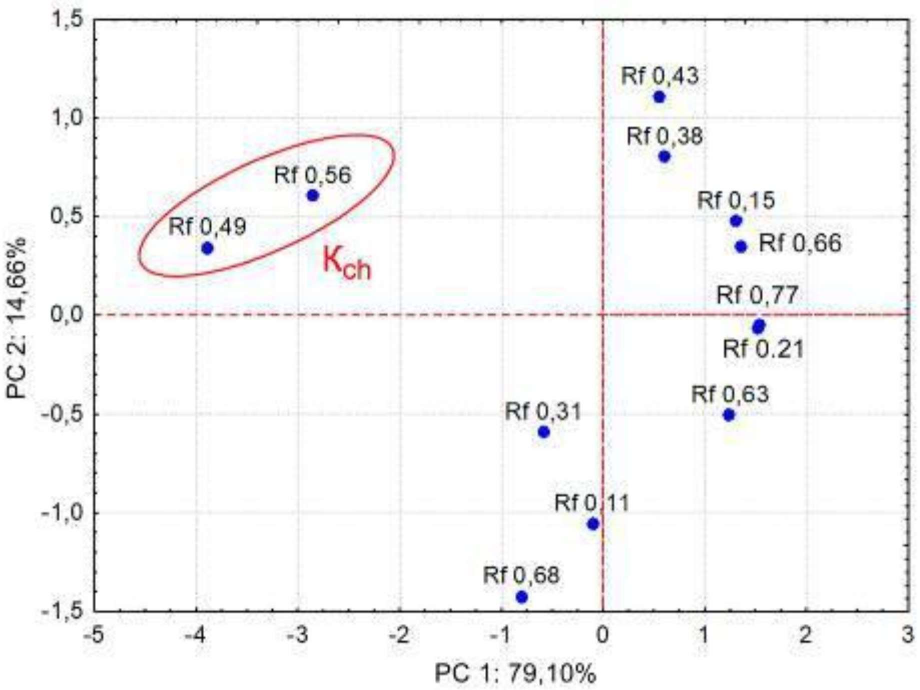

Fig. 5. Principal components analysis of biochemical profiles of *Sambucus nigra* leaves

The ratios and fractions of substances in the total amount of phenolic compounds, analyzed with the above method, also showed this ratio (Kch) value to increase correspondingly with higher recreational pressure on forest and park ecosystems (Fig. 6).

Fig.6. Indexes of physiological state of *Sambucus nigra* plants according to biochemical markers in different habitats of the National Nature park "Holosiivskyi"

Thus, it is established that high sensitivity of certain chains of phenylpropanoid synthesis and formation of individual phenolic components are characteristic of the Norway maple and elderberry plants. Some of the individual compounds, in particular CA, are considered sensitive biochemical markers of plant physiological status, as well as indicators for determining the levels of recreational pressure on forest and park ecosystems.

Plants are an important component of environmental monitoring as indicators of the state of the ecosystem (De Temmerman *et al*., 2001, Mehlhorn, 1991, Micieta, 2000, Batic, 1999, Lorenzini, 2000). At the same time, not all plant species can be used as indicators of anthropogenic pressure. Choosing the indicator species depends on the factors on which the monitoring is focused. They should occur in all monitoring areas and have characters sensitive to environmental changes. According to the data obtained in the forest-park urban ecosystems in the zone of mixed forests, leaves of *Sambucus nigra* and *Acer platanoides* plants were quite sensitive to recreational pressure. These species are typical representatives of forest ecosystems, sufficiently resistant to adverse factors, such as acid soils with increasing free aluminum content. They also are shade-resistant and resistant to recreational pressure. A wide range of phenolic compounds, with variant and conservative components, contained in the leaves of studied species allows using them as markers of the physiological state of plants (Manquion-Cerda K., 2018).

Conclusions

The content of individual phenolic compounds is related to the general process of phenylpropanoid synthesis. Hence, not only the synthesis of individual compounds, but also their ratios in the total phenol content are informative. Using variable components of phenolic synthesis with high antioxidant potential and their ratios to the total phenol amount are an important element in the evaluation of the general physiological state of plants. These biochemical parameters are not suitable for rapid analysis of the state of forest ecosystems, but can be used for studies of stress factors. The specific absorption spectrum of UV radiation of phenolic compounds (in particular chlorogenic acid) may have wide prospects for remote spectral studies of forest and park ecosystems, as well as agroecosystems.

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2. PLANT PROTECTION AND FOOD SAFETY

PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITIES IN VARIOUS TYPES OF CARROT EXTRACTS

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Abstract

The objective of this study was to evaluate antioxidant activities of vegetable extracts *Dancus carota* L., grown in Serbia. Different experimental models have included the determination content of total phenolics, total flavonoids and antioxidant activities of extracts. From the same material, two extracts were obtained by various methods: maceration and ultrasonic extraction. The highest content of phenolic compounds was detected in *D. carota* L. 50.42 mg GAE/g, ultasonic extract. The lowest content of phenolic compounds shown *Dancus carota* L. macerat, 17.45 mg GAE/g. The obtained antioxidant activities are in correlation with the content of phenolic components. On the basis of the results obtained, extract of were found to serve as a potential source of natural antioxidants due to their marked activity. The obtained results may be useful in the evaluation of new dietary and food products.

Keywords: *D. carota L, maceration, ultrasonic extraction, phenolic compounds*.

Introduction

Different parts of plants (roots, leaves, flowers, fruit, stem, bark) have been successfully used to treat numerous diseases (Beninger and Hosfield, 2003). Owing to their antioxidant activity, they can influence a number of physiological processes, thus protecting the organism from the damaging effect of free radicals and inhibiting the development of unwanted microorganisms. Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (Yanishlieva *et all.*, 2006). Our body is exposed to a large number of foreign chemicals everyday . The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells. The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases. Antioxidants can significantly delay or prevent the oxidation of easily oxidizable substances (Macheix and Fleuriet, 1998). Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions but do not convert free radicals. However, synthetic antioxidants, such as butylated hydroxyltoluene (BHT) and butylated hydroxyanisole (BHA), known for their ability to terminate the chain reaction of lipid peroxidation, have been proven to be carcinogenic and to cause liver damage (Prieto *et all.*, 1999). The use of plants in the food industry in place of synthetic preservatives, antioxidants or other food additives has significantly increased over the last few years due to their ability to produce biologically active substances (Merken and Beecher, 2000). There is growing interest toward natural antioxidants from herbal sources (Beninger and Hosfield, 2003).

Material and Methods

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). The plant material used in the experiment included is grown under plastic-covered greenhouse conditions in Cacak.

Spectrophotometric measurements were performed using an MA9523-SPEKOL 211 UV-VIS spectrophotometer (ISKRA, Horjul, Slovenia).

Plant materials (20 g) were macerated in a mixture of 95% ethanol (250 ml) and 0.5% glacial acetic acid at room temperature for 24 hours. The resulting macerate was filtered and the maceration procedure was repeated once. The extracts obtained were combined and concentrated until dry in a rotary vacuum evaporator to produce the (E_1) extract [5]. The (E_2) extract was obtained by ultrasound-assisted extraction using a Brason B-220 ultrasonic bath (Smith-Kline Company, USA).

The typical procedure involved ultrasound-assisted extraction of crushed plant material with 95% ethanol over a period of 1 hour.

Total phenols were estimated using the Folin-Ciocalteu method (Duh *et all.,* 1999). Plant extracts were diluted to a concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and 2 mL of $NaHCO_3$ (7.5%), (Yan *et all.*, 2006). After heating for 15 min at 45˚C, the absorbance was measured at 765 nm in a spectrophotometer against blank sample. Total phenols were determined as gallic acid equivalents (mg GA/g extract), and the values are presented as means of triplicate analyses (Hsu *et all*., 2008). The method used by (Brighente *et all*., 2007), was adopted with suitable modifications from (Daker *et all*., 2008). DPPH (8 mg) was dissolved in C_2H_5OH (100 mL) to obtain a concentration of 80 mg/mL. Serial dilutions were carried out with the stock solutions (1 mg/mL) of the extracts. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 510 nm. Ascorbic acid (AA), gallic acid (GA) and butylated hydroxytoluene (BHT) were used as reference standards and dissolved in methanol to make a stock solution at the same concentration (1 mg/mL), (Yanishlieva *et all.,* 2006). Control sample was prepared containing the same volume without test compounds or reference antioxidants (Tandon *et all*.,1995). Ninety-five percent ethanol was used as blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

% inhibition = $[\,(A_c - A_s) / A_c]\ 100$.

Results and Discussion

The total phenolic content of the (E_1) ultrasonic extraction 50.42 mg GAE/100g of sample, and that of the (E_2) maceration extract was 17.45 mg GAE/100g of sample. The results obtained were calculated as average values of five parallel measurements. High values of antioxidant activity were identified, being 92.67 % and 89.67 % for the E_1 and E_2 extracts, respectively. Figures 1. and 2. show graphic presentation of the antioxidant activity of the E_1 and E_2 extracts.

Fig. 1. Antioxidant activity of the E_1 extract

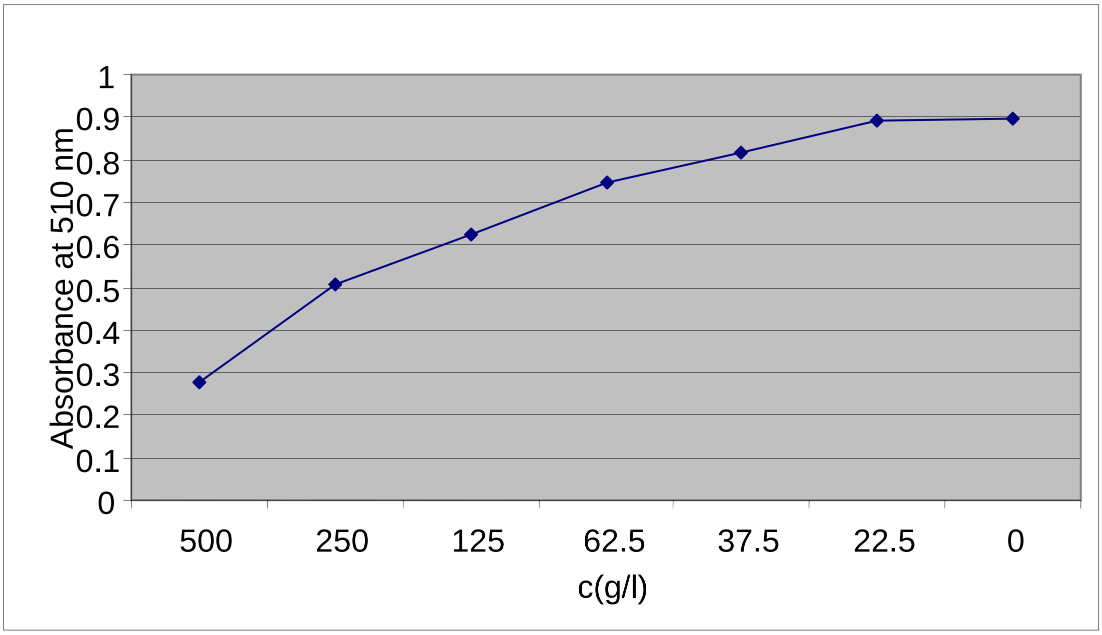
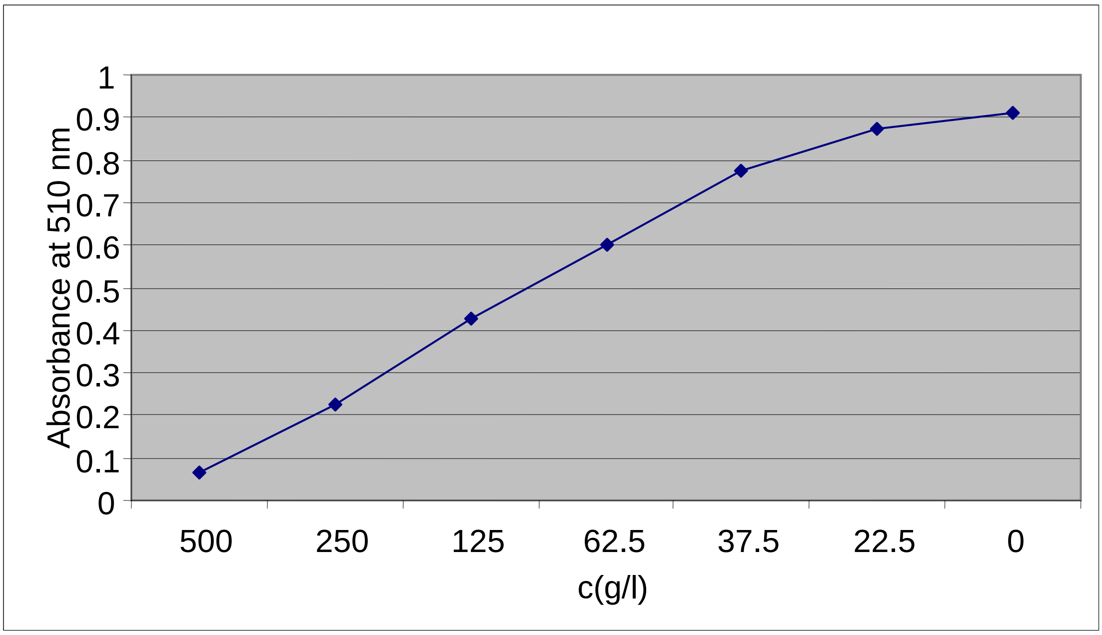


Fig. 2. Antioxidant activity of the E_2 extract



The DPPH capacity values were calculated relative to rutin, trolox and quercetin (Table 1). The values were calculated based on the graph representing dependence between standard concentration (mg/g DPPH) in the X-axis and capacity of DPPH radicals (%) in the Y-axis.

Rutin c= (% DPPH- 1.29481) / 0.37472
Troloks c= (% DPPH- 0.24856) / 0.38094
Quercetin c= (% DPPH- 12.09185) / 5.2032

Table 1. DPPH mg/g relative to rutin, trolox and quercetin

extract	mg/g DPPH	standard
Ethanol E_1	0.24385	Rutin
Ethanol E_2	0.24118	Rutin
Ethanol E_1	0.24261	Trolox
Ethanol E_2	0.23999	Trolox
Ethanol E_1	0.01549	Quercetin
Ethanol E_2	0.01529	Quercetin

Conclusions

The importance of this study lies in a preliminary examination of whether carrot extracts can be used as a source of natural preservatives in the food industry. Results show significant antioxidant activity in the vegetable extracts tested. Moreover, the E_1 and E_2 extracts were found to have significant antioxidant activity which correlated with the total phenolic content. Total phenols, hence and antioxidant activity, are dependent upon the method and time of extraction, which is most likely due to their instability. Therefore, this fact should be considered when selecting and obtaining natural antioxidants.

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CHANGES IN GERMINATION AND PRIMARILY GROWTH OF THREE CULTIVARS OF FENNEL UNDER APPLICATION OF DIFFERENT DIATOMITE TREATMENTS

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Abstract

Fennel is a plant belonging to the *Apiacea* (*Umbelliferae*) family, and used by humans for a long time for medicinal purposes. In order to evaluate the influence of different materials on germination and seedling growth of three cultivars of fennel, an experiment was conducted in 2018. There cultivars of fennel were Isfahan, Yazd, and Shiraz in main plots, and three materials including 100% soil, 50% diatomite + 50% soil, and 100% diatomite in subplots, were analyzed in a split plot experiment based on a randomized complete block design (CRBD) with three replications. The highest total germination percentage, coefficient of velocity of germination, coleoptile length, fresh coleoptile weight and dry coleoptile weight was related to Isfahan. The maximum speed of germination, mean germination time, fresh length and dry leaf weight was achieved in Shiraz cultivar. The higher values of total germination percentage, speed of germination and mean germination time were related to 100% soil, while application of 50% of soil + 50% of diatomite had obtained the maximum values of radicle length, coleoptile length, fresh coleoptile weight, dry leaf weight and dry coleoptile weight. The maximum values of coefficient of velocity of germination and fresh leaf weight was achieved in application of 100% diatomite. It seems that application of 50% soil + 50% diatomite and Isfahan and Shiraz cultivars have a great potential of seed germination of seedling growth.

Keywords: Fennel, Diatomite, Germination, Seedling Growth

Introduction

The aromatic and medicinal plants are important in all over the world (Heidari et al., 2014; Ogbaji et al., 2018; Shahrajabian et al., 2018; Shahrajabian et al., 2019a; Shahrajabian et al., 2019b; Shahrajabian et al., 2019c; Soleymani and Shahrajabian, 2018). Fennel (*Foeniculum vulgare Mill*.) had aromatic, medicinal and flavouring properties, and it is rich in various biological active ingredients (Ozcan and Chalchat, 2010; Soleymani and Shahrajabian, 2012a;Badgujar et al., 2014; Gama et al., 2014). It is cultivated in different parts of the world such as Europe, the middle east and Asia (Sadeghpour et al., 2015). Inappropriate seed germination is a common phenomenon which may cause a great concern for crop production, especially medicinal and aromatic crops (Khoshkharam et al., 2010; Shahrajabian et al., 2018).Seed vigor can be defined as the ability of the seed to germinate and become established under less than optimal conditions, or to survive a series of environmental stresses during germination (Soleymani et al., 2011; Balla et al., 2012; Soleymani et al., 2013). Diatoms are the most diverse group of phytoplankton ranging in the size from a few micrometers to a few millimetres and exist either as single cells or chains of cells (Aksakal et al., 2013; El-Sherif et al., 2018). Huang et al. (2012) concluded that diatomite is a siliceous sedimentary rock with a porous structure, low density, high surface area and excellent thermal resistance, and it is readily available and environmentally friendly in sustainable agriculture.

El-Sherif et al. (2018) found that diatomite can improve the growth and mitigate the negative effects of salinity stress, and also reduce the negative effects of water stress. Yong et al. (2018) mentioned that favourable temperatures and water supplies are the most important conditions required for germination and seed emergence. Unfortunately, in Iran the available researches and studies on diatomite and application of this technology for medicinal crop cultivation is limited. Therefore, the aim of this research was to evaluate changes of seed germination and seedling growth characteristics of fennel cultivars in centre of Iran to different managements of diatomite application.

Materials and methods

Diatomite and three varieties of fennel were obtained from Agricultural Research Centre, Isfahan, Iran. There cultivars of fennel were Isfahan, Yazd, and Shiraz in main plots, and three materials including 100% soil, 50% diatomite + 50% soil, and 100% diatomite in subplots, were analyzed in a split plot experiment based on a randomized complete block design (CRBD) with three replications in a green house at Mojgan Agricultural Company, Mahmood abad, Isfahan, Iran (Latitude 32 $^{o}52^{/}$ N, Longitude $51^{o}34^{/}$ E and 1660 m elevation) in 2018. 50 seeds per pots were planted. Germination percentage, germination speed (GR), mean germination time to complete germination (MGT), and coefficient of velocity of germination (CVG) were measured with equations number 1, 2, 3 and 4, respectively.

Germination percentage= Number of germinated seed /100 (1)

GR= $\Sigma \frac{N}{\Sigma(n \times g)}$ (2)

Where, n is the number of germinated seed on growth day and g is the number of germination seeds.

MTG= $\Sigma \frac{NiDi}{N}$(3)

N: total seed number, Ni: number of germinated seed per day, and Di: the number of required days for germination.

CVG= $\frac{1}{MTG}$ (4)

Germinated seeds were carried out by using three replicates of 50 seeds and other seeds were cultured to determine dry matter. Radicle and coleoptileof germinated seeds were measured for length and weight characteristics. To this purpose, 10 seeds were chosen randomly, within those germinated first. The selected seedlings were oven-dried for 48 hours at 65 ^{o}C for dry weight measurement. Analysis of variance (ANOVA) was used to evaluate the significant differences. The Multiple Range Test of Duncan performed the separation means ($P<0.05$). All statistics was performed with the SAS statistical software.

Results and discussion

The influence of cultivar was significant on total germination percentage, speed of germination, radicle length, coleoptiles length, fresh leaf weight, fresh coleoptile weight, dry leaf weight and dry coleoptile weight. Except fresh coleoptile weight, dry leaf weight and dry coleoptile weight, all experimental characteristics namely, total germination percentage, speed

of germination, mean germination time, coefficient of velocity germination, radicle length, coleoptile length and fresh leaf weight was significantly influenced by material components.
The highest total germination percentage (75.99%) was belonged to Isfahan cultivar which had meaningful differences with other cultivars. The total germination percentage in Yazd and Shiraz cultivar was 59.86% and 63.82%, respectively. The appropriate seed germination is very important for final successful productions of crops (Shahrajabian et al., 2011; Soleymani and Shahrajabian, 2017). The higher speed of germination was related to Shiraz cultivar, followed by Yazd and Isfahan. Although, there was a significant difference between Isfahan and Shiraz, both of these cultivars had no significant differences with Yazd. The maximum and the minimum mean germination time was achieved in Shiraz and Isfahan cultivar, respectively. Uniformity and increase percentage of seedling emergence in direct seedling planting significantly influence the yield and quality of crops (Khoshkharam et al., 2010; Soleymani and Shahrajabian, 2012b; Soleymani and Shahrajabian, 2012c; Ogbaji et al., 2013; Shahrajabian et al., 2013; Soleymani et al., 2016). Although, the higher value of coefficient of velocity of germination was obtained for Isfahan cultivar compare to those of other cultivars, no significant differences were found between treatments. The higher radicle length was related to Yazd (2.60 cm), followed by Isfahan (2.50 cm), and Shiraz (2.43 cm). On the one hand, Isfahan had significant difference with Shiraz; on the other hand, its difference with Yazd was not significant. The highest and the lowest coleoptiles length was related to Isfahan (6.50 cm), and Yazd (3.66 cm), respectively, which had meaningful differences with other. The higher fresh leaf weight was related to Shiraz (0.55 g), followed by Isfahan (0.51 g), and Yazd (0.31 g), respectively. Although, there was no significant difference between Isfahan and Shiraz, both of these cultivars had meaningful difference with Yazd. The maximum and the minimum fresh coleoptile weight were belonged to Isfahan and Yazd cultivar, respectively. Fresh coleoptile weightin Isfahan, Yazd and Shiraz was 0.60 g, 0.38 g, and 0.47 g, respectively. The maximum and the minimum dry leaf weight was related to Shiraz and Yazd, respectively, which had meaningful difference with each other. However, no significant difference was found between Isfahan and Shiraz cultivar. The higher dry coleoptile weight was achieved in usage of Isfahan, followed by Shiraz and Yazd, respectively. All differences between treatments were significant (Table 2). The maximum and the minimum total germination percentage and speed of germination were achieved in usage of 100% soil, and 100% diatomite, respectively, which had meaningful differences with each other. The highest and the lowest mean germination times were related to 100% soil, and 50% soil + 50% diatomite, respectively. All differences between treatments were meaningful. Application of 100% soil had obtained the lowest coefficient of velocity of germination and radicle length which had meaningful differences with other treatments. Delayed and reduced germination and seedling emergence which cause non-uniform and establishment, therefore seedlings subject to soil-borne pathogens (Nourimand et al., 2011; Shahrajabian and Soleymani, 2017; Shahrajabian et al., 2017; Abdollahi et al., 2018). The higher values of coefficient of velocity of germination and radicle length were observed in 50% soil + 50% diatomite and 100% diatomite, respectively. The maximum and the minimum coleoptile length was related to 50% soil + 50% diatomite and 100% diatomite respectively. All differences between treatments were meaningful. The higher values of fresh leaf weight was related to 100% diatomite, followed by 50% soil + 50% diatomite and 100% soil respectively, which had significant differences with each others. Application of 50% soil + 50% diatomite had obtained the highest values of fresh coleoptile weight, dry leaf weight and dry coleoptile weight. There were not any significant differences for fresh coleoptiles weight, dry leaf weight and dry coleoptile weight between treatments. The minimum fresh coleoptile weight and dry coleoptiles weight were achieved in 100% of application of soil (Table 2).

Table 2. Mean comparison for total germination percentage (%), speed of germination, mean germination time, coefficient of velocity germination, radicle length (cm), coleoptile length (cm), fresh leaf weight (g), fresh coleoptiles weight (g), dry leaf weight (g), and dry coleoptile weight (g).

Treatment	Total Germination Percentage	Speed of Germination	Mean Germination Time	Coefficient of Velocity Germination	Radicle Length	Coleoptile Length	Fresh Leaf Weight	Fresh Coleoptile Weight	Dry Leaf Weight	Dry Coleoptile Weight
Cultivar (C)										
Isfahan (1)	75.99a	20.56b	2.98a	0.87a	2.50a	6.50a	0.51a	0.60a	0.048a	0.028a
Yazd (3)	59.86c	25.02ab	3.01a	0.85a	2.60a	3.66c	0.31b	0.38c	0.041b	0.019c
Shiraz (2)	63.82b	28.25a	3.02a	0.84a	2.43b	5.54b	0.55a	0.47b	0.049a	0.022b
Material (M)										
100% soil (M1)	85.78a	34.39a	3.49a	0.70c	2.30c	5.34b	0.49c	0.47a	0.039a	0.021a
50% soil + 50% diatomite (M2)	80.63b	24.72b	3.03b	0.79b	2.80a	6.01a	0.55b	0.49a	0.041a	0.023a
100% diatomite (M3)	33.25c	12.73c	3.12c	1.08a	2.46b	4.37c	0.56a	0.48a	0.038a	0.022a

Common letters within each column do not differ significantly.

Conclusion

Recently, growing and cultivation of medicinal plants is an important sector in agriculture and is the main source of extracting and producing materials for manufacturing current drugs. Germination ability and percentage of medicinal crops are of fundamental importance, influencing the viability of the plants developing from the grains. Diatomite (DE) is a sedimentary rock primarily composed of the fossilized remnants of unicellular fresh water plants known as Diatoms. Fennel has a long history of herbal uses as not only medicine, but also food. Fennel also is a widespread annual, biennial or perennial medical herb and it depends on the variety. The highest total germination percentage, coefficient of velocity of germination, coleoptile length, fresh coleoptile weight and dry coleoptile weight was related to Isfahan. The maximum speed of germination, mean germination time, fresh length and dry leaf weight was achieved in Shiraz cultivar. The higher values of total germination percentage, speed of germination and mean germination time were related to 100% soil, while application of 50% of soil + 50% of diatomite had obtained the maximum values of radicle length, coleoptile length, fresh coleoptile weight, dry leaf weight and dry coleoptile weight. The maximum values of coefficient of velocity of germination and fresh leaf weight was achieved in application of 100% diatomite. All in all, on the basis of results, it can be concluded that application of 50% soil + 50% diatomite and Isfahan and Shiraz cultivars have a great potential of seed germination of seedling growth.

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ANISE SEED GERMINATION AND PRIMARILY GROWTH UNDER VARIOUS TREATMENTS OF GIBBERELLIC ACID, BENZYLADENINE AND KINETIN

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Abstract

Seed dormancy is one of the major problems in agricultural studies, especially for medical plants. Anise (*Pimpinella anisum L.*) is an important medical plant with dormant seed and it is established and distributed only in its natural habitats. In order to evaluate the effects of some pretreatment factors on primary growth and germination characteristics of Anise, an experiment was conducted as Factorial layout within completely randomized design with four replications. Pre-chilling treatments were 0, 15, 30 and 45 days treatments and hormone treatments were GA_3 (Gibberellic Acid), BA (Benzyladenine), KI (Kinetin), GA_3+BA, GA_3+KI, BA+KI, GA_3+BA+KI, KNO_3, H_2SO_4 and distilled water as a control treatment. Prechilling treatment effects on coleoptile and radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index were meaningful. Different hormone treatments had significant influence on coleoptile and radicle length, seedling length, germination percentage, mean time germination, germination rate and seed vigor index. The highest germination percentage and germination rate was related to usage of BA+KI. The higher values for radicle length and uniformity of seed germination were achieved in application of BA and KI, respectively. Moreover, application of GA_3+BA+KI had obtained the highest seed vigor index. It seems that application of endogenous GA_3+KI and BA+KI concentration, which is provided mostly by chilling treatment, is the most effective factor for breaking the seed dormancy. On the basis of the results, usage of 45 days moist prechilling accompanied with application of GA_3+KI and BA+KI in Esfahan cultivar was appropriate.

Keywords: Seed dormancy, Seed germination, Seedling growth, Anise.

Introduction

Plants, herbs and their secondary metabolite constituents have a long history of use in traditional and modern western medicine (Soleymani and Shahrajabian, 2012; Ogbaji et al., 2013; Ogbaji et al., 2018; Shahrajabian et al., 2019a; Shahrajabian et al., 2019b; Shahrajabian et al., 2019c). Anise (*Pimpinella anisum L.*) is a flowering plant in the family Apiaceae native to the eastern Mediterranean region, west and Southwest Asia (Ibrahim Doaʾa Anwar, 2017). Anise is also famous in traditional Chinese medicine (TCM), Ayurveda and Unani medicine. Anise has been used for different purposes in traditional medicine of Iran (Shojaii and Abdollahi Fard, 2012). Seed priming treatments have been employed to accelerate germination, seedling growth and yield in most seeds under normal and stress conditions (Shahrajabian et al., 2011; Soleymani and Shahrajabian, 2012; Shahrajabian et al., 2013; Mahdavi, 2016; Soleymani et al., 2016). Seed germination can be controlled by many factors like natural germination and growth inhibitors (Bhardwaj et al., 2016; Shahrajabian et al., 2017; Shahrajabian et al., 2018; Soleymani and Shahrajabian, 2018). These are the derivatives of gibberellic acid (GA_3), abscisic acid (ABA), cinnamic acid, kinetin (KI), bonzyladenin (BA), coumarin, jasmonic and etc. The variation in seed dormancy and seedling emergence are controlled by environmental conditions. The origin of research into Gibberellins can be traced to Japanese plant pathologists who were investigation the causes of the bakane (foolish seedling) disease that seriously lowered to the yield of rice crop in Japan, Taiwan and some other Asian countries (Patel and Mankad, 2014). Gibberellic acid is a plant growth hormone that has important role in seed germination (Patel and Mankad, 2014). It has been reported that the stimulating effects of GA_3 on seed germination are not similar in all crop species (Bell et al., 1995). GA_3 has been also reported to promote growth in cotton, rice and in some halophytes under saline conditions (Lin and Kao, 1995). Tsygankova et al. (2016) confirmed specific auxin-like, cytokinin-like and minor gibberellins-like effect of synthetic heterocyclic compounds on cell division, cell proliferation, cell elongation and cell differentiation that are the basic processes of plant growth and development. Gibberellins eliminated the chilling requirements of peach and apple seed and increased their germination (El-Barghathi and El-Bakkosh, 2005). Primed with plant hormones such as gibberelin could improve quality of seeds and germination in seeds (Shekari et al., 2015). Liopa-Tsakalidi et al. (2011) also suggest that germination and seedling growth of 11 species responded differently to different levels of GA_3. Fernandez et al. (2002) revealed that cold stratification has a direct influence on production of gibberellins (Gas) in seeds of *Arabidopsis thaliana*. Exogenously applied GA overcomes seed dormancy in several species (Hassan and Fardous, 2003). Hormone priming increased antioxidant enzyme activity and decrease amount of reactive oxygen space (Shekari et al., 2015). Sharifi and Pouresmael (2006) concluded that only cold treatments such as gibberellic acid, cytokinin, potassium nitrate, washing and light treatments are not useful. It has been reported that GA is effective in breaking seed dormancy in snowberry (Rosner, 2002). Nkomo and Kambizi (2010) noted that prechilling followed by exposure to a temperature higher than 30^{o}C encourages germination of *C. Olitorious* seeds. Rouhi et al. (2010) concluded that applying 500 ppm concentration of GA_3 and 0.1 of KNO_3 resulted in higher germination in waterlily dormant seeds. Plant hormones are used in breaking seed dormancy (Gupta et al., 2008). Cytokinin and auxin are the most common plant growth regulators used in in vitro culture of plat tissues (Eudes et al., 2003; Abu-Romman et al., 2015). Cytokinins constitute a major class of plant growth regulator that is involved in a wide range of physiological processes (Davies, 1995). Cytokinins have a stimulatory or an inhibitory role in different development processes, such as control of apical dominance in the shoot, root growth and branching, leaf senescence, and chloroplast development. In spite the fact that Anise is an important and expensive medicinal and spice plant, not enough information is available on the effects of moist pre-chilling and application of hormones on different cultivars of it. So, the aim of this study is survey the certain effects of different treatments to stimulate seed germination and seedling growth of Anise.

Material and Methods

In order to evaluate the influence of some pretreatment factors on primary growth and germination characteristics of anise (*Pimpinella anisum L.*), an experiment was conducted as Factorial layout within completely randomized design with four replications at Research laboratory of Mojgan Agricultural Company, Mahmood abad, Isfahan, Iran (Latitude 32 ^{o}52$^{/}$ N, Longitude 51^{o}34$^{/}$ E and 1660 m elevation).

Pre-chilling treatments were 0, 15, 30 and 45 days treatments and hormone treatments were GA_3 (Gibberellic Acid), BA (Benzyladenine), KI (Kinetin), GA_3+BA, GA_3+KI, BA+KI, GA_3+BA+KI, KNO_3, H_2SO_4 and distilled water as a control treatment. First, seeds were surface sterilized in 1.5% (w/v) sodium hypochlorite solution for 15 minutes and then rinsed three times sterile distilled water. For each treatment, 4 Petri dishes were used and 30 seeds were put into each of them, then, each Petri dish were covered with 10 mm of each specific treatment. In the first trial, seeds were chilled for 15, 30 and 45 days, and after that, seeds were soaked and treated with 10 hormone treatments. In the second experiment, seeds were treated without pre-chilling treatments. In the thirds experiment, seeds treatments were done with poly ethylene glycol. Equation number one and number two was used to calculate germination percentage and germination rate, respectively.

Germination percentage= Number of germinated seed / 100 (1)

1) $GR = \Sigma \frac{N}{\Sigma(n \times g)}$ (2)

Where, n is the number of germinated seed on growth day and g is the number of germination seeds Analysis of variance (ANOVA) was used to determine the significant differences.
Uniformity of seed germination and mean time for seed germination (MTG) was evaluated by equation number 3 and 4. Seed vigor index was calculated by equation number 5.

Uniformity of seed germination= $\dfrac{1}{\dfrac{\sum (D-\bar{D})^2 \times N}{\sum N}}$ (3)

$MTG = \dfrac{\Sigma(nd)}{\Sigma n}$ (4)

n: The number of germinated seed in the specific day.
d: The number of days from the beginning of germination.
∑n: The total number of germinated seed.

Seed vigor index: Germination percentage × mean of seedling length (mm)(both coleoptile and radicle) / 100 (5)

The Multiple Range Test of Duncan performed the separation means ($P<0.05$). All statistics was performed with the SAS statistical software.

Results and Discussion

Prechilling had significant impact on coleoptile length, radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index. All experimental characteristics expect of uniformity of seed germination was affected by hormone treatments. The highest coleoptile length was related to 45 days chilling (2.45 mm) which had meaningful differences with other treatments. Although, the higher value of radical length was obtained for 45 days (0.8485 mm) chilling, its difference with 30 days chilling was not meaningful. The minimum coleoptiles (0.7536 mm) and radical length (0.6819 mm) was related to control treatment. The higher values of seedling length (2.98 mm), and germination percentage (70.02%) was obtained for 45 days of chilling followed by 30 days, 15 days and control treatment. There were significant differences in seedling length and germination percentage between 45 days of chilling and other treatments. Control treatment had obtained the highest mean time for germination (11.18) which had meaningful differences with 30 days and 45 days pre-chilling, although, its difference with control treatment was not meaningful. The maximum value for uniformity of germination rate (5.87%), and seed vigor index (2.244) was achieved in 45 days pre-chilling which had meaningful differences with other treatments. Both germination rate and seed vigor index was increased significantly from control treatment to 45 days pre-chilling. The maximum and the minimum uniformity of seed germination was related to control treatment (0.2165), and 15 days pre-chilling (0.1075), which had no meaningful differences with each other (Table 1). Gupta et al. (2008) reported that prechilling treatment also improved seed germination in Isabgol. Shekari et al. (2015) concluded that GA_3 priming treatment had a significant effect on germination percentage and number of normal seedlings. The highest coleoptiles length (1.663 mm), and seedling length (2.574 mm) was related to application of GA_3+KI and the minimum one was observed in KNO_3. Patel and Mankad (2015) concluded that low concentrations of GA_3 influence all developmental and physiological processes in plants. The maximum and the minimum radical length was related to application of BA (1.7232 mm), and KNO_3 (0.5286 mm), respectively, which had meaningful differences with each other. Benzyladenine (BA) at a high concentration was also shown to be effective in shoot regeneration in *P. vulgaris* (Malik and Saxena, 1992). Application of BA + KI had obtained the highest value for germination percentage (66.33%) and germination rate (4.126%), which had significant differences with other treatments. Sawan et al. (2000) demonstrated that kinetin application improved seed viability and seedling vigour as shown by lengths of the hypocotyls, radical and the entire seedling, as well as seedling fresh weight. Gibberellic acid is also known to play an essential role in seed germination, stem elongation and flower development (Sharma et al., 2006). The maximum and the minimum mean time for germination was achieved in usage of BA + KI (66.33), and KNO_3 (25.45), respectively. Narra et al. (2010) also found that the seedling under the GA_3 influence showed enhanced germination, seedling elongation and dry weight accumulation on *Trachyspermum ammi*. Although, the higher value for uniformity of seed germination was related to KI (0.1523), followed by other treatments, there were no significant differences between treatments. Application of GA_3+BA+KI had obtained the highest seed vigor index (1.545), and the minimum one was related to application of distilled water (0.4076) (Table 1). Gupta et al. (2008) concluded that GA has shown promising effect in breaking seed dormancy with accelerated seed germination (speed of germination, vigor index) and seedling growth (seedling dry weight).

Table 1- Mean comparison for coleoptile length (mm), radicle length (mm), seedling length (mm), germination percentage (%), mean time for germination, germination rate (%), uniformity of seed germination and seed vigor index.

Treatment	Coleoptile length	Radicle length	Seedling length	Germination percentage	Mean time for germination	Germination rate	Uniformity of seed germination	seed vigor index
Prechilling (day)								
0	0.7536d	0.6819bc	1.463c	14.22d	11.18a	0.3626d	0.2165a	0.3287d
15	1.000c	0.5625c	1.554c	35.28c	11.00a	1.489c	0.1075a	0.6328c
30	1.340b	0.7635ab	2.133b	53.74b	7.81b	3.356b	0.1336a	1.239b
45	2.145a	0.8485a	2.986a	70.02a	5.52c	5.87a	0.1352a	2.244a
Hormone								
GA_3	1.352bc	0.8112abc	2.185b	37.91d	10.44a	2.533d	0.1471b	1.056ab
BA	1.428abc	1.7232bcd	2.143bc	61.22b	9.862a	3.837b	0.1066b	1.365ab
KI	1.536ab	0.8687ab	2.385ab	47.33c	9.575a	3.199b	0.1523b	1.351ab
GA_3+BA	1.345bc	0.6835cd	2.036bc	52.26b	10.61a	3.007bc	0.1474b	1.263bc
GA_3+KI	1.663a	0.9123a	2.574a	40.23cd	10.66a	2.673cd	0.1132b	1.484ab
BA+KI	1.425abc	0.7348be	2.176bc	66.33a	9.74a	4.126a	0.1035b	1.464ab
GA_3+BA+KI	1.563abc	0.8140abc	2.356ab	54.00b	10.28a	3.179b	0.1202b	1.545a
KNO_3	0.7537d	0.5286e	1.343d	25.45e	6.40b	1.855e	0.1148b	0.4661de
H_2SO_4	1.256c	0.5573de	1.915c	25.69e	6.26b	1.632e	0.1242b	0.6842d
Distilled water	0.8223d	0.5647de	1.465d	25.68e	5.48b	1.987e	0.1247b	0.4076e

Common letters within each column do not differ significantly.
GA_3= Gibberellic Acid; KI= Kinetin; BA= Benzyladenine

Conclusion

Seed germination is a complex physiological processes that response to environmental signals such as light, water and other factors. Also, Seed germination is very important to know the germination pattern of a plant, especially the medicinal plants. Prechilling treatment effects on coleoptile and radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index were meaningful. Different hormone treatments had significant influence on coleoptile and radicle length, seedling length, germination percentage, mean time germination, germination rate and seed vigor index. Prechilling treatment for 45 days had obtained the highest coleoptile and radicle length, seedling length, germination percentage, germination rate and seed vigor index. While control treatment had obtained the maximum mean time for germination and uniformity of seed germination. Application of GA_3+KI had obtained the highest coleoptile length, seedling length, and mean time for germination. The highest germination percentage and germination rate was related to usage of BA+KI. The higher values for radicle length and uniformity of seed germination were achieved in application of BA and KI, respectively. Moreover, application of GA_3+BA+KI had obtained the highest seed vigor index. All in all, in conclusion, it was shown that GA_3, KI and BA had greatly enhanced the germination parameters in terms of germination percentage, seedling elongation and other characteristics.

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INTERCROPPING AND ORGANIC FERTILIZERS WITH COMPARATIVE ADVANTAGES IN SUSTAINABLE AGRICULTURE FOR MORE STABLE AGRICULTURAL SYSTEM

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Abstract

Sustainable agricultural system is the best way to provide needs of people today and future generations. In sustainable agricultural system, agricultural yields increase with intercropping due to higher growth rate, reduction of seeds, pests and diseases and more effective use of resources. Intercropping is a one of the most important way to increase diversity in an agricultural ecosystem. Intercropping has a long history. Intercropping systems could be more stable systems of agricultural practices than mono cropping. The most important advantages of intercropping are increasing production, greater use of environmental resources, significant reduction of pests, diseases and weeds damage, improve soil fertility and increase in nitrogen, stability and uniformity yield. Integrated use of synthetic and organic fertilizers may lead to development of sustainable crop production. This method also can increase the efficiency of chemical fertilizers and of course reduce their use. Green manures are legume crops like clover and others that are able to add nitrogen by fixation to the soil and then reduce the need for commercial nitrogen. Moreover, planting green manures is an effective means of adding nitrogen to the agricultural land. The biological nitrogen fixation is also play an important role in sustainable agricultural systems. In sustainable agricultural system, fertilizers, livestock manure and cover crops are important parameters in productive agricultural systems to have stable food. All in all, in sustainable agricultural system, intercropping included, fertilizers, livestock manure and cover crops are important parameters in productive agricultural systems to have stable food.

Keywords: *Intercropping, Chemical Fertilizer, Manure, Sustainable Agriculture, Stable System.*

Introduction

The rapid increases in human population and exploitative use of non-renewable resources have worsened food shortages (Amini et al., 2012; Esfandiary et al., 2012; Soleymani et al., 2012a,b; Shahrajabian et al., 2017a; Shahrajabian et al., 2012b,c,d; Ogbaji et al., 2018; Shahrajabian et al., 2018; Soleymani et al., 2018; Yong et al., 2018; Shahrajabian et al., 2019a,b,c,d,e,f,g). Chemical fertilizers play a dominant role in agriculture development (Shahrajabian and Soleymani, 2017a,b). Most scientists believe that increasing yield per ha is a major way for increasing crops yield (Ogbaji et al., 2013; Soleymani et al., 2016; Soleymani and Shahrajabian, 2017; Yong et al., 2017). Due to high costs and poor accessibility of inorganic fertilizers to resource-poor farmers, other inputs are oftentimes proposed as alternatives (Shahri et al., 2011; Soleymani and Shahrajabian, 2012d; Shahrajabian et al., 2013). It is believed that much of deficient plant nutrients could be supplied to soils through organic matters while short falls are made up with mineral fertilizers (Ogbaji et al., 2013). Farmyard manure (FYM) contains very small amount of major nutrients and even difficult to obtain required quantity of FYM and also involve large transportation. It also maintains the soil physical and chemical condition and improves the overall ecological balance of the crop production system. It also reduced use of external inputs and implies on reliance on self regulating ecosystem process. When the livestock fodder is scarce, results in impaired livestock nutrition and health condition. In forage production, considering chemical position of forage crop is so important.

Intercropping

Intercropping is known as a practice, which can improve the utilization of available resources and cause yield advantages and increases yield stability compared to sole cropping (Soleymani et al., 2011g; Soleymani and Shahrajabian, 2012a,b). Intercropping is a sustainable practice used in many developed and developing countries and an essential element of agricultural sustainability. Symbiotic N_2 fixation (SNF) in legumes is a fundamental process for maintaining soil fertility continued productivity of organic cropping systems. One of the benefits of intercropping is, having high potential to extrapolated crop combinations. Intercropping legumes with non legume crops is a common practice in the world. Sole annual legumes, cereals and intercropping mixture of annual legumes and cereals are intensively cultivated in the world for forage production. Recently intercropping gained an increasing interest in an attempt to substantiate functional biodiversity for agriculture production and to reduce chemical inputs use. The introduction of a living cover crop during a cash crop growth cycle (relay intercropping) may help to preserve biodiversity, increase soil organic matter content and carbon sequestration and provide nutrient recycling. Soil organic matter associated with microbial activity plays a major role in the nutrient cycling process in soil leading to enhanced nutrient availability. Soleymani et al. (2012c) reported that there has been a rapid increase of fertilizer application in recent years to achieve high yields in Iran, but studies showed that intercropping cause yield advantage and better nutrition uptake. They have found that intercropping of berseem clover and forage corn in low input farming system, nitrate accumulation in clover was suitable for animal 's grazing. They concluded that mix cropping legumes with cereal and grasses species had been applied to enhance nutrition value, supply energy and protein on both crops; mixture of cereal and legumes offer a sustainable alternative to maintain efficient farming systems with reduced environmental impacts. Soleymani et al. (2012d) mentioned that green manuring is an age-old practice used for supplying the nitrogen to crop plants; the intensive cropping system, heavy input technology, environmental degradation and other related problems again encouraged its re-inclusion in plant nutrient supply system. They did conclude that residue burning accompanied with usage of triticale as a green manure was the best choice to achieve high quality, but for obtaining the most fresh forage yield and biological yield of forage corn, triticale plantation can be replaced by barley cultivation.

Fertilizer

Low-input farming systems such as arable organic farming, often have limited access to nitrogen, and this often limits the productivity of these systems (Soleymani et al., 2010; Soleymani et al., 2011f; Soleymani and Shahrajabian, 2012e; Soleymani et al. 2012a; Shahrajabian et al., 2020). Minimal or no fertilizer input had caused serious nutrient depletion, which coupled with the low fertility status of soil and it is the major limiting factor to crop production. Nitrogen supply increases plant growth such as increasing nitrogen supply enhances both shoot and root, and plant growth inhibited by low nitrogen (Soleymani and Shahrajabian, 2011; Shahrajabian et al., 2011). Supplemental nitrogen application, is needed to optimize plant production and minimize production cost (Soleymani et al., 2013), however, the application of excessive amounts of nitrogen can cause the accumulation of toxic levels of nitrate (NO_3^-) in plants (Khoshkharam et al., 2010; Soleymani and Shahrajabian, 2013). In most commercially available fertilizers, the concentrations of fertilizer active ingredients rapidly diminish prior to sufficient plant uptake due to degradation (e. g. chemical, photochemical and biological), volatilization, leaching, adsorption or land immobilization (Broumand et al., 2010). Nitrate toxicity in forage plants can pose chronically or acute in livestock.

Manure

Organic farming, which evolved in 1980s, is one way to solve the current farming problems, in this method, manure and green manure use instead of chemical fertilizers (Soleymani et al., 2011b; Soleymani et al., 2011e; Soleymani et al. 2012b). Manures are very variable products, often difficult to apply accurately and release nutrients in the soil at a desirable rate. Some studies have shown that farmyard manure applied alone or in combination with inorganic fertilizers was effective in maintaining soil fertility under continuous cultivation. Applying farm manure increased cation exchange capacity (CEC), organic carbon and water holding capacity of the soil and nutrient availability. Dairy manure is an excellent source of nitrogen for crops and can easily fulfill the nitrogen requirement. To get satisfactory results well-composted manure must be used, because it is usually free of weed seeds and has a better nutrient balance. It has been noted that application of organic manure has a more lasting beneficial residual effect that can remain significant up to four seasons when compared with inorganic fertilizers whose residual benefit do not last beyond season.

Green manure

The typical organic production is characterized by extended rotations involving leguminous crop green manure and organic amendments utilization (Lynch et al., 2008). Canali et al. (2010) noted that supply of nitrogen from the soil, which consist of nitrogen mineralized from organic soil matter and crop residue is an important and variable contributor of nitrogen to potato crop production. Without organic farming, food security will be hampered (Sarker and Itohara, 2010). Legumes are often grown for incorporation into soil as a green manure providing benefits such as off season soil cover, stimulated soil biological activity and improved plant nutrition (Soleymani et al., 2011d). Most interests has been attached to the legume 's ability to furnish subsequent crops with readily available nitrogen (N).

Conclusion

Sustainable agriculture means a shift from monoculture to intercropping. In another word, intercropping means the agricultural cultivation of two or more crops in the same space and at the same time. Sustainable farming also means self-sustaining, low-input and energy-efficient agricultural systems. Biodiversity is the main key and strategy for sustainable agriculture. In intercropping system, there is normally one main crop and one or more added crops, with the main crop being primary importance for food and forage production. The most important aim and advantage of intercropping is to produce a higher yield on a given piece of land by appropriate use of the available growth resources that may not be utilized by each single crop grown alone. There are different types of intercropping but the most important types are, row intercropping, strip intercropping, mixed intercropping and relay intercropping. Intercropping system may lead to soil conservation, improvement of soil fertility, and improvement of forage quality, and reduction of pest and diseases. The intercropping systems are old and widespread applications in low-input agricultural systems, and they were common for so many countries before the modernization of agriculture. There are both direct and indirect facilitative interactions of intercropping systems. Intercropping systems can cause more effective use of resources by providing symbiotic nitrogen from legumes or making available inorganic phosphorus fixed in soil because of lowering of pH via nitrogen fixing legumes. Also, more efficient water usage in intercropping systems was suggested by so many researchers. Intercropping practices are the most productive when intercrops of different growth period are used so that their maximum requirements for growth resources occur at different times. Intercropping is the best way of introducing more biodiversity into agro-ecosystems and results have shown that increased crop diversity may increase the number of ecosystem service provided. It is also the best way to ecological balance, more utilization of resources, increase the quantity and quality of agricultural products and significant reduction damage and lost by pests, diseases and weeds. On the basis of multiple advantages of intercropping especially in the terms of sustainable agriculture and organic farming, it is more clear that intercropping is more reasonable than sole cropping systems. Application of organic and synthetic fertilizers to soil would provide multiple benefits for improvement of soil chemical, physical and biological properties leading to improved crop yield. Integrated use of synthetic and organic fertilizers lead to development of sustainable crop production. Also, this may improve the efficiency of synthetic fertilizers and reduce their usage. Integrated use of organic and synthetic fertilizers is a good method to improve crop productivity and sustain soil quality and fertility. In sustainable agricultural system, fertilizers, livestock manure and cover crops are important parameters in productive agricultural systems to have stable food. In this review study, it is informed about the importance and use of the intercropping system, manure and chemical fertilizers in agricultural production to have a stable and sustainable agricultural production.

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PROSPECTIONS FOR FOOD BASED LURES IN MASS TRAPPING OF *BACTROCERA DORSALIS* ON *CHRYSOPHYLLUM ALBIDUM* IN OSUN STATE, NIGERIA

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Abstract

The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae**)** is an invasive pest in many countries across the globe, including Nigeria. The potentials of three food-based lures in mass trapping of *B. dorsalis* on *Chrysophyllum albidum* were assessed during 2019 fruiting season in three selected villages in Osun state, Nigeria. The food lures included: pineapple juice, orange juice, banana juice, Methyl Eugenol (standard check) and control (water). The lures were baited with cypermethrin applied at 40ml/trap/week, while methyl eugenol was applied at 10 ml/trap/week. Data collected were subjected to ANOVA, and significant means were separated using Turkey's Honestly Significant Difference (THSD). The results showed that *B. dorsalis* was trapped on *C. albidum* in all the study sites. The number of *B. dorsalis* trapped was higher during the ripening period of *C. albidum* in all locations. The percentages of trapped flies after 10 weeks were 70.85% - 79.50% (Methyl Eugenol) >7.86% - 11.09% (Orange juice) > 8.95% - 11.05% (Pineapple juice)> 2.52% - 6.41% (Banana juice)> 0.60% - 1.09% (control) at the study sites. There were no significant differences (P > 0.05) on the density of *B. dorsalis* trapped in all locations. However, the densities of trapped *B. dorsalis* significantly (P<0.05) differed among the different treatments in all location. All the food-based lures significantly (P<0.05) trapped higher flies than control. The food-based lures tested had potential in trapping *B. dorsalis,* hence increased dosage and application frequency could be used to control *B. dorsalis* in homestead trees and home gardens.

Keywords: *Fruit fly, attractants, mass trapping, control, African star apple.*

Introduction

Fruit flies are a prevalent pest in most of Africa; affecting domestic and export fruits exploitation (De Meyer *et al.,* 2010). The *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) fruit fly complex comprises more than 75 species and is one of the crucial pest complexes in global agriculture (Clarke *et al..*, 2005). *B. dorsalis* is a pest regarded as a significant threat for biosecurity among the fruit fly species (CABI, 2015; EPPO, 2015). The species in the complex are highly polyphagous and invasive in nature enabling them to infest a wide range of fruits (Goergen *et al.*, 2011). The pest is known in association with almost 80 host plant species (De Meyer *et al.,* 2012), but African star apple (*Chrysophyllum albidum*) is one the main host of *B. dorsalis in* West Africa. (Goergen *et al.* 2011). African star apple is an indigenous wild fruit tree with enormous potentials for plantation establishment; the fruit/seed could serve as an alternative carbohydrate or energy foodstuff (Okigbo, 1978; Okafor, 1981). The fruits are good sources of vitamins, irons, flavours to diets and raw materials to some manufacturing industries (Adisa, 2000; Bada, 1997; Okafor and Fernandes, 1987; Umelo, 1997) *C. albidum* is reported to be a renewed tree in the agroforestry system and is cultivated in Nigeria, Uganda, Niger, Cameroon, Ivory Coast republics on a commercial scale (Adewusi and Bada, 1997).

The fruit pulp of *Chrysophyllum albidum* contains 21.8 mg/100 g ascorbic acid and the skin contains 75 mg/100 g while Edem and Miranda 2011 reported 446 mg and 239 mg/100 g for pulp and skin respectively (Adesina, 2000; Achinewhu,1983). In Nigeria, children and pregnant mothers consume *C. albidum* ripe fruits, taking a great advantage from.

Production of quality and safe fruits including *C. albidum* has been hampered by the infestation of the fruit flies, especially the *B. dorsalis* since its arrival in Nigeria.

In most parts of Africa, the smallholder farmers, which are responsible for producing the bulk of the fruits, rarely apply any control measures against fruit flies resulting in significant economic losses.

The use of synthetic insecticides for pest control is, conversely, associated with various environmental problems such as contamination, adverse effects on non-target organisms and the development of resistance (Croft, 1990). Female Tephritids require a post-eclosion protein meal for ovarian development and egg maturation (Drew and Yuval 2000). Consequently, fruit fly detection, monitoring, and suppression strategies have relied strongly on the use of food-based attractants baited with various toxicant.

The bait spray strategy significantly reduces the amount of pesticide needed for fruit fly control and has been used successfully in eradication campaigns (Roessler, 1989). According to Mediouni-Ben *et al*., (2010) the use of the mass trapping technique by female-targeted and male-targeted lures can also be included as a component of an Integrated Pest Management (IPM) program for Fruit flies. Therefore, this study evaluates the potentials of three food base attractants or Methyl Eugenol in mass trapping of *B. dorsalis* on *C. albidum* as a management practice.

Materials and methods

Experimental site

The study was carried out in Ife South, Osun state South West Nigeria during the Chrisophyllum fruiting season of 2019. If South is a Local Government Area in Osun State, It has an area of 730 km² located within latitude 7°11′00″N and longitude 4°42′00″E (GMT) with annual rainfall from about 1.300 mm to 1.500 mm, average relative humidity of about 80 to 85 %, and average yearly temperature of 26.2 °C. Three local sites were selected for the study, and they include; Akerodolu, Agodi and Orisunbare

Preparation of food-based bait:

Pineapple, orange and banana food bait were prepared by peeling 1kg of each the fruit and blending it into a smooth slurry paste using an electric kitchen blender. The juices were extracted separately with 1 litre of water for each and sieved with muslin cloths to obtain a homogenous solution as described by Ugwu *et al.*, (2018). The banana paste was prepared by blending 1 kg of banana with 500 ml of water. The prepared samples were refrigerated until when used.

Experimental setup

Lynfield trap (LT) was used to trap the flies. Lynfield trap is a bucket type trap composed of a cylindrical plastic container with four equidistant holes on the upper third, and the lid of the trap contains a hook to which an ME dispenser such as Invader Lure must be fitted (Copeland, 2012) There were five treatments including control; they include Methyl Eugenol, Pineapple juice bait, Orange juice bait, Banana paste and control (water). Cypermethrin (2 ml) was added to each attractant to knock down the trapped flies.

Placement of traps

Three trees were selected from each location at a distance of 10 m from each other to obtain three independent replicates per site. Five traps were hung per tree in all the sites. Forty milliliters of prepared food baits were taken with the aid of 10 ml injection syringe and carefully dropped on 0.5 gm of absorbent cotton wool and placed at the bottom of the trap while twenty milliliters of Methyl Eugenol were used following the same procedure. Each lure was replicated three times per location in a Complete Randomized Block Design (CRBD) using each tree as a sampling unit. Five traps were hung on each tree at 10 m above the ground within the tree canopy (Ugwu *et al.,* 2018)

Data collection and analysis

Data were collected on the number of fruit flies trapped per trap every week in all the locations for ten weeks consecutively. The trapped flies were taken to the laboratory for counting, identification and sexing. Data collected were transformed using square root transformation (X + 0.5), then subjected to ANOVA and significant mean separated at 5% level using THSD.

Results and discussion

Effects of treatments on the density of *Bactrocera dorsalis* trapped at Akeredolu

The number of *B. dorsalis* trapped at Akeredolu village is shown in Table 1

The result showed that Methyl Eugenol was the most effective attractant for *B. dorsalis* at Akeredolu village. It recorded a mean population density of 65.62 (79.50%) of adult *B. dorsalis* per trap after ten weeks. Methyl Eugenol was followed by pineapple juice bait with a mean density of 7.41 (10.17%) per trap at ten weeks. The weekly catches of flies by the lures per trap revealed that Methyl Eugenol significantly (p<0.01) trapped the highest number of flies throughout the period of the study. The number of trapped *B. dorsalis* increased with the progress of fruit ripening, and a peak was on the 9th week of the experiment. A higher population of flies were recorded in correspondence of *C. albidum* fruit ripening from 7 to 10 week in February 2019

Table 1. The Population Density of *B. dorsalis* Trapped on *C. albidum* at Akeredolu Village

Treatments	1	2	3	4	5	6	7	8	9	10	Mean
Methyl eugenol	4.57a	4.06a	2.72a	2.94a	5.11a	7.23a	9.19a	9.70a	10.17a	9.93a	65.62a
Orange juice	0.33b	0.80b	1.28ab	0.00b	0.58b	0.82b	0.33b	0.00b	0.00b	2.35b	6.49b
Pineapple juice	0.67b	1.14b	1.52ab	0.67b	0.47b	0.67b	0.00b	0.47b	0.00b	1.80b	7.41b
Banana	0.67b	0.00b	0.00b	0.47b	0.00b	0.47b	0.00b	0.47b	0.00b	0.00b	2.08c
water	0.00b	0.00b	0.00b	0.00	0.00b	0.00b	0.00b	0.94b	0.00b	0.00b	0.94d
Sig. level	**	**	*	**	**	**	**	**	**	**	**

Means followed by the same letter within the column are not significantly different, *= significant at 0.05 level of probability; **= significant at 0.01 level of probability by Turkey's test

Effects of treatments on the density of *Bactrocera dorsalis* trapped on *C. albidum* at Orisunbare Village.

The number of *B. dorsal*is trapped on *C. albidum* at Orisunbare village is shown in Table 2

The population density of *B. dorsalis* flies recorded at Orisunbare village followed a similar trend with that of Akeredolu village. The highest population of *B. dorsalis* were trapped on Methyl Eugenol with a mean occurrence of 65.92 (73.58%) per trap in 10 weeks. Methyl Eugenol was followed by orange juice bait with a mean number of 9.33(10.41%) per trap in 10 weeks. Methyl Eugenol significantly (p<0.01) caught a higher number of *B. dorsalis* flies from week one to week ten. Orange juice bait trapped a higher number of *B. dorsalis* than other food-based attractants, although it did not differ significantly with the population trapped by pineapple juice bait. All the food-based lures significantly (p<0.01) caught a higher number of flies than control (water). Higher densities of *B. dorsalis* flies were trapped during the peak of *C. albidum* fruit ripening from week 7-10 in February.

Table 2. The population density of *B. dorsalis* Trapped on *C. albidum* at Orisunbare Village

Treatments	1	2	3	4	5	6	7	8	9	10	Mean
Methyl eugenol	4.23a	3.77a	3.78a	4.50a	5.22a	4.66a	10.25a	7.10a	11.63a	10.78a	65.92a
Orange juice	1.49b	1.14b	0.00b	0.00b	0.67b	0.00b	0.33b	0.58b	0.00b	5.12b	9.33b
Pineapple juice	1.14b	0.80b	0.00b	0.00b	1.05b	0.00b	0.33b	0.58b	0.00b	4.20b	8.02b
Banana	0.33b	0.33b	0.00b	1.27b	0.82b	0.81b	0.00b	1.94b	0.00b	0.00c	5.50c
water	0.00b	0.47b	0.00b	0.00b	0.00b	0.33b	0.00b	0.00b	0.00b	0.00c	0.80d
Sig. level	**	**	**	**	**	**	**	**	**	**	**

Means followed by the same letter within the column are not significantly different, **= significant at 0.01% level of probability by Turkey's test

Effects of treatments on the density of *Bactrocera dorsalis* trapped on *C. albidum* at Agodi Village

The population density of *B. dorsalis* tapped by the different attractants at Agodi village is shown in Table 3. Methyl eugenol significantly ($p<0.05$) recorded the highest number of *B. dorsalis* flies throughout the study period with a mean density of 79.30 (70.85 %) per trap in 10 weeks, followed by orange juice bait with a mean value of 12.41 (11.09%). There was no significant ($p>0.01$) between the orange juice bait and pineapple juice bait on the density of flies trapped in this location. However, all the food-based attractants significantly($p<0.01$) stuck a higher number of flies than control. *B. dorsalis* density was similarly higher from 7th – 10th week (February) during the study in this location.

Table 3. The population density of *Bactrocera dorsalis* trapped on *C. albidum* at Agodi village

Treatments	1	2	3	4	5	6	7	8	9	10	Mean
Methyl eugenol	6.71a	5.86a	5.06a	5.67a	5.77a	7.88a	10.30a	8.21a	11.97	11.87a	79.30a
Orange juice	2.20b	0.67b	0.00b	0.94b	0.00b	0.00b	2.00b	1.45b	0.00b	5.15b	12.41b
Pineapple juice	0.80b	0.67b	0.00b	1.22b	1.39b	1.74b	0.91b	1.32b	0.00b	4.32b	12.37b
Banana	0.33b	1.15b	0.67	1.15b	0.00b	0.00b	1.82b	2.05b	0.00b	0.00c	7.17c
water	0.00b	0.67b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.67d
Sig. level	**	**	*	**	**	**	**	**	**	**	*

Means followed by the same letter within the column are not significantly different,*= significant at 0.05 level of probability; **= significant at 0.01level of probability by Turkey's test.

Percentage population of *B. dorsalis* trapped on *C. albidum* at the three sites of the study areas

Bactrocera dorsalis were trapped in all the three study sites selected for the study (Fig. 1) There were no significant differences ($p \geq 0.05$) on the population density of *B. dorsalis* trapped in all the locations. However, the highest percentage of *B. dorsalis* flies were caught at Agodi village (39.40%), followed by Orisunbare village (31.54%) while Akeredolu village trapped the least density of *B. dorsalis* flies (29. 06%)

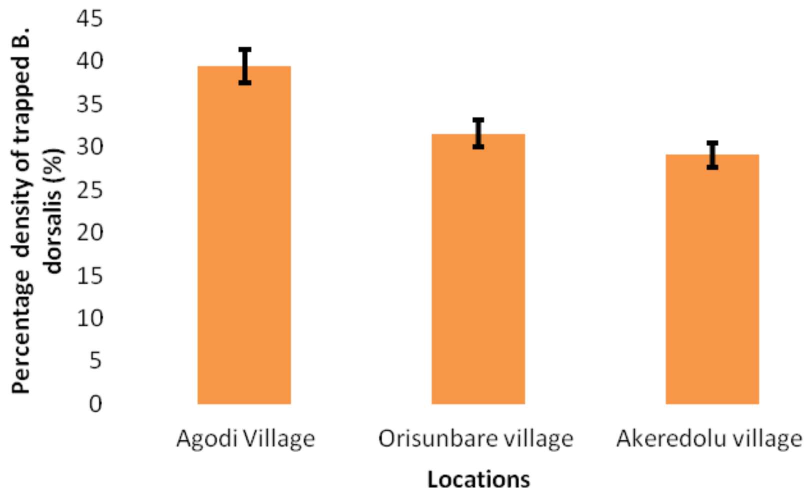

Fig. 1. **Mean percentage density of *B. dorsalis* trapped on *C. albidum* at the three study sites**

Percentage density of female and male *B. dorsalis* trapped on *C. albidum* trees at the three study sites.

Female and male *Bactrocera dorsalis* were trapped in all the study sites (Fig. 2)

Methyl Eugenol trapped only males *B. dorsalis* at the three study sites while food-based lures and control trapped both female and male flies. The percentage density of male *B. dorsalis* trapped by Methyl Eugenol was significantly ($p<0.05$) higher than other treatments All the food-based lures significantly ($p< 0.05$) caught a higher number of females than male *B. dorsalis*.

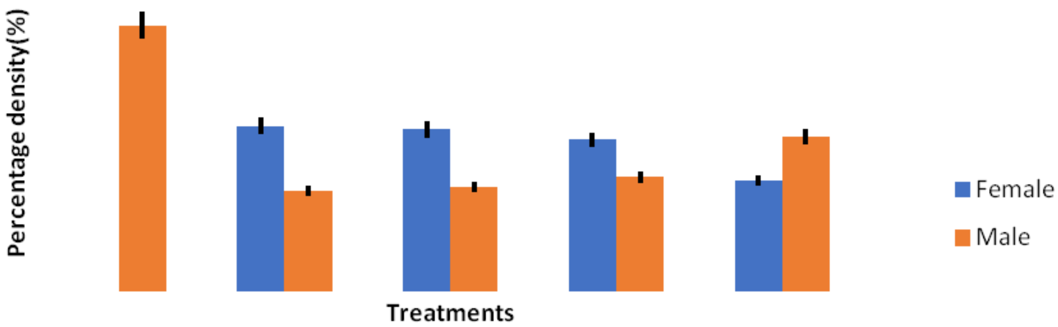

Fig.2. Percentage density of female and male *B. dorsalis* trapped at the three study sites

The study has shown that all the food-based lures evaluated attracted adult *B. dorsalis.* Although there were disparities in the level of attraction by different lures assessed. The results support the earlier findings by Vargas *et al*. (2003) that reported the type of protein in a food-based bait could influence the attractiveness of the lure to fruit flies.

Methyl Eugenol acts as a para pheromone attracting more *B. dorsalis* flies than the food-based lures did. Moreover ME trapped only male flies while food based lures were moderately effective and trapped both male and female *B. dorsalis* This report supports the earlier report by Russell Messing (1999) that parapheromone lures (methyl eugenol, cue-lure, ceralure, trimedlure and latilure) attract males only and every fruit fly species in Hawaii is attracted to a different kind. Methyl Eugenol has earlier been reported to be very effective in mass trapping *Bactrocera* spp. in mango and established to be the most effective technique among the various fruit fly management strategies tested using ME trap plus Bait (Ishaq *et al.* (2004), Stonehouse *et al.* (2005) and Jiji *et al.* (2009).

Correspondingly, Ekesi *et al.* (2014) reported that Methyl Eugenol is a male annihilation lure for *B. invadens*, and it attracts only males. Ugwu and Shuaib, (2018) and Ugwu *et al*., (2018) also reported that methyl eugenol trapped only male *B. dorsalis* while food-based attractants caught both sexes on guava and mango homestead trees

Bactrocera dorsalis were trapped on *C. albidum* trees in all the locations during the study, indicating that *C. albidum* is among the most suitable hosts of *B. dorsalis* complex as reported by Goergen *et al.* (2011) where he listed *C. albidum* among a wild host of *B. dorsalis* complex. In correlation to *B. dorsalis* host rang*e*, it has also been reported that the host range of *B. dorsalis* in Africa consists of more than 72 plant species of both wild and cultivated crops spread across 28 families (Goergen *et al.* (2011; Umeh and Onukwu, 2016)

Protein source is a significant constituent in the food baits, and commercial lures have been used to trap *B. cucurbitae* (Fabre *et al*., 2003) and *B. dorsalis* (Alyokhin *et al*., 2000; Cornelius *et al*., 2000)

Conclusion

This study has contributed to the knowledge about the presence of *B. dorsalis* in Osun state Nigeria. *C. albidum* has proved to be one of the major wild hosts of *B. dorsalis*, and food-based lures have shown potential for trapping of *B dorsalis.* Thus, the study is vital for the development of efficient management strategies against fruit flies, hence increased dosage and application frequency could be used to manage *B. dorsalis* infestations in homestead trees, orchards and home gardens. The study also confirmed the selective and robust response of *B. dorsalis* to Methyl Eugenol and food-based attractants and their ability to infest and develop in *C. albidum* fruits.

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PHYTOCHEMICAL CHARACTERISTICS OF DIFFERENT MALTS AND POSSIBILLITIES FOR THEIR APPLICATION IN FUNCTIONAL BEVERAGES

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Abstract

The development of new assortments of beverages with high biological value and functional effect on human health is a new trend in the industry. Therefore, malt, a major raw material in brewing, is of particular importance. Malt has a high biological value and some of its components (phenolic compounds, catechins, ferulic acid, and etc.) have a high antioxidant capacity. The aim of this study was to investigate the phenolic content and antioxidant capacity of 8 malt types (2 Pilsner, 2 Vienna, 2 Wheat, 1 Munich and 1 Pale ale) and wort produced from them. The total phenolic content, phenolic acid and flavonoid phenolic compounds were determined. The total phenolic content varied between 0.88 and 6.66 mg GAE/g dw for malt and 263.57 and 412.65 mg GAE/L for wort. The results for phenolic acids and flavonoid phenolic compounds were almost equal both for malt and wort. The antioxidant activity was determined by the radical scavenging assay (DPPH) and ferric reducing antioxidant power (FRAP). The DPPH radical scavenging activities varied from 1.21 to 9.31 μM TE/g dw for malt and 492.39-6105.68 μM TE/L for wort, respectively. The results from FRAP assay ranged from 1.35 to 28.56 μM TE/g dw for malt and between 192.08 and 473.76 μM TE/L for wort, respectively. The results obtained were used for a discussion on the possibilities for the production of wort-based functional beverages.

Keywords: *malt, phenolic compounds, antioxidant activity, functional beverages*

Introduction

Beer is the oldest and one of the most consumed alcoholic beverages in the world. It is a natural product prepared from four main ingredients (malt, hops, water and yeast), each of which has a number of benefits for human health (Carvalho et al., 2016).

Malt, which is made mostly from barley through a malting process, is essential for the beer quality. It provides a certain amount of starch, as well as contributes to the organoleptic characteristics and colour of the beer. Malt plays a major role in the oxidative stability of beer, as its various components have antioxidant capacity. Malt can contribute to about 95% and 86% of the antioxidant capacity of dark and pale beers, respectively. The antioxidant capacity of malt is also important for human health by protecting against various diseases - cancer, nervous and cardiovascular diseases (Cechovska et al., 2012; Vanderhaegen et al., 2006; Landete 2013; Carvalho et al., 2016). The antioxidant capacity of malt is not only due to the barley polyphenols but also to a number of components generated during kilning as reductones and Maillard reaction products (MRPs) (Rivero et al., 2005; Carvalho et al., 2016). Different phenolic compounds have been identified in barley and malt, including flavan-3-ols, proanthocyanidin oligomers, hydroxycinnamic acid derivatives, and low amounts of flavonols. The amount of some of them changed during malting but others withstand the process (Dvorakova et al., 2008; Leitao et al., 2012; Carvalho et al., 2016). The higher antioxidant activity of malts was mainly attributed to the formation of MRPs upon heating, which is positively correlated with their color (Chandra et al., 2001; Coghe et al., 2003; Carvalho et al., 2016).

The aim of this study was to investigate main brewing characteristics and antioxidant capacity of different malt types. The results obtained were used for the selection of methods of modeling the mixture composition which will be used for production of wort-based functional beverages.

Material and Methods

Malts

In the present work, eight different malts, produced 2017-2018, were used. Four of them were produced by Weyermann®, Germany - Pilsner, Vienna, Wheat, and Munich 1, (indicated with W) and the other four were produced by Best Malt, Germany - Pilsner, Vienna, Wheat, and Pale Ale (indicated with BM).

Reagents

Folin-Ciocalteu reagent, gallic acid, caffeic acid, quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-*s*-triazine), $FeCl_3 \cdot 6H_2O$, and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased by Sigma-Aldrich. Hydrochloric acid was purchased by Merck, Germany. All the other reagents were of analytical grade.

Main brewing characteristics of malts

The main brewing characteristics of malts (Table 1) were determined according to standard methods of European Brewery convention (Analytica EBC, 2007).

Extraction procedures

Malt extracts were produced by mixing 10 g of malt and 40 mL of 80% (v/v) methanol. After staying overnight the mixture volume was made to 50 mL with methanol, homogenized, filtered (Whattman No.1) and stored at -20 ºC. If it is necessary, extracts were diluted properly with purified water before analyses. Wort was produced by Congress mashing (Analytica EBC, 2007) and stored at -20 ºC before use. Wort was diluted with methanol in proper ratio, left for 30 minutes and filtered (Whattman No.1).

Phenolic content of malt and wort

The content of total phenolic compounds was determined by the Folin–Ciocalteu (FC) method, as described by Dvořáková et al. (2008) with slight modifications. 1 mL of sample, 4 mL of Folin-Ciocalteu working solution, 5 mL of sodium carbonate (7.5%, w/v) were introduced into a test tube. This solution was agitated and left to stand for 1 h. The absorbance at 765 nm was determined in a Shimadzu UV-VIS1800 spectrophotometer (Kyoto, Japan) against blank sample prepared with purified water. The calibration curve was performed with gallic acid, and the results are expressed as mg of gallic acid equivalents (GAE)/g dw for malt and mg GAE/L for wort. The total phenolic compounds, total phenolic acids and total flavonols were determined by modified Glories method as described by Mazza et al. (1999). Briefly, the sample (1 mL) was pipetted in a test tube and 1 mL 0.1% HCl in 95% ethanol (v/v) and 18.2 mL 2% HCl (v/v) were added. The solution was thoroughly mixed and allowed to stand for approximately 15 min before reading the absorbance with a spectrophotometer against blank sample prepared with purified water. The absorbance (A) at 280 nm was used to estimate total phenolic content, $A_{320\ nm}$ was used to estimate phenolic acids, and $A_{360\ nm}$ was used to estimate flavonols. The calibration curves for total phenolic compounds, total phenolic acids and total flavonols were constructed by using gallic acid, caffeic acid and quercetin as standard, respectively.

Antioxidant capacity of malt and wort

The antioxidant activity of malt and wort was measured by DPPH method as described by Dinkova et al. (2014) with minor modifications. Briefly, 250 µL of sample were added to a

2.25 mL DPPH solution in methanol (6×10^{-5} M); the mixture was left for 15 min (kept in the dark at room temperature) so that a reaction could take place, and then the absorbance at 517 nm against blank sample with purified water was read. The control sample was made with methanol. The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2\cdot3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3\cdot6H_2O$ solution. The fresh working solution was prepared by mixing acetate buffer, TPTZ solution, and $FeCl_3\cdot6H_2O$ solution in ratio 10:1:1. Extracts (150μL) were allowed to react with 2850 μL of the FRAP solution for 4 min in the dark condition. Readings of the coloured product were then taken at 593 nm against blank sample prepared with methanol. For both of the analysis for antioxidant activity the results were determined from a calibration curve using Trolox as standard and the results were expressed as μM Trolox equivalents (TE)/g dw for malt and μM TE/L for wort.

Statistical analysis

The results of all analysis were expressed as the mean ± the standard deviation of three replicates.

Results and Discussion

Main brewing characteristics of malts

The results of the analyses of main brewing characteristics of malt are summarized in Table 1.

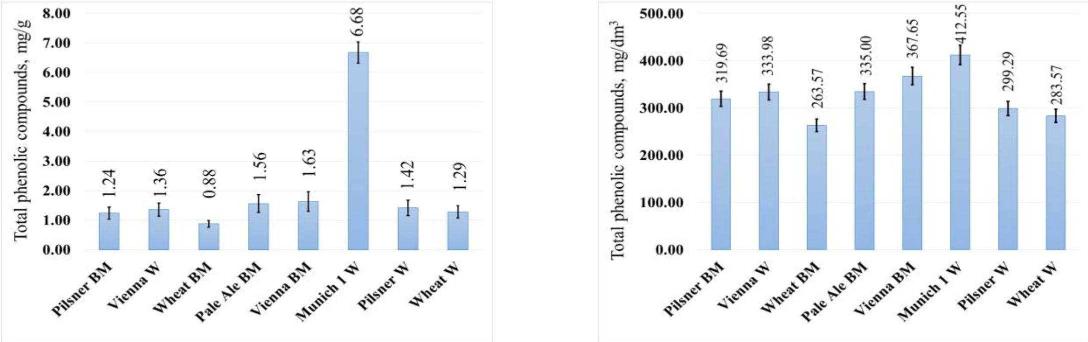
Table 1. Main brewing characteristics of malts

Malt type	Moisture, %	Extract of wort,°P	Extract of malt, %	pH of wort	Color of wort, EBC units
Pilsner W	5.20±0.13	8.69±0.05	80.83±0.58	5.73±0.02	3.2±0.3
Pilsner BM		8.42±0.05	78.09±0.69	5.72±0.02	3.3±0.3
Vienna W		8.50±0.05	78.85±0.61	5.75±0.02	8.1±0.5
Vienna BM		7.75±0.02	71.38±0.85	5.65±0.02	8.0±0.5
Wheat W		8.12±0.03	75.09±0.71	5.6±0.02	5.3±0.4
Wheat BM		8.07±0.03	74.57±0.73	5.62±0.02	5.1±0.4
Pale Ale BM		8.18±0.03	75.70±0.71	5.65±0.02	6.5±0.4
Munich 1 W		8.79±0.05	81.90±0.61	5.63±0.02	12.5±0.7

The results for malt analyzes showed that all malts could be used up to 100% for the production of the different beer types. Wort extract ranged from 7.75 °P to 8.79 °P, which corresponded to malt extract of 71.38% to 81.90%. The pH of wort obtained was relatively high - about 5.6-5.7 units. The color of the wort obtained varied within the range of 3.2-12.5units, depending on malt types.

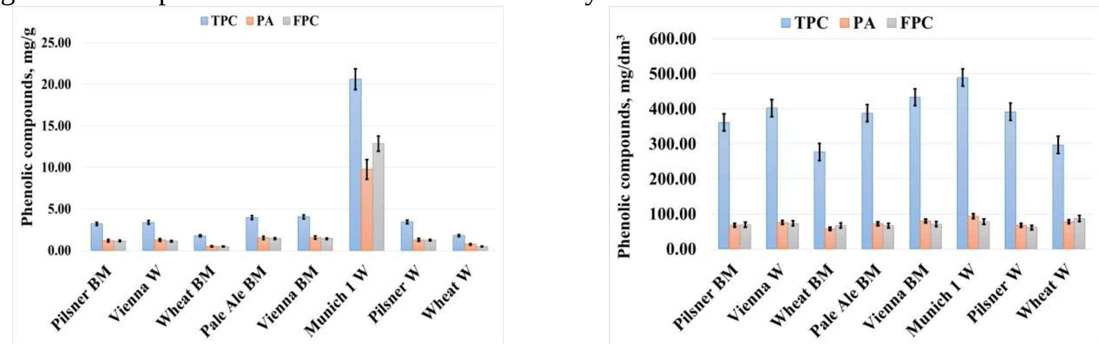
Biological value of malts

The biological value of malts was determined by three main indicators: phenolic compounds content, DPPH radical inhibition and FRAP method. The phenolic compounds content of malt and wort is shown in Figure 1 (determined by FC reagent) and Figure 2 (determined by the modified Glories method). The phenolic compounds in malt determined by FC reagent varied from 0.87 mg/g dw to 1.56 mg/g dw for pale malt types, while the dark malt Munich 1 had a content of 6.66 mg/g dw. The results for total phenolic compounds determined by the modified Glories method were about 3 times higher, as the presence of both oxidized and non-oxidized phenolic compounds was taken into account. Interestingly, the highest results for the phenolic compounds content in Munich 1 were likely to be due to the higher kilning temperature that provoked the release of a portion of the cell wall-bound phenolic compounds or their polymerization (Carvalho et al., 2016).



a) malt b) laboratory wort

Figure 1. Total phenol content in malt and laboratory wort



a) malt b) laboratory wort

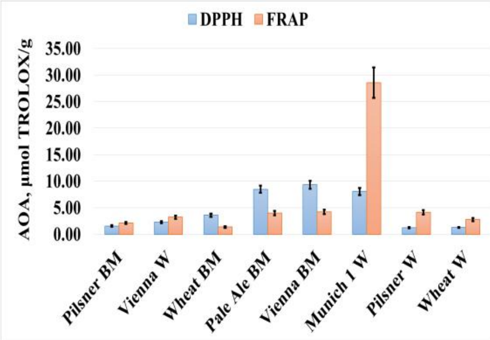
TPC – total phenolic compounds; PA – phenolic acids; FPC – flavonoid phenolic compounds

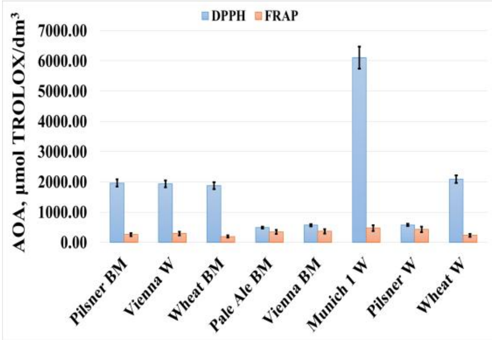
Figure 2. Phenolic compounds in malt and wort

The data for total phenolic compounds in wort in Figure 1 and Figure 2 did not show significant differences. It can be explained that during mashing a part of the phenolic compounds remained bound to the cell walls because of rapid inactivation of enzymes at higher temperatures than 45 ºC (Wannemacher et al., 2018). The data in Figure 2 show almost equal content of phenolic acids and flavonoid phenolic compounds in malt and wort, respectively. The results confirmed the observations made by Szwajgier (2009) that free phenolic acid content in wort was significantly lower than the corresponding free phenolic acid content in malt. The results for antioxidant activities of malt and wort are presented in Figure 3. The data shows that malt had a high antioxidant capacity, which increased with the increase in time and/or kilning temperature. The lowest antioxidant capacity had Pilsen and wheat malts because of relatively low formation of melanoidins which together with phenolic compounds contribute to the malt antioxidant capacity. The highest activity showed Munich 1. Therefore, it can be suggested that the increase in the malt colour (Table 1) had a positive correlation to the antioxidant capacity. During mashing, the components that determine the antioxidant capacity of the malt are extracted into the wort. Here, however, they undergo changes that negatively affect the biological value, as part of the phenolic compounds form precipitates with the proteins. This was the main reason for the observed reductions in the antioxidant activity in some of the laboratory wort (Figure 3b). The low formation of melanoidins during mashing led to the slight increase in the antioxidant capacity in some of the variants. It is interesting to note that all the laboratory wort had greater capacity with respect to the inhibition of the DPPH radical compared to the ferric reducing ability. Again, the highest antioxidant potential showed wort produced by Munich 1 malt.

Possibilities for the production of functional wort-based beverages

The production of new types of beverages requires a systematic approach that combines the knowledge of the process technology and the data on the biological activity of the various malts. Therefore, the best approach is to use a planned experiment for modelling the mixture composition. Mixture design is a very effective method of determining the proportions of variables (ingredients) of a blend. The output varies depending on the proportions but the total remains constant as 1(Buruk Sahin et al., 2016).

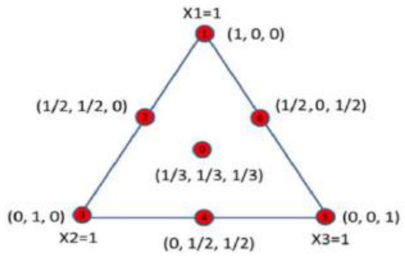


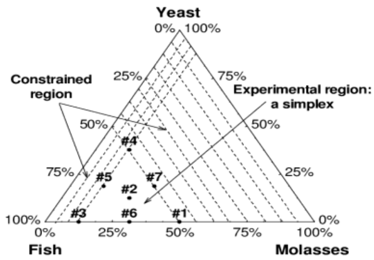


a) malt б) laboratory wort

Figure 3. Antioxidant activity of malt and laboratory wort

A simplex-centroid method with and without constrain is an interesting variant (Figure 4). The method without constrain is suitable for mixtures with 3 or 4 components. The increase in ingredients requires a reduction in the number of attempts, which results in constrains to be used. Constrains may be not only mathematical. In brewing, the main malt used is pale barley malt, which amount has to be over 40%. On the contrary, the wort obtained with higher portions of special malts, which also possess high biological activity, will be very viscous and difficult for lautering. On the other hand, the increase in some special malts portion leads to the problems with beer sensory profile. Therefore, in this study the biological potential of a group of pale and low-coloured malts was investigated and they will be used as the basis for mixtures design. In this case, these malts will be over 40% of the mixtures composition and the other special malts having high biological potential will be limited to such quantities that simultaneously provide a high antioxidant capacity of the beverage and allow the production in a semi-pilot and pilot scale.





a) without constrains b) with constrains

Figure 4. Simplex-centroid method for mixture design

Conclusions

The biological value of eight malts, which can be used up to 100% for brewing, was determined by three main indicators: phenolic compounds content, DPPH radical inhibition and FRAP method. The results showed that the antioxidant capacity increased with the increase in time and/or kilning temperature of malt. Therefore, the increase in the malt colour had a positive correlation to the antioxidant capacity. The results obtained will be used for the mixture design. The mixture will include over 40% of pale and low-coloured malt and the other malts will be dark (specialty) malts with high antioxidant activity.

Acknowledgement

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CONTROL TRIALS OF ANABOLIC HORMONE RESIDUES IN TISSUES OF WILD AND FARMED NILE TILAPIA IN EGYPT

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Abstract

Due to the excessive use of growth promoters in fish production and its possible hazards for humans, our study focusses on monitoring and control attempts regarding their residues in Nile tilapia fish. A total of eighty random samples of Nile tilapia were collected from different Nile canals and markets in El-Menofia governorate, Egypt, for estimation of Methyltestosterone (MT) and Trenbolone acetate (TB) residues by using enzyme-linked immune sorbent assay (ELISA). In the present study, the MT and TB hormone residues were in acceptable levels and without exceeding the maximum permissible limits MPLs (2ppb) of codex (2007) and European Commission "EC" (1999), respectively; except MT residues level in small size farmed tilapia; as 49 % of samples were unacceptable and exceeded MPLs of codex (2007). These results provided no proof for illegal hormones use but did not exclude the possible misuse of hormones. Routine monitoring of these hormones as a food quality and health control measure is needed. Application of various cooking methods (frying and grilling) on Nile tilapia of each category (n =3) exhibited that cooking methods positively reduced residues of MT and TB. The obtained results revealed that the most effective cooking methods for reducing the levels of such hormone residues were grilling (78.8% and 82.05% for MT and TB, respectively) followed by frying (34.8% and 53.85% for MT and TB, respectively).

Key words: *Nile tilapia, methyl testosterone, trenbolone acetate***,** *frying, grilling.*

Introduction

Hormones are common additives, to increase meat production by stimulating the protein synthesis and improving the feed conversion. The most serious potential hazards arising from using of anabolic steroids are tissue residues of these substances and their metabolites, where the effect of these residues is greater on human as it can cause early puberty for girls and boys, liver tumors, cell carcinoma and increase embryo mortality. Also, long-term consumption of anabolic hormones can lead to breast and ovarian cancer. Many countries' regulations clearly define residual limits for these compounds in food. The most common compounds used are the natural anabolic agents(testosterone) and the synthetic anabolic agents (17α-methyl testosterone and trenbolone acetate) which aremale sexual hormones, in low doses also present in females, promote bone/muscle growth and anabolic effects. They are synthetically produced anabolic and androgenic steroid hormones. Ministerial Decree NO. 2655/2003: Prevents the use of"testosterone hormone" in hatcheries for its harm onhealth. Hormones as growth promoters was prohibited for aquaculture in the EU with a strict and limited exceptional use for therapeutic and breeding uses. In other countries such as the US, Canada, Brazil, Australia and New Zeeland hormones are commonly used legally in stock farming (Zhai, et al., 2009 and Commission Regulation, 2010). The determination of anabolic steroid residues faces some difficulties due to their very low concentration in muscle especially after illegal application and their short half life time. So, it needs a powerful, sensitive and efficient method for detection as ELISA (XU et al., 2006). Fish is often treated by various cooking methods before consumption. The cooking process produces fish with desirable sensory qualities, while also minimizing nutrient loss and ensuring the destruction of microbial pathogens. During cooking, the heat treatment and evaporation of water affect the physical and chemical changes in fish, and therefore digestibility is increased due to protein denaturation (Asmah et al., 2014). Cooking processes (frying and grilling) generally decreased the bio-accessibilities of trace or toxic elements or residues. So, fish may be eaten raw, boiled or steamed to improve nutritional values for essential elements and may be eaten fried and grilled to diminish the toxicity and health risk from toxic elements (He et al., 2010). Due to the excessive use of growth promoters in fish industry. Our study focusses on firstly, monitoring of testosterone and trenbolone hormone residues to figure out if these residues fall in the accepted MRLs; and secondly measuring the effect of various popular cooking methods in reducing or elimination of such residues in meat. Moreover, selecting the ideal cooking method for effective reduction of hormone residues in fish prior to consumption.

Materials and Methods

Determination of hormone residues (Manual kits ELISA R-Biopharm AG, Darmstadt, Germany):

Collection of fish samples: Eighty fillet samples of Nile tilapia (farmed and wild, 40 samples of each, 20 large sized fish (205:275g) and 20 small sized fish (90:125g) for each group) were collected from different Nile canals and markets in Al-Menoufia governorate, Egypt, for determination of their contents of hormonal residues level (Methyl testosterone and Trenbolone acetate)based on wet weight (mg/kg). The samples were transported to the laboratory at about 4°C.

Preparation of samples: Skin and scales were removed from fish, and ten grams of the ground muscle was homogenized with 10ml of 67 mM Phosphate buffer (pH 7.2) by mixer for 5 min.

Extraction of samples: Two grams of homogenized sample were mixed with 5ml of tertiary butyl methyl ether (TBME) in acentrifugal screw cap vial and shaken vigorously by shaker for 30-60 min. The contents were centrifuged at 3000 rpm for 10 min. The supernatant was kept and the extraction with TBME was repeated. The supernatants were combinedand evaporated by N_2 evaporator then the dried extract was dissolved in 1mLof 80% methanol. The methanolic solution was diluted with 2ml of 20 mM Phosphate buffer and applied to a RIDA C18 column (solid phase extraction column with C18 end-capped sorbent of an average particle size of 50µm**)** for filtration of the samples then the filtrate was used in ELISA kit.

Test procedures were done according to the chart enclosed in the kits of RIDA^{R} and RIDS screen. R is register trademarks of R-Biopharm AG. Manufacture: R-Biopharm AG, Darmstadt, Germany. R-Biopharm AG is ISO certified.

Application of Heat treatment (Experimental Part): The main purpose of the present work is to investigate the effects of certain common cooking methods (grilling for 15 min. and frying for 10 min.) on the concentration and stability of the hormones in the positive samples. Accordingly, 3 positive samples of fish containing low, medium (around permissible limit) and high concentrations of Methyl testosterone and Trenbolone acetate were subjected to the frying and grilling methods of cooking used at home. Sample weighing 10 g, with thickness 2.5 cm and core temperature of 71.5°C were used. Accordingly, heat treatments as frying in neutral oil at 190°C for 10 minutes and grilling for 15 minutes were applied on the positive samples which proved to be polluted by such hormonal residues to determine the efficacy of each cooking method on the stability of the such dangerous residues (El-Bagory et al., 2017).

Statistical Analysis: The results were statisticallyevaluated by application of student t-test according to Feldman et al. (2003).

Results and Discussion

Methyltestosterone: Fish should be safe and without harmful substances for human health. Anabolic agent used for various purposes in animal husbandry tend to leave residues and this causes some problems in consumer health (Nazli et al., 2005). MT hormone was detected in all samples of wild and farmed Nile tilapia, in which the mean concentration of MT hormone was 0.96±0.08 ppb and 1.52±0.14 ppb in small and large size wild Nile tilapia respectively. On the other hand, the mean concentration of MT hormone was 3.48±0.27ppb and 1.93±0.19 ppb in small and large size farmed Nile tilapia respectively (table 1). These results were agreed with that obtained by Hemmat et al. (2015) and Ezzat (2015) who found the MTmean value 2.057±0.200 ppb and 2.06±0.20 ppb respectively, by using ELISA. Our results were higher than that obtained by Hegazy (2007) and Marzouk et al. (2016) who found the mean testosterone value 0.535±0.03 ppb and 0.753 ppb respectively. On the other hand, higher results were obtained by El-Asaly (2004) and El-Neklawey et al. (2009) who found the meantestosterone value 4.22±1.1 ppband 6.946 ppb respectively, in tilapia farmed fish. The present study takes testosterone level in wild tilapia as a guide to determine the hazard concentration of testosterone in farmed tilapia.

Table (1): Statistical analysis and acceptability of MethyltestosteroneTrenbolone acetate levels
(ppb) in theexaminedsamples of wild and farmed Nile tilapia (n=20).

Size / Fish meat	Small		Large +		°†Acceptability %			
	Mean ± S.E*		Mean ± S.E*		Small		large	
	MT	TA	MT	TA	°MT	†TA	°MT	†TA
Wild Nile tilapia+	0.96 ± 0.08	0	1.52 ± 0.14	0	100	100	100	100
Farmed Nile tilapia	3.48 ± 0.27	0.37 ± 0.03	1.93 ± 0.19	0.18 ± 0.01	51	100	100	100

S.E* = standard error of mean + = Significant differences (P<0.05) as indicated by student t-test. °MPL of Methyltestosteroneaccording to codex (2007) is 2 ppb.
†MPL of Trenbolone according to European Commission "EC" (1999) is 2 ppb.

MT hormonedetected in ourwild tilapia may attributed to normal hormone secreted by fish glands. Also, the MT hormone detected in farmed tilapia may attributed to widely use of synthetic MT in fish production in Egypt (Hegazy, 2007). We must put in our mind that wild Nile tilapia samples have male fish (high testosterone) and female fish (low testosterone) but farmed Nile tilapia samples have nearly all male samples. So, the present study suggests that no public health hazard from consumption of large size farmed Nile tilapia agreeing with Pandian and Kiran Kumar (2003) and Rizkalla et al. (2004) who concluded that no potential hazards from fish feed MT as fries. Long food consumption with MT and TA hormone residues may lead to chronic diseases including breast and prostate cancer, thyroid disease, obesity and diabetes, endometriosis, uterine fibroids, and infertility (Aksglaede, et al., 2009).
Trenbolone acetate: it was not detected in wild Nile tilapiabecause it is a synthetic anabolic hormone added only on fish farms for their benefit of all-male and anabolic properties; while the mean concentration value of TA hormone in farmed Nile tilapia was 0.37±0.03ppb and 0.18±0.01 ppb in small and large size tilapia samples respectively (Table 1). However, all farmed Nile tilapia are accepted and not exceed the MPL of trenbolone stipulated by EC (1999). All wild and farmed Nile tilapia were accepted and fit for human consumption

according to the EC MPLs (Table 1). This result agreed to some extent with results obtained by Hemmat et al., (2015) and Ezzat (2015) who found the mean value of TB as 0.227±0.007ppb and 0.23±0, respectively. TA hormonal residues in tilapia may be attributed to wide use of synthetic androgen (Hotchkiss and Nelson, 2007). Our results proved that hormones residues in small farmed tilapia have high hormonal residue than large one, so they should rear hormone treated tilapia fries to adult size for at least five months to ensure zero hormone residue remains in fish.

Effect of different cooking methods on hormones residues in fish: Different ways of heat treatment is applied to fish in to enhance their flavor, taste and to increase their shelf-life (Oluwaniyi and Dosumu, 2009).Therefore, use of proper cooking processes that have a higher temperature and longer time can lead to the most reduction of residues and can provide an additional safety margin for consumer (El-Bagory et al., 2017). The effect of heat by different cooking methods (frying and grilling) on level of testosterone hormone residues of tilapia flesh proved to be clearly reduced in a ratio of 34.8% with frying from (in three trails). While, by grilling, the level of testosterone hormone decreased with reduction percent of 78.8% (Table2). These results were higher than that obtained by Ezzat (2015) who noted the reduction percent of testosterone hormone by frying were 2.67±0.15 ppb and by grilling to 2.86±0.15 ppb, also by Yassein (2010) with frying to 1.62% and by El-Khaky et al. (2012) with frying to 2.7%. During fish cooking, chemical and physical reactions take place that improve or impair fish value.

Table (2): Effect of different cooking methods on Methyltestosterone and Trenbolone acetate residues level (ppb) in Nile tilapia tissues.

Trial	Control		Frying				Grilling			
			Content		Reduction %		Content		Reduction %	
	MT	TA	MT	TA	MT	TA	MT	TA	MT	TA
1	1.92	0.17	0.63	0	67.2	100	0	0	100	100
2	2.28	0.32	1.47	0.09	35.5	71.9	0	0	100	100
3	5.44	0.69	4.18	0.45	23.1	34.7	2.05	0.21	62.3	69.6
Mean	3.21	0.39	2.09	0.18	34.8	53.8	0.68	0.07	78.8	82.05

Cooking induces water loss, but in turn increases its lipid content and only some fat is lost in case of oily fish. (Sveinsdóttir et al., 2009). The percent of reduction mean of trenbolone residues in hormone containing tilapia after frying were 53.8% for three examined samples and after grilling the ratio reached 82.05%. (Table 2). The best cooking methods to minimize the hormone residues in fish are microwaving, grilling or cooking in oven. However, grilling was found to be the best method (Zeitoun and Ahmed, 2011). We concluded that grilling is the best method of cooking in reduction of hormonal residues.

Conclusion

There are no maximum residue limits (MRLs) established for banned anabolic hormones from fish intended for human consumption in Egypt. However, the situation is more complicated with respect to natural hormones. Synthetic MT and TA hormone residues were not exceeded the MPLs except MT level in small size farmed tilapiawhich exceeded MPLs. So, we suggest that the public health hazard may come only from consumption of small size farmed tilapia. The level of residues of synthetic anabolic steroids should be rationally considering endogenous steroids naturally occurring in fish tissue. Cooking methods (frying and grilling) have positive effects on degrading MT and TB residues; grilling proved to be better than frying as a cooking method. Monitoring system should be introduced to control hormonal residues in fish and ensure consumers safety.

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LEGAL REQUIREMENTS FOR PREPARATION AND PRODUCTION OF FOOD IN BOSNIA AND HERZEGOVINA WITH SPECIAL REFERENCES ON THE ENTITY OF THE REPUBLIC OF SRPSKA

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Abstract

Consumers expect the food they consume to be safe and acceptable to use. Food-borne illnesses are uncomfortable, while in worst cases they may have a deadly outcome. Food-induced epidemics may have a negative impact on trade and tourism, which can lead to a decline in primary producer and processor income, then to an increase in unemployment and ultimately even court proceedings. The food safety area in Bosnia and Herzegovina is currently regulated by legal and by-law acts that are in line with the EU legislation and standards of Codex Alimentarius. In addition, at the level of the Entities of the Republic of Srpska, as well as the Entities of Federation of Bosnia and Herzegovina, there are food laws that are in line with existing state-level laws and EU regulations. The system of good manufacturing practice is implemented in the entire territory of Bosnia and Herzegovina, ie in the Republic of Srpska and the Federation of Bosnia and Herzegovina. These standards are very important in food production and manipulation, as they guarantee quality and safety. In the Republic of Srpska this segment is regulated by the law and the food regulations, which the manufacturers are obliged to implement. The essence of the concept of health security is contained in constant efforts and concrete planning activities to position, define and timely eliminate any dangerous phases or situations in the entire cycle of production of agricultural and food products. The standard has become necessary due to the significant increase in diseases caused by food contamination, both in developed countries and in developing countries. There is a large number of laws and regulations in the territory of Bosnia and Herzegovina that define food production and manipulation. Businesses are required to apply the HACCP standard, and a large number of manufacturers also have the ISO 22000 standard. The main objective of the paper is to define and monitor the application of the HACCP system and the ISO 22000 standard on the territory of Bosnia and Herzegovina, with particular emphasis on the Republic of Srpska entity. In addition, the paper presents a fragmented presentation of the above mentioned system and standards in relation to the European Union with the aim of establishing the position of Bosnia and Herzegovina in the application of the HACCP system and the ISO 22000 standard.

Key words: *consumer, producer, primary production, food regulations, standards.*

Introduction

The application of international standards on the market is an essential element in the process of improving company competitiveness. Customer care, healthy and safe food and environmental standards are just some of the conditions that modern business demands from food manufacturers. (Đorđević et al., 2011) Therefore, it has become an important imperative for food-related companies to implement and implant system management focused on the safety, legality and quality of their products (Verano and Ponce, 2008).

Consumers have the right to expect that the foods they consume are safe and acceptable to use. Food-born illnesses are, in the least, unpleasant, while in the worst cases they may have a deadly outcome. Food-induced epidemics may have many other consequences, such as adverse trade and tourism effects, can lead to a decline in primary producer and processor revenue, then to an increase in unemployment and eventually even to court proceedings. Food spoilage has costly consequences and reduces the confidence of consumers who expect food to be safe and quality (Mačkić et al., 2010).

HACCP represents a systemic preventative approach to ensuring food safety. HACCP is based on the identification and analysis of specific endangered health risks and the establishment of preventive measures in food production and transport that eliminate or reduce the risk to an acceptable level. This system is based on estimates and is embedded in the whole production process (Bunch, 2006). ISO 22000 is a new international comprehensive standard for food safety management systems. It sets a set of general health food safety requirements that apply to all food chain organizations and is recognized globally, these universal standards harmonize key requirements and overcome the difficulties of different standards related to food safety in terms of region, country, activities of organization and types of food (Živković, 2012). In the territory of Bosnia and Herzegovina, or both of its Entities and the Brcko District, the application of the HACCP system is widespread because laws at all levels of government are based on its principles.

The main objective of the paper is to define and monitor the application of the HACCP system and the ISO 22000 standard on the territory of Bosnia and Herzegovina, with particular emphasis on the Republic of Srpska entity. In addition, the paper presents a fragmented presentation of the above mentioned system and standards in relation to the European Union with the aim of establishing the position of Bosnia and Herzegovina in the application of the HACCP system and the ISO 22000 standard.

Integrated processes in food security

The right to safe food is one of the fundamental human rights and the obligation of each country to ensure that the health of its citizens is not endangered by the use of health-defective food products. Food safety today has become a world problem, which is particularly pronounced in the conditions of intense trade in food items on the international market. While sometimes the consequences of food poisoning were mostly limited to the immediate environment of poisoning agents, today the cause may be in one, and victims in many other countries. Extended food supply chains are prolonged, and more and more rapid preparation products are available, without additional heat treatment, further increasing the dangers present. The "integrating" way of thinking is increasingly used not only for food security but also for commercial reasons (Bunch, 2006). Measures to be taken in the process of production, maximum permissible concentrations for certain contaminants, procedures and methods of monitoring the contagion in certain areas, as well as basic elements for legislative regulation in the area of limiting or prohibiting the use of certain resources or processes in the food production process (Milosavljević, 2014). On the basis of such findings, risk management procedures, monitoring and monitoring programs for certain contaminants or groups of landowners, agricultural crops, final products and foodstuffs of plant and animal origin have been prescribed. However, the control of final products, no matter how comprehensive and rigorous, could not prevent relatively frequent incidents and food poisoning not only microbiological but also chemical and physical agents. In addition to the classical nature of the quality control and the health of the final products, it required a lot of time (due to the length of the individual analyzes) and significantly slowed the production process and food turnover (Stojanović et al., 1994). Therefore, in the early 1990s, a wide-ranging application of preventive system approach used in food production as a quality assurance system for food safety was introduced. This concept, known as HACCP, was developed in 1959 for the purposes of space research, and in practical application as an integrated system in the process of producing foodstuffs from "field and farm to market" in most western countries has been introduced since 1991. In essence, the HACCP system is a scientifically based, rational and systematic approach to the identification, assessment and control of risks during the process of production, processing, preparation and use of food, with the aim of ensuring that food is safe for the consumer, that is, unacceptable risk to health. In livestock production, the balance is shifted to integrated systems focusing on preventive and proactive actions, emphasizing the importance of intervention in the places where the cattle are fed, with the aim of providing better hygiene and the quality of meat and milk. In this respect, in Europe, such veterinary actions on farms are being demonstrated by applying HHSP herd health monitoring programs. These programs significantly contribute to protecting people from zoonoses and food-borne diseases. Together with the HACCP system, this program provides maximum health food safety (Pribisic, 2014). The integrated concept of food safety is the only way to achieve the basic goals, a high level of consumer, animal and environmental protection. The key factor is the knowledge of consumers to respect and protect their interests and concerns, not only from the point of view of health, but also to animal welfare and environmental protection. The new philosophy in food inspection that is developing in the European Union is based on self-control. It is accepted that it is in the best interest of the industry to undertake and implement all necessary measures to ensure the health and quality of food at the consumer level. It is therefore expected from the industry to provide, by using a proactive systematic methodology, that the finished product is a reliable pre-established quality and level of health care. All the actions take place in a transparent and documented way, so that they gain consumer confidence and enable them to obtain an official certificate of production and finished products. HHSP at the level of livestock production, and HACCP at the level of processing industry, meet the health-safety criteria, and ISO standards guarantee product quality (Milosavljević, 2014).

The basics of food safety standards in the European Union

Current European legislation requires the use of a proactive and systematic approach to business to ensure food security through the implementation of an appropriate control system. However, very often consumers are exposed to various risks ranging from poisoning, which can lead to more serious health complications and even death. What is the importance of this branch of industry in the European Union territory is the data that the Food and Beverage Industry is leading in the industry sector with annual production of 800 billion euros. This production accounts for 15% of total production, employing 2.8 million workers, of which 30% in small and medium-sized enterprises. Only the agricultural sector generates 220 billion euros annually, which is equivalent to 7.5 million full-time employees. The export of agricultural products, food and beverages to the EU level generates income of 50 billion euros annually. (Coleman et al., 2000). Starting from the importance of food, on 12 January 2000, the EU Commission adopted the baseline and action plan given in the document titled "White Paper on Food Safety". At the beginning of 2002, an "Independent Food Authority" was formed within the European Commission, and a network of National Agencies and Scientific Authorities was set up, whose basic task is to implement the adopted action plan, the compilation of the accompanying laws and food safety standards. At the EU level, the basic principle has been adopted that the law must rest on the aspect of food production and control "from farm to farm", and standards for security must be based on three key aspects (Milosavljević, 2014): economic, social and environmental consequences. The European Union regulated the marketing of food products with many of the most important documents (Bunch, 2006): The European Parliament and Council Regulation and the General Food Law. This law covers all phases of production, processing and distribution of food for humans and animals, except for primary production, preparation, handling and storage of food in households (intended for household consumption and consumption, ie not intended for sale on the market). "Foodstuff" (food) means any substance or product, processed, partially processed or unprocessed, intended for human consumption or is reasonably supposed to be consumed by humans. This includes beverages and any substance, including water, which is intentionally incorporated into food during its production, preparation or processing (Bunch, 2006).

The following institutions are responsible for food safety in the EU (Novaković et al., 2014): **European Commission**, within the framework of the General Directorate for Health and Consumers; **The European Parliament**, in addition to the Committee on Environmental Protection, Public Health and Food Safety (ENVI), includes twenty more committees; **The European Food Safety Agency** is the cornerstone of the European food safety net. It provides scientific counseling services and information on possible and existing risks to nutrition. EFSA is independent and acts separately from the European Commission, the European Parliament and the EU Member States. Within the Agency there is a scientific committee through ten scientific and expert panel groups.

Legal obligations for food preparation and production in Bosnia and Herzegovina with a special reference to the Republic of Srpska entity

According to the Food Act that is implemented in the territory of Bosnia and Herzegovina under the term food, we mean any substance or product that is processed, partially processed or unprocessed, intended for human use or can be expected to be used by humans. The term food includes both a drink, a chewing gum, a nutritional additive, and any other substance intentionally incorporated in food during its production, preparation or processing. In order to introduce a modern integrated system in Bosnia and Herzegovina, it is necessary to draft a new legislation harmonized with EU legislation, ie the *Acquis communautaire*, as well as consumer protection and placing producers in an equal position. In Bosnia and Herzegovina, a number of outdated executive regulations in the field of food that have been taken pursuant to the Decree-Law on the Takeover of Federal Laws and Other SFRY Laws, Applicable as Republic Laws and Other By-Laws, are currently in force. Pursuant to Articles 16 and 17 of the Food Act, the Council of Ministers of BiH, upon the proposal of the Agency, in cooperation with the competent authorities of the Entities and the Brcko District of BiH, issues valid regulations on the application of any process in production, processing, processing and distribution affect the hygiene and health of food and feed. According to Article 72 of the Food Act, the Council of Ministers of BiH, at the proposal of the Agency, shall issue new food regulations, labeling and food advertising regulations, regulations on traditional food reputation, originality and geographic origin descriptions, as well as other regulations from this area. In accordance with the provisions of Article 54 of the Food Act, the Agency initiates, prepares and organizes the drafting and implementation of the provisions of the Act with the aim of: protecting consumer interests, allowing consumers to make choices regarding the food they consume and protect the interests of the producer. The regulations on implementation, based on the said members of the Food Act, establish requirements related to: the obligations of the subjects in the food business with regard to quality; classification, categorization and name of food; organoleptic properties and composition of food; manufacturer specification type and quantity of raw materials, additives and other substances used in food production and processing; technological processes applied in food production and processing; sampling methods and analytical methods to control food quality; additional or specific information that should be included in the food declaration and are of interest to the consumer; marks of traditional food reputation; originality and geographic origin of food; new food, as well as other necessary regulations in this area.

The food safety area in BiH is currently regulated by legal and by-law acts that are in line with the EU legislation and standards of *Codex Alimentarius*. When it comes to the state level, there are two laws on food:

1. The Food Act ("Official Gazette of BiH", No. 50/04),
2. The GMO Act ("Official Gazette of BiH", no. 23/09).

In addition, at the level of the Entities of the Republic of Srpska, as well as the Entities of Federation of BiH, there are food laws that are in line with existing state-level laws and EU regulations. Also, at the state level there is a large number of regulations that determine this sector. There is still a certain number of regulations taken over from the SFRY. The area of veterinary medicines and pesticides in food of animal origin is regulated by the Ordinance on maximum permitted levels of veterinary medicines and pesticides in animal products ("Official Gazette of BiH", No 6/09). In addition to the aforementioned regulations, there are also rule-of-law rules that are aligned with the state level and are fully applicable in both Entities.

The concept of good manufacturing practice (DPP) system in Bosnia and Herzegovina with a special reference to the Republic of Srpska entity

Good manufacturing practice presents a number of recommendations that are desirable to be carried out in the manufacture, processing, storage and supply of food to prevent its microbiological, chemical or physical contamination. In other words, good manufacturing practice points to what needs to be done to prevent food contamination, and when and who should to do it (Mačkić et al., 2010). Good manufacturing practice does not refer to certain harmful factors, and the loss of control will not always and indisputably directly jeopardize the health of consumers but will increase the relative risks.

Areas in which good manufacturing practice is realized are (Henson and Jaffe, 2011):

- staff (including their assignments, job description, organizational structure and hygiene training),
- premises (including location and layout, design, construction aspects, maintenance, work environment, light, temperature, humidity),
- equipment (including shape, maintenance and calibration),
- raw materials for production (including live animals, packaging materials, food ingredients and chemicals),
- product traceability,
- services (including sanitation, waste disposal, supply of electricity, water, cooling steam),
- documentation.

The system of good manufacturing practice is implemented in the entire territory of Bosnia and Herzegovina, ie in the Republic of Srpska and the Federation of Bosnia and Herzegovina. These standards are very important in food production and manipulation, as they guarantee quality and safety. This segment in the Republic of Srpska is regulated by the law and the food regulations, which the manufacturers are obliged to implement.

The concept of good hygenic practice system (DHP) in Bosnia and Herzegovina with a special reference to the Republic of Srpska entity

As part of good manufacturing practice, cleaning and hygiene are of particular importance and are considered to be the main elements of good hygiene practice. Good hygiene practice can be described as a set of procedures to ensure a clean, sanitary environment for the production, processing, storage and supply of food products. Companies to meet these requirements must determine control measures to ensure compliance with the following requirements hygiene requirements, such as hygiene of personnel, facilities, equipment, and specific requirements (Popelka et al., 2005). In other words, good hygiene practice determines what needs to be done in connection with cleaning and hygiene, as well as when and who should perform these tasks. it is necessary to continually perform disinfection or sanitary program, maintain building and equipment maintenance, and conduct cross-contamination control during production, including people, surfaces, air and segregation of raw materials and processed products (Raspor, 2007). The areas covered by the program of good hygiene are (Hayburn, 2014): cleaning of the facility / section and equipment; health and cleanliness of staff performing food-related tasks; purity of raw materials for production, including live animals; ensuring that all means of hygiene and other chemicals are properly packaged, labeled, stored and applied in accordance with their intended purpose and documented procedures.

Healthy food safety is a guarantee that food will not harm the consumer when prepared and / or consumed in accordance with its purpose. According to the new legal regulations of Bosnia and Herzegovina, in compliance with the process of harmonization with the legal regulation of the European Union, the food business operator (The Food Act of BiH) is responsible for the health of food safety. Food monitoring requirements should be met, which means that food business entities must establish such a system that they can identify the "step forward and step backwards" in the chain: the barrel - the manufacturer - the distributor - the consumer. Food business entities must further ensure that all phases of production, processing and distribution of food that they are under their control meet the requirements related to the hygiene prescribed by this Law and the relevant regulations adopted pursuant to this Act. They must also carry out regular controls of hygiene conditions at all stages of production, processing and distribution of food, except at the level of primary production and related activities, in each facility under their control, by carrying out a preventive self-control procedure developed in accordance with the principles of the Hazard Analysis System and Critical Control Points (7 HACCP Principle) (Milosavljević, 2014). For example, a good hygienic practice program for hospitality includes: sanitary technical and hygienic conditions, cleaning, pest control, maintenance of equipment, personal hygiene, occupational health, waste disposal, staff training, supply, delivery and food reception, food storage, food control, food handling in a safe manner, foods that require special attention when preparing, withdrawing products, verifying, controlling health / microbiological food safety (Zdravković, 2014). By introducing and applying the principles of good hygiene practice, good manufacturing practices and self-control systems based on the principles of HACCP system, the quality and safety of food can be ensured and guaranteed. The difference between the code of good hygiene practice and the HACCP system is that the HACCP represents one step more, since the application of this system keeps records and that there is a so-called recall procedure with HACCP, which implies signing up of food from the market is related to large manufacturing systems (Zdravković, 2014).

In the Republic of Srpska, the Ordinance on food hygiene was published in the Official Gazette of Republika Srpska, no. 39 of 10 May 2018. It has defined a number of provisions relating to food business in all phases of production, processing and food traffic under the control of the entity. According to this rule, food hygiene implies the measures and conditions required to control the risk and ensure the eligibility of food used for human consumption in accordance with its purpose. Food business subject to special regulations in the Republic of Srpska is obliged to carry out hygiene measures related to (RS Ordinance on Food Hygiene, 2018): meeting the microbiological criteria for food, the procedures necessary to meet the objectives set out in this Ordinance, the fulfillment of conditions regarding the control of food temperature, maintaining the cold chain, sampling and analysis.

HACCP system

Hazard Analysis and Critical Control Point (HACCP) is a food safety system that is based on the analysis and control of potential biological / microbiological, chemical and physical hazards that are exposed to raw materials, possible hazards to the handling, production, distribution and consumption of end products. Its application implies compliance with the standard operating procedures and instructions that reduce the risks to food safety. HACCP is based on identifying and analyzing specific hazards and identifying preventive measures that eliminate or reduce the risk of production and the emergence of potentially dangerous foodstuffs to an acceptable measure (Bošnjak, 2009). This system has the task of (The Food Security Agency of Bosnia and Herzegovina, 2012): establishing, evaluating and controlling the dangers that could affect food safety, the system manages the quality and safety of food based on prevention, everyone is trained to know what, how, when and why to do it in order to prevent food risks and also their own responsibility so that the end user consumes healthy and safe food. Established hygiene practices are essential for monitoring the situation throughout the entire food chain, from primary production to final product. Its application prevents contamination and provides an adequate food handling environment.

Implementation of the HACCP system in the territory ofBbosnia and Herzegovina with a specific reference to the Republic of Srpska entity

A large number of economic entities in the territory of Bosnia and Herzegovina, ie the Entities of the Republic of Srpska and the Federation of Bosnia and Herzegovina, apply the HACCP system for food production, as manufacturers are obliged to implement them. Thus, according to the food act at all administrative levels of the state, food business entities, in addition to the entity performing the activity of primary production, establishes and implements in all phases of production, processing and food transport a self-control system, based on good manufacturing practice principles and good hygienic practices, risk analysis and critical control points - HACCP. The principles of the HACCP system in the Republic of Srpska are fully applied, as defined by the Republic of Srpska Food Regulations. Companies applying this system, in the territory of the Republic of Srpska, as well as throughout Bosnia and Herzegovina, go through the same procedures as all other entities in the European Union market. For this reason, HACCP is the standard whose settings must meet in order for companies to own it.

Implementation of ISO 22000 in the territory of Bosnia and Herzegovina with a special reference to the Republic of Srpska entity

There is a standardization institute in Bosnia and Herzegovina. The Institute proposes a standardization strategy in Bosnia and Herzegovina, prepares and publishes the standards of Bosnia and Herzegovina, represents and presents Bosnia and Herzegovina in international, European and other standardization organizations, and carries out the tasks deriving from international agreements and membership in these organizations. It participates in the preparation of technical regulations, develops and establishes an information system on the standards of Bosnia and Herzegovina, organizes and conducts specialized training of personnel in the field of standardization. He also deals with publishing and publicity activities in the field of standardization (Popović, 2012). In the field of conformity assessment, it participates in the establishment and maintenance of the certification and homologation system in accordance with the European model. It defends Bosnia and Herzegovina in European and international conformity assessment organizations (EOTC and EUROLAB, etc.) to form an association of test laboratories and associations of calibration laboratories and organizes education in the area of assessment of compliance. Also, it organizes various sourcing assistants for the implementation of ISO standards and the HACCP system.

Standardization is the activity of establishing rules for general and multiple use, which relate to existing or potential problems, in order to achieve optimal degree of regulation in a particular area (Filatov, 2016). Standardization activities consist primarily of preparation, formulation and issuing standards, and enabling standard application. Important benefits of standardization are improving the benefits of products, processes and services for the foreseen purposes, preventing barriers to trade and facilitating technological cooperation (Živković, 2012). In order for a particular economic entity to comply with standardization, it is necessary to officially verify that all requirements related to the standard, as well as company objectives and policies are met. Certification is carried out by an independent international body certification. The certification process is much more complicated than the certification of the HACCP system, with the acquisition of the ISO 22000 certificate giving a great reputation to the company and recognized at international level (Bijelić, 2015). This is a set of international standards that encompasses all organizations in the food chain and defines the requirements of the Food Safety Management System. The ISO 22000 standard is based on the principles of the HACCP system for obtaining the final food product, fully respecting the safety standards: monitoring, studying and describing the production process for preventing or establishing controls in case of food safety risks, conducting analysis, assessing and establishing control, management and disposal resources. For this reason, it is important that business entities from Bosnia and Herzegovina, in addition to the mandatory HACCP system, also approach the implementation of ISO 22000 standard in food production, as it will provide a much better competitive position on the market (Zdravković, 2014).

Conclusion

The essence of the concept of health security is contained in constant efforts and concrete planning activities to position, define and timely eliminate any dangerous phases or situations in the entire cycle of production of agricultural and food products (from primary production of basic and auxiliary raw materials to immediate consumption of finished products). The standard has become necessary due to the significant increase in diseases caused by food contamination, both in developed countries and in developing countries. In addition to health risks, food-borne diseases can significantly increase economic costs, including medical treatment, job absences, insurance payments, and statutory compensation. As a result, several countries have developed national standards for safe food supply, and certain food and beverage companies and groups have developed their standards or programs to control their suppliers (European Commission, 2014). The existence of good hygienic and manufacturing practices, as well as the HACCP system, ISO 22000 standards, have led to a new higher level of food production process. So good hygienic and production practices can be said to be the lowest in the observed set of measures to protect food production. Next, the HACCP system is more comprehensive than the previous two measures. Finally, the most comprehensive is the ISO 22000 standard, which assures the safety and security of food production. There is a large number of laws and regulations in the territory of Bosnia and Herzegovina that define food production and manipulation. Businesses are required to apply the HACCP standard, and a large number of manufacturers also have the ISO 22000 standard. When the Republic of Srpska entity is in question, there is also a large number of laws and regulations defining this area and in compliance with state-level laws and regulations. It is also important to point out that laws and regulations in the production of food are being implemented in the territory of Bosnia and Herzegovina by the European Union. First of all because of its export to its market and the emergence of Bosnia and Herzegovina to become its full member.

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EFFECT OF DIFFERENT THICKENING AGENTS ON FREEZE-THAW STABILITY OF FOOD SYSTEMS WITH MODIFIED MAIZE STARCH

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Abstract

The stability of food systems is an essential characteristic for determining the quality of products. Freeze–thaw stability is an important property that is used to evaluate the ability of starch to with stand undesirable physical changes occurring during freezing and thawing. We analyzed the effect of different thickening agents on freeze-thaw stability of food systems with modified maize starch. It was made four systems with modified maize starch and combinations of starch and guar gum, starch and xanthan gum, and starch and pectin. The choice of starch quantity and thickening agent originates from the initially uniform viscosity of the resulting systems. The received food systems were storage at 8±1 °C and -18±1 °C for four days. The structural-mechanical properties and stability of the investigated samples were analyzed. The addition of a thickening agent reduced the amount of the used starch and increased the stability of the systems. The sample with 0.09 % xanthan gum and 3 % starch had the best result in regard to shear-resistance, and the worst result was found in sample with 0.14 % pectin and 3 % starch, storage at 8±1 °C. However, the different storage type of the samples influenced on their stability and thixotropic properties, which can be an indicator of choosing the type of storage.

It was measured the value of the separated liquid (in %) of the samples. The results shown that only the sample №1 (only with starch) had a separated liquid. Incorporation of a thickening agent is an indicator of increasing the retrograde stability of the starch.

Keywords: *Food systems, Thickening agents, Structural-mechanical properties, Freeze-thaw stability*

Introduction

Hydrocolloids are water-soluble, high molecular weight polysaccharides that serve a variety of functions in food systems including enhancing viscosity, creating gel-structures, film formation, and control of crystallization, inhibition of syneresis, improving texture, and encapsulation of flavors and lengthening the physical stability (Dickinson, 2003; Anwar et al., 2015). These functional ingredients are widely used in dairy products, canned foods, bakery products, salad dressings, beverages, sauces, soups and other processed foodstuffs to improve textural characteristics, flavor and shelf life (Sahin and Ozdemir, 2004).

The foremost reason behind the ample use of hydrocolloids in foods is their ability to modify the rheology of food system. This includes two basic properties of food system namely, flow behavior (viscosity) and mechanical solid property (texture). The modification of texture and/or viscosity of food system help to modify its sensory properties, and hence, hydrocolloids are used as important food additives to perform specific purposes. Various food formulations like soups, gravies, salad dressings, sauces and toppings use hydrocolloids as additives to attain the desired viscosity and mouth feel. Considering their role in the adjustment of viscosity and texture of food formulations, several studies have been conducted in various food systems employing different hydrocolloids either singly or in combination (Saha and Bhattacharya, 2010). Starch is the most commonly used hydrocolloid thickener, the reason being it is relatively cheap, abundant and possibly it does not impart any noticeable taste if used at a low concentration of 2 to 5%. Further, as starch is a common ingredient of many foods we encounter, addition of starch does not offer any foreign taste which may be true for different gums. It is mainly the hydrocolloid providing a base texture in soups and sauces. Thickening of sweet and sour sauces with various polysaccharide combinations like potato starch-xanthan gum and oat starch-xanthan gum has been studied (Gibinski et al., 2006).

Generally, gums and stabilizers have non-Newtonian rheology and they impart non-Newtonian character to the emulsions even when the amount of the dispersed phase is low (Krstonošić et al., 2015).

Upon freezing, however, water in the foods transforms into ice, often resulting in physical stress to the food matrix. When a frozen food is thawed for consumption, the moisture is readily separated from the matrix and it causes softening of the texture, drip loss, and often deterioration of overall quality (Rahman, 1999). The ability of starch to withstand the undesirable physical changes during freezing and thawing has been commonly termed "freeze-thaw" stability and can be used as an indicator of the tendency of starch to retrograde. When a starch paste or gel is frozen, phase separation occurs with the formation of ice crystals. On thawing, the paste or gel will continue to be composed of a starch-rich and starch-depleted aqueous phase (Karim et al., 2000). Freeze-thaw stability may be simply evaluated by gravimetric measurements (Charoenrein and Preechathammawong, 2012).

In this case it was analyzing the effect of different thickening agents on freeze-thaw stability by their structural-mechanical properties and the value of the separated liquid of the investigated samples. The goal of the research is to make a more freeze-thaw stable sweet-sour system with a lower starch content and determined initial viscosity.

Material and Methods

Preparation and storage of systems

The model systems were produced at the University of food technology`s laboratory in Plovdiv, Bulgaria, by mixing the following basic components: sugar 30%, apple vinegar (6 % solution) 15%, starch in combination with different hydrocolloids in quantity shown in Table 1 and water to 100 %. It was used Acetylated distarch adipate from waxy maize starch E 1422 combined with guar gum (GG), xanthan gum (XG) and pectin (P), to created a product with the same initial viscosity. The control sample (№1) was only with modified starch.

Table 1.Quantity of used thickening agents depending on the sample

Ingredients:	Sample №1, %	Sample №2, %	Sample №3, %	Sample №4, %
Starch	3.4	3.0	3.0	3.0
Hydrocolloids:				
-guar gum	-	0.11	-	-
-xanthan gum	-	-	0.09	-
-pectin	-	-	-	0.14

Each sample was prepared by mixing all components with water, homogenized and heated at temperature of 85 °C for about 7 minutes. After cooling and storage the systems were analyzed. The samples were divided into two. First parts of the samples were storage at 8±1 °C, and the others were frozen at -18±1 °C for four days following the method of Arocas et al., 2009. After frozen storage, the samples were thawed at room temperature and heated to 30 °C. The measurements were performed on the same day.

Measurement the value of the separated liquid (syneresis in %)

After storage the tempered samples were put in test tubes and centrifuged of 5000 rpm for 15 min (Sodhi and Singh, 2003). For analyze it was used a centrifuge Hettich Zentrifugen EBA 200 (Germany). The separated liquid was removed and weighed. Syneresis was expressed as weight percent of the decanted liquid phase.

$$\text{Syneresis} = (W_1 \ / \ W_0).100 \quad (1)$$

where: W_1 is The separated liquid, g; W_0 is the centrifuged sample, g.

Measurement of the structural-mechanical characteristics

The measurements were conducted at 30 °C with rotational viscometer "Reotest 2" (Germany) with shear rate in the range from 0.33 to 145.8 s^{-1}. The major rheological characteristics were determined - dynamic viscosity (η, Pa.s), yield stress (τ_0, Pa), consistency coefficient (k, Pa.s) and the flow behavior index (n). The dynamic viscosity (η) was calculated using the formula:

$$\eta = \tau / D \qquad (2)$$

where: τ is the shear stress, Pa; D is the shear rate, s^{-1}.

The thixotropic areas were also calculated. The Herschel–Bulkley equations (Rao, 1999) were fitted to obtained results.

Statistical analysis

All results were expressed as the mean ±standard deviation (SD). The experimental data were subjected to analysis of variance, at the confidence level of $p = 0.05$, using ANOVA. The test of Tukey was used for determination of the statistically significant differences between values of the samples storage at the same temperatures.

Results and Discussion

The value of the separated liquid (syneresis in %)

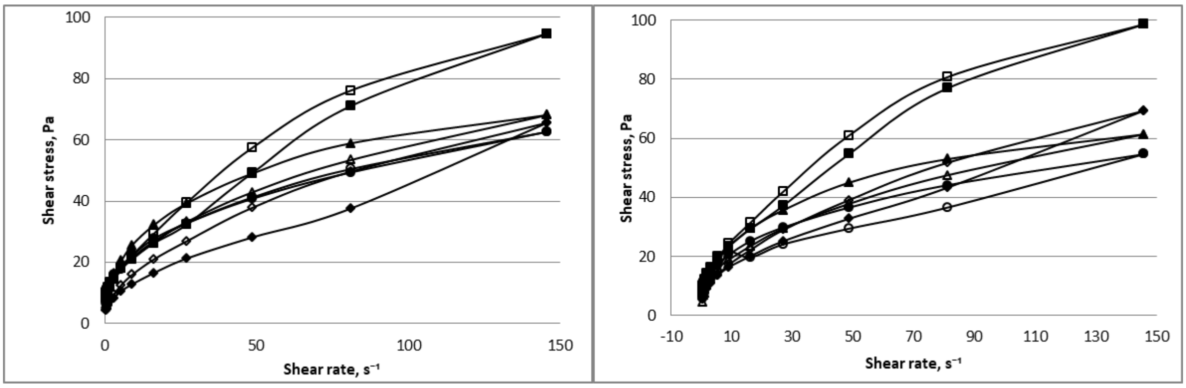
The measured value of the separated liquid is an indicator for the retrogradation (syneresis) properties of the used starches.

The results shown that only the sample №1 (only with starch) had a separated liquid in a quantity of: for the non-frozen sample: 8.721±0.16% released liquid and for the frozen sample: 9.000±0.08% released liquid. There were no significant differences in sample values stored in different conditions. The other samples had no separated liquid, which means that they are more stable than sample №1.

Structural-mechanical characteristics

The structural-mechanical properties are an important characteristic for all foods products, determine the quality, and it is essential information to the economic design of the most suitable food process equipment and operation that can be selected. Rheological properties of the samples were studied at 30°C.

The rheograms of the analyzed samples are shown in Figure 2. It is obvious that the samples represent Non-Newtonian fluids, which is evident from the shape of these flow curves.



(A) (B)

Figure 2. Rheograms of the systems, storage at 8±1°C (A) and freeze-thaw samples, storage at -18±1°C (B):
Sample 1 - □ (upward curve), ■ (downward curve); Sample 2 - Δ, ▲;
Sample 3 - ○, ●; Sample 4 - ◊, ♦;

Flow curves for all systems presented narrow hysteresis loops indicating rather changes in the structure of them on the shear applied.

The power law model parameters for the samples are given in Table 2, along with correlation coefficient (R^2). The multiple correlation coefficients, R^2, informed about, generally, very good fitting of the Herschel–Bulkley model and it`s obtained varied from 0.9620±0.048 to 0.9989±0.033, typical for the Herschel–Bulkley models.

$$\tau = \tau_0 + k.D^n \qquad (3)$$

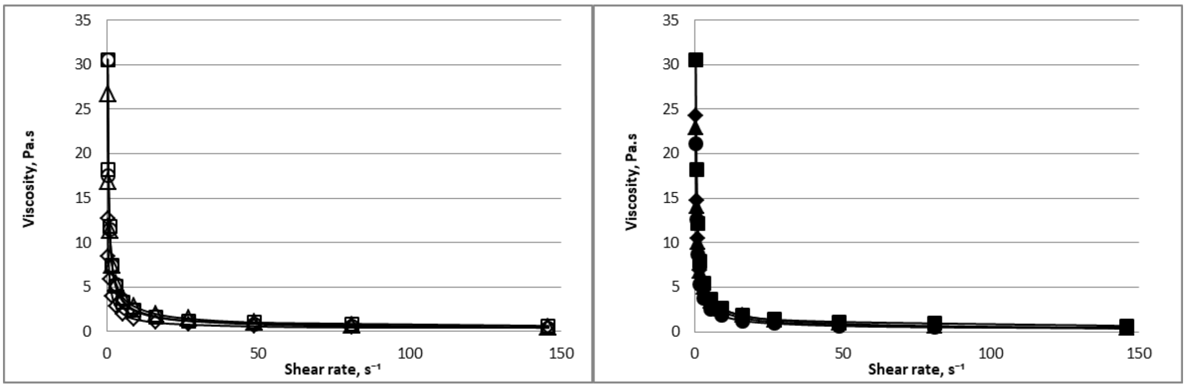
where τ is the shear stress, Pa; D – is the shear rate, s^{-1}; τ_0 - is the yield stress, Pa ; k – is the consistency coefficient, Pa.s; n – is the flow behavior index.

Table 2. Parameters of Herschel–Bulkley models for flow curves of the systems

Storage	Sample №	τ_0 (Pa)	K ($Pa.s^n$)	n (-)	R^2	τ_0 (Pa)	K ($Pa.s^n$)	n (-)	R^2	Hysteresis loop area (Pa. s^{-1})
		Upward curve				Downward curve				
After 96 h at 8°C	1	9.40± 0.47a	11.10± 0.55a	0.383± 0.018ac	0.9632± 0.048a	7.00± 0.35b	10.27± 0.51a	0.422± 0.021ac	0.9742± 0.049a	-778.4± 63.4a
	2	7.00± 0.35b	11.79± 0.59a	0.357± 0.017ab	0.9946± 0.030a	4.50± 0.23a	9.32± 0.47a	0.392± 0.019ab	0.9986± 0.042a	631.6± 13.9b
	3	8.60± 0.43c	12.00± 0.60b	0.312± 0.015c	0.9854± 0.049a	5.10± 0.26a	9.88± 0.49a	0.366± 0.018b	0.9989± 0.033a	8.3 ±1.3c
	4	2.80± 0.14a	5.73± 0.29a	0.425± 0.021b	0.9756± 0.039a	2.20± 0.11c	6.01± 0.30b	0.467± 0.023c	0.9951± 0.046a	4860.4± 243.02d
After 96 h at -18°C	1	9.00± 0.45e	12.08± 0.60d	0.380± 0.019ab	0.9614± 0.049b	6.30± 0.32e	10.73± 0.54d	0.429± 0.021d	0.9856± 0.049b	-466.3± 23.2a
	2	7.00± 0.35ab	10.37± 0.52c	0.366± 0.018ac	0.9964± 0.023b	4.00± 0.32d	7.75± 0.39c	0.411± 0.020d	0.9983± 0.036b	748.1± 12.3b
	3	7.40± 0.37ac	10.43± 0.52c	0.322± 0.016ed	0.9958± 0.040b	5.40± 0.27f	8.20± 0.41c	0.352± 0.017e	0.9833± 0.038b	864.8± 13.9c
	4	6.20± 0.31bd	8.40± 0.42e	0.362± 0.018bd	0.9620± 0.048b	3.80± 0.19d	7.62± 0.38c	0.416± 0.020d	0.9865± 0.048b	-720.1± 12.7d

*Parameters in columns for different systems denoted which have the same letters do not differ statistically at the level of confidence p = 0,05. Comparison is made between the values of system storage at the same temperatures.

Yield stress is an important characteristic. The high values of yield stress, τ_0, from the Herschel–Bulkley model, pointed to a high stability of the structure of the. Sample №1 had the highest value of τ_0, followed by sample №3, and the lowest – Sample № 4. This dependence is valid for the two types of storage, so the high stability of structure have the samples №1 and №3. Viscosity functions data showed that the values for flow behaviour indices, n, were below 1, which was indicative of the pseudoplastic nature (Bayod et al., 2008). The smaller n values determine the greater departure from the Newtonian behavior (Sengül et al., 2005). That parameter usually decreased on storage (Sikora et al., 2007; Sharma et al., 2014), which is not observed to depend on the storage temperature. Consistency coefficient, K, from the Herschel–Bulkley model can also be used as a criterion of viscosity. In terms of that coefficient, all of the systems have closely viscosity except Sample №4 at 8±1°C, which was the least viscous. The data shown that the narrowest hysteresis loop area were found for the sample №3 storage at 8±1°C, followed by sample №1, storage at -18±1°C, so the structure of such sauces was the strongest and shear-resistant. Sample №4, storage at 8±1°C had the highest value of the hysteresis loop area which means the weakest and the lowest shear-resistant. This dependence was not observed at freeze-thaw samples. Moreover, the freeze-thaw storage of the samples influence on their stability. Only in the sample №2 there was no considered change in the stability. It was observed that freeze-thaw sample №4 changed its thixotropic properties, from thixotropic to anti-thixotropic. Anti-thixotropy, is also known as rheopexy was examine by Dewar and Joyce, 2006. It is just opposite to thixotropy of solutions or suspensions. Viscosity is also defined as the main rheological property of the different food systems. The viscosity values of the samples as a function of the shear rate (D) are shown in Figure 3.



(A) (B)

Figure 3. Changes in the apparent viscosity by shear rate, storage at 8±1°C (A) and freeze-thaw samples, storage at -18±1°C (B):
Sample 1 - □ (system A) , ■ (system B); Sample 2 - Δ,▲; Sample 3 - ○, ●; Sample 4 - ◊, ♦;

It was obvious that the shear rate increased and the viscosity decreased. These facts showed that all systems have closely viscosity variations. These samples have pseudoplastic properties because they flow with applying the external impact.

Conclusions

This study showed that the addition of a thickening agent reduced the amount of the used starch and increased the stability of the systems towards retrogradation as well as the structural-mechanical stability. Only the sample with starch had a separated liquid which was an indicator for reducing the retrograde properties of starch. This analyzes and results showed that the type of storage affects both the stability and the thixotropic behavior of the samples as the sample with guar gum had least dependence of the type of storage. The best result had the sample with xanthan gum and starch, and the worst - with pectin, storage at 8±1°C. A change in thixotropic properties was detected in a sample with pectin. As far as stability was concerned, the freeze-thaw treatment shown that the sample with pectin changed its thixotropic properties.

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TOXIGENIC POTENTIAL OF *ASPERGILLUS PARASITICUS* ISOLATES ORIGINATING FROM MAIZE GRAIN IN SERBIA

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Abstract

Maize is one of the most susceptible crops to mycotoxins in the world. In relation to mycotoxins, the greatest attention has been paid to aflatoxins, because of their potential for carcinogenicity and other health issues in humans and animals. *Aspergillus flavus* and *A. parasiticus* produce aflatoxins in many economically significant crops in both, fields and storages. Since *A. parasiticus* isolates originating from Serbia caused significant direct losses as a result of maize grain infection and potential contamination with aflatoxins, it is necessary to establish their toxigenic potential. Aflatoxins were produced by almost all *A. parasiticus* strains. Obtained results indicate that there is a great diversity in the production of all individual aflatoxin. The concentrations of aflatoxin B1 were ranged from 33.05 to 7361.03 μg kg^{-1}, while the presence of aflatoxin G1 was in the range of 5.13-6666.12 μg kg^{-1}. The existence of atoxigenic isolates of this species can be significant for Serbia, as they have been increasingly applied as biocontrol agents for virulent strains of fungi. The use of these isolates as biological agent in plant protection should be estimated. Prevention is the most important and economically most beneficial practice in the decrease of fungal growth and mycotoxin production.

Key words: *A. parasiticus, toxigenic potential, aflatoxins, maize.*

Introduction

Maize is one of the most cultivated cereals worldwide ranking first in Serbia. The maize production satisfies national needs. At the same time it is a strategic product intended for export. The major aim of maize production is stable and high yields but also a high nutritional value of grain, which is at the same time health safe. *A. parasiticus* does not only degrade maize grain quality, but also synthesises toxic substance - aflatoxins, which are, from the aspect of human and animal health the key group of mycotoxins. At the same time, the study of the content of the most important individual and total aflatoxins synthesised by this species, emphasises an overall impact of *A. parasiticus* on yield components and maize health.

Aspergillus contamination has been occasional under agroecological conditions prevailing in cereal-growing regions of Serbia. However, frequent occurrence of high temperatures and prolonged droughts favour increased frequency of *Aspergillus* spp., therefore our assumption was that this pathogen might cause certain problems in Serbia. The species *A. parasiticus* was isolated from maize grain for the first time in Serbia in the 2012 growing season (Stanković *et al.*, 2015). Additionally, the isolates obtained from wheat grains were also identified as *A. parasiticus*, for the first time under climatic conditions of Serbia in 2017 (Nikolić *et al.*, 2018).

Maize is commonly known as a crop very susceptible to mycotoxins. Due to their capability to cause not only cancers but also durable complications in and animal health, aflatoxins, produced by *A. parasiticus*, represent a distinctive group of mycotoxins. *A. parasiticus* synthesises four aflatoxins (AFB1, AFB2, AFG1 and AFG2), but not CPA (Amaike and

Keller, 2011). Since mycotoxins cause enormous destructive effects their amounts are legally regulated in foodstuffs in Europe and our country (Kos, 2015).
The aim of this study was to establish aflatoxins accumulation from pathogenic and toxigenic *A. parasiticus* isolates originating from maize, because *A. parasiticus* causes damages on maize grain, which is also contaminated with aflatoxins.

Materials and methods

Ten *A. parasiticus* isolates were evaluated for levels of aflatoxin contamination.

Sample preparation for the mycotoxin assessment

The aflafoxin production was determined in isolates inoculated in the centre of the 9-cm Petri dish with a dense conidial suspension and grown on PDA as single colonies. The incubation of cultures was done at 28±1°C in the dark for 5 days (Abbas *et al*., 2004).

HPLC, extraction and quantitation of aflatoxins

According to Abbas *et al*. (2004), the contents of PDA dishes were put into a tube to collect fungal biomass, which was placed in glass vials of a known weight and weighed again. Fungal biomass was extracted with a solvent mixture acetonitrile-water (90:10, v/v) in the ratio of 100:1, v/m. A reciprocal shaker was used to shake vials for 30 min at high speed. A 1-mL aliquot of extract was removed and centrifuged at 12 000 g for 10 minutes. The HPLC-fluorescence detection method was used to confirm the presence of aflatoxins in the supernatant (AOAC Official Method 994.08). Data are reported as the mean value of three independent injections.

Results and Discussion

Toxicological characterisation

The quantitative and qualitative analysis of the production potential by the HPLC method has shown that there is a high variability in the concentration of individual aflatoxins (B1, B2, G1 and G2) among isolates.
Aflatoxin production capability of all *A. parasiticus* used in this experiment was analysed with chromatographic technique, HPLC-FLD. In our study, aflatoxins were produced by almost all *A. parasiticus* strains. The MRIZP isolates (MRI 2Ap, MRI 3Ap, MRI 5Ap, MRI 7Ap, MRI 8Ap) produced aflatoxins B1, B2, G1 and G2, except of the isolates MRI 6Ap and MRI 10Ap that did not produce aflatoxins B2 and G2, and MRI 1 that did not produce aflatoxins G2. Some isolates was unable to produce any aflatoxin (MRI 4Ap, MRI 9Ap). We pointed out the existence of isolates that synthesized aflatoxin G1 at higher concentrations, compared to aflatoxin B1 (MRI 2Ap, MRI 3Ap, MRI 5Ap) (Table 1).

The average synthesis of aflatoxin B1 was 3467.60 μg kg^{-1}, which ranks this species into strong AFB1 producers. The average synthesis of aflatoxin B2 was 257.69 μg kg^{-1}. The average synthesis of aflatoxin G1 was 2749.89 μg kg^{-1}, which classify this species into strong AFG1 producers, while the average synthesis of aflatoxin G2 was 59.31 μg kg^{-1}.

The highest concentrations of AFB1 were detected in MRI 1Ap isolates (7542.19 μg kg^{-1}), while at least concentrations were observed in MRI 10Ap (33.05 μg kg^{-1}) isolates. The highest concentrations of AFG1 were detected in MRI 5Ap (6666.12 μg kg^{-1}). The lowest concentrations of this mycotoxin were detected in the MRI 7Ap (5.13 μg kg^{-1}) isolates (Table 1). On the basis of the obtained results, there is a great diversity in the production of all individual aflatoxin.

Table 1. Different concentrations of *Aspergillus parasiticus* isolates

Isolates	B1	B2	G1	G2
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
MRI 1Ap	7542.19	995.41	3205.56	nd
MRI 2Ap	4409.10	143.97	5357.14	68.92
MRI 3Ap	5708.12	150.01	6495.07	80.05
MRI 4Ap	nd	nd	nd	nd
MRI 5Ap	5918.46	254.03	6666.12	98.25
MRI 6Ap	89.67	nd	15.06	nd
MRI 7Ap	5198.68	403.79	5.13	128.02
MRI 8Ap	5776.73	629.68	5746.97	217.83
MRI 9Ap	nd	nd	nd	nd
MRI 10Ap	33.05	nd	7.89	nd

nd – not detected

The results of the HPLC method indicated the existence of statistically very significant positive correlations between the production potential of AFB1 and AFG1 in isolate *A. parasiticus* (r = 0.74**), while the correlation between production potential AFB2 and AFG2 was significant (r = 0.37*) (Table 2).

Table 2. Correlation between the synthesis of aflatoxins B1, B2, G1, G2 in the *Aspergillus parasiticus* isolate obtained by the HPLC method

Mycotoxin	Correlation coefficient (r)	
	AFG1	AFG2
AFB1	0.74**	
AFB2		0.37*

** Statistically significant difference (p <0.01)
* Statistically significant difference (p <0.05)

A. parasiticus isolates showed high production potential of B and G type aflatoxins in studies performed by Vaamonde *et al*. (2003), Rodrigues *et al*. (2009) and Baquião *et al*. (2013).
Nikolic *et al.* (2017) analysed the concentrations of individual aflatoxins of the group G, established by the use of the HPLC-FLD method, varied from 8.03 to 7421.58 μg kg^{-1} for AFG1 and from 0 to 395.18 μg kg^{-1} for AFG2. Furthermore, Nikolic *et al.* (2018) studied the potential of the production of *A. parasiticus* isolates that originated from grain of wheat cultivated under different agro-climatic conditions in Serbia and found out that all isolates had the ability to synthesise AFB1, AFB2 and AFG1 in the concentration range of 4859.15-7361.03 μg kg^{-1}, 142.81-1543.8 μg kg^{-1} and 3085.11-7191.62 μg kg^{-1}, respectively. One isolate did not synthesise AFG2, while remaining ones synthesised this aflatoxin in the concentration ranging from 86.92 to 181.76 μg kg^{-1}. However, these authors established two isolates that synthesised AFG1 in greater concentrations than AFB1.
During testing of the production potential of the *A. parasiticus* isolates, Donner *et al.* (2009) recorded the average concentrations of AFB1 (90-2092 μg kg^{-1}) and AFG1 (99-3450 μg kg^{-1}). The range of concentrations of AFB1 and AFG1 synthesised in these tests amounted to 90-4957 μg kg^{-1}, and 0-6131 μg kg^{-1}, respectively.

On the occasion of studying the potential of the total aflatoxins of the *A. parasiticus* isolates originating from Nandi County in Kenya, Okoth *et al.* (2012) detected a significantly higher range of concentration variations (9982-13662 μg kg^{-1}). Concentrations of individual aflatoxins ranged from 603 to 11077 μg kg^{-1} (AFB1), 208 to 2876 μg kg^{-1} (AFB2), 24 to 5982 μg kg^{-1} (AFG1) and from 0 to 7798 μg kg^{-1} (AFG2). The production potential of isolates, originating from Makueni County, indicated significantly higher values of synthesised both total aflatoxins (22-55419 μg/kg) and individual aflatoxins: AFB1 (22-15139 μg kg^{-1}), AFB2 (0-3141 μg kg^{-1}), AFG1 (0-28728 μg kg^{-1}) and AFG2 (0-8411 μg kg^{-1}). These authors did not find out differences in increased synthesis of AFG1 in comparison to AFB1.

Okun *et al.* (2015) recorded significantly higher concentrations of the total aflatoxins (1742-1670000 μg kg^{-1}) synthesised by *A. parasiticus* isolates. Almost all isolates were capable to produce aflatoxins of the B and G group - there were just two isolates that synthesised aflatoxins only of group B.

Baquiao *et al.* (2013) established average concentrations of individual aflatoxins synthesised by this species in nutlets in Brazil: AFB1 - 6172 μg kg^{-1}, AFB2 - 184 μg kg^{-1}, AFG1 - 16.776 μgkg^{-1} and AFG2 - 313 μg kg^{-1}.

In Portugal, Rodrigues (2011) recorded that the majority of observed isolates (86%) showed a typical profile of the species *A. parasiticus*, meaning that they are strong producers of aflatoxins B1 and G1 and that they do not produce CPA. Nonetheless, studies showed that 8 isolates (4%) did not synthesise aflatoxins of the group G, as well as that 7% of isolates synthesised aflatoxin G1 in higher concentrations than aflatoxin B1.

Rodrigues (2011) recorded results obtained on the existence of isolates that did not synthesise aflatoxins. Horn *et al.* (1996), Vaamonde *et al.* (2003), Barros *et al.* (2006) pointed to *A. parasiticus* isolates that occurred naturally, but had no aflatoxin synthesis-ability, and ranged from 3 to 6%.

Studies carried out within the present paper indicated isolates that did not synthesise aflatoxins B1, B2, G1 and G2 or if they did the concentrations were very low and their values were impossible to determine. The existence of atoxigenic isolates of this species can be a significant gain for Serbia, since they are increasingly applied in the biological control of virulent fungal strains.

Conclusions

Gained results confirm the capability of this fungus to produce mycotoxins. Further studies are required to improve existing strategies for prevention and control of these economically important pathogens. Preventive actions and good practices at harvest can reduce the impact of *Aspergillus* ear rot on yield and grain quality. Certain isolates of *A. parasiticus* did not synthesize aflatoxins and their use as biological agents in plant protection should be estimated. If global climate changes persist to affect cereal-growing regions in Europe, this pathogen will further develop and the danger will be even greater. If climate changes could be precisely foreseen these changes and fungal infection and subsequent aflatoxin contamination could be accurately linked, then predicting and dealing with this emerging risk would be less difficult to quantify.

Acknowledgements

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COMPARATIVE EVALUATION OF MOLECULAR METHODS USED FOR FUSARIUM STRAINS IDENTIFICATION IN THE WEST PART OF ROMANIA

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Abstract

In a top of the most devastating fungi all over the world the fourth and fifth positions are occupied by fungi from the Fusarium genus. They produce toxins which cause significant quantitative losses in many agricultural crops and introduce into the food chain some dangerous toxins which are a serious threatening to food safety. The main plants affected are cereals, which are actually the most important source of food for both humans and animals. Therefore the development of reliable, fast and cost effective methods for *Fusarium* strain identification based on gene/DNA fragments evaluation is of great importance. In our studies, after fungal isolation from wheat samples collected from different locations, the DNA was extracted and purified, followed by specific amplification (PCR) or sequencing. First, the primers which amplify specific genes for *F. graminearum, F..culmorum, F..proliferatum and F. verticilloides* were used in a qualitative PCR. Following amplification of DNA from 58 single spore colonies with the *F. graminearum* species-specific primer, 39 positive and 19 negative results were obtained. The amplifications with the other specific primers were not successfully. Therefore another identification method was necessary. According to the literature data for the unknown samples the elongation 1 factor (TEF) genes were amplified, sequenced and compared with the databases *Fusarium*-ID. Therefore the precise identification was possible. It has been shown that some negative samples for the *F. graminearum* - specific primers are still part of this specie, indicating the possibility of genetic polymorphisms that degrade the binding sites of some primers. For a safe and accurate identification the sequencing for specific genes is recommended.

Keywords: *Fusarium, strain identification, molecular methods, DNA sequencing*

Introduction

In a top of the most devastating fungi all over the world the fourth and fifth positions are occupied by fungi from the *Fusarium* genus, namely *Fusarium graminearum* and *Fusarium oxysporum* (Dean *et al*., 2012). Also, of the 101 plant species of economic importance (www.apsnet.org/online/common/search.asp), at least 81 are associated with a disease caused by *Fusarium* sp. The climate change in recent years has had a strong impact on *Fusarium* species. Increasingly unpredictable oscillations of rainfall, humidity and temperature levels have led to an increase in plant vulnerability, an increase in pathogenicity, aggression, and toxicity of these fungi. All these changes have caused significant quantitative losses in agricultural crops, but also the introduction of strong toxins into the food chain, which is a serious threat to food safety. The main plants affected are cereals, which are actually the most important source of food for both humans and animals.

Mycotoxins produced by *Fusarium* sp., including deoxynivalenol, zearalenone and fumonisins, are considered dangerous food contaminant with hepatotoxic, nephrotoxic and neurotoxic effect. Acute toxicity results in the rapid installation of immediately visible and sometimes fatal manifestations, while chronic effects are more misleading and difficult to classify, resulting in serious economic losses caused by declining domestic livestock productivity.

Identification based on morphological traits is difficult, time-consuming and requires experienced specialists. For this reason, different methods of molecular analysis have been developed, which are fast with high accuracy (O'Donnell *et al.,* 2000, Aoki and O'Donnell, 1999; O'Donnell, 2004, Geiser *et al*., 2001, Ward *et al*., 2002).

The most common molecular technique is based on polymerase chain reaction (PCR), which is very useful in identifying and analyzing a particular region of the genome (Seifert, 2009). In contrast to traditional methods, this is very specific and allows identification of the pathogen even from impure crops. The selection of the region to be amplified is determined by three factors: DNA sequences should include a region highly conserved to allow the design of specific PCR primers; the same sequences have to show some differences in order to distinguish one species from another, and in the specialized databases should be sufficient information about the sequences (Geiser *et al*., 2004; Sampietro *et. al.,* 2010**;** Ioja-Boldura *et. al.,* 2012). Once the target region is selected, the samples can be amplified with species-specific primers - a positive amplification result means confirmation of the specie for which the primers were designed or in the direction of identifying the toxicogenic strains, the so-called chemotype can be determined.

Another method for molecular identification of Fusarium species is based on the DNA sequencing technique. This assumes the selection of a specific locus, considered as a marker in these identifications, which could be subjected to the sequencing (Geiser et al., 2004). Repeated attempts to find the most convenient marker allowed the discovery of the gene encoding the elongation factor EF-1 alpha which contains the information of an essential part of the protein biosynthesis translation mechanism. This turned out to be an excellent tool for identifying *Fusarium* species, and since September 2003 a database has been created containing TEF sequences of thousands of *Fusarium* strains. This information has been made available to researchers around the world through the Fusarium-ID site that currently contains 77 species, 1844 strains, 5558 sequences (http://isolate.fusariumdb.org/guide.php).

The aim of this paper was the development of reliable, fast and cost effective methods for Fusarium strain identification based on gene/DNA fragments evaluation.

Material and Methods

As biological materials 58 samples of wheat grains collected from different granaries were analyzed, collected in 2017 from different farms from the West part of Romania..

The grains, sterilized for 1 min with NaClO 3% and washed twice with sterile distillated water were transferred on PDA (Potato Dextrose Agar) medium and maintained at 25°C, with a photoperiod of 12/12 light/dark. After 10 days the growth mycelium have been used for development of a single-spore culture. For this purpose the serial dilution method described by Hansen & Smith (1932) was applied. A small amount of mycelium was introduced into sterile water and diluted until reaching the concentration of 10^2 cells/ml, or 4-5 macroconidia in a water drop, determined with the Burker counting chamber. The solution was transferred on PDA (Potato Dextrose Agar) medium and single spore were further on isolated and analyzed (Leslie *et al*., 2006). The mycelium required for DNA extraction was harvested after 5 days from the single-spore culture initiation. 100 mg of each sample was used for DNA extraction, using the Plant/Fungi DNA extraction kit (Norgen). The quality and concentration of DNA samples were evaluated by spectofotometric method (Nanodrop 8000) and standardized at 20 ng /µl.

Next, genes specific to each species of Fusarium were identified by amplification with specific primers (Table 1),

Table 1. The primer sequences and amplification conditions for *Fusarium* specie identification (Sampietro *et. al.*, 2010**)**

Fungal specie	Primer sequences 5'…3'	Primers annealing conditions (temp/time)	number of cycles	Amplicon size
F. graminearum	GTTGATGGGTAAAAGTGTG CTCTCATATACCCTCCG	53ºC/ 30sec	25	230
F. culmorum	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	65°C/ 30sec	25	450
F. proliferatum	CGGCCACCAGAGGATGTG CAACACGAATCGCTTCCTGAC	65°C/ 30sec	25	500
F.verticillioides	CGCACGTATAGATGGACAAG CACCCGCAGAATCCATCCATCA	65°C/ 30sec	25	700

In all cases, the reaction volume was 25 µl containing 50 ng of DNA. The reaction mixture was made according to the manufacturer's instructions with the KAPA2G RobustHotStart ReadyMix kit (KAPA BIOSYSTEMS, Boston, USA) and amplified with Mastercycler ProS (Eppendorf), The resulting PCR products were separated in 1.5% agarose gel at room temperature, with a voltage of 90 volts. After about 30 minutes the products were visualized and photographed in UV light (UVP, England).
In the second step of molecular analysis the gene sequencing was followed up. The primers used for the amplification of the TEF (elongation factor EF-1 alpha) gene were first developed by O'Donnell et al. in 1998, known as ef1 ($^{5'}$ATGGGTAAGGAGGACAAGAC $^{3'}$), and ef2 ($^{5'}$GGAAGTACCAGTGATCATGTT$^{3'}$). These primers amplify a gene region of approximately 700 bp, marking three introns that occupy about half of the TEF gene (Geiser et al., 2004). The composition of the amplification mixture was similar to that described above and the specific primers annealing temperature was 53ºC.
The amplification products were separated by 1.5% agarose gel electrophoresis, with a voltage of 100. The gel fragments containing the fragments of interest with the expected size of 700 bp were excised under UV light and purified using the PureLink kit, Quick Gel Extraction & PCR Purification Combo Kit (ThermoFisher Scientific). The concentration of purified DNA samples was determined by the spectrophotometric method (Nanodrop 8000, Thermo Scientific). The purified DNA was sequenced by Macrogen Company, Holand. Once the nucleotide sequences were established, it was inserted into the Fusarium-ID database as well as the NCBI Genbank for Fusarium specie identification.

Results and Discussion

The 58 *Fusarium* samples, originated from the single-spore culture colonies isolated and morphologically identified (Bozac *et. al.*, 2014), have been evaluated with the PCR technique, namely the extracted and purified DNA was amplified with the primers specific for different *Fusarium* species.
The isolated DNAs were subjected to amplification with the primers for *F. proliferatum*, *F. verticilloides* and *F.culmorum*, without positive results. Lack of amplification may indicate that the strains do not belong to the mentioned species or the chosen primers were not suitable for the studied material. Following amplification of the 58 DNA samples with the *F. graminearum* species-specific primers, 39 positive and 19 negative results were pointed out (Fig. 1).

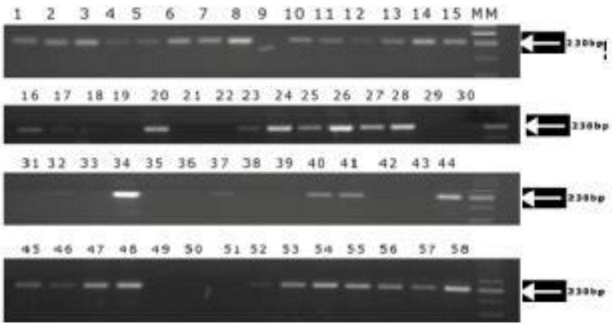

Fig. 1. The agarose gel (1.5%) electrophoresis for the *Fusarium* DNA samples amplified with *Fusarium graminearum* (Fg) specific primers

All the positive samples with the Fg primers which were previous identified as *Fusarium graminearum* based on morphological analysis have not been further analyzed. The 19 negative samples (9, 17, 18, 19, 21, 22, 29, 30, 31, 33, 35, 36, 38, 39, 42, 43, 49, 50, 51) were further evaluated along with 2 positive samples (37, 47) used as control.
The obtained results were compared with the previous morphological evaluations. Therefore, in the case of 14 samples from the negative ones, the morphological identification of the *Fusarium* species was not possible. Two of the samples (19 and 36) were identified as *Fusarium gramineraum* following morphological analyzes, with macroconidia with 5-6 septa with a tipical basal cell without microconidia, with pink/orange colour, even they were not amplified with the Fg primers. Other two samples (38, 42) were identified as *Fusarium equisety* with long, thin macroconidia, with a very long apical cell, dirty yellow colour and one strain was identified as *Fusarium avenaceum* (22), with long, slights curved, thin macroconidia, with orange colour.
For all of the 19 negative samples with the Fg primers together with the two samples considered as control the DNA was amplified with the primers specific for the TEF gene (ef1/ef2) (Fig. 2).

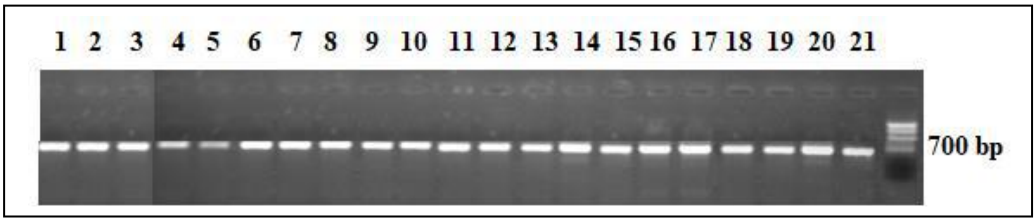

Fig. 2. The fragments amplified by the ef1/efr primers, seoarated by 1.5 agarose gel electrophoresis

Legend. 1- 9, 2- 17, 3- 18, 4- 19, 5- 21, 6- 22, 7- 29, 8- 30, 9- 31, 10- 33, 11- 35, 12- 36, 13- 38, 14- 39, 15- 42, 16- 43, 17- 49, 18- 50, 19- 51, 20- control 37, 21- control 47

All the fragments were excised from the gel, the DNA was purified and sent for sequencing to Macrogen company (Holand). All the sequences were compared with Fusarium Id and NCBI databases. The results, jointly with the morphological ones were presented further on (Table 2).
It was pointed that of the 14 samples for which the morphological identification of species were not possible, 6 belong to the F. *graminearum* species, even if amplification with Fg-specific primers did not occur. In addition, it was determined that 5 samples (9, 17, 39, 50 and 51) belong to the species *F. proliferatum*, one sample is *F. verticilodes* (49), one *F. subglutinas* (29) and one *F. andyiaze* (43). For two samples, morphological identified as *F.*

graminearum (19, 36) the specie was confirmed by sequencing, even if the amplification with Fg primers failed. For the samples which were morphological identified as *F. avenaceum* (22) and *F. equisety* (38 and 42) the results were correlated with the sequences evaluation.
For the samples used as control (37 and 47) all the evaluations methods had the same result - *F. graminearum.*

Table 2. Correlation of results obtained through morphological, PCR and sequencing evaluations

Nr.	**Sample**	**Fg amplification**	**The morphological identification**	**The molecular identification (TEF gene)**		
				Specie	**Similarity NCBI %**	**Similarity Fusarium ID %**
1.	**18**	-	*Fusarium* sp.	*F. graminearum*	99	99.68
2.	**21**	-	*Fusarium* sp.	*F. graminearum*	99	99.68
3.	**30**	-	*Fusarium* sp.	*F. graminearum*	99	99.28
4.	**31**	-	*Fusarium* sp.	*F. graminearum*	99	99
5.	**33**	-	*Fusarium* sp.	*F. graminearum*	99	98.5
6.	**35**	-	*Fusarium* sp.	*F. graminearum*	91	-
7.	**9**	-	*Fusarium* sp.	*F. proliferatum*	98	99.35
8.	**17**	-	*Fusarium* sp.	*F proliferatum*	99	96,58
9.	**39**	-	*Fusarium* sp.	*F. proliferatum*	99	99.38
10.	**50**	-	*Fusarium* sp.	*F. proliferatum*	98	98
11.	**51**	-	*Fusarium* sp.	*F. proliferatum*	99	99
12.	**49**	-	*Fusarium* sp.	*F. verticilloides*	99,2	99.2
13.	**29**	-	*Fusarium* sp.	*F. subglutinans*	91	-
14.	**43**	-	*Fusarium* sp.	*F. andyiaze*	98	98
15.	**19**	-	*F. graminearum*	*F. graminearum*	99	99
16.	**36**	-	*F. graminearum*	*F. graminearum*	99	99
17.	**22**	-	*F. avenaceum*	*F. avenaceum*	99	99
18.	**38**	-	*F. equiseti*	*F. equiseti*	99	99
19.	**42**	-	*F. equiseti*	*F. equiseti*	99	99
20.	**37**	+	*F. graminearum*	*F. graminearum*	98	98.5
21.	**47**	+	*F. graminearum*	*F. graminearum*	99	99

Conclusions

Our results pointed out that if in the first stage the results of the morphological analysis are confirmed by amplification with specific primers, species identification can be considered accurate. When morphological analysis did not have precise results, it is recommended to evaluate the sequences specific to the TEF gene. It is possible to get negative results for the amplification with species-specific primers because of polymorphism that can distort primers binding sites.
Therefore, molecular analysis could be considered to be an important tool in identifying *Fusarium* species, and sequencing the TEF gene and comparing results with international databases as a sensitive, specific, accurate and fast method.

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EVALUATION OF SPRAY QUALITY USING WATER SENSITIVE PAPERS AND SIMULATED TREE CANOPY

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Abstract

There are many problems due to remaining of excessive pesticide residues on agricultural products and risk of environmental pollution. Therefore, it is necessary to use new methods to enhance the spraying technique and quality. In this research, the number of droplet and volume of consumed chemical were evaluated for two sprayers (turbine and lance-nozzle) and two sizes of peach tree (small and large) using water sensitive papers and simulated tree canopy. Droplet diameters was considered in four categories as 0-150, 150-300, 300-450 and >450 micro meter. Data were analyzed based on randomized completely design by factorial test at five replications. Results showed that type of sprayer, droplets diameter and their interactions have significant effect on canopy size but volume of chemical used in large tree canopy was 16.5% more than small tree canopy. Also, in air blast sprayer the volume of chemical was about 101% and the droplet number was 17% more than lance-nozzle sprayer. Mean comparison of data showed that there is a significant difference between number of droplets and volume of chemicals at different particle size, as the maximum number and the minimum volume was obtained at 0-150 micro meter. Mean of total droplet number with turbine sprayer at range of 150-450 micro meters was 42.2% that considers about 84.7% of chemical volume. For lance-nozzle sprayer, mean value of droplet number at the same range of diameter was about 49.9% as it considers 46% of chemical volume. In general when we use turbine sprayer, the consumption is about 50% less than lance-nozzle sprayer.

Keywords: *Spraying, water sensitive paper, air blast sprayer, spray droplet diameter.*

Introduction

The most important component of food security in the community is healthy food. At present, in Iran, about 25 to 27 thousand tons of different types of pesticides are used annually at a level of about 16 million hectares (taking into account the number of application per unit area) (Plant Protection Organization, 2018). Spraying is effective in protecting the product, but the quality depends on the correct choice of sprayer type and how it works (Derksen *et al.*, 2010).
Use of water-sensitive paper cards to indicate or quantize the distribution of poison is one of the most commonly used methods in agriculture to measure the diameter and density of particles per unit area. These cards, which have a yellowish surface, become blue spots as a result of the collision of droplets of venom (Khot *et al.*, 2011). The color changed cards can be evaluated by image analysis (Cunha *et al.*, 2012; Zhu *et al.*, 2011a). Water-sensitive cards to determine the appropriate volume of poisons (Zhu *et al.*, 2011c), evaluation of intraocular canine venom concentration for the development of variable rate sprayers (Jeon *et al.*, 2011), spray output control for proper pest control (Zhu *et al.*, 2011a) or determining the pattern of poison distribution in aerosol sprayers (Fritz *et al.*, 2006). In a research, using the water-sensitive paper, the amount and distribution of solution droplets, the volume of spray solution, surface area, diameter of droplets and number of droplets on citrus trees were evaluated (Salyani *et al.*, 2013). Peyman *et al.* (2011) determined the number and diameter of pesticide particles by scanning water-sensitive cards using the artificial neural network. In a study, water sensitive card was used at three altitudes of the ground and four depths of tree canopy (Sanchez *et al.*, 2012). Zhu *et al* (2011b) evaluated portable scanners, surface coverage and

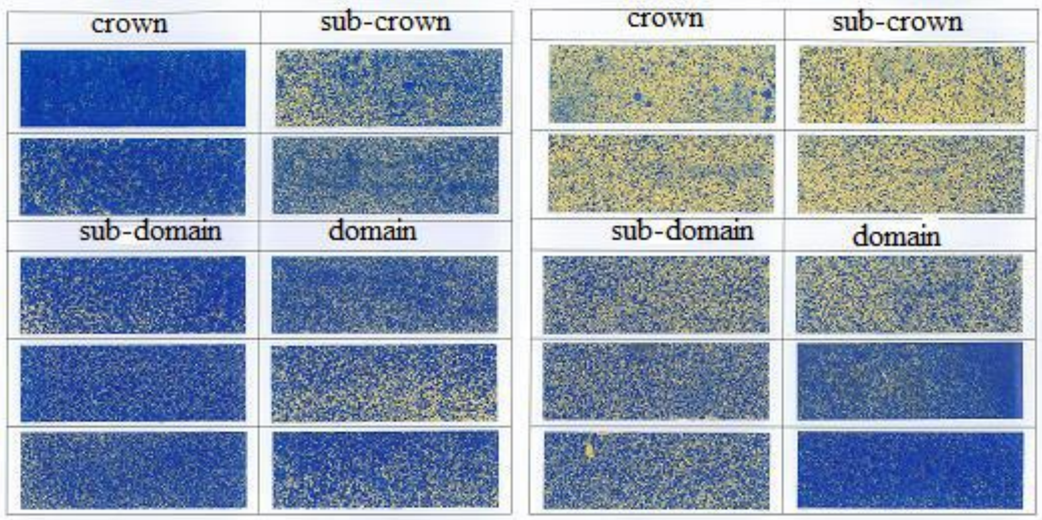
distribution patterns on water-sensitive paper. Fuentes *et al.* (2015) tested a blown air sprayer in a densely packed olive garden and found that the parameters of poison coating, the amount and distribution of poison droplets in different areas of the tree umbrella were affected by the volume of spraying and the intensity of the poison flow. Therefore, the aim of this study was to determine the volume, diameter and number of soluble particles per unit area using water sensitive papers and simulated tree canopy.

Material and Methods

Figure 1 shows spraying on the simulated tree canopy using lance-nozzle and turbine sprayers. The tractor was first placed in the right direction and about five meters before the tree plant. The engine speed was set at 2250 rpm and the pump pressure was 25 bars. The angle and amount of spray nozzles were regulated and controlled in the same way as spraying. Spraying five meters before the start of the tree and five meters later it was cut off. After the spraying was completed as a first repetition, after about 15 minutes, the cards were collected from the tree canopy and sorted.

Fig. 1. Spraying on the simulated tree canopy using two methods

All collected cards were individually scanned each time with a 1200 dpi professional scanner (CanoScanLide, Canon, Japan), and each group was stored in a special file. Due to the fact that the diameter and number of particles are different, four ranges of 0-150, 150-300, 450-300 and more than 450 microns for particles were defined. The scanned cards were separated by 5 x 5 mm (5000×5000 micron) using Photoshop image processing software environment and then magnified 10 times. After the segmentation, the number of particles was counted according to the defined range. Finally, the particles of the same domain were numbered in a special layer with a certain color and the information was recorded in the relevant tables. An example of collecting and sorting cards is shown in Fig. 2.



Lance sprayer Turbine sprayer

Fig. 2. Typical particle distribution on water sensitive paper for two methods of spraying

Results and Discussion

The particle number and volume per unit area

The number and volume of solution consumption per unit area, according to the type of tree and type of sprayer, are shown in Fig. 3. The comparison of averages indicates that the number of particles for tree type is not significant, but spraying method has a significant effect on the number of particles sprayed per unit area, so that the number of particles in turbine sprayer was found to be about 17% more than the lance type. This indicates that the number of particles in the surface area of water-sensitive cards is independent of tree canopy, but the turbine spray disperses more particles per unit area of tree than the lance sprayer. The number of particles in different diameters has a significant difference, so that about 83% of the number of particles in the range of 0-150 microns was observed and the lowest number was in the range of more than 450 micron.Due to the fact that in the turbine spray the intensity of the blown air for powdering and spreading the solution is used, therefore, most of the particles are found to have fewer diameters due to the air flow and collisions, resulting in a more uniform distribution of the solution per unit area.

There is a significant difference between the two types of sprayers in the large tree, with the highest number of particles per unit area when using a turbine sprayer and the smallest number of particles associated with the use of lance sprayer. The highest amount of soluble consumption for large tree and lance sprayer and the lowest for small tree with turbine spray were obtained. Since the turbine spray can withstand the turbine wind through a wide range of canopy trees, the size of the tree has no effect on the number of particles sprayed and the amount of solution consumption.

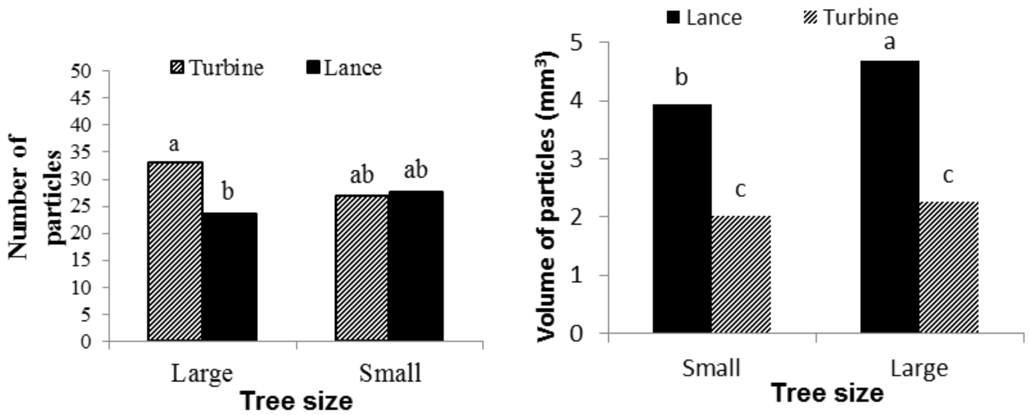


Fig. 3. Interaction of number and volume of particles on two size of tree and two types of sprayer

The highest and lowest amount of the consumed solution volume was for large trees and particle diameter greater than 450 microns and 0-150 microns, respectively (Fig. 4). The same result was obtained for lance sprayer (Fig. 5). About 1.4 to 2% of particles in a turbine sprayer have a diameter of more than 450 microns, which is not suitable for spraying. This value for lance sprayer in small trees is 11.4% and in large trees is 17.6%. The maximum and minimum number of solution particles was obtained for a turbine spray with a range of 0 to 150 microns and >450 microns, respectively (Fig. 5). One of the indicators of effective spraying is the finer content of sprayed particles, resulting in more toxins in the leaf area of the trees. Therefore, turbine sprayers are preferred over lance type. The difference of about 97% between these two particle sizes in the lance sprayer shows that most of the particles sprayed with these type of sprayers, are coarse and for this reason, when using them, the uniformity of particle spraying and their coating surface relative to the turbine type is much less. Hermosilla et al. (2012) reported that the use of turbine sprayers reduced solution consumption about 54% and subsidence into the canopy was about 40% more than lance sprayers. Fuentes et al. (2015) showed that in air blown sprayers spraying efficiency, the penetration into the canopy of the tree and the uniformity of spraying on the leaves are significantly higher than that of non-air assistant sprayers.

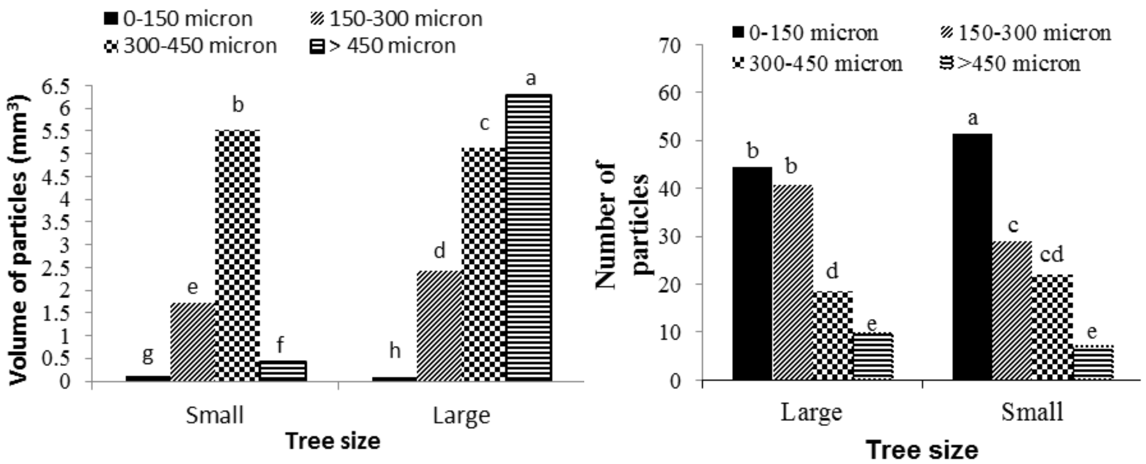


Fig. 4. Interactions of tree and sprayer type on the number and volume of particle on unit area

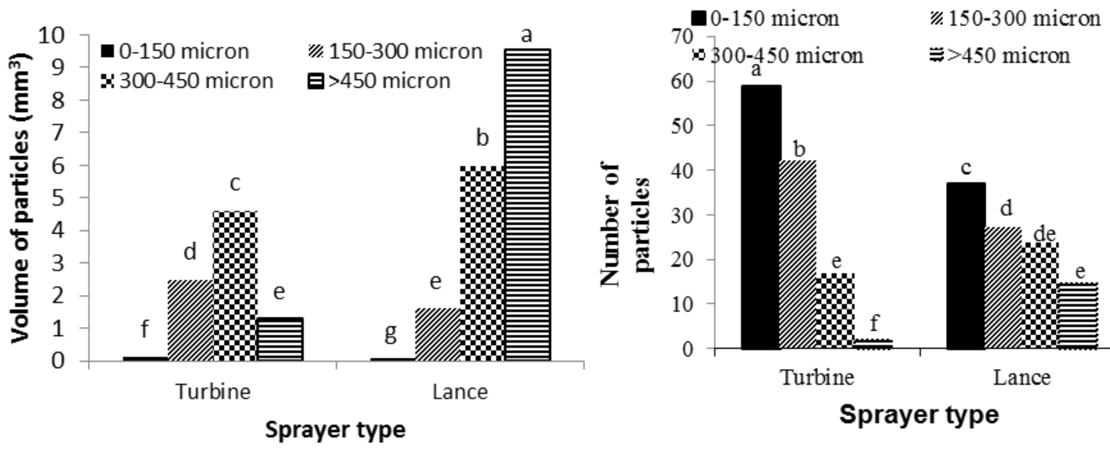


Fig. 5. Effect of sprayer type and particle size on volume and number of particles

Analyzing the effective range of particle diameter and volume

As we know, the most types of pesticides are mostly effective in the range of 150-450 micron diameter. By analyzing the diameter and particle size data, the cumulative percentage of each domain and the separation of the range of 150-450 microns with a range of less than 150 and more than 450 microns can be calculated and compared with the solution volume in these two domains (Fig. 6). Regarding the total number of particles on water-sensitive cards, it was determined that the total number of particles on the cards in small and large trees was 46.1% and 52%, respectively, with turbine spray in the range of 150-450 microns and this number was about 89.6% and 79.8% of the amount of solution consumed, respectively.

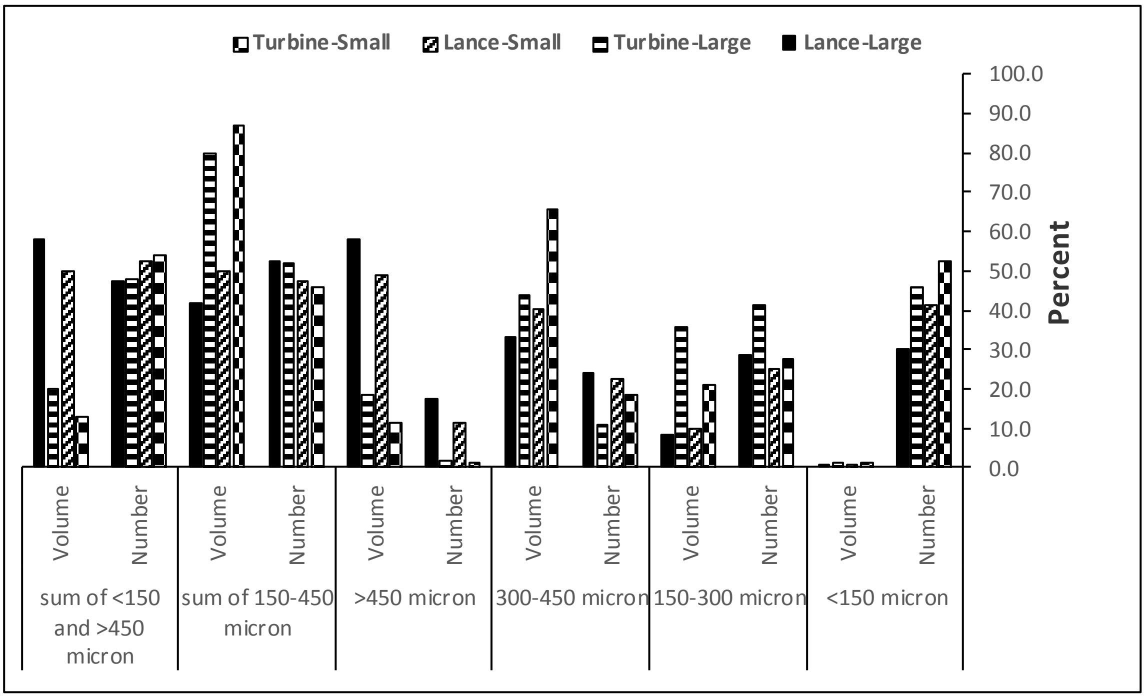


Fig. 6. Comparison of cumulative percentage of particle diameter

Conclusions

The type of sprayer and size of tree have significant effect on volume of chemical used in spraying. In turbine sprayer the number and volume of chemical droplet was more than lance-nozzle sprayer. More than 80% of chemical volume was at the range of 150-450 micro meters. For lance-nozzle sprayer, mean value of droplet number at the same range of diameter was about 49.9% as it considers 46% of chemical volume. In general when we use turbine sprayer, the solution consumption is about 50% less than lance-nozzle sprayer.

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TESTING ANTIBACTERIAL ACTIVITIES OF *SEMPERVIVUM TECTORUM*

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Abstract

Since their discovery in the twentieth century synthetic antibacterial drugs, penicillin, streptomycin and, other antibiotics have significantly influenced the reduction of the risk of contagious diseases. On the other hand, bacterial infections (respiratory and urinary tract infections, meningitis, sexually transmitted infections) are increasingly frequent in recent years, mainly due to the resistance of bacteria to synthetic antimicrobial drugs. In recent decades, there is an increasing need for natural and non-toxic antimicrobial substances, which is conditioned by the more frequent development of microorganisms resistance to synthetic antimicrobial drugs.

Natural preparations made from fresh plant material and biologically active compounds isolated from various plant species that have been used in folk medicine for centuries can represent valuable resources for the production of new natural remedies.

The aim of this paper is to test the antibacterial activity of *Sempervivum tectorum* on the tested bacterial isolates : pure *Sempervivum tectorum* juice, then in combination with honey and the action of the honey itself and to determine the type of action. The results of the work have confirmed the antibacterial activity of *Sempervivum tectorum* on seven clinical isolates, with a growth inhibition zone from 9.00 mm to 24.33 mm. Also, the obtained results show that *Sempervivum tectorum* represents a potential source of new compounds with antibacterial activity.

Key words: *Sempervivum tectorum, antibacterial, natural preparations*

Introduction

The use of plants in medicine and pharmacy instead of synthetic drugs has significantly increased in recent years. Phytotherapy is today most prevalent in underdeveloped countries, so it is estimated that between 60% and 80% of the world's total population relies on the use of herbs (Tucakov 2010). Medicinal herbs represent a significant source of medicines and medicinal raw materials (Harvey, 2000). Nowadays, thanks to modern methods and contemporary laboratory techniques, medicinal, aromatic and other useful plants, are being studied in more detail from all aspects in the search for new and better medicines and medicinal products. There are still completely or insufficiently unexplored plant species which leads to inexhaustible possibilities of modifying natural substances with polysynthesis in order to obtain compounds of better physiological and pharmacological properties. (Savikin *et al*., 2013,, Nanjan, 2010). The chemical constituents present in medicines of plant origin or plants themselves are part of the physiological functions of the plants and therefore they are believed to have better compatibility with the human body. Natural products from plants are rich sources of medicinal substances used for centuries to treat various diseases. It is well known that the use of synthetic drugs, in addition to their primary function, also has a secondary effect that may be even more dangerous than the disease which is treated. Herbal remedies contain natural substances that can promote health and alleviate the disease, have better patient tolerance and are relatively cheaper (Savikin *et al*., 2013, Sen *et al*., 2009;

Ramchoun *et al.,* 2009; Kamboj, 2000). Chronic administration of nonsteroidal anti-inflammatory drugs used to treat pain, high temperatures, inflammation, rheumatic and cardiovascular diseases can lead to gastric or duodenal damage and severe complications such as gastrointestinal bleeding and perforation (Kamboj, 2000). *Sempervivum tectorum* is a plant commonly known as houseleek, ayegreen, ayron, bullock's eye, hens and chickens, jupiter's beard, liveforever, sengreen and thunder plant. It is mainly grown as a decorative plant with medicinal properties. The name *Sempervivum* originates from the Latin word semper- to live, because the plant lives on the ground where most plants do not survive, it is very resistant to drought and high temperatures. (Muselin *et al*., 2014, Alberti *et al*., 2012, RHS 2008). It originates from Mexico, but it has been grown in our area for 9 centuries. It has been known since ancient times and is mentioned in the documents of Charles the Great (742-814) as "a plant that everyone grows on roofs of houses". It was believed to protect a house from fires and lightning struck. Because of this myth (lat. Tectorum- on the roof) it got this Latin name (Baum et al. 2005). Our people also believe that houseleek protects the house and household members from evil spirits so there is a saying that '' it's better to have a houseleek on the house than two watchdogs in front of it'' (Perić S. 2008). It is a succulent perennial terrestrial plant from the *Crassulaceae* family with a diameter of 10-20 cm and a strong root system. It has pinkish white flowers. Its leaves are fleshy, juicy and elliptical with a pointed tip. (Harvey, 2000, Muselin *et al*., 2014). Houseleek is widespread throughout southern Europe, especially in Peloponnese, Alps and the Dinaric Alps. In folk medicine, whole-fruit juice (*Sempervivum tectori succus*) or only leaf (*Sempervivum tectori folium*) is used against burns, herpes and ulcers. The leaves have an astringent and sour taste without odor. Houseleek in the form of a tincture is used as a homeopathic remedy against throat inflammation (Milovanović, 1975, Alberti *et al*., 2012). *S. tectorum* is a plant that is mainly used in folk medicine and its medicinal properties have not yet been fully explored. Its medicinal substances contain malic and formic acid, AHA acids, tannins, calcium malate, polysaccharides, flavonoids, alkaloids, as well as a smaller amount of resin, and there are some substances that are still unexplored (Karabegović *et al*., 2017., Blažević *et al*., 1992 a,b,c, Blažević *et al*. 1994.,Sentjurc *et al*., 2003). These ingredients have medical properties and houseleek is commonly used in the treatment of various conditions such as cysts, hemorrhoids, ear infections, diarrhea and urinary tract infections, ulcers (Rashid *et al*., 2014, Alberti *et al*., 2012., Bremness, 1996, Tierney *et al*., 2005). It is used in the form of juice, lining, ointment, tincture or tea. Due to its antibacterial, anti-inflammatory and antiseptic properties it is a great first aid to burns, insect's stings and bites, ulcers, and pain because it provides rapid relief and sedation. It is also used for removing warts and foot corns (Grieve 1994). *S. tectorum* is rich in flavonoids, which are responsible for its healing and beneficial effect in various inflammatory processes. Polyphenols isolated from the leaves of *S. tectorum* are the main agents responsible for antibacterial effects (Abram, 1999). Alkaloids act regenerative, palliative and as an anti-inflammatory, while tannins exhibit an adrenergic, antimicrobial, antipruritic, anti-inflammatory, antioxidant, and uv-protective property (Rovčanin *et al*., 2015, Khlifi *et al*.,2011., Šentjurc *et al* 2003., Abram and Donko, 1999.) Today, this plant is mostly used in the cosmetics industry for the preparation of sunscreen and after shave preparations, as well as in baths and shampoos. It is found in all anti-age and sunscreens preparations because it is rich in AHA acids. Houseleek has a long history of human use and there are no side effects (except it can be an emetic in high doses) or drug interactions and it is good against swallowing and water retention. The famous English herbalist Culpepper pointed out that *S. tectorum* is good for all internal and external inflammations, because its juice cools and reduces thirst and it is especially good in severe inflammation (eg erisipelas) and greatly relieves pain and gout. (Duke, 2002). In traditional medicine, *S. tectorum* is mostly used in otitis and ulcers, and therefore we have tested *S.tectorum* antibacterial activity on bacterial cultures isolated from the clinical material of humans and animals. *S. pseudintermedius* is the dominant type of coagulase-positive staphylococci on the skin and the main cause of pyoderma and otitis externa in dogs. Dermatoses represent a large percentage of dog diseases in veterinary practice. Uropathogenic *Escherichia coli* is the primary cause of urinary tract infections, while *S. aureus* is one of the main causes of inflammation of the throat, nose and ear. The aim of the study is to examine the antibacterial activity of *Sempervum tectorum* against clinical isolates of certain bacterial cultures. and to determine the type of action.

Material and Method

Sempervivum tectorum juice, domestic acacia honey and mixture of honey and *Sempervivum tectorum* juice were used as materials. *Sempervivum tectorum* juice is obtained by extracting juice from leaves that are very juicy in the summer. The mixture of houseleek and honey is made using the traditional recipe; 300g of leaves are chopped and mixed with 300g of honey. The mixture was left in a cold place for seven days with occasional stirring.

Test microorganisms

For antibacterial activity of *S. tectorum* the following seven bacterial cultures isolated from the clinical material (human isolates) were used to test antibacterial activity of *S. tectorum* : ß hemolytic *E. coli* (urine), coagulase positive staphylococci (throat), *E. coli* (urine) and *S. aureus*$_s$ (nasal blisters) *S. pseudintermedius*$_n$ (swab of a dog's ear), *S. pseudintermedius* (ureter swab) and one *Staphilococcus* spp. (swab from a dog's skin). Cultures were inoculated in a nutrient medium and incubated at 37^0C for 18h. Petri plates with the appropriate substrate (Müeller - Hinton agar) were inoculated with 0.1 ml of a bacterial suspension with a concentration of 10^5cfu / ml.

Test method

An agar diffusion method was used (Bauer 1966) by placing a metal cylinder of 9 mm in diameter on a solid sterile substrate (Müeller-Hinton agar-MHA). 100 μL of plant and honey mixture, 100 μL of *S.tectorum* juice, and 100 μL of honey were dropped on the disks by micropipet. A disc with 100 μL of 96% ethanol was used as a control. The ability of a strain to grow and multiply depends on its susceptibility to the tested substances, and if the effect is present there is a clear translucent zone formed around the disk in which there is no growth of microorganisms. Petri plates were incubated for 24h at a temperature of 37 ° C. Three repetitions were performed and results were obtained after 18-24h incubation by determining the inhibition zone diameter and the mean value was calculated.

Type of action

The type of action was also determined. To see if *S. tectorum* has bacteriostatic or bactericidal properties, a small amount of the agar was added from the zone of inhibition and added to the nutrient broth. Incubation was carried out at 37ºC for 24h. if after incubation the broth is clouded, the effect is bacteriostatic, that is, if the broth remains clear, the effect is bactericidal.

Results and Discussion

In the paper, the antibacterial activity of *S.tectorum* is examined in the form of mixture of plants and honey, honey itself, and pure plant juice.The results of the work are shown in Chart 1.

The results of this study showed that *S. tectorum* possesses a specific antibacterial activity against tested clinical isolates. Growth inhibition zone ranged from 9.00 mm to 24.33 mm. The mixture of *S.tectorum* and honey was antibacterial with an inhibition zone of 12.33 mm to 24.33 mm. The honey had antibacterial activity against tested bacterial isolates with inhibition zones of 10.00 mm to 23.33 mm.

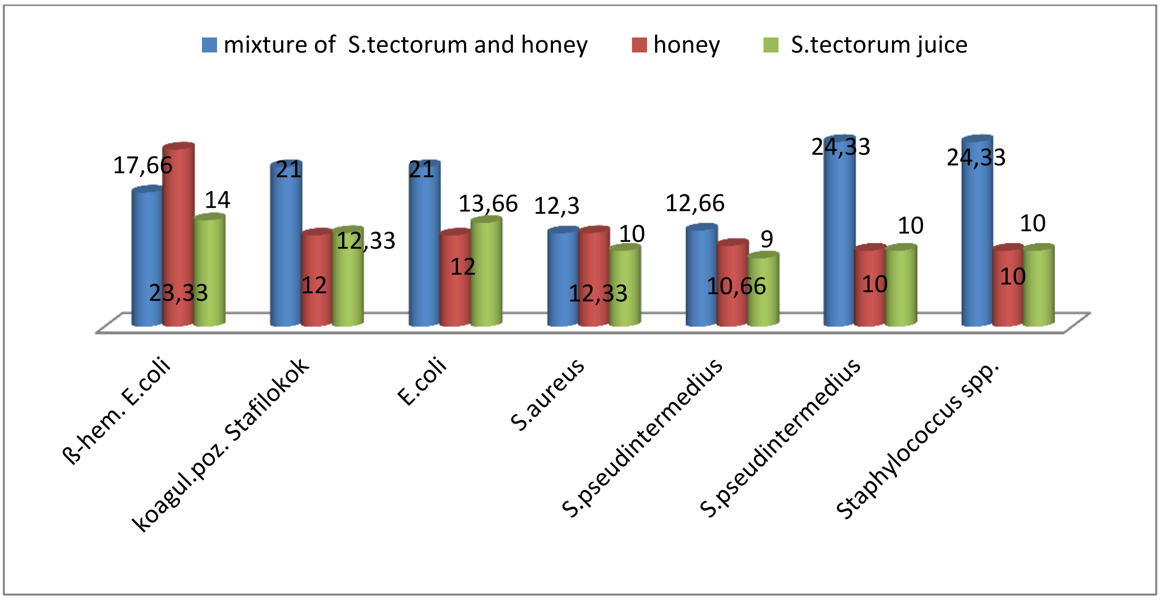

Chart 1. Antibacterial activity of Sempervivum tectorum to clinical isolates

It is evident that only ß-hemolytic *E. coli* honey showed stronger antibacterial activity with a 23.33 mm inhibition zone. The antibacterial activity of pure juice *S. tectorum* was quite uniform for *S. aureus*, *S. pseudintermedius*$_s$, *S.pseudintermedius*$_n$ and *Staphilococcus* sp. with an inhibition zone of 9.00 mm to 10.00 mm, while somewhat more pronounced antibacterial activity was observed in ß-hemolytic *E. coli*, *E. coli*, and coagulase-positive *Staphylococcus* with an inhibition zone of 12.33 mm to 14.00 mm. The results of the work are in accordance with the work of other researchers (Rovčanin et al., 2015, Gao et al., 2012, Abram et al 1999, Stojkovic et al., 2015) that examined the chemical composition and antibacterial activity of *S. tectorum*.

The results of the study justified the use of this traditional herbal remedy itself or in combination with honey for certain (otitis, gastritis, ulcer, dermatitis and the like) bacterial diseases.

Antibacterial activity of *S. tectorum* may vary from partial to full inhibition of bacterial growth, so it exhibits bacteriostatic or bactericidal activity. *S. tectorum* exhibited stronger bactericidal activity against tested clinical isolates, while the honey was more bacteriostatic, as shown in Chart 2

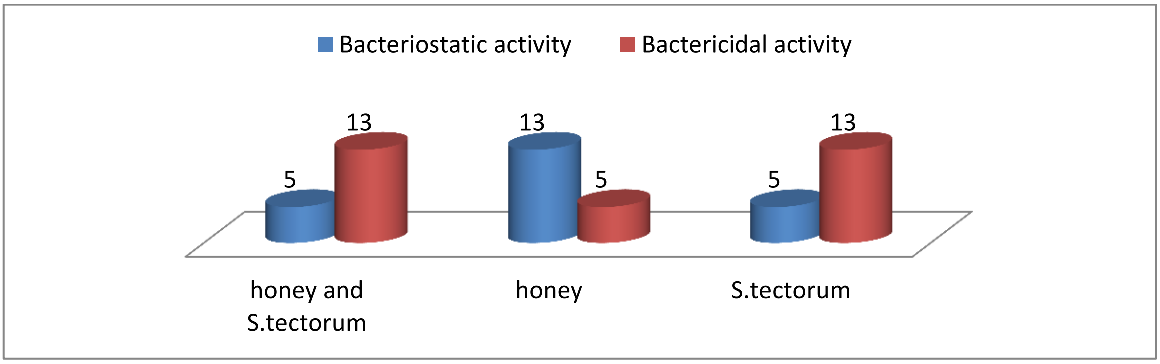

Chart 2. Bactericidal and bacteriostatic activity S.tectorum

Conclusion

The obtained results show that *Sempervivum tectorum* is a potential source of new compounds with antibacterial activity. Continued use of antimicrobial drugs has led to many bacteria becoming resistant and modern lifestyle has led to the emergence of a large number of stress-induced illnesses, which imposes the need for isolation and identification of antimicrobial compounds from plant sources that are pharmacologically powerful and have a low or no side effect of use in both medicine and the food industry. Increasing knowledge about phyto-constituents as biologically active substances and their inclusion in everyday use and nutrition can provide sufficient support to the human and animal body in combating many diseases. The present research is the basis for further testing of the biological activity of plants from the *Crassulaceae* family.

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EFFECTS OF ZINC OXIDE NANOPARTICLES ON *SOLANUM LYCOPERSICUM* L. AND VIABILITY OF *TENEBRIO MOLITOR* (TENEBRIONIDAE)

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Abstract

Recently, the number of greenhouse plants (cucumbers, strawberries, tomatoes) has grown. Pathogens become the main enemy in this system of cultivation. The main pests of tomato plants include insects. These pests cause fading of leaves and necrosis. Leaves become yellow, lose turgor and finally degraded. Moreover, some insects produce honeydew, which allows the development of fungal diseases. In view of the EU's rejection of the introduction of new genetic methods (CRISPR), it is necessary to look for new ways how to protect plants from pests and the associated sustainability, which is most important for agricultural production. The main objective of this work was to test nanocomposite materials and their toxicity for individual pathogens and the plant itself. In this work, we tested metal nanoparticles on the growth of Solanum lycopersicum L. and viability of model pathogen Tenebrio molitor. Basic biochemical analysis of stress markers was performed as part of NPs plant toxicity analysis. Mainly, the total content of carotenoids, polyphenols, flavonoids, chlorophylls, antioxidants was associated with abiotic stress. Samples were analyzed using ambient and chromatographic techniques with a mass detector, spectrophotometer with 96-well plate reader. During the experiment, we used a different concentration of Zinc oxide nanoparticles (ZnO NPs). Larvae T. molitor was sprayed with nanoparticles 3 times a week, and the greatest effect had the highest concentrations of ZnO NPs, where the mortality was 80 %.

Keywords: *Tomato, Zinc-oxide, nanoparticles, Tenebrio molitor.*

Introduction

Nanotechnology is one of the potentially effective options for significantly strengthening the global agricultural production needed to meet the future demands of a growing population. Nanoparticles, materials usually 1-100 nm in size, have a number of specific properties, based on their size, shape and structure (Jeevanandam, Barhoum et al. 2018). Zinc is one of the essential elements playing an important role in many plant pathways. Its deficiency in plants results in persistence of growth, sterility and susceptibility to phytopathogens (Duffy and Défago 1997, Helfenstein, Pawlowski et al. 2015).

Zinc oxide nanoparticles (ZnO NPs) belong to the most used nanostructures of metal oxide in practice and research (Mishra, Mishra et al. 2017). Due to their small size, ZnO NPs are better absorbed by the plants and depending on the used concentration ZnO nanoparticles can have both, positive or negative effects (Lin, Xing et al. 2008, Doolette, Read et al. 2018, Akanbi-Gada, Ogunkunle et al. 2019). Zinc oxide nanoparticles are widely used in many application in the agricultural industry (McClements and Xiao 2017) and can be used as potential pesticides in protecting plants from various pathogens, as well as fertilizers in foliar treatment of plants.

Tomato (*Solanum lycopersicum* L.) is the most widely vegetable in the global production system (Testa, Trapani et al. 2014). Belongs to the order of *Solanales* and family *Solanaceae*. Botanically, tomato is not a vegetable but is a fruit berry (Bergougnoux 2014). After potato, consumption of tomato surpasses all other vegetables, making it one of the most popular garden crops. During the last 10 years, area and production of tomato cultivation are continuously increasing. Major problems in tomato production are pathogens (insects, bacterias, viruses, fungi). *S. lycopersicum* L. contains primary metabolites (protein, sugar, lipids) and the number of secondary metabolites (vitamin A, ascorbic acid, antioxidants, alkaloids and polyphenols) which are considered to reduce the risk against cancer and cardiovascular diseases (Rao and Agarwal 2000).
The aim of the experiment was to test zinc oxide nanoparticles on larvae *T. molitor*, as potential insecticides, as well as testing phytotoxicity of ZnO NPs on tomato plants. Although *T. molitor* is not a primary tomato pest, it will be used as a model for our research.

Material and Methods

Preparation of Zinc oxide nanoparticle (ZnO NPs) solution:
Solutions of ZnO NPs were prepared by mixing distilled water and stock solution of the commercial ZnO NPs (Houston, USA) at five different concentrations (285 mg/L, 95 mg/L, 40,71 mg/L, 850 mg/L and 2 g/L). The size of the nanoparticles was 20-30 nm. Solutions were sonicated for 10 minutes before use.
Cultivation of tomato (*Solanum lycopersicum* L.) and larvae *Tenebrio molitor*:
Larvae and plants were cultivated separately. Larvae *Tenebrio molitor* were bought in an animal shop and were cultured under laboratory conditions in Petri dishes.
Tomato seeds were sown in Grodan® cubes size 2,5 x 2,5 x 4 cm. After two weeks plants were transferred to flowerpots with soil. Tomato was cultivated in cultivation boxes with the optimal condition. During the experiments, plants and larvae were sprayed three times per week with the aforementioned concentration of nanoparticles.
Preparation of plant samples for analysis:
After lyophilization, 5 mg of each sample was homogenized by homogenizer in 1 mL of 80% methanol. In the case of chlorophylls and carotenoids determination instead of methanol, 96% ethanol was used. Incubation was followed for 1 hour at 55 °C. Each sample was analyzed three times.
Determination of flavonoids:
The total flavonoids were determined by a colourimetric method (Zhishen, Mengcheng et al. 1999). Rutin (RE) was used as the standard. The result was expressed as an equivalent in mg rutin on 1 g of dry weight (DW).
Determination of polyphenols:
The phenolic content was detected by Folin-Ciocalteu assay, based on the reduction of a phosphowolframate – phosphomolybdate complex by phenolic compounds to form blue-coloured products (Koşar, Dorman et al. 2005). Gallic acid (GAE) was used as the standard. The result was expressed as an equivalent in mg/GAE/g DW.
Estimation of total antioxidant capacity (TAC):
The total antioxidant capacity of extracts was investigated by phosphomolybdenum assay, according to the methods published before (Alam, Bristi et al. 2013). Like a standard sample, Trolox was used.
Determination of chlorophylls (a and b) and total carotenoids:
Chlorophylls (a and b) and total carotenoids were measured spectrophotometrically at 470, 649, 665 nm. The extract of each sample (200 μL) was pipetted into the holes of the spectrophotometric plate. Chlorophylls a (C_A) and b (C_B) and carotenoids (C_Z) were calculated as (Hynstova, Sterbova et al. 2018):

C_A – *chlorophyl a* = (13,95 x absorbance 665 nm) – (6,88 x absorbance 649 nm)
C_B – *chlorophyll b* = (24,96 x absorbance 649 nm) - (7,32 x absorbance 665 nm)

$$C_Z - carotenoids = \frac{(\mathbf{1000\ x\ absorbance\ 470\ nm}) - (\mathbf{2{,}05\ x\ CA}) - (\mathbf{114{,}8\ x\ CB})}{\mathbf{245}}$$

Mortality of larvae *Tenebrio molitor*:
Mortality was measured by Henderson-Tilton's formula (Henderson and Tilton 1955).
Statistical analysis:
Each sample had 3 biological repetitions. All data were expressed as a mean of standard deviation. The data were determined by one-way ANOVA variance test followed by T-test at $p < 0.05$.

Results and Discussion

The effects of various ZnO NPs concentrations were monitored in this study. Differences in the synthesis of secondary metabolites in *S. lycopersicum* L. plants were observed by biochemical analyses, but larval mortality was observed with the naked eye. The plants were grown for 5 weeks. Application of ZnO NPs, took place a week with a subsequent one-day pause. Plants treated with the highest concentration of ZnO NPs 2g/L were the highest and had the largest leaves in comparison with other plants treated with smaller concentrations.
Effects of ZnO NPs on chlorophylls and carotenoids content:
ZnO NP showed a slight effect on the overall content of plant pigments in the treated variant compared to the control. Figure 1 (A) shows that the difference between the control sample and the sample treated with 95 mg/L ZnO is statistically significant ($p < 0{,}05$) with estimated concentration for chlorophyll a of 51.45 ± 3.91 and 66.62 ± 2.28, respectively. Other samples did not show a significant difference from control.
Figure 1 (B) shows the overall amount of chlorophyll b. The chlorophyll b concentration increased in the sample treated with 95 mg/L ZnO (27.58 ± 0.97) versus the control sample (20.27 ± 1.54) with a statistic difference ($p < 0{,}01$).
Singh, Singh et al. (2016) observed the effect of Zinc sulphate and ZnO nanoparticle to *Elaeagnus angustifolia*. They concluded that at lower concentrations ZnO NPs had a positive effect on the plant pigments production but the highest concentration resulted in a decrease. Statistically non-significant differences in carotenoid content were observed in all samples, as shown in Figure 1 (C). Similarly, the highest concentration of carotenoid shows a sample of 95 mg/L ZnO NPs compared to the control. Liu, Zabaras et al. (2009) monitored the carotenoid content of tomatoes under the influence of UV-C and found out that the carotenoid content was increased by the ageing of tomatoes and UV radiation. Faizan, Faraz et al. (2018) observed the effect of ZnO on photosynthetic activity, and results show that a concentration of 8 mg/L maximizes photosynthetic activity.

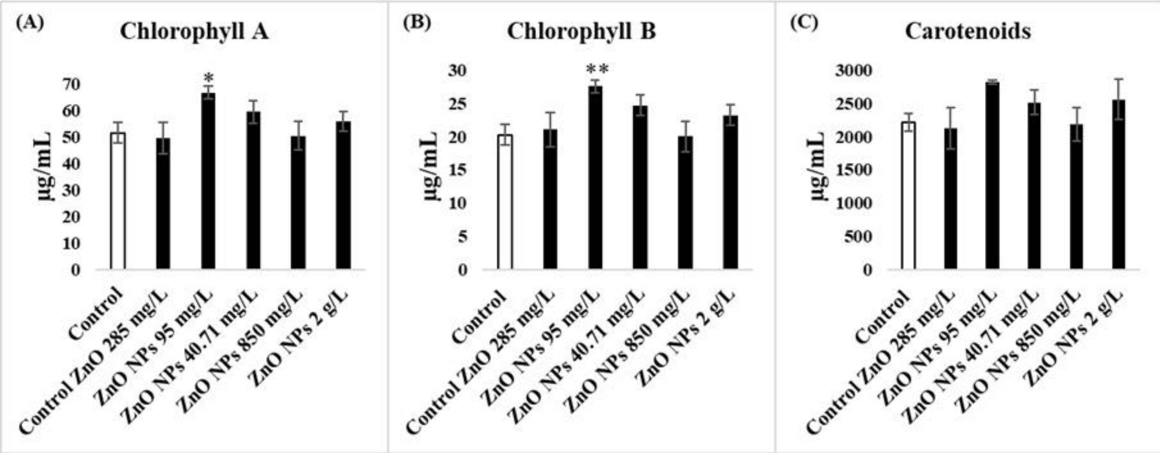

Figure 1 *Plant pigments treated by different concentration of ZnO nanoparticle A) Total chlorophyll a, B) Total chlorophyll b, C) Total carotenoids*

Effects of ZnO NPs on secondary metabolites (flavonoids, polyphenols and total antioxidant capacity):

The total amount of flavonoids, polyphenols and total antioxidant capacity was determined spectrophotometrically. The statistical analysis revealed a decrease in flavonoid content compared to control (Figure 2 (A)).

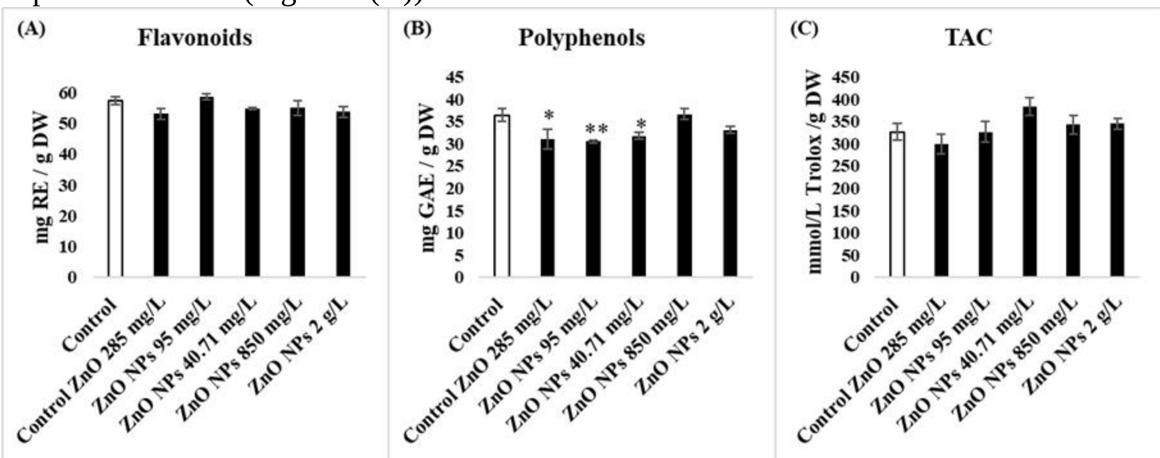

Figure 2 *Plant secondary metabolites by different concentration of ZnO nanoparticle A) Contain flavonoids expressed as mg RE g DW, B) Contain polyphenols expressed as mg GAE g DW, C) Total antioxidant capacity expressed as mmol/L Trolox g DW*

In the case of polyphenols content Figure 2 (B), statistical differences ($p < 0,05$) in polyphenols decreasing were found in the samples 285 mg/L (31.10 ± 2.24) and 40.71 mg/L (31.82 ± 0.80). The decrease was also in the sample of 95 mg/L (30.53 ± 0.41) with statistical differences ($p < 0,01$). Results of total antioxidant capacity were standardized on Trolox and expressed per mmol/L Trolox / g DW. Figure 2 (C) shows the TAC and here is seen that the nanoparticles do not lead to ROS (Reactive oxygen species) formation. Most of the samples had the same values as the control (327.16 ± 19.10). Pinheiro, Alegria et al. (2015) observed the effect of stress factors on the production of phenolic compounds and found out that greater stress leads to the production of secondary metabolites. Stress induces the formation of ROS in plants, which leads to an increase in antioxidant production (Ma, Wallis et al. 2014). Plants synthesize secondary metabolites that function to protect cells from oxidative stress.

Mortality of larvae *Tenebrio molitor*:

Nanoparticles have been successfully tested against a wide range of pathogens such as insects, bacteria and fungi (Benelli 2016). *T.molitor* larvae were used for the experiment because of their easy availability in the shops. They were cultured in laboratory conditions during 120h. During the experiment, larvae were sprayed three times a week with different concentrations of ZnO NPs and then mortality was monitored. During the first 48 hours of ZnO nanoparticles, they did not have any effect on the larvae. This can be explained, that the nanoparticles ZnO are slowly getting to the inside of larvae. The first effect of mortality occurred after 72 h from the first spraying. Total mortality during 120h is shown in Figure 3.

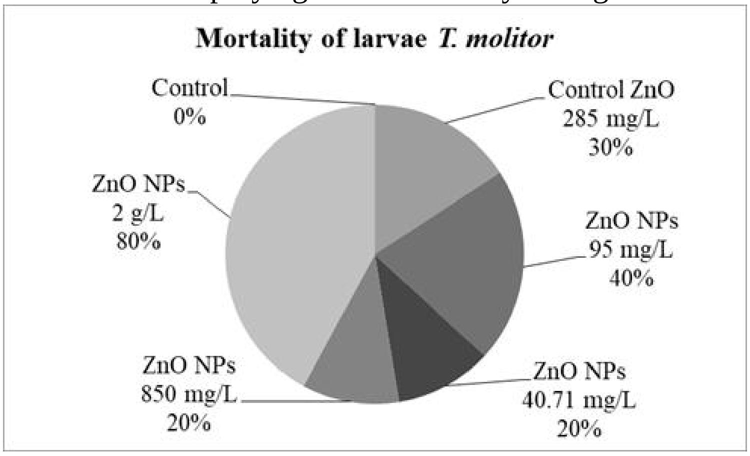

Figure 3 *Mortality of larvae Tenebrio molitor during 120h*

Mortality of the control sample, sprayed with water, during 120 h was 0%. Water was found to have no effect on the larvae, which was expected. The most significant effect had the sample treated by 2 g/L of ZnO NPs, where 80% of larvae died within 120h. Dead larvae had very dry skin, probably because of dehydration caused by the treatment with nanoparticles. Chakravarthy, Kandakoor et al. (2012) reported the inorganic silver nanoparticles and TiO_2 against *Spodoptera litura* Fab. under laboratory conditions had an insecticidal effect. Silica nanoparticles are equally effective and exhibit dose-dependent insecticidal activity. Insect dead bodies have been found to be extremely dehydrated and killed all *S. litura* larvae (Debnath, Mitra et al. 2012).

Conclusion

Nanotechnology is the emerging technology which found its wide application in the field of agriculture. ZnO NPs are gaining attention as pesticides and fertilizers because they are able to supply needed nutrients for the plant and also to protect the plant from the harmful pests. Further experiments, based on the use of nanoparticles will be performed and their efficiency toward different tomato pests will be explored.

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VEGETABLE OILS: DO THEY HAVE ANTIOXIDANT ACTIVITY?

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Abstract

The main aim of this paper is to start the discussion about the potential antioxidant activities of oil extracts and oils from various vegetable raw materials. Many publications reported that the seed oils and essential oils possess the high antioxidant activities. In our opinion it is not so. We want to share the results of own investigations and our view at this topic. We tested: a) oils extracts from seeds of apricot, grape, peach, pumpkin, fenugreek, white bryony, sea buckthorn and rosehip; b) walnut oils (eight samples of oil); c) essential oils from needles of juniper and pine. Antioxidant activities of oils and oil extracts were appreciated by two spectrophotometric methods: method based on model oxidation reaction of N,-diphenyl-n-phenylenediamine by azo-bis-(isobutyronitrile) as the initiator of free radicals; and procedure of DPPH free radicals scavenging. Trolox was used as standard antioxidant. Tested oil extracts from seeds did not show antioxidant capacities; on the contrary, they took part in reaction enhancing of free radical oxidation. Walnut oils demonstrated a neutral effect against free radicals. They did not enhance and did not reduce oxidative processes. Thus, the antioxidant capacities of walnut oils also were not identified. Freshly obtained essential oils from needles of juniper and pine possessed the antioxidant activity, which decreased by 2-8 times during one mount of storage. The oil extracts, vegetable and essential oils have others nutritional values and should not be considered as antioxidant. Moreover, to extend shelf-life they need to be fortified by antioxidants.

Keywords: *oil extract, vegetable, essential oil, antioxidant activity*

Introduction

Vegetable oils are valuable sources of high-calorie fats and essential fatty acids, phospholipids, carotenoids and other physiologically active components. Many publications reported that the seed oils and essential oils possess the high antioxidant activities (Poiana et al., 2009; Xie et al., 2015; Jorge et al., 2016; Akbari et al., 2018; Shinagawa et al., 2018). Taking into account the beneficial effects of antioxidants on human health, many researchers and manufacturers endeavour to find the high antioxidant activity of vegetable oils. In this regard, in 2007, the Armenian company Hagenas LLC asked us to study the antioxidant properties of oil extracts from various seeds, which were intended for sport nutrition. The results of these investigations were not so positive as to be shared in a wide public publications or commercial promotion. That is why we can write about it just now. Subsequent testing of other vegetable oils allowed us to confirm that the quality of the vegetable oils should not be focused on its antioxidant activity. Some authors, describing the qualitative parameters of cold-pressed oils from vegetable seeds, studied the antioxidant activity of methanolic extracts from the corresponding seeds, but no these oils (Kulaitiene et al., 2018), which is not the same thing. The purpose of this paper is to start the discussion about the potential antioxidant activities of oil extracts and oils from various vegetable raw materials based on our comparative researches.

Materials and methods

The oil extracts from seeds of apricot, grape, peach, pumpkin, fenugreek, white bryony, sea buckthorn and rosehip were produced by HAGENAS LLC (Yerevan, Armenia); and were stored during one year at 20±2ºC. In plus the samples of grape seed oils and beta-carotene oil solution were purchased from Moldovan markets.

The walnut oil samples were obtained by cold pressing using an electrical press (Model PSU-125) in laboratory of the Technical University of Moldova. Tatarov & Popovici (2014) described the general scheme of procedure for walnut oil extraction. The eight samples of walnut oil obtained in different times were tested.

The essential oils from needles of *Juniperus sabina* (juniper), *Pinus sylvestris* (scots pine) and *Pinus nigra* (black pine) were extracted by hidrodistillation using Ginsberg method. (Rassem et al., 2016) in laboratory of Institute of Genetics. Physiology and Plant Protection.

The chemicals: ethanol, N,-diphenyl-n-phenylenediamine, azo-bis-(isobutyronitrile), 2,2-diphenyl-1-picrylhydrazyl, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) of HPLC, spectrophotometric or analytical grade were supplied by Sigma-Aldrich.

Spectrophotometric methods for antioxidant activity determination of oils and oil extracts were used. One of them, the method that was proposed for standardization of individual and complex herbal remedies, including hydrophobic ones (Dadali &Dadali, 2015). The essence of the method consists in applying a model oxidation reaction of N,-diphenyl-n-phenylenediamine (DPPD) dissolved in dimethylformamide (DFA). Azo-bis (isobutyronitrile) was used as the initiator of free radical oxidation of DPPD. The result of the oxidation reaction is the formation of N, N-diphenyl-n-benzoquinone-diimine (DPBQD), which has a stable chemical structure and could be quantified at 450 nm. The reaction was carried out at a temperature of 60°C for 4-5 hours. Other method was widely reported procedure that based on scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Clarke et al., 2013) with absorption at 515 nm. Trolox was used as standard antioxidant substance and antioxidant activity was expressed in trolox equivalent (TE).

***The statistical analysis** of the results was carried out by EXCEL software. All assays were performed at least by triplicate.*

Results and discussion

During 4-5 hours of the reaction for oxidation of DPPD free radicals, the DPBQD formation and accumulation had the greatest approximation according the Beer-Lambert's law (R^2=0.951-0.998). The linear dependence of DPBQD absorbance on time, that very evident is shown in fig.1, allowed us to recalculate its content. The amount of accumulated DPBQD in reaction with tested samples was compared with amount of DPBQD accumulated in reaction without antioxidants (control). Thus, using the curves of absorbance the antioxidant activity of standard substance - trolox (fig.1a) and beta-carotene (fig. 1b) was calculated. The freshly prepared solution of trolox in concentration of 0.39 mg/ml inhibited 75–80% of free radicals, while the oil solution of beta-carotene in concentration of 0.01% scavenged only 5-10% of free radicals.

As shown in fig.1 the accumulation of DPBQD in reaction with Armenian oil extracts from grape (fig.1a) and fenugreek (fig.1b) seeds was proceeded at an increased rate. The tested oil extracts contributed to a greater accumulation of DPBQD or, in other words, they increased the number of free radicals involved in the reaction as an initiator of free radical oxidation. According our results, the oil extracts of fenugreek and grape seed enhanced of the reaction of free radical oxidation of DPPD to DPBQD by 3.6 and 2.4 times, respectively. Akbari with coauthors (2018) reported that the fenugreek seed oil possesses the high antioxidant activity. Index of antioxidant activity IC_{50} (concentration that inhibit 50% of free radicals) determined by DPPH radical scavenging procedure was equal to 172.6±3.1 μg/ml (Akbari et al., 2018).

This fact can be explained by excellent prevention of powdered seeds and extracted oil from air and light oxidation before determination of antioxidant activity. Moreover, the oil extraction was performed so that the polyphenolic content of oil (38.97±0.34 mgGAE/g) was analogic to water-ethanolic extracts of fenugreek seed (Melikyan et al., 2008). It is known (Kulaitiene et al., 2018) that antioxidant activity directly depends on the content of polyphenols in vegetable extracts, but it is necessary to indicate that these substances are soluble in water (no fat soluble).
Many authors (Shi et al., 2003; Ivanova & Casian, 2008; Ross et al., 2011) described the rich content of polyphenols in grape seeds. However, the property of grape seed oils to exhibit the antioxidant activity (from 53.41 to 205.52µM TE/100g) was saved through maintaining the samples at temperature -20°C into amber glass bottles and under nitrogen gas (Shinagawa et al., 2018). The highest values of antioxidant activity and polyphenols amount were obtained foe the fresh unrefined grape seed oil (Poiana et al., 2009). The pumpkin seed and orange seed oils after extraction were also stored under nitrogen gas at -18°C and oils with liquid nitrogen were analyzed (Jorge et al., 2016; Kulaitiene et al., 2018), which is not quite suitable for public markets.

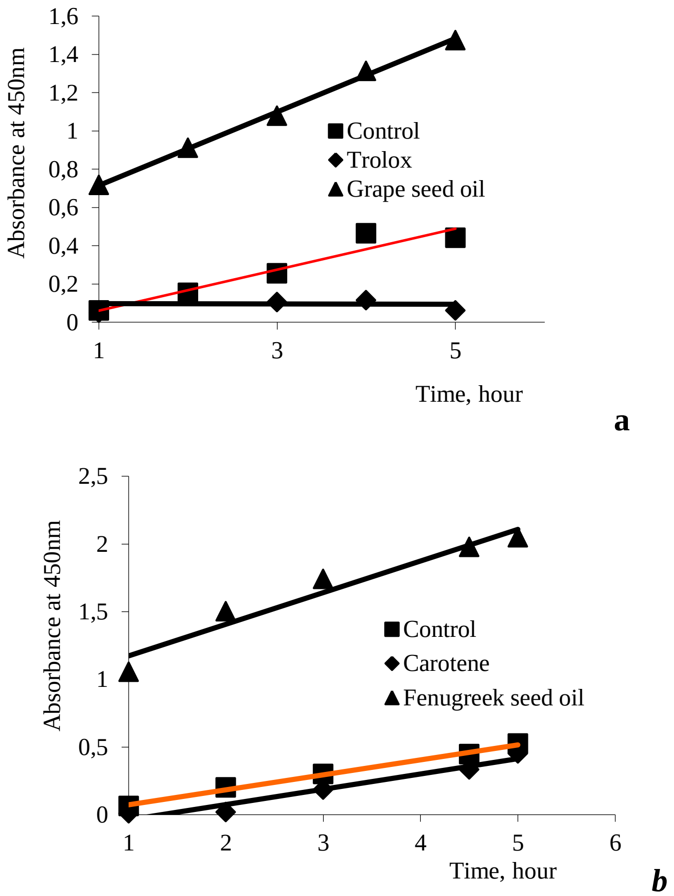

Figure 1. Dependences of DPBQD absorbance on time in reactions without antioxidant (control), with antioxidant trolox (a) and in presence of oil extracts: carotene (b), Armenian grape (a) and fenugreek seeds (b).

The other tested by us oil extracts from seeds also exhibited no antioxidant properties. Taking into account their capacities to increase the reaction of free radical oxidation (an increase by calculated % compared to control) the oil extracts from seeds can be represented by the following sequence: fenugreek (363%) > grape (Armenia) (238%) > white bryony (229%) >

sea buckthorn (138%) > rosehip (136%) > pumpkin (97%) > peach (65%) > grape (Moldova) (39%) > apricot (25%). Thus, the studied oil extracts from various seeds took part in the reaction and contributed to the enhancement of free radical oxidation of the reaction components.
The presence of walnut oils (it were tested eight samples of oil) in the reaction mixture did not significantly increase or decrease the oxidation processes (fig.2). Three samples showed the radical scavenging activity equal to 7.14-13.39%, two samples increased the content of oxidation products by 14.29-16.07%, and three samples practically did not differ from the control (0.89-1.79%). This fact indicated that the presence of walnut oil does not interfere with the normal course of the reaction. Since the values of radical scavenging activities, constituting less than 20% in this method are not statistically significant, it can be argued that walnut oils do not exhibit antioxidant activity. Therefore, it was showed, that these oils were unstable during the storage (Tatarov et al., 2017), and to increase their shelf life they needed to be fortified with some antioxidant ingredients.

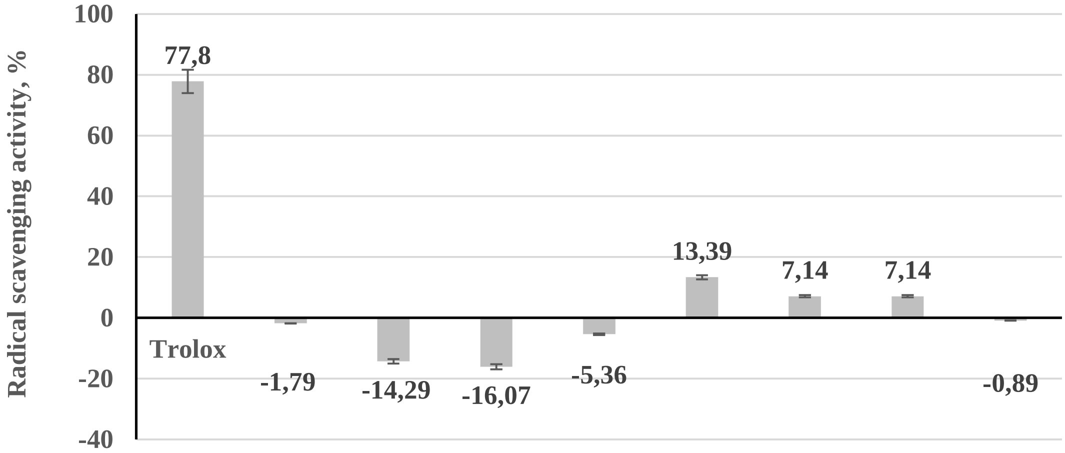

Figure 2. Radical scavenging activity (%) of trolox and various samples of walnut oil (total 8) determined by DPBQD method

It necessary to mention, that the main components of chemical composition of vegetable oils are saturated and unsaturated fatty acids, which easily are exposed to oxygen oxidation and according their chemical structures could not act as antioxidant. Protection against oxidation of oils is achieved by the action of antioxidants both natural (γ-tocopherol, β-carotene, lycopene) and synthetic (butylated hydroxytoluene), which are soluble in lipids. Influences of γ-tocopherol content on resistance during storage of vegetable oils were reported (Guseva et al., 2010; Radvzievska & Melnik, 2016). Moreover, the dependences of antioxidant activity values on total content of antioxidants (γ-tocopherol and carotenoids) are the extreme character and can be described by the function of the standard normal distribution. Because of that, the maximal antioxidant activity demonstrates only in certain concentration intervals of antioxidant substances contained in vegetable oils (Lobaeva et al., 2004).
Index of antioxidant activity IC_{50} for trolox determined by DPPH method was equal 140.02±0.24μg/ml. The essential oils from needles of juniper, scots and black pine collected in different areas of Slovak Republic (SR) and Republic of Moldova (RM) possessed the antioxidant activity. The antioxidant activities of tested essential oils expressed in trolox equivalent (mgTE/g) were equal to: 1.79±0.08 for *Juniperus sabina* (SR), 1.57±0.07 for *Juniperus sabina* (RM), 1.20±0.03 for *Pinus sylvestris* (RM); 1.63±0.02 for *Pinus nigra* (RM).

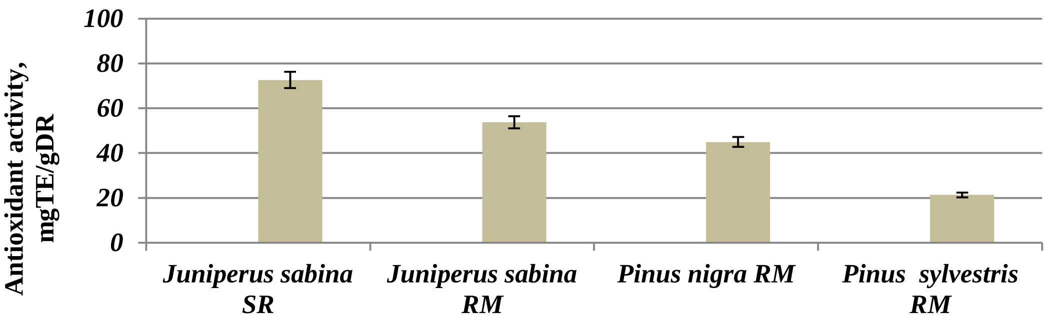

Figure 3. Antioxidant activity in trolox equivalent of water-ethanolic extracts from needles of juniper and pine determined by DPPH method

The essential oils from needles of six China endemic Pinus taxa exhibited antioxidant activity, which authors name as “acceptable” (Xie et al., 2015). However, they reported that antioxidant activities of pine oils were lower than some popular plant species such as Camellia sinensis and Oenocarpus bacaba. Low antioxidant activity of essential oils from different parts of Juniperus spp. was been explicated by specific constituents of the oil, which can be inactive in DPPH test, and the trace amounts of compounds that act as antioxidants (Emami et al., 2010). Samusenko A.L. (2011) concluded that the antioxidant activity of essential oils in a complex way depends on oils constituents, concentration and ratio between the most active components.

In our experiments, the trolox equivalents of essential oils were 17-40 times less than analogical indexes of water-ethanolic extracts from the same vegetal materials (fig. 3). It should be to mention that the storage of essential oils during one mount led to decreasing 2-8 times of their antioxidant activity. Due to the high content of no antioxidant biologically active constituents (for example α－pinene), the juniper essential oil, as natural product, has a perspective in ecological agriculture as insecticide against harmful insect species ((Elisovetcaia & Brindza, 2018) in medicine as a potential anti-tumor drug, in food and cosmetic industries as donor of aroma and odour (Salamon & Petruska, 2017).

Conclusion

The high nutritional and biological value of vegetable oil extracts and essential oils is due to the content of biologically active components, such as polyunsaturated essential fatty acids, terpenes etc., which do not act as antioxidants. Some vegetable oil extracts contain fat-soluble antioxidant compounds (tocopherols, carotenoids) which are useful and at the same time consumed to prevent oils from oxidation. Because of this, many long-stored vegetable oils lose or do not possess antioxidant capacity. To extend the shelf life of vegetable oils, they must be enriched with fat-soluble antioxidants in concentrations and ratio determined for the predicted shelf life

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HAZARDS RELATED TO FOOD SAFETY

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Abstract

Today, it is clear that the concept of food production and food safety must be dealt with together. It is not possible to talk about a commercial success when food safety is not applied. Reliable food cannot be obtained at all stages of production unless necessary control measures are taken. In this case, public health hazards can enter the food chain from many different points and may adversely affect production. Therefore, the entry point or points of each hazard should be determined and necessary precautions should be taken at these points. Contagion occurring at one point in the food chain also affects the next stage of production. Thus, even if the other steps of the food chain are successful, the result is negative. What is the concept of food chain, which has an important place in food safety? 'Farm to table' 'or' 'from field to fork' expressed in the food chain until it covers all the processes available to consumers, starting from primary production. Chemical contaminants (industrial residues, remnants of pesticides, remnants of veterinary drugs), biological agents (bacteria, viruses, parasites) and physical contaminants (nails, glass pieces, stones, bristles, etc.) effect chain process negatively. It impairs food safety and endangers public health. In this review article, it is aimed to give information about the physical, chemical and biological hazards that threaten food safety.

Keywords: *Food Safety, Public Health, Physical Hazards, Chemical Hazards, Biological Hazards.*

İntroduction

Food safety hazards are a preventable public health problem that cause foodborne diseases. Because of these hazards, diseases and deaths occur every year around the world. Food safety hazards are classified as chemical, biological and physical hazards. Food-borne diseases occur as a result of consuming foods that contain these hazards. In excess of 250 distinct kinds of infections, microbes, parasites, poisons, metals and prions are related with foodborne diseases in people. In spite of the fact that infections are in charge of over half of foodborne maladies, hospitalizations and passings are normally brought about by bacterial specialists. Indications shift from gentle gastroenteritis to perilous neurological, hepatic and renal disorders (Nyenje & Ndip, 2013). Albeit a large portion of the foodborne diseases cases are mellow and constrained in zones, genuine cases can result in high mortality and morbidity in unsafe gatherings. High hazard bunches for foodborne ailments incorporate babies, youngsters, the old and immunosuppressed people. The battle against sanitation risks is tested by the globalization of the nourishment advertise, environmental change, and the adjustment in human utilization, for example, the decision of new and negligibly prepared sustenances (Schelin *et al*., 2011). Food safety hazards negatively affect general wellbeing and the national economy. Food safety hazards and associated outbreaks can lead to the closure of food outlets or the food industry, resulting in job loss for employees. Thus, individuals and communities can be adversely affected. Local foodborne diseases episodes can turn into a worldwide danger. Foodborne diseases flare-ups are as often as possible revealed at national and universal level, underlining the significance of sanitation (WHO, 2011). The most evident food safety hazards are protozoa, viruses and bacteria. Other food safety hazards incorporate compound chemical residues and physical contaminants. Elements that assume a job in the study of disease transmission of rising foodborne issues incorporate changes in pathogens, advancement, urbanization and new ways of life, issues in wellbeing frameworks, existing learning, convictions and practices, statistic structure, travel and movement, sustenance and creature exchange, neediness and contamination (Magkos *et al*., 2006). Foodborne problems are likely to become even more important in the coming years.

Biological Contaminantions

Bacteria

Numerous normal instances of the runs are brought about by bacterial diseases brought about by ingestion of contaminated food. Aversion of these illnesses should concentrate on great individual cleanliness by all food processors (food chain), including the sustenance buyer. Some bacterial illnesses, for example, Bacillus anthracis, bovine tuberculosis and brucellosis, are especially identified with animal foods. (Bari & Yeasmin, 2018). Some bacterial agents related to food safety hazards are described below.

Escherichia coli O157 is a pathogenic microorganism called enterohemorrhagic *E. coli* (EHEC). This microorganism produces toxins known as verotoxins. Dairy cattle are the principal reservoir. Contamination to humans occurs with consumption of crude or half-cooked meat items and contaminated foods such as raw milk. Freshly squeezed apple juice, yogurt, cheese, salad and cooked corn are shown as a wellspring of contamination. Fecal contamination of water and food, cross-contamination during food processing, person-to-person contact can lead to infections. Symptoms include bloody and non-bloody diarrhea. It additionally causes chronic difficulties, for example, hemolytic uremic syndrome (Ferens & Hovde, 2011).

Listeria monocytogenes have been isolated from a variety of environments such as rotting vegetation, soil, animal feed, sewage and water. Impervious to various ecological conditions. It can develop at low temperatures up to 3 ° C. Milk and cheese can be found in a wide assortment of crude and handled foods such as meat (including poultry) and meat products, seafood and fish products. Even in storage conditions, it can survive and survive (Ajayeoba *et al*., 2016). L. monocytogenes is in charge of contaminations of immunocompromised individuals, including pregnant ladies, babies and the older. Initially, it causes meningitis, encephalitis or septicemia. At the point when pregnant ladies are tainted, they can cause premature birth, stillbirth or preterm labor (Bari & Yeasmin, 2018).

Most sporadic diseases with *Campylobacter jejuni* are related with the utilization of defiled poultry. Some *C. jejuni* episodes are related with crude milk or chlorine-free water utilization. Campylobacteriosis can prompt Guillain-Barré disorder, a reason for pendulous loss of motion (McCarthy & Giesecke, 2001). Poultry, cattle, pigs, sheep, rodents and birds are reservoirs of *C. Jejuni* (Whiley *et al*., 2013).

Viruses

Fecal-oral diseases can sully people from tainted foods. Foodborne viral contaminations for the most part have a brooding time of 1 to 3 days. They cause self-restricting sicknesses in people (that is, they recuperate normally), yet extreme diseases and even passings can once in a while happen. Rotavirus is a typical reason for spewing and watery looseness of the bowels in the gathering of viral diseases that reason viral gastroenteritis (VGE). Lack of hydration is a conceivable result except if proper rehydration treatment is utilized (Bari & Yeasmin, 2018). Some viral agents related to food safety hazards are described below.

Viral hepatitis brought about by hepatitis A and E infections is transmitted by the fecal-oral course. Hepatitis A differs from other viral agents due to its long-term incubation period (2-6 weeks), its capacity to spread to the liver outside the stomach and digestive organs. It usually causes jaundice. It can sometimes cause chronic liver dysfunction. Hepatitis E infection

(HEV) more often than not enters the body with crude shellfish polluted with water or foods, particularly waste water (van der Venter, 2000).
Noroviruses are a gathering of infections that reason "stomach influenza" or gastroenteritis in people. Most foodborne norovirus infections have been related with direct pollution of food by a sustenance processor just before utilization (Achson *et al*., 2002). Noroviruses are found in feces or vomits of infected persons. It is transmitted to people when expending foodpolluted with norovirus, contacting surfaces or items tainted with norovirus, and after that carrying the hands to the mouth, direct contact with someone else who is contaminated and symptomatic. Noroviruses can make due on practically any surface, including entryway handles, sinks, handrails and dishes. It is progressively regular in winter and influences all age gatherings (Bari & Yeasmin, 2018).
Rotavirus is a noteworthy reason for extreme looseness of the bowels (gastroenteritis) in youngsters, particularly in creating nations. Approximately 95% of children develop infection until the age of 5 (Festini *et al*., 2010). It is estimated that around 600,000 children die annually worldwide due to rotavirus infection. Mortality rates are high in India, Nigeria, China, Pakistan, Congo, Ethiopia and Bangladesh (Bernstein, 2009). Rotavirus infection is clinically more important in children, usually between the ages of 3-35 months, and the first infection is the most severe. Rotaviruses are exceedingly infectious and are frequently transmitted by the fecal-oral course. Since the infection is steady in the earth, disease may happen by individual to-individual contact or by contact with food, water and sullied surfaces. Rotavirus disease is believed to be restricted to the gastrointestinal tract. However, rotavirus has also been reported in nasopharyngeal secretions (Azevedo *et al*., 2005).

Parasites

There are three primary classes of parasites that can cause infection in people. These include protozoa, helminths and ectoparasites (Bari & Yeasmin, 2018). Some parasitic agents related to food safety hazards are described below.
Toxoplasma gondii is delegated protozoa and its essential hosts are felines. Human infection occurs in contact with cat feces. What's more, rodents can be delivered by crude or half-cooked meat from meat from middle has, for example, pigs, dairy cattle, goats, chickens and feathered creatures. Toxoplasmosis in people for the most part creates mononucleosis-type manifestations, yet buttran-splacental disease can cause fetal passing on the off chance that it happens from the get-go in pregnancy. In immunocompromised people, contamination may cause pneumonia, myocarditis, meningoencephalitis, hepatitis, chorioretinitis, or mixes thereof. Cerebral toxoplasmosis is every now and again found in AIDS patients (van der Venter, 2000).
Taenia Saginata (Bovine tapeworm) is a helminth that is a causative agent of tapeworm disease (Gebrie & Engdaw, 2015). Youthful types of tapeworm are found on infected herbs. When these herbs are consumed by the animals, the eggs contaminate the animals and tapeworms develop in the muscles of the animals. People become contaminated when they eat crude or half-cooked meat. Grown-up tapeworms create in the small digestive system of humans, and parts of tapeworms containing eggs are transmitted to the environment by human feces. The cycle continues in this way (Qekwana *et al*., 2016).

Chemical Contaminantions

Chemical contaminants can be found in buildups of pesticides and veterinary drugs, defilement from natural sources (water, air or soil contamination), cross sullying or pollution during food handling, relocation from bundling material to food and/or utilization of unapproved food additiv (Bari & Yeasmin, 2018).

Pesticide Residues

The utilization of pesticides, for example, bug sprays, fungicides and herbicides has turned into an essential piece of present day horticulture to improve harvest yield and quality by controlling different vermin, infections and weeds (Maksymiv, 2015). The enlistment of new pesticides is a carefully controlled procedure that evaluates their poisonous quality and ecological effect and decides the most extreme remaining points of confinement (resiliences) in crude and/or prepared items (Damalas & Eleftherohorinos, 2011). There are more than 1400 pesticides known. Some of them should not be used because they threaten public health. Some should be used within certain limits. Older pesticides should be reassessed based on available scientific data (Bari & Yeasmin, 2018).

Veterinary Drug Residues

Veterinary drugs are synthetic substances that have experienced an extensive enrollment procedure bringing about the change of greatest buildup limits/resiliences in sustenance of creature root. Significant classes of veterinary medications incorporate anti-infection agents, anthelmintics, coccidiostats, nonsteroidal calming drugs, tranquilizers, corticosteroids, beta-agonists and anabolic hormones. These medications directed to live creatures may stay as buildups in creature tissues (Bari & Yeasmin, 2018). Liver and kidney natural capacities are touchy to the remaining parts. A few anti-infection agents, for example, penicillin, can cause serious unfavorably susceptible responses in delicate people. This is a significant explanation behind deciding buildup restricts in creature determined food. Another significant purpose behind constraining the utilization of anti-infection agents in creatures utilized in food generation is to decrease the danger of pathogenic microorganisms being impervious to anti-microbials. Most veterinary prescriptions don't have intense toxicological concerns, yet a few substances, for example, nitrofurans, chloramphenicol, clenbuterol and diethylstilbestrol are restricted in numerous nations since they are cancer-causing (Mohsina, 2011).

Environmental Residues

Synthetic substances, for example, dioxins, chlorinated biphenyls, furans and overwhelming metals can contaminate the earth because of modern exercises (Kataria *et al.*, 2015). From nature, these synthetic substances can enter the evolved way of life through plants or creatures and cause different medical issues. These are viewed as issues that emerge in nations that are in the beginning times of industrialization. Instances of natural contaminants entering the evolved way of life incorporate overwhelming metals, polychlorinated biphenyls (PCBs), "dioxins" (polychlorinated-dibenzo-dioxins and dibenzofurans), tireless chlorinated pesticides (eg, DDT, aldrin, dieldrin, heptalor, mirex, chlorine).), brominated fire retardants (particularly poly-brominated-diphenyl ethers), polyfluorinated mixes, polycyclic fragrant hydrocarbons (PAHs), perchlorate, pharmaceutical and individual consideration items or haloacetic acids (Bari & Yeasmin, 2018).

Physical Contamination

Physical hazards are defined as all kinds of foreign substances that are undesirable in food (glass pieces, plastic, bone, paper, stone, soil, wood, metal pieces, hair, nails, cigarette ash, flies, insects, radioactivity and visible dirt). Physical hazards arise from faulty and incomplete applications applied at many points of the food chain during production, consumption. In addition, these dangers bring microbiological risks in some cases, give an idea that food is not produced under hygienic conditions, affect consumer choice and may cause public health problems (Aydınol *et al.*, 2015).

Conclusions

Sanitation risks may cause maladies that reason indications, for example, sickness, spewing and diarrhea. Be that as it may, it can likewise cause infections, for example, malignancy, renal or hepatic inadequacy, cerebrum and nerve issue. These ailments might be increasingly genuine in youngsters, pregnant women, the old and those with feeble invulnerable frameworks. Be that as it may, expanding foodborne episodes represent a worldwide risk to power and raise the requirement for further work to avert foodborne illnesses.

As sanitation is a typical duty of all partners all through the evolved way of life, there is a significant requirement for instruction and preparing in the aversion of foodborne illnesses among sustenance producers, providers, chiefs and general society. What's more, all through the natural way of life, all partners need to work intimately with governments to help distinguish and actualize sanitation procedures and strategies that will give safe sustenance to the total populace.

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THE EFFECTS OF PROBIOTICS ON PUBLIC HEALTH

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Abstract

In our intestines, only digestion and absorption is not realized. Furthermore, the microbial activity required for a healthy life is carried out by microorganisms in our gut. There are 10^{14} microorganisms of more than 500 different species in the gastrointestinal tract. In determining the composition of these microorganisms, the place of probiotics is very important. Microorganisms used as probiotics; *Lactobasillus* species, *Bifidobacterium* species, *Bacillus* species, *Pediococcus* species, *Streptococcus* species, *Bacteriodes* species, *Propionibacterium* species, *Leuconostoc* species, some molds and yeasts. The most commonly used microorganisms among these microorganisms are *Lactobasillus* species and *Bifidobacterium* species. These microorganisms were selected and characteristics such as being reliable, stable, colonizing the intestines, competing with carcinogenic and pathogenic bacteria, producing antimicrobial bacteria, being able to produce resistance to diseases, being resistant to antibiotics, staying alive and stable in storage conditions for a long time heve been studied. This review article aims to give information about the positive effects of probiotics on public health.

Keywords: *Probiotics, Public Health, Microorganisms*

İntroduction

Useful microorganisms in the body play an important role in adapting to human life and environment. The relationships between body cells and microorganisms have made human beings dependent on a wide variety of microorganisms. Factors that disrupt this relationship can damage the human body and cause various disorders. With modern life, the relationship between mammalian cells and microorganisms has weakened, and less common diseases have become more common in the past. Probiotics contribute to the prevention of diseases by strengthening this weakened relationship. Probiotics are useful microorganisms that, when taken orally, in sufficient amounts reach the digestive system and affect one's health in a positive way. These microorganisms affect the health of the person by regulating the balance in the intestinal flora. The word "probiotic" is derived from the Greek word için for life. Foods that contain enough live probiotic microorganisms (108cfu / g) until the end of their shelf life are called probiotic foods. In line with these expressions, a significant number of microorganisms were isolated and classified as probiotic. Some of these probiotics have been tested in humans and animals for the purpose of treating or preventing various diseases, disorders and syndromes. There are various strains of microscopic organisms that have probiotic properties in all microorganisms named probiotics. For instance, 23 unique strains in *Lactobacillus* are in the probiotic class, while in *Bifidobacterium* this number is 5, in *E. coli* 2, *Bacillus*, *Streptococcus*, *Enterococcus* and *Lactococcus* this number is one. Moreover, there is *Saccharomyces boulardii* yeast with probiotic properties (Goldin, 2011).

Probiotics and Public Health

Lactose Intolerance

The sickness happens because of the abatement in the movement of the lactase compound in the intestinal mucosa. This diminishing in movement results in a decrease in lactose ingestion. This is brought about by inadequate assimilation, gas, swelling, stomach spasms and moderate or serious loose bowels. The ailment is increasingly regular in the old. The utilization of dairy items, particularly milk, by wiped out individuals is confined. A few examinations have demonstrated that the lactase chemical delivered during yogurt creation is powerful in the intestinal tract alongside yogurt utilization (de Vrese *et al*., 2001; Kim & Gilliland, 1983; Kolars *et al*., 1984; Savaiano *et al*., 1984). Living beings utilized for the generation of yogurt *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus*. A portion of the patients with lactose narrow mindedness were nourished with yogurt and some of them were given milk. There was a critical decline in hydrogen levels in the breath of yogurt-encouraged patients. The degree of hydrogen in breath mirrors the intestinal microflora digestion of non-absorbable lactose in the small digestive system and is along these lines found in the colon where the microflora is available in high fixations (Kim & Gilliland, 1983). In another investigation, it was accounted for that individuals taking 18 g of lactose in yogurt had 67% less hydrogen in their breath contrasted with a similar portion of lactose given in milk (Kolars *et al*., 1984). An examination of intestinal duodenal desires from yogurt-expending subjects demonstrated huge degrees of lactase in the duodenum. In 2005, it was presumed that probiotics were not successful in the treatment of lactose prejudice (Levri *et al*., 2005). Notwithstanding, a few scientists have contended this might be because of the sort or nature of probiotics utilized in treatment. For instance, lactobacilli with low lactase levels can be possibly jumbling. The strains chose for the creation of yogurt must have the elevated amounts of lactase required for the proficient arrangement of yoğurt (Goldin, 2011).

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is an articulation utilized for intestinal irritation. This classification incorporates Crohn's illness, ulcerative colitis and peevish inside disorder. Probiotics have noteworthy potential in IBD treatment and avoidance of repeats. There are a set number of concentrates on the advantageous impacts of probiotics in the treatment or easing of IBD side effects. Probiotics have been accounted for to help alleviate manifestations in Crohnis sickness from E. Coli (Malchow, 1997). The expansion of Lactobacillus salivarius to drain and organization to interleukin (IL) - 10 knockout mice fundamentally decreased aggravation in the secum and colon contrasted with milk nourished knockout mice (O'Mahony *et al*., 2001). It is a calming cytokine that causes colonic irritation when IL-10 level is practically zero. This investigation showed that probiotics can influence the colonic invulnerable framework and forestall low IL-10 levels. In another investigation, L. salivarius and Bifidobacterium longum subsp. IL-10 knockout mice treated with the mix of infantis have been accounted for to cause an abatement in sickness seriousness. The seriousness of the illness was surveyed over a 6-week time frame with weight reduction, colon pathology, and outward presentation (McCarthy *et al*., 2003).

Cholesterol Lowering Effect

A few examinations have announced that probiotics lessen complete serum cholesterol and/or low thickness lipoprotein (LDL) cholesterol. The outcomes are dubious and frequently conflicting. Bringing down LDL cholesterol will effectsly affect diminishing the danger of coronary artery infection and lethal myocardial dead tissue. Because of human investigations which affect plasma cholesterol levels in fermenteddairy items, it has been accounted for that absolute cholesterol diminished between 5.4-23.2% and LDL diminished between 9-9.8% (Anderson & Gilliland, 1999). Yogurt with typical and L. acidophilus and B. animalis subsp lactis was tried to analyze serum total cholesterol in a randomized hybrid gathering of 14 individuals, including a 6-week nourishing period and a 4-week washout period. There was a huge lessening in total cholesterol levels in yogurt with L. acidophilus and B. animalis subsp lactis (Ataie-Jafari *et al*., 2009). Scarcely any subjects took an interest in cholesterol contemplates and the term was commonly constrained to about a month and a half. In view of in vitro and creature thinks about, a few systems have been proposed to decrease serum cholesterol by probiotics. These are the ingestion or absorption of cholesterol by probiotics (Walker & Gilliland, 1993).

Treatment of Urogenital Infections

Vaginal contaminations are brought about by operators, for example, *Candida*, *Trichomonas*, or microorganisms, for example, *Gardnerella vaginalis* and *Mycoplasma hominis*. Urinary tract contaminations are increasingly regular in ladies. These diseases are generally brought about by *E. coli*, *Chlamydia* and *Candida*. Ordinary sound ladies have around 50 unique sorts of microorganisms in the vaginal verdure. In an investigation of 10 pre-menopausal ladies, intravaginal lactobacilli were regulated to ladies and a lessening in urinary tract contaminations was accounted for (Goldin, 2011). Yogurt containing *L. acidophilus* has been accounted for to diminish *Candida*-inferred vaginitis triple (Hilton *et al*., 1992). Concentrates on the treatment and/or counteractive action of urogenital diseases with probiotics are exceptionally restricted. In any case, there are specialists attempting to plan explicit probiotics to be managed orally to anticipate or decrease the rate of urogenital contaminations.

Cancer Treatment and Prevention with Probiotics

Because of their metabolic movement, probiotics may impact the etiology of colon malignant growth and tumors in different areas. Probiotics have been accounted for to diminish intestinal bacterial proteins engaged with enactment of procarcinogens (Hosoda *et al*., 1996). Probiotics can likewise deliver short-chain unsaturated fats that can be defensive in the colon. Mouse studies have demonstrated that probiotics hinder the arrangement of anomalous tomb foci in the colon. The mix of inulin in addition to B. longum has been accounted for to decrease synthetically incited strange grave foci by 74% (Rowland *et al*., 1998). Inulin alone diminished anomalous sepulchers by 21%. Mice encouraged with a blend of oligofructose, inulin, LGG, and B. animalis subsp lactis were altogether less helpless to azoximetane-incited colon tumors (Marotta *et al*., 2003).

Conclusion

When utilizing or endorsing probiotics, consideration must be paid to the probiotic definition, including live, dead, joined arrangements or items thereof, the successful portion to be utilized, and the sort of infection focused on. It is unimaginable to expect to gauge the particular impacts and/or dosages of a specific probiotic and sum up these properties to different portions and/or strains of probiotic microorganisms. The Food and Drug Administration of the United States as of now does not direct probiotic items and does not give any administration oversight to quality control. In this manner, the genuine number of living creatures in business items might be very not quite the same as that promoted. In synopsis, future enormous scale clinical preliminaries that control dosing, suitability and other basic factors will be urgent to give the logical proof important to decide the viability of expanding probiotic use.

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THE INFLUENCE OF ABIOTIC FACTORS IN DEVELOPMENT OF CODLING MOTH AND ITS NATURAL ENEMIES IN SOUTHERN SERBIA

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Abstract

The codling moth (*Cydia pomonella* L.) is the most economically significant pest found in apples, pears, quinces, peaches and walnuts in southern Serbia. The research was carried out with the aim to determine the presence of this species in the monocultural apple plantations, apple plantations with mixed varieties of apple trees, abandoned apple plantations and on apple trees that were part of the spontaneous flora. The monitoring was also carried out on the individual apple trees grown on infields, whereby some of them were chemically protected and others were not. The standard entomological methodology used for the research was divided into the field observation (pheromone traps, trunk bands made of corrugated cardboard, visual examination, the branch beating method, entomological isolators) and the laboratory growth (the growth of interwoven larvae, the growth of the collected plant organs with eggs hatched on them, taxidermy, labelling, determination and collecting of the material gathered in the field). The results of the research showed that the variability of abiotic factors affected the length of the codling moth's developmental phases. It also affected the numerousness of codling moths. These are the identified natural enemies of the codling moth: parasite species of codling moth from the Hymenoptera order (Ichneumonidae, Braconidae), species known as moth (Tortricidae) parasites from Hymenoptera and Diptera orders, predator species from the following orders: Dermaptera (Forficulidae), Neuroptera (Chrysopidae), Coleoptera (Coccinellidae) and Heteroptera (Miridae).

Key words: *Cydia pomonella L., abiotic factors, natural enemies, southern Serbia.*

Introduction

Serbia has favorable agroecological conditions for the growth of continental fruit species (Mišić, 2005). Apple is the most significant fruit that is of big economic importance because of its capability to adapt to natural conditions and high quality of its yield (Blagojević, 2000). Weather change can affect the development of apple production in a specific way. In order to overpower the adverse impact of drought and fruit stress caused by extreme meteorological changes, it is necessary to use high-level agro-pomotechnical measures.

Modern strategy of fruit production for control of harmful organisms requires producer's expertise in terms of knowing agrobiocenosis, biology and ecology of each individual species as well as their interaction (Nikolić *et al.*, 2018). Thus the development of harmful organisms is enabled while the possibility of increasing of many helpful organisms is improved. This also enables the production of unpolluted, health-wise safe yields (Milić *et al.*, 2011).

Apple is being attacked by many pests, such as insect species from the Tortricidae family. The most represented pest is codling moth (*Cydia pomonella* L.), one of the most economically harmful insect species in fruit growing. Fighting for its survival, codling moth feeds on the yields the man is also interested in (Almaši *et al.*, 2004).

Its appearance is recorded in monocultural orchards, mixed orchards, neglected trees or trees that can be found next to planted apple orchards and are not treated, and wild fruits. In southern Serbia, it can also be found on pear, quince, peach and walnut yields (Nikolić, 2015).

The goal of the research was to determinate the influence of variable abiotic factors over the length of developmental phases of codling moth (hatching of the first eggs and drilling of infant larvae into the fruits, as one of the most important stages of the development cycle of codling moth). The goal was to determinate the presence of the natural enemies of codling moth in orchards with different protection systems and in variable localities.

Material and Methods

The research was carried out in fruit plantations in the territory of southern Serbia in 8 localities (with different altitude) in the following districts:

1. Jablanica District: Beli Potok (272 m), Donje Stopanje (253 m), Leskovac (230 m) and Strojkovce (266 m);
2. Nišava District: Niš (237 m);
3. Toplica District: Prokuplje (285 m);
4. Pčinja District: Vladičin Han (389 m) and Prekodolce (299 m).

In all orchards except in locality of Strojkovce, chemical protection has been performed. The number of pests approximately close to the main plantations in neglected untreated orchards, on trees of houses in the surrounding or on wild trees, was also recorded for the comparison of the presence of codling moth and its natural enemies.

During the research period (2006-2008), the weather (air temperature and rainfall) was being analysed in the weather stations in: Leskovac (for Jablanica District), Niš (for Nišava and Toplica District), Vranje (for Pčinja District).

The standard entomological methodology (Group of authors, 1983) was used for the research necessary for the field observations (visual examination, pheromone traps, trunk bands made of corrugated cardboard, the branch bearing method, entomological isolators) and the laboratory processing of material gathered on the field (the growth, taxidermy, labelling, determination and collecting) (Nikolić, 2015).

Results and Discussion

The codling moth was registered in all localities of the three yeard research in the territory of southern Serbia. Except on apple fruits, its damage was determined on peat, quince, peach, and walnut. Its presence with a different intensity in plantations of apples, but also pear, quince, peach, walnut and sweet cherry, is confirmed in researches of other authors (Tadić, 1957; Ciglar, 1989; Stamenković *et al.*, 2006, Kutinkova, 2010).

The codling moth is a thermophilic species, to which growth periods with high temperatures and a lack of rainfall are suitable. With the appealing conditions for its development, it has multiple generations and causes bigger economic damages. The characteristics of weather conditions in southern Serbia in the period of the research are the following:

- the year 2006 was a pretty warm year with a quantity of rain in a normal state;
- the year 2007 was one of the hottest years recorded in Serbia, with heatwaves and unevenly rainfalls during the year;
- the year 2008 was extremely hot and quite dry, with fewer exceptions than 2007.

The activity and the length of the developmental phases of *C. pomonella* are in direct correlation with weather changes, distribution and quantity of the rainfall in vegetation period. The average monthly temperature between April and October had the highest variable recorded in the year 2007, and the lowest in 2006 in three weather stations (Figure 1a). The sum of rainfall in the period between April and October was the highest in the year 2006 in three weather stations, and lowest in 2007 in Vranje, as well as in 2008 in Leskovac and Niš (Figure 1b). During the research weather conditions with stimulating characteristics for the codling moth were also registered, such as mechanical damage of the fruit from hail and drying of the fruit due to the high temperature, periodical rotting and decreasing of fruits.

Damaged and softer fruits are an easier target to attack by caterpillars of codling moth and the damage itself is doubled.

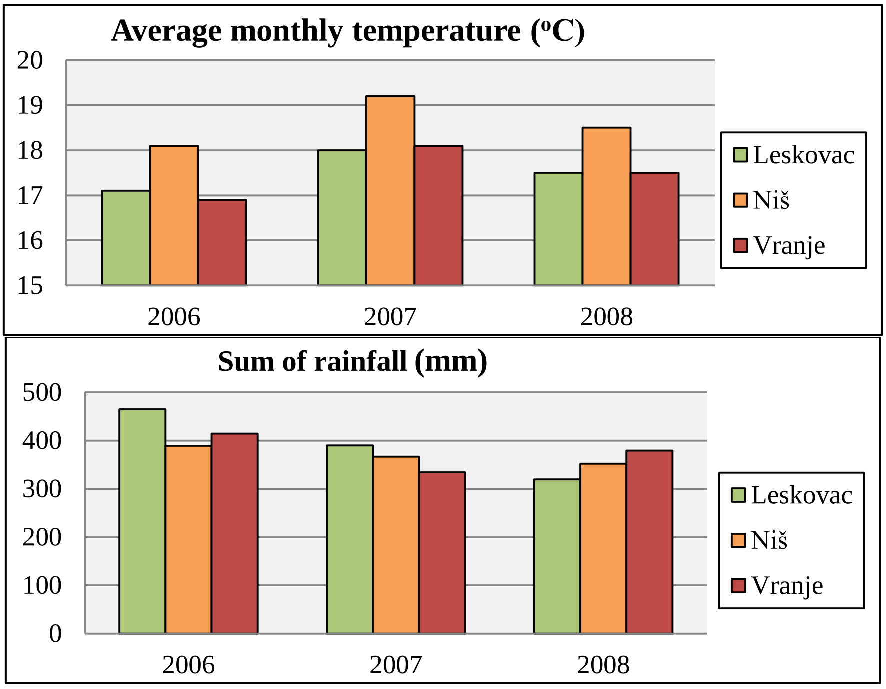

Figure 1. Weather conditions in southern Serbia in the period between April and October in years 2006 to 2008: a) Average monthly temperature (ºC), b) Sum of rainfall (mm)

Weather conditions were extremely in favor of the development of this species. Two generations per year in each locality were determined, while in some localities of Jablanica district it was recorded prolonging of the flight and extension of the development of the second generation of codling moth: in year 2006 in localities Donje Stopanje T (treated orchards) and UT (untreated orchards), Leskovac T, Strojkovce UT, in year 2007 in localities Beli Potok UT, Donje Stopanje T and UT, and in year 2008 in locality Donje Stopanje T and UT (Nikolić, 2015).

Multiple authors in Serbia and neighbor countries indicate an easy adaptation of codling moth on weather conditions and its big potential of reproduction (Tadić, 1957; Maceljski, 1973; Thalji, 2002; Lazarevska *et al.*, 2005; Pajač – Živković, 2012; Nikolić, 2015).

The variability of abiotic factors affected the length of the developmental phases of the codling moth and its number. Laying and hatching of the first eggs and drilling of infant larvae into the fruits is of big importance for the representation of the complete development cycle of the codling moth, as well as for overlapping of developmental phases from different generations (Table 1). The first appearance of the eggs and hatched larvae happened a few days earlier in the localities that were chemically treated in comparison to the chemically untreated localities (Nikolić, 2015).

First hatched eggs in the period of examination were determined on April 29th, 2006 in locality Beli Potok, on April 30th, 2007 in Donje Stopanje and on April 24th, 2008 in Donje Stopanje. First drilling of larvae in the fruits was registered in the locality of Beli Potok (May 14th, 2006, May 21st, 2007, and May 16th, 2008).

Examinations of autochthonous useful entomophauna of *C. pomonella* are in the interest of every fruit production area. Determination of differences of the faunistic composition of natural enemies of codling moth, depending on the locality and implemented protection system, give a different mark to a specific area and to apple production in general. The role of every determined natural enemy cognates in the possibility that they are used as a factor for regulation of the number of the pest. Reduction of its population is possible, keeping in mind that the codling moth can be exposed to the attack of the entomophagous parasites and predators in every developmental phases (Lazarevska *et al.*, 2005).

Table 1. Evolution phases of *Cydia pomonella* in southern Serbia in period 2006 to 2008

Year		2006				2007				2008			
Locality	Gen.	I		II		I		II		I		II	
	Stad.	Egg	Larvae	Egg	Larvae	Egg	Larvae	Egg	Larvae	Egg	Larvae	Egg	Larvae
Beli Potok	T	**April, 29**	**May, 14**	**June, 28**	July, 14	May, 5	**May 21**	**June, 19**	**July, 25**	April, 30	**May, 16**	July, 9	**July, 22**
	UT	May, 2	May, 18	July, 3	July, 18	May, 6	May, 27	June, 20	July, 28	May, 4	May, 18	July, 15	July, 23
Donje Stopanje	T	May, 3	May, 19	June, 30	**July, 8**	**April, 30**	May, 25	June, 23	July, 29	**April, 29**	May, 20	**June, 26**	July, 26
	UT	May, 8	May, 23	July, 6	July, 10	May, 4	May, 28	June, 27	**July, 30**	May, 6	May, 22	June, 29	July, 26
Lesko-Vac	T	May, 10	May, 22	July, 2	July, 9	May, 7	May, 26	July, 3	July, 29	May, 4	May, 19	July, 5	July, 25
	UT	May, 13	May, 26	July, 8	July, 12	May, 11	May, 28	July, 8	July, 29	May, 10	May, 23	July, 10	July, 28
Stroj-Kovce	UT	May, 9	May, 25	July, 7	July, 14	May, 8	May, 26	June, 28	July, 28	May, 5	May, 24	July, 4	July, 25
Niš	T	May, 3	May, 16	June, 29	July, 25	-	May, 28	July, 6	July, 28	April, 30	May, 26	July, 3	July, 24
	UT	May, 4	May, 17	June, 6	July, 28	-	May, 23	**July, 13**	**July, 30**	May, 2	May, 25	July, 9	July, 24
Prokup-lje	T	May, 9	May, 19	July, 4	July, 20	April, 29	May, 24	July, 5	July, 26	May, 6	May, 23	July, 5	July, 23
	UT	May, 12	May, 22	July, 8	July, 26	May, 8	May, 26	July, 11	July, 28	May, 10	May, 26	July, 9	July, 25
Vladičin Han	T	May, 14	**May, 27**	July, 12	July, 25	**May, 13**	**May, 30**	-	July, 27	May, 12	**May, 29**	July, 15	July, 26
Preko-dolce	T	**May, 16**	May, 26	**July, 17**	**July, 29**	-	May, 28	-	July, 26	**May, 17**	May, 28	**July, 18**	**July, 29**

(T – treated orchards; UT – untreated orchards)

The registered natural enemies of the codling moth in the conducted researches can be clustered in the following groups:

➢ Parasite species *C. pomonella* from Hymenoptera order (Ichneumonidae: *Pristomerus vulnerator* Panc. and *Trichomma enecator* Rossi. and Braconidae: *Ascogaster quadridentata* Wels. and *Chelonus annulipes* Wesm.);

➢ Parasite species Tortricidae from Hymenoptera order (Ichneumonidae: *Itoplectus maculator* F., *Itoplectus alternans* Grav., *Phaeogenes invisor* Thumb., Braconidae: *Agathus laticorpa* Telenga and *Apanteles* sp.) and from Diptera order (*Actia pilipennis* Fall.);

➢ Predator specias from the followig orders:

- Dermaptera (Forficulidae: *Forficula auricularia* L. and *Forficula smyrnensis* Audinet-Serville);
- Neuroptera (Chrysopidae: *Chrysopa carnea* Steph., *Chrysopa perla* L. and *Dichochrysa ventralis* Curtis);
- Coleoptera (Coccinelidae: *Coccinella septempunctata* L., *Coccinella quatuordecimpustulata* L., *Adalia bipunctata f. Tybica* L., *Adalia bipunctata f. Sexpustulata* L., *Adalia decempunctata* L., *Chilocorus renipustulatus* Scriba, *Hippodamia variegate* Goeze, *Oenopia conglobate* L., *Platunaspis luteorubra* Goeze, *Propylea quatourdecimpunctata* L., *Psyllobora vigintiduopunctata* L. and *Scumnus frontalis* Fabr.);
- Heteroptera **(**Miridae: *Deraecoris ruber* L.).

The presence of the natural enemies of codling moth is encountered in the researches of multiple authors (Lacey, Unruh, 2005; Lazarevska *et al.*, 2005; Andreev *et al.*, 2006; Nikolić, 2015). The codling moth eggs parasitised by the Trichogramma species were identified on both chemically treated and chemically untreated fruits in Prokuplje and Vladičin Han during all three years of the research. These parasits were indicated by Tadic (1957) in his researches.

C. pomonella falls under the species with a lesser number of generations, smaller reproductive capability and species with specific parasites and predators that regulate its number (Krnjajić, Injac, 2000; Almaši *et al.*, 2004; Lazarevska *et al.*, 2005).

Conclusions

The codling moth was present in all examined localities in the territory of southern Serbia with a different intensity. Economically, it is the most significant pest of apple, but also of pear, quince, peach and walnut.

The variability of abiotic factors during the vegetation affected the length of the codling moth's developmental phases and its number. The activity and the length of the developmental phases of *C. pomonella* are in direct correlation with weather changes, distribution and quantity of the rainfall in vegetation period. The mechanically damaged fruits from hail and drying of the fruit due to the high temperature are an easier target to attack by caterpillars of codling moth and the damage itself is greater.

In the conducted researches the following were registered as the natural enemies of the codling moth:

- Parasite species *C. pomonella* from Hymenoptera order (Ichneumonidae and Braconidae),
- Parasite species Tortricidae from Hymenoptera and Diptera orders,
- Predator species form following orders: Dermaptera (Forficulidae), Neuroptera (Chrysopidae), Coleoptera (Coccinellidae) and Heteroptera **(**Miridae).

The role of every determined natural enemy cognates in the possibility that they are used as a factor for regulating the population of the codling moth.

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EFFECT OF MODE PACKAGING AND STORAGE ON SENSORY PROPERTIES AND COLORS IN LETTUCE

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Abstract

The increased need for fresh vegetables has led to the application of new technologies for the preservation of quality during storage. Lettuce is a type of vegetable that is consumed often and in relatively large quantities**.** The objective of this study was to determine the effect of storing and keeping sensory properties of lettuce using packaging in normal atmosphere and modified atmosphere (MA). The research was done with one control group and two experimental groups. The control group consisted of lettuce stored in normal atmosphere in the refrigerator and the experimental group included lettuce stored in MA, (98% nitrogen and 2% oxygen). During 25-day storage period, the sensory properties and colour were evaluated using a spectrophotometer (CM-5, Konica Minolta). Colour was measured on the outer, middle and inner leaves of the lettuce and ribs, under storage conditions of relative humidity of 95% and temperature 4°C. The colour parameter L* in the control group, changed from 61.92 at the beginning to 68.59 at the end of the experimental period. An increase in L* value in lettuce is a sign of decreased intensity of the green color due to the appearance of yellow pigments. Sensory evaluation determined that there was significant difference ($p = 0.05$) in the overall impression in the MAP, normal atmosphere and control samples.
MAP enables the preservation of quality and sensory properties for a longer period of time (5 days).

Key words: *Green lettuce, MAP, normal atmosphere, color change, sensory rating.*

Introduction

Lettuce is one of the most popular types of vegetables available almost all year round. It is a rich source of bioactive substances important for human health, which serve to prevent many diseases. The most important bioactive substances contained in lettuce include chlorophyll, poly-phenols and flavonoids, carotenoids, vitamins (tocopherol, ascorbic and folic acid) and minerals, especially magnesium (Nicolle et al., 2005; Hamu ł ka, et al., 2005; Kenny and O'Beirne, 2009; Murillo et al., 2010; Perucka et al., 2011). Today, there is a growing demand for fresh, naturally preserved and high quality food products that are treated as physically and chemically as possible during production. The life of the product is influenced by physical, chemical and biological factors (Lee et al., 1997; Mattheis and Fellman, 2000).

The shelf life and maintenance of freshness is determined by monitoring the parameters of the chemical composition, physical, sensory and microbiological properties during the time when the lettuce maintains the properties appropriate for its consumption. Apart from the storage conditions of the product, choosing the right packaging material is just as important as choosing the right gas mixture inside the package (Ballantyne et al., 1988; Kader, 2002).

In cases where the lettuce is exposed to uncontrolled conditions of relative humidity and temperature, the main reason of lettuce deterioration is water loss, which causes degradation of cell walls and affects quality properties such as texture, discoloration, enzymatic darkening after picking (Alzamora et al., 2000; Rodrigues et al., 2013). In a sensory evaluation of lettuce, the external appearance has a great influence on the choice of the product. According to Almeida (1995) and Gonzales et al. (1999), instrumental color determination can be used to establish quality standards in fresh or processed products. Colour can also be used as a factor to determine the shelf life of a product when studying color variations during storage (Lee et al., 1996). Chlorophyll is responsible for the green color of the plants. Because their chemical structure is unstable, they are easily degraded, resulting in degradation products that alter the perception and quality of food (Lopez-Galvez et al., 1997; Schoefs, 2002). This aspect is entirely related to the purchase of these products by consumers; a reduction in chlorophyll content leads to market losses, thereby doing research is an attempt to find methods for better preservation .

Material and Methods

The tested lettuce samples, *Iceberg*, were stored at 4℃ and 95% relative humidity. The chamber temperature and relative humidity were measured using a digital thermometer with a „Tesko AG 922" thermocouple. Samples were stored in MRC LTD, PGI-550V 0 to +70℃. The samples were tested for color on 1, 4, 7, 10, 12, 14, 16, 18, 20, 23, 25 days, while sensory tests were performed for 1, 10, 20 and 25 days.

Method of growing lettuce

Production of seedlings was carried out in early August, and planting in early September in an open field in Gornja Bioča, in the production area of the company "BH food". About 5000 *Iceberg* lettuce seedlings are planted at 2000 m^2, with an average daily temperature of 25℃ to 30 ℃.

The harvest was done on the 65^{th} day after planting. Immediately after sowing, the seedlings were treated with *Previcur* for protection. Savings were made three times during production with different NPK ratios and irrigation every 3 days at 0.4 l per seedling.

Lettuce packing method

The lettuce was packaged at „BH Food" in MAP and in a normal atmosphere with „Multivac" packer. When packing in MAP, a gas mixture (O_2: N_2: CO_2) (2: 98: 0) was used by the manufacturer's Messer, which was pressed into polyethylene bags (0.07 mm - throughput level), dimension 240 x 340. The packaging program was set to 50 MB per pack. When packing in a normal atmosphere, the same bags as the modified atmosphere were used.

Determination of color

CM-5 (Konica Minolt) spectrophotometer was used to determine the color. The changes inthe color of lettuce was evaluated using the CIELAB system at light source D65. Observation of color change was done on ribs and leaves, with three repetitions, on the outer, middle and inner part of the lettuce head. Mean values were taken to determine the color coordinates of L* (brightness), a* (redness) and b* (yellow).

Determination of sensory properties

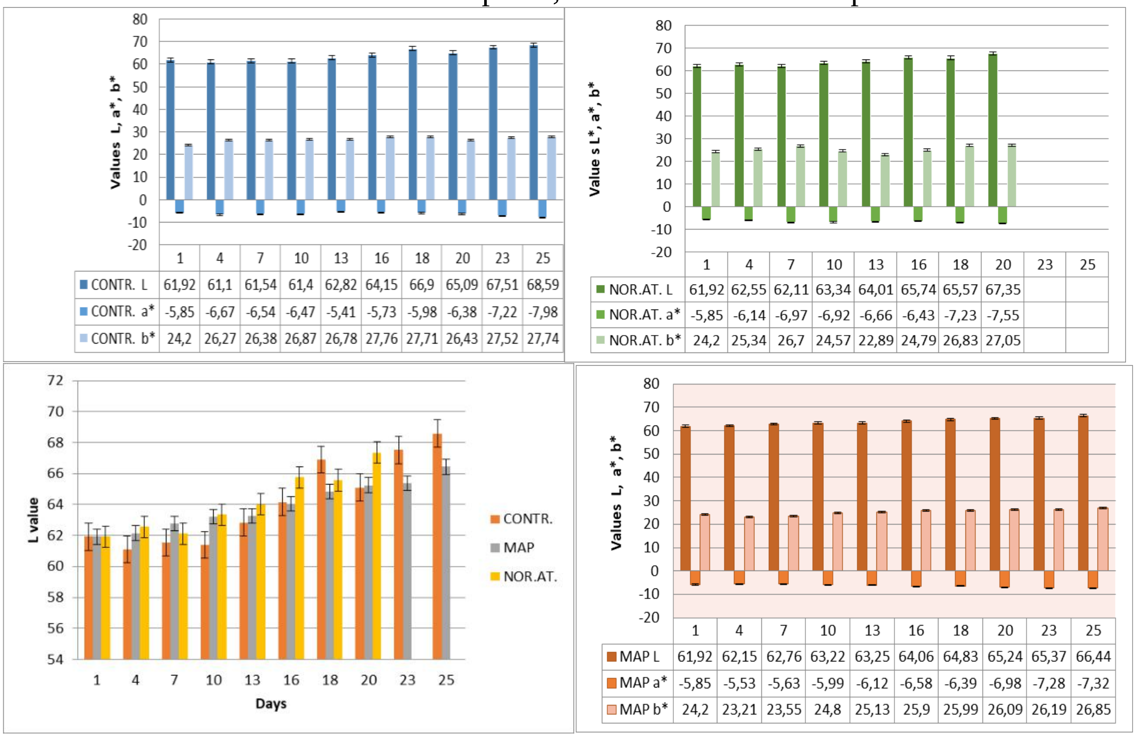
Sensory evaluation of lettuce was performed by ten trained evaluators, in the laboratory for heat treatment. Samples of lettuce heads were encrypted and prepared on white plastic plates. The method of sensory evaluation of lettuce is described, where the sensory properties of the product are evaluated on the basis of the proposed quality criteria, (from 5 for the intrinsic quality to 1 unacceptable). Color, odor, taste, texture and appearance were evaluated.

Statistical processing
Experimental data were analyzed with a statistical package (SAS Ver. 9.0). One-way analysis of variance (ANOVA) was performed for each type of package to obtain a statistical estimate of the evolution of parameters during storage at a confidence level of p = 0.05. For sensory processing, the Mann-Whitney U nonparametric test was used to evaluate significant differences in the dependent variable.

Results and Discussion

The loss of quality due to the appearance of yellow, rusty brown, head damage and fading were slightly higher in the lettuce control samples and the ones packed in a normal atmosphere than in MAP. The color parameters L*, a* and b* of lettuce samples, as well as the values of total color change measured during storage in the refrigerator for 25 days at a relative humidity of 95%, represent the mean of three measurements from three parts of the lettuce head (outer, middle and of the inner part). The values of the lettuce color parameters are shown in Figure 1.

Figure 1. Values of L*, a*, b* and ΔE with error bars for lettuce leaf during storage under normal atmosphere, MAP and control samples.



	1	4	7	10	13	16	18	20	23	25
CONTR. L	61,92	61,1	61,54	61,4	62,82	64,15	66,9	65,09	67,51	68,59
CONTR. a*	-5,85	-6,67	-6,54	-6,47	-5,41	-5,73	-5,98	-6,38	-7,22	-7,98
CONTR. b*	24,2	26,27	26,38	26,87	26,78	27,76	27,71	26,43	27,52	27,74

	1	4	7	10	13	16	18	20	23	25
NOR.AT. L	61,92	62,55	62,11	63,34	64,01	65,74	65,57	67,35		
NOR.AT. a*	-5,85	-6,14	-6,97	-6,92	-6,66	-6,43	-7,23	-7,55		
NOR.AT. b*	24,2	25,34	26,7	24,57	22,89	24,79	26,83	27,05		

	1	4	7	10	13	16	18	20	23	25
MAP L	61,92	62,15	62,76	63,22	63,25	64,06	64,83	65,24	65,37	66,44
MAP a*	-5,85	-5,53	-5,63	-5,99	-6,12	-6,58	-6,39	-6,98	-7,28	-7,32
MAP b*	24,2	23,21	23,55	24,8	25,13	25,9	25,99	26,09	26,19	26,85

*Source: Author's elaboration based on lab measurements

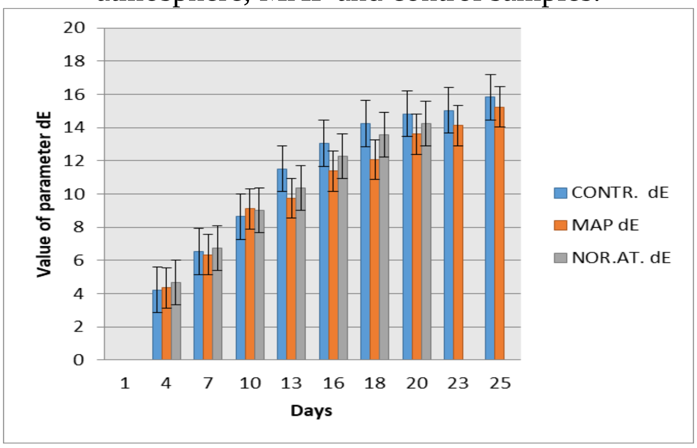
Figure 1 shows the changes in the color parameters L*, a* and b* during the storage at different packing conditions (MAP, normal atmosphere and control sample) at 4℃ and 95% air humidity. Treatments of packing had a very little effect on the color of lettuce, measured by L*, a*, b*, which is not statistically significant with a 95% probability. These results confirm the stability of chlorophyll content during cold storage and agree with those reported by Lopez et al., (1997) and Managa (2018). Individual analysis of color parameters reveals which parameter influenced the most the overall change in the color of lettuce and under which treatment is the highest change. The largest changes were measured in keeping the lettuce in the refrigerator without packaging, that is, in the control sample, whereas by the sensory evaluation the sample was stored in a normal atmosphere after 20 days, judged to be unacceptable for consumption due to the unbearable smell of fermentation and taste changes, which were evaluated by the lowest grade. The largest changes in the L* parameter occurred from 16 days, when a change of more than 1.5 was observed in the control sample and normal atmosphere, while the changes in the MAP were more uniform. The difference between the first and the last day in the MAP was 4.52, while in the control it was 6.67 and in the normal 5.43. An increase in the value of the parameter L* indicates an increase in the luminance (brightness) of the sample. Changes in the color of lettuce are not defined by the standard. Although there is no scale to determine the quality of lettuce on the basis of color, it can be concluded whether the measured changes are significant or not. Sharma (2003), state that changes in color parameters greater than 3 are significant. In all three samples, an increase in the + b* parameter indicating yellow was measured during the storage process. Major changes were measured in the storage of control samples 3.54, while in the normal atmosphere they were 2.85 and in MAP 2.65. An increase in the value of the + b* parameter occurs continuously over 25 days of storage. Changes in the -a* parameter on all three samples were small and did not affect the color change much. The value of a* of first, to day 25 for the control sample changed by 2.13 and for MAP by 1.47.

The quality lettuce packed in MAP, normal atmosphere and control sample prepared from whole *Iceberg* heads up to 13 days at 5 °C was similar. Afther 13 days of storage lettuce in normal atmosphere, CO_2 injury resulted in severe discoloration and tissue softening. The use of MAP delayed the development of a change lettuce color, for several days (Brecht et al. 2003; Singh 2010).

Changes in the value of the total color

The occurrence of discolouration in fresh leafy vegetables has been reported as a severe problem for the food industry as a whole (Chiesa, 2003). Losses due specifically to cut-surface discolouration are hard to quantify although anecdotal evidence suggests that this can account for substantial customer (Hunter et al., 2017). The analysis of variance did not confirm the influence of the experimental factors on the change in brightness of lettuce samples, as well as the parameters a* and b* on the change in the values of these parameters. The results of the total color change, which contains all three parameters, which is why this size and the best indicator of color change are also included in the analysis. The results of the analysis are shown in Figure 2. Major changes in ΔE during storage were measured in control samples, which is a consequence of water loss of transpiration and drying of the outer leaves (Kader, 2002).

Figure 2. Values of ΔE with error bars for lettuce leaves during storage under normal atmosphere, MAP and control samples.



*Source: Author's elaboration based on the lab measurement

Sensory evaluation was performed to compare the main visual-qualitative attributes with the measured physicochemical parameters over the life of the lettuce. Ratings during storage showed good correlations with changes in the color parameters tested. Error bars are used that express a potential error, relative to the point or marker for the given data set. Average mean scores of the ten assessors are given in Table 1.

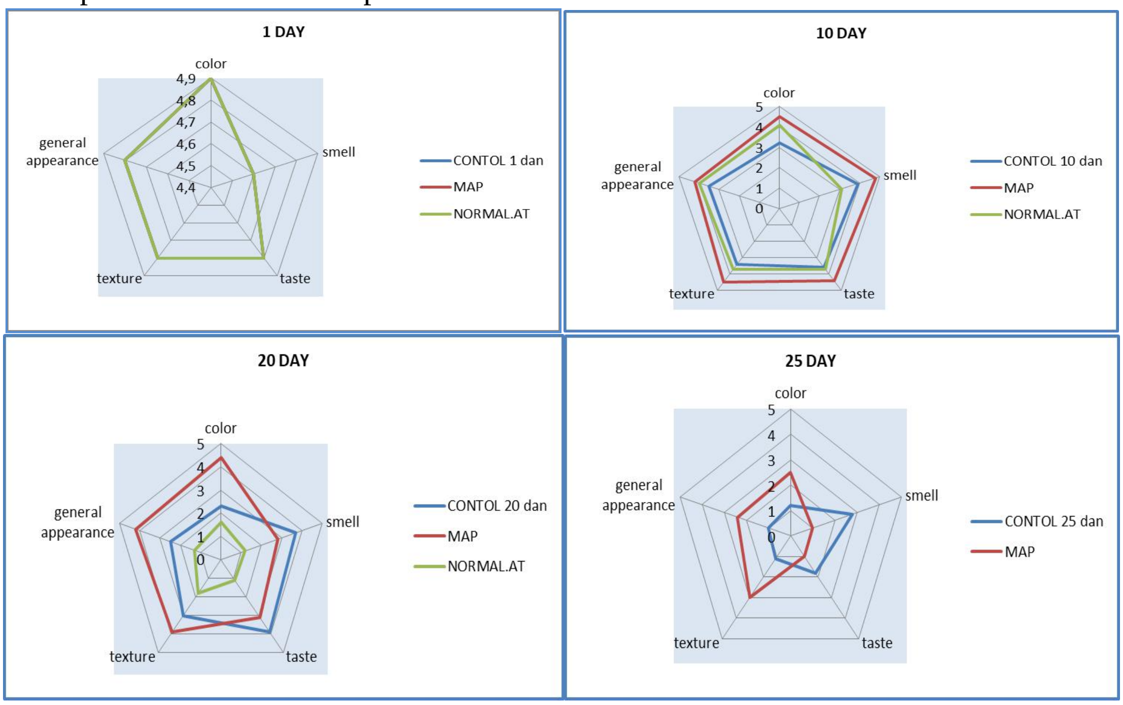
Table 1. Sensory evaluation of lettuce

SAMPLES	CONTROL				MAP				NORMAL AT.		
	DAYS										
CARACTERISTICS	1	10	20	25	1	10	20	25	1	10	20
color	4,9	3,2	2,3	1,2	4,9	4,5	4,4	2,5	4,9	4,1	1,6
smell	4,6	3,9	3,7	2,8	4,6	4,8	2,8	1	4,6	3,1	1,2
taste	4,8	3,6	3,9	1,8	4,8	4,4	3,1	1	4,8	3,7	1,1
texture	4,8	3,4	3	1,1	4,8	4,5	3,9	3	4,8	3,7	1,8
general appearance	4,8	3,5	2,5	1	4,8	4,2	4,2	2,4	4,8	4	1,3
Average	4,78	3,52	3,08	1,58	4,78	4,48	3,68	1,98	4,78	3,72	1,4

*Source: Author's elaboration based on the questionnaire survey results.

Specifically, lettuce packed in a normal atmosphere were rated as unacceptable after 20 days, while packed in MAP had the highest ratings up to 25 days when the evaluators stated that the smell and taste were not specific to the product, which was the case with the products in the normal atmosphere of the 20th day.
Overall visual quality scores were generally at or above the minimum acceptable value for salability at the ''Best if Used by Date'' (12—16 days after processing) (Lopez-Galvez; 1997). Another problem that arose during storage was the appearance of browning, which was expressed in packs of normal atmosphere. The problem with the control sample was drying the outer leaves and leaving a poor visual impression.

Figure 3. Demonstration of sensory evaluation by day for lettuce stored in MAP, normal atmosphere and control sample.



*Source: Author's elaboration based on the questionnaire survey results.

According to Chitarra and Chitarra (2005), aging is considered to be a period of the life cycle of a plant when catabolic processes (degradation), which are responsible for tissue death, prevail, as the ability of plant synthesis is very limited in the final stages of life. Analyzing the results, the most sustained mixture of gases into which lettuce was packaged showed a modified atmosphere (Figure 3). Regarding the general appearance parameter at storage day 25, lettuce in MAP represented a mean of 2.4; while the continuity of the sample was graded 1. The smell and taste of the sweet packed in MAP for 25 days were graded 1. Texture and color are the parameters that the graders rated higher, (3 textures and 2.5 colors). An overall acceptability score of three was considered an acceptable limit for sales or consumption (Zhan et al., 2012; Rodrigues et al., 2013). Analysis of variance revealed that there was a statistically significant difference between samples packed with MAP and control ($p = 0.05$) in overall impression as well as in color and texture based on the sensory evaluation of the evaluator. For the other parameters tested, odor and taste, no statistically significant difference was found between the samples.

Conclusion

Lettuce stored in different packages (normal and modified atmosphere and control sample), had a very little effect on the color, measured by L*, a*, b*, which is not statistically significant with a 95% probability. Sensory evaluation provided a comprehensive picture confirming that the most durable lettuce was packaged in a modified atmosphere. The sensory quality of lettuce was reduced from day 1 to day 10 of storage, in control samples, while a sudden decrease in quality was observed in the normal atmosphere on day 20th, when lettuce was considered unsuitable for consumption, due to its extremely unpleasant odor and taste. MAP enables the preservation of quality and sensory properties for a longer period of time (5 days), as confirmed Brecht et al., 2003; Singh, 2010; Hunter, 2017.

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LABORATORY GERMINATION AND EPIPHYTIC MYCOPHLORA OF VARIOUS GENOTYPES OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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Abstract

Seed quality is an indication of thesuitability for sowing. The species composition of the seed mycophlora is an important factor for seed sowing qualities. This study aimed to determine the species composition of the epiphytic mycophlora developing on the seeds of different genotypes of sorghum and their influence on the sowing qualities of the seeds, in 2018. Seeds of 5 plants of each sorghum genotype were collected to determine the species composition of pathogens growing on their surface. The collected seeds were transported to the phytopathological laboratory of Shumen University and analyzed for the presence of epiphytic microflora on them, and to study their seed qualities - germination and germination energy. The seeds germination and the germination energy is determined in line with the standard methods. All genotypes under laboratory conditions showed germination above 85%. The identification of phytopathogens is performed by the wet chamber method, by microscopic diagnosis and using different methods of isolation. It was found that *Alternaria alternate* (Fr.) Keissl. Was the most common fungus growing on the surface of the seed among all 7 isolated species, referring to 6 genus: *Alternaria alternate; Alternaria solani*, *Fusarium moniliformae*, *Helminthosporium turcicum*, *Peronospora sorghu*, as well as the causative agents of the *Aspergillus* and *Mucor* species.This pathogen is capable of damaging seeds and sprouts, thereby aggravating the sowing properties of sorghum seeds. When introducing new species of agricultural cultures in specific region information on seeds germination, seed-borne diseases eventual manifestation and development is explicitly important. In this connection, the present research is directed towards the germination and germination energy of the seeds, as well as the mycoflora of different sorghum genotypes, cultivated in Shumen region.

Key words:*Sorghum, Epiphytic micophflora, Qualities of seeds, Alternaria alternate.*

Introduction

The composition of the seeds' mycoflora is determined as an aggregate of all types of microorganisms, colonizing the sheath and the internal parts of the seeds, such as viruses, bacteria and microfungi. Part of those microorganisms are pathogenic and they are referred as pathogenic microflora in literature (Stancheva, 2010). In accordance with researchers on seeds pathology (Bakan, 2002; Dawar, 1991; Thakur et al., 2007; Little, et al., 2012) the microorganisms, colonizing the seeds endosperm, infect the seeds systematically and other microorganisms occupy the surface of the seeds and infect the healthy plants under suitable conditions. The results from tests, made by the above-cited authors, show that this is a variety of pathogenic microorganisms, adherent to different groups. According to (Stancheva, 2010) the surfaces of the agricultural seeds may be colonized also by microorganisms, which do not cause any visible pathological changes in the seeds, they do not participate in diseases transmission and they are determined as non-pathogenic microflora. The microorganisms colonizing the seeds as pathogenic or non-pathogenic microflora are predominantly representatives of different types of bacteria and fungi (Sharma et al., 2015).

All transmitted sorghum seed-borne diseases affect the growth and the productivity of the crop. (Stancheva, 2010). The infected seeds are characterized with reduced germination and rotting; local and systematic infections are observed in later stages of the plantation development. (Isakeit and Jaster, 2005; Ramathani, 2010; Kikindonov et al. 2013). The only way to reduce to minimum the risk of transmission of seed-borne diseases and the introduction of new types or strains is the good knowledge of the pathogens biology and the modes of transmission from vegetation to vegetation (Degefu,1990). Some fungi, infecting the sorghum and corn seeds – representatives of *Fusarium*, *Aspergillus*, *Rhizoctonia*, *Penicillium*, *Helminthosporium*, attack and destroy the seeds' endosperm by eating the starch inside, leading to reduced germination and incapacity to develop normal sprouts (Stancheva, 2010; Cook, 1980; Domsch, Degefu,1990) Representatives of *Pythium* and *Peronospora* species, attack the young seedlings and lead to rotting of the roots and the bases of the plants at the initial phases of their development, which leads to reduction of the crop (Isakeit and Jaster, 2005). Examinations on the seeds' microflora of the cereal grain crops show that the colonization of the seeds by representatives of the following species: *Fusarium* and *Alternaria* decrease the production efficiency (Stancheva, 2010; Bakan, et al., 2002). The authors consider that those pathogens, under certain conditions, may associate in pathogenic complexes causing withering, root and stem rotting for the whole vegetation. The *Fusarium* and *Alternaria* produce mycotoxins, deteriorating the quality of the seeds and make it unusable as fodder. Stancheva (2010) describes *Fusarium* (*Fusarium moniliforme* J. Sheld; *Fusarium graminearum*) and *Alternaria*, (mainly *Alternaria alternate)* species as the causative agents of a complex pathological syndrome, showing pathologic symptoms on the seeds, the vegetative organs and the roots: stem rotting – manifestation through the phases of milk and dough-ripe maturity, head rotting. **Figure 1** shows pictures of the causative agents of those symptoms. The manifestations starts after the milk-dough-ripe maturity and the symptoms continue to develop during the storage phase as well, such as rotting.

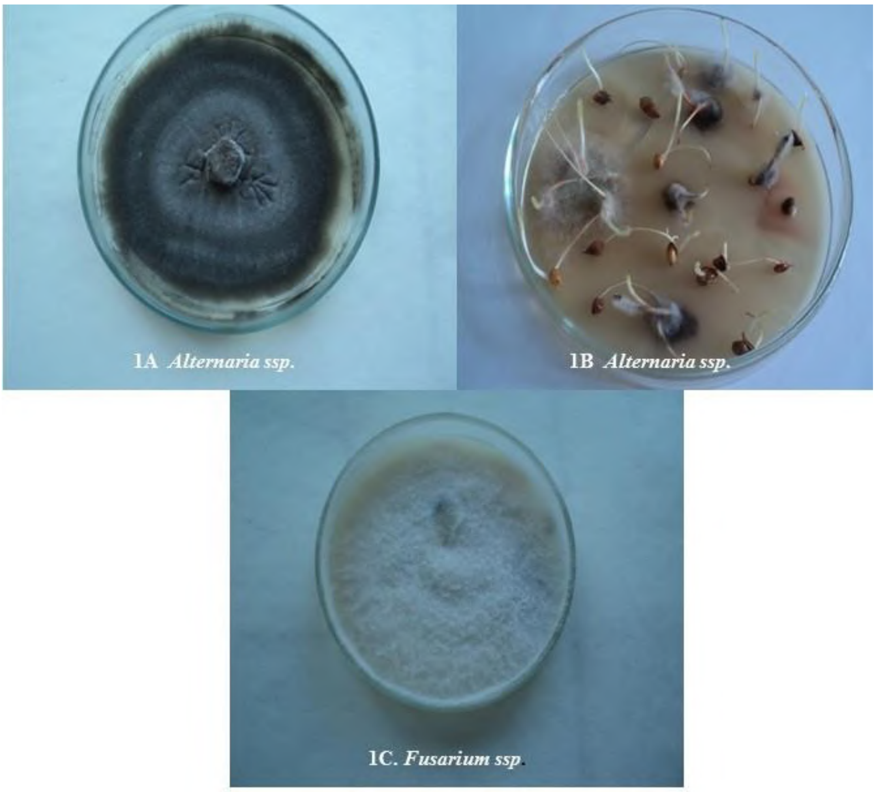

Figure1. Pathogens that cause diseases in sorghum.
(Image by Krasimira Tanova)

The pathogenic fungi - *Fusarium moniliforme*J. Sheld**,** *Fusarium culmorum*, *Penicillium* ssp.are the causative agents of those pathologies (Stancheva, 1996; Stancheva, 2009; Andreeva and Tanova, 2011).
When introducing new species of agricultural cultures in specific region information on seeds germination, seed-borne diseases eventual manifestation and development is explicitly important. In this connection, the present research is directed towards the germination and germination energy of the seeds, as well as the mycoflora of different sorghum genotypes, cultivated in Shumen region.

Materials and methods

In January and February 2019 an examination on germination and epiphytic microflora constitution is conducted in the phytopathology laboratory of the Natural Science Faculty of the University in Shumen, Bulgaria. The subject of the examination are 7 sorghum varieties, 6 of them were by RAGT, seeds producer: DODGGE, FLAGG, GGUSTAV, ANGGY, ISEBERGG, GOLDEN and one of them, Maxired, is from the selection program of Shumen Agricultural Institute, serving as standard. The varieties were included in a demonstration, which took place in 2019 on the territory of the Shumen Agricultural Institute (Kikindonov et al. 2013). Seeds from 5 plants from each variety are provided on random basis, for the purposes of determination of the germination and the analysis of the present epiphytic microflora.
Standard phytopathology methods are used for the determination of the sub epidermal mycoflora pathogens, therefore each version includes 3 repetitions with 100 pcs. seeds (Popkova, 1989, Popkova et al., 2005). The surface of the seeds is sterilized with 0.01 % Hg CI_2 in advance. On the 7^{th} day after the incubation the quality and quantitative composition of the seeds mycoflora is measured (Stancheva, 2010).
The seeds germination and the germination energy is determined in line with the standard methods of the Executive Agency in Species Testing, Approbation and Seed Control of the Bulgarian Ministry of Agriculture. Binocular microscope Optika-B150 is used for the microscopic examinations.
The accuracy of the difference between the compared estimates of each version of measurements is valued through the means of the so called marginal difference. The number of the seeds, contaminated with different species fungi is reported in %. The results of the laboratory analysis are statistically processed through the means of a variation analysis of the qualitative signs (Zapryanov, 1983).

Results and Discussions

Figure 2 presents the results from the genotypes sowing qualities. Visible from the results presented, the germination energy is highest with the seeds from the DODGGE and GGOLDEN materials, respectively 86.50 % and 87.85 % and they show higher values than the seeds of the controlled variety – Maxired.
The lowest index is with the seeds of GGUSTAV, ANGGY, FLAGG ANGGY и ISEBERG varieties, showing lower germination energy in comparison with the control version of the seeds. The seeds germination under laboratory conditions shows differences as per the tested genetic origin, as the seeds from the analyzed genetic origins show higher values than the control version. The maximum reported value is for the FLAGG seeds, respectfully 96.25 % and the lowest one with the ANGGY-86-75%.

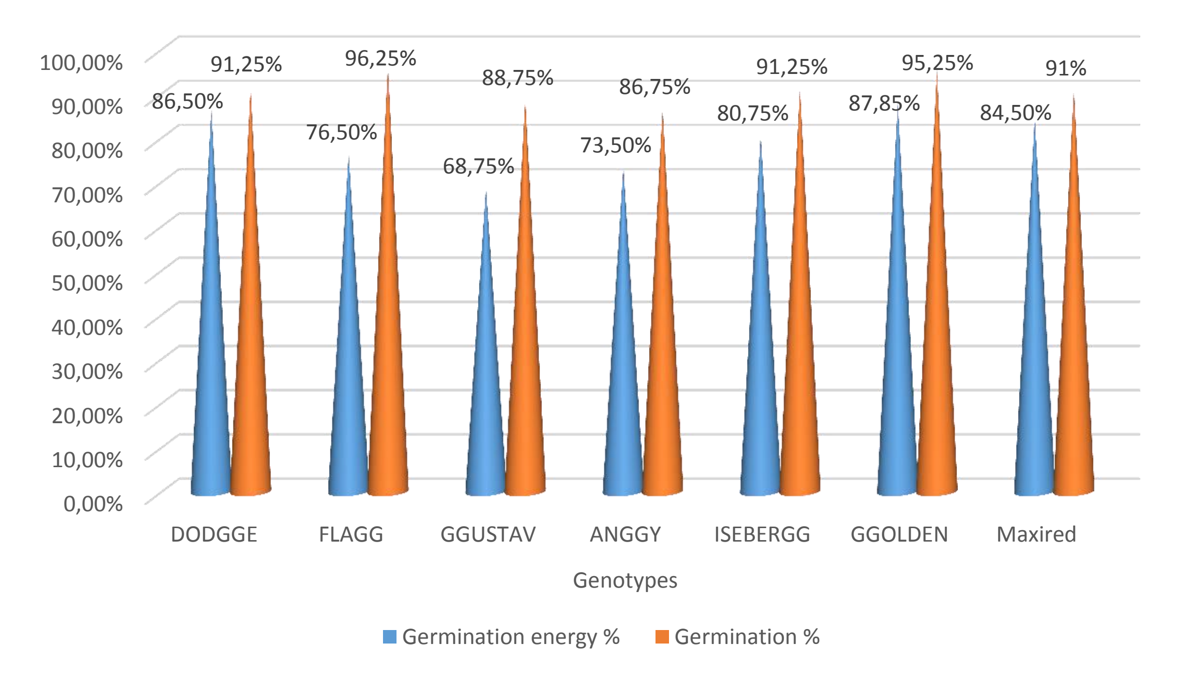

Figure. 2 Germination and germination energy of various genotypes sorghum

Table 1. Reliability of differences between genotypes studied and control

Germination energy						
Genotypes	DODGGE	FLAGG	GGUSTAV	ANGGY	ISEBERGG	GGOLDEN
Maxired	**t>2,58**	**t>3,29**	**t>3,29**	**t>3,29**	**t>3,29**	**t>3,29**
Germination						
Genotypes	DODGGE	FLAGG	GGUSTAV	ANGGY	ISEBERGG	GGOLDEN
Maxsired	**t<1,96**	**t>3,29**	**t>3,29**	**t>3,29**	**t>3,29**	**t>3,29**

t<1,96	not credibility
t>1,96	P = 5%
t>2,58	P = 1%
t>3,29	P = 0,1%

High values of this index, above the control seeds values, we encounter with the following varieties: DODGGE and GGOLDEN, which show a value higher than the control one and they are showing to be the seeds with best germination under laboratory conditions. **Table 1.** show the differences between the Maxired control variety and the remaining varieties in terms of germination energy. The germination index shows unreliable difference with the first variety, DODGGE.

Seven fungi species are determined by the surface and sub epidermal mycoflora, referring to 6 genus. They have different frequencies of presence, depending of the seeds origin. Two of them are causing the alternariosis disease (*Alternaria alternate; Alternaria solani*), fusariosis (*Fusarium moniliformae)*, septoriosis (*Helminthosporium turcicum*), dawny mildew - *Peronosclerospora sorghu*, as well as the causative agents of the *Aspergillus* and *Mucor* species. The research data is presented in **table 2**. Most frequently present istheAlternaria *alternate*, available in the mycoflora of all origins.

Table 2. Frequency of isolated seminal mycophlora in%

Genotype	Pathogens							Root tip necrosis
	Alternaria alternate	*Alternaria solani*	*Aspergillus ssp.*	*Mucor ssp.*	*Helmintosporium turcicum*	*Fusarium monilifor*	*Peronosclespora sorghum*	
	1	**2**	**3**	**4**	**5**	**6**	**7**	
DODGGE	21	0	9	0	0	0	0	18
FLAGG	30	0	8	0	5	10	0	18
GGUSTAV	29	0	10	0	12	17	0	19
ANGGY	32	0	1	4	7	0	14	4
ISEBERG	47	42	0	0	13	0	13	0
GGOLDEN	74	10	0	6	6	0	0	0
Maxired	47	26	0	0	30	0	15	0

P = 5%; P = 1%; P = 0,1%

Most frequent presence of this pathogen is ascertained for the GGOLDEN, ISEBERGG and MAXIRED varieties -47-74%. Lowest frequency of presence is ascertained for DODGGE-21%. Contamination with the other causative agent of the alternaosis- *Alternaria solani* is ascertained only in the mycoflora of GGOLDEN, ISEBERGG and MAXIRED in moderate degree – 10 – 24 %.

The causative agent of the fusariosis -*Fusarium moniliformae*is found in two origins - GGUSTAV, frequency of presence- 17 % and *Helminthosporium turcicum*– ascertained in all origins, besides DODGGE, as the frequency in the mycoflora composition is within the limits from 5 to 30%. The causative agent of the downy mildew for the FLAGG is with encounter frequency of 10% and the one for the septoriosis - *Peronospora sorghum*is present in the mycoflora composition of 3 varieties and ecounter frequency between 10-15%. The share of the varieties of genus *Aspergillus ssp.* and *Mucor ssp.*is comparatively lower. In four of them a sprout necrosis is encountered, due to the unfavorable combination of pathogenic species, as it is best displayed with the GGUSTAV. It is noticed that the control Maxired does not show sprout necrosis, which would be due to the genotype sustainability.

Conclusions

The results gained provide grounds for the following conclusions:

- All tested seeds show better laboratory germination than the MAXIRED variety.
- The mycoflora of the sorghum seeds analyzed consists of 7 types of pathogenic fungi, referred to 6 genus. Key importance is given to the causative agent of the alternariosis, fusariosis, septoriosis.

The combination of pathogens in the seeds damages the germs and the deterioration of seed qualities.

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EFFICIENCY AND THE POSSIBILITY OF USING ZEOLITE AND APATITE IN PURIFYING WATER FOR IRRIGATION AND REMEDIATION OF CONTAMINATED SOIL

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Abstract

Knowledge of the mobility of heavy metals and radionuclides, such as Pb, Cd, Zn and U, represents one of the goals of protection, regulation, rational use and irrigation of agricultural soils from the aspect of safe food production. The aim of this work was investigation of efficiency of natural mineral materials based on zeolite and apatite from domestic deposits in the mobility of heavy metals and radionuclides in the waters and soils of the different physico-chemical characteristics (pseudogley and chernozem). The affinity, efficiency, zeolite adsorption mechanisms and apatite adsorption precipitation were determined in a constant-pressure column system at 300 mg l^{-1} for different pH values (5.00 and 7.00) of the basic contaminated solution (Pb, Cd, Zn, U) at time intervals of 30, 60, 90, 120, 180 minutes. In all experiments, significant changes in the pH of the filtrate occurred. The most significant changes in the pH of the filtrate, minimal fluctuations in the time interval, at pH = 5.00, recorded the basic solutions of Pb (7.69-7.87) and U (7.77-7.93) during leakage through the column with apatite, while slightly lower changes for Cd and Zn were observed. Changes also occurred in the column with the zeolite, but with a much lower intensity compared to the column of apatite, and with the trend of changes U> Pb> Cd> Zn. The trend of changes between apatite and zeolite also occurred in the basic contaminated solution with initial pH=7.00. Zeolite and apatite adsorption/precipitation processes successfully immobilized Pb at both tested pH values of stock solution. Our investigation showed that in colonies, apatite better immobilized U, zeolite is better for immobilization of the Cd, while for the Zn both materials showed a very similar affinity. Zeolite and apatite were added in amount of 20 gkg^{-1} soil to reduce the content of water-soluble and easily accessible forms of Pb, Cd and Zn in uncontaminated and contaminated soil.

Keywords: *soils, water for irrigation, zeolite, apatite, heavy metals*

Introduction

The partial damage and destruction of land and water are a major problem for many countries in the world today, especially in food production. Land and water are the main natural resource not only in food production but as an environment with which human are in constant contact. According to that, it is a very serious problem for both: professional and socio-political organizations how to protect these irreplaceable natural resources. Increasingly stringent conditions and rules to produce health food are provided from "HACCP" and "GAP" concepts. It is predicted that Serbia will not be able to export any agricultural product without a certificate of land quality as a "critical place" in primary agricultural production after 2020 (Grubišić, 2017). Usage of nonmetallic mineral materials is important "in-situ" technology for remediation of toxic metals and radionuclides. The materials used for this purpose must be efficient, inexpensive, available in tonnes, and applicable in different mediums (water, soil).
The most commonly used materials are iron oxides, zeolites, apatites, MgO, carbonates, compost, peat, cottonseed flour and lime (Conca, 1997, Brown *et al.*, 1996).

"In situ" technology is a successful and efficient passive method for the sanitation of contaminated groundwater and surface water as well as soil. It involves in some way control-management combining chemical, biological and physical remediation processes. The use of "in situ" technology has advantages in comparison to the conventional technologies for remediation due to it requires no maintenance and minimal operation (Knox *et al.,* 2000, NATO/CCMS U.S. EPA, 2002).
It is known that zeolites (from the clinoptilolite group) can be successfully used for immobilization of Cs, Sr, Cu, Cd, Pb, and Zn in soil and water (Alther, 2005, Ming & Mumpton, 1989). Ma *et al.*, 1993 showed that metal stabilization is efficient in combination with apatite especially for Pb, Zn, Cu, Cd, Ni, U, Ba, Cs, Sr, Pt, Th and other elements.
The usage of minerals for adsorption and precipitation processes in soils and waters is highly dependent on the pH of the medium, the presence of carbonate, organic matter, Fe-Mn oxides as well as the mechanical composition and type of soil mineral clay (Mission *et al.*, 2009). Prior to the in-situ remediation procedure and the use of mineral raw materials, it is necessary to carry out the procedure under laboratory conditions in order to determine the fillers (adsorbents, precipitators), the amount of material, the shelf life, the methods efficiency, etc. Based on experimental results and equations it can be calculated efficiency (K) and capability (Cap) of the various additives for immobilization and outdoor application in field conditions (Jang *et al.*, 1998).
The aim of the study is to investigate the efficiency and possibility for application of zeolites and apatites for remediation of contaminated groundwater, irrigation water and soil.

Material and Methods

Efficiency of mineral raw materials (zeolites and apatites) in a mobility of toxic metals and radionuclides are investigated in Institute for Technology of Nuclear and other Mineral Raw Materials. Experiment was carried out in glass columns (diameter 4 cm), with controlled constant flow. The mass of the clinoptilolite zeolite in the column was 30 g, granulation of 0.8-2.0 mm. The weight of the second mineral supplement-apatite was 30 g, granulation <100 microns. Due to the low filtration coefficient of micronized material, the apatite was mixed with 300 g of purified and calcined quartz sand (ratio 1:10). In this way, a complete control of the constant-pressure filtration coefficient of 200 ml/h for both mineral adsorbents was performed (Figure 1a).

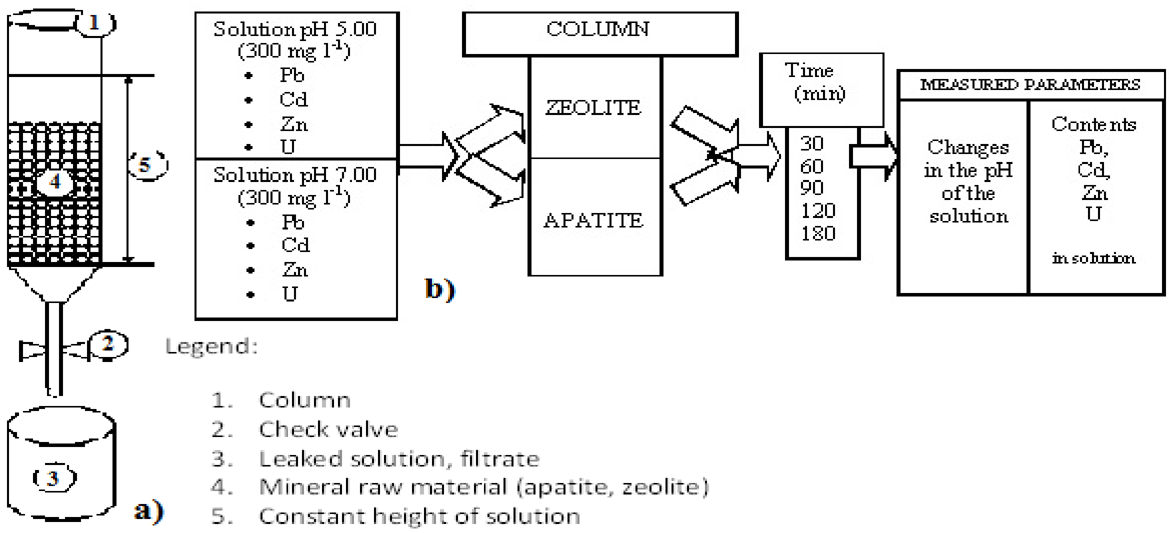

Figure 1 – Schematic veiw of: column a), column experiment b)

The concentration of toxic metals (Pb, Cd, Zn) and radionuclides (U) used for column experiments was 300 mg/l. Toxic metals solutions were prepared from acetates and radionuclide solution Uran-a was prepared using uranyl nitrate. The pH value of prepared solutions were adjused at pH 5.00 and pH 7.00. Prepared solutons were passed through a system of columns filled with mineral additives (zeolite, apatite). The samples are collected at different period times: 30, 60, 90, 120, and 180 min. In this way, 4 x 100 ml and 1 x 200 ml solutions for chemical analysis at the 5 different intervals based on the filtration coefficient were collected (Figure 1b). The second part of experiments involved adding of 20 g kg^{-1} zeolites and apatites in the soil (pseudogley pH=5.55, sand chernozem pH=7,05). In prepared soils the portion of 50 mL solution with 2 mL Pb-Zn-Cd syntetic solution were added. The concentration of syntetic solution was: Pb=50 mg cm^{-3}, Zn=30 mg cm^{-3}, Cd=1 mg cm^{-3}. After first and thirtieth days, water-soluble and accessible forms of added toxic metals (Pb, Zn. Cd) as well as their total content in prepared soil samples were determined.

Results and Discussion

The affinity of mineral adsorbents in reducing the mobility of toxic (Pb, Cd, Zn) metals and radionuclides (U) at different pH values (pH 5 and pH 7) of the solution was determined at the first part of experiments.

The change of pH value of a basic toxic solution at pH 5 in a column system with apatite and zeolite after passing through column are shown in Table 1. As can be seen from Table 1, the filtrates with the highest pH value at pH 5 have the basic solutions of Pb and U which passed throught apatite column at all investigated intervals (t1-t5). Also, the diferences in pH value between investigated solutions are minimal: Pb (7.75-7.80) and U (7.77-7.93) (Figure 1).

Slightly lower intensity of pH value change was detected in the basic solution at pH 5 contaminated with Zn and Cd.

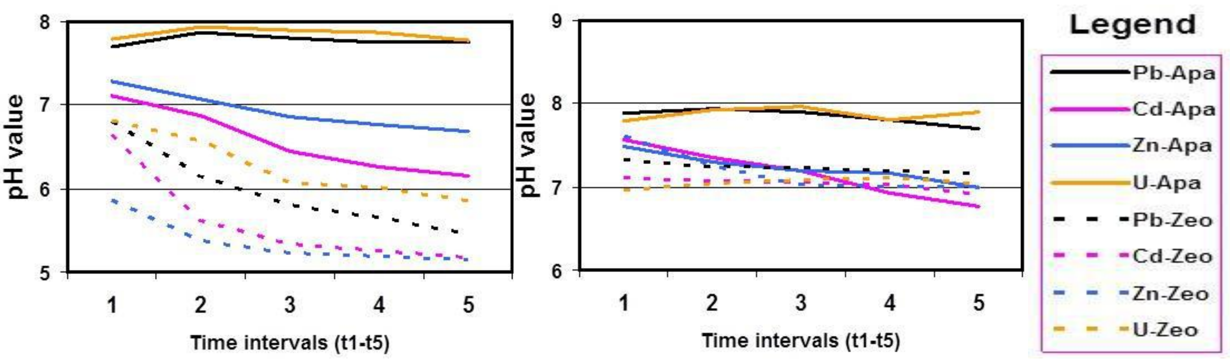

Figure 1. The change of pH value of basic solution (pH 5, left), (pH 7, right)with toxic metals after passing through column with apatite and zeolite

That solutions of Cd and Zn (pH 5) which passed through the apatite column, have lower pH of the filtrate and record a linear, slight decrease in the pH of the filtrate in Zn basic solution (7.28-6.68) and slightly faster in Cd basic solution (7.11-6.15) Knox *et al*. (2003).

The change of pH value of a basic toxic solution (Pb, Zn, Cd, U), pH 5, are also deceted in a column system with zeolite. As can be seen, the lower pH change intensity but with same distribution of contaminant (U, Pb, Cd, Zn) in comparison with experimental results from apatite column was observed.

Also, the pH value change was detected after passing of basic contaminated solution (Pb, Zn, Cd i U), pH 7, through apatite-zeolite column (Figure 1, right.).

The pH value changes at pH 7 are significantly less than the basic solution pH 5, and the variations range is in interval from 6.77 to 7.96. The same trend with the pH 7 solution (the largest pH changes) occurred in the Pb and U filtrate obtained through an apatite column. The

pH of the filtrate is quite uniform for the apatite column for contaminated solutions with Pb (7.70-7.94) and U (7.79-7.96). The obtained filtrate from the apatite column for Cd and Zn have linear decrease in pH over all investigated intervals t1-t5: from 7.56 to 6.77 and from 7.49 to 7.00 for Cd and Zn, respectively (Matusik *et al.*, 2012).
Zeolite adsorbent, as a filter in the columns, influenced the pH changes of the basic contaminated solutions (Pb, Zn, Cd, U), pH 7, but with a much smaller effect compared to the same conditions where the filter was apatite. Zeolite had a greater influence on the filtrates when the initial contaminated solution with toxic metals (Pb, Zn, Cd, U) was acidic.
Zeolite and apatite can successfully mobilize Pb at pH 5, stock solution through adsorption/precipitation processes (Figure 2). The U-ion is better immobilized by apatite than by zeolite, while Cd is better immobilized by zeolite and has a very similar affinity for immobilization of Zn over a 180-min in the acidic medium of stock solution, pH 5.
Zeolite and apatite, at the pH = 7.00 are very efficient in reducing the mobility of Pb, at all investigated intervals (t1-t5).

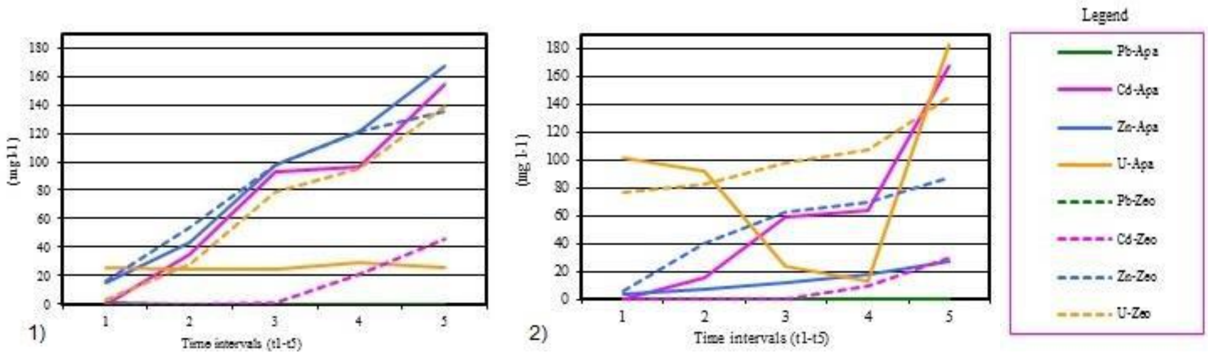

Figure 2. Dynamics of adsorption-precipitation of toxic metals and radionuclides in time, concentration of contaminants in stock solution was 300 ppm, pH 5 (1) pH 7 (2)

Zeolite is very efficient adsorbent for Cd, especially in the first three time intervals (Figure 2). Compared to zeolite, the efficiency of apatite is significantly lower for Cd immobilization. Zn stock solution (pH 7) after passing through apatite and zeolite columns was well imobolized. The Zn content in filtrate is lower than in the acidic stock solution.
The efficiency of zeolite and apatite for U imobilization in neutral stock solution (pH 7) is significantly reduced compared to the acidic stock solution (pH 5)
Immobilization, the process of precipitation of U ions was very slow under neutral reaction conditions, it took 60 minutes (t2) to initiate Ca ions from apatite and create stable complexes. The efficiency of zeolites in the immobilization of uranium ions in the neutral pH medium is weak, the U content in the filtrate increase linearly during the investigated time from 76.66 (t1) to 145.33 mg l/1 (t5).
The efficiency of adsorbent/precipitator mineral resources in the soil-toxic element system in two soil types (Table 1) was investigated in the second part of experiment.
The same amount of added toxic elements (Pb, Cd, Zn) in pseudogley and sand chernozem had a different influence on the content of readily available forms of the same elements, so the content of readily available Pb is 146.0 mg kg^{-1} in sand chernozem, while Pb content in pseudogley is 195.0 mg kg^{-1}. The content of readily available Cd in contaminated soil depends on the pH value, on th pseudogley Cd content is higher (2.70 mg kg^{-1}), while on sand chernozem is lower (1.12 mg kg^{-1}). The similar results were obtainet for the content of readily available Zn: in pseudoglej the content was 121.0 mg kg^{-1} while in sand chernozem was 47.7 mg kg^{-1}, as well as for the readily available form Pb (in pseudogley the content was 195.0 mg kg^{-1} while in sand chernozem 146.0 mg kg^{-1}).

Table 1. Content of water-soluble and easily accessible forms of toxic metals in soil, 24 hours after contamination with Pb, Cd, Zn

Soil type	Variant	Toxic element, (TE), (mg kg^{-1})					
		Pb		Cd		Zn	
		H_2O	DTPA	H_2O	DTPA	H_2O	DTPA
Sand Chernozem	Control (K)	0.2	1.2	0.07	0.44	0.16	0.60
	K+Apatite	0.2	0.6	0.08	0.22	0.14	0.38
	K +Zeolite	0.2	0.9	0.08	0.24	0.08	0.33
	K+Toxic Element	1.5	146	0.20	1.12	0.87	47.7
	K+Apatite+TE	1.4	110	0.18	0.95	0.12	29.5
	K+ Zeolite+TE	1.2	96	0.14	0.95	0.24	32.1
Pseudogley	Control (K)	0.1	2.2	0.08	0.26	0.10	1.24
	K+Apatite	0.05	1.8	0.08	0.22	0.08	0.98
	K +Zeolite	0.1	2.1	0.06	0.21	0.08	0.94
	K+ Toxic Element	1.0	195.0	0.16	2.70	0.82	121.0
	K+Apatite+TE	0.4	69.7	0.15	1.40	0.36	59.8
	K+ Zeolite+TE	0.8	79.2	0.15	2.10	0.56	85.8

The content of readily available forms of toxic metals Pb, Cd, Zn is much higher in the acidic pH of the pseudogley soil (pH in H_2O, 5.55) than in the soil of neutral pH (pH in H_2O, 7.05) in sand chernozem. According to experimental results it can be concluded that zeolite and apatite are efficient as reducants of the content of water-soluble and easily accessible forms of Pb, Cd and Zn in uncontaminated and contaminated soil, regardless of soil conditions (pH, mechanical composition, mineralogical composition, chemical composition), which is of great importance for the remediation of agricultural land. Also, a small difference between the forms of toxic metals for the 720 hour duration of the experiment is statistically insignificant. This indicates that there was no increase in the water-soluble and readily available forms of toxic metals, suggesting strong adsorption/precipitation process performed by zeolite and apatite.

Conclusions

The minelal raw materials (zeolite and apatite) from the territory of Serbia can be successfully used in groundwater and soil remediation technologies by using them as fillers for semi-permeable reactive barriers (PRB). These adsorbent precipitators are efficient in the column system in reducing the mobility of Pb, Zn, Cd and U at different pH values (pH 5.00 and 7.00). In this way, the wide use of non-metallic mineral raw materials from the territory of Serbia was once again confirmed. Zeolite and apatite can be successfully used as minerals for reducing the mobility of Pb, Cd, Zn and U in pseudogley and sand chernozem soils as well as surface irrigation waters. Short equilibrium time, efficiency, high sorption capacity, degree of stabilization are the main advantage for application of these materials from domestic raw material in the technology of immobilization of harmful elements in soil in a simple, inexpensive and successful procedure.

Acknowledgements

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USAGE OF PARSLEY POWDER AS AN ALTERNATIVE CURING AGENT IN FERMENTED SUCUK PRODUCTION

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Abstract

Sucuk is one of the most consumed traditional Turkish meat products and is a dry, cured, fermented and produced from beef meat, animal fat, curing ingredients and various spices. Nitrate and nitrite, which are used as curing agents in the production of sucuk, are very effective in extending the shelf life of product and in the formation of characteristic color and flavor. However, nitrate and nitrite cause toxic effects on human health. The control and reduce of nitrate and nitrite content in cured meat products is very important for the quality of the products and food safety. On the other hand, nowadays, consumers demand foods that do not contain chemical additives so that current studies focus on the usage of plant-derived natural additives as alternative curing agents. In this study two different groups of fermented sucuk were produced containing C: 100 ppm sodium nitrite (control group) and P: 1.17% parsley powder (100 ppm nitrate equivalent). The effects of parsley powder as an alternative curing agent on residual nitrate and nitrite contents, lipid oxidation, pH values, color and sensory properties of fermented sucuk during storage at 4°C for 45 days were investigated. After 30 days of storage, there was no nitrate in the P group. At the end of storage C and P groups had 2.23 and 2.86 ppm sodium nitrite contents, respectively. C group had lower pH values (4.89-5.05) than P (5.16-5.29). Overall acceptability scores of the sucuks containing parsley powder were higher than the control samples.

Keywords: *Alternative curing, Fermented sucuk, Parsley powder, Residual nitrate, Residual nitrite.*

Introduction

Fermented meat products are widely consumed in the world and sucuk is one of the most important fermented meat products (Karaçıl and Tek, 2013). Fermented sucuk is obtained by mixing ground meat and fat with salt, spices, curing ingredients and filling it into the intestines and ripening sucuk dough at a certain temperature and humidity (Gökalp *et al.* 2004). Nitrate and nitrite are widely used as curing ingredients in production of meat products. Nitrate and nitrite have been used in meat products for improvement of characteristic colour and flavour, preventing of lipid oxidation and antimicrobial effect on foodborne pathogens (Sindelar *et al.*, 2007). Despite these important roles in cured meat products, nitrate and nitrite have negative effects on human health. Nitrite reacts with secondary amines in the body and nitrosamines are formed. Nitrosamines are highly carcinogenic and/or mutagenic compounds (Connolly and Paul, 2001; Roberts and Dainty, 1991). Consumers are demanding natural foods that do not contain chemical additives because of their negative effects on health. This demand of consumers can be met by uncured or naturally cured meat products (Alahakoon *et al.*, 2015; Sebranek and Bacus, 2007; Winter and Davis, 2006). Nowadays, there are increasing studies to remove or reduce the amount of nitrate/nitrite from the formulations and to reduce the amount of residual nitrite in the final product by using plant-derived additives in meat products (Turp and Sucu, 2016). Vegetables have an important potential as natural nitrate sources in cured meat products. Vegetable powders and juices are commercially available and can be used in the production of natural meat products (Sebranek and Bacus, 2007). However, in order to perform the effects of nitrate

on meat products, it must be reduced to nitrite. The reduction of nitrate to nitrite is carried out by microorganisms present in the natural flora of meat, or by microorganisms capable of reducing the added nitrate (Sindelar *et al.*, 2007).
The concentration of nitrate in vegetables varies between 10 and 10000 mg/kg (Özdestan and Üren, 2010). Spinach, carrots, celery, lettuce, beet, dill, leek and parsley are vegetables with high concentrations of nitrate source (Erkmen et al., 1990). Some studies have been carried out on the use of vegetables such as spinach, celery (celery powder and celery juice) and leek as natural nitrate sources in meat products (Horsch *et al.*, 2014; Jackson *et al.*, 2011; Kim *et al.*, 2017; Krause *et al.*, 2011; Magrinyà *et al.*, 2009; Sindelar *et al.*, 2007; Tsoukalas *et al.*, 2011).
The average nitrate content of fresh parsley is 134.63 ppm (Matallana Gonzalez *et al*., 2010). There are few studies using parsley powder as nitrate source in meat products. Riel *et al.* (2017) used parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausages. The other study is reduction in the amount of synthetic nitrite in fermented sausages by using parsley powders (Yüzlü, 2018). The aim of the present study is to determine the possibility of the use of parsley powder as natural curing agent (alternative to nitrite) in Turkish fermented sucuk.

Material and Methods

Fresh parsley was obtained from a local market in Konya, Turkey. After the parsley was washed, it was dried under natural conditions (in laboratory). The dried parsley was ground and parsley powder was obtained. It was sterilized for 2 hours at 120 °C.
Two different groups of fermented sucuk were produced containing C: 100 ppm sodium nitrite (control group) and P: 1.17% parsley powder (100 ppm nitrate equivalent). Sucuk batters were produced the following formulation per kg of beef meat: 0.35 kg beef fat, 0.04 kg garlic, 0.02 kg NaCl, 1.5 g dextrose, 45 g spice mixture (red pepper, black pepper, cumin, pimento, and clove). Each batter was inoculated with mixture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-T03, Christian Hansen, Hoersholm, Denmark) at a level of 10^7 CFU/kg of sucuk batter. Salt containing 100 ppm sodium nitrite was used for the production of control group and 1.17% (100 ppm nitrate equivalent) parsley powder was added to sucuk formulation for the group of P. Sucuk batters were filled into the casings and were placed in climatic room for fermentation and drying. The climatic room conditions were 24 °C, 90% relative humidity and 0.5 m/sec air flow rate for the first day. On other days, the temperature and relative humidity were gradually reduced until the moisture content of sucuks decreased to 36-39%. Sucuk samples were vacuum packed and stored at 4 °C for 45 days.
pH, color properties ($L*$, $a*$ and $b*$), TBARS, penetrometer, residual nitrate and nitrite analyses of the sucuk samples were conducted at 0, 15, 30 and 45 days of storage. Residual nitrate and nitrite contents were determined also in sucuk batters (before fermentation). Nitrate and nitrite contents were also determined at the beginning of the fermentation in sucuk batters. Sensory analysis of samples was performed on the 15th day of storage.
The pH values of the samples were determined according to AOAC (2000). Color analysis were conducted with colorimeter (Minolta, Japan) according to Hunt *et al.* (1991). The method described by Ockerman (1985) was used to determine the lipid oxidation (TBARS) of sucuks. Hardness of samples were determined using Penetrometer (K-936, Koehler Instrument Company, US) according to Anonymous (1975). Residual nitrate and nitrite contents of samples were determined according to the method described by Cortesi *et al.* (2015). Sensory analysis was conducted by using the method described by Gökalp *et al.* (2015).Results were analyzed by two-ways analysis of variance (ANOVA) using MINITAB 16.0 for Windows. Comparisons of mean values were made using the Tukey test.

Results and Discussion

pH, TBARS, *L**, *a**, *b** and penetrometer values of sucuks are given in Table 1. pH values of sucuks decreased with increasing storage days. Sucuks including parsley powder had higher pH values than control group. Similarly, Jin *et al.* (2014) reported that containing red beet powder increased the pH values of emulsified pork sausages. However, some researchers indicated that plant-derived natural additives such as leek, celery products, parsley extract powder caused decrease in pH of cured meat products (Eisinaite *et al.*, 2016; Fista *et al.*, 2014; Riel et al., 2017).

TBARS value is an indicator of lipid oxidation which is one of the important quality parameters for meat and meat products. TBARS values of samples were expressed as mg malondialdehide (MA) per kg of sample. TBARS values of samples increased with storage days but this increase was not significant ($p>0.05$). The group of P had the higher TBARS values than control group at 0 and 15 days of storage (Table 1). After 15 day storage, there was no difference between the groups of C and P in terms of TBARS values. This could be partly explained that on the 15th day, most of the nitrate was reduced to nitrite which plays an important role in preventing lipid oxidation (Sindelar *et al.*, 2007).

Table 1. pH, TBARS, *L**, *a**, *b** and penetrometer values of samples

	Day	Samples	
		Control (C)	Parsley (P)
pH	0	5.06±0.01^{Ab}	5.29±0.01^{Aa}
	15	5.00±0.01^{Ab}	5.24±0.00^{Ba}
	30	4.90±0.01^{Bb}	5.17±0.00^{Ca}
	45	4.89±0.03^{Bb}	5.16±0.00^{Ca}
TBARS (mg MA/kg sample)	0	0.39±0.02^{Ab}	0.45±0.05^{Aa}
	15	0.42±0.01^{Ab}	0.48±0.05^{Aa}
	30	0.43±0.02^{Aa}	0.51±0.06^{Aa}
	45	0.45±0.01^{Aa}	0.53±0.07^{Aa}
*L**	0	45.57±1.08^{Ba}	42.03±1.14^{Ba}
	15	51.04±0.52^{Aa}	44.87±0.71^{ABb}
	30	48.84±0.37^{ABa}	48.31±0.46^{Aa}
	45	51.21±0.21^{Aa}	47.81±0.63^{Ab}
*a**	0	15.11±0.73^{Aa}	13.24±0.58^{Ab}
	15	14.79±0.30^{Aa}	12.11±0.17^{ABb}
	30	14.43±0.05^{Aa}	11.20±0.15^{Bb}
	45	13.85±0.53^{Aa}	11.52±0.06^{ABb}
*b**	0	17.41±0.92^{Aa}	13.28±0.01^{Ab}
	15	17.34±0.60^{Aa}	13.22±0.84^{Ab}
	30	18.52±0.52^{Aa}	12.14±0.30^{Ab}
	45	19.77±1.67^{Aa}	11.90±0.24^{Ab}
Penetrometer value (1/10 mm)	0	533.90±3.57^{ABa}	542.80±1.25^{Aa}
	15	555.00±11.18^{Aa}	549.20±5.14^{Aa}
	30	510.30±2.13^{Ba}	511.90±0.05^{Ba}
	45	513.00±8.02^{Ba}	480.50±2.16^{Ca}

Mean ± standard error

$^{A-B}$ Within the same column, values with different uppercase superscripts indicate significant differences ($p < 0.05$).

$^{a-b}$ Within the same row, values with different lowercase superscripts indicate significant differences ($p < 0.05$).

Parsley powder addition affected the lightness (*L**) values of the samples on the 15th and 45th day. *L** values of the group P were lower than control samples in these days. While *L** values of samples increased during storage for the group of P, the highest *L** values were observed at

15 and 45 days of storage for control group. Storage time did not affect the redness (*a**) values of control sucuks while *a** values of P group samples were significantly ($p<0.05$) different. The group of P had lower *a** values than control group due to the dark green color of parsley powder. Yellowness (*b**) values of the samples were not different during storage days. Parsley powder addition decreased the *b** values of sucuks. Similarly, Sucu and Turp (2018) reported that usage of beetroot powder as natural curing agent decreased the *b** values of fermented sucuks.

Penetrometer value indicates the tenderness of samples. There is an inverse relationship between the penetrometer value and the hardness of the sample. Parsley powder addition did not affect the penetrometer values of samples (Table 1.). Lower penetrometer values (decrease in tenderness) were determined in all groups at 30 and 45 days of storage. These results were in accordance with studies indicating that beetroot powder did not affect the textural properties of fermented sausages (Sucu and Turp, 2018) and red beet powder addition did not change the texture of emulsified pork sausages (Jin *et al.*, 2014).

Nitrate and nitrite contents of sucuk batters at the beginning of fermentation were determined as 9.65 and 5.04 ppm for the control group and 43.81 and 6.28 ppm for the group of P, respectively. The presence of nitrate in the control group is thought to be the cause of spices (Özcan and Akbulut, 2007). Figure 1 shows the residual nitrate and nitrite levels of sucuks during storage days. Final products had no nitrate in control group (C) while the group of P included 16.26 ppm nitrate on 0 day of storage. After 15 day of storage, nitrate content was also depleted in the group of P. The reason for the difference between the groups in terms of nitrate levels can be explained as follows: P group was produced with nitrate (plant-derived nitrate) and C group was cured using containing conventional (chemical) sodium nitrite. Residual nitrite levels of samples were ranged between 2.23 and 3.57 ppm during storage. Residual nitrite levels of the samples decreased during storage time (except on day 30 of P group). After 15 days of storage an increase in nitrite level was observed on day 30 due to the reduction of nitrate in the P group. Control group had lower nitrite contents than the group of P. Similarly, Horsch *et al.* (2014) and Sullivan *et al.* (2012) reported that hams including celery powder and celery juice concentrate had higher residual nitrite contents than control samples.

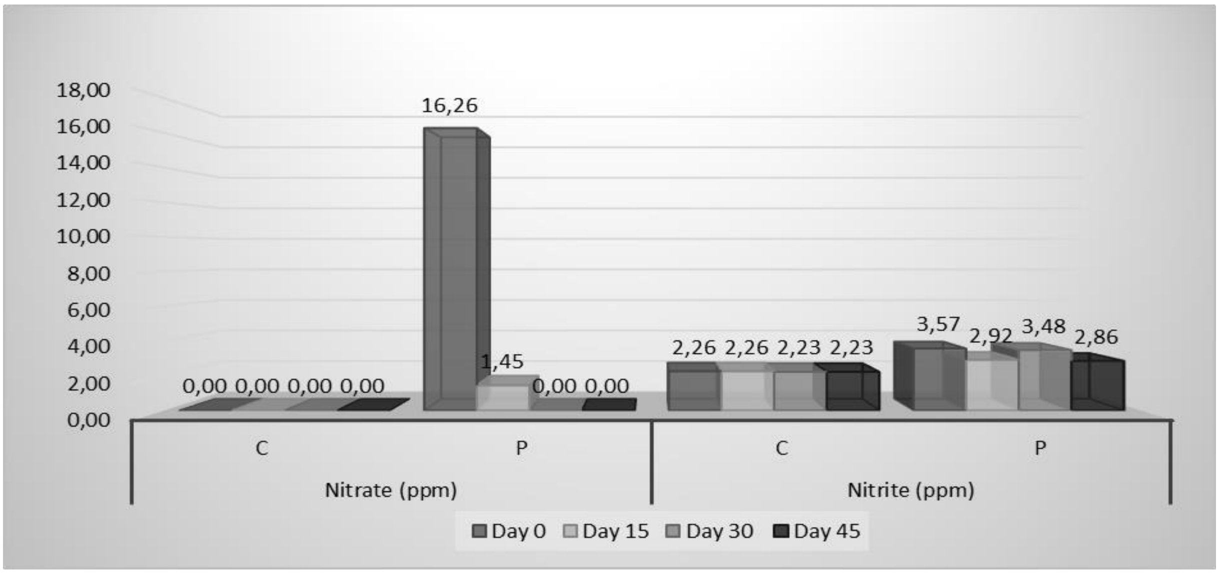

Figure 1. Residiual nitrate and nitrite levels of sucuks

Figure 2 indicates the scores of sensory parameters (odor, color, flavor, texture and overall acceptability) of sucuks. Panelists gave higher scores to group P in terms of flavor (8.07), texture (7.80) and overall acceptability (7.87). The differences between odor and color scores were not statistically significant (p>0.05).

Figure 2. Odor, color, flavor, texture and overall acceptability scores of samples

Conclusions

pH values of sucuks including parsley powder were higher than control. After 15 day storage, there was no difference between the groups of C and P in terms of TBA values. Parsley powder addition decreased the lightness and redness values of sucuks. The tenderness of samples was not affected the parsley powder. After 30 days of storage, there was no nitrate in the P group. At the end of storage C and P group had 2.23 and 2.86 ppm nitrite contents, respectively. Flavor, texture and overall acceptability scores of P group were higher than control group.

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BIOCONTROL OF STRAWBERRY GREY MOLD USING PEPPER EXTRACTS

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Abstract
Grey mold is an important yield loss causing disease in strawberry around the world. The growing resistance of the pathogens requires the continuous formulation of new plant protection products and one of the natural resources of bioactive ingredients are plants. Black pepper and allspice (pimento) are commonly used spices for foods. However, they also have active substances which provide these spices and their fruits aromatic, taste or medicinal properties. This gives a potential for antifungal activity of the extracts of these spices. The aim of the research was to investigate the inhibition of *Botrytis cinerea* growth by black pepper (*Piper nigrum* L.) and allspice (*Pimenta dioica* L.) extracts. The research was carried out at LAMMC Institute of Horticulture, Lithuania. Extracts from dried fruits of spices were produced using subcritical CO_2 extraction. Firstly, inhibition of *B. cinerea* was examined by mixing different concentrations of investigated extracts with PDA and inoculating each plate with 6 mm diameter, 7-day old pathogen disc. Secondly, strawberry leaves were sterilized and placed in Petri dishes with filter paper and 5 ml of sterile water. Leaves were sprayed with mixtures of extracts with sterile distilled water. Then each strawberry leave was inoculated with 9 mm diameter, a 7-day old disc of *B. cinerea.* Both experiments were incubated separately at 22±2 ^{o}C in the dark and evaluated after 2, 4 and 7 days. The results showed that allspice extract demonstrated higher inhibition of *B. cinerea* than black pepper extract both on the PDA and on strawberry leaves.

Keywords: *allspice, antifungal, black pepper, inhibition, pimento.*

Introduction
Botrytis cinerea cause grey mold of strawberries and is responsible for high yield losses in case of the occurred disease (Adebayo et al., 2013; Rasiukevičiūtė et al., 2019). The resistance of the pathogens is growing because of extensive use of chemical compounds in plant protection and the changes in *B. cinerea* genome may be provoked by conventional chemical fungicides (Valiuškaitė et al., 2010; Abbey et al., 2019), which will result in new strains and inefficiency of many plant protection measures against this strawberry pathogen. The resistant pathogens could also be spread through countries with the propagating material (Rasiukevičiūtė et al., 2018). Known as a diverse pathogen, *B. cinerea* insists on changing measures to control it. Finding a natural source of active ingredients for plant protection products may solve resistance problem (Dayan et al., 2009) and let to avoid the contamination of the environment, including human food, with chemical residues as it is known as biodegradable and non-toxic material (Abdel-Kader et al., 2011).
Black pepper (*Piper nigrum* L.) and allspice (*Pimenta dioica* L.) are used as spices for food making and in traditional medicine for their strong aromatic and antioxidant properties (Lorenzo-Leal et al., 2018). The major components of black pepper volatile oil are trans-caryophyllene, limonene and sabinene (Singh et al., 2004; Teneva et al., 2016; Hikal, 2018), meanwhile allspice consist mainly of eugenol or α-terpineol (Song et al., 2016; Chaudhari et al., 2018). Alkaloid piperine gives the bitter taste of black pepper. It is already stated that this component and black pepper oil had antimicrobial properties against several bacteria strains

and foodborne fungi (Nikolic et al., 2015; Teneva et al., 2016; Hikal, 2018). It was determined allspice extract (microwave assisted) ability to inhibit bacteria from food (Lorenzo-Leal et al., 2018). The growth of human pathogen *Staphylococcus aureus* was inhibited by the tannins, isolated from allspice leaves (Al-Harbi et al., 2017) and whole extracts from allspice leaves showed potential to be effective against several human pathogenic fungi (Bhat and Raveesha, 2016).
Some information about activity against plant pathogens could also be found in the literature. Previous studies (Sagitov et al., 2011) reported that black pepper extract reduced wilt (*Fusarium oxysporum* f.sp. *lycopersici*.) symptoms and disease severity on tomato plants. Moreover, black pepper essential oil was effective *in vitro* against *Fusarium graminearum*, and acetone extract was effective against *Penicillium viridcatum* and *Aspergillus ochraceus* (Singh et al., 2004).
There is a lack of studies on the effectiveness of allspice extract and few studies on black pepper extract against plant pathogens thus we aimed to determine whether these extracts have potential to be used in biocontrol of strawberry grey mold (*Botrytis cinerea*).

Materials and methods

The research was carried out at Laboratory of Plant Protection, Institute of Horticulture, LAMMC, Lithuania, in 2019. Dried grinded black pepper, and pimento was purchased from spice suppliers (Saldva, Lithuania). The 10 kg of each material was extracted with liquid CO_2 under these parameters: 40 bar pressure, 10 ^oC temperature and duration 6 hours. The extracts were collected, stored in the refrigerator at 5 ^oC and used in further experiments. Single spore isolate of *B. cinerea* was used in this study. The monoculture was obtained from infected strawberry fruits and identified by PCR (Rasiukevičiūtė et al., 2018). Inhibition of *B. cinerea* was examined by mixing 1000-2000 μl/l concentrations of investigated extracts with PDA. Petri dishes with extracts were inoculated with 6 mm diameter, 7-day old pathogen disc and incubated 7 days at 22 ± 2 ^oC in the dark. Evaluations of *B. cinerea* colony diameter were made 2, 4 and 7 days after inoculation. The diameter of the fungal colony was recalculated to percentage inhibition, using formula (Cherkupally et al., 2017):

Inhibition (%) = (C-T)/C x 100,
there
C – diameter of the pathogen colony in control, cm;
T – diameter of the pathogen colony in treatment, cm.

The inhibition of grey mold infection on detached strawberry leaves was examined with 6 ml/l, 12 ml/l concentrations of black pepper extract (BE) and 20 ml/l, 40 ml/l concentrations of allspice extract (PE). The extracts were mixed with 1% Tween 80 and sterile distilled water. Detached strawberry leaves were rinsed in 70% ethanol 2 min. and in sterile distilled water 1 min. Leaves were placed in Petri dishes with filter paper and 5 ml of distilled water, sprayed with 10 ml of a prepared mixture, inoculated with 9 mm diameter, 7-day old disc of *B. cinerea*, incubated 7 days at 22 ± 2 ^oC in the dark. Evaluations of *B. cinerea* colony diameter were made 2, 4 and 7 days after inoculation and data calculated as percentage inhibition.
Obtained data were analysed using SAS Enterprise Guide 7.1. programme. ANOVA procedure was performed, and means of four replicates were compared by Duncan test at a probability level of 95%.

Results and Discussion

Inhibition of *B. cinerea* colony growth on PDA is presented in Figure 1. Data varied between the investigated extracts and concentrations. Allspice extract had higher inhibition of *B. cinerea* and reached 84.56% at 2000 µl/l and inhibited the pathogen growth by 74.71% at 1400 µl/l and 1800 µl/l. The lowest inhibition by allspice extract was 56.32% at 1000 µl/l. Some information about the impact of allspice extract or essential oil on the various pathogens could be found. Previously, allspice leaves, extracted with petroleum ether and chloroform, was found to inhibit the growth of human pathogenic fungi *C. albicans*, *Microsporum canis* and *Microsporum gypseum* (Bhat and Raveesha, 2016). Furthermore, it entirely reduced the growth of mycotoxins producer *Aspergillus flavus* (Chaudhari et al., 2018). Essential oil of allspice had antifungal effect against *F. oxysporum*, *Fusarium verticilioides*, *Penicillium brevicompactum*, *Penicillium expansum*, *A. flavus*, *Aspergillus fumigatus* (Zabka et al., 2009). Our results could extend the effectiveness of allspice extract with strawberry pathogen *B. cinerea*.

Although Singh et al. (2004) stated that the essential oil of black pepper inhibited the growth of *F. graminearum* for 100% while the acetone extract was 100% effective against *A. ochraceus* and *P. viridicatum*, we found that black pepper extract was not so effective with the highest 46.76% inhibition of *B. cinerea* growth. The other concentrations suppressed the pathogen growth similarly and varied from 40.59% to 44.26%.

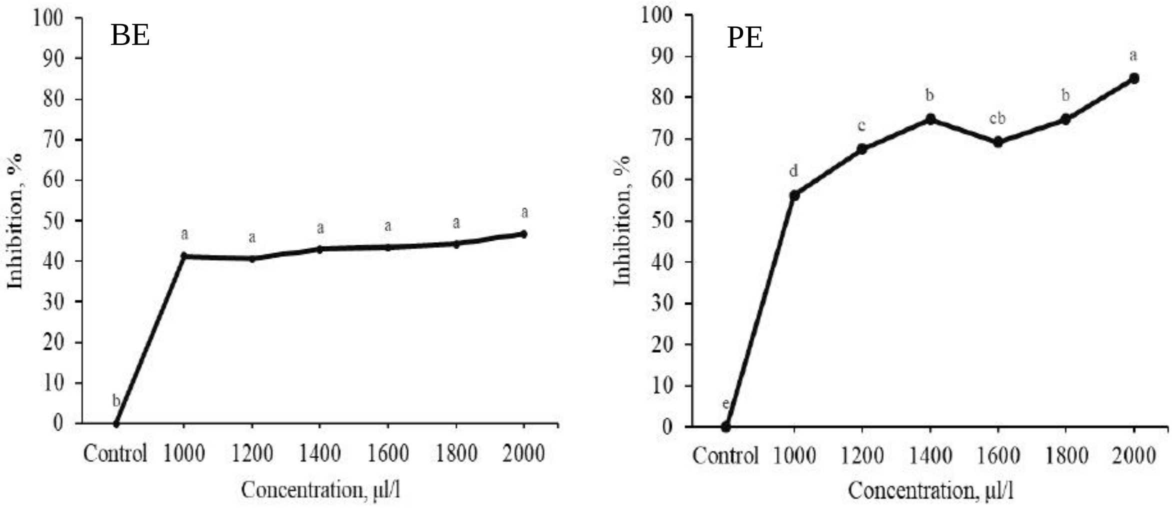

Figure 1. Percentage inhibition of *B. cinerea* colony growth by black pepper (BE) and allspice (PE) extracts *in vitro*

Control of grey mold on detached strawberry leaves was investigated by spraying leaves with the black pepper and allspice extracts. Inhibition of *B. cinerea* colony growth is shown in Figure 2. Only allspice extract reduced infection of grey mold on strawberry leaves, and the result is significant exclusively on the concentration of 40 ml/l, which distinguished from others with 91.89% inhibition 4 DAI. Besides, the mentioned concentration managed to maintain the inhibition of the pathogen growth 7 DAI (79.34%). The 20 ml/l of allspice extract demonstrated weak inhibition compared with control. As expected, black pepper extract had the lowest inhibition, and this result confirms the result of PDA. There is still a need of information about black pepper and allspice extracts effectiveness against grey mold on strawberry leaves. However, black pepper extract was found to reduce the spread of wilt infection on tomato plants after spraying with 4% concentration and showed less effect at 0.5% concentration (Sagitov et al., 2011). Results of our research are in contrast, as black pepper extract had even increased the growth of *B. cinerea* compared to control at 12 ml/l and is considered as ineffective against infection of grey mold on detached leaves. Presumably, the results of our and previous study may differ because of the different composition of the tested plant material due to the used extraction methods. Eugenol and its derivatives were found to inhibit *B. cinerea*, as these compounds are know for their antifungal activity through various mechanisms (Olea et al., 2019). Aguilar-Gonzalez et al. (2015) stated that *B. cinerea* was suppressed *in vitro* and on strawberry fruits by clove (*Syzygium aromaticum*) essential oil with more than 80% of eugenol and its derivatives in its composition. Our study results support the effectiveness of allspice extract and the idea of eugenol as one of the main components of it.

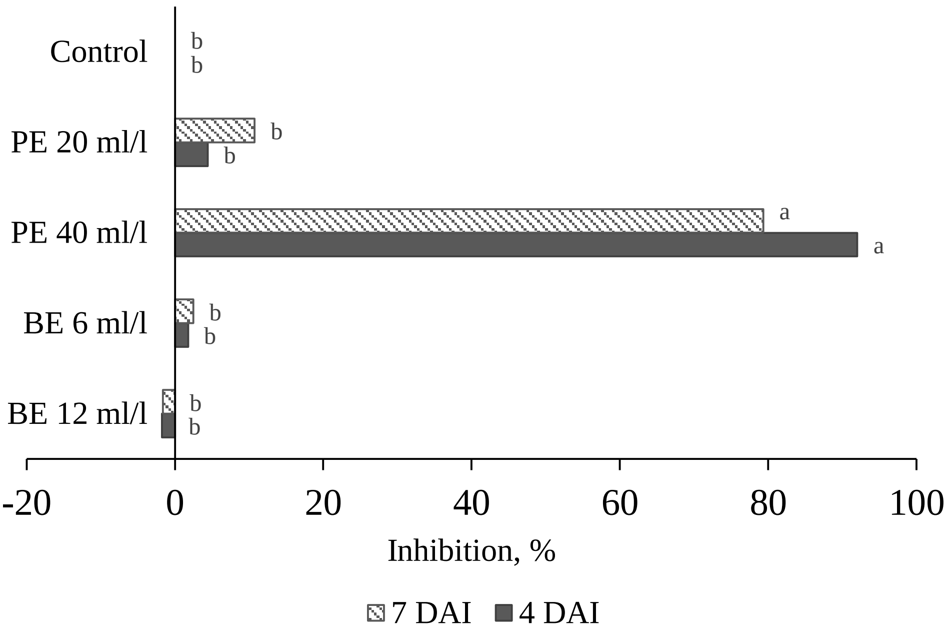

Figure 2. Percentage inhibition of *B. cinerea* colony growth by black pepper (BE) and allspice (PE) extracts on detached strawberry leaves 4 days after inoculation (4 DAI) and 7 days after inoculation (7 DAI)

Conclusions

Allspice inhibited the growth of the strawberry pathogen *B. cinerea in vitro* from 56.32% to 84.56% and black pepper extract consequently from 40.59% to 46.76%. Allspice extract significantly reduced infection of grey mold on detached strawberry leaves. Meanwhile, black pepper extract was not sufficient. To sum up, allspice extract was stronger measure against strawberry pathogen than black pepper extract both *in vitro* and on detached strawberry leaves.

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MONITORING OF THE SAFETY OF WILD -GROWING FRUITS FROM DIFFERENT REGIONS OF KAZAKHSTAN

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Abstract

The article presents the results of environmental monitoring of wild plant species that are growing in various regions of the Republic of Kazakhstan during 2015-2018.The aim of the research was to study the species diversity of fruit and berry crops of natural flora, contamination of theirberriesby heavy metals.The geobotanical characteristic of wild-growing species of fruit crops was compiled by the route survey method. In the laboratory species of wild-growing plants were identified and a list of species biodiversity were compiled.It was established that the composition of biological diversity includes 34 species of fruit and berry crops from 10 families.In laboratory conditions, the fruit crops contamination by heavy metalssuch asPb, As, Cd, Zn, Cu, Mg has been studied.The completed studies on environmental safety were aimed at studying the gross concentrations of heavy metals in berries. The results of the study of fruits contamination by heavy metals showed that their existance was noted in all the selected samples.*Berberissibirica* Pall.accumulates increased concentrations of Pb (0, 042 mg/kg), *Ribesrubrum* L., *Fragariavesca* L., *Loniseraaltaica* Pall.- As(0.019-0.038 mg/kg); *Crataegus sanguine* Pall. Cd (0.04 mg/kg).It was determind that the number of the accumulation of heavy metals in the examined fruits did not exceed the limits of permissible levels.The results of research characterize the wild berries of the regions of Kazakhstan as safe for widespread use in food and medicinal purposes.

Keywords: *ecology, natural flora, biodiversity, fruits, food security*

Introduction

Now in the world the steady trend of increase in demand for vegetable raw materials and it types of products is observed. Knowledge of the population leads to increase in number of consumers of phytoraw materials, expansion of the range of food, medicinal, perfumery and cosmetic products in economic use. Among forest resources the wild-growing wild berries tooka big place as foodstuff. Their number includes many officinal and vitamin plants (Development Initiatives, 2017.; Termentzi et al. 2006)

The biodiversity of flora of forest fruit crops of Kazakhstan considerably varies, as well on structure and number of various taxons (types, sorts, etc.), and on geography. The major factors causing distribution of plants are confinedness of types to climatic zones and high-rise belts. Estimated works of experts showed that the resource potential of wild-growing fruits is sufficient for satisfaction of domestic demand for vegetable raw materials. In the region of the Kazakhstan part of Altai there is the considerable specific variety of flora and the vegetable resources applied in alternative and traditional medicine (Kotukhov, 2015).

In different regions of Kazakhstan various industrial enterprises of the Republic of Kazakhstan having the considerable technogenic impact on natural ecosystems and health of the population (Alinov, 2017) adjoin to forests. Thus, carrying out biocomplex researches of fruit and berry plants of the woods in these territories is necessary for ensuring food safety of the population, perspective for preparation. Taking into account the above mentioned data, studying of questions according to the resource capacity and environmental safety of wild-growing fruit and berry plants of the regions of Kazakhstan available to consumers became the purpose of the conducted researches.

Material and Methods

Forwarding field researches are conducted by us in forests Eastern (suburbs of Ridder, Pikhtovsky and Riddersky forestries), Northern Kazakhstan (suburbs of the State national natural park "Burabay"). In work the standard geobotanical and resource knowing methods were used. Route reconnoitring methods studied cenopopulationof fruit and berry plants (Bykov, 1957). In field conditions geobotanical methods determined features of body height, development, occurrence and abundance of plants (Bykov, 1970). The visual assessment of quantity of individuals was carried out on G. Drude's scale (Determination methodics, 1986). Identification of plant species was carried out on "Flora of Kazakhstan" (Flora of Kazakhstan,1956-1966). The nomenclature of types was provided according to Cherepanov S.K. report (Cherepanova, 1995).

Objects of research were wild-growing types of berries of the forest territories in Kazakhstan prepared by local population for use as food. Tests of berries of a mountain ash Siberian (*Sorbus sibirica* Held.), guelder-roses ordinary (*Viburnum opulus* L.), bilberries ordinary (*Vaccínium myrtíllus* L.), sea-buckthorn krushinovy (*Hippophae rhamnoides* L.), currants red (*Ríbes rúbrum* L.) were selected during expeditions in 2016-2018 in territories of forest ecosystems of the Kazakhstan part of Altai, Akmolaregions. The scheme of the experimental platforms of forest territories in Kazakhstan is provided on Figure 1. For assessment of the current ecological state of the studied plant species the methods of radiation control of a surrounding medium were used (Instruction on ground examination, 1989).

Figure 1. Location of the experimental sites in the regions of the Eastern and Central Kazakhstan.

Concentration of heavy metals in the tests of medical herbs were defined according to the requirements for carrying out an atomic absorption spectrometry (Interstate standart, 1996). On the basis of the received characteristics the assessment of food safety of the specified types was carried out.

Results and Discussion

Long-term monitoring of climatic conditions of East part of Kazakhstan shows that the remoteness from oceans and a mountainous terrain is defined by degree of continentality, humidification and a temperature schedule during all annual cycle of climatic parameters of the region. Weather conditions of winter are defined by the Mongolia-Siberian atmospheric high bringing cold weather within five months. In spring and winter northeast, east winds prevail. Average height of snow cover on open spaces reaches 50-60 cm with a depth of a freezing of the soil from 40 to 119 cm. The sum of an annual atmospheric precipitation varies on average from 400 to 550 mm. Average temperature of winter is-12,6ºC with short-term frosts in the range 35-45ºC. In Kokshetau mountain region (the northern region) average annual temperature fluctuates in limits 1-2ºC, absolutely maximum temperature reaches to-51ºC, absolutely minimum temperature equals +41ºC. Height of snow cover reaches 30 cm, the amount of precipitation reaches 400 mm (National report, 2018).

In Table 1 the data including information on administrative location of registration platforms, geographical coordinates of the area, height above sea level, exposure dose rate (EDR) are consolidated.

Table 1. Main parameters of the conditions of the experimental sites in places of growth of fruit and berry plants in Kazakhstan

Region	Location of registration platforms	Geographical coordinates	Height above sea-level, m	EDR, mkSv/h
Eastern Kazakhstan	Ridder forest farm, Central forest area, 26 block, 17 division	$50^{0}22^{1}25^{II}$n.l. $83^{0}55^{I}44^{II}$e.l.	1171	0,17
	Ridder forest farm, Chernoubinskoye forest area, 83 block, 70division	$50^{0}22^{1}25^{II}$n.l. $83^{0}55^{I}44^{II}$e.l.	1182	0,19
	Ridder forest farm, Zhuravlinskoye forest area, 18 block, 64 division	$50^{0}22^{1}25^{II}$n.l. $83^{0}55^{I}44^{II}$e.l.	1140	0,14
	Pikhtovskiy forest farm, Butakovskoye forest area, 38 block, 40 division	$50^{0}22^{1}25^{II}$n.l. $83^{0}53^{I}54^{II}$e.l.	1083	0,22
	Pikhtovskiy forest farm, Suburban forest area, 84 block, 16 division	50º20'26" n.l. 83º45'24" e.l.	1117	0,18
Northern Kazakhstan	Burabay region, Southern forest area, 26 block	$52^{0}56^{I}41^{II}$n.l. $70^{0}17^{I}05^{II}$e.l.	452 m	0,14
	Burabay region, SNNP «Burabay», Priozernoye Forest area, 51 block 15 division (glade) adjoin division 9	$53^{0}00^{I}95^{II}$n.l. $70^{0}21^{I}34^{II}$e.l.	385 m	0,11

In east regions of Kazakhstan during inventory of the higher vascular plants about 2500 species are established that makes about 44% of total number of species of the higher plants. The list of medical herbs of the Kazakhstan part of Altai includes 783 species 131 families where 87 species are successfully applied in traditional medicine, considerably the bigger number is used in alternative medicine (Kotuhov, 2015].

The total area of studied territories in Akmola region in SNNP "Burabay" occupied with fruit and berry cultures is subdivided into zones: limited economic activity – 3958.8 hectares; tourist – 261.9 hectares; reserved – 620.9 hectares; ecological stabilization – 2372.2 hectares.

In the territory of east Kazakhstan 66 hectares of the territory of the mountain woods are examined. It is established that in the territory of the East Kazakhstan region free air of Ridder city in general is characterized by the increased pollution level. In general, around the city average concentration of dioxide of sulfur made-1.2 MAC, ozone-1.7 MAC, other pollutants – didn't exceed maximum allowable concentration. The background radiation varied 0.11-0.22 mkSv/hwithin two areas. On average in area the radiation gamma hum noise made at the level of 0.13 mkSv/hand was in tolerance limits.
The surveyed registration platforms are carried to the following types of the wood: larch-grassy, fir birch mixed herbs, birch forest mixed herbs. Distribution of berry wild plants in various regions of Kazakhstan is characterized by inhomogeneity. A bigger specific variety is noted in forest and forest-steppe zones. Forest vegetable communities are presented by wide specific structure. By geobotanical researches it is established that 33 species from 9 families of fruit and berry cultures are a part of biological diversity. They are almost widely used in the food purposes, many of them are successfully applied in traditional medicine, separate types (bilberry, dogrose, hawthorn) are applied in pharmaceutics and medical practice (Kubentaev S.A.,2018). Fruit and berry cultures in Kazakhstan grow on plains of northern, east part of the republic and in mountain forests (the Altai, theTarbagatai, the Dzungarian Alatau, the Ulytau). Berry thickets meet on forest edges, on crude shrubby thickets, gorges, stony slopes. In a natural environment many types of wild-growing berries grow on loops of slopes and in deep hollows of a steppe zone.
Possible wind-dust-tranfer can promote accumulation of ecologically dangerous pollutants in fruits of plants. The industrial enterprises, the motor transport are the main sources of the environment of regions pollution (National report, 2018). Considering this fact, for ensuring environmental safety of phytoraw materials, the regions prepared in forest ecosystems, environmental monitoring of quality of the prepared products is carried out. During the research we defined concentration of heavy metals in the studied species of berry plants and the comparative analysis of compliance of raw materials to standards of the maximum-permissible concentration (MAC) is made. Data of results of researches are consolidated in table 2.

Table 2. Central cases of heavy metalscontent in berries, mg/kg

Name of culture	Region	Pb	As	Cd	Cu	Zn	Mg
MAC		0.4	0.2	0.03	5.0	10.0	-
Crataegus sanguine Pall.	Northern Kazakhstan	0.029	0.017	0.003	3.58	6.70	31.72
Crataegus sanguine Pall.	Eastern Kazakhstan	0.030	0.018	0.040	4.84	9.30	32.70
Fragari avesca L.	Northern Kazakhstan	0.023	0.019	0.001	2.4	8.70	29.56
Fragari avesca L.	Eastern Kazakhstan	0.040	0.020	0.004	1.8	9.02	32.50
Berberis sibirica Pall.	Eastern Kazakhstan	0.042	0.019	0.005	2.9	8.90	35.8
Hippophae rhamnoides L.	Northern Kazakhstan	0.031	0.012	0.004	2.9	7.3	25.4
Hippophae rhamnoides L.	Eastern Kazakhstan	0.033	0.021	0.002	3.8	8.8	36.1

Lonisera altaica Pall.	Eastern Kazakhstan	0.028	0.021	0.004	3.2	7.6	34.7
Ribes rubrum L.	Northern Kazakhstan	0.004	0.002	0.004	3.4	9.0	35.8
Ribes rubrum L.	Eastern Kazakhstan	0.029	0.038	0.002	4.2	9.02	36.6

Results of the experiments showed that the range of accumulation of the most dangerous heavy metals in wild berries was in limits: Pb - 0.004-0.042 mg/kg, As- 0.002-0.038 mg/kg, Cd - 0.001-0.040 mg/kg. Wild-growing berries of forest territories of east Kazakhstan accumulate a little high concentration of pollutants, than the fruits collected in forests of northern regions. But distinctions donot have significant sizes. It is possible to explain it with the fact that registration platforms of northern areas are located in a zone of especially protected areas, and the potent industrial enterprises of the republic in the east are concentrated. However, registration platforms of east area are located in the depth of forests at the considerable distances from the industrial enterprises.
The comparative analysis of safety of wild-growing berriesfruits as food and medicinal raw materials showed that noted levels of heavy metals don't exceed values of threshold limit values. These results allow carrying out their preparation for practical application. We noted the single case of insignificant excess of impurity by cadmium of tests *Crataegus sanguine* Pall berries (Table 2). By results of the executed research it is possible to draw an important basic conclusion about the wild-growing berries prepared in forest ecosystems of Kazakhstanregions meets the standards of maximum-permissible concentration accepted in the republic. This conclusion is caused by the fact that the chosen experimental platforms are located at the considerable distances from polluters.

Conclusion

Thus, we executed researches on assessment of environmental safety of fruit and berry plants of various regions in Kazakhstan available to locals and suppliers. Among the used types of phytoraw materials the most widespread 6 types of the wild-growing berries which are widely used by consumers of the region were selected. Results of the executed researches supplement information on the quality of food of forest not wood products of regions in the territory of Kazakhstan for needs of pharmacy stretch, individuals, industrial enterprises.

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COMPARING OF PREEMERGENT WEED CONTROL TECHNOLOGIES IN SOYBEAN (GLYCINE MAX)

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Abstract

Soybean is one of our most important protein crops, with worldwide production reaching 320 million tons. It is grown in Hungary at 60,000 ha in the southern part of the country. Its yield is currently around 180,000 tons. Currently it is forbidden to cultivate genetically modified soybean in Hungary, which is why Hungarian soy production has a great value. Current legislation does not allow all of the preemergence herbicides to be used, as well as the use of post-emergence herbicides. Soybean is a very bad weed supressant at the initial stage of development. During this period, herbicide control is warranted, for which at present there are not enough active substances available. The aim of our experiment was to investigate the effects of five preemergent herbicides on soy weeds in field trials. We also examined the phytotoxic effect of the herbicides on soy. The tested active ingredients were s-metolachlor, metobromuron, flumioxazine, metribuzin, and dimethenamid-p + s-metolachlor. The weeds in the experimental area were: *Echinochloa crus-galli*, *Datura stramonium*, *Chenopodium album*, *Ambrosia artemisiifolia*, *Hibiscus trionum*, *Sorghum halepense*, *Cirsium arvense*. During the evaluation, we examined the effect of each active substance on the weeds mentioned earlier. The active substances were classified according to official regulations based on herbicidal efficacy and phytotoxicity.

Keywords: *Soybean, weed control, preemergence, field experiment.*

Introduction

Soybean protein is more than 25% of the total vegetable protein produced in the world. In the period 2016-2017, the total amount of soybean in the world reached 320 million tons. In recent years, the area and quantity of soy have grown in the world (Bányai, 2015).
We have information about the exact area of soy sowing since the 1930s. By 1939, 13 million tonnes of soy has been produced worldwide. At that time, 72% of the beans was produced in China and the rest in other parts of the world, mostly in the USA.
Today, soy is grown on over 100 million hectares worldwide. The main reason for its spread is that it has become the most versatile industrial crop (Balikó et al. , 2007).
In 2018, Brazil take the lead from the US as the world's largest soybean producer, with net total 118 million ton. Total production in 2017 was 338-339 million ton.
From the *Fabaceae* family, the soy has the highest amount, biologically complete protein content (36%). The most important amino acids are found in it, such as lysine, valine or leucine. It can be used to replace animal protein in human food. In addition to its valuable protein content, it also increases the importance of having a fat content of 18%. Its oil is almost 100% digestible, which is an excellent food oil (Radics, 2012). Of our cultivated plants, soybeans play an extremely important role. It has an exceptional role mainly in animal nutrition (poultry, pigs) but also in human nutrition. The great advantage is that it can be easily inserted into the crop rotation, thus reducing the production of cereal crops (Balikó, 2015). Looking at yields, we produced 181,000 tons of soybeans in 2016, ranking 4th in Europe. Only GMO-free soybeans can be produced in Hungary, which makes us competitive in Europe.

Soy has poor weed suppression during its initial development and has a lot of dangerous, hard to control weeds. Soybean production without weed control is not profitable.
Soybean weed control can be done in mechanical, agrotechnical methods, and also with herbicides, that we can use in pre-emergence and post-emergence. Chemical weed control can only be done with the knowledge of weed conditions and we also choose technology based on it (Balikó et al., 2005).
The main problem with the weed control using herbicides, that we have a low number of active ingredients, and the low herbicide tolerance of the crop. We can successfully protect against perennial weeds in the previous year on a cereal fields. However, post-emergence treatments show good herbicidal efficacy (Bárány, 2017).
In order to select herbicides acting on the soil, we need to know the soil's humus content and boundness. The one-year-old monocots are first-class weeds in soy. When selecting herbicides, the longer duration of action is given priority, because the germination time of soy is long. In areas infected with perennial monocotyledons, stubble management is absolutely necessary.
Preemergent technology is used after sowing and before the germination of the weeds. The herbicides sprayed to the soil and sometimes rinsed into a few centimeters of the top layer of soil, from which the majority of weed seeds germinate (Reisinger, 2000). The advantage of this method is that it can be used in any soil tillage system. Its disadvantage is that its effect depends on the amount of rain that has fallen (at least 10 mm) (Dobszai-Tóth, 2014).
The preemergent herbicides are effective against the weeds, tha germinate from the upper part of the soil (monocotyledonous and dicotyledonous plants also), but they are ineffective against the weeds that germinating from a deeper soil layer, such as *Ambrosia artemisiifolia, Abutilon theophrasti* or *Datura stramonium* (Kádár, 2016).
The aim of our experiment was to investigate the effects of five preemergent herbicides on soy weeds in field trials.

Material and Methods

In our study, authors analyzed the differences between herbicidal efficiencies against mono- and dicotyledonous seeds. Against perennial weeds authors did not evaluate herbicidal efficacy, knowing the spectrum of action of the active ingredients. During this study, the following formulations (Table 1) were delivered preemergently in 2019 in 2019.

Table 1. The active ingredients and the herbicides, that were used

Name of the herbicide	**Active ingredient**
Dual Gold	960 g/l S-metolachlor
metobromuron	500 g/l metobromuron
Pledge 50W	500 g/kg flumioxazine
Sencor 600 SC	600 g/l metribuzin
Wing -P	212,5 g/l dimethenamid-p+250 g/l pendimethaline

The active ingredient metobromuron has been previously authorized for use in herbicide testing in soya in recent years.
Untreated control plots did not receive herbicide treatment and were used to compare the herbicidal efficacy of each preparation. Treatments were done in four replicates, and the plots

were randomized. Size of plots: 8m x 3m, 24m^2. The weeds and their development on untreated control plots were recorded at the time of each evaluation. The average weed coverage was determined on 1 m^2 by counting weed species.
On the untreated control plots, we recorded the weeds according to the Balázs-Ujvárosi shooting scale.
Table 2 shows the dosage of the herbicides sprayed to the fields.

Table 2.: Threatments, dosages and method of application

Number of threatment	Threatment	Dosage (l, kg/ha)	Method of application	
1.	Unthreated control			
2.	Sencor 600 SC	0,55	A	preemergence
3.	Dual Gold	1,6	A	preemergence
4.	Wing -P	3,5	A	preemergence
5.	Pledge 50W	0,06	A	preemergence
6.	Pledge 50W	0,08	A	preemergence
7.	metobromuron	3	A	preemergence
8.	metobromuron	4	A	preemergence

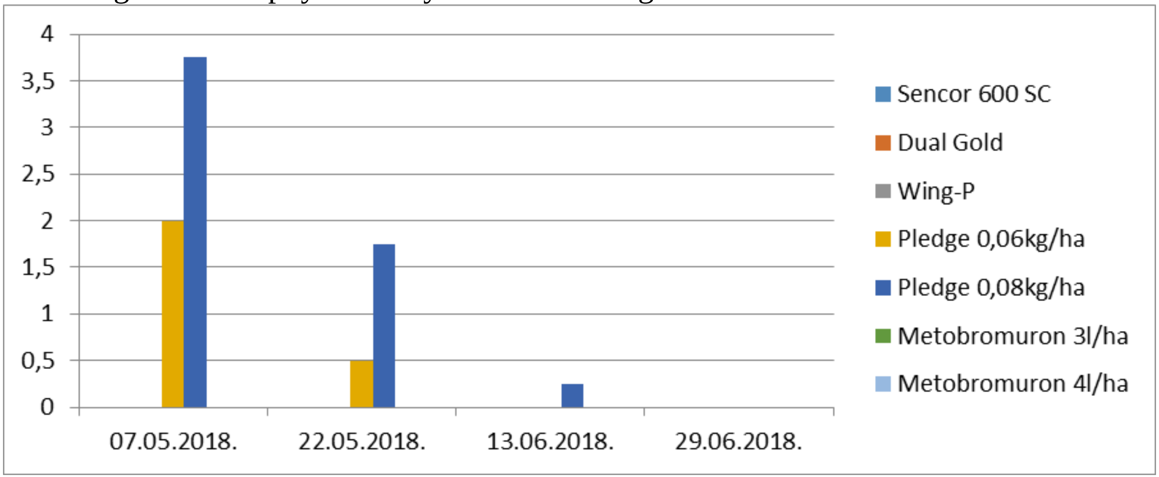
Treatment took place on the 4th day after sowing, when the soy had not yet germinated.
Results were evaluated by one-factor analysis of variance at 5% significance level ($p < 0.05\%$). Hypothesis H1 is that there it is at least one herbicide that has significantly different herbicidal properties. Hypothesis H1 is accepted if p is less than 0.05.

Results and Discussion

The experiment was evaluated for herbicidal efficacy and phytotoxicity according to the official Herbicide Testing Methodology (FVM, 2004).
The first evaluation was performed after weed control, on the untreated control plots (day 8 after treatment) and then on days 23 and 45 of post-treatment.
Between 29 April and 13 May, 19 mm of rain fell, so the preemergic agents were able to exert their herbicidal effects.
All products except *Hibiscus trionum* were properly removed by Dual Gold. Slightly weaker efficacy was seen with Wing-P.
The threatments against *Ambrosia artemissifolia* with the exception of Dual Gold had good efficacy.
With the exception of Dual Gold, other formulations against *Chenopodim album* showed herbicidal efficacy above 90%. We can conclude from this that if the *Hibiscus trionum, Ambrosia artemisiifolia* and *Chenopodium album* in our area are predominant, we do not use S-metolachlor as a preemergent active ingredient. S-metolachlor mainly kills monocotyledon seedlings.
Good efficacy against *Datura stramonium* was observed only at two doses of Sencor 600SC and Pledge 50WP. The other active ingredients are practically ineffective against the tested weed.
Against Echinochloa crus-galli only the Dual gold and Wing-P has very good herbicidal efficacy. The other herbicides have weak herbicidal efficacy against the weed.

Phytotoxicity was observed only with the use of the Pledge 50WP formulation of flumioxazine in the 1-2 leafy state of the soya (Figure 1.), but did not affect the subsequent development and yield of the crop. The weed control efficiencies decreased slightly on the last evaluation date (June 29), and their effects are not exerted during the entire vegetation period. In our study, we concluded that based on preemergent (basic) treatments, mono- and dicotyledonous weeds cannot be efficiently controlled.

Figure 1. The phytotoxicity of the active ingredients in different evaluation dates.



There is a high risk of using preemergent formulations, because they need a 10-20 mm rain in precipitation within 2 weeks of spraying. They are less effective if they do not reach the root zone of weeds, and on the other hand, preemergic herbicides kill mono- and dicotyledon weeds, that germinates in the upper part of the soil layer. They are less effective against weeds germinating from deeper soil layers.

Preemergent threatment is therefore necessary, but it is not enough to maintain the crop in the whole vegetation period, so there is a need for postemergent management as well. The advantage of post-emergence spraying is that we already see the weeds on the board, so we can choose the right active ingredient and preparation for effective protection. We need to recognize the weeds in a few leafy states, because it is advisable to protect against dicotyledons in a 2-4 leaf state, and against the monocotyledons in a 1-3 leaf state. If the spraying is delayed and the weeds are at a more advanced stage, the defense may become ineffective.

Postemergent weed management is especially needed in years when there is no precipitation after the application of preemergent agents, and thus the management is ineffective.

Conclusions

The portion of soy protein in the consumption of the animal feed proteins is 35%, and soy beans exceed 20% of the total plant protein produced in the world. From these informations it can be seen that soybeans play an outstanding role among the cultivated plants. The role of soybeans is extremely important in animal nutrition as well as in human nutrition, and we cannot neglect it.

The biggest challenge of soy cultivation is weed control, weeds directly and indirectly pose a serious threat, thus putting the soy's cultivation and profitability at high risk. The extra costs of weed control greatly increase the costs of cultivation. Soybean suppressed by weeds produce less and the high weed infectation make it difficult to harvest. Soybean production is significantly determined by the appropriate weed control technology. It can not be produced profitably without herbicide usage.

Soybean is both damaged by annual and perennial monocotyledonous and dicotyledonous weeds. Nowadays in hungary there are few problems with the number of active ingredients that can be used. Preemergently only 7, post-emergence 8 active ingredients can be used. The production and placing on the market of potential new active substances can take up to 10 years, which further complicates weed control, and the continuous withdrawal of the active ingredients does not help the plant protection of the soy.
S-metolachlor and pendimethalin + dimethenamid-p are the most effective against seedlings. Flumioxazine and metribuzin are the most effective against weeds that are constantly germinating, but they can be relieved by post-emergence treatment. Metribuzin, flumioxazine, and metobromuron showed the best results against dicotyledonous weeds.
Our studies have shown that although the initial development of soybean is facilitated by pre-emergence weed control under favorable weather conditions, it should not be based on pre-emergence spraying. In a rainless period, preemergent management is ineffective, so post-emergence management is inevitable. In order to keep the soybean free of weeds throughout the vegetation period, post-emergence treatments are also required.

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CONTENT OF Cu, Zn, Co, Ni, Cr IN SOIL AND FRUITS OF APPLE AND PLUM

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Abstract

In plants, some metals play important role as micronutrient components, significant for growth in low concentrations. If present in higher concentrations than necessary, they can be phytotoxic and cause product contamination. Analysed soils are classified from clay loam to heavy clay, medium to high supply with soil basic fertility parameters, acid to neutral soil reaction. Analysing total content of Cu, Zn, Co, Ni, Cr in soils under apple and plum plantations, an averaged higher content of Cu (13,88 mg kg^{-1}), Zn (33,78 mg kg^{-1}), Co (22,05 mg kg^{-1}), Ni (52,38 mg kg^{-1}) has been measured under plum plantations and the content of Ni (9,55 mg kg^{-1}) under apple plantations. Results of the examination on the presence of the above elements in fruits have shown differences in relation to the content in the soil. Content of Cu, Zn, Co, Cr have had higher average values in fruits of apple and the Ni content has been higher in plums, with higher measured values in individual fruits of plum Cu 3,11 mg kg^{-1} of dry matter), Zn (4,08 mg kg^{-1} of dry matter), Ni (1,32 mg kg^{-1} of dry matter). Adoption of metal from the soil depends on the pH value of the soil, organic composition, the soil type and the concentration of metals in the soil itself. It also depends on the percentage of metal adoption through the plant root, water, metabolic capacity, ion absorption and other. Monitoring the content of metals in soil and fruits is significant in sustainable land use and health food safety.

Keywords: *Metals, Apple, Plum, Soil fertility, Health food safety.*

Introduction

Fruit growing in Serbia is ever increasing with increased area under intensive fruit plantations. Most prevalent are plantations of stone fruits among which two thirds is under plum. Plum has long been the leading fruit species though apple, which is grown on 23.737 ha is dominant in modern production. In European scales, Serbia ranks 12th in areas under apple (Keserović et al., 2014).

For achieving high yields and production of health safe fruits, of particular importance is to choose land as a major source of nutritional substances necessary for plant growth and fruiting. Chemical analysis of soil is important for monitoring conditions of the environment and legislation. (Merry, 2010). Modern growing systems demand soil fertility monitoring but also the analysis of other nutrients of calcium, magnesium, sulphur and microelements: iron, manganese, zinc, copper, boron and molybdenum which plants need in smaller quantities than macroelements. Excess nutrients and those with no physiological significance belong to the group of hazardous and deleterious substances among which the most common are: Cu, Zn, Ni, Pb, Al, Cr. Natural content of hazardous and deleterious substances in soil is of geochemical origin and mostly so negligent that it has no significant effect on agroecosystem contamination. Hazardous and deleterious substances deriving from anthropogenic sources in soil are found in forms that are easily accessible. Accessibility of nutrients and their further transport in plants is affected by mechanical composition, soil reaction, humus content, calcium carbonate content and available phosphorous in the soil.

Different fruit types have different needs for adoption of biogenic elements. Deposition of certain elements is also different so in some plants, the largest concentration is in the root, in

others, in leaves or buds while the maximum allowable concentration in fruits is determined based on different parameters including human medicine. Absorption and accumulation of heavy metals in plants depend on bioaccessibility and contents in the soil, genetic properties of plants, edaphic and other ecological factors. These differences are mostly conditioned by plants' genetic base or other parameters.
The aim of the investigation is determining effects of Cu, Zn, Co, Ni, Cr presence in soil on the content of the same elements in fruits of plum and apple.

Material and Methods

Investigations were carried out in September 2018 by sampling of the soil in the rural area of Čačak, in apple and plum orchards of the following cadastral municipalities: Baluga Trnavska, Trnava, Prislonica, Pridvorica, Vranići, Gornja Gorevnica, Vranići and Miokovci. Sampled orchards were marked by GPS coordinates: N from 4352028 to 4357480 and E from 2016305 to 2027156. Sampling depth was 0-30 cm. Analysed soil under plum plantations was classified as clay loam and light clay with 15.80-27.50% share of clay fraction and 42.10-58.00% of physical clay. Soils under apple plantations are classified as heavy clays on three sites with about 46.0% share of clay fraction and 71.40-79.20% physical clay. In apple orchards in Baluga Trnavska, the soil is classified as clay loam. Agrochemical characteristics of soil are analysed at the laboratory of the Fruit Research Institute, Čačak using the following methods: pH value in H_2O and 1N KCl-u (potentiometrically); humus (method by Kotzman); total nitrogen (method by Kjeldahl); easily accessible phosphorous and potassium (AL method, P_2O_5- colourmetrically, K_2O flame photometrically). The samples were previously dried at ambient/room temperature and sieved through a sieve ≤2 mm. The total content of macro and micro elements is specified by mineralization of soil samples with HCl and H_2SO_4, and the contents of the same elements in fruits upon a modified procedure (Morais et al., 2017) and reading on AAS (Perkin Elmer, 2018).

Results and Discussion

Numerous studies point to the influence of mechanical composition, soil reactions, humus content on the availability of nutrients. Strongly acidic soils are poor in available forms of macroelements and certain microelements for plants, while at the same time these soils contain higher amounts of ions of Al, Fe and Mn, available to plants, which in high concentrations, have toxic effect (on plants) (Dugalić et al., 2008). Research results (Tab. 1) have shown that the analysed soil is of weakly acid to weakly alkaline reaction. Average values of the active soil acidity (pH/H_2O) in the humus horizon (0-30cm) under plum and apple plantations is about 6.82. Substitutional soil acidity (pH/KCl) was higher in soils under apple plantations, which is caused by the mechanical composition of soil. Acidification process can be regarded as a primary cause of the reduced productivity of agricultural land (Mrvić et al., 2012), which was in the past substantially accelerated by anthropogenic factor, primarily increased emission and deposition of acidic contaminants, improper use of mineral fertilizers and other (Sparks et al., 2002). Humus supply is low to medium, of higher average values under apple plantations of 3.09%. Total nitrogen is, on average, of medium content up to 0.16%. The content of easily accessible phosphorous varies on the sampled parcels. The lowest value 4.15 mg/100g of soil is in acid reaction soil in Prislonica while the content of easily accessible P_2O_5 is high in other parcels. Under conditions of increased soil acidity, phosphorus as one of the most important macronutrients in the forms bound with Al and Fe becomes inaccessible to plants (Barber, 1995). The investigated soils are optimally supplied with easily available K_2O. The research results of all analysed parameters are consistent with the results of Milivojevic et al., 2017.

Table 1. Basic soil fertility

Site	Populated area	pH		$CaCO_3$	Humus	Total N	AL-P_2O_5	AL-K_2O
		H_2O	KCl	%	%	%	mg/100g adl	mg/100g adl
1	Baluga Trnavska	7.43	6.68	0.69	1.00	0.05	30.53	17.3
2	Trnava	6.47	5.78	0.69	3.59	0.18	45.42	55.4
3	Prislonica	5.82	4.68	0.00	2.24	0.11	4.15	34.8
4	Prislonica	7.50	6.60	1.57	5.54	0.28	57.88	52.5
	average	**6,81**	**5,94**	**0,74**	**3,09**	**0,16**	**34,50**	**40,0**
6	Pridvorica	6.43	5.82	0.82	3.89	0.19	74.81	55.8
7	Vranići	7.35	6.55	2.33	1.12	0.06	47.17	29.9
8	Gornja Gorevnica	6.89	6.23	1.37	4.18	0.21	26.04	34.1
9	Miokovci	6.60	5.86	0.00	2.65	0.13	29.06	31.3
	average	**6,82**	**6,12**	**1,13**	**2,96**	**0,15**	**44,27**	**37,78**

*parcels under apple (1-4)
*parcels under plum (6-9)

In soils with alkaline reaction, mobility and accessibility of phosphorus, potassium, magnesium, iron, manganese, boron, cobalt, copper and zinc is decreased. Exceptions are (found in) molybdenum, sulphur, nitrogen, which are better adopted with an increase of pH value of the soil solution. Results of the content of macro and micro elements are shown in tab. 2. The average Cu content in the analysed parcels ranged from 9.2-25.3 mg kg^{-1}. According to the literature (Pendias Kabat et al., 2001) zinc content in soil should not be higher than 200-300 ppm. It is believed that high values of the content of total copper (> 60 mg kg^{-1}) are caused by fungicide application based on copper compounds (Schramel et al., 2000; Pietrzak and McPhail, 2004; Wightwick et al., 2006; Rusjan et al., 2007). The research results of the total copper content in smonitsas of Western Serbia (Milivojević et al. 2017) amounted to somewhat higher values in relation to our investigations. The content of Co was 12.8-31.0 mg kg^{-1}, and the content of Zn varied considerably between the analysed parcels 7.3-59.4 mg kg^{-1}.

Table 2. Content of micro elements in soil

No. of sample	**Cu**	**Co**	**Zn**	**Cr**	**Ni**
	mg kg^{-1}				
1	10,4	12,8	26,5	10,2	60,8
2	9,2	18,5	51,8	9,8	30,8
3	17,2	21,3	13,0	8,9	63,9
4	17,5	18,5	21,5	9,3	2,9
average	**13,58**	**17,80**	**28,2**	**9,55**	**39,6**
6	8,8	16,0	34,9	9,2	35,2
7	9,8	16,4	7,3	9,2	11,2
8	11,6	24,8	33,5	9,0	50,8
9	25,3	31,0	59,4	10,1	112,3
average	**13,88**	**22,05**	**33,78**	**9,38**	**52,38**

*parcels under apple (1-4)
*parcels under plum (6-9)

The presence of the total Cr did not show greater interval variation from 8.9-10.2 mg kg^{-1}, in contrast to the presence of Ni, with the content from 2.9-112.3 mg kg^{-1}. Boskovic Rakočević et al., 2014: found lower values of the Cu and Zn content in the soil, and the lower value of Zn in the fruits of plums, and an approximate value of Cu in relation to our investigations. Minerals and toxic element concentration in fruit are often variable and impacted by the species and cultivar, climatic conditions, geological origin of soil, the usage of fertilizers and other agricultural chemicals, plant growth stage and soil elements availability (Ekholm et al., 2007). The usage of fertilizers and pest control chemicals, urban industrial activities, and irrigation and fertilization type may affect the accumulation of microelements in soils (He et al., 2005). Microelements which have a role of regular enzyme functioning may also have adverse effects on human health depending on the intake quantity.

Investigation results show that, on average, the content of all analysed metals in apple fruits is higher in relation to plum fruits. However, looking at individual samples, there are deviations from previously mentioned. Cu content in the fruits apples ranged from 1.63-2.87 mg kg^{-1}, and in plum fruits 1.18-3.11 mg kg^{-1}. Miclean et all., 2000 report that the Cu content in agricultural products should be between 4 and 15 ppm, and according to the limit values of the World Health Organization, it is 3 mg kg^{-1} and less. The presence of Co in certain samples is below the level of detection to a content of 0.55 mg kg^{-1}. Zn is present in the amounts of 1.88-2.95 mg kg^{-1} in apple and 0.50-4.08 mg kg^{-1} in plum. According to the FAO/WHO (1984) allowable Zn in fruits is up to 27.4 ppm. The content of Zn in our investigations is within the range of optimal values whereas the Cu content is above the values allowed according to FAO/WHO and optimal values, according to Miclean et all., 2000. There are not many data on levels of cobalt (Co) in foods of plant origin in the scientific literature. The available data showed low levels of this micronutrient, often under 0.001 mg/100 g.

Table 3. Content of micro elements in fruits

No of sample	**Cu**	**Co**	**Zn**	**Cr**	**Ni**
	mg kg^{-1}				
1	2,87	<0,05	2,95	0,85	1,21
2	1,63	0,55	2,89	0,46	0,53
3	2,26	0,23	1,88	1,60	0,71
4	1,85	**0,44**	1,95	0,26	0,63
average	**2,15**	<0,05	**2,42**	**0,79**	**0,77**
6	1,72	0,05	4,08	0,49	0,76
7	3,11	<0,05	0,50	0,36	1,32
8	1,22	0,32	2,72	0,46	1,17
9	1,18	**0,21**	2,22	1,08	2,21
average	**1,81**	**0,21**	**2,38**	**0,60**	**1,37**

*apple fruits (1-4) *plum fruits (6-9)

Results of Osmanovic et al., 2014, show significantly higher levels of Zn and Cu, and the approximate values of Cu in the fruits of plum in relation to our investigations. Hegheđűş-Mindru Ramona et al., 2014 found similar values of the Cu content in fruits of apple and plum, and the content of Zn and Co. was higher in our investigation results.

Average contents of Cr and Ni in apple is of approximately values (0.77-0.79 mg kg^{-1}), and in plum fruits the content of Ni is increased. FAO/WHO, 2001 anticipates a limit of 2.3 ppm for the content of Cr, which is within the limits of our values. An upper tolerance limit for the fruits of Ni is 0.5 mg to 100 g^{-1} of Ni and the values are within the allowable value for our investigations. Fruits usually present Ni between <0.004 and 0.05 mg 100 g^{-1} (Szefer and Grembecka, 2007). The recommended daily intake of Ni is in the range of 302–735 µg (Roychowdhury et al., 2003). A RDA for Cr is not well defined, but it is considered to be between 25–35 µg/day, fruits and vegetables being the major dietary contributors of Cr intake. Increased heavy metal concentrations in food may adversely affect human health due to the predominating soil-plant pathway for their entry into the human body (Liu et al. 2013).

According to the data from the studies on phytotoxicity (Kabata - Pendias, 2011), Ni is, unlike others, a very mobile element in plant (Cr), and its transfer to fruits is the result of the phloem mobility. Accessibility of heavy metals, as well as their adoption by plants, depends on a number of factors, primarily on their overall and available content in soil, followed by soil reaction, organic matter content, oxidation-reduction conditions and other (Adriano, 2001; Kabat - Pendias, 2011), while the uptake of heavy metals depends on the plant type too (Overesch et al., 2007). Particularly outstanding is the influence of soil pH where the higher the acidity the greater the adoption of metal and vice versa, i.e. alkalinity increase leads to metal immobilization.

Conclusions

Investigation results show that the analysed soil is slightly acid to slightly alkaline. Humus supply is low to medium, of higher values, on average, under apple plantations with 3.09%. Total nitrogen is, on average, of medium content up to 0.16%. The content of easily available phosphorus differs on sampled parcels with the lowest values 4.15 do 74,81 mg $100g^{-1}$ of soil P_2O_5. The investigated soils are optimally supplied with easily available K_2O.

Total content of deleterious and hazardous substances in soil shows that the average Cu content in the analysed parcels ranged from 9.2-25.3 mg kg^{-1}. The content of Co was 12.8-31.0 mg kg^{-1} and the content of Zn varied considerably between analysed parcels, 7.3-59.4 mg kg^{-1}The presence of the total Cr did not show greater variation in the interval from 8.9-10.2 mg kg^{-1}, in contrast to the presence of Ni, having a content from 2.9-112.3 mg kg^{-1}.

The presence of heavy metals in fruits of apple and plum show that Cu in apple fruits ranged from 1.63-2.87 mg kg^{-1}, and in fruits of plum 1.18-3.11 mg kg^{-1}. The presence of Co is in certain samples below the level of detection up to 0.55 mg kg^{-1}. Zn is present in the amounts from 1.88-2.95 mg kg^{-1} in apple and 0.50-4.08 mg kg^{-1} in plum. Average content of Cr and Ni in apple is of approximate values (0.77-0.79 mg kg^{-1}), and in plum fruits, an increased Ni content is noticeable.

Availability of metals, as well as their adoption by plants, depending on their total and available content in soil, the reaction of soil, which has a significant effect, the content of organic matter, the oxidation-reduction conditions and the presence of heavy metals depend on the fruit type as well. In order to obtain health-safe food and assess the amount of fruit consumption, it is necessary to analyse each sample of soil and fruit individually.

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EFFECTS OF TEA WOOD ESSENTIAL OIL ON *TRIBOLIUM CONFUSUM* DU VAL. AND *ORYZAEPHILUS SURINAMENSIS* L.

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Abstract

Tribolium confusum Du Val. and *Oryzaephilus surinamensis* L. are important storage pests which cause a significant decrease in yield. For controlling storage pests, chemical pesticides have been used for years. However, their application can leave residues in food, have a negative influence on human or animal health and manifest high ecotoxicological influence on the environment. Therefore, there is a justifiable need for safe and eco-friendly measurements against these two storage pests. One of the ways is using essential oils or plant extracts. The aim of this study was to explore the contact, the contact – digestive and the repellent effects (Y tube olfactometer) of tea wood essential oil (*Melaleuca alternifolia*) on *T. confusum* and *O. surinamensis*, applied at concentrations of 0.5%, 1% and 2%. Methanol and water were used for the control treatment. The experiment was performed in four replications with 10 insects in each, at a temperature of 25±1˚C and 45–70% RH. Effects were determinated 24, 48 and 72h after the application. Tea wood essential oil caused the highest mortality of *T. confusum* (95%) in the concentration of 2% after 72h in contact test, while in the contact–digestive test, the mortality ranged from 22.5 to 80%. Essential oil of tea wood did not have such a good influence on *O. surinamensis*. The highest mortality of *O. surinamensis* was in contact-digestive test with efficiency of 52.5% after 72h, while in the contact test caused mortality in the range of 15-50%. Tea wood oil showed an excellent repellent activity to both species in concentration of 2%.

Keywords: *Tribolium confusum, Oryzaephilus surinamensis, tea wood, essential oil.*

Introduction

Storage pests are important pests worldwide of many crops, including flour, grain, stored food or other stored organic matter in warehouses, stockrooms, grocery stores, homes, and silos. These pests can make damage by completely destroying products or partially damaging it what causes less quality of the products, especially grains. These damaged grains have less or no ability to germinate what causes significant economic loss. It is estimated that around 30-35% of the yearly harvest in the world is decreased because of damages caused by stored pests (Dubey *et al.*, 2008; Pavela, 2016). The growing population of humans requires a greater amount of available food and there is constant importance of protecting and preserving stored products in order to reduce losses from harvesting to consuming food. Reduce of harms of stored products was achieved by using chemical pesticides for years. However, uncontrolled and more frequent uses of chemical pesticides caused many problems, like leaving toxic residues in food, having a negative influence on human or animal health and manifesting high ecotoxicological influence on the environment (Wilson & Tisdell, 2011). Therefore, there is a justifiable need for safe and eco-friendly measurements against storage pests.

One of the possibilities is using essential oils or plant extracts. Application of botanical insecticides has a long history even if their use was suppressed by new synthetic pesticides. Botanical insecticides were even used in ancient lands like Greece, India, and China (Isman, 2006). Many essential oils have insecticide activity. It is hard to determine essential oils mode of action because these oils are a complex mix of secondary metabolites with volatile characteristics. Separation and extraction of the active substance in the aim of studying the mechanisms is a difficult task (Wu *et al.,* 2015). Tea tree (Picture 1a) essential oil derived from the Australian native plant *Melaleuca alternifolia* (*Myrtaceae)* shows antifungal, antibacterial and insecticide activity (Arweiler *et al*., 2000; Hammer *et al.,* 2003). A monoterpene - terpinen-4-ol is the main component in tea tree essential oil which shows fumigant toxicity against the pests of stored grains (Liao, 2018).

Picture 1: a) Tea tree (http://hennacaravan.com); b) *Tribolium confusum (*https://e-insects.wageningenacademic.com) c) *Oryzaephilus surinamensis* (https://keys.lucidcentral.org)

Tribolium confusum (Jacquelin du Val) or confused flour beetle (Picture 1b) is a cosmopolitan insect which attacks and infests numerous grains or grain products (Park, 1934; Ajayi *et al*., 2019). It is very similar to the red flour beetle - *Tribolium castaneum* and the destructive flour beetle - *Tribolium destructor*. Confused flour beetle feeds on broken grains, grain dust, flour, cereals, rice, pasta, dried fruits, nuts, and beans but cannot feed on whole grain (https://entnemdept.ifas.ufl.edu). Grains infested by this pest have reduced weight, lower quality, bad baking properties followed by displeasing smell which reduced the marketability (Vayias, 2006).

Oryzaephilus surinamensis (Linnaeus) or saw-toothed grain beetle (Picture 1c) is a pest on whole grain and grain products but feeds on other products too, like dried fruit, chocolate, cereals, tobacco, nuts, and spices making them unsaleable and unappealing (Pricket *et al.*, 1990). There is a similar species called *Oryzaephilus mercator* or Merchant grain beetle. One of the main differences is the fly ability of *O. mercator*. The main problem of their infestation is the contamination of food with feces and dead bodies (Lyon, 1991).

Material and Methods

The experiment was conducted during 2019 at the Laboratory for Biological Research and Pesticides, Department of Phytomedicine and Environmental Protection, Faculty of Agriculture, University of Novi Sad. Laboratory studies were conducted to assess the effect of tea tree oil against confused flour beetle and saw-toothed grain beetle. In the study commercially available essential oil is used. Adults of *T. confusum* and *O. surinamensis* were obtained from laboratory cultures maintained in the dark in incubators at 25±1˚C and 70-80% RH. *T. confusum* was reared on wheat flour and *O. surinamensis* on cereals. The repellent effect of two solution of essential oil (1% and 2%) was evaluated using a Y - tube olfactometer. After 24h, the tested repellency was determined and expressed by the pre-referendum index (PI). Effect of essential oils on the mortality of these two pests was tested using two different methods for contact and contact - digestive treatment. The essential oil, firstly dissolved in methanol, was applied at concentrations of 0.5, 1 and 2%. The bottom of the Petri dish was treated with 0.3 ml of essential oil solution with different concentrations in the contact test and let to dry at the room temperature. In the contact digestive test, firstly are made flour disks at the bottom of the Petri dish and treated with all 3 solutions (0.3 ml). Of each species, separately, 10 adult insects were placed into Petri dishes. The experiment was set in four replicates. Methanol and water were used for the control treatment. Petri dishes were incubated in a thermostat at 25°C and 45-70% RH. Mortality was checked after 24, 48 and 72h. Datas were processed with Statistica 10 statistical software. One-way analysis of variance (ANOVA) of mortality was used to determine the statistical significance compared with the controls ($p<0.01$ were considered significant).

Results and Discussion

The results of this bioassay with tea tree essential oil in different concentrations on *T. confusum* and *O. surinamensis*, depending on the mode of exposure, are presented in Tables 1-4. Tea wood essential oil caused the highest mortality of *T. confusum* in the concentration of 2% after 72h in contact test, where mortality ranged from 57.5-95%. In contact-digestive test the mortality ranged from 32.5 to 80% after 72h. The lowest mortality was in contact-digestive test in concentration of 0.5% after 24h where only 5% were dead.

Table 1. Effect of essential oil on *T. confusim* in contact-digestive test

Time of exposure	**Mortality of *T. confusum***				**F value**	**P**
	Concentration (%)					
	0.50%	1%	2%	Control		
24h	22.5±1.26 b	32.5±1.71 b	65±1.29 b	0±0 a	18.9**	p<0.01
48h	30±0.82 b	47.5±2.22 b	75±1.91 b	0±0 a	17.1**	p<0.01
72h	32.5±1.50 c	62.5±2.87 bc	80±2.22 c	2.5±0.5a	9.9**	p<0.01

Table 2. Effect of tea tree essential oil on *T. confusim* in contact-digestive test

Time of exposure	**Mortality of T. confusum**				**F value**	**P**
	Concentration (%)					
	0.50%	1%	2%	Control		
24h	5±0.57 a	42.5±1.7 b	62.5±0.96 c	0±0 a	34.7**	p<0.01
48h	52.5±1.26 c	65±1.29 bc	75±1.73 b	0±0 a	28.5**	p<0.01
72h	57.5±1.26 b	90±2.0 c	95±1.0 c	2.5±0.5a	42.4**	p<0.01

Essential oil of tea wood did not have such a good influence on *O. surinamensis*. The highest mortality of *O. surinamensis* was in contact-digestive test with efficiency of 52.5% after 72h in concentration of 2%, while in the contact test mortality was in the range of 15-50%. The highest mortality (50%) of *O. surinamensis* in contact test was achieved in concentration of 1%.

Table 3. Effect of tea tree essential oil on *O. surinamensis* in contact-digestive test

Time of exposure	**Mortality of *O. surinamensis***				**F value**	**P**
	Concentration (%)					
	0.50%	1%	2%	Control		
24h	17.5±0.5 b	25±0.57 b	35±2.08 b	0±0 a	11.3**	p<0.01
48h	20±0.95c	35±1.73 bc	42.5±1.25 b	0±0 a	10.1**	p<0.01
72h	25±1.0 b	45±1.29 b	52.5±0.95 b	2.5±0.5a	20.95**	p<0.01

Table 4. Effect of tea tree essential oil on *O. surinamensis* in contact test

Time of exposure	**Mortality of *O. surinamensis***				**F value**	**P**
	Concentration (%)					
	0.50%	1%	2%	Control		
24h	20±4.0 b	15±1.73 bc	17.5±1.25 c	0±0 a	0.63 nz	p=0.61
48h	35±4.72a	27.5±1.89 a	25±1.29 a	0±0 a	1.33 nz	p=0.3
72h	40±4.54 ab	50±1.41 b	40±0.81 ab	2.5±0.5a	2.97 nz	p=0.07

During the study it was found that tea tree has the repellent effect at both concentrations (1% and 2%). The oil showed an excellent repellent activity to both species in concentration of 2% (Table 5).

Table 5. Repellent activity

Tea tree essential oil			
	PI 1%	PI 2%	Repellent activity
T. confusum	-0.4	-1	Repellent
O. surinamensis	0	-1	Repellent

-1.00 to -0.10 repellent activity; -0.10 to +0.10 neutral activity; +0.10 to +1.00 attractant activity

In the laboratory studies of Callander and James (2012) the effect of tea tree oil (terpinen-4-ol chemotype) was assessed against different stages of the *Lucilia cuprina,* where formulations containing 1% oil caused 100% mortality. Klauck et al., (2014) evaluated the insecticidal and repellent effects of tea tree and andiroba essential oils on *Haematobia irritans* and *Musca domestica*. Tea tree oil at a concentration of 5.0% had 100.0% efficacy after 12 h of exposure to *M. domestica*. Cutler *et al*., (1996) explored the effect of thirteen essential oils on *T. confusum*. Eucalyptus oil showed the very best results. The induced mortality was 100%. Liao *et al.,* (2016) determined but not with certainty that tea tree essential oil acts on the mitochondrial respiratory chain in insects. In the study of Liao *et al.*, (2018) it was established that tea tree has fumigant toxicity on *T. confusum* which increases over time with the mortality of 98.86%. *Ocimum gratissimum* essential oil showed insecticidal activity against *O. surinamensis* (LC50 0.19 µL/L) (Campolo et al., 2018). Monoterpenoid, 1,8-Cineole, which can be found in tea tree essential oil possess strong larvicidal, insecticidal, fumigant toxicity repellent and antifeedent activities against *O. surinamensis* (Padalia et al., 2015).

Conclusions

Essential oils have substantial potential for pest management in stored places and in the fields. These compounds reduce negative impacts on human health and the environment. Tea tree essential oil shows insecticidal and repellent activity against many insects, as shown in this study on T. *confusum* and *O. surinamensis.* The better effects were on *T. confusum* than on *O. surinamensis*, with mortality range of 57.5-95% in contact test after 72h. Highest mortality of *O. surinamensis* was in contact-digestive test with efficiency of 52.5% after 72h in concentration of 2%. Essential oils from plants are natural and biodegradable sources of bioinsecticides which are expected to spread use in the near future.

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THE EFFECT OF SOME CHEMICAL ADDITIVES ON THE FOAMING PERFORMANCE OF THE PASTEURIZED LIQUID EGG WHITE

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Abstract

The foam stability of egg white changed between 46.7(0.5 mg/kg phospholipase at 24h) and 64.6 (0.5 mg/kg phospholipase.48 h). When tartaric acid was added to egg white in the rate of 5 %, an increasing in foam capacity and stability was observed. In the use of sodium acid pyrophosphate **(**SAPP), especially on performance effects, it was seen that the similar results to citric acid and tartaric acid were obtained. In control group 1., while the effect on foam capacity is 200 units, due to the fact that it was not been form cream of lump of dough. In group 2, whose contamination of egg yolk is low, it was seen that it was only 40 units. In the use of citric acid, that important effects on foam capacity can be provided on condition that the content of egg yolk is kept at a certain levels. In control group 1 having low contamination, while the effect on foam capacity is 200 units, due to the fact that it does not been form cream of lump of dough, in group 2, whose contamination of egg yolk is low, it was seen that it was only 40 units. In the rates of tryethyl citrate of 0.1 -1 ml/kg, in the studies on two different control group, in pH and brix, any variation was not observed. While foam capacity values change between 640 and 690, and in stability values ranged from 60.3 to 67.4. It is considered that the rate of maximum using was around 0.2 ml/kg. Triethyl citrate protects egg white against the detrimental effects of egg yolk.

Keywords: *Egg whites, foamming, food addivites, performance of egg white*

Introduction

Eggs are used in the preparation of many food products. The most well-known uses of eggs are based on the liquid eggs coagulate or solidify when heated (cakes, breads, crackers); whipping of egg white produces lighter and airier products (meringues, angel cake); and emulsifying egg yolk phospholipids and lipoproteins produces mayonnaise, salad dressing and sauces (Davis and Reeves, 2002). A foam is a colloidal dispersion in which a gaseous phase is dispersed in a liquid or solid phase. Food foams are dependent on the surface activity and film forming properties of specific protein components (Kinsella, 1984). Many proteins are too hydrophobic or too hydrophilic for good foaming properties, and so chemical or enzymatic modification of them makes them more surface active. Foaming agents generally used in the food industry are modified natural proteins of soy, casein, egg white, whey, whey protein isolate lactoglobulins, lyzosyme (Mita *et al.*, 1978; German and Phillips, 1994;Narchi *et al.*, 2007; Patino *et al.*, 2008; Kralova and Sjöblom, 2009). Protein film stability and elasticity vary due to the fact that proteins consist of different amino acids so that different intermolecular interactions ocur (German and Phillips, 1994; Kralova and Sjöblom, 2009). Hen eggs are very well known foaming ingredients (Kamat *et al.*, 1973). They produce large volume, stable foams which coagulate during heating. When egg white was beaten, air bubles were trapped in the liquid albumen does. During the beating of egg white, the air bubbles, decrease in size and increase in number, and the translucent albumen takes on an opaque but moist apperance. As increased amounts of air are incorporated, the foam becomes stiff and loses its flow properties (Lowe, 1955). Overwhipping insolubilizes too much of the ovomucin and lowers the elasticity of the bubbles (MacDonnel *et al.,* 1955). If the first foam is beaten for a long period, the darinage liquid exhibits poor whipping properties (Forsythe and Bergquist, 1951). The greatest amount ovomucin is removed with the first whipping (Macdonnell *et al.,* 1955) but about one third of the ovomucin can be recovered form the drainage after the third whipping (Cunningham, 1976). In addition to ovomucin, lysozyme, globulins A1 and A2 and conalbumin are retained in the foam (Cunningham, 1976). The structure of egg albumen allows it to perform well in foams and each component carries out a different function (Stadelman ve Cotterill, 1994). Globulins are excellent formers but the foaminess is significantly affected by the protein interactions with ovomucin, lysozyme, and, to a lesser extent, ovomucoid, ovotransferrin, and ovalbumin (Johnson and Zabık, 1981). The aim of this study is facing the egg whites industry, the biggest problem is yolk-white distinction, during the egg yolk with contamination as a result of decrease foam capacity and stability, to investigate the food additives that may be used for improvement.

Material and Methods

Pasteurized egg whites were provided from ANAKO Liquid Egg Industry in Konya in Turkey. Pasteurized egg whites were used in the study because of hygiene.

Different properties are different batch production reference egg whites are used for each additive and at different times because of the realization of the trial, the control group showed changes as a result of egg whites. Two different control group has been compared result for each additive.

Kitchen Aid Professional sample is taken and transferred to the mixer. 6th cycle in the mixer for 1 minute, blended for 2 minutes at the 10th cycle. After the beating, the foam formed is transferred to measuring cup to 1000 ml. Leaves no residue in the mixer vessel is attempted whenever possible (Yavuz and Özcan, 2016). For measuring, sample was heated to 20^{o}C.

Foaming capacity relative reading of the following formula

%RWC= V*100/75

Foam do not fall, after measuring whipping capacity, and at the end of an hour amount of leakage weigh and calculated whipping stability by the Formula:

S: Whipping stability

%S= (1 - W/77,25) x 100

pH measurements was used in table-top type device. Device was calibrated by the buffer solutions for 4.01, 7.0 and 10.0. pH meter probe was pressed into the sample immersed in pH measurement button and wait 40 sec. After measuring the pH value was recorded as time runs out on the screen. For brix, refractometer at 20 °C the calibration was done with distilled water. The prepared egg white samples were incubated in a warm water bath until 20 °C. For Brix, the refractometer was used. Reading sample is brixs% (MacDonnell *et al.,* 1955).

200 ml egg white's samples was heated at 20 °C. About 200 g was provided to dissolve by the addition of sugar. Sugar and egg white mix to 4th cycle for 1 min and 10th cycle for 4 minute were mixed. After blending, caliper measurement was taken from the high portion of non-container mixer. So, merinque dough performance was measured (Yavuz and Özcan, 2016).

Results and Discussion

Effect of phospholipase enzyme on egg white performance at different times are given in Table 1. With addition of phospholipase to the environment, there were not any important deviation in pH and Brix (Table 1).

Table1. Effect of phospholipase enzyme on egg white perfornance at different times

Samples	pH	Brix (%)	Foam capacity (%)	Foam stability (%)	Heigh of meringue crema(cm)
Control	8.9	14.1	513	51.7	-*
0.25 ml /kg phospholipase 24h	8.9	14.1	1066	54	7.3
0.25 ml/kg phospholipase 48h	8.9	14.1	1066	54.7	7.5
0.5 ml/kg phospholipase 24h	8.9	14.1	1100	51	7,9
0.5 ml/kg phospholipase 48h	8.9	14.1	1113	56,4	8,3

*cremadid not form

In term of the capacity of foam, an important increase occurred in those treated compared to the control (Table 1). In Brix, an important variation was not observed, and it was considered that the decrease of 0.2 units in the first control group may be related to the precision of instrument (Yavuz and Özcan, 2016). Foam capacity values of samples changed between 513% (control) and 1113% (0.5 ml/kg phospholipase for 48h. The highest foam capacity was established in the sample with addition of 0.5 mg of phospholipase for 48 hours (Table 1). It is considered that this increase may probably be due to protein interaction. When regarded to the foam stability, it was seen that there was a fluctuation depending on the concentration of enzyme added and the time. In general, foam stability ranged from between 46.7 (0.5 mg/kg phospholipase for 24h) and 64.6 (0.5 mg/kg phospholipase for 48 h). When the height of lump of dough compared to the control, an important increase was observed, and maximum height of lump of dough became 9.3 (0.5 mg/kg phospholipase for 48 h). At the beginning, egg white having a foam capacity of 1200 mg/100 g, with addition of egg yolk of 0.3%. It was seen that foam capacity regressed to 300 ml/100 g and that phospholipase enzyme, added at the rate of 200 ml/ton, rearranged the foam capacity at 0-4 º C and improved it at the levels of 1200 ml/100 g. In foam stability, it was observed that pure egg white of 87 ml/ 60 min with contamination of 0.3% egg yolk, regressed to 35 ml/60 min, and with phospholipase enzyme, that there was again an improvement at the levels of 80-85 ml/60 min. While 50 units of improvements in stability are provided and 900- 1000 units in foam capacity, in this study, the amounts of improvements remained at lower levels. As the reasons for this, it was considered that the foam capacities and stabilities were low. Contamination of egg yolk was higher than the study we made a comparison. In addition, it was thought of that this would result in low improvement rates.
On condition that the content of egg yolk is kept at a certain level, when tartaric acid is added to egg white in the rate of 5 %, an increasing in foam capacity and stability were seen. The effect of tartaric acid on egg white performance at different times are shown in Table 2.

Table 2 . The effect of tartaric acid on egg white performance at different times

Samples	pH	Brix (%)	Foam capacity(%)	Foam stability (%)	Heigh of meringue cream (cm)
Control 1	8.8	13.8	659	56.2	7.7
1% cream tartar	8.33	14	720	65.7	8.1
3% cream tartar	7.5	14.1	700	70.9	6.7
5% cream tartar	7.0	14.1	770	68.8	7.1
10% cream tartar	6.3	14.2	713	66.7	6.3
12 %cream tartar	5.8	14,3	650	64.5	7,4

In the uses over 5%, also with the effect of increasing acidity, the losses were again seen in the foam values. In view of this, in the uses of cream of tartar, it is considered that pH should be lowered below the levels of 7-7.5. In addition, in egg whites, whose the concentration of egg yolk is high (Control Group 2), it did not show any effect on lump of dough performance and it was seen that the formation of cream of foam could not possibly be provided. Foam stability of egg White were determined between 56.2% (control) and 70.9% (3% cream tartar. In heated white egg, by using lactic acid, hydrochloric acid, or potassium acid tartrate (tartaric acid), it was reported that pH should be kept around 6.0 – 6.5 (Slosberg, 1948). However, due to leakage in "lump of dough" product, reducing a beaded structure forming near it cannot be provided by potassium acid tartrate (Hester and Personius, 1949). About the quantity of the use of potassium acid tartarate /tartaric acid), also in our study, pH related literature results were reached. In egg white having the rate of 10%, determined at pH 6.12, some decreases occurred in foam capacity, foam stability, and the height of cream of lump of dough, which are performance values.

In the use of SAPP, especially on performance effects, it was seen that the similar results to citric acid and tartaric acid were obtained. Again, if the first performance of control group at a certain level, some improvements were observed; otherwise, it was seen that it did not have any positive effect on cream of lump of dough but considerable increase in the values of stability and foam was not observed. For being able to obtain maximum result, maximum rate of use should be around 3% (Table 3) due to the fact it is an acidic agent, pH ranges in a wide range.

Table 3. The effect of sodium acid pyrophosphate on egg white perfornance at different times

Samples	pH	Brix (%)	Foam capacity(%)	Foam stability (%)	Heigh of meringue crema(cm)
Control 1	8.9	14.0	533	44	-*
0.6 %SAPP	8.7	14.0	533	52	-
1%SAPP	8.6	14.0	626	68.6	6.2
3 %SAPP	7.9	14.0	683	57.8	6.2
5 %SAPP	7.5	14.0	608	46.8	6.0

*cremadid not form

In the use of citric acid, that important effects on foam capacity can be provided on condition that the content of egg yolk is kept at a certain levels (Table 4). In control

Group 1 having low contamination, while the effect on foam capacity was 200 units, due to the fact that it does not form cream of lump of dough, in Group 2, whose contamination of egg yolk is low, it was seen that it was only 40 units (Table 4). For increasing foam properties of duck egg, lemon juice was used (Rhodes, 1960).. Apparently, it was seen that, addition of acid, influencing ovomucin, reduced whisking time. In angel cake, made with acid added duck egg, it was seen that a better result was obtained compared to that made with chicken white egg white (Rhodes et al., 1960). Acid increases the internal brightness of cake and acid and acid salts develop stability. In addition, acid and acid salts develop foam stability of albumin (Grewe and Child, 1930). Also in our study, in control 2, while foam capacity falls with the amount of citric acid that increase, it was seen that there was an increase in foam stability. Again, in control 1, while height of cream of lump of dough falls, seeing that there was not any decrease in stability and that acid has an effect increasing the stability, an equivalent result to literature was obtained.

Table 4.The effect of citric acid on egg white performance at different times

Samples	pH	Brix (%)	Foam capacity(%)	Foam stability (%)	Heigh of meringue crema (cm)
Control	13.0	8.92	1000	54.6	6.9
0.1% citric acid	12.4	7.14	1133	68	7.0
0.3% citric acid	12.8	6.03	1200	69	6.8
Control2	13.4	9.0	720	45.2	-*
0.1 % citric acid	13.4	7.4	760	48.6	-
0.3% citric acid	13.4	6.4	640	51.6	-

*cremadid not form

In the rates of tryethyl citrate of 0.1 -1 ml/kg, in the studies on two different control group, in pH and brix, any variation was not observed. But its positive effects was seen on the performance criteria. In foam capacity, the values between 640 -690 were identified and in stability, the values ranging 60.3-67.4. It is considered that the rate of maximum use was around 0.2 ml/kg (Table 5).

Table 5. The effect of triethyl citrate on egg white performance at different times

Samples	pH	Brix (%)	Foam capacity (%)	Foam stability (%)	Heigh of meringue crema (cm)
Control	9.1	14.1	666.5	60.6	6,5
0.1 ml/kg triethyl citrate	9.1	14.1	715	62.9	6.5
0.2ml/kg triethyl citrate	9.1	14.1	825	64.5	6.7
0.5 ml/kg triethyl citrate	9.1	14.1	715	63.9	6.0
1 ml/kg triethyl citrate	9.0	14.2	700	64.2	6.0

Triethyl citrate protects egg white against the detrimental effects of egg yolk. While it reduces whisking time in white eggs that contains yolk or not, its detrimental effect on cake was only seen as increase of cake volumes made with white egg containing egg yolk (Yavuz and Özcan, 2016) As indicated in the literature, also in our study, it was seen that triethyl citrate provided improvement in even Control 2, whose contamination of egg yolk is high. In foaming agents such as citric acid and SAPP, since cream of lump of dough did not form, in white eggs, in which it is considered that it contains high yolk contamination, while the desired improvement cannot be provided, triethyl citrate, even if its cream height is low, it is possible for it to form a firm cream. In the other additives used, while improvements in control group show a variability compared to the beginning concentration and the amount of egg yolk contamination, in NaOH, these variabilities were not seen in the considerable rates. (Yavuz and Özcan, 2016).

Conclusion

When tartaric acid was added to egg white in the rate of 5 %, an increasing in foam capacity and stability was observed. In the use of sodium acid pyrophosphate **(**SAPP), especially on performance effects, it was seen that the similar results to citric acid and tartaric acid were obtained. In the use of citric acid, that important effects on foam capacity can be provided on condition that the content of egg yolk is kept at a certain levels. Triethyl citrate protects egg white against the detrimental effects of egg yolk.

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CHEMICAL PROTECTION OF WINTER WHEAT AND ITS ENVIRONMENTAL IMPACT IN DIFFERENT SOIL TILLAGE SYSTEMS

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Abstract
The aim of the study was to assess the environmental impact of chemical protection of winter wheat in three soil tillage systems: conventional tillage, reduced tillage and direct sowing. The study was conducted in 15 farms in the Wielkopolska region (Poland), in the years 2015-2017. The highest intensity of winter wheat protection, in terms of the number of chemical protection treatments was in the conventional tillage (4.1), followed by the reduced tillage and the direct sowing (3.4 and 3.1, respectively). The largest consumption of active substances was found in the reduced tillage system (2.16 kg a.s./ha). Smaller quantities of active substances were used in the conventional tillage (2.04 kg a.s./ha) and the direct sowing (1.43 kg a.s./ha). In the analyzed soil tillage systems, the costs of winter wheat protection per hectare ranged from 272 to 353 PLN/ha. The direct sowing was characterized by the lowest cost of plant protection products used. Among the analyzed tillage systems, the most negative values of the potential toxicity index had the reduced tillage and the conventional tillage (-68.1 and -55.5 points, respectively) which resulted from larger consumption of active substances with properties associated with higher environmental threats. In turn, less negative points had the direct sowing (-39.5 points). The environmental impact of chemical protection of winter wheat in the studied tillage systems was mainly related to a high risk of volatilisation of substances into the atmosphere, followed by surface water contamination and leaching into groundwater, while there was the lowest risk of bioaccumulation.

Keywords: *Plant protection products, Costs, Potential toxicity index, Soil tillage systems, Winter wheat.*

Introduction
Winter wheat (*Triticum aestivum* L.) is a dominant crop in cereals production in Poland. In 2018, the area under its cultivation was over 1.9 million hectares and production amounted to 8.3 million tonnes (CSO, 2019b). Wheat is cultivated primarily in a conventional tillage system. This system also known as traditional, based on ploughing allows to cover crop residues or organic and natural fertilizers, reduce the losses of fine soil particles and nutrients, achieve good soil scarification, deepening the growth zone of the root system and destroying root-weeds. However, when it is being intensively carried out then contributes to a degradation of the soil environment by accelerating the mineralization of organic matter, increasing the risk of wind and water erosion, and soil drying (Mikanová *et al.*, 2012; Stanek-Tarkowska *et al*., 2018). A large consumption of fuel in conventional tillage system as well as bad weather conditions like drought which more and more often cause difficulties in carrying out soil tillage operations before sowing lead to greater interest in no-ploughing tillage systems (Smagacz and Madej, 2012). The main difference with respect to conventional tillage system consists in replacing a plow by other machines such as cultivator with rigid tines to till to a depth of 10-15 cm or total abandonment of soil tillage and adopting so called direct sowing in the soil with maintained crop residue on the soil surface. The beneficial effects are economic savings and protection of the environment due to energy savings, smaller use of

machinery and thus reductions in consumption of natural resources, fossil fuels and air pollution from fuel combustion (Smagacz, 2012; Sørensen *et al*., 2014).
Plant protection products are widely used in wheat protection. Oerke (2006) estimated the potential loss in wheat cultivation without any protection treatments is around to 55% and the actual loss in case of low efficient wheat protection amounts to 30%. Since 2014, plant protection in Poland must comply with principles of the integrated pest management (IPM). The IPM recommends taking into account primarily non-chemical methods and limiting the intensity of plant protection product use to the necessary minimum. Despite these challenges chemical method plays a dominant role due to a high efficiency in limiting crop losses caused by pests activity (Podleśna *et al*., 2018). According to scientific literature, the use of no-ploughing tillage systems may lead to an increase in the occurrence of diseases and pests (Bankina *et al*., 2018; Hurej *et al*., 2012; Małecka-Jankowiak *et al*., 2015). This is related to more intensive plant protection and its higher cost in comparison with conventional tillage (Jankowiak and Małecka, 2008). Higher use of plant protection products is associated with more harmful effects on various elements of the environment e.g. air, soil, ground water, surface water and living organisms. Moreover, this impact may vary dependent on the potential toxicity of active substances of plant protection products (Holka, 2017).
The aim of the study was to evaluate a cost and a potential environmental impact of chemical plant protection in winter wheat under different soil tillage systems.

Materials and Methods

The study was conducted in 15 farms, located in the Wielkopolska voivodship (Poland), during the period 2015-2017. The chemical protection of winter wheat in three soil tillage systems: conventional tillage, reduced tillage and direct sowing was analyzed. Detailed data on chemical protection treatments and the purchase prices of plant protection products were collected from the surveyed farms. Assessment of the impact of plant protection products on the environment was performed using the potential toxicity index, which results from the selected physico-chemical properties and quantities of active substances (Lewis and Tzilivakis, 1998). The following properties of active substances were taken into account: octanol-water partition coefficient, solubility in water, Henry's constant and ground-water ubiquity score index. Each of the variables indicates one of the four different potential environmental risks, which are the components of the potential toxicity index (Figure 1).

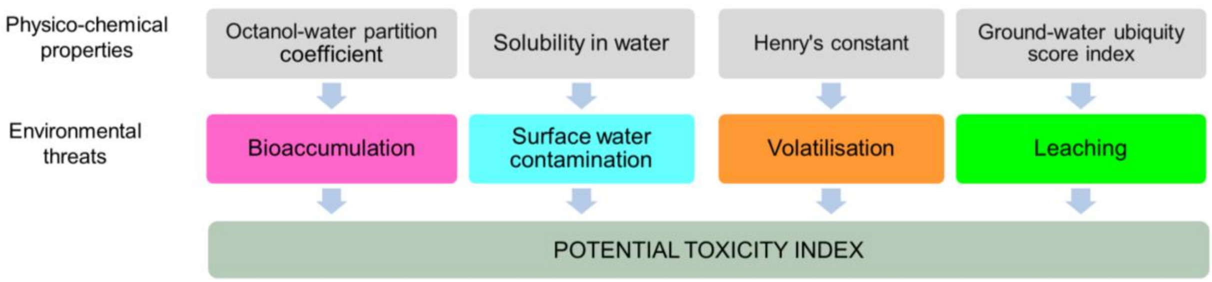

Figure 1. Scheme of assessment of a potential toxicity index of plant protection products.

The potential toxicity index was calculated as follows:

$$EMA = \sum_{i=1}^{n} \left(E_i \cdot Q_i \right),$$

where:
E_i - sum of points obtained by the i-th active substance on the basis of its physico-chemical properties,

Q_i - the amount of the i-th active substance used, in kg/ha,
n - the number of active substances.
The point assessment of each active substance was performed based on the properties data (EU Pesticides Database, 2019; Pesticides Properties Database, 2019), and the criteria adopted (Bieńkowski, 2011).

Results and Discussion

Figure 2 shows the differences in the number of chemical protection treatments in winter wheat between the soil tillage systems. The most treatments were carried out in the conventional tillage system (4.1), followed by the reduce tillage (3.4), and the direct sowing (3.1). The most treatments were based on fungicides (their share was in the range from 48.1% in the direct sowing to 41.5% in the conventional tillage) and herbicides (ranged from 28.7% in the direct sowing to 39.0% in the conventional tillage). There were smaller numbers of insecticide treatments (they ranged from 9.9% in the direct sowing to 14.9% in the reduced tillage) and growth regulator treatments (from 7.3% in the conventional tillage to 13.3% in the direct sowing). Hołaj (2011) also noted 4 chemical protection treatments of winter wheat in the conventional tillage.

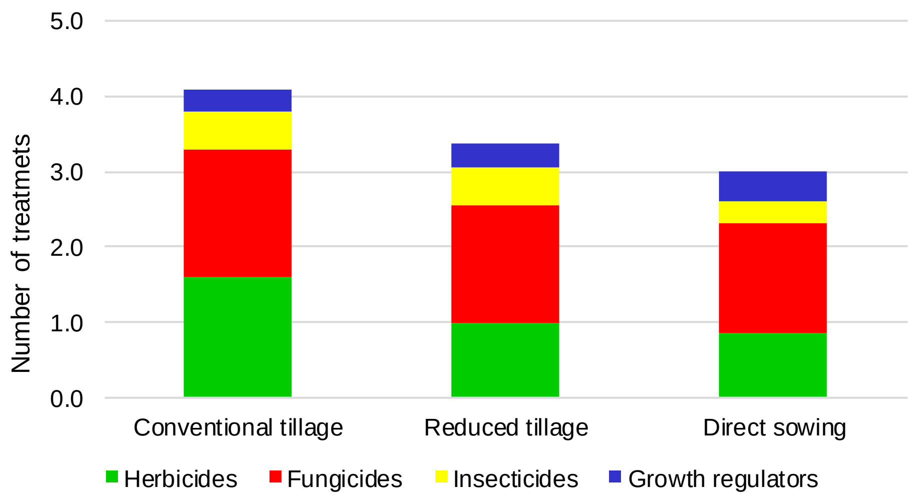

Figure 2. Number of plant protection treatments in winter wheat according to the soil tillage systems (averages for the years 2015-2017).

The largest consumption of active substances was in the reduced tillage (2.16 kg/ha) (Figure 3). Slightly lower amounts of active substances were used in the conventional tillage (2.04 kg/ha) and markedly lower in the direct sowing (1.43 kg/ha). Among the soil tillage systems, herbicides had the largest share of plant protection products used (ranging from 36.1% in the direct sowing to 64.8% in the conventional tillage), followed by fungicides (ranging from 29.3% in the reduced tillage to 39.8% in the direct sowing). In addition to this, protection of winter wheat in the conventional tillage was characterized by smaller use of growth regulators than in other soil tillage systems. The Central Statistical Office of Poland (CSO, 2019a) reported lower average consumption of active substances for winter wheat grown in the country, amounted to 1.32 kg per 1 ha. In the studies of Jankowiak *et al.* (2012), an increase in the consumption of plant protection products was related to area structure of farms. The obtained results indicated more intensive crop protection in terms of the consumption of plant protection products in the reduced tillage. As it was observed in the study this system is more frequently used by larger farms which are furnished with appropriate machinery equipment and run intensive crop production (Townsend *et al.*, 2016).

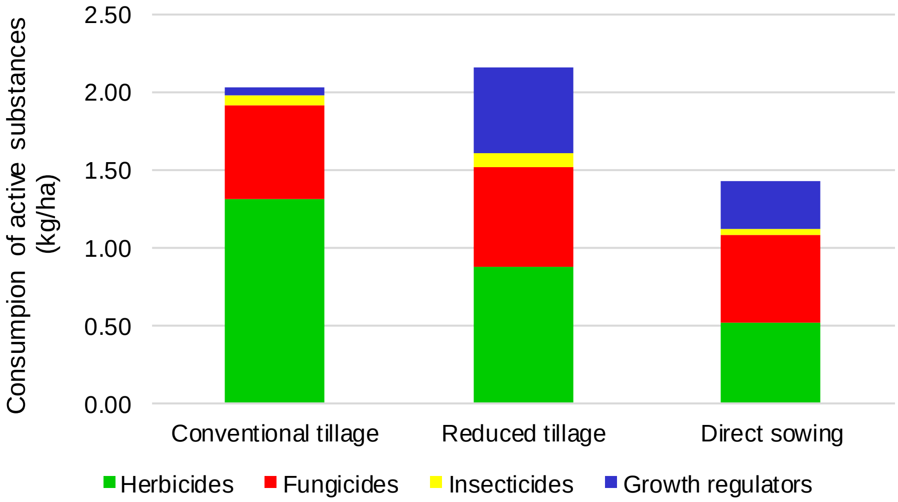

Figure 3. Consumption of active substances in winter wheat according to the soil tillage systems (averages for the years 2015-2017).

The higher costs of plant protection products were in the reduced tillage and in the conventional tillage (Table 1). The direct sowing achieved the lowest cost. The total costs resulted mainly from the use of herbicides and fungicides. On average, the cost of plant protection products for winter wheat in the selected farms in Lublin Voivodeship amounted to 386 PLN/ha (Bojarszczuk and Podleśny, 2017). Smagacz (2012) noted higher overall costs of winter wheat protection in the direct sowing (836 PLN/ha) in comparison with the conventional tillage and the reduced tillage (in both cases amounted to 769 PLN/ha). In the study of Gaworski *et al.* (2013) the costs of plant protection products in winter triticale production in the traditional tillage was 162 PLN/ha and in the direct sowing 216 PLN/ha. In England, costs of winter wheat protection were higher in farms never plough (Townsend *et al*., 2016)

Table 1. Cost of plant protection products used in winter wheat in different soil tillage systems, in PLN/ha (averages for the years 2015-2017).

Type of plant protection products	Soil tillage system		
	conventional	reduced	direct sowing
Herbicides	137.90	113.90	99.10
Fungicides	172.90	217.00	145.90
Insecticides	8.30	14.20	7.50
Plant growth regulators	34.00	10.20	20.40
Total	353.20	355.30	272.90

The soil tillage systems examined differed in respect to the potential toxicity of chemical protection. The results presented in Figure 4 show that the most negative score of the potential toxicity index, indicating the largest use of plant protection products with high toxicity to the environment, had the reduced tillage (-68.1 points), followed by the conventional tillage (-55.5 points). In turn, the least harmful impact of chemical plant protection on the environment was found in the direct sowing as evidenced by the lowest score of the index (-39.5 points). The relative contribution of particular components to the total value of the index was similar between the analyzed soil tillage systems. The greatest environmental risk was driven by volatilisation, followed by surface water contamination and leaching. Risk bioaccumulation of the substances in the tissues of living organisms was of minor importance. In earlier study, the average value of the multi-criteria index of the impact of plant protection on the environment for cereals in conventional tillage amounted to 62.4 (Holka, 2017). Bieńkowski (2011) obtained an average value of the index in farms specializing in field crops equal to -39.4 points. In this study, volatilisation was also found to be the main contributor to the multi-criteria index profile of potential toxicity of chemical protection.

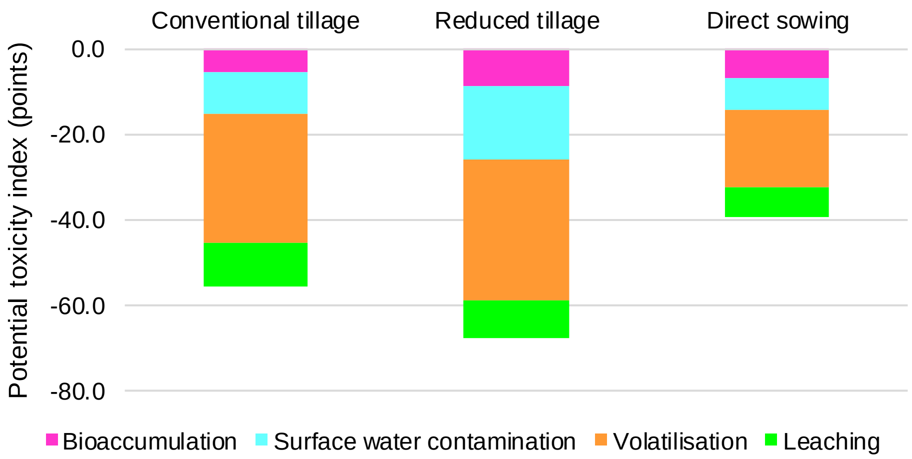

Figure 4. Potential toxicity index and its components calculated for the chemical protection of winter wheat in the analyzed soil tillage systems (averages for the years 2015-2017).

Conclusions

Winter wheat in the reduced tillage system was associated with the highest intensity of plant protection products used, and at the same time it had the most negative impact on the environment. Environmental impact of plant protection was expressed by the multicomponent index of potential toxicity attributable to basic physico-chemical properties of active substances. Practicing direct sowing in wheat cultivation turned out to be most advantageous with respect to number of spray application, environmental impact and cost of chemical protection.

Acknowledgment

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ANTIBACTERIAL ACTIVITY OF PROPOLIS EXTRACTS FROM GREECE AND REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

Propolis (bee glue) is a mixture of various amounts of wax and resin that honey bees collect from leaf buds or tree barks and bushes. Usually these are poplar trees, ash tree, apple, birch, chestnut, etc. Bees disinfect and protect their hive against unwanted external influences, such as fungi, microorganisms, smaller animals (mice, worms, ants), but they also protect hives against cold, moisture and flows. Propolis is a complex content that result from mixing of natural plant derived and bee released elements and compounds. The proportion of the various materials found in the propolis that related to its place and time of collection but, in general, raw propolis is estimated to have a composition of around 50% of vegetable resins, 30% of wax, 10% essential oils, 5% pollen, and 5% of various organic compounds. Propolis is lipophilic in nature, rigid, fragile and brittle material when cold; but when temperature rises, it becomes soft, pasty, gummy and adhesive property and be sticky. It is used in the treatment of infections, and prevents throat inflammation, paradentosis, etc. The aim of this study was to investigate the antibacterial effects of two alcoholic solutions of propolis originating from Greece and from Republic of Srpska and their effect on clinical isolates of *Staphylococcus pseudintermedius* (isolates from the nose, skin and ear of the dogs and rabbits) and to determine the type of action.The results of work confirmed the higher antibacterial activity of alcoholic solution of propolis originating from the Republic of Srpska with an inhibition zone of 10.66 mm to 23.33 mm, and the range of action for propolis solution originating from Greece was from 10.00 mm to 19.00 mm. Alcoholic propolis solution originating from Republica of Srpska showed greater bactericidal activity, while the propolis originated from Greece have stronger bacteriostatic activity.

Keywords: *propolis, antibacterial properties*

Introduction

Propolis is a mixture of different amounts of beeswax and resins that obtained by the honeybee from different natural plants, especially from flowes and leaf buds. The color of propolis varied from yellow to be more darker brown according to the origin of the resins. Fresh propolis extracted from the hive is a soft, sticky mass and curing at room temperature. The flavor, color and chemical properties depend on the type of plants that are in the bee's radius. Propolis has a pleasant smell of plant buds, honey, wax and vanilla, bitter taste, sticky on the touch and if it is kept longer receives a dark color (Pušnik, 2016). The meaning of the word propolis, according to some interpretations, comes from the Greek: "pro" mean, in front of and "polis" mean city. There is an opinion that the name derives from the Greek and Latin words "propolis" which means to smudge or to smooth out. A honey bee collects propolis and carries it back to the nest on her hind legs. By cutting and chewing the resin and with bee saliva they affection the composition of propolis. Bees use propolis alone or with beeswax in the construction and adaptation of their nests, which ensures purity and sterility, protection against pests and external factors. The dead bees and pests are covered with propolis and thus preventing their decomposition, thereby eliminating the source of potential infection of the bee's society (Toreti, 2013; Kuropatnicki *et al*,. 2013; Pušnik, 2016;).The ancient Egyptians used it as a medium for mummification of pharaohs (Bankova et al., 2000). Bee Propolis has a long history of medicinal use, dating back to 350 B.C., the time of Aristotle and he also mentions propolis in his work "Historia animalium" ("Animal speech") as naturally medies for treating skin lesions, wounds and infections (Toreti, 2013), while the ancient Greeks blended propolis with vegetable oils and made perfumes (Bogdanov and Bankova, 2012). Propolis is used by ancient Inca civilization, against inflammatory processes and to treating a fever (Bogdanov and Bankova, 2012). The ancient Romans used propolis for wound dressings and according to some data dating from that time, propolis had a higher price than honey (Toreti, 2013). Between the 17th and 20th centuries, propolis began to be used intensively in Europe because of his antibacterial properties (Monti *et al.*, 1983). In the 20th century and in the beginning of this century, the use of propolis in the pharmaceutical, food and cosmetic industry is growing, and accordingly it grows interest in examining the chemical composition and biological properties of propolis. Propolis has a complex chemical composition that has not yet been fully tested. It contains about 50% vegetable resins, 30% wax, 10% essential oils, 5% pollen and 5% of various organic compounds. It contains flavonoids, organic acids, polyphenols, terpenes, esters, polysaccharides, minerals, vitamins, aldehydes, coumarins and potentially present foreign substances (Pietta *et al.* 2002; Rusak, 2008; Bankova *et al*. 2008; Bogdanov and Bankova 2012 Graikou *et al.* 2016). The type and amount of biologically active compounds in propolis depends on the chemical composition of the plant resin, which is directly related to the climatic and phytogeographical characteristics of the regions of origin, and therefore propolis from different climatic zones are different from each other (Marcucci, 1995). Propolis exhibits a wide range of biological properties, and antibacterial activity is the most important feature, as confirmed by a large number of studies (Bankova *et al*., 1995; Zwolan and Merest, 2000; Havsteen, 2002; Bankova, 2009; Bogdanov, 2012; Kročko *et al.*, 2012;;). Antibacterial (bactericidal and bacteriostatic) propolis activity has been studied on a large number of bacterial species (*Streptococcus haemolyticus*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Bacillus anthracis*, *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, etc.). Propolis has good effects on fungi: *Candida albicans*, *Candida tropicalis*, *Candida crusei*, *Torulogis glaerata*, *Aspergilus* spp., *Cryptococcus neoformans*, and others. The strongest antibacterial effect is on *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and others (Park *et al.*, 2005; Mavri *et al*., 2012; *Osés et al*., 2016, Saeed *et al.*, 2016, Graicou *et al*., 2016,). Propolis stops the development of a influenza and herpes virus (Adenovirus, Coronavirus, Herpes Symplex, Influenza A and B virus, Rota Virus, etc.) (Schnitzler *et al*., 2010). Propolis has a great advantage over synthetic drugs because it does not lead to bacterial resistance (Mladenov and Radosavović, 1998; Banskota *et al*., 2001; David et al., 2012; Gressler *et al*., 2012), is not toxic, does not damage the normal intestinal flora, increases the antibacterial effect of ciprofloxacin, penicillin, amoxicillin, ampicillin, cefalexin and tetracycline (Oxyxil *et al.*, 2005; Orsi *et al.* 2012,). Sometimes, propolis is more effective than commercially available drugs (Krell, 1996). Propolis possesses good antibacterial, antiviral, antimicrobial, antioxidant, anti-cancerogenic, immunomodulatory, hepatoprotective, cardioprotective and antiallergic properties (Galvao *et al.*, 2007; Sforcin, 2007;; Fischer and Vidor, 2007; Bankova, 2009 Bogdanov, 2012; Toretti, 2013; Pušnik, 2016; Saeed *et al.*, 2016;).

Staphylococcus pseudintermedius is a normal resident of the skin and mucosa and can be isolated from the nose, mouth, pharynx, forehead, hips and anus of healthy dogs and cats. *Staphylococcus pseudintermedius* is the main cause of skin and ear infections, postoperative wounds in dogs and cats and other tissues (van Duijkeren, 2011). *Staphylococcus*

pseudintermedius is a Gram-positive bacterium called *Staphylococcus intermedius* for the first time described by Hajek in 1976. as a coagulase-positive staphylococci.
S. pseudintermedius has been described from dogs, cats, horses and parrots using the molecular method, and it has been proposed that isolates from dogs, if no molecular methods for identification of such isolates are available, are attributed to this species (Bond and Loeffler, 2012). It is presumed that most of the isolates that were formerly referred as *S. intermedius* are most likely belonging to the species *S. pseudintermedius*, at least in terms of isolates originating from dogs. It is also likely that infections in people who were in contact with dogs were caused by *S. pseudintermedius*. There is an increasing number of cases showing serious invasive infections with *S. pseudintermedius* in humans such as bacteraemia (Talan *et al*. 1989; Goldstein, 1992; Lee, 1994), pneumonia, sinusitis (Gerstad *et al*., 1999), otitis externa (Kempker *et al.*, 2009), nail infection (Tanner *et a*l., 2000), mastoiditis (Pottumarthy et al., 2004), brain abnormalities (Kikuchi *et al.*, 2004), skin absorption (Atalay *et a*l., 2005), bacteremia complicated with septic arthritis (Kelesidis and Tsiodras, 2010).
The aim of this work is to explore the antibacterial characteristic of two samples of propolis originating from Greece and Republic of Srpska and there influence on eleven isolates of *Staphylococcus pseudintermedius* and also to determine the type of action.

Materials and methods

Material

In this study we used two samples of 20% ethanolic solution of propolis from different origin (Greece and Republic of Srpska).

Test cultures

For testing the antibacterial activity of propolis we used bacterial cultures of *Staphylococcus pseudintermedius* (eleven isolates) isolated from a various clinical materials (6 samples from the ear swabs, 4 skin swabs and 1 nose swab) from 10 dogs and 1 rabbit. The cultures were seeded in a nutrient broth and incubated at 37 ºC for 18 h. Petri plates with the appropriate substrate (Müeller-Hinton agar) were seeded with 0.1 ml of bacterial suspension in concentration of 10^5cfu/ml.

Methods

To examine the effects of propolis on selected bacterial cultures, we used agar diffusion method on sterile solid-nutrient medium (Müeller-Hinton agar). Metallic cylinders of 9 mm diameter were placed on the surface of a solid nutrient medium where's a certain pure bacterial culture was previously sown. 100 µl of propolis was placed in the cylinders with the micropipette. As a control, 100 µl of 96% alcohol was put in the one cylinder. The plates were incubated for 24 hours at a temperature of 37 °C. For each bacterial culture, three repetitions were made and the average value for each bacterial culture was calculated.
We also determined the type of action of propolis. To see if propolis has bactericidal or bacteriostatic effect, a small amount of agar was taken from the inhibition zone and added to the nutrient broth. Incubation was carried out at 37ºC for 24 h. After incubation presence of dark blur in broth tell us about bacteriostatic effect, or if, the broth remained clear, about bactericidal effect.

Results and discussion

Products based on propolis come on the market in different shapes, but in our market is the most common alcoholic solution of propolis concentration of 20% and more.
Antibacterial properties of two samples of propolis alcoholic solution originating from Greece and Republic of Srpska were tested on eleven isolates of *Staphylococcus pseudintermedius*

from skin swabs (4 isolates), ear swabs (6 isolates) and nose swab (1 isolate) of 10 dogs and 1 rabbit, and the results are shown in Table 1.

Table 1. Antibacterial activity of alcoholic solutions of propolis on *Staphylococcus pseudintermedius*

Staphylococcus pseudintermedius	Inhibition zone in mm	
	20% ethanol solution of propolis (R. Srpska)	20% ethanol solution of propolis (Greece)
1	18.33	13.33
2	10.66	10.00
3	15.66	**0.00**
4	23.33	19,00
5	18.33	**0.00**
6	12.66	11.66
7	12.00	16.66
8	12.00	**0.00**
9	15.00	10.66
10	**0.00**	10.00
11	**0.00**	16.66

1, 2, 3, - dog skin swabs, 4 - rabbit skin swabs, 5, 6, 7, 8, 9, 10 - ear swabs, 11 - nose swab

As can be seen from the results, a solution of propolis originating from the Republic of Srpska showed greater antibacterial activity on most tested bacterial isolates compared to the Greek propolis solution. Inhibition zones for the propolis solution originating from Republic of Srpska ranged from 10.66 mm to 23.33 mm, while the range of action for the propolis solution originating from Greece was from 10.00 mm to 19.00 mm.

The alcoholic solution of propolis originating from the Republic of Srpska did not appear to be inhibitory for two isolates (one ear swab and one nose swab) while the alcoholic solution of Greek propolis acted inhibitively on both these isolates with inhibition zones of 10.00 mm and 16.66 mm. The alcoholic solution of Greek propolis did not appear to inhibit the isolation of *Staphylococcus pseudintermedius* isolated from the skin swab and two isolates from the ear. Different biogeological factors influence on the different chemical composition of the plant material available to the bees for the production of propolis, and propolis from different climatic zones are different one from another. Despite the differences in the chemical composition of different types of propolis, propolis exhibits a strong antibacterial effect, and this is supported by a large number of publications (Bogdanov, 2012).

The differences in the antibacterial activity of alcoholic tincture propolis from Greece and the Republic of Srpska are in line with the results published in the literature and are probably dependent on the geographical area from which the bees collected honey (Stepanović *et al.*, 2003, Adewumi *et al.*, 2016). Phenolic acids and flavonoids significantly contribute to the antimicrobial activity of propolis (Pepelnik *et al.*, 2004; Mercan *et al.*, 2006., Cabral *et al.*, 2009; Junior *et al.*, 2012., Toreti, 2013., Neves *et al.*, 2016;;). Also, the propolis activity is influenced by the way of conservation, freshness, content of ingredients and method of application (Haynes *et al.*, 2011., Tasleem *et al.*, 2011., Wolska *et al.*, 2016.). The results of this study have shown that both propolis exhibited good antibacterial activity against the tested Gram-positive isolates of *Staphylococcus pseudintermedius*, which is in agreement with the results of other researchers (Stepanović *et al.*, 2003., Bogdano, 2012,). To see whether propolis has a bactericidal or bacteriostatic effect from the inhibition zone, a small piece of agar is taken and added to the nutrient broth. The obtained results are shown in Figure 2.

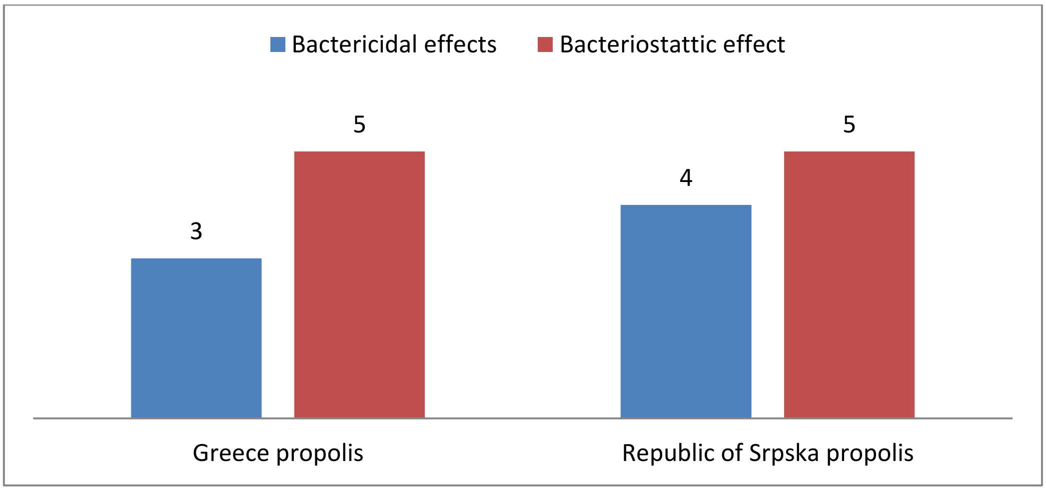

Figure 2. Bacteriostatic and bactericidal effect of propolis

As can be seen from the attached chart, both alcoholic solutions of propolis exhibited a greater bacteriostatic activity on the examined bacteria.

An alcoholic solution of propolis originating from the Republic of Srpska has exhibited greater bactericidal activity compared to Greek propolis.

The results of this work confirm the bactericidal and bacteriostatic effect of alcoholic tincture of propolis, which is in line with research conducted by other researchers (Havsteen, 1983.; Bonvehi *et al.*, 1994., Oksuz *et al.*, 2005., Salas *et al.*, 2014 ;).

Conclusions

The results of the work showed that the propolis solution originated from Republic of Srpska had inhibitory effect on clinical isolates of *Staphylococcus pseudintermedius* with inhibition zones of 10.66 mm to 23.33 mm, while the extent of action for propolis solution originating in Greece was from 10.00 mm to 19.00 mm. Propolis solution originated in Greece acted bacteriostatic with 63.63% and propolis from the Republic of Srpska in 54.54% of the isolates.

Bactericidal activity in 27.27% of isolates showed a propolis solution originating in Republica of Srpska, and a propolis solution from Greece at 18.18% of the isolates. Based on the obtained results, the use of propolis as a natural antibacterial medicament against *Staphylococcus pseudintermedius* can be recommended. The use of propolis in pharmaceutical, cosmetic, nutritional and other purposes determines his diverse chemical composition, as well as experimental scientific research. There is very little research about use of propolis for medical purposes, which provide scientifically-based results, and we must continue this resarch in the future.

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INFLUENCE OF INOCULATION OF *Q. CERRIS* SEEDLINGS WITH A ROOT PATHOGEN *P. PLURIVORA* ON THE PERFORMANCE OF *L. DISPAR* LARVAE UNDER EXTREMELY ELEVATED CO_2 LEVEL CONDITIONS

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Abstract

A large number of organisms like pathogens and herbivores are related to different tree species. They could be often found together on the same plant host. The elevated CO_2 levels can also influence the changes in plant metabolism, which can influence the plant-herbivore relations caused by the pathogens. This paper presents the results of influence of biotic stress, caused by inoculation of Turkey oak (*Qercus cerris*) seedlings with root pathogen (*Phytophthora plurivora*), on the performance of the gypsy moth (*Lymantria dispar*) larvae in elevated CO_2 conditions in extreme scenario which predicts concentration of up to 1000 ppm in the future. Influence of inoculation of seedlings with a root pathogen on the performance of gypsy moth larvae was examined by analysis of covariance (ANCOVA) with plant status (exposure to inoculation, with or without biotic treatment) and environmental conditions (ambient or elevated CO_2 level) as independent variable, and growth rate (GR) and relative growth rate (RGR) as dependent variables. Plant height and larval weight before treatments were used as covariates. Fisher's least significant difference test was used to examine the differences in mean values of individual treatments. ANCOVA showed a statistically significant influence of CO_2 concentration on both parameters, GR and RGR of the larvae. Inoculation with the root pathogen and covariates (larval weight and plant height) as a source of variation, individually, did not influence the performance of the larvae statistically significantly. Interaction between the biotic and environmental conditions had a significant influence on the RGR, but not on the GR.

Keywords: *gypsy moth, root pathogen, Turkey oak, elevated CO_2, tree-trophic interactions*

Introduction

Forests are structurally and functionally complex biotopes rich with species (Messier *et al.*, 2013). More than 25% of all herbivorous arthropods live in tree crowns (Strong *et al.*,1984). Increase in CO_2 level directly influences the trees physiological processes (Traw *et al.*, 1996). The response to this, especially in the content of nitrogen in the leaf tissue, varies from species to species (Peterson *et al.*, 1999). That is why herbivorous organisms face the significantly altered chemical composition of the plant organs that ultimately affects their growth and development (Hättenschwiler and Schafellner, 2004) due to the changes in the plants resistance to pathogens and insects (Henn and Schopf, 2001).

Increased CO_2 concentration directly influences the photosynthesis process, when, with the aid of sunlight and water, plants transform the surplus CO_2 to oxygen and carbohydrates which constitute the plant tissues (Lindroth *et. al.* 1993), while increasing the total forest productivity (Eamus and Jarvis, 1989). By doubling the CO_2 concentration, the plants increase the photosynthetic and water use efficiency by 30-50% (Running and Nemani, 1991). However, the nitrogen content in the leaves which are exposed to increased concentration of

CO_2 decreases, while the tannin content in the same leaves increases (Traw *et al.*, 1996). Since the beginning of the industrial revolution the CO_2 concentration has risen by 30 ppm by 1958, and from 1958 to 2014 by 90 ppm. The total amount increased from 280 to 400 ppm (Besermenji, 2007). Compared to the reference period from 1961 to 1990 an increase in temperature between 1.5 and 4.8 °C is expected by the end of the 21st century (Doljak and Petrović, 2015). It is predicted that the CO_2 concentration will be around 700 ppm by the end of the 21st century (Popović *et al*., 2009).
Previous studies on the influence of elevated CO_2 content in the atmosphere on different forest tree species, and indirectly on the tree pests, were conducted with CO_2 concentrations between 500 and 700 ppm. These concentrations correspond with the most probable scenarios of the increasing of concentration of this gas by the end of the 21st century (van Vuuren *et al*., 2011). However, pessimistic scenarios which predict an increase in CO_2 levels up to 900-1000 ppm in the atmosphere by the end of 21st century were not studied so far. One of the aims of this research was to determine the indirect influence of the root pathogen and increased CO_2 content on herbivorous organism. For this purpose, two-year old Turkey oak (*Quercus cerris* L.) seedlings, gypsy moth (*Lymantria dispar* L.) larvae, and a root pathogen (*Phytophthora plurivora* T. Jung & T. I. Burgess) were subjected to the experiment in this study.

Material and methods

Plant material: Fourty Two-year old Turkey oak (*Q. cerris*) seedlings grown the previous year from acorns that were collected in the vicinity of Belgrade were used for the experiment. The seedlings were grown on peat and perlite substrate (70:30 ratio), in PVC bags of 750 cm^3.
Inoculum: Inoculum was prepared according to Jung *et al*. (1996), where 500 cm^3 of fine vermiculite and 40 cm^3 of millet seeds were measured in one-liter Erlenmeyer flasks. Then, 350 ml of liquid V8 substrate was added to the mixture. The V8 substrate was prepared with 200 ml/l of V8 juice (Biotta, Swiss), 3 g/l of CaCO3 and 800 ml/l of distilled water (Jung *et al*., 1996). After mixing, the mixture was sterilized in an autoclave for 20 minutes at 120°C. Fresh, 3-5 days old *P. plurivora* cultures were taken from the collection and developed on V8-agar media (200 ml/l of V8 juice, 20 g/l of agar (Torlak, Serbia), 3 g/l of CaCO3 and 800 ml/l of distilled water. After inoculation with pieces of fresh *P. plurivora* colonies, the Erlenmeyer flasks were closed with a sterile plug made out of wadding and aluminum foil. Incubation was at room temperature in the dark for 4-6 weeks, until the inoculation. Before the inoculation itself, the inoculum was washed in sterile distilled water, so that the nutrients and sugar from the substrate that could be a carrier of bacterial contaminants, were removed.
Inoculation: The inoculation was conducted by adding of ca. 40 cm^3 of inoculum to the plants rhizosphere, following methodology of standardized soil infestation test (Jung *et al*., 1996). After that the plants were immediately flooded for a period of 72 hours.
Insect material: Gypsy moth egg masses were collected in an oak forest in the vicinity of Bor. They were kept in a freezer at a temperature of 4°C until the spring 2018. Before the beginning of the experiment they were moved to temperature of 25°C in order to induce hatching. After hatching, the larvae were fed with artificial food in petri dishes (120 x 15 mm) at a temperature of 23°C, Rh 75% and light regime 15:9 (day: night) until molting to the fourth larval stage.
Experiment design: For testing the influence of elevated CO_2 and inoculation with a root pathogen on gypsy moth larvae which were intermediated with Turkey oak seedlings, four experimental groups were formed (ten larvae on ten plants per group): LCA (gypsy moth larvae that consumed the leaves of Turkey oak seedlings exposed to ambient CO_2 concentration), PLA (gypsy moth larvae that consumed the leaves of Turkey oak seedlings which were inoculated with a root pathogen in conditions of ambient CO_2 concentration), LCE (gypsy moth larvae that consumed the leaves of Turkey oak seedlings that were exposed

to elevated CO_2 levels) and PLE (gypsy moth larvae that consumed the leaves of Turkey oak seedlings that were inoculated with a root pathogen in conditions of elevated CO_2 levels).
Statistical analysis: Influence of inoculation with *P. plurivora* on the performance of gypsy moth larvae was examined by analysis of covariance (ANCOVA) with plant status (exposure to inoculation, or without biological treatment) and environmental conditions (ambient or elevated CO_2 level) as independent variable, and growth rate (GR) and relative growth rate (RGR) as dependent variables. Plant height and larval weight before treatments were used as covariates. Fisher's least significant difference test was used to examine the differences in mean values of individual treatments.

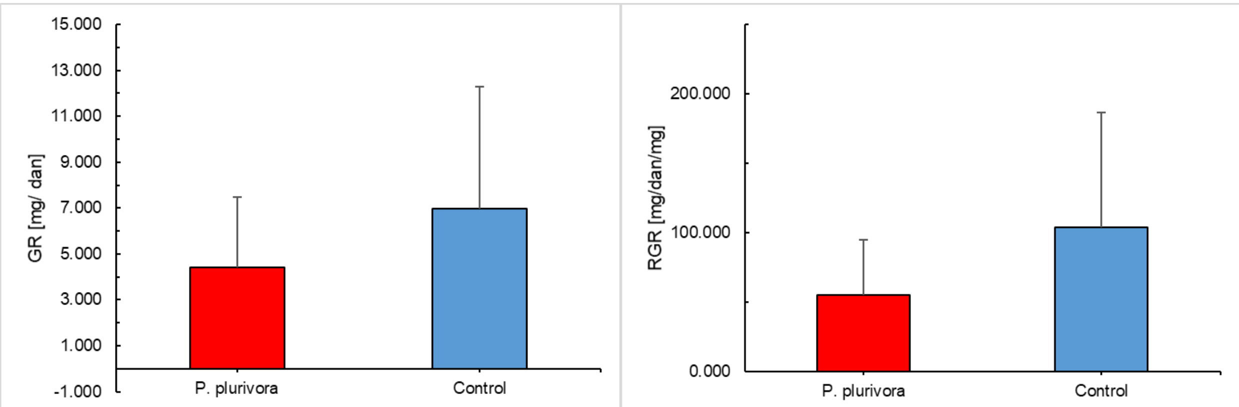
Results and discussion

Results of the research on influence of inoculation with *P. plurivora* and elevated CO_2 level on the performance of the gypsy moth larvae are presented in the Table 1, and Graphs 1, 2 and 3. Results of the analysis of covariance (ANCOVA) showed a statistically significant influence of CO_2 concentration on both parameters, growth rate (GR) and relative growth rate (RGR) of the gypsy moth larvae. *Phytophthora plurivora* and covariates (larval weight and plant height) did not influence the performance of the gypsy moth larvae statistically significantly. Interaction between the biotic and environmental conditions had a significant influence on the RGR (Table 1).

Table 1. Results of the analysis of covariance of the examined influence of inoculation with *P. plurivora* and environmental conditions (ambient and elevated CO_2 level) on the performance of the gypsy moth larvae

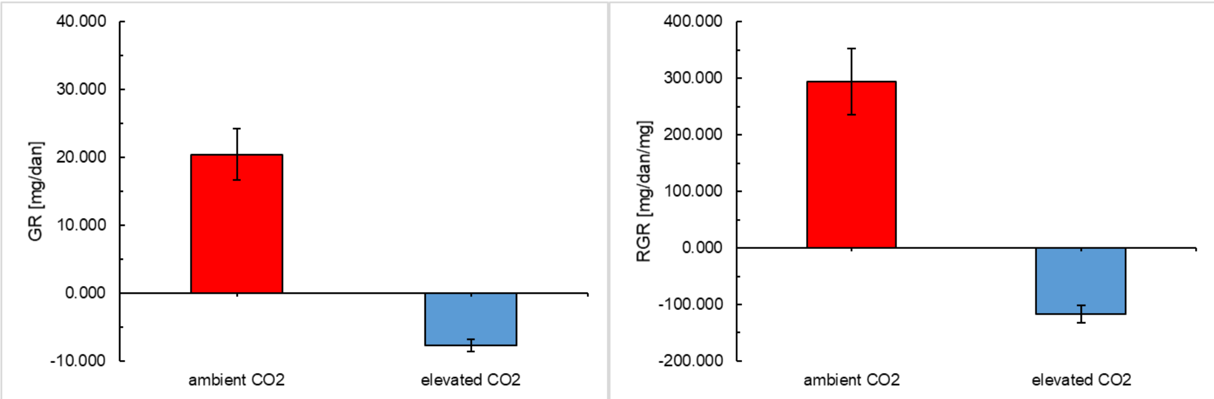
Source of variation	GR				RGR		
	d.f.	MS	F	P	MS	F	P
P. plurivora	1	2.12	0.02	0.9008	23.19	0.00	0.9766
CO_2	1	**7236.71**	**53.94**	**0.0000**	**1642196.24**	**61.82**	**0.0000**
P. plurivora * CO_2	1	326.80	2.44	0.1284	**157925.12**	**5.94**	**0.0205**
Larval weight	1	74.79	0.56	0.4607	94658.88	3.56	0.0682
Plants height	1	1.32	0.01	0.9216	489.44	0.02	0.8929
Error	32	134.16			26564.58		

*Bold values indicates a statistically significant difference



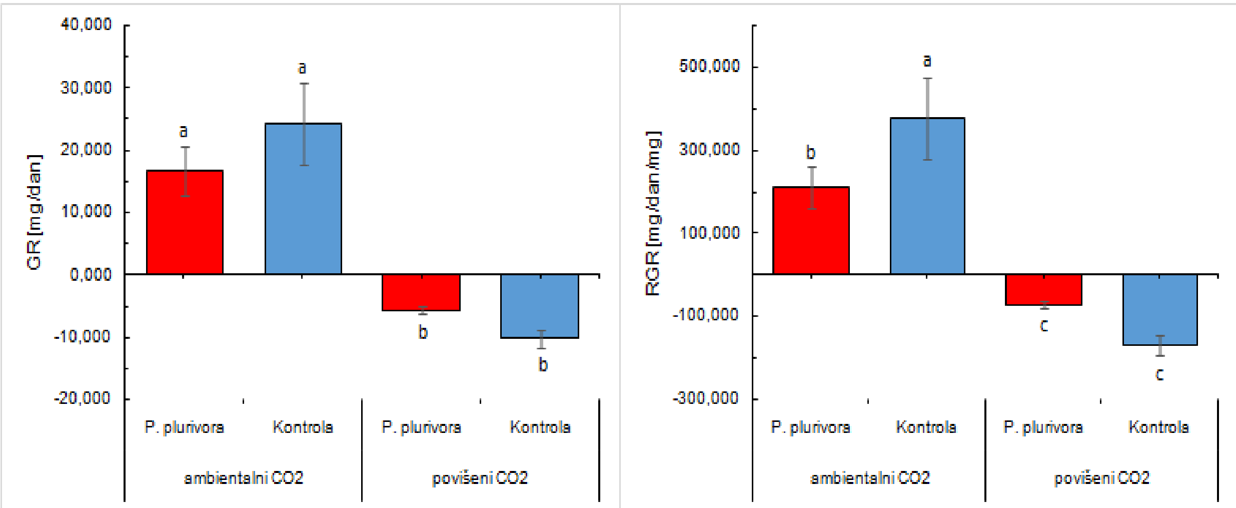
Graph 1. Influence of inoculation with *P. plurivora* on (a) growth rate (GR) and (b) relative growth rate (RGR) of the gypsy moth larvae

GR and RGR of the larvae of the LCA and LCE group compared to the PLA and PLE groups was 37% and 47% higher, respectively, but those differences were not statistically significant (Table 1, Graph 1).



Graph 2. Influence of CO_2 concentration on (a) growth rate (GR) and (b) relative growth rate (RGR) of the gypsy moth larvae

Influence of CO_2 on the performance of the gypsy moth larvae was statistically significant for both GR and the RGR (Table 1, Graph 2a, b). Larvae of the LCA and PLA groups gained 20 mg day^{-1} on average, while those of the LCE and PLE groups lost 7 mg day^{-1} on average (Graph 2a). Larvae of the LCA and PLA groups had a positive RGR of 294 mg day^{-1} mg^{-1}, and those of the LCE and PLE groups had a negative RGR of -116 mg day^{-1} mg^{-1} (Graph 2b).



Graph 3. Influence of CO_2 concentration and inoculation with *P. plurivora* on (a) growth rate (GR) and (b) relative growth rate (RGR) of the gypsy moth larvae

Larvae of the LCA group compared to those of the PLA group had a 31% greater weight. The GR of the larvae of the PLE group was 45% greater that of those from the LCE group (Graph 3a). The larvae of the LCA group had a 44% higher RGR than the larvae of the PLA group, while the RGR of the larvae of the PLE group had a 57% higher than of those in the LCE group (Graph 3b).

Inoculation of the Turkey oak seedlings with a root pathogen *P. plurivora* caused a negative effect on the growth of the gypsy moth larvae in this experiment, while Milanović, *et al.* (2015) noted a positive influence on the growth of the gypsy moth larvae which consumed the leaves of red oak (*Quercus rubra* L.) that were taken from the trees naturally infected with the same pathogen. These findings point out that the relations between a pathogen and an herbivore depends on the plant which mediates them. Also, these differences might be explained with the influence of the exposure length to the inoculum. Namely, in this experiment we used artificial inoculation and short-term exposure of the Turkey oak roots to the *P. plurivora*, and additional experiments with longer soil infestation tests are required to clarify the differences in these findings. Besides the influence of a root pathogen, the intermediary influence of CO_2 level on the gypsy moth larvae was also examined in this study. Numerous studies investigated the influence of CO_2 on diet and development of insects on different tree species (Traw *et al.*, 1996; Henn and Schopf, 2001; Hättenschwiler and Schafellner, 2004; Wang *et al.*, 2009). Hättenschwiler and Schafellner (2004) conducted a research on the diet of the gypsy moth larvae on adult trees in natural stands with elevated CO_2 levels (530 μ mol mol^{-1}). RGR of the larvae which consumed the leaves of the sessile oak (*Quercus petraea* (Mattuschka) Liebl.) exposed to elevated CO_2 levels was 30% lower that the RGR of the larvae which consumed the leaves of the trees exposed to ambient CO_2 level (370 μ mol mol^{-1}). Also, Wang *et al.* (2009) determined that on Mongolian oak (*Quercus mongolica* Fisch.) and birch (*Betula platyphylla* Sukaczev), at elevated CO_2 level, the GR of the gypsy moth larvae was 44.4% lower and the RGR 4.8%, respectively. The results of our experiment are even more drastic in terms of the reduction of GR and RGR in conditions of elevated CO_2 concentration because the recorded negative values, viz., the larval weight did not grow, but they actually lost weight. However, RGR of the larvae which consumed the laves of common hornbeam (*Carpinus betulus* L.) exposed to elevated CO_2 level increased by 29% compared to the RGR of the larvae which consumed the leaves of the control trees at normal CO_2 level. The RGR of the larvae that consumed the leaves of beech (*Fagus moesiaca* L.) did not show any significant decrease or increase at elevated CO_2 conditions (Hättenschwiler and Schafellner, 2004). By comparing these results, we can see that the larval development depends more on the host plant than on the CO_2 concentration (Hättenschwiler and Schafellner, 2004). Based on the above, we can conclude that the indirect influence of increased CO_2 concentration on gypsy moth larvae depends on the mediator tree species. This claim is backed up by the results of Traw *et al*. (1996) who monitored the feeding of gypsy moth larvae on yellow birch (*Betula allegheniensis* Britt.) and gray birch (*Betula populifolia* Marsh.) in the conditions of normal (350 ppm) and elevated (700 ppm) CO_2 levels. Their results indicate that the elevated CO_2 content does affect the gypsy moth larvae feeding on the leaves of gray birch, while when feeding on the leaves of yellow birch, the mass of the females decreases.

Conclusion

Based on the results of our research we can conclude that:

- In the conditions of increased CO2 concentration, inoculation with the root pathogen positively influences the growth of the gypsy moth larvae.
- In the conditions of ambient CO2 concentration, inoculation with the root pathogen negatively influences the growth of the gypsy moth larvae
- The increased CO_2 concentration does not only change the relations between the herbivores and their host plants, but also the relations between different groups of organisms which share the same host plant.

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DIFFERENT ASPECTS OF NON-STANDARD FOLIAR FERTILIZERS BASED ON AMINO ACIDS, PHYTOHORMONES AND PLANT EXTRACTS

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Abstract

We studied different aspects of application of foliar non-standard fertilizers based on amino acids, phytohormones and plant extracts. The trials were carried out at the level of the seedlings, individual plants grown in semi-controled conditions and plants grown in the field. Various energetic and thermodynamic parameters were analyzed, then the chemical composition (mineral elements, different sugars, secondary metabolites, etc.), as well as parameters of plant growth and their yield, in order to better assess the impact of these fertilizers on crops. We found that on maize seedlings it works by changing the content of various elements, then the polyphenol profiles, as well as thermodynamic parameters, where this effect does not only depend on the dosage of the fertilizers, but also on the corn genotype. We also found that the fertilizers affect the energetic and thermodynamic parameters of individual maize plants, as well as the parameters of plant growth. The most significant and most diverse results were obtained by analyzing the yield and components of the yield of many different crops (crop, fruit, vegetable), as well as their chemical composition (mineral elements, different sugars, secondary metabolites, etc.) in terms of improving nutritive quality. It was noticed that these fertilizers greatly affect the content of microelements, starch and crude proteins in maize and barley, sugar and polyphenol content in various fruit trees, as well as in soybeans, in which we note that in certain agroecological situations these fertilizers have led to spectacular magnification yields of different crops, but there were also situations when they did not have any positive effect on crop yields. Overall, the early treatment of cultivated plants with non-standard fertilizers greatly affects the vigor of seedlings of these plants, which is of great importance for crop yield. These fertilizers also significantly affect the quality of crop yield in terms of improved chemical composition of edible parts of these plants.

Keywords: *Amino acids, brassinosteroid (BRs) phytohormones, plant extracts, plant protection, resistance of plants to stress, biofortification*

Introduction

Unlike "classical" fertilizers, which are used as agro-technical measures to supply plants with certain elements, their treatment of non-standard fertilizers is primarily based on the intensification of plant metabolism, either due to their treatment with specific metabolites (eg, amino acids), either phytotohormones, or plants extracts, containing all these substances. This acceleration of plant metabolism occurs due to increased synthesis of protective substances, more intensive adoption of some important nutrients or due to the presence of additional signal substances outside. This path affects not only the yield of crops in quantitative terms, but, more importantly, their reaction to specific agroecological situations of abiotic, biotic and xenobiotic stress, as well as the change in some qualitative yield parameters in terms of increasing the nutritional value of economically usable parts of plants. Thus, more effects are achieved, which do not necessarily have to be related only to crop mineral nutrition, but also to their increased resistance to stressful situations, and also to the possibility of bio-conditioning of crops in a kind of organic food production. Unlike our previous release, which relates only to corn and fertilizer based on brasinosteroid phytohormones (Waisi *et al*., 2015), we give a broader overview of crops and types of applied non-standard fertilizers.

Materials and Methods

Plant growth and yield parameters are described in our previous work (Waisi *et al.*, 2015). The thermodynamic parameters are defined in usual manner (Sun, 2002), and their calculus is described in our previous works (Waisi *et al*., 2017a, 2018). Total polyphenols, starch, sugars, crude proteins, oils are described as in our previous work (Waisi et al., 2015), where a more detailed description of the methods of quantification of total polyphenols, proteins and polyphenolic fractions and antioxidative plant tissue capacity is given as in another our work (Đurović et al., 2019), while the description of the quantification of certain sugars was given in an PhD thesis (Waisi, 2016). The quantification of the elements was performed using the AAS method (Waisi *et al*., 2015, with more detailed in Waisi *et al*., 2017b).

Results and discussions

In our announcement (Nikolić and Waisi, 2012), we examined the results from micro-trials, wich where set up in 2011 in two apple orchards located at northern part of Serbia. Plots were treated with combinations of half of the usual dose of mancozeb and tebuconazole fungicides as a controla, and also same treatments combined with brassinosteroid (BRs) based preparation and also with non-standard fertilizers based on amino acids and plant extracts. First, we evaluated the usual parameter of yield of fruits, and the apples were sampled for determination of content of reducing sugars in extracts of fruit pulp. Also we assesed efficacy of these procedures to plant protection od apple leaves and fruits from notorious phytopatogenic fungus *Venturia inaequalis* (Stevanović *et al*., 2012). In Obrenovac trial evaluated yield/ ha of BRs treated apples is same as in control plots, with comparable pomological and fruit quality parameters of apple. In Šid trial evaluated yield/ ha of BRs treated apples for almost a quarter more then apple yield from control plots (treated by half and full doses of fungicides) and other treatments, also with comparable pomological and fruit quality parameters of apple fruits (data not shown). From plant protection view, our procedures is also satisfy with 78,71% and 77,69% protection efficacy of BRs+half fungicide doses treatment in leaves and fruits (against 84,17% and 87,90% efficacy from full fungicide doses treatment) at Obrenovac, which is satisfactory result. In *Šid locality* we got similar results, whith also satisfactory results (not shown). Our results are very similar to findings by other researchers (Khripach *et al*., 2000).

Also, we examined influence of non-standard fertilizers on yield and yield components in soybean and barley. During 2012 sezone 3 soybean genotypes (ZP-015, "Nena", and "Laura" with low content of Kunitz-trypsin inhibitor protein) was treated with a non-standard fertilizers, as type of biofortification. By this approach we found that it is in lesser extent affected by alterations in P_{phy} (content of phytic phosphorus), as important factor which restrain availability of mineral nutrients. Only at the Zn level, this dependence is significant, where lowering in P_{phy} increase parallel Zn concentration in grains. Moreover, the influence of β-carotene is significant for availability of mineral nutrients, but more important is that its increase is linked with parallel Fe increase, mainly in grains with higher weight, as part of better yielding potential. It is significant to underline that ratio between P_{phy}, β-carotene and mineral nutrients could be altered in some degree by application of foliar fertilizers with potentially higher availability of minerals, but it also depends on soybean variety. 24-epibrassinolide (24-EBL) based preparation and the plant extract ("Zircon") were efficient for decrease of mentioned ratio for ZP-015 and "Nena" grains, as well as some plant extracts ("Zlatno inje" and "Zircon") were efficient for "Laura". Also, in soybean is very significant correlation between 1,000 grain weight (as important yield component) and grain content of β-carotene and Zn (Dragičević *et al*., 2016b). At a beginning of seasons 2013 and 2014 we sown hull-less barley (*Hordeum vulgare* L. var. *nudum*; cv. "Apolon"), after that in the spring of the years, we treated the crop with BRs based preparation, and with other non-standard fertilizers (based mainly on plant extracts and other bioregulators). After harvesting in the summer we assessed yield (at 14% grain moisture content; kg ha^{-1}) and determined by standard methods different chemical ingredients from barley grains. Obtained results (Dragičević *et al*., 2016a) indicate that year affects barley grain yield and its chemical composition, with the highest impact obtained for Si under harsh climate conditions. Applied treatments was the most effective for grain yield and increase of grain quality mainly across reduction of P_{phy}/β-carotene ratio and increase of GSH content, thus increasing potential bioavailability of the mineral elements. What is more, stress present in high precipitation amount (in 2014) could be mitigated by application of *an* fertilizers by increasing potential bioavailability of P, Mg, Ca and Fe. Generally, BRs preparation influenced content of P_i, Zn and Fe, and other fertilizers mainly affected potential availability of some other mineral elements (Ca, Mn, Si and GSH).

From previous field trials carried out on one fruit and two field crops we indicated that *beetwen* other non-standard fertilizers, preparation based on BRs affects not so much yield, as to yield quality or chemical composition (Nikolić and Waisi, 2012; Dragičević *et al*., 2016a, 2016b) and protection to the crops in stress conditions (Stevanović *et al*., 2012).

In our paper (Đurović *et al*. 2019) the influence of different non-standard fertilizers on the content of polyphenolic acids and proteins in soybean seed was monitored. It is also followed the antioxidative capacity of soybean seed (as important soybean nutritional feature) by various methods: Total phenol content (TPC; not shown), free radical scavenging activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radikal assay, ferric reducing ability of plasma (FRAP) assay as a reducing power of soybean extract (Figure 1), and the Briggs-Rauscher (BR) reaction method (Figure 2). All examined plant-extract-based products expressed significant changes in the total phenolic contents and antioxidant activities of the soybean flour extracts. An exception was the treatment with "Cropmax", which only caused a decrease in TPC and antioxidant activities determined by the DPPH and FRAP methods. All other treatments showed a positive influence on the TPC, DPPH and FRAP methods, which is in accordance with several studies (Danilčenko *et al*., 2017; Verkleij, 2012) that showed a positive influence of biofertilizers (based on plant extracts) on the yield, growth and antioxidant activities of different plant species. The results of the post-hoc Tukey test showed that all treatments significantly effected a change in TPC as compared to the control sample. A statistically significant difference in antioxidant activity was not found between the samples treated with "Ekofus" and "Vegard", while all treatments in relation to the control sample exhibited statistically significant differences in antioxidant activity as measured by both DPPH and the FRAP methods. Inhibitory effects after the addition of aqueous extracts of soy flour to active Briggs-Rauscher (BR) mixture were reported (Cervellati *et al*., 2000). However, to the best of our knowledge, this is the first time that soybean treated with products based on plant extracts during vegetation were analyzed in the oscillatory Briggs-Rauscher reaction. It is well-known that the BR reaction method provides a "larger antioxidant picture" and can also show a synergistic effect (Milos and Makota, 2012).

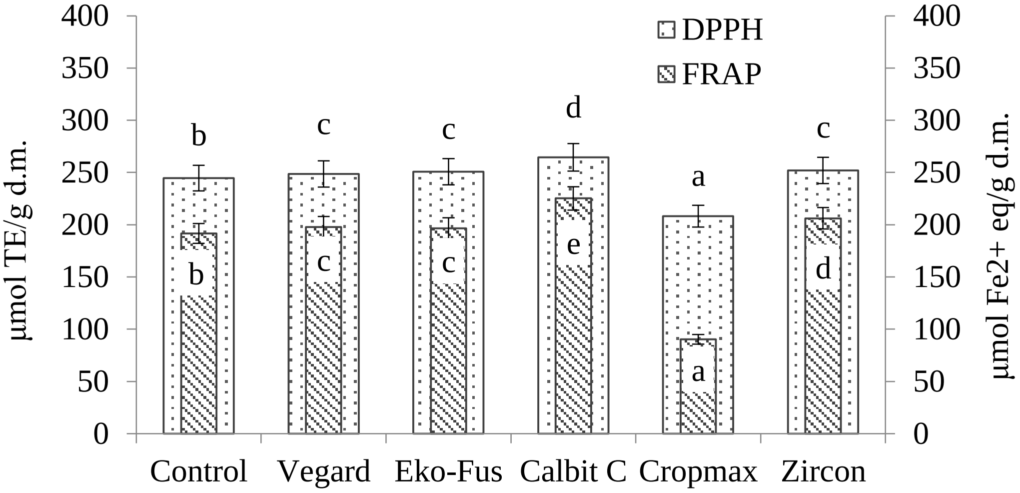

Figure 1. Antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) assay in soybean seeds treated with plant extract products. The values followed by the same letters are not significantly different at the 0.05 level

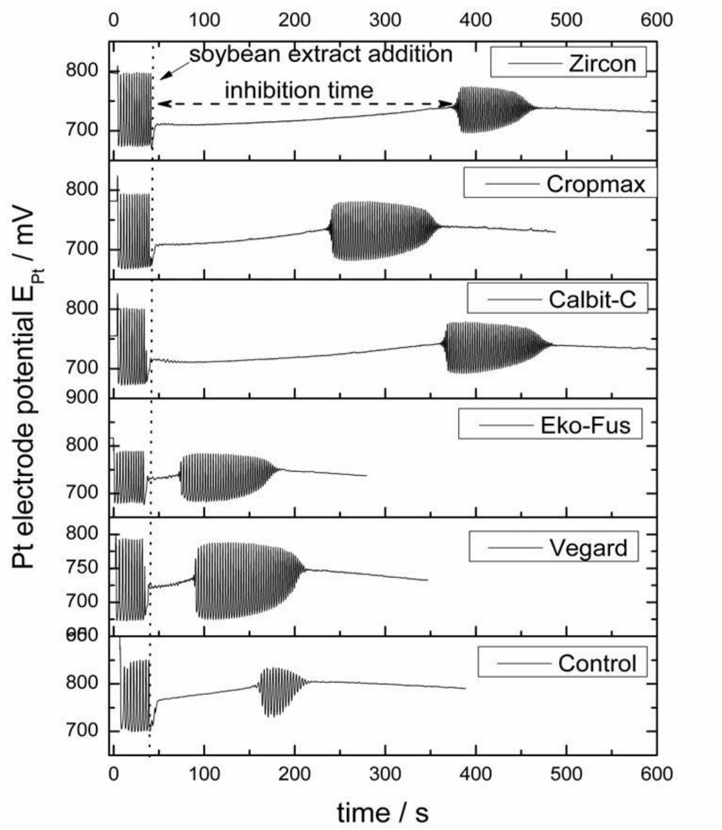

Figure 2. The Briggs-Rauscher oscillograms obtained with particular soybean extract addition (100 µl) after 30 s from oscillatory reaction beginning. The initial concentrations of reactants for BR reaction were $[CH_2(COOH)_2]_0$ = 0.0789 mol/dm^3, $[MnSO_4]_0$ = 0.00752 mol/dm^3, $[HClO_4]_0$ = 0.0300 mol/dm^3, $[KIO_3]_0$ = 0.0752 mol/dm^3 and $[H_2O_2]_0$ = 1.2690 mol/dm^3.

Therefore, the results obtained by the BR reaction method demonstrated the synergistic effect (of phenolic compounds and proteins) in soybean treated with "Cropmax", as well as a more favorable outcome of soybean treatment with "Calbit-C" and "Zircon". This indicated that not only phenolic compounds participated in the inhibition of the oscillatory regime, but also other molecular species (such as proteins and some ions), which influenced the BR reaction, possibly by building and/or stabilizing macromolecular structures in plant cells.
Also we tested (Waisi *et al*., 2017) a brassinosteroid (BRs) phytohormone based fertilizer and their influence on plant growth and microelements accumulation in seedlings of two maize genotypes. It was found that BRs influencing both germination and growth of maize hybrids ZP 704 and ZP 434 at their lower concentrations. Hybrids divergently reacted to exogenous treatment by brassinosteroids. Lower BRs concentrations stimulated seed germination and growth of seedlings, but high concentrations inhibited these processes. Considering that germination percentage, whole plant mass and their *innitial* height as a vigour parameters of plants, seedlings treated with various concentrations of brassinosteroids will probablly have better chance for growth and field establishment. It was found that BRs is affecting redistribution of elements in young plants. Elements could be linked with initial growth and it was assumed that in the case of hybrid ZP 704, poorer emergence of shoots is influenced with lower Zn concentration, since it is known that the Zn and plant growth are correlated. In case of the lowest concentration of brassinosteroid (BRs) phytohormone is obvious blocking distribution of Cu, and these could mean that maize plants could achieve optimum of growth in polluted soils. Low values of ratio of photosynthetic pigments for both hybrids are confirming photosynthetic inactivity. Results are implicating from our findings that maize treated with BRs could be grown at soil polluted with heavy metals due to its ability to remove or block accumulation of toxic elements, especially in shoot.

Conclusions

Overall, the early treatment of cultivated plants with non-standard fertilizers greatly affects the vigor of seedlings of these plants, which is of great importance for crop yield. These fertilizers also significantly affect the quality of crop yield in terms of improved chemical composition of edible parts of these plants.

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COMPARISON OF THE BIOACTIVITY OF PROPOLIS EXTRACTED BY OLIVE OIL PRODUCED BY DIFFERENT PRODUCTION PROCESSES

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Abstract

Virgin olive oil is obtained from the fruits of the olive tree (*Olea europea*) by mechanical or other physical means, under conditions that do not cause any changes to the oil. Important factors in the production of good quality olive oil are the harvesting period, maturity of the fruit, the mode of harvesting (hand picking, nets, other means), storage of olives before processing, leaf removal, mode of crushing and kneading, and the system of extraction. Some oils, such as virgin olive oil, are used without further treatment, but most are refined in some measure before use. The refining processes remove undesirable materials, but may also remove valuable minor components which are antioxidants and vitamins such as carotenes and tocopherols. In this study, propolis was extracted with virgin, refined and riviera olive oils in different condensations. Then, the total phenolic, antioxidant and antiradical activities of these extracts were measured. Total phenolic content of the extracts ranged from 462.03-1780.49 mgGAE/100g, antioxidant activity 9.27-27.39 mgAAE/g and antiradical activity 57.52-83.32%. According to the results, the highest activity was observed in virgin olive oil extract.

Key words: Propolis, olive oil extract, total phenolic content, antioxidant activity

Introduction

Propolis is a bee product with high resin content which is used as a building material and provides hygiene in the hive by adding wax and bee secretions to the resin collected from the poplar tree buds. Although the chemical composition of propolis is highly dependent on its geographical and plant origins, crude propolis is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%) and other organic compounds (5%). Phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes and alcohols are important organic compounds found in propolis (Gomez-Caravaca et al. 2006).

Since propolis is not used in the food and pharmaceutical sector in its raw form, it is usually extracted with ethyl alcohol, propylene glycol and glycerol. However, due to the restriction of the use of alcohol for religious reasons, alcohol intolerance and children, researchers have sought to find new solvents. One of these solvents is olive oil. In olive oil, more than 200 different chemical compounds have been identified, including sterols, carotenoids, triterpenic alcohols and phenolic compounds. Phenolic compounds are also the main antioxidants found in extra virgin olive oil containing both hydrophilic and lipophilic phenols. The main phenolic subclasses found in olive oil are phenolic alcohols, phenolic acids, flavonoids and ligands (Bendini et al. 2007; Servili et al. 2009). To date, many reports have shown that phenolics found in extra virgin olive oil show strong antioxidant properties and can prevent oxidative stress in brain tissue (Beauchamp et al., 2005; Visioli et al., 2002; Petroni et al. 1995). Some epidemiological studies have shown that the consumption of olive oil has an effect on diseases such as cardiovascular diseases, cancer and rheumatoid arthritis (Guasch-Ferre et al., 2014; Pelucchi et al. 2010; Berbert et al., 2005).

In this study, total phenolic content of propolis olive oil extracts prepared by using different production methods with olive oil (cold press, rivyera, refined) and different concentration of propolis samples were determined by Folin Ciocalteu method and antiradical activity by DPPH method and antioxidant content were determined by phosphomolibdenum test.

Material and methods

Propolis sample

Propolis samples were obtained in 2016 by hand collected from the beekeepers in the region where the poplar trees are common in Bünyan district of Kayseri. In the extraction of propolis with olive oil, cold pressed, refined and rivera olive oils were prepared in 10, 20, 30 and 40% concentrations.

Preparation of Propolis Extraction

10, 20, 30 and 40 grams of propolis were weighed to be 100 ml v/w with olive oil obtained by different production methods.

Determination of total phenolic content

Total phenolic content in the propolis was determined by Folin-Ciocalteu method and applied spectrophotometrically (Singleton and Rossi, 1965). The propolis samples were weighed to 1 g, prepared with 4 mL (diluted 4-fold in a 1: 4 ratio) methanol (Merck KGaA, Darmstadt, Germany) and thoroughly dissolved by vortexing. 1 (Sigma Aldrich, Darmstadt Germany). The concentration of the samples was taken as 200,000 ppm stock. Propolis samples were prepared in 5 replicates. From a 40 stokL stock propolis sample, 2400 µL distilled water, 200 µL undiluted Folin-Ciocalteu (Sigma Aldrich, Darmstadt Germany) reagent, 600 µL Sodium carbonate (20% Na2CO3 Merck Millipore KGaA, Darmstadt, Germany) solution and finally 760 distL distilled water was added to the experiment was established. For two controls, 40 yerineL of methanol was placed in place of the sample and incubated at room temperature and in the dark for 2 hours. The absorbance of the resulting mixtures was then read on the spectrometer (Varian Cary, United State) against corn at a wavelength of 765 nm, and the total phenolic content was given in mg gallic acid equivalent (GAE) / 100 g propolis.

Determination of Total Antioxidant Capacity

Antioxidant capacity of propolis samples were determined by phosphomolybdenum method (Prieto et al., 1999). One g of the propolis sample was weighed and vortexed by addition of 9 mL of methanol to allow complete dissolution. 1 (Sigma Aldrich, Darmstadt Germany). 2.1290 g of sodium phosphate (Merck Millipore KGaA, Darmstadt, Germany) was weighed in a beaker and dissolved in some amount of purified water, 0.9887 g of ammonium molybdate (Merck Millipore KGaA, Darmstadt, Germany) was weighed in a separate beaker and dissolved in a quantity of purified water, weighed in 11.7696 g of sulfuric acid . Sodium phosphate and ammonium molybdate were dissolved in a measuring tape with the aid of distilled water, and the weighed sulfuric acid was slowly added. It was then made up to 200 ml with distilled water and made ready for use.

400 µL of the sample liquid aliquot from the stock sample was mixed with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and placed in a stand in a 95 ° C water bath (Julabo®, Seelbach/Germany) for 90 minutes. The reaction was stopped by cooling in water and the absorbance of the samples was read spectrophotometrically at 695 nm wavelength.

Determination of Antiradical Activity

The free oxygen scavenging activities of propolis extracts were determined using DPPH (2,2 diphenyl-1-picrylhydrazyl) method with some modifications (Gyamfi et al., 1999). The propolis samples were weighed to 1 g, prepared with 4 mL (diluted 4-fold in a 1: 4 ratio) methanol (Merck KGaA, Darmstadt, Germany) and thoroughly dissolved by vortexing. 1 (Sigma Aldrich, Darmstadt Germany). The concentration of the samples was taken as 200,000 ppm stock. Propolis samples were prepared in 5 replicates. Weighed 0.0049 g of DPPH reagent (Sigma Aldrich, Darmstadt, Germany) in a plastic weighing vessel, dissolved with 20 mL of methanol, and finished again with 125 mL of methanol. The concentration of the samples was taken as 200,000 ppm stock. 100 µl of propolis samples and 3900 µl of DPPH reagent were mixed and the mixture was kept at room temperature in the dark for 2 hours and its absorbance was read spectrophotometrically at 517 nm wavelength.

% Values of antiradical activity; % inhibition = [(Abs control-Abs sample) / Abs control) * 100].

Statistical Analysis

The SPSS 11.00 for Windows software package was used for ststistical analyses. Data were given in the form of arithmetical mean values and standard deviations. One-way analysis of variance was performed, and variant groups were determined by means of the Duncan test.

Results and Discussion

The total phenolic content, antioxidant and antiradical activities of propolis extracts obtained with olive oil at different concentrations and different production properties are shown in Table 1. The total phenolic content of propolis extracts obtained in 10-40 % concentration using refined olive oil varied between 462-03-1650.98 mgGAE/100 g. Antioxidant activity was 9.27-22.04 mgAAE/g and antiradical activity ranged between 78.71-81.79 %. In the extracts made using cold pressed olive oil, total phenolic content was determined between 633.76-2064.74 mgGAE/100 g, antioxidant activity 12.74-27.39 mgAAE/g and antiradical activity 69.61- 82.21%. The total phenolic content, antioxidant and antiradical activity of the extracts using other solvent riviera olive oil were determined as 550-51-1780.49 mg GAE/100 g, 10.03-20.61 mgAAE/g and 57.52-81.19 % respectively.

Table.1. Bioactive properties of olive oil extract of propolis

Concentration	Total phenolic content (mg GAE/100 g)	Antioksidant activity (mg AAE/g)	Antiradical activity (% inhibition)
% 10 P + RAZY	$462.03 \pm 16.37^{a*}$	9.27 ± 0.18^{a}	78.71 ± 0.58^{d}
% 20 P + RAZY	888.76 ± 55.50^{e}	16.90 ± 0.19^{d}	80.18 ± 0.09^{e}
% 30 P + RAZY	1609.11 ± 6.96^{h}	17.98 ± 0.07^{e}	$82.00 \pm 0.99f^{g}$
% 40 P + RAZY	1650.98 ± 26.30^{h}	22.04 ± 0.32^{h}	$81.79 \pm 1.11f^{g}$
% 10 P + SSZ	633.76 ± 31.03^{c}	12.74 ± 0.22^{c}	69.61 ± 0.88^{b}

% 20 P + SSZ	1208.01±28.40^{f}	18.77±0.14^{f}	81.62±0.23f^{g}
% 30 P + SSZ	1621.93±26.10^{h}	23.75±0.38^{i}	81.85±0.14f^{g}
% 40 P + SSZ	2064.74±53.16^{j}	27.39±0.37^{j}	82.21±0.40^{g}
% 10 P + RIZY	550.51±13.80^{b}	10.03±0.24^{b}	57.52±0.33^{a}
% 20 P + RIZY	725.61±26.42^{d}	12.67±0.27^{c}	76.69±0.72^{c}
% 30 P + RIZY	1540.92±14.10^{g}	18.55±0.13^{f}	83.32±0.72^{h}
% 40 P +RIZY	1780.49±39.13^{i}	20.62±0.17^{g}	81.19±1.01^{f}

*: Different letters in the same column represent different statistical groups ($P < 0.05$). Values; mean ± SD. P: propolis SSZ: Extra virgin olive oil, RAZY: refined olive oil , RIZY: riviera olive oil

As a result, it was found that cold pressed extra virgin olive oil had the highest values in all concentrations in terms of total phenolic content and antioxidant and antiradical activity.
As a result of the regression analysis;
Total phenolic substance - Antioxidant activity R = 0.943 R^2 = 0.890,
Total phenolic agent - Antiradical Activity R = 0.655 R^2 = 0.428,
Antioxidant activity-Antiradical Activity R = 0.643 R^2 = 0.414 was determined to have a positive correlation between total phenolic content and anitoxidant and antiradical activity. Similarly, a positive correlation was found between antioxidant and antiradical activity.
Although extraction of propolis with alcohol and its derivatives is seen as a simple and effective method, it has a sharp resinous taste and limitations in its use in the cosmetic and pharmaceutical industries. In addition, ophthalmology is not considered appropriate for medical use as in patients with otorhinolaryngology, pediatrics, diabetes and cancer, and people with alcohol intolerance (Kubiliene et al., 2015). In water extraction of propolis, it is reported that phenolic compounds are 10 times lower than ethanolic extraction due to the low water solubility of the active components of propolis (Mello et al., 2010; Matta et al., 2004). For this reason, researchers have started to obtain high activity propolis extract by using non-chemical natural solvents other than ethyl alcohol, propylene glycol and glycerol. Therefore, the dissolution of propolis with vegetable oils and the biological activities of oil-soluble phenolic compounds are of interest. In this research, total phenolic, antioxidant and antiradical activities of propolis extracted with extra virgin olive oil were found to be higher than those obtained by other methods.
Olive oil obtained by chemical extraction can only be used for consumption after refining. A refining process is to purify the extracted oil from any residual solvent and other impurities. Refined olive oil; it lacks vitamins, polyphenols, phytosterol and natural ingredients (Kamm et al. 2001). Extra virgin olive oil is more expensive than other olive oils, but contains the highest level of polyphenols (kalogeropoulos ve Tsimidou, 2014). Due to the removal of free fatty acids, extra virgin olive oil has additional flavor, aroma and light color. Olive oil is mostly composed of triacylglycerols (98-99%). Triacylglycerols (TGA) are various groups of glycerol esters with different fatty acids. The predominant fatty acid found in TGAs with olive oil is monounsaturated oleic acid. The fatty acids that form the remainder of the olive oil TGAs are palmitic acid, linoleic acid, stearic acid and palmitoleic acid. Extra virgin olive oil contains abundant lipophilic or amphiphilic microcomponents, including phytosterols, squalene, tocopherols, phenolic compounds, terpenic acid derivatives (Baskou, 2009;

Luchetti, 2002; Ramirez-Tortosa et al., 1997). Phenolic compounds are formed as phenolic acids or alcohols, oleuropein derivatives, ligands and flavonoids. The content of polyphenols in olive oil ranges from 50 to 1000 mg / kg (Fragaki et al., 2005; Grossi et al., 2014; Luna et al., 2006).

It is a known fact that the total phenolic content of propolis varies depending on the plant source of the propolis, the season, the extraction method of propolis and the solvent used, but has a strong biological activity. Because, considering its use in nature, resin, which is the main substance of propolis, protects the plant bud, propolis created by honey bees by adding saliva and wax protects the honey bees.

Although there are many studies on the total phenolic, antioxidant and antiradical activity of ethanolic and water extract of propolis, there is little research about these activities of olive oil. Kubiliene et al. (2015) water, propylene glycol, alcohol and olive oil used as a solvent in the research propolis + water extract (W1) 5 hours at room temperature, propolis + PEG + water extract (W2) at 70 ° C, propolis + olive oil extract (A1) at room temperature The propolis + PEG + olive oil + water extract (A2) was extracted at 70 ° C and the propolis ethanol extract (EEP) was extracted at room temperature. As a result of this extraction method, the total phenolic content of the extracts were determined to be 1.6, 10.7, 0.5, 9.5 and 12.7, respectively. According to our research results, total phenolic content of propolisin olive oil extract was determined to be between 6.34-20.65 mg/ml GAE. In addition, as the concentration of propolis increased, total phenolic content increased. The differences in the results may be due to the extraction method and the nature of the solvents used. Because the olive oil used in our research was cold-pressed extra virgin olive oil, the duration of extraction with propolis was one week and the extraction temperature was 40 ° C.

Conclusion

In conclusion, it is necessary to select the appropriate extraction method in order to obtain high phenolic content of propolis in olive oil extract. In other words, in the extraction of propolis, besides the factors related to propolis, solvent, extraction time and temperature also gain importance in total phenolic content.

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NUTRITIONAL CONTENT OF BEE BREAD (PERGA) FROM NEW DISCOVERED HONEYBEE PRODUCTS

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Abstract

The basic nutrient needs of honeybee (*Apis mellifera* L.) are nectar, pollen, and water. While nectar is the source of carbohydrates for honeybees, pollen is a source of protein, lipid and vitamins. The pollen collected by forager honeybees is brought to the hive by putting the honey bee secretions and pressing the pollen basket. Then, with the help of other young bees in the hive, beebread is poured into the honeycomb cells and covered with a small amount of honey and bees wax to prevent spoilage. This mixture is subject to chemical modification by the action of different enzymes, microorganisms, moisture and temperature (35-36 C). This chemically modified pollen is called "bee bread". Bee bread is consumed by adult bees and larvae as fed. The chemical structure of production of bee bread in Turkey was identified in this study. Chemical analyzes were performed according to the methods recommended by AOAC. Accordingly, the ash, protein, carbohydrate, dietary fiber, fat and energy content of bee bread were determined as 8.14g / 100 g, 13.56 g / 100 g (Nx6.25), 30.60 g / 100 g, 18.18 g / 100 g, 21.69 g / 100 g and 408 kcal / 100 g, respectively. There is a need for research on perga, which is rich in nutrients and has bioactive properties.

Key words: *Bee bread, perga, chemical analysis, nutritional content*

Introduction

Living things need nutrients to grow, reproduce and obtain energy. The nutrients of honey bees are nectar and pollen. Honey bee larvae hatching feed on nutrients prepared by young worker bees. These nutrients contain the necessary nutrients for the development of larvae and are rich in protein. A larva needs 4-6 mg of nitrogen to grow. For the development of honey bee larva, amino acids such as cystine, aspartic acid, asparagine, leucine, phenylalanine, arginine must be present in their nutrients. While carbohydrates give energy, vitamins are effective in the development of food glands. Water is necessary for honey bees as well as all living organisms. The content of food in honey bees gains great importance in caste differentiation (Brodschneider and Crailsheim, 2010).

For adult honey bees, honey contains a high percentage of carbohydrates, while the protein content is very low. Pollen is essential for the growth of young worker bees, the development of tissue, muscle and food glands. However, without pollen, it is impossible for colonies to breed offspring. A worker bee needs 3.21 mg of nitrogen to become mature, which is met by 145 mg of pollen. In order to meet protein and amino acid needs, a normal colony collects pollen between 20-60 kg per year. (Brodschneider and Crailsheim, 2010).

Pollen is collected by worker bees older than 21 days who have completed their hive tasks. The season in which the colonies collect pollen most intensively during the year is the spring months and the period in which the offspring activity is highest. Pollen collection activity is provided by the baskets located behind the tibia of the honey bees. The honey bee takes pollen from the flower with its upper jaw and gets it on its tongue with its lower jaw. It is then moistened by vomiting honey and placed in the pollen basket on the hind legs with the help of middle legs. The pollen carried to the hive is discharged to the honeycomb cells with the help of other worker bees in the hive. It is covered with a small amount of honey and wax. Pollen, honey, saliva enzymes and temperature effect in the honeycomb start the chemical process

and the pollen is fermented. This pollen, whose chemical structure is differentiated, is called "bee bread" (Vásquez A, Olofsson, 2009; Anderson et al. 2014).
Bee bread contains about 20 % protein, 24-35 % carbohydrate, 3 % lipid, 3% vitamin and mineral. Bee bread (perga) has a alarga variety of minerals and high quantities of iron, cobalt, phosphorus, calcium, and it is one of the richest natural foods containing selenium. It is also contains all the essential amino acids that the human body cannot bisynthesize suc as phenylalanine, leucine, valine, isoleucine, arginine, histidine, lysine, methionine, threonine, and tryptophan, vitamins such as A, B1, B2, B3, B6, B12, C, PP, E, D, K, H, ensymes such as amylase pigments, phosphatase sucrose, flavonoids and caratenoids, Also it is a nourishing supplementt rich in phyophormones, flavonoids, amino acids, minerals, and other biological compounds with bioavailability at least three times more than pollen (Ceksteryte et al., 2012; Kaplan et al., 2016; Tomas et al., 2017).
In this research, the nutrient content (ash, protein, carbohydrate, dietary fiber, lipid, and energy) of bee bread was determined.

Material and Methods

Bee bread samples
Bee bread was obtained from Nutral Therapy Company in Kayseri, Turkey and was stored at -20 C°.

Chemical analysis
Chemical analysis of bee bread (ash, crude fat and crude protein) was carried out using standard AOAC methods 920.153, 991.36 and 960.52, respectively. Moisture content was measured using a vacuum oven model VO200 (Memmert GmbH+Co. KG, Schwabach, Germany) at 60 °C and weighing until a constant mass. The ash content was measured gravimetrically after incineration at 550 °C and weighing. The total protein content was calculated by multiplying the nitrogen content by nitrogen to protein conversion factor of 6.25. (AOAC, 2000). All analyses were made in triplicate and the results were expressed in g per 100 g of fresh bee bread.
Determination of oil content was carried out using the ISO 659:2009 standard method. The bee bread samples were homogenized using a stainless steel blender (Waring, Atlanta, GA, USA). A mass of 2 g of sample was weighed accurately into a glass beaker and mixed with 100 mL of 4 M HCl. Then the content was heated at 100 °C and stirred for 15 min. After cooling to room temperature the solution was washed three times with 25 mL of distilled water. The sample was filtered through a filter paper, which was dried at 105 °C in an oven for 1 h. The extraction of oil from bee bread samples was carried out with diethyl ether at 50 °C for 3 h by automated Soxhlet extractor (VELP Scientifica, Usmate (MB), Italy).
Statstical Analysis
The SPSS 11.00 for Windows software package was used for ststistical analyses. Data was expressed in mean values of three replicates and expressed as mean ± SD..

Results and Discussion

According to the results of our study, ash, protein, carbohydrate, dietary fiber and fat content of dried bee bread were 8.14, 13.56, 30.60, 18.18, 21.69 g / 100g, respectively. Barene et al. (2015) study, the nutrient content of bee bread; Proteins were found to be 20.30–21.70% Fats 0.67–1.58% carbohydrates 24.40–34.80%. Compared to this research, the reason for the difference in protein and fat values may be due to the use of dried bee bread in our study. Zuluaga et al. (2015) determined that ash content of bee bread obtained from Colombia was 2.19-2.60, lipid content was 1.65-5.50% and protein content was between 19.1-27.3%. In another study, Tomas et al. (2017) determined the amount of ash, protein, carbohydrate, fat

and energy in 17 bee samples collected from Portugal as 2.1-7.7, 14.1-20.9, 63-78, 3.6-16.8% and 397-468 kcal, respectively. In another study from Turkey by Kaplan et al. (2016) the moisture fractions of the samples were between 11.4 and 15.9%, the mass fractions of ash were 1.9 to 2.5%, the fat from 5.9 to 11.5% and protein from 14.8 to 24.3%.
The nutrient content of bee bread samples obtained from different geographic regions is different. Possible reasons for this are; different flora, processing processes, for example, fresh /dried.

Table 1. Nutrient content of bee bread

Nutrients	**Content**
Ash	8.14±0.2 g/100g
Protein	13.56±0.6 g/100g (Nx6.25)
Carbohydrate	30.60 ±1.2 g/100g
Dietary fiber	18.18 ± 0.4 g/100g
Fat	21.69±0.8 g/100 g
Energy	408 ± 1.6 kcal/100 g

An increasing number of researcher appreciate the therapeutic effect not only of honey, but also of other products such as bee polen, bee bread, royal jelly, apilarnil, and bee venom with wide application in apitherapy. Bee bread (perga) is a unique product, which is very important not only for humans, but also for the bees. It is not always easy to get it, and the price is several times higher than the price of honey and bee pollen.
Honeybees pack the components in the cells of the honeycomb, then cover the mixture with bee wax and honey (Barene et al., 2015). Then covered and preserved pollen is subject to lactic fermentation in the environment of beehive. Fermented bee pollen is called the"bee bread"(Fuenmayor et al., 2014; DeGrandi et al. 2013). It is known that, bee bread is characterized by a higher nutritional value than pollen,better digestibility, and richer chemical composition (Habryka,Kruczek, and Drygas, 2016). Moreover, it is better absorbed by the human body than pollen since the nutritional components of bee bread are partially fermented and are more easily assimilated in human organism (Barene et al.,2015). Bee bread contains considerably larger amounts free amino acids. The presence of all the essential amino acids, perga is characterized by better composition than many valuable products obtained based on animal proteins. It contains about 30% protein on average (Degrandi-Hoffman et al.,2016). Roulston and Cane (2000) revealed that, the content of starch in pollen is in the range of 0–22%. Carbohydrates constitute between 24 and 34% (Barene et al.,2015). Perga is more biologically active and easily digestible due tothe high content of easily digestible sugars, fat, vitamin-mineral components, and a higher proportion of free amino acids when compared to pollen (Nagai et al., 2004; Trzybiński, 2005). Bakour et al. (2019) demonstrated that carbohydrates (74.82 ± 0.04 g/100 g bee bread) followed by proteins (19.96 ± 0.08 g/100 g bee bread) were the main macronutrients in bee bread. They were found for ash (3.32 ± 0.08 g/100 g bee bread) and fat (1.90 ± 0.086 g/100 g bee bread), revealing an overall energetic contribution of 396.2 ± 0.4 kcal/100 g bee bread).
Zuluaga et al. (2015) described that the lipid content ranged from 1.65% to 5.50% of bee bread. Andelkovic et al (2012) in Serbian bee bread samples, reported that the lipid content ranged between 4.51% and 4.92%. Moreover, the protein content in bee bread samples from Colombian and Serbia ranged from 27.6% to 29.9%, respectively (Andelkovic et al., 2012; Zuluaga et al., 2015). The differences found between the samples could be due to the floral origin of the bee bread (Urcan et al., 2018).

Conclusion

People are paying more attention to their health in a developing and changing world. Nutritional activities are no longer just a necessity, but have become an activity aimed at preventing or treating diseases. People now understand the importance of natural life and natural nutrition rather than chemicals. Bee products are one of the most important of these natural food groups. Bee bread, which contains protein, vitamins, minerals and carbohydrates, is one of the important nutrients with high nutritional value.

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PRESENCE OF DEOXYNIVALENOL IN BREAD IN SERBIA DURING 2018-2019

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Abstract

Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect wheat, corn, rice, oats, barley and other grains in the field or during storage. DON affects animal and human health causing vomiting, acute temporary nausea, diarrhea, abdominal pain, headache, dizziness and fever. Wheat flour and wheat flour-based products, such is bread take an essential place in Serbian diet which raises exposure level of total population in cases of higher contamination. The objective of this study was to evaluate the presence of DON in wheat flour bread. In this study, a total of 210 samples of wheat flour bread were collected in the period of 2018-2019. All samples were analyzed for DON by enzyme-linked immunosorbent assay. DON was detected in 47 out of 210 wheat flour bread samples (22.38%), at levels ranging from 81 to 214 μg/kg. The maximum contamination level of DON (214 μg/kg) in this study was found in wholemeal bread. These results suggest not very high percentage of contaminated samples. However, the level of contamination was higher in wholemeal bread than in white bread, which raises a risk for consumers of bread made of whole wheat flour.

Key words: Deoxynivalenol, bread, ELISA.

Introduction

Wheat bread is a staple food prepared by baking a dough of flour and water usually leavened with yeast, which is widely consumed around the world (Dewettinck *et al.*, 2008).

Unfortunately, wheat like many other cereals is susceptible to fungal attack and possible mycotoxin contamination, which affects wheat-based products, as well. The presence of mycotoxins is often associated with chronic or acute mycotoxicoses, therefore their occurrence in cereals is of great concern worldwide . Approximately 25% of cereals produced in the world are contaminated with mycotoxins (Charmley *et al.*, 1995).

A great variety of fungi can produce mycotoxins, however several *Fusarium* species are predominant pathogens on cereals in both temperate and semitropical areas and present major concern for all European cereal growing areas (Bottalico, 1998). The percentage of contamination on the worldwide level for some *Fusarium* toxins, such is DON, is considered to be much higher than 25% (Bullermann, 1996).

Deoxinivalenol (DON, vomitoxin) is a natural-occuring mycotoxin, type B-trichothecenes produced mainly by strains of *F. graminearum*, a food-borne fungi widely spread in crops. DON is considered to be one of the most important mycotoxins in wheat and wheat based products. It affects both animal and human health by causing gastro-intestinal problems followed by diarrhea and vomiting (Kushiro, 2008).

Although, occurrence and prevention of DON has been intensively studied, there is a small number of studies conducted in Serbia on retention of DON after harvest and during processing. It is important to know survival rate of mycotoxins during processing in order to evaluate the risk that Fusarium mycotoxins might pose to the consumer. To achieve this it is necessary to study both the occurrence of the mycotoxins in primary agricultural crops such as wheat and the effect of processing and food manufacturing on their concentrations in the retail products (Hazel and Patel, 2004). While considerable data are available to show the frequency and levels of Fusarium mycotoxins, particularly DON, occurring in wheat there is much less information on their transfer to wheat-based products such as bread, cakes, pastries and biscuits at the retail point (Scudamore *et al.,* 2009).

Wheat flour and wheat flour-based products, such are bread, pasta, pastry and cookies represent approximately 26% of Serbian market basket and hold and essential place in Serbian diet (Škrbić *et al.,* 2012).

The objective of this study was examination and determination of the presence of DON in wheat flour bread collected from Serbian producers in order to determine the levels of contamination in different types of wheat flour bread.

Materials and Methods

Reagents and chemicals

RIDASCREEN FAST DON SC (R-Biopharm), a competitive enzyme immunoassay for quantitative analysis of DON in cereals, malt and feed was used according to manufacturer's instruction (RIDASCREEN FAST DON SC Art.No.:R5905). Distilled water was used for the extraction.

Collection of samples

From September 2018 until April 2019, 210 samples of white bread and wholemeal bread were collected from 50 Serbian producers, as a part of the food safety control. Of total number of samples 150 were white bread, and 60 were wholemeal bread. Before analysis, the samples were stored at 4-6 °C and protected from light.

Sample preparation

Collected samples were prepared and analyzed in accredited laboratory for testing food and feed safety Jugoinspekt Beograd. All samples were thoroughly homogenized. Namely, 5 g of each sample of white bread and wholemeal bread was extracted by shaking with 100 ml of distilled water manually for 5 minutes. After shaking sample extracts were filtered through Whatman No.1 filter. 50 μL of the filtrate was used for further analysis according to RIDASCREEN FAST DON SC manual.

Instrumental conditions

The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the DON concentration in the sample. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s. Using method was validated (LoD = 75 μg/kg, Recovery = 92%).

Statistical analysis

All obtained data were analyzed using SPSS 15.0 software (SPSS, IBM corporation, USA).

Results and Discussion

The results on occurrence of deoxynivalenol in white wheat flour bread, whole wheat flour bread are given in Table 1.

DON was detected in 18 out of 150 white bread samples (12.00%), at levels ranging from 78 to 176 μg/kg. The average and median values obtained for DON in white bread were 101 and 85 μg/kg, respectively. Of 60 samples of whole meal bread 29 was contaminated by DON (48.33%), at levels ranging from 118 to 214 μg/kg. The average and median values obtained for DON in wholemeal bread were 113 and 94 μg/kg, respectively. The maximum contamination level of DON (214 μg/kg) in this study was found in wholemeal bread. None of the white bread nor wholemeal bread samples exceeded the limit of 500 μg/kg set by Serbian regulative for allowed presence of DON in wheat flour bread („Sl. glasnik RS", br. 22/2018 - Official Gazette of the RS no. 22/2018). The obtained results are in compliance with the level of contamination of bread showed in a study report done by EFSA. (Scientific report of EFSA, 2013). Higher levels of DON found in wholemeal bread are due to the fact that the distribution of DON is not uniform in the milling fractions (Abbas *et al.*, 1985). Abbas *et al.* (1985) found that the highest concentration was in bran, followed by reduction flour and break flour, which proves that the invasion of fungus into the wheat is not uniform, as well. Trigo-Stockli *et al.* (1996) in the similar study, reported that DON levels were highest in the bran (3.4 mg/kg) and lowest in the flour (1.5 mg/kg), as well. This could be due to the fact that after milling most of the concentration remains in outer layers (Tanaka *et al.*, 1986).

Table 1. Occurrence of deoxynivalenol (DON) in white bread and wholemeal bread

Commodity	DON (µg/kg)				
	No. of positives/total	Average value [a]	Median value	max value	Interval of concentration (contaminated samples)
white bread	18/150	124	122	176	81 - 176
wholemeal bread	29/60	169	189	214	88 - 214

a) Arithmetic mean. Values below the detection limit (75 µg/kg) are set to have concentration of half of detection limit

Conclusion

The presence of DON was detected in 47 out of 210 analyzed samples of white bread and wholemeal bread. Higher percent of contaminated samples was detected among wholemeal bread samples (48.33%) than in white bread samples (12.00%). The maximum contamination level of DON (214 µg/kg) in this study was found in wholemeal bread. All of the wheat flour bread samples are in compliance with Serbian regulative („Sl. glasnik RS", br. 22/2018 - Official Gazette of the RS no. 22/2018). These results suggest a high percentage of contaminated samples among wholemeal bread samples, which raises a risk for consumers. These data are also important for the realization of a 'Total Diet study' (TDS). The TDS can be a complementary tool to estimate the population dietary exposure to DON across the entire diet by analyzing main foods prepared 'as consumed'. A provisional tolerable daily intake (TDI) for DON was set in 2002 by the Scientific Committee for Food (SCF) at 1 µg/kg body weight (b.w.) per day.

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Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja („Sl. glasnik RS", br. 22/2018) (Sr) Rule book on the maximum allowable amount of residues of plant protection products in food and feedstuff and on food and feedstuff for which shall be determined the maximum allowable amount of residues of plant protection products (Official Gazette of the RS No. 22/18) (En)

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FUNGAL BIOMASS AND MYCOTOXIN IN KERNELS OF *AVENA* L.

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Abstract

The fungal biomass (DNA) of *Fusarium culmorum* Sacc. (FC) and content of mycotoxin deoxynivalenol (DON) were determined in *Avena* spp. kernels. Kernels were harvested from panicles *A. strigosa, A. ludoviciana, A. byzantina, A. fatua, A. sativa* and *A. nuda* after artificial inoculation by fungi. The plants panicles were infected with FC by spray + polyethylene (PE) bag cover 48 h. method during flowering. Quantification of pathogen DNA in samples was carried out by Real-Time PCR method using ABI PRISM® 7000 machine in MicroAmp optical 96-well plates. A commercial competitive enzyme-linked immunosorbent assay (ELISA kit) was used to determine the DON concentration in samples. The lowest content of fungal DNA was found in kernels from *A. byzantina* (0.043 ng kg^{-1}) and the highest content from tested genotypes was found in *A. sativa* (1.725 ng kg^{-1}). A high positive correlation by Pearson ($r = 0.857$; $P = 0.000$) was found between fungal grain infection (FI = % DNA in kernels) and the content of DON. The cultivar PS-201 was the most affected (FI = 4.288%; DON = 37.2 mg kg^{-1}) genotype. In contrast, the *A. byzantina* and one breeding line from *A. nuda* had the lowest FI and accumulated low DON content. The amount of fungal DNA in grain was used as an indicator of the infection degree and the content of DON in kernels showed the level of oat resistance to DON accumulation.

Keywords: *Avena spp., Fusarium culmorum, deoxynivalenol, fungal DNA*

Introduction

Fusarium head blight (FHB) is disease of crop caused by different *Fusarium* species. Infections of spikes lead to yield reductions and contamination of kernels by fusarium mycotoxins. The consumption of *Fusarium*-contaminated cereals and cereal-derived products may cause the poisoning known as mycotoxicosis. Several studies have confirmed that the mycotoxins, present in animal feed, have toxic effects on animals and similar effects can be expected in humans, e.g. cytotoxic effects were detected in human liver cells (Königs *et al.*, 2008).

In the cultivation of oats, the fungus can be attacked by fungi belonging to the genus *Fusarium* spp. and the subsequent accumulation of mycotoxins in the kernels produced by the fungi during infection. Several surveys on the natural occurence of mycotoxins in oat kernels grown in Europe show that oat kernels are most frequently contaminated with fusarium mycotoxins deoxynivalenol (DON), T-2 toxin and HT-2 toxin (Nathanail *et al*., 2015; Hietaniemi *et al*., 2016; Hofgaard *et al*., 2016; Martin *et al*., 2018). In Europe, they are most often responsible for producing DON species *F. culmorum* (FC) and *F. graminearum* (FG) but recently, FG has been spreading Northward in Europe displacing the closely related FC. This shift may be due to changing agricultural practices, climate change and increased maize cropping (Yli–Mattila *et al.* 2013). Increased prevalence of oat samples with above-the-limit DON levels was observed in the sites of the largest European growers of this crop, e.g. in Russia (Gavrilova *et al*., 2016), Norway (Hofgaard *et al*., 2016), Sweden (Fredlund *et al*., 2013) and Finland (Hietaniemi *et al*., 2016).

Searching for resistant genotypes and evaluation of their resistance to fungal pathogens is one of the main goals of plant breeding worldwide as many fungal pathogens produce mycotoxins harmful for people and animals. Evaluation of resistance can be done by modern methods of molecular biology such as real-time PCR for quantification of fungal pathogen DNA in combination with quantification of mycotoxins by ELISA method after artificial inoculation of tested genotypes as genotypes differ also in resistance to mycotoxin accumulation. Quantification of *F. culmorum* DNA using highly specific and sensitive real-time PCR in comparison with quantification of DON using ELISA was successfully done in wheat and barley and significant correlation was found (e.g. Leišová *et al.*, 2006; Hudcovicová *et al.*, 2012). However, this relationship can be influenced by environmental conditions.
The objective was found out infection degree and resistance to DON accumulation of different oat genotypes after artificial inoculation with fungi *Fusarium culmorum* by DNA content and amount of DON.

Material and Methods

Plant material: *Avena strigosa, A. ludoviciana, A. byzantina, A. fatua, A. sativa* (cultivars and breeding lines: Vaclav, Vojtech, Vit, Norik, Dalyup, PS-201) and *Avena nuda* L. (cultivars and breeding lines: Dunajec, Inovec, Važec, Hronec, SV-5, PS-223, PS-215, PS-218, PS-219).
Inoculum production: The fungal colonies of *Fusarium culmorum* was grown on potato dextrose agar (PDA) plates at 25 ºC for twenty-one days in dark. Approximately 1ml conidial suspension of the inoculum (5×10^6 per mL) was applied to each oat panicle.
Greenhouse experiment: The experiment was established with 30 seeds from each genotype of *Avena* spp. These seeds were sown in 3 pots per treatment (5 seeds in one pot). The panicles of *Avena* spp. were sprayed with FC inoculum in three replicates and then were covered with plastic bags for 48 h. In the mature stage, panicles were collected from each *Avena* spp. and kernels were manually separated.
Deoxynivalenol content: A commercial competitive enzyme-linked immunosorbent assay (ELISA kit) was used to determine the DON concentration in oat samples (Ridascreen Fast DON; RBiopharm, Darmstadt, Germany) with limit of detection < 0.2 mg kg^{-1} (mg kg^{-1}) and limit of quantification 0.2 mg kg^{-1} (mg kg^{-1}). The absorbencies (of the wells) were determined photometrically at 450 nm (MRX II. Dynex Technologies. Chantilly. Virginia. USA).
Quantification of pathogen DNA in oat seeds was carried out by real-time PCR method using ABI PRISM® 7000 machine (Applied Biosystems, Foster City, USA) in MicroAmp optical 96-well plates (Applied Biosystems). We used TaqMan TM probe FC92s1 5´FAM 3´MGB and primer pair Fc92s1 specific for *F. culmorum* according to Leišová *et al.* (2006). Reactions were carried out in 25 µl reaction volume consisting of 12.5 µl TaqMan Universal PCR Master Mix (Applied Biosystems), 300 nM each primers, 200 nM TaqMan MGB probe (labelled with FAM fluorescent dye) and 25 ng of DNA in 1 µl. Conditions of PCR were following: 95 ^{o}C for 10 min., 40 cycles: 95 ^{o}C for 15 s, 60 °C for 1 min. As standards for standard curves we used five dilutions of pure *F. culmorum* DNA (1, 10, 100, 1000 and 10 000 pg μl^{-1}) in triplicate in every run. For evaluations of results we used ABI PRISM® 7000 software (Applied Biosystems), in which unknown samples are quantified from measured Ct values by interpolation using the regression equation derived from standard curves.

Results and Discussion

The mean DON level among the selected genotypes ranged from 2.32 to 37.20 mg kg^{-1} (Figure 1). The genotype *A. ludoviciana* showed the highest and *A. byzantina* the lowest mycotoxin levels of all tested genotypes. Our results correspond with the results published by Gagkaeva *et al.* (2017) from an experiment in which sixty-six accessions belonging to *Avena*

spp. were infected by isolates of FC and where *A. byzantina* accumulated the low amount of DON. Among the cultural species *Avena*, *A. nuda* had 54.33 % lower mean DON content (10.66 mg kg^{-1}) than *A. sativa* (23.34 mg kg^{-1}).
Figure 2 shows that *A. byzantina* has the lowest FI (0.04320 %) of all genotypes. The highest mean FI (1.8426 %) was found out in kernels of *A. sativa*. The fungal grain infection of *A. byzantina*, *A. strigosa* and *A. nuda* ranged from 0.04 % to 0.39 % and it was 5.5 times less than FI of *A. fatua*, *A. ludoviciana* and *A. sativa* (mean FI ranged from 1.23051 % to 1.84266 %).

Figure 1. Deoxynivalenol content (mg kg^{-1}) in *Avena* spp. after artificial inoculation with *Fusarium culmorum* Sacc.

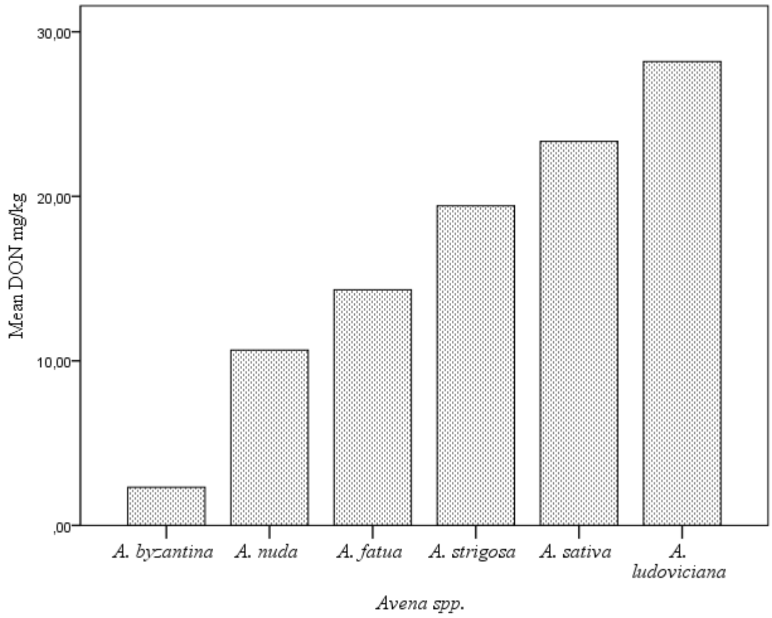
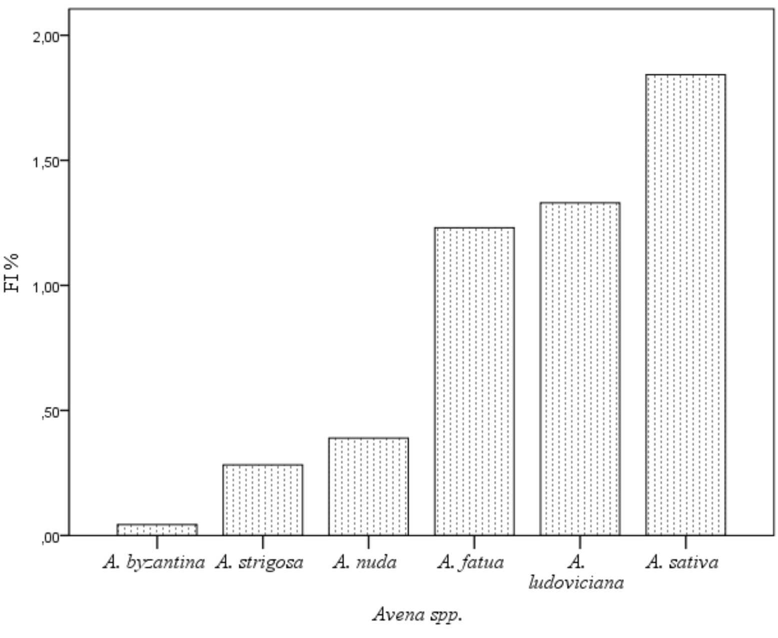


Figure 2. Fungal grain infection (FI = % DNA in kernels) in *Avena* spp. after artificial inoculation with *Fusarium culmorum* Sacc.



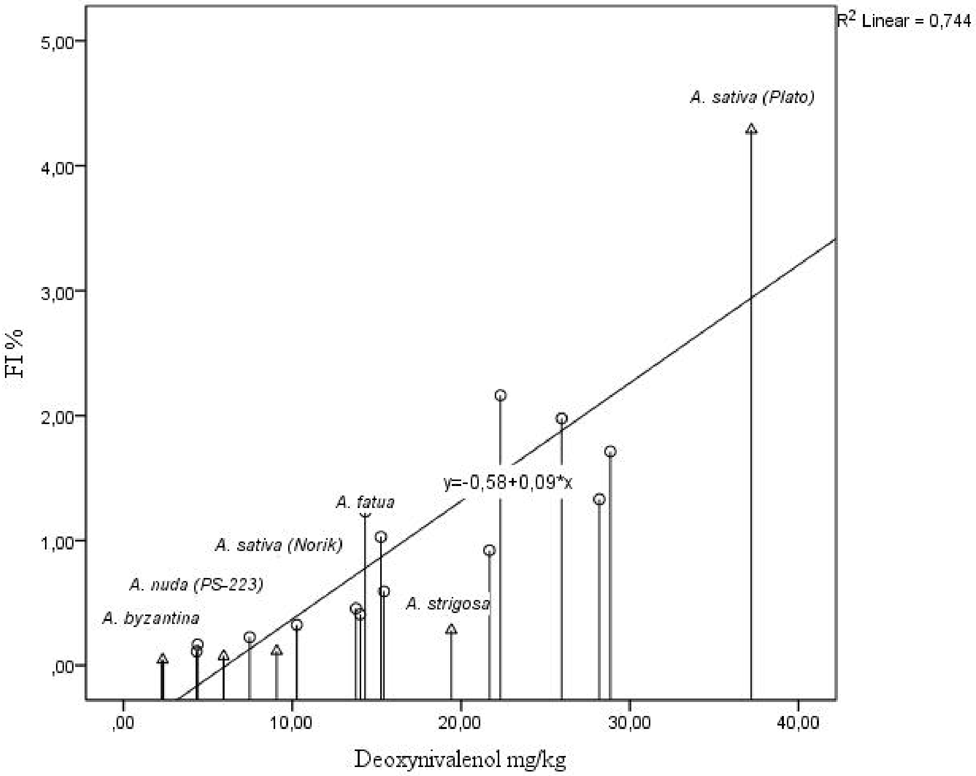
The results in Table 1 show that DON content in kernels of *A. sativa* ranged from 10.27 mg kg^{-1} to 37.20 mg kg^{-1} (Table 1). The lowest DON content was found in kernels of cultivar Norik (Figure 3), and it had the lowest FI from *A. sativa* genotypes. This cultivar cumulated lower mycotoxin DON than some *A. nuda* tested genotypes where DON content ranged from 4.33 mg kg^{-1} to 21.68 mg kg^{-1}. This finding is significant information for breeders because it is known that more DON accumulates in the hulles oat kernels than in the naked oat kernels. It has been found that the most DON is accumulated in the hulls (Šliková *et al.*, 2010), which are analyzed together with the kernels in the hulless oat varieties. Of the genotypes of *A. nuda*, kernels of SV-5 contained the lowest DON.

Table 1. Means of fungal DNA, fungal infection (FI) and deoxynivalenol (DON) contents in kernels of genotypes *Avena* spp. after artificial infection fungi *Fusarium culmorum* Sacc.

Avena spp.	Genotype	*F. culmorum* DNA (ng $1g^{-1}$)	DNA infection percent (FI %)	Mean DON (mg kg^{-1})
A. strigosa	*A. strigosa*	0.408832	0.28244	19.42
A. ludoviciana	*A. ludoviciana*	0.631671	1.33264	28.19
A. byzantina	*A. byzantina*	0.042532	0.04320	2.32
A. fatua	*A. fatua*	1.958003	1.23052	14.32
A. sativa	Vaclav	0.513167	0.59312	15.43
A. sativa	Vojtech	1.260435	2.16124	22.33
A. sativa	Vit	1.585367	1.71132	28.84
A. sativa	PS-201	4.214207	4.28796	37.20
A. sativa	Norik	0.325975	0.32416	10.27
A. sativa	Dalyup	2.453461	1.97780	25.96
A. nuda	Dunajec	0.449139	0.40816	14.02
A. nuda	Inovec	0.641262	0.45596	13.77
A. nuda	Važec	0.884419	0.92012	21.68
A. nuda	Hronec	0.163563	0.22604	7.47
A. nuda	SV-5	0.060386	0.11084	4.33
A. nuda	PS-223	0.093741	0.07260	5.93
A. nuda	PS-215	0.079499	0.11344	9.07
A. nuda	PS-218	0.203138	0.16744	4.39
A. nuda	PS-219	1.469698	1.02920	15.25
Mean			0.91820	15.79
Control		0.006498	0.00560	

A high positive correlation by Pearson (r = 0.862; P = 0.000) was found between the fungal infection and the content of DON (Figure 3). The ideal genotypes with good resistance characteristics should have low average DON content and low FI. From the tested set *A. byzantina* and PS-223 had low FI and DON content. The results suggest that *A. strigosa* is more susceptible to DON accumulation in kernels than other tested genotypes. This genotype has relatively low FI and high DON. On the other hand, we found genotypes such as *A. fatua* with relatively lower DON content and higher FI than other tested genotypes (Figure 3).

Figure 3. The scatter plot from deoxynivalenol (mg kg^{-1}) content and fungal grain infection (FI = %) in *Avena* spp. kernels after artificial infection fungi *Fusarium culmorum* Sacc.



Hautsalo *et al.* (2018) described the current knowledge of oats resistance to Fusarium head blight (FHB) and several resistance related traits. Few studies or knowledge of oat resistance to *Fusarium* have been published so far. Currently, some resistance-related loci are known that allow the selection of oat genotypes with resistance to FHB (He *et al.*, 2013; Bjørnstad *et al*., 2017). Genes that directly affect DON resistance have not yet been described in *Avena* spp, but some wheat genes associated with DON resistance and DON response have been discovered (Walter *et al.*, 2008; Hofstad *et al.*, 2016). Testing genotypes against *Fusarium* showed that within *Avena* spp. it is possible to find genotypes that accumulate low DON after artificial infection. Very important knowledge in breeding oats for resistance to *F. graminearum* and low accumulation of DON was reached in Norway; there were identified lines of *A. sativa* with consistently low DON (and early heading) (Bjørnstad *et al*., 2017).

Conclusions

Kernels were analysed for DON and fugal biomass contents on a set of different genotypes of oats (*Avena* spp.). The lowest DON content and FI were found in kernels *A. byzantina* from the tested set of oat genotypes. There is cultivar Norik (*A. sativa*) and breeding line PS-223 (*A. nuda*) that had the lowest DON content and FI from cultural species, too. The results obtained by the quantification of *Fusarium* in kernels can be helpful for evaluating genotypes for *Fusarium* infection. The evaluation of genotypes for FHB resistance by the quantification of pathogen is more than just using the DON content.

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CONTROL OF APHIDIDAE IN CHRYSANTHEMUM UNDER GREENHOUSE PRODUCTION

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Abstract

Significant pests on ornamental plants in the greenhouses are aphids (Aphididae), such as *Myzus persicae* which cause decreased growth, shriveling of the leaves and transmit plant viruses. The trials were conducted in 2018 by standard OEPP methods at locality Budisava (Vojvodina) in chrysanthemum crop (cultivar Multiflora) in the greenhouse. The pesticide preparations based on dimethoate (400 g a.i./L, SL) were applied at a concentration of 0.1%, bifenthrin 0.05% (100 g a.i./L, EC) and chlorpyrifos + bifenthrin (400 + 20 g a.i./L, EC) at a concentration of 0.1%. Pesticides were foliar applied, using a backpack sprayer with water consumption of 500 L/ha when chrysanthemums were in the flowering stage. The efficiency of the insecticide was calculated by Henderson & Tilton and the significance of differences was determined by ANOVA for a confidence interval of 95%. Before the treatment, the average number of aphids ranged from 133.7 to 161.7. Two days after the application of the insecticides, the number of *M. persicae* was significantly lower in comparison to the control, and the efficacy was 92.8-95.9%, while seven days after the treatment efficacy was 94.5-96.4%. Fifteen days after the application, the efficacy was still high in the range of 93.2-95%. Aphididae populations have shown high sensitivity to these insecticides but this certainly does not exclude the necessity of including them in the monitoring of susceptibility to the most commonly used insecticides, since aphids are capable of developing resistance rapidly.

Key words: *Flowers, greenhouse, Aphididae, insecticides.*

Introduction

The production of ornamental plants represents a valuable potential in agricultural production, landscape architecture, and other similar services. Good climatic conditions, geothermal resources, and a skilled workforce are just some of the preconditions (Glavendekić, 2013). In the Republic of Serbia in 2015, the production of flowers occupied an area of 445 ha, and in 2017 on 1057 ha, but from 2017 the land from the gardens was included in the total used agricultural land (Statistical Yearbook, 2018). The increasing interest in decorative plants in the last two decades has resulted in increased production of these plants in our country as well. Besides increasing production and profitability, it has also led to its modernization, which has caused certain production problems (Balaž *et al*., 2013). First of all there were problems with pests and pathogens. Greenhouses besides creating optimal conditions for plant growth, also create an optimal environment for phytophagous insects, mites and other harmful species. Greenhouses provide immeasurable amounts of plant food as well as a lack of predators, parasitoids and other natural enemies. Harmful insects that have migrated through various openings to greenhouses have all the favorable biotic factors for the rapid growth and development of populations, and as a result, a large number of phytophagous insects gains status as an invasive species on plants grown in greenhouses. Pests occurring in greenhouse are well adapted to living conditions and their populations can only be controlled by integral measures of protection. Their number is constantly increasing by importing the reproductive and planting material of ornamental plants. Aphididae are harmful species that attack a large number of plants used as food but also occur on ornamental plants. They occur in the open field as well as in a protected area. *M. persicae* occurs on ornamental plants such as carnation, chrysanthemum, Christmas star and roses (Petaković and Glavendekić, 2013). It can occur in large populations on young shoots of plants, resulting in a stressful condition, the plant begins to vein and may lose growth. Aphids feed on the host's plant juices but the most harm they cause by transmitting the viruses. Peaches aphids are, as some experts believe, the largest vectors of the viruses in the world. The aim of this study was to determine the degree of chrysanthemum protection in greenhouse conditions against aphids (Aphididae), using preparations based on dimethoate, bifenthrin, and chlorpyrifos + bifenthrin.

Material and methods

The trials were conducted in 2018 by standard OEPP methods (Anonymus, 2004; Anonymus, 2012; Anonymus, 2014) at locality Budisava (Vojvodina Authonomus Province, Serbia) in chrysanthemum crop (cultivar Multiflora) in the greenhouse. The pesticide preparations based on dimethoate (400 g a.i./L, SL) were applied at a concentration of 0.1%, bifenthrin 0.05% (100 g a.i./L, EC) and chlorpyrifos + bifenthrin (400 + 20 g a.i./L, EC) at a concentration of 0.1%. Pesticides were foliary applied, using a backpack sprayer with water consumption of 500 L/ha when chrysanthemums were in the flowering stage.

Experiment in Budisava was set on 18.10.2018. The trials were set in four replicates in a randomized block design. Four assessments were made based on the number of aphids on 10 previously marked shoots per replication: 1) before the treatment, 2) two days after the treatment, 3) eight days after the treatment, 4) 15 days after the treatment.

The efficiency of the insecticides was calculated by Henderson & Tilton (Wentzel, 1963) and the significance of differences was determined by ANOVA for a confidence interval of 95%.

Results and discussion

The results of testing the efficacy of insecticides based on dimethoate, bifenthrin, and chlorpyrifos + bifenthrin in greenhouse production conditions for controlling *M. persicae* are shown in Tables 1-2. Based on the abundance estimate just before the trial was set up (Table 1), the average abundance of *M. persicae* by variants ranged from 133.7 to 161.7.

Table 1. Average number of green peach aphids (*M. persicae*) and insecticide efficacy (Budisava, 2018.)

Insecticides (%)	Before treatment		two days after the treatment		
	$\bar{x}$	±Sd	$\bar{x}$	±Sd	E%
dimethoate (0.1)	151.2 a	20.8	10.2 b	4.62	94.3
bifenthrin (0.05)	137.2 a	25.3	11.5 b	2.61	92.8
chlorpyrifos + bifenthrin (0.1)	161.7 a	38.7	7.75 b	2.50	95.9
control	133.7 a	24.9	157.5 a	11.3	
LSD 5%	42.11		10.14		

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy

Two days after application of dimethoate, bifenthrin, and chlorpyrifos + bifenthrin preparations, the abundances of *M. persicae* was significantly lower than the control. The efficacy of the tested preparations ranged from 92.8 to 95.9% and they are at the same level of significance. The number of Aphids, eight days after the treatment, was at a significantly lower level compared to the control. The efficacy of the tested preparations was 94.5-96.4%. The number of green peach aphids 15 days from the application of the insecticides is significantly lower than the control. The preparation efficiency was still high and ranged from 93.2 to -95.0% (Table 2).

Table 2. Average number of green peach aphids (*M. persicae*) and insecticide efficacy (Budisava, 2018.)

Insecticides (%)	eight days after the treatment			15 days after the treatment		
	$\bar{x}$	±Sd	E %	$\bar{x}$	±Sd	E%
dimethoate (0.1)	9.50 b	2.64	95.6	15.2 b	4.02	93.2
bifenthrin (0.05)	6.75 b	1.71	96.4	10.5 b	5.92	95.0
chlorpyrifos + bifenthrin (0.1)	12.5 b	1.29	94.5	13.7 b	4.03	94.3
control	189.5 a	32.2		199.5 a	8.35	
LSD 5%	26.04			9.39		

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy

In *M. persicae,* two ways of developing resistance are most commonly present: increased detoxification of insecticides and change of site sensitivity. This is the reason why resistance can be developed by a large number of insecticides with different mode of action. Biochemical methods for determining resistance have shown that the apparently reduced sensitivity of green peach is developed to the most commonly used insecticide groups, ie. that resistance has been developed to a certain extent, but that the studied populations of *M. persicae* in Serbia do not yet belong to the category of extremely resistant Aphids (Vučetić *et al*., 2007). As a result of intense exposure to the insecticides, *M. persicae* has developed resistance to numerous insecticides including neonicotinoids (imidacloprid), organophosphates (chlorpyrifos), carbamates (carbaryl and thiodicarb) and synthetic pyrethroids (deltamethrin) in many countries worldwide (Bass *et al*., 2014; Puinean *et al*., 2013; Slater *et al*., 2012).

In order to evaluate the resistance of *M. persicae* populations to seven insecticides, Tang *et al*. (2017) examined the sensitivity of 11 populations collected from eight provinces in China. Toxicity analysis showed that *M. persicae* populations developed several levels of resistance to each tested insecticide. Population from field conditions has developed high levels of resistance to beta-cypermethrin and cypermethrin, while resistance to bifenthrin remains low. According to bifenthrin, 7 of the 11 studied populations of *M. persicae* showed resistance (12-38 times).

Conclusions

The studied population of *M. persicae* in chrysanthemum under greenhouse conditions showed high sensitivity to insecticides based on dimethoate, bifenthrin and chlorpyrifos + bifenthrin. However, since Aphids belong to the group of insects that are able to develop resistance relatively quickly, these insecticides should not be used alternatively with other preparations from the chemical groups of organophosphates and pyrethroids.

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APPLICATION OF CHITOSAN-BASED COATING IN LEMON FRUIT CONSERVATION

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Abstract

The fruit characteristic such as nutrition, favour and firmness deteriorated during the process of storage and lead to the decay of the fruit. Lemon is one of the most important citrus fruit and is cultivated in many countries including Iran. The effects of chitosan-clay nano-composite coating on the quality properties of lemon fruit during storage were investigated. Chitosan- clay solution was prepared by composition 1% clay, 2% chitosan. The lemon fruit were dipped into the chitosan-clay composition and then dried at environment temperature. Experiments were carried out at four storage times with coated and uncoated samples. The physical, chemical and mechanical properties of lemon fruit were measured during the storage. The results showed that the pH of lemon fruit juice increased significantly ($p<0.05$) for coated and uncoated samples. Coated samples had the lower TSS (Total Soluble Solid) and higher TA (Titratable Acid) rather than uncoated samples. The inner strength of coated samples was higher because of the higher firmness and peel shear force and also the lower punch force. As a result, the coated fruits had more resistance to the applied force than the uncoated fruits. Coating was more effective in maintaining lemon firmness at prolonged storage. Thus, the coated samples had the most resistance to fungal disease. Finally, it should be indicated that resistance to fungal diseases, interior tissue strength and quality properties of fruit were increased by using the nano composite coating. Consequently, it reduced the percentage of weight loss and maintained the fruit quality longer.

Keywords: *Citrus, Lemon, Mechanical properties, Nano composite*

Introduction

Among the citrus, lemon is the third most important Citrus species after orange and mandarin (Porat *et al*., 2000), and is a subtropical fruit of high commercial value on the international fruit market. It has a split skin and very juicy which can easily suffered by green mold and skin shrinkage. It is extremely sensitive during the storage (Chien *et al*., 2007). Similar to deciduous fruit, lemon fruits are not held in the cold storage and must be harvested with delay from the tree. There are several exceptions to this statement such as reduction of physical disorders, humidity control environment, usage of coating material, disturb carbon dioxide and employing ethylene in cold storage which increase the shelf life (Miller, 1980).

One effective strategy for maintaining product quality properties and keep it fresh is using nanotechnology. It has many applications in agriculture science including: recycling of waste from agricultural products and maintain product quality and properties during food processing and storage process. Nano-coating and nano-composites is a way to increase the shelf life of fruits and vegetables (Henriette, 2009). Nanotechnology has many applications in relation to maintenance of food and particularly their packaging and in some cases nano achievements are applied in practice (Chaudhry *et al*., 2008 and Chen *et al*., 2006).

Consumers interest in nutrition and food safety and also to environment stimulated the in the field of edible coatings. Renewable sources of forming the coating layers are quite available (Baldwin, 2007). The main reason for applying coatings on fruits is the loss of fruit juice in the post-harvest stages. Juice loss usually occurs in the vapor phase. Water vapor permeability describes the movement of water vapor inside layer covered in different temperatures and moistures. (Hugh and Krochta, 1994).

In recent years, many layers and edible coating of food items like candies, fruits and fresh vegetables and processed meats are commonly used (Baldwin, 2007). Chitosan-clay coating has been considered in recent years. Chitosan is a polysaccharide that is non-toxic and is biodegradable (Warayuth *et al*., 2010). It is derived from chitin and is known as anti fungicide. It is edible coating and has significant inhibitory properties in microbes, fungi and pathogenic factors growth (Muzzarelli *et al*., 1990). Nano-composition of chitosan and clay can be obtained by method of clay-particle nano disturb in chitosan solution. Advantages of these compounds are the delay in the loss of moisture; reduce in evaporation and breathing rate, changes in material tissue properties (Muzzarelli *et al*., 1990; Warayuth *et al.*, 2010). Chitosan film has been used to coating of crops such as strawberries, mandarin and cherry (Fornes *et al*., 2005; Martınez-Romero *et al*., 2006; Ribeiro *et al*., 2007). Nowadays studies are focused on the expansion of technology to improve food quality and its durability.

Therefore the aim of this research was to investigate the effects of chitosan-clay nanocomposite coating during the different storage period on the physical, chemical, and mechanical properties of lemon fruit.

Material and Methods

The mature Lemon fruit (140) used in this experiment was provided randomly from citrus orchard in Qaemshar, Mazandaran, Iran. The samples were brought to the laboratory immediately after harvesting. Chitosan- clay solution was prepared by composition 1% clay, 2% chitosan and 25 cc acetic acid then dispersed in 1L of distilled water (Casariego *et al.*, 2009). Chitosan and clay were bought from Aldrich LTD.

Fruit were washed with tap water, dried in room temperature and dipped into the chitosan-clay composition by hand. The surface of coated fruit was dried at environment temperature for 12 h then fruit were stored at about 8^{o}C and 75-80% relative humidity for 63 days. Experiments were carried out at four storage times, 0, 21, 42 and 63 days, with coated and uncoated samples.

The physical, chemical and mechanical properties of lemon fruit were measured during storage. The measurements were done in Citrus Research Central of Sari Agricultural Sciences and Natural Resources University.

The chemical parameters such as titratable acid (TA), total soluble solid (TSS) and pH of fruit juice were measured. Total soluble solid is quality index and TSS were measured with digital refract meter (ATAGO- Palette) in range 0- 20%.

Mechanical test comprised of fruit compression, peel shear and punch test. All tests were performed by Instron machine (Hounsfield Co., England, Model 4400), 60 mm/min speed and 250 KN load cell.

The experiment is performed randomly by factorial design 2*4 (2 coatings and 4 storage times) and 3 replicate. Data analysis and figure drawing was done by SPPS and Excel soft respectively. Differences were regarded as significant when the p-values were less than 0.05.

Results and Discussion

The pH values of juice fruit is illustrated in Table 1. It was increased in the coated and uncoated treatments during the storage which is consistent with the results of Martin-Diana *et al.*, 2009). The pH amount only was significant ($p<0.05$) within the first 3 weeks of storage but it turned to not significant at remained storage period for two treatments. The results showed that the pH of coated samples generally were higher than the uncoated samples. It shows that the coating of lemon fruits could be able to increases the pH of lemon juice.

As it is known, fungal attack the citrus when the pH juice is low. Also the pH of juices has been associated to microbial spoilage (Del Caro *et al*., 2004; Cortes *et al.*, 2008). Thus, by increasing fruit resistant, the quality will be maintained at high level.

Table 1: The amount of pH, TSS and TA for coated and uncoated samples in during storage

During Storage(day)	Coated (Chitosan- Clay) Samples			Uncoated Samples		
	TA	**TSS**	**pH**	**TA**	**TSS**	**pH**
0	2.97^{a}	8.83^{a}	5.88^{a}	2.97^{a}	8.83^{a}	5.88^{a}
21	2.77^{ab}	8.97^{a}	6.33^{b}	2.63^{a}	10.97^{a}	6.33^{b}
42	2.69^{ab}	10.20^{b}	6.37^{b}	2.21^{a}	10.93^{a}	6.37^{b}
63	2.5^{b}	10.67^{b}	6.32^{b}	2.33^{a}	11.07^{a}	6.32^{b}

* the same letter in the table for any column shows that the means had no significant difference ($p<0.05$)

Total Soluble Solid (TSS) measurement

According to Table 1, the TSS of coated samples significantly ($p<0.05$) increased but no significant difference in Brix^{o} were detected for uncoated samples over the storage period. The Brix^{o} values were about 8.83 to 11.07 in the uncoated fruit. It was higher in coated fruit samples (8.83-10.67). Baldwin *et al.* (1995) reported that coated fruits had slightly lower Brix^{o} than uncoated fruit. So lemon fruits with chitosan- clay coating have lower respiration and moisture losses. Therefore, coated treatment by using of chitosan- clay had protected fruit.

Total Acidity (TA) measurement

The TA of the lemon juice significantly ($p<0.05$) decreased by chitosan- clay coating in during storage. The reduction of TA had no significant difference ($p>0.05$) for uncoated samples (Table 1). According to the data, the TA rates for coated samples were higher than uncoated samples. The TA of fruit juice were reduced over the storage time which confirmed by El-Zeftawi (1976). So the coated samples had lower TA than uncoated samples due to higher TSS.

Mechanical Properties

Firmness was measured as a percentage of deformation. According to the data presented in Table 2, the coating application did not decrease firmness values of lemon fruit. For prolonged storage the differences found between the coated and uncoated lemon samples and it decreased for uncoated. The decrease in firmness of fruits is in agreement with the results which reported by Singh and Reddy (2006) for oranges.

Table 2: The amount of firmness, puncture and cutting force for coated and uncoated samples during the storage

During Storage(day)	Coated (Chitosan- Clay) Samples			Uncoated Samples		
	Shear Force	Puncture Force	firmness Force	Shear Force	Puncture Force	firmness Force
0	84.01^{a}	27.64^{a}	260.13^{a}	84.01^{a}	27.64^{a}	260.13^{a}
21	72.67^{a}	21.59^{b}	258.92^{a}	70.18^{ab}	26.79^{a}	215.23^{a}
42	71.22^{a}	21.75^{b}	256.75^{a}	55.81^{bc}	25.61^{a}	169.20^{a}
63	70.73^{a}	20.88^{b}	254.38^{a}	36.47^{c}	25.34^{a}	165.18^{a}

* the same letter in the table for any column shows that the mean amount had no significant difference ($p>0.05$)

The firmness force for the coated lemons (260.13 to 254.358 N) was generally higher than uncoated samples (260.13 to 165.18 N) during storage, but there was no significant difference ($p>0.05$) in firmness of all samples. This might be because of a metabolic delay. Consequently, fruit softening was also delayed, at coated fruit (Singh and Reddy, 2006). When storage was prolonged, coatings were more effective in maintaining lemon firmness. So the coated fruits were more resistant to the applied force than uncoated fruits.
The puncture force decreased from 27.64 to 25.34 N and 27.64 to 20.88 N for uncoated and coated fruits, respectively (Table 2). No significant changes ($p>0.05$) observed in puncture force for uncoated fruits, but puncture force changed significant ($p>0.05$) within the first 3 weeks of storage for coated samples. The puncture force of lemon fruit in the chitosan- clay coating was lower than uncoated samples. The similar trends were also explained by Singh and Reddy (2006).
The peel shear force significantly ($p<0.05$) decreased in coated samples (Table 2). The amount of peel shear force for uncoated sample was significantly ($p<0.05$) lower than coated samples (Table 2). Intercellular spaces result in less cell wall material per volume of tissue and less contact area between cells. Both factors could cause the tissue to be less resistant to shear stress (Harker, *et al.*, 1997). Therefore non coating sample has lowest stiffness because of less contact area between cells.

Conclusions

Investigation of lemon quality properties showed that coated samples have the higher pH and TA and lower TSS rather than uncoated samples. Thus coated samples have the most resistance to fungal disease and have the lower percentage of weight loss. Also studying, mechanical properties showed that inner strength of coated samples was high because of the higher compression; peel shear force and having the lower punch force. Lemon coating did not reduce the firmness loss and more effective in maintaining lemon firmness at prolonged storage. So the coated fruits have more resistance to the applied force than uncoated fruits. Chitosan has been demonstrated to be able to inhibit the growth of some fungi. Finally, it should be concluded that the coating by chitosan-clay proposed in this paper not only promotes fruit moisture retention while maintaining fruit quality.

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ANTIMICROBIAL ACTIVITY OF PGPR ACTINOBACTERIA

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Abstract

Actinobacteria are filamentous bacteria, commonly found in nature and especially in the soil, and are the most prolific of all microorganisms as producers of bioactive molecules. They are responsible for 70% of natural antibiotics known in the world, 55% of which are produced by actinobacteria belonging to the genus *Streptomyces*. However, with the appearance of resistant pathogenic strains, several antibiotics have become little or no effective. One of the research strategies adopted for rare genera isolation, producers of new antibiotics, is the exploitation of particular ecosystems in order to isolate rare strains. In this investigation, we studied the antibacterial and antifungal activities of six actinobacterial strains, already isolated from the semi-arid rhizospheric soil of eastern Algeria. Different rhizospheres were explored. Among the six isolates, four belonged to the genus *Streptomyces* and two were assigned to the rare genus *Nocardiopsis*. In fact, the six strains were tested for their antifungal activity against seven phytopathogenic fungi. The six isolates were then tested against phytopathogenic bacteria. The results showed that among the six actinobacteria, five strains (Lac1, Lac3, Vic8, Pru14 and Pru16) inhibited all phytopathogenic fungi. Only isolate Pin10 was unable to inhibit *M. nivale*. The six antagonistic strains showed more or less important antibacterial activities. Thus, *S. griseus* Lac1 exhibited the best inhibitory activity. This study confirmed not only the antimicrobial activity of *Streptomyces* strains, but also revealed interesting antibacterial and antifungal activities of the two rare strains belonging to the genus *Nocardiopsis*.

Key words: *Antimicrobial activity, Streptomyces, Nocardiopsis*.

Introduction

Excessive use of chemical fungicides can lead to environmental pollution and the emergence of pathogens resistant to specific fungicides. Plant protection products are responsible for various health problems in humans and animals (Gerhardson, 2002). Phytopathogenic antagonist microorganisms have been discovered and some of them are already used as biological control agents against plant diseases (Prapagdee *et al*., 2008). Actinobacteria are well known for their metabolic capacities, especially *Streptomyces* spp. which produces thousands of antibiotics (Berdy, 2015). They appear to be good candidates for biological control (El-Tarabily and Sivasithamparam, 2006). Actinobacteria are Gram-positive, most are saprophytes (Khamna *et al*., 2009) and are abundant in the rhizosphere (Sardi, 1992). This environment has been considered as a rich source for the isolation of biocontrol agents and plant growth promoters (PGPR) (Jiménez-Esquilin and Roane, 2005; Yilmaz *et al*., 2008).
The aim of this study was to assess the *in vitro* antagonism of six strains of rhizospheric actinobacteria (*Streptomyces* and *Nocardiopsis*) against phytopathogenic fungi *Verticillium dahliae*, *Fusarium culmorum*, *Drechslera teres*, *Microdochium nivale*, *Bipolaris sorokiniana*, *Botritys fabae* and *Fusarium oxysporum*. These strains have also been tested against plant pathogenic bacteria (*Erwinia carotovora* and *Agrobacterium tumefaciens*).

Materials and methods

Antibacterial activity

The method used was the modified technique of Anibou *et al* (2008). The agar cylinders were cut from well sporulated actinobacteria cultures. They were placed on the Petri dishes already inoculated, in mass by the bacterial strains (10^8 cells/ml). The cultures were first incubated for 18 hours at 4°C to allow the diffusion of bioactive substances and then incubated at 30°C. Antimicrobial activity was assessed by measuring the inhibition zone (mm) after 48 hours of incubation. The phytopathogenic bacteria tested were isolated from fruit trees (apple and raspberry) and vegetable crops (potato) in Quebec: *Agrobacterium tumefaciens*, *Erwinia. carotovora* subsp. *carotovora* 824, *E. carotovora* subsp. *amylovora* 1111, *E. carotovora* subsp. *amylovora* 998, *E. carotovora* subsp. *amylovor*a 1024 and *E. carotovora* subsp. *atroseptica* 750.

Antifungal activity

Actinobacteria are streaked, on PDA medium in the center of the Petri dish (145 mm diameter), into a 6 mm large central streak. These cultures are incubated at 28°C for 8 days. Two discs (7 mm diameter) of the 8-day-old fungi culture are placed on each side of the actinobacteria streak. The distance between the fungal discs and the edge of the Petri dish was 15 mm. For each fungal strain, a control treatment was performed, with two discs placed on the PDA medium without actinobacteria. All dishes were incubated at 28°C for 5 days. Every day the diameter of the fungal colony was measured for all treatments including the control (Soares *et al.*, 2006).

Statistical analysis

The data were analyzed with repeated measures analysis of variance (ANOVA) followed by the least significant difference (LSD) test using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC) (Ryan and Kinkel, 1997; Alves-Santos et al., 2002).

Results and discussion

Antibacterial activity

The results of the antagonism test against phytopathogenic bacteria, belonging to the genus *Erwinia* (*E. carotovora* subsp. *amylovora* 1111, *E. carotovora* subsp. *amylovora* 998 and *E. carotovora* subsp. *amylovora* 1024) revealed an inhibitory activity of two strains Lac1 and Pru14. By comparison of the inhibition zones, those obtained with strain Lac1 (32 to 34 mm) are more important than those obtained with strain Pru14 (17 to 20 mm). *E. carotovora* subsp. *carotovora* subsp. 824 and *E. carotovora* subsp. *atroseptica* 750 were found to be resistant. Lac1, Vic8 and Pru14 strains exhibited antibacterial activity against *Agrobacterium tumefaciens*, the diameters of the inhibition zones are 21, 18 and 21 mm, respectively (Table 1, Figure 1).

Table 1. Antimicrobial activity against phytopathogenic bacteria.

Phytopathogenic bacteria	**Actinobacterial strain**[1]		
	Lac1	**Vic8**	**Pru14**
E. carotovora subsp. *carotovora* 824	n.i.[2]	n.i.	n.i.
E. carotovora subsp. *atroseptica* 750	n.i.	n.i.	n.i.
E. carotovora subsp. *amylovora* 1111	33	n.i.	19
E. carotovora subsp. *amylovora* 998	32	n.i.	17
E. carotovora subsp. *amylovora* 1024	34	n.i.	20
Agrobecterium tumefaciens	35	18	21

[1] No inhibition zone was obtained with strains Lac3, Pru16 and Pin10; [2]: n.i.: no inhibition.

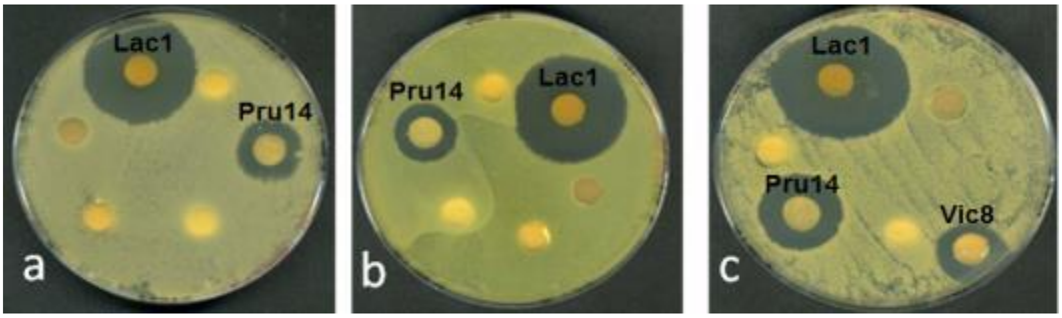

Figure 1. Result of antibacterial activity, (a) *E. carotovora amylovora* 1111; (b) *E. carotovora amylovora* 998; (c) *Agrobecterium tumefaciens*

Antifungal activity

The results of the antagonism test are presented in Figures 2 and 3. Among the six antagonistic actinobacteria strains, five of them (Lac1, Lac3, Vic8, Pru14 and Pru16) inhibited all phytopathogenic fungi. Only Pin10 strain was unable to inhibit the growth of *M. nivale*. Lac1 was found to be the most effective in the control of mycelial growth of *D. teres*, *M. nivale*, B. *sorokiniana* and *B. fabae* with a growth inhibition of 59.72%, 72.19%, 65.09% and 52.33%, respectively. The strain Pru14 was effective in reducing the mycelial growth of *F. culmorum* (60.15%), while Pru16 was the best antagonistic strain for the control of *V. dahliae* and *F. oxysporum* with growth reductions of 50.81% and 62.77%, respectively. Lac1 and Pru16 strains showed the highest antagonistic activity.

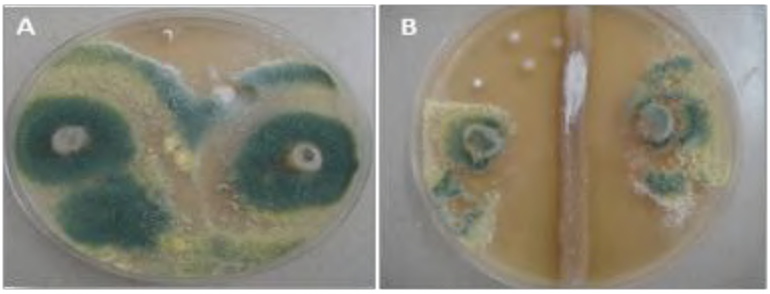

Figure 2. Result of the antifungal activity of isolate Vic8 against *V. dahliae*, (a) the control; (b) antagonism test.

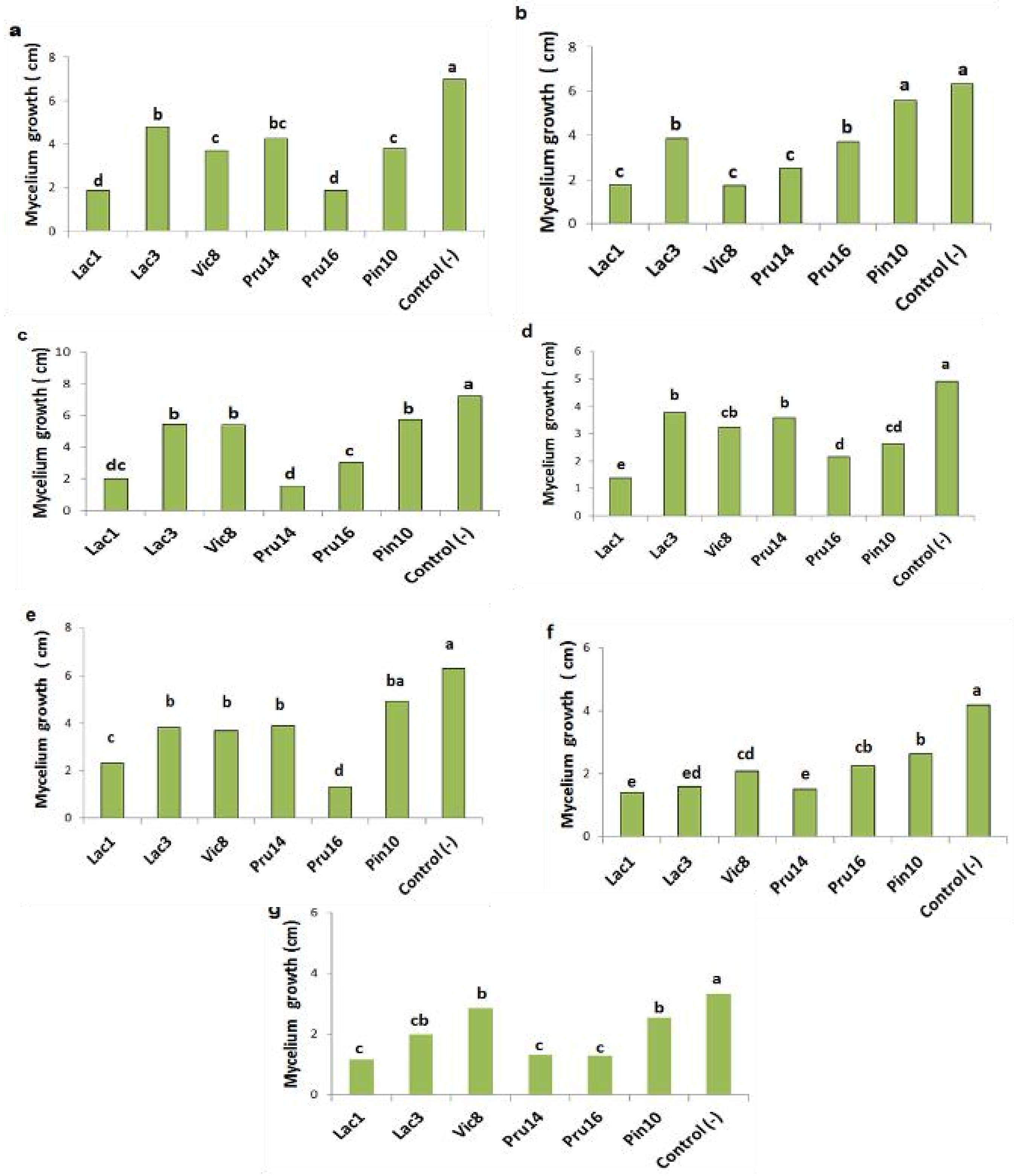

Figure 1. Effect of antagonistic actinobacterial isolates on mycelium growth of phytopathogenic fungi. Values from bars with the same letter do not differ statistically (LSD). (a) *F. oxysporum*, (b) *M. nivale*, (c) *F. culmorum*, (d) *D. teres*, (e) *V. dahlia*, (f) *B. sorokiniana*, (g) *B. fabae.*

Actinobacteria are characterized by their metabolic activities, and PGPR bacteria often showed biocontrol properties. It has been reported that nearly 5% of rhizobacteria can

increase the growth of plants and protect them against pathogens such as bacteria and fungi. Actinobacteria are well known to produce secondary metabolites and extra-cellular enzymes with interesting antibiosis (Khamna *et al.,* 2010). Thus, several studies have examined theirs *in vitro* antibiosis activity against phytopathogenic bacteria (de Oliveira *et al.,* 2010).
In the present investigation, six actinobacterial strains were evaluated against phytopathogenic bacteria and fungi. These strains were obtained from rhizospheric soils, at first selected for their PGPR traits (acide 3-indole acétique, sidérophores and phosphate solubilization) (Aouar *et al.,* 2019). Antifungal activity was detected in five strains that inhibited all fungi. Among the active strains, one strain belonging to a rare genus (*Nocardiopsis*) could produce interesting antifungal molecules. *In vitro* assays performed against phytopathogenic bacteria revealed different antibiosis abilities among the six actinobacteria tested as potential biocontrol agents. While *E. carotovora* subsp. *carotovora* 824 and *E. carotovora* subsp. *atroseptica* 750 appeared resistant to all actinobacteria, Lac1 showed the most significant inhibitory activity. This strain belongs to *Streptomyces griseus* species from which several antibiotic compounds with antagonistic activities against Gram-positive (streptomycin, novobiocin and griseoviridin) and Gram-negative bacteria (griseorhodin) have been isolated.
Many studies have focused on the exploration of antimicrobial activity of actinobacteria. The results of Oskay *et al* (2004) indicated that 34% of the 50 actinobacteria isolates were active against at least one tested microorganism; *A. tumefaciens*, *E. carotovora* subsp. *amylovora*, *Pseudomonas viridiflova*, *Clavibacter michiganensis* subsp. *michiganensis*, *B. subtilis, K. pneumoniae*, *Enterococcus feacalis*, *S. aureus*, *E. coli* and *Sarcina lutea*. The study by Yilmaz *et al* (2008), involving 55 actinobacteria strains, isolated from different rhizospheres, showed the antimicrobial activity of 22 strains. This activity is more pronounced on Gram-positive bacteria than on Gram-negative bacteria. In addition, about 18% of actinomycetes showed antifungal activity.

Conclusion

This study highlighted the antifungal and antibacterial potential of six strains of rhizospheric actinobacteria belonging to two genera *Streptomyces* and *Nocardiopsis*, previously selected for their PGPR traits. Thus, except the Pin10, the other strains inhibited all the phytopathogenic fungi. The Lac1 strain has the best antifungal activity. In addition, the Lac1, Pru14 and Vic8 strains have inhibited the growth of phytopathogenic bacteria. Among these strains, Lac1 exhibited the most significant antibacterial and antifungal activity. Among active strains, one was affiliated with the *Nocardiopsis* genus. Members of the *Nocardiopsis* genus could be promising candidates leading to the development of interesting biocontrol products.

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INVESTIGATION OF THE OXIDATION PRODUCTS OF THE OILS OF THE LATEST NON-OILY SUNFLOWER HYBRID SEEDS

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Abstract

Sunflower seed hybridization proceeded in two directions: towards an increase in oil content (oily sunflower hybrid seed) and an increase in protein content (non-oily or confectionary sunflower hybrid seed). Sunflower oil hybrids are used for oil production, while non-oily seeds are primarily used as a source of protein, however they contain significant amounts of oil (about 30%) and are a potential raw material for oil production.

The oxidative stability of three different cold-pressed oils obtained by confectionary sunflower hybrid seed was studied by monitoring the content of oxidation products. Samples were tempered at 63±2°C for a period of 8 days and the peroxide (PV) and anisidine value (*p*-AnV) as well as the content of the conjugated dienes and trienes in the initial samples and after 4th and 8th day of the exposure to the test were determined. The best oxidative stability in the initial sample had the oil of hybrid NS-H-6304. The PV was 2.20±0.02 mmol kg^{-1}, while the *p*-AnV was 0.47±0.06. The worst oxidation characteristics in the initial sample had NS-H-6971 oil (PV=3.98±0.05 mmol kg^{-1}, *p*-AnV=1.52±0.34). After 8 days, the lowest PV was found in the sample NS-H-6971 (54.70±4.10 mmol kg^{-1}), while the highest value was obtained in the sample NS-H-6488 (72.88±6.07 mmol kg^{-1}). The lowest *p*-AnV after 8 days was 1.60±0.02 (NS-H-6488) while the highest value of 6.56±0.04 was found in the sample NS-H-6791.

Keywords: *Sunflower oil, non-oily hybrids, primary and secondary oxidation products*

Introduction

Non-oily (confectionary) sunflower hybrid seed and oily sunflower hybrid seed have significant difference in chemical composition as well as the appearance of seed. Confectionary hybrid seed has lower oil content (30-35%) and higher protein content (about 40%) (Kaya *et al*. 2008; Hladni *et al*., 2011). Sunflower seed contains small amounts of antinutritive compounds (eg protease inhibitors, cyanogen, lectins, etc.) (Gassmann, 1983; González-Pérez and Arellano, 2009; González-Pérez, 2015). The amino acid composition of sunflower seed protein, with the exception of a small amount of lysine, is in accordance with the requirements of the FAO/WHO for human nutrition (Gassmann, 1983; Raymond *et al*. 1991).

Confectionary sunflower hybrid seed compared with oily sunflower hybrid seed has larger seed with a thicker hull, most commonly colored (black and white). Hull represents 40-45% of the total seed mass, it is poorly attached to the kernel and is easily removed (Jovanović, 2001; Gonzales-Perez and Vereijken 2007).

Oxidative stability of the oil represents the ability of vegetable oils to resist the oxidation process during production, storage and manipulation. It determines the quality and shelf life of the oil. The oxidative stability of the oil is influenced by many factors: fatty acids composition, antioxidants, the content of free fatty acids, mono and diglycerides, conditions of production, storage and manipulation, etc. Some of these factors are influenced by the seed hybrid used to produce oil. The aim of this paper is to examine the oxidative stability of sunflower oils obtained from the latest non-oily sunflower hybrids.

Material and Methods

Material: Hybrid seeds are a secondary filial (F2) generation of confectionary sunflower hybrid seed. Samples NS-H-6791, NS-H-6488 and NS-H-6304 were grown under conditions of small-plot trials in 2017 (Vojvodina, Serbia). Six months after harvesting the seed was cleaned and stored under normal storage conditions and without prior peeling and pressed with a screw press. The oil temperature at the output of the press was 60-70°C. Samples of cold pressed oils were taken after 72 hours of agitation at room temperature for the sedimentation, decantation and oil filtration through plain, wrinkled laboratory filter paper.

Methods: Samples of oil have been exposed in the laboratory conditions at moderate temperatures (63±2°C), in the presence of air, without the presence of light according to the methodology described by Pokorny *et al*. (1985). It was measured in 50 ml of each sample and subjected to test conditions for a period of 8 days. After the 4^{th} and 8^{th} day, the samples were taken and analysed and compared with the initial samples. The content of primary oxidation products is determined on the basis of the peroxide value (PV) (ISO 3960:2017) and specific absorbances at a wavelength of 232 nm, i.e. the content of conjugated dienes (ISO 3656:2011/Amd 1:2017). Based on anisidine value (ISO 6885:2016) and specific absorbances at a wavelength of 270 nm, i.e. the content of conjugated triens (ISO 3656:2013/A1:2017), the content of secondary oxidation products has been determined. The fatty acids composition in oils is determined by the application of gas chromatography - mass spectrometry (GC-MS) (ISO 12966-1:2014, ISO 12966-2:2017) using the HP 5890 gas chromatograph with HP 5971A mass detector ("Hewlett Packard", USA). All determinations were done in three replications, the results are tabulated as the mean value ± standard deviation, and only the mean value for the graphic representation was obtained. Statistical processing of the obtained results and graphical representations were made using Microsoft Excel 2010 (Microsoft, Washington, USA) and Statistica 13.0 (StatSoft, Tulsa, USA)

Results and Discussion

Tested oil samples are obtained from linoleic hybrid seed so the variation in fatty acid composition is minimal (Table 1).

Table 1. Fatty acid composition of the tested samples

Fatty acid (%)	NS-H-6791 % w/w	NS-H-6488 % w/w	NS-H-6304 % w/w
C 14:0	0.05±0.00	0.06±0.01	0.06±0.01
C 16:0	5.91±0.03	6.07±0.28	6.17±0.07
C 16:1	nd	nd	nd
C 18:0	4.57±0.05	3.80±0.16	4.00±0.06
C 18:1c	30.04±0.11	32.70±0.09	31.40±0.08
C 18:2c	57.65±0.03	55.70±0.34	56.91±0.24
C 20:0	0.33±0.02	0.24±0.00	0.26±0.00
C 20:1	0.16±0.01	0.14±0.00	0.14±0.02
C 22:0	0.88±0.04	0.94±0.13	0.74±0.03
C 24:0	0.41±0.03	0.35±0.07	0.32±0.04

nd – not detected

The highest content of linoleic fatty acid (C18:2) was determined in the NS-H-6791 sample and amounted to 57.65±0.03%. In the same sample the smallest content of oleic acid (C18:1) was also determined, which amounts to 30.04±0.11%. The smallest content of linoleic fatty acids of 55.70±0.34% and the highest content of oleic acid 32.70±0.09% were determined in the sample NS-H-6488. In the sunflower oil samples, the content of linoleic (C18:2) and oleic acid (C18:1) is very strongly correlated according to the literature data (Oštrić-Matijašević and Turkulov, 1980). The NS-H-6791, NS-H-6488 and NS-H-6304 sunflower hybrids examined in cold-pressed sunflower oil also identified a very strong negative correlation (C18:2 = -0.732 × C18:1 + 79.712, R = -0.989) between the content of linoleic and oleic acid.
The highest peroxide value (3.98±0.05 mmol kg^{-1}) and content of conjugated dienes (4.02±0.01) was determined in the initial sample NS-H-6791 while the lowest peroxide value (2.20±0.02 mmol kg^{-1}) was obtained in NS-H-6304 initial sample and the lowest conjugated dienes content (3.15±0.01) was found in the initial NS-H-6488 sample. In the initial sample NS-H-6488 the value of the peroxide number was 2.87±0.03 mmol kg^{-1}, and the content of conjugated dienes was 3.68±0.01 in the NS-H-6304 initial sample. After 4 days of exposure samples at a temperature of 63±2°C, peroxide values ranged from 29.85±1.00 mmol kg^{-1} (NS-H-6791) to 31.13±1.33 mmol kg^{-1} (NS-H-6488). The content of conjugated dienes after 4 days ranged from 9.22±0.02 (NS-H-6304) to 12.94±0.01 (NS-H-6791). After 8 days, the highest peroxide value of 72.88±6.07 mmol kg^{-1} was determined in the sample NS-H-6488 while the lowest value of 54.70±4.10 mmol kg^{-1} was determined in the NS- H-6791 sample. The content of conjugated dienes after 8 days of exposure to the test conditions ranged from 18.81±0.01 (NS-H-6791) to 21.28±0.01 (NS-H-6304).
In the tested samples, a strong linear correlation (R = 0.990-0.994) was obtained between the content of the conjugated dienes and the peroxide value (Figure 1, Table 2).

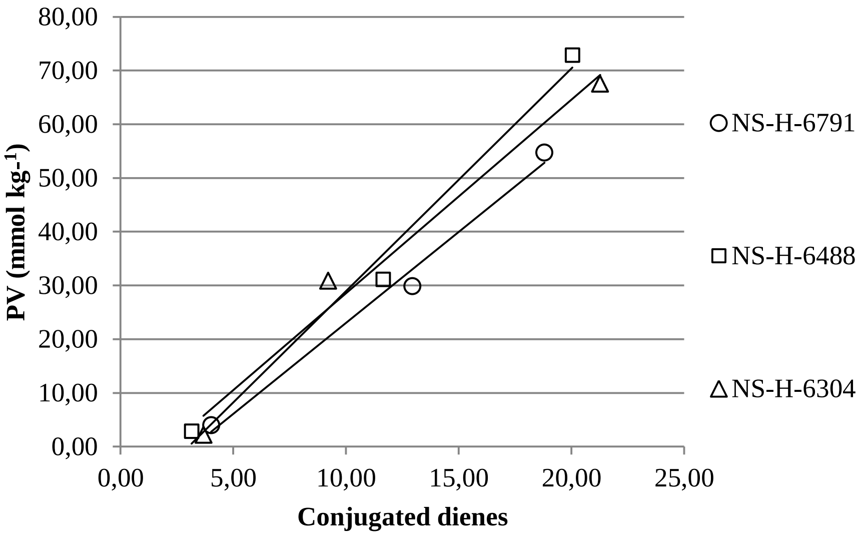

Figure 1. Linear dependence of the peroxide value and content of conjugated dienes

Table 1. Parameters of the linear dependence of the peroxide value and content of conjugated dienes

Sample	PV = a × Conjugated dienes + b			
	a	b	R^2	R
NS-H-6791	3.387	-10.869	0.989	0.994
NS-H-6488	4.141	-12.478	0.987	0.993
NS-H-6304	3.606	-7.5358	0.981	0.990

Romanić *et al*. (2018) examined the dependence of the peroxide value and conjugated dienes in sunflower oil obtained from oil hybrids under the same conditions and obtained high values of coefficients of determination (R^2 = 0.994-0.999).
The highest anisidine value in the initial samples (1.52±0.34) was determined in the sample NS-H-6791. After 8 days exposure of the sample to a moderate temperature, the anisidine value of 6.56±0.04 was determined. The lowest anisidine value in the initial samples was determined in the sample NS-H-6304 and was 0.47±0.06. After 8 days of the test the anisidine value was 2.44±0.64. The highest content of conjugated trienes in the initial samples (0.54±0.00) was determined in the NS-H-6791 sample, in which after 8 days was determined the maximum value of conjugated trienes content (1.22±0.01). The lowest value of conjugated triene content was determined in the NS-H-6488 initial sample and amounted to 0.37±0.01. In the same sample, the minimum content of conjugated trienes of 0.54 ± 0.01 was found after 8 days of the test.
Between the peroxide values and conjugated trienes content in the tested samples was found a significant and strong linear correlation with correlation coefficients between 0.405 and 0.806, as shown in Figure 2 and Table 3.

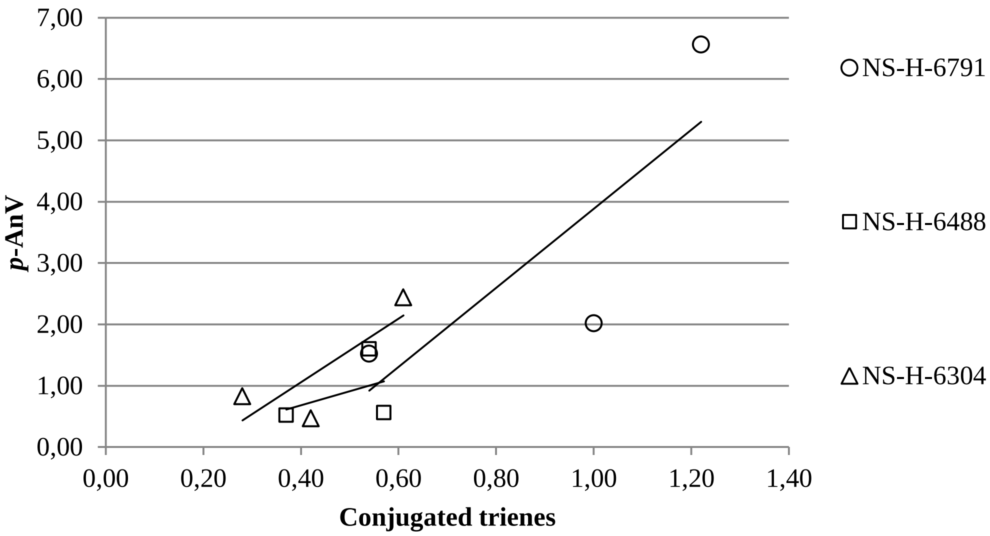

Figure 2. Linear dependence of anisidine values and content of conjugated trienes

Table 3. Parameters of the linear dependence of the anisidine values and content of conjugated trienes

Sample	*p*-AnV = a × Conjugated trienes + b			
	a	b	R^2	R
NS-H-6791	6.445	-2.529	0.649	0.806
NS-H-6488	2.298	-0.240	0.164	0.405
NS-H-6304	5.196	-1.022	0.673	0.820

Romanić *et al*. (2018) examined the dependence of the anisidine number and conjugated trienes on sunflower oil obtained from oil hybrids under the same conditions and obtained similar values of coefficients of determination (R^2 = 0.164-0.717).
Based on the peroxide and anisidine values, as well as the content of conjugated dienes and trienes in the initial samples, and after 4 and 8 days of the test, clustering was performed using

the *Ward's method* and the differences between the samples were expressed as Euclidean distance values (Figure 3).

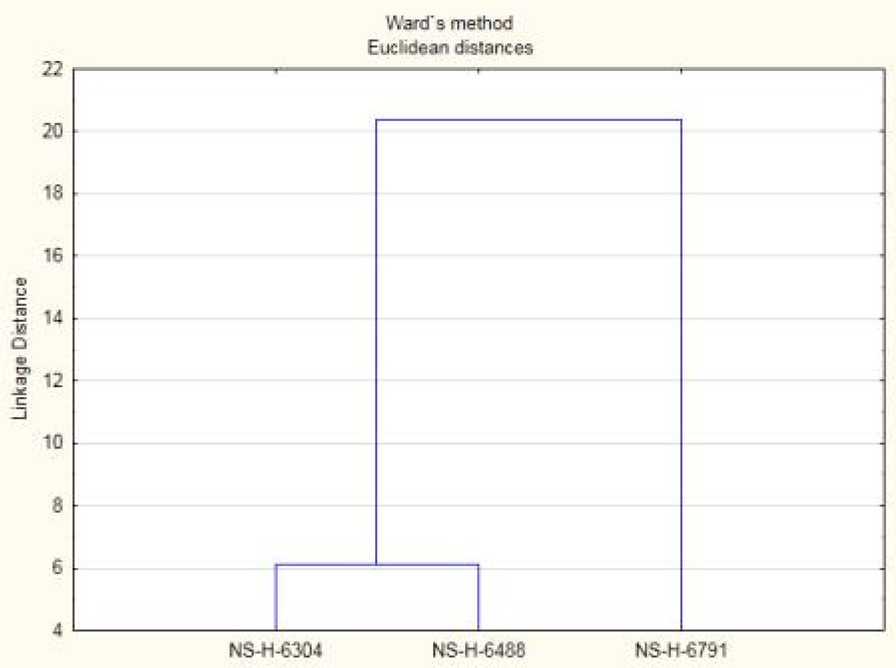

Figure 3. Hierarchial dendogram analysis of oxidation products of tested samples

The NS-H-6304 and NS-H-6488 samples are less different by oxidative stability than the NS-H-6791 sample. The largest difference was found between samples NS-H-6488 and NS-H-6791 and was 19.1 (expressed as Euclidean distance). A somewhat lower difference was found between samples NS-H-6304 and NS-H-6791 and was 14.5, while the smallest Euclidean distance was found, as mentioned, between samples NS-H-6304 and NS-H-6488 and amounted to only 6.1.

Conclusions

Based on cluster analysis, it is concluded that the NS-H-6791 sample varies considerably by oxidative stability compared to the other two samples. The highest peroxide value in the initial sample was found in this sample and also the lowest peroxide value after 8 days of exposure to the test. The peroxide value in this sample increased 13.74 times, while for samples NS-H-6488 and NS-H-6304 it increased 25.39 and 30.71 times, respectively. The NS-H-6791 anisidine value increased 4.32 times during test exposure, while for samples NS-H-6488 and NS-H-6304 this increase was 3.08 and 5.19 times, respectively.

Aknowlegment

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THE EFFECTS OF WATER EXTRACTS OF XERANTHEMUM CYLINDRACEUM SIBTH. ET SM. ON SEED GERMINATION OF THREE WEED SPECIES

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Abstract

The present study focused on inhibitory effects of water extracts of flowers, leaves, stems and roots of *Xeranthemum cylindraceum* Sibth. et Sm. on the total germination percentage, germination rate and germination rate index of chickweed (*Stellaria media* [L.] Vill.), common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.). Water extracts were made by soaking 10 g of plant biomass in 100 ml of distilled water on a shaker for 24 h. Batches of 50 (*A. artemisifolia* and *S. media*) or 30 (*A. theophrasti*) seeds were germinated in 0%, 10%, 20% and 50% extract solutions in three replicates. The extract of *X. cylindraceum* flower did not affect the germination percentage of test species but it did affect the germination rate and germination rate index of all three species. The highest concentration of leaf extract significantly reduced chickweed germination percentage, while it had no corresponding inhibitory activity on the other two species. Its 10% concentration even stimulated the germination of *A. theophrasti* seeds. Leaf extracts had inhibitory effects on the germination rate and germination rate index of all three species. Stem extract of *X. cylindraceum* had no effect on any germination parameter of common ragweed seeds. However, it had inhibitory effect on the germination rate and germination rate index of chickweed, and on germination rate of velvetleaf. Root extract acted inhibitory on all three germination parameters of chickweed seeds, and on the germination rate of velvetleaf. It had no inhibitory activity on the seeds of common rageweed. Based on available literature, the present study is the first to show that *Xeranthemum cylindraceum* has allelopathic properties.

Keywords: *alellopathy, Xeranthemum cylindraceum, water extracts, weed seeds, germination inhibition.*

Introduction

Successful cultivation of crop hybrids resistant to diseases and insects, in combination with evolving integrated pest management systems, have enabled reductions in damage caused by fungicides and insecticides, but the use of herbicides has continued to expand worldwide (Bhadoria, 2011). Research into the allelopathic properties of various plants has intensified over the past several decades both for scientific purposes and with the idea of introducing allelochemicals in agricultural production in order to eliminate or at least reduce harmful effects of excessive herbicide use. Based on available literature, allelopathic properties of *Xeranthemum cylindraceum* Sibth. et Sm. (syn. *X. foetidum* Mnch., *Xeroloma foetida* Cass.) have not yet been studied. The plant is widespread in Serbia and frequent on roadsides, in arable fields, vineyards, sunny grasslands and on rocky terrain (Gajić, 1975).
The present study focused on testing the inhibitory effects of water extracts of flowers, leaves, stems and roots of *X. cylindraceum* on total germination, germination rate and germination rate index of chickweed (*Stellaria media* [L.] Vill.), common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.) seeds.

Material and Methods

X. cylindraceum plants were sampled in the vicinity of Krušedol Monastery, Mt. Fruška Gora in northern Serbia in July 2017. They were dried before flowers, leaves, stems and roots were partitioned. Water extracts were made by soaking 10 g plant biomass in 100 ml of distilled water on a shaker for 24 h.

Seeds of the test plants were sampled in Belgrade's Zemun outskirts (Serbia) over the 2017 season, depending on their respective maturation periods. *A. artemisifolia* and *A. theophrasti* seeds were sterilized for 10 min in 10% water solution of bleach (industrial sodium hypochlorite, minimum concentration 40 g active chlorine/l) and stratified for four weeks at 4±2°C, and for 10 min at 65±5°C, respectively. *S. media* seeds were neither sterilized nor stratified. Batches of 50 (*A. artemisifolia* and *S. media*) or 30 (*A. theophrasti*) seeds were germinated in 0%, 10%, 20% and 50% extract solutions in three replicates. The seeds were germinated in the dark, and illuminated for 5-10 min during counting sessions at 1-3 days intervals. Chickweed seeds were germinated at 18±1 °C, and seeds of the other two species at 28±1 °C.

Germination properties were assessed based on: total germination (TG), germination rate (GR) and germination rate index (GRI). Total germination (TG) is the percentage of germinated seeds at the end of experiment. Germination rate (GR) is the sum of the quotients of germinated seed number since previous counting session and number of days since the beginning of experiment. It is calculated using a modified equation by Maguire (1962): $\mathbf{GR=\sum(Gi/ti)}$, where **Gi** is the number of seeds germinated since the last counting session on day **i**, and **ti** is the number of days since the beginning of experiment until day **i**. Germination rate index (**GRI**) is the product of **GR** and the quotient of germinated seed number by the end of experiment (**G**) and total seed number (**N**): $\mathbf{GRI=GR(G/N)}$.

Statistical analyses were performed using the STAT-GRAPHICS software, version 4.2 (STSC Inc. and Statistical Graphics Corporation, 1985-1989, USA). The percentage data were *arcsin* transformed before statistical analysis. The data were subjected to one-way analysis of variance (ANOVA). Differences between means were evaluated by Fisher's LSD test calculated at the confidence level of $P \leq 0.05$. The results of statistical analysis are marked with appropriate letters in the figures, and samples marked with the same letters have no significant statistical difference.

Results and Discussion

The effects of water extracts of different *X. cylindraceum* organs on germination dynamic of chickweed (*Stellaria media*) seeds were most evident in leaf extracts (Figure 1b). Their concentrations of 20% and 50% delayed germination start by a day, and the raching of germination plateau by two and one day, respectively. All concentrations of *X. cylindraceum* water extracts delayed the germination of chickweed seeds, compared to their germination in distilled water, but only the highest concentration (50%) delayed the reaching of germination plateau by a day (Figure 1a). Germination start was not delayed by stem extracts, while seeds germinating under 50% concentration reached germination plateau two days later than control seeds (Figure 1c). Only the highest concentration of root extract delayed the start of germination of chickweed seeds (Figure 1d).

Common ragweed (*Ambrosia artemisiifolia*) seeds generally showed no change in germination dynamic, except at the highest concentration (50%) of *X. cylindraceum* water extract, which delayed germination plateau by two days (Figure 2). The analysis of germination parameters suggest similar conclusions as only the highest concentrations of flower and leaf water extracts affected the GR and GRI (Table 2).

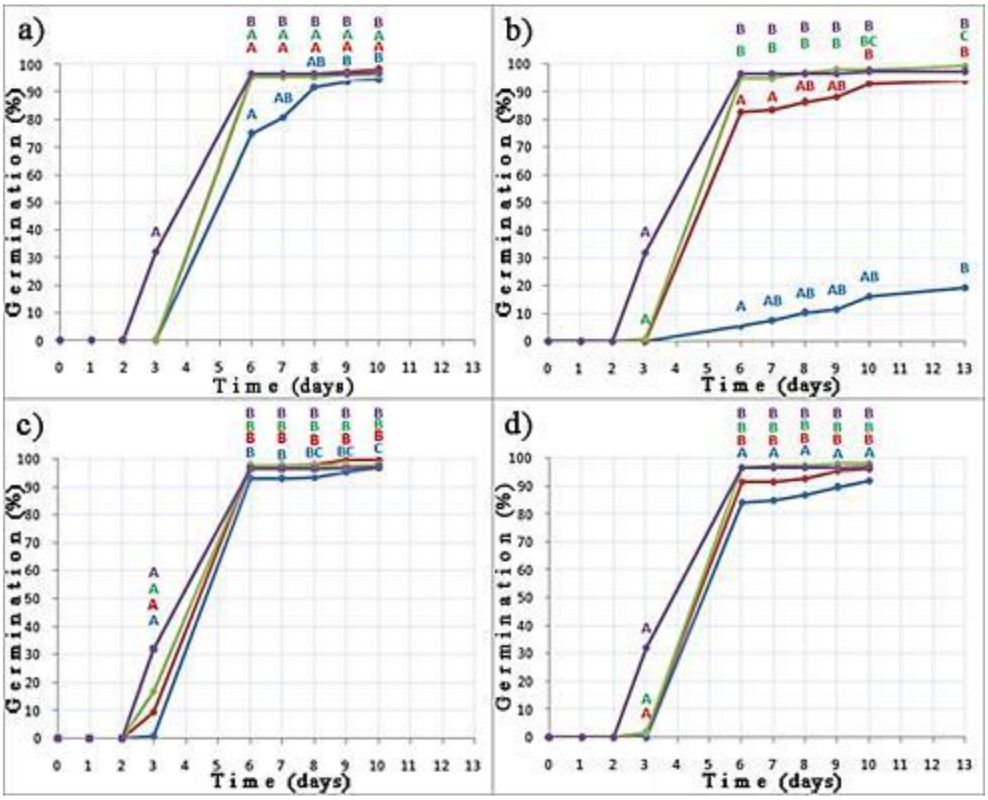

Figure 1. Dynamic of germination of *Stellaria media* seeds in different concentrations of *Xeranthemum cylindraceum* flower (a), leaf (b), stem (c) and root (d) water extracts. Different colours are used to distinguish different concentrations: purple – 0% (control), green - 10%, red - 20%, blue - 50%. Letters of the same colour show significant difference in the percentage of germinated seeds over time in extracts of corresponding concentrations.

The dynamic of velvetleaf (*Abutilon theophrasti*) seed germination changed only under the influence of leaf extract. Concentrations of 20% and 50% delayed the start of germination by one day, while 10% and 50% concentrations also delayed germination plateau by a day (Figure 3).

The effects of extracts on seed germination of the test species generally varied depending on plant organs used and their extract concentrations, as well as on the plant species whose seeds were tested (Tables 1, 2 and 3). These findings are in agreement with previous reports showing that allelopathic effects tend to differ depending on extract source, extract concentration and target species (Tabrizi and Yarnia, 2011; Pirzad *et al*., 2012; Suwitchayanon and Noguchi, 2014.; Zhang *et al*., 2018).

Leaf extracts of *X. cylindraceum* demonstrated the strongest effect on seed germination of the test species in this experiment. Leaf extracts had inhibitory effects on the GR and GRI of all three species (Tables 1, 2, 3). The highest concentration of leaf extract significantly reduced the TG of chickweed seeds, while it had no corresponding inhibitory activity on the other two species (10% concentration of leaf extract even had a stimulating activity on the TG of *Abutilon theophrasti* (Table 3)). Many studies have revealed stronger inhibitory activity of leaf extracts on seed germination or early root or shoot development and shoot growth, compared to extracts of other plant organs (Chung and Miller, 1995b; Naderi and Bijanzadeh, 2012; Suwitchayanon and Noguchi, 2014; Zhang *et al*., 2018).

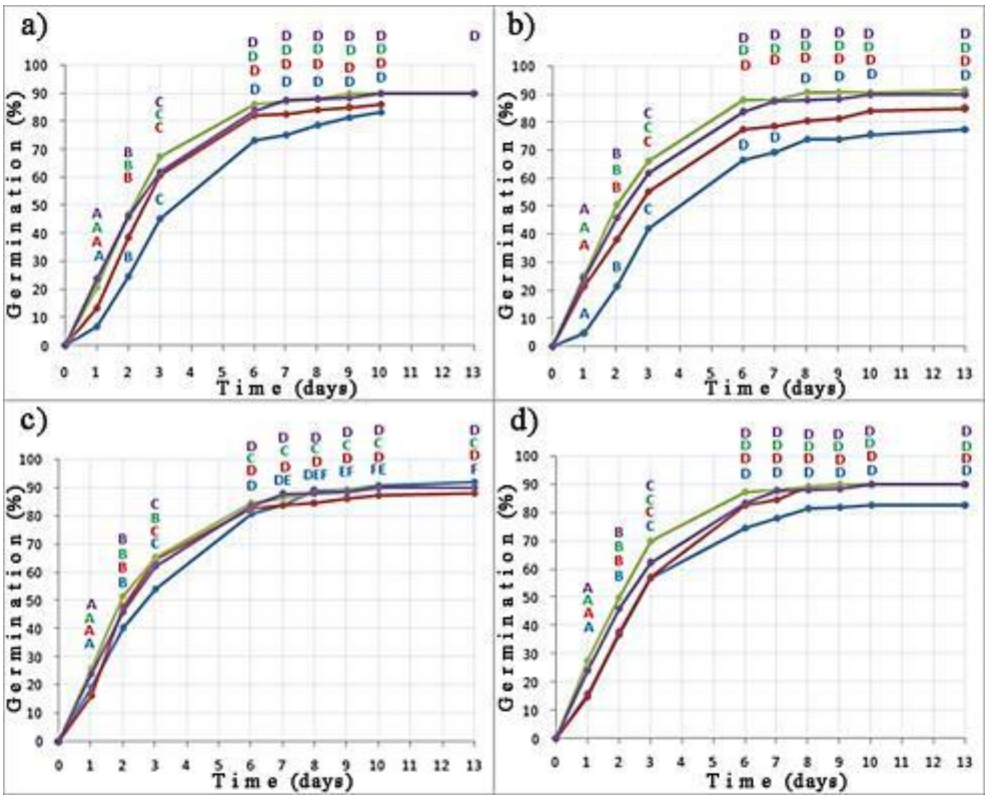

Figure 2. Dinamic of germination of *Ambrosia artemisiifolia* seeds in different concentrations *Xeranthemum cylindraceum* flower (a), leaf (b), stem (c) and root (d) water extracts. See Figure 1 for markings.

The extracts of *X. cylindraceum* flower did not affect the TG of test species but did affect the GR and GRI of all three species. Studies of allelopathic activity of plant organs probably focus the least attention to the flower, which usually has no distinct activity, compared to the other plant organs. However, other examples have also been reported. Chon (2004) showed the highest inhibition of alfalfa (*Medicago sativa*) seedling growth in extracts of flowers and leaves, followed by stems and roots of common thistle (*Cirsium pendulum* Fisch.).

Of all parts of sorghum (*Sorghum bicolor*), its stem extracts have shown the highest allelopatic effect on mung bean (*Vigna radiata*) seedling growth (Moosavi et al., 2011). On the other hand, Chung and Miller (1995a) ranked autotoxic effects of water extracts of alfalfa plant parts as: leaf (greatest), seed, root, flower and stem (least). The stem extract of *X. cylindraceum* in our present study had no effect on any germination parameter of common ragweed seeds. However, it had an inhibitory effect on GR and GRI of chickweed, and on GR of velvetleaf.

Root extract of *X. cylindraceum* acted inhibitory on all three germination parameters of chickweed seeds, and on the GR of velvetleaf. It had no inhibitory activity on common rageweed seeds.

The results of this experiment infer that *X. cylindraceum* owns allelopathic properties. The species is known to contain cyanogenic glicosides (Nahrstedt and Schwind, 1992), which have allelopathic activity (Fateh *et al.*, 2012). Recent studies have targeted the chemical composition of *X. cylindraceum* plant parts sampled in Serbia (Dekić *et al*., 2015; Gavrilović *et al*., 2018). The detection and identification of lactonitrile indicated the presence of cyanogenic glycosides in *X. cylindraceum* (Dekić *et al*., 2015).

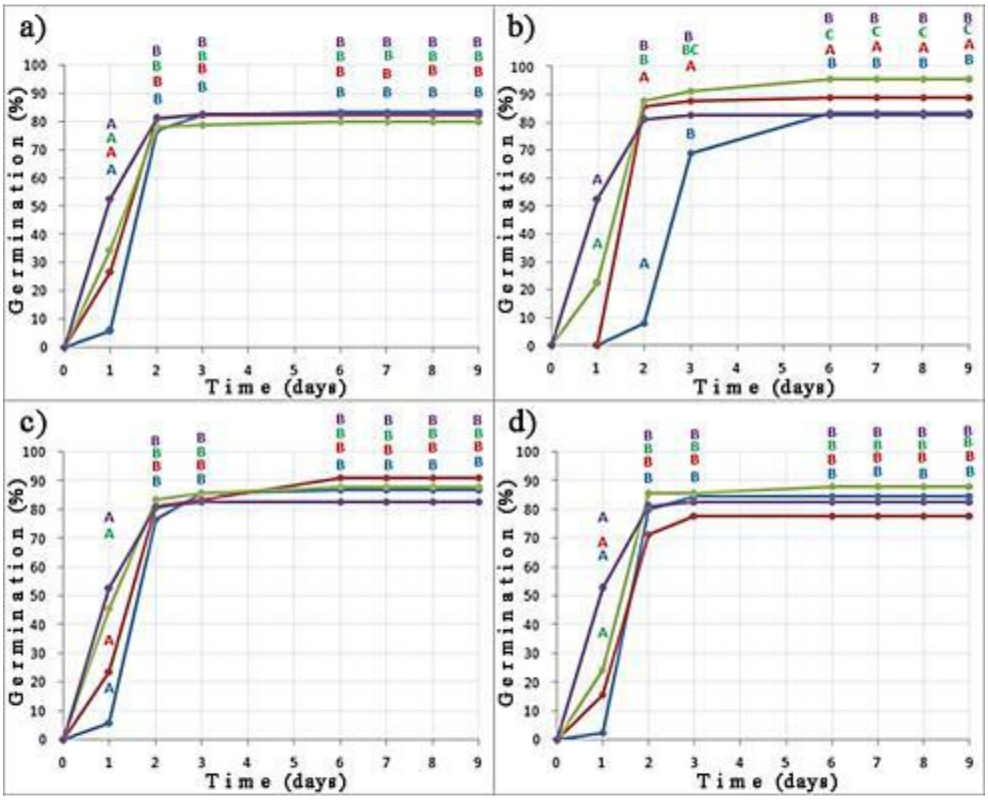

Figure 3. Dynamic of *Abutilon theophrasti* seed germination in different concentrations of *Xeranthemum cylindraceum* flower (a), leaf (b), stem (c) and root (d) water extracts. See Figure 1 for markings.

Table 1. Effects of water extracts of different *Xeranthemum cylindraceum* organs on total germination (TG), germination rate (GR) and germination rate index (GRI) of *Stellaria media* seeds.

Extr.	Flower extract						Leaf extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	97	A	10.73	B	10.41	B	97	BC	10.73	C	10.41	D
10	96	A	7.98	A	7.66	A	99.3	C	8.19	B	8.14	C
20	98	A	8.11	A	7.95	A	94	B	7.5	B	7.05	B
50	94.7	A	7.5	A	7.12	A	19.3	A	1.19	A	0.28	A
Extr.	Stem extract						Root extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	97	A	10.73	C	10.41	C	97	B	10.73	B	10.41	C
10	97.3	A	9.5	B	9.25	B	98	B	8.25	A	8.09	B
20	99.3	A	9	B	8.93	B	96	AB	7.99	A	7.67	AB
50	96.7	A	8	A	7.73	A	92	A	7.45	A	6.87	A

Table 2. Effects of water extracts of different *Xeranthemum cylindraceum* organs on total germination (TG), germination rate (GR) and germination rate index (GRI) of *Ambrosia artemisiifolia* seeds.

Extr.	Flower extract						Leaf extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	90	A	22.38	B	20.16	B	90	AB	22.38	B	20.16	B
10	90	A	22.08	B	19.93	B	91.3	B	23.58	B	21.56	B
20	86	A	18.68	B	16.09	AB	84.7	AB	20.03	B	16.9	B
50	83.3	A	14.21	A	10.31	A	77.3	A	12.62	A	9.94	A
Extr.	Stem extract						Root extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	90	A	22.38	A	20.16	A	90	A	22.38	AB	20.16	AB
10	90	A	23.6	A	21.37	A	90	A	24.28	B	21.84	B
20	88	A	20.52	A	18.12	A	90	A	18.92	A	17.12	AB
50	82	A	20.07	A	18.49	A	82.7	A	18.37	A	15.2	A

Table 3. Effects of water extracts of different *Xeranthemum cylindraceum* organs on total germination (TG), germination rate (GR) and germination rate index (GRI) of *Abutilon theophrasti* seeds.

Extr.	Flower extract						Leaf extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	82.5	A	20.17	B	16.75	B	82.5	A	20.17	C	16.75	C
10	80	A	17	AB	13.62	AB	95.6	B	17.05	BC	16.3	C
20	82.2	A	16.28	AB	13.41	AB	88.9	A	13.11	B	11.67	B
50	83.3	A	12.94	A	10.82	A	83.3	A	8	A	6.7	A
Extr.	Stem extract						Root extract					
(%)	TG (%)		GR		GRI		TG (%)		GR		GRI	
0	82.5	A	20.17	B	16.75	AB	82.5	A	20.17	B	16.75	A
10	87.8	A	19.67	B	17.34	B	87.8	A	16.61	AB	14.65	A
20	91.11	A	16.28	AB	14.81	AB	77.8	A	13.67	A	10.84	A
50	86.7	A	13.28	A	11.54	A	84.4	A	12.78	A	10.8	A

Conclusions

The effects of water extracts of flower, leaf, stem and root of *X. cylindraceum* on the germination of chickweed (*Stellaria media*), common ragweed (*Ambrosia artemisiifolia*) and velvetleaf (*Abutilon theophrasti*) seeds were tested. Based on available literature, this was the first study on allelopathic properties of *X. cylindraceum.* Leaf extracts perceptibly affected the seed germination dynamic of *S. media* and *A. theophrasti*, but not *A. artemisiifolia*. The other extracts demonstrated considerably weaker effects on seed germination dynamic. Extracts of the examined organs did not show strong inhibitory effect on TG, except leaf extract which exceptionally reduced the percentage of TG of *S media* seeds (below 20%). Flower and leaf extracts reduced GR and GRI in all three weed species. Stem and root extracts did not affect the germination of common ragweed, while they only reduced GR data for *A. theophrasti*. The study for the first time showed that *X. cylindraceum* owns allelopathic properties. It was shown to act inhibitory at variable degrees on the seed germination of all three tested weed species.

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BIOCONTROL POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST ROT CAUSING FUNGI OF WHITE YAM (*DIOSCOREA ROTUNDATA* POIR*)* TUBERS

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Abstract

Biological control potential of *Trichoderma harzianum* in the control of postharvest fungal pathogens of *Pepa* white yam tubers in storage was carried out for two years. Rotted *Ogoja* and *Pepa* white yam tubers were collected from farmers' barns in Zaki-Biam, Benue State, Nigeria. Pathogenicity tests conducted on healthy *Pepa* yam cultivars after fourteen days of inoculation revealed that the tubers were susceptible to *A. flavus*, *F. moniliforme* and *P. expansum*. Treatments comprised either inoculation of yam tubers with *A. flavus*, *F. moniliforme* and *P. expansum* alone or paired with *T. harzianum* as well as a control where the tubers were neither inoculated with antagonist nor with fungi pathogens and were stored for five months between December, 2015 and April 2016 and between December, 2016 and April, 2017. Results got in the first year of storage showed that tubers treated with fungi pathogens alone caused mean percentage rot of between 8.89% (*P. expansum*) and 20.00% (*A. flavus*) while those treated with *T. harzianum* alone produced only 2.22%. In the paired treatments, mean percentage rots were between 4.44% (*P. expansum* × *T. harzianum*) and 6.67% (*A. flavus* × *T. harzianum*). The findings in the second year revealed 13.33% (*P. expansum*), 22.22% (*A. flavus*) and 4.44% (*T. harzianum*) in the alone treatments while paired treatments produced mean rot of between 4.44% (*P. expansum* × *T. harzianum*) and 8.89% (*A. flavus* × *T. harzianum*). The results revealed that *P. expansum* was the most antagonized while *A. flavus* was the least inhibited. The findings revealed that *T. harzianum* (biological control agent) was more effective in inhibiting the growth of *A. flavus*, *F. moniliforme* and *P. expansum* in the first year of storage compared with the second year of storage. The antagonist therefore has biological potentials in controlling fungi pathogens of yam in storage.

Keywords: *Biocontrol, Trichoderma harzianum, Zaki-Biam, Pathogenicity Test, Aspergillus flavus.*

Introduction

Trichoderma harzianum is an antagonistic fungus that is widely recognized as an effective biocontrol agent for a range of important airborne and soil borne plant pathogens (Papavizas, 1985; Harman *et al.*, 2004; Mokhtar and Aid 2013). The genus *Trichoderma* is known to be promising members against soil-born plant parasitic fungi. Rot of crop produce have been known to reduce quantity and quality of yam both in the field and in storage (Amusa *et al.*, 2003) and fruits (Amusa *et al.*, 2003).

Studies conducted in different parts of the country have shown that fungal rot is the greatest cause of tuber loss in storage (IITA, 1993; Amusa *et al.*, 2003). These pathogenic organisms associated with yam rot in Nigeria are: *Aspergillus flavus*, *A. niger*, *Botryodiplodia theobromae, Colletotrichum spp, Fusarium oxysporum, F. solani, F. moniliforme, Penicillium purpurogenum, P. digitatum, P. oxalicum, Rhizoctonia sp, Rhizopus nodosus* (Markson *et al.*, 2012; Ogunleye and Ayansola, 2014; Gwa *et al.*, 2015; Gwa and Akombo, 2016; Shiriki *et* *al.,* 2015, Gwa and Abdulkadir, 2017, Gwa and Nwankiti, 2017a). Rot of yam tubers in storage is caused by up to 30 different pathogenic fungi (Ikotun, 1989 and Nahunnaro, 2008). There are different methods of control of these pathogens such as chemical, use of natural plant extracts as well as biological control method. Chemical control is fast but with lots of adverse effects such as killing beneficial micro and macro organisms, risk to humans, pollution of the environment (Yadav, 2010; Lakshmeesha *et al.,* 2013a). The use of plant extracts in controlling these pathogens of crops has proven to be effective (Taiga *et al.,* 2008; Nweke 2015; Gwa and Akombo 2016; Gwa and Nwankiti 2017a). Biological control using fungi belonging to the genus *Trichoderma* such as *T. harzianum*, *T. viride* and the genus *Pseudomonas* such as *P. syringae, P. chlororaphis* as well as *Bacillus subtilis* and *Gliocladium roseum* has been considered very effective as bio- control agents in controlling postharvest and storage rots of yam tubers (Okigbo, 2004; Okigbo and Emeka, 2010; Gwa *et al.,* 2016; Gwa and Nwankiti, 2017b, Gwa and Ekefan, 2017; Gwa and Abdulkadir, 2017). The bio control agents have no phytotoxic effects, target specific, eco-friendly with no pollution problem as well as promote plant growth (Mausam *et al.,* 2007; Harman *et al.,* 2004). The study therefore, focuses on the capabilities of *T. harzianum* as a bio control agent of postharvest rot causing fungal pathogens of yam tubers as alternative to synthetically used fungicides.

Materials and Methods

Source of *T. harzianum*

T. harzianum was collected from yam pathology unit, University of Ibadan, Nigeria. Stock cultures of the isolate were aseptically prepared and maintained on slants of acidified potato dextrose agar (PDA) in McCartney bottles and stored for subsequent studies.

Collection of rotted and healthy yam tubers

Ogoja and Pepa varieties of white yam (*D. rotundata*) tubers showing various degrees of rot symptoms were collected from yam farmers' barns in Zaki-Biam, Benue State, Nigeria which lies between longitudes 9^o $25'$ and 9^o $28'$E, and latitude 7^o $32'$ and 7^0 $35'$N respectively. The yam tubers were packaged in sterile polyethylene bags to avoid wounding before taken and to Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria for subsequent isolation and identification of pathogens two days after collection. The medium used for isolation of the pathogens was Potato Dextrose Agar (PDA).

Isolation and identification of fungi associated with rots of yam tubers

Tubers were washed in running tap water and were cut into approximately 2 x 2mm from the advancing edge of lesion with sterile scalpel. The cut tissues were surface sterilized for 2 minutes in 5 % Sodium hypochlorite solution in order to remove surface contaminants. The pieces were then rinsed in four successive changes of sterile distilled water and dried on sterile filter paper (Gwa and Nwankiti, 2017a). Four pieces of the sterilized tissues were plated out on the solidified potato dextrose agar (PDA). The plates were neatly covered with mastic tapes and incubated at ambient room temperature (30±5°C) for 7 days and growths were observed daily for the development of fungi. Sub-cultures of growing fungi mycelial were identified after 7 days of incubation when pure cultures were fully established (Gwa and Nwankiti, 2017a). The grown pure cultures were used for identification of the fungi with the aid of a compound microscope and identification guide (Navi, *et al.,* 1999; Burgess *et al.*, 2008).

Pathogenicity test of the isolated fungi

B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum P. expansum and *Pestalotia* sp isolated from the rotted yam tubers were inoculated into healthy *Pepa* yam tubers. Healthy yam tubers were washed under running tap water and surfaced sterilized in 5 % Sodium hypochlorite solution for 2 minutes. The tubers were rinsed in four successive changes of sterile distilled water. Cylindrical discs of 5 mm were removed from the tubers using a sterile cork borer. Mycelial discs of each fungus measuring 4 mm in diameter were taken from 5 day-old cultures of each of the fungi and each fungal disc was put into a hole in each of tubers. Same procedure was replicated for the control experiment except that sterile agar discs were used instead of the inoculum obtained from the fungi in the holes created in the tubers (Gwa *et al.,* 2017). Petroleum jelly was used to completely seal the remaining holes to prevent contamination by other pathogenic organisms. The inoculated yam tubers were replicated three times for each of the pathogens and control experiments. A total of 27 tubers of Pepa yam varieties were used in this experiment (three tubers for each of the eight pathogens and three tubers for control). The treatments were completely randomized and incubated for 14 days at ambient room temperature (30±5^{o}C) under sterile condition to allow for growth and establishment of the fungi organisms after which the tubers were examined for infection and disease development by cutting transversely at point of inoculation. Disease symptoms produced by artificial inoculation of the yam tubers with the pathogens after the incubation period were compared with those observed on the naturally infected tubers initially collected from farmers' barns. The fungi were re-isolated from the inoculated diseased yam tubers and cultured on PDA plates. The morphology of each pathogenic fungus was compared with that of the original culture obtained from the naturally infected tubers.

Preparation of fungal spore suspension and culture of *T. harzianum*

Spores suspensions of *A. flavus*, *F. moniliforme* and *P. expansum* and the antagonist; *T. harzianum* were prepared from 5 days old cultures grown on Potato dextrose agar (PDA) plates. Conidia from the surface of agar plate from these fungi were scrapped with a sterile glass rod to dislodge the spores (Nduagu *et al.*, 2008) and were each re-suspended in 1L of sterile distilled water containing 5% Tween 80 (Ismet *et al*., 2012). The spore suspensions obtained were filtered through four folds layer of sterile cheesecloth into a sterile 1000ml Pyrex glass beaker. The suspension concentrations were determined by using an improved Neubauer haemocytometer (model BS 748) and adjusted to 1×10^{6} spores per ml.

Determination of the interaction between rot fungi (*A. flavus, F. moniliforme* and *P. expansum*) and biological antagonist (*T. harzianum*) on healthy *Pepa* white yam tubers

Healthy white yam tubers of Pepa cultivar (*D. rotundata*) were used for storage when applied with different treatments. Treatments comprising *A. flavus, F. moniliforme* and *P. expansum* were each paired with *T. harzianum* separately to determine their effects on rot development in Pepa white yam tubers during storage. The tubers were also inoculated with each of the fungal isolates separately without *T. harzianum* as a bio control agent. Yam tubers without fungal isolates and *T. harzianum* served as the control. *T. harzianum* was paired with the three pathogenic fungi and yam tubers were inoculated separately according to the following inoculation regime (Okigbo and Emeka (2010):

(a) Uninoculated yam tubers (control);
(b) Tubers inoculated with *A. flavus* alone;
(c) Tubers inoculated with *P. expansum* alone;
(d) Tubers inoculated with *F. moniliforme* alone;
(e) Tubers inoculated with *T. harzianum* alone;
(f) Tubers inoculated with *T. harzianum* and *A. flavus* simultaneously;
(g) Tubers inoculated with *T. harzianum* and *P. expansum* simultaneously;
(h) Tubers inoculated with *T. harzianum* and *F. moniliforme* simultaneously;

Three tubers formed a treatment; each of the eight treatments was replicated three times giving a total of nine tubers per treatment. 72 tubers of yams were used in this experiment for the eight different treatments. The suspension for each of the treatments was poured in a hand sprayer and the yam tubers were sprayed accordingly (Sarma 1984; Wilson and Pusey, 1985). The yam tubers were arranged in completely randomized design and stored at ambient room temperature (30 ± 5 ^{0}C) for five months for two years. Record of rotted tubers were kept on periodic basis and cumulative percentage rot during storage of yam tubers that were inoculated with *T. harzianum* and the post harvest pathogens of yams in different combinations were calculated at monthly interval for five months according to the method described by Dapaah, (2013), thus, calculated as follows;

$$Percentage\ rot\ (\%) = \frac{N}{T} \times \frac{100}{1}$$

Where,
% = Percentage rot of tubers
N = Number of rotten tubers at the time of evaluation
T = Total number of tubers stored for the treatment

Data analysis

Data that were collected from the different treatments were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12, Graph Pad Prism 6 for trend graphs and significant means for each measured parameter were separated using Fisher's least significant difference (FLSD) ($P\leq 0.05$) (Cochran and Cox, 1992).

Results and Discussion

Isolation and identification of pathogenic fungi from rotted yam tubers

B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum P. expansum and *Pestalotia* sp. were identified from the rotted Ogoja and Pepa white yam tuber cultivars. These fungi are rot-causing pathogens of *D. rotundata* in different parts of Nigeria (Ogunleye and Ayansola, 2014; Okigbo *et al.,* 2015, Gwa and Akombo, 2016; Gwa and Nwankiti, 2017b). The occurrence of *B. theobromae*, *A. flavus*, *A. niger*, *F. oxysporum* in high numbers in major yam producing areas in Nigeria have been previously demonstrated by Ogunleye and Ayansola (2014), Okigbo *et al.*, (2015) and Shiriki *et al.,* (2015).

Pathogenicity tests carried out on the healthy Pepa white yam tubers produced typical symptoms of the pathogens isolated from the naturally infected tubers and the re-isolation of identical fungi from the artificially inoculated rotted yam tubers fulfils Koch's postulates and establishes the pathogenicity of the yam fungi isolates. The tubers that were treated without mycelial from different fungi (control tubers) did not produce rot suggesting the absence of inoculum in the bored yam tissues. Table 1 shows the result of inoculation of healthy white yam tuber of Pepa with *T. harzianum* and rot-causing fungi alone and in combination for two years. There was no significant difference ($P\leq 0.05$) in mean percentage rot in the treatments for the five months storage period between for the two years. However, *P. expansum* was most antagonized followed by *F. moniliforme* while the least antagonised pathogen was *A. flavus*. The result revealed that *T. harzianum* was more antagonistic in the first year of storage compared with the second year of storage (Table 2). Similar results were obtained by Markson *et al.,* (2012) and Nikolajeva *et al.,* (2012) who showed that *Trichoderma* species are useful in the control of rot fungi of fruits, vegetables and tuber diseases.

Result obtained by Okigbo and Emeka (2010) showed the biological control of rot-inducing fungi of water yam (*D. alata*) using *T. harzianum*, *Pseudomonas syringae* and *P. chlororaphis* which they both revealed that the three antagonists significantly inhibited the growth of *B.*

theobromae and *F. solani* on stored yam tubers. Result obtained by Okigbo (2005) showed the inhibitory potentials of *Bacillus subtilis* in controlling post harvest fungal pathogens of yam tubers in storage

The use of *T. harzianum* in controlling postharvest fungal pathogens of yam tubers in storage for five months is similar to the result obtained by Okigbo and Ikediugwu, (2000) that used a single application of *T. harzianum* and protected yam tubers in storage for up to 6 months. Though *T. harzianum* was able to reduce rot pathogen infections, the effect of the antagonist pairing with *P. expansum* was more potent than the inhibition recorded between the interaction of *T. harzianum* and *F. moniliforme* and *P. expansum*. The difference in antagonistic potency could probably be due to production of antifungal phenolic compounds which function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity and limiting the growth of these pathogens (Anita *et al*., 2012).

The tubers that were neither treated with pathogenic fungi nor antagonist (control tubers) showed between 8.89 % and 15.56 % rot in the first and second year respectively after five months of storage similar to the result obtained by Ekundayo and Naqvi (1972) who reported losses in yam tubers due to rots pathogens to be between 10 % and 15 % in the first three months of storage. The results however, disagreed with studies conducted by Okigbo and Ikediugwu (2000) who estimated an average of between 20 and 39.5 % of stored tubers lost to rot pathogens. Arinze (2005) and Okigbo *et al*., (2009b) reported that about 50% reduction of the total stored tubers has been reported lost to diseases within the first 6 months of storage in Nigeria. The findings revealed that *T. harzianum* (biological control agent) was more effective in inhibiting the growth of *A. flavus, F. moniliforme* and *P. expansum* in the first year of storage compared with the second year of storage. The difference in the antagonistic potential of *T. harzianum* could probably be due to favourable environmental condition such as adequate moisture or relative humidity which increased the interaction of the rot-causing fungi with the host yam tissues and decreased the potentials of *T. harzianum* in the second year. It has been reported that fungal species occurred more abundantly in the more humid months where the environmental conditions favoured the production of inoculum more than in the drier less humid period (Ekundayo, 1986; Agrios, 2005).

Table 1: Cumulative percentage rot of Pepa white yam tubers inoculated with bio control agent (BCA) and the postharvest fungi pathogens of white yam in different combinations for five months

Treatment	**Period of Storage**					
	Dec., 2015	**Jan., 2016**	**Feb., 2016**	**Mar., 2016**	**Apr., 2016**	**Mean**
1st Storage Period						
Control	0.00±0.00	0.00±0.00[b]	11.10±11.10	11.10±11.10	22.20±22.20	8.89±5.11
A. flavus alone	0.00±0.00	22.20±11.10[a]	22.20±11.10	22.20±11.10	33.33±0.00	20.00±4.36
F. moniliforme alone	0.00±0.00	0.00±0.00[b]	11.10±11.10	11.10±11.10	22.20±11.10	15.56±6.40
P. expansum alone	0.00±0.00	0.00±0.00[b]	11.10±11.10	33.30±19.20	33.30±19.20	8.89±3.94
T. harzianum alone	0.00±0.00	0.00±0.00[b]	0.00±0.00	0.00±0.00	11.10±11.10	2.22±2.22
A. flavus X T. harzianum	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	22.20±11.10	6.67±3.56
F. moniliforme X T. harzianum	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
P. expansum X T. harzianum.	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
LSD	-	*11.78*	*23.55*[ns]	*35.33*[ns]	*40.80*[ns]	*18.25*[ns]
2nd Storage Period						
	Dec., 2016	**Jan., 2017**	**Feb., 2017**	**Mar., 2017**	**Apr., 2017**	**Mean**
Control	0.00±0.00	11.10±11.10	22.20±11.10	22.20±22.20	33.30±19.20	15.56±6.40
A. flavus alone	0.00±0.00	11.10±11.10	11.10±11.10	33.33±0.00	44.40±11.10	22.22±5.31
F. moniliforme alone	0.00±0.00	0.00±0.00	11.10±11.10	22.20±22.20	33.30±19.20	17.78±6.40
P. expansum alone	0.00±0.00	11.10±11.10	11.10±11.10	33.30±19.20	33.30±19.20	13.33±6.34
T. harzianum alone	0.00±0.00	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
A. flavus X T.harzianum	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	11.10±11.10	8.89±3.94
F. moniliforme X T.harzianum	0.00±0.00	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	8.89±3.94
P. expansum X T.harzianum.	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	11.10±11.10	4.44±3.03
LSD	-	*20.40*[ns]	*28.85*[ns]	*45.61*[ns]	*44.07*[ns]	*19.71*[ns]

Means on the same column with different superscript are statistically significant ($P \leq 0.05$). ns = not significant

Table 2: Mean percentage rot of Pepa white yam tubers inoculated with *T. harzianum* (BCA) and the postharvest fungi pathogens of white yam in different combinations for two Years

Treatment	**Time of Storage**		**T-Value**	**Df**	**P-Value**
	1st Year	**2nd Year**			
Control	8.89±5.11	15.56±6.40	-0.81	26	0.42
A. flavus alone	20.00±4.36	22.22±5.31	-0.32	26	0.74
F. moniliforme alone	15.56±6.40	17.78±6.40	-0.60	23	0.55
P. expansum alone	8.89±3.94	13.33±6.34	-0.25	28	0.80
T. harzianum alone	2.22±2.22	4.44±3.03	-0.59	25	0.55
A. flavus X T.harzianum	6.67±3.56	8.89±3.94	-0.42	27	0.67
F. moniliforme X T.harzianum	4.44±3.03	8.89±3.94	-0.89	26	0.37
P. expansum X T. harzianum.	4.44±3.03	4.44±3.03	0.00	28	1.00

Conclusion

The result has demonstrated that *T. harzianum* has potentials to control *A. flavus, F. moniliforme* and *P. expansum* in post harvest Pepa white yam tubers. It is therefore, concluded that the application of the antagonist will provide better alternative measures in reducing rot of yam tubers in storage compared with the use of synthetically produced fungicides which are in many cases destructive to the ecosystem, expensive, non target specific and toxic to the applicator as well as beneficial organisms.

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INFLUENCE OF MACERATION TIME OF GRAPE POMACE CABERNET SAUVIGNON ON EXTRACTION KINETICS OF SOME POLYPHENOLS AND ANTI-DPPH RADICAL ACTIVITY OF WINES

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Abstract

The influence of five different maceration times on extraction of some polyphenols and anti-DPPH radical activity of wine samples were investigated. In focus were: caffeic acid, syringic acid and *p*-coumaric acid. Grape variety Cabernet Sauvignon was harvested in the state of technological maturity. Phytosanitary state: 100% health, sugar in must 23% and total acid in must 6.8 g/l. Alcohol fermentation and maceration was carried out by microvinification method using the pigeagé system. Sulphur dioxide was added as $K_2S_2O_5$ (10g/100 kg crushed grapes), enzymatic preparation Caractére (Car) (Enartis, Italy) with pectolytic, hemicellulase and *ß*-glycosidase activity and wine yeast *Saccharomyces cerevisiae* (BDX, Lallemand, Canada) in the amount of 20g/hl. Liquid parts were separated from the start of fermentation (3, 5, 7, 14, 21 day, respectively), and fermented without contact with pomace (seeds and skin). Control sample obtained first day, without maceration. Determination of the amount of caffeic, syringic and *p*-coumaric acids was performed using Waters Acquity UPLC H-Class with mass detector. It was found that the dynamics of extraction during alcohol fermentation all three phenolic compounds take place exponentially. Maximal amount of extraction for caffeic acid was at 13th day (2.851 mg/l), for syringic acid was at 11th day (1.096 mg/l) and for *p*-coumaric acid at 12th (1.335 mg/l) day of maceration. For anti-DPPH radical activity, the highest potential (5.8%) was found for wine macerated 21day, and lowest (40.66%) was found for wine macerated 3 days. Addition of enzymes and longer maceration leads to better extraction of phenolic compounds and to higher antiradical activity.

Keywords: *Maceration time, Phenolic compounds, Extraction, Antiradical activity.*

Introduction

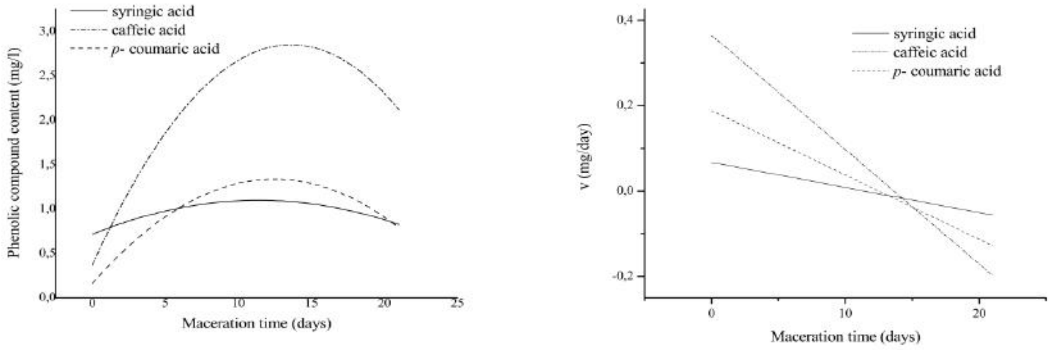
The polyphenolic compounds in wine consist in two classes of components (flavonoids and non-flavonoids) and depend on the grape variety, vineyard location, cultivation system, climate, soil type, vine cultivation practices, harvesting time, production process and ageing (Di Majo et al., 2008). Phenolic compounds come from various parts of grape bunches and are extracted during winemaking and play a very important role in enology because of their contribution to wine sensory properties and antioxidant properties. Grapes and wine contain benzoic and cinnamic acids which belong to group of phenolic acids. Benzoic acids include *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid, syringic acid, salicylic acid and gentisic acid**.** Cinnamic acids include *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid. In grapes, they are mainly present as linked glycoside, from which they are released by acid hydrolysis, and esters (gallic and ellagic tannins), from which they are released by alkaline hydrolysis. Free forms are more prevalent, mainly in red wine, due to the hydrolysis of these combinations (Ribereau-Gayon et al., 2006; Sun et al., 2011). During maceration and alcohol fermentation these compounds turn into wine from solid parts of grapes. Among other factors, the optimum pomace contact time needed to achieve the proper level and composition of phenolics in wine depends on the desired wine style and cultivar. The antioxidant compounds present in wine are derived almost exclusively from grapes and have been identified as phenolic acids, flavonols, monomeric catechins and anthocyanidins (Damijanic et al., 2012). There are a lot of winemaking techniques for better extraction phenolic compounds from solid parts of grapes such as increased maceration temperature (thermovinification), cold maceration, must or grape freezing, addition of some enzymatic preparations, prolonged maceration time etc. Addition of pectolytic enzymes improve juice yield and clarification by breaking down the pectins that provide the form structure of the berry (Lopez et al., 2009; Kelebek et al., 2009). The polyphenolic molecules have a functional role because they behave as antioxidants against the free radicals and show a physiologic role as well. In fact, they increase the antioxidant capacity in the human body after red wine consumption (Di Majo et al., 2008). The first objective of this study was to determine the moment during maceration when the maximal amounts of certain phenolic compounds were extracted. The three phenolic compounds i.e., caffeic acid, syringic acid and *p*-coumaric acid were in the focus of the study. Also, the interest of the study was to evaluate the anti-DPPH radical activity of these red wine samples obtained with different maceration time and addition of enzymatic preparations.

Material and Methods

Grape variety Cabernet Sauvignon was harvested in the state of technological maturity at the Oenological station Radmilovac of Faculty of Agriculture, Zemun, Belgrade, Serbia. Phytosanitary state: 100% health, sugar in must 23% and total acid in must 6.8 g/l. Alcohol fermentation with maceration was carried out by microvinification method at temperature of 25°C using the pigeagé system. Sulphur dioxide was added as $K_2S_2O_5$ (10 g/100 kg crushed grapes). Yeast *Saccharomyces cerevisiae* (BDX, Lallemand, Canada) in the amount of 20 g/hl and Enzyme Caractére (pectolytic, hemicellulase and ß-glycosidase activity) (Enartis, Italy) in the amount of 2 g/hl were used. Liquid parts were separated from the start of fermentation (3, 5, 7, 14, 21 day, respectively), and fermented without contact with the solid phase (seeds and skin). After the alcoholic fermentation, the wines were racked for the first time in December 2016. Second racking was done in March 2017 after which the wines were bottled into 750 ml bottles and stored until analysis. In order to detect and quantify the phenolic compounds in wine samples of Cabernet Sauvignon variety, it was necessary to separate them from the accompanying components, concentrate and purify. Solid phase extraction has been experimentally found to be the most suitable. Determination of the amount of caffeic acid, syringic acid and *p*-coumaric acid was performed using the Waters Acquity UPLC H-Class with mass detector (Waters TQ (Tandem Quadrupole, WAT-176001263). All samples were analysed three times. Anti-DPPH radical activity of the wine samples was evaluated as previously described (Blois, 1958). The obtained results were expressed as a reciprocal value I (%) multiplied by 100.

Results and Discussion

It was found that dynamics of the extraction of phenolic compounds during maceration and alcohol fermentation took place exponentially. Maximal extracted amount for caffeic acid was at 13th day (2.851±0.118 mg/l), for syringic acid was at 11th day (1.096±0.088 mg/l) and for *p*-coumaric acid was at 12th (1.335±0.214 mg/l) day of maceration (Figure 1a).



a) **b)**

Figure 1. Extraction dynamics (a) and kinetics (b) of syringic acid, caffeic acid and *p*-coumaric acid during 21 day of maceration.

According to Francesca et al. (2014), that studied these phenolic acids, during prolonged maceration, noticed increasing of its amounts until 20th day and in our research that is a few days earlier. There are many factors that could affect extraction of phenolic compounds such as temperature, yeast, enzymes etc. (Ribereau-Gayon et al., 2006).

One more parameter which can be defined during maceration is extraction rate of certain phenols from pomace to must or wine. For different phenols there are different extraction rates. In our study, the highest extraction rate had caffeic acid (0.02095) than *p*-coumaric acid (0.0151 mg/day) and the lowest had syringic acid (0.005892 mg/day). This depend of temperature, yeast, enzymes etc. (Figure 1b).

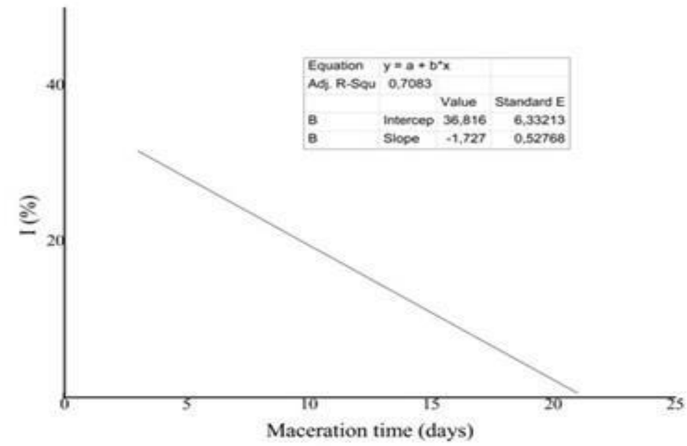


Figure 2. Anti-DPPH radical activity of wine samples during 21 day of maceration with enzyme Caractére.

Antioxidant capacity was measured by radical scavenging assay (DPPH). During prolonged maceration there is an enrichment of phenol content, what consequently improve antioxidant capacity of wine samples. This could explain the significant increase in the antioxidant capacity of Cabernet Sauvignon wine samples during prolonged skin maceration. Higher alcohol level improved the extraction of phenolic compounds leading to better antioxidant capacity of wine. With increasing total phenols content there is increasement of antioxidant properties of wine samples (Ribereau-Gayon et al., 2006). Maceration with this enzyme showed that wine sample macerated 21 day had the highest anti-DPPH radical activity (5.8%) and the lowest was observed for wine macerated 3 days (40.66%) (Figure 2).

Conclusions

In the past many researchers try to find the best way for extraction phenolic compounds from pomace to must or wine. In this study we showed one way for maceration grapes variety Cabernet Sauvignon. It is concluded that prolonged maceration lead to increasement of syringic acid, caffeic acid and *p*-coumaric acid. For this phenolic acids maximal extractions were until 13th day of maceration. The highest extraction rate was evaluated for *p*-coumaric acid. Also, prolonged maceration lead to better anti-DPPH radical activity so that wine sample obtained at 21st day had the highest.

Acknowledgements

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INTERSPECIFIC IDENTIFICATION OF SOME APHID SPECIES BASED ON THE MITOCHONDRIAL CYTOCHROME OXIDASE I (mtCOI) PARTIAL GENE

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Abstract

Aphid species identification based on host plant and morphological traits is difficult for immature aphids, sometimes even for adult aphids and in other cases for biotypes that have no or very small differences in morphological characters. However, precise identification of aphids is necessary in agriculture because of differences between species and biotypes in virus transmission efficiency or insecticides susceptibility. Fortunately, since the development of molecular techniques, mitochondrial DNA sequences were used for species discrimination and even for intraspecific differentiation between populations. In this study we performed the cytochrome c oxidase subunit I (COI) mitochondrial barcode region on 17 *Aphis gossypii* and 13 *Aphis spiraecola* populations collected from citrus, hibiscus, zucchini, potato and pepper in addition to 03 samples of *Pterochloroides persicae* collected from peach tree. Sequence analysis of the partial mtCOI gene of 709 bp fragment for the three studied species showed the utility of mtCOI as species-distinguishing molecular marker that can be used as reliable species identification of aphid species. In contrast, intraspecific discrimination was not evidenced. The two species of Aphidinae subfamily were identified in a neighbor-joining tree. Mean intraspecific sequence divergence in Aphidinae subfamily was of 6.4%, ranging from 5.8% to 7%. However, the mean interspecific variation between subfamilies (Aphidinae and Lachnidae) was higher than 10% with a range of 9.4% to 10.6%. Intraspecific variations of *A. gossypii* and *A. spiraecola* populations were insignificant with a very low clonal diversity level that varied from 00% to 0.3% and from 00% to 0.9% respectively. This molecular test evidenced that the mtCOI partial gene is a powerful marker to solve the taxonomic ambiguities that the morphological identification cannot decipher.

Key words: *Aphids, Identification, mtCOI, Reliability, Molecular*.

Introduction

The genus *Aphis* is the most species rich genus among Aphididae, with approximately 560 species known in the world (Favret, 2005). Compared to other genera, the species in the genus *Aphis* have remarkable diversity in their host plant utilization (Heie, 1986; Von Dohlen and Teulon, 2003) and display various types of lifecycles; monoecy on woody or herbal plants and heteroecy (Kim *et al.,* 2010). However, due to their rapid radiation, most of the congeneric species are often cryptic in their morphology (Kim *et al.,* 2010). Before molecular tools development for study evolutionary relationships studies, there was little data on the phylogeny of *Aphis* species because of their similar morphologies. Phylogeny within the Aphidini tribe has been partially based on changes in host plant utilization during the evolution of aphids (Komazaki *et al.*, 2010). Morphology and host plant association are significant sources of aphid classification. Heie (1986) asserted that, identification using the morphological characters and host-plant affiliation are more reliable than those based on morphological data alone. However, identification could be restricted by substantial intraspecific variation (Margaritopoulos *et al*., 2000). There are many reports of using mitochondrial DNA to study aphid phylogeny (Stern, 1995, Turcinavicien *et al*., 2006, von Dohlen *et al*., 2006) for species discrimination (Raboudi *et al*., 2005, Valenzuela *et al*., 2007) and even for intraspecific differentiation between aphid populations (Boulding, 1998, Anstead *et al.*, 2002). As a result, they are widely used to solve the taxonomic ambiguities of insect pests. In the light of this, we have decided that it will be very useful to carry out molecular identification work on two economically important aphid species in the study area, *Aphis gossypii* and *A. spiraecola* populations collected on different crops in the Chlef Valley. This study will verify the effectiveness of the partial mitochondrial mtCOI gene as species identification marker and its ability to detect possible intraspecific variations existing within populations of the same species.

Material and methods

Molecular characterization of aphid's populations

In order to assess the reliability of the Mitochondrial Cytochrome Oxidase I (mtCOI) partial gene fragment to identify interspecific and intraspecific variation within the Aphidinae subfamily. A genetic approach was performed on Five (5) *A. gossypii* populations (Ag_{CITR}, Ag_{HIBI}, Ag_{COUR}, Ag_{POMM} and Ag_{POIV}) sampled from citrus (*Citrus sinensis* (Osbeck, 1765): Rutaceae), hibiscus (*Hibiscus syriacus* (L, 1661): Malvaceae), zucchini (*Cucurbita pepo* (L, 1753): Cucurbitaceae), potato (*Solanum tuberosum* (L, 1753): Solanaceae) and pepper (*Capsicum annuun* (L, 1753): Solanaceae) respectively, and five (5) populations of the green citrus aphid *A. spiraecola* collected from the same plant hosts, characterized respectively by As_{CITR}, As_{COUR}, As_{POMM}, As_{HIBI} and As_{POIV}. One (1) population of the giant black bark aphid *Pterochloroides persicae* (Pp) (Hemipetra: Lachninae) sampled from peach trees (*Prunus persica* L. Batsch) was added as out of group to assess genetic variation degree between subfamilies. All aphid samples were stored in 70% ethanol at -20°C. Selected available mtCOI sequences were retrieved from NCBI GenBank and used in this study for intraspecific and interspecific variation of the three studied species.

DNA Extraction and PCR

Aphids stored in 70% ethanol were rinsed twice with RNase and Protease free molecular biology water and then with 100 µl of dellaporta extraction buffer. Five adult apterous aphids were randomly selected from each sample. Total nucleic acids were extracted using the protocol described by Dellaporta *et al* (1983). The Mitochondrial Cytochrome Oxidase I (mtCOI) partial gene fragment (709pb) was amplified using universal barcode primers (LCO1490F) 5̀GGT CAA CAA ATC ATA AAG ATA TTG3̀ and (HCO2198) 5̀TAA ACT TCA GGG TGA CCA AAA AAT CA3̀ (Folmer *et al*, 1994; Zhang and Hewitt, 1997; Cocuzza *et al*, 2008; Komazaki *et al*, 2010; Sharma and Koabayashi, 2013; Zhang *et al*, 2014, Lee *et al*, 2015 and Lokeshwari *et al*, 2015). Synthesized at Integrated DNA technologies IDT, Coraville, Iowa, USA. PCR was performed in a thermocycler (BIORAD C1000©) with the following cycling parameters: five cycle of 94°c for 40s, 45°c for 40s, 72°c for 60s, followed by 35 cycles of 94°c for 40s, 51°c for40s, 72°c for 60s and 72°c for 10mn as final extension. PCR was performed was performed in a 25 µl total reaction volume containing 0.5 µl of each primer, 2.5 of PCR buffer, 0.5 µl of dNTPs, 0.25 µl taq DNA polymerase (Roche diagnostics international LTD). The amplified products were resolved in 1% agarose gel, stained with ethidium bromide (0.6µl/ml). PCR products were submitted to further mtCOI sequencing. Homology tests were carried out using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), compared with published sequences available in the NCBI, and matched with corresponding region of mitochondrial COI. All the sequences generated in the present study and retrieved sequences corresponding to COI, were aligned with the multiple sequence alignment software CLUSTAL OMEGA at nucleotide level. Phylogenetic tree was generated using neighbor-joining method (NJ) (Saitou et Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004). Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016) .

Results and Discussion

In total, 33 aphids' population were included for COI data set, among them 17 *Aphis gossypii* samples of Ag_{CITR}, Ag_{HIBI}, Ag_{COUR}, Ag_{POMM} and Ag_{POIV}, 13 *Aphis spiraecola* samples characterized by As_{CITR}, As_{COUR}, $As_{POMM,}$ As_{HIBI} and As_{POIV} and three (3) *Pterochloroides persicae* samples found on peach trees. Sequencing of the partial mtCOI gene yielded a 709 bp fragment for *A. gossypii*, *A. spiraecola* and *P persicae* (Fig 1). Comparison of the replicate sequences for the 33 samples showed no mismatch, indicating no sequencing errors. No pseudo-genes were amplified in any of the sequences subjected to analysis, as indicated by the absence of stop codons. A BLAST search for the sequences showed highest hits for the respective species. The sequences obtained were deposited in NCBI-GenBank with accession numbers MH632726 to MH632734.

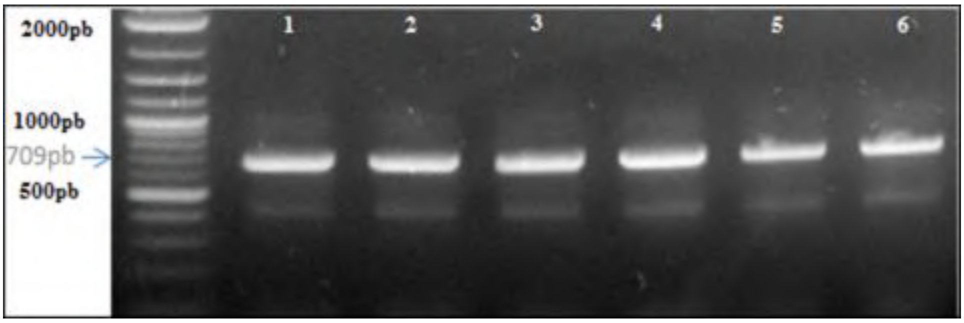

Figure 1. Polymerase chain reaction products (709pb) were amplified using universal primers set: LCO1490F-HCO2198. PCR products were separated in a 1% agarose gel and stained with ethidium bromide.

The partial sequence of the mtCOI (709bp) of *A. gossypii*, *A. spiraecola* and *P. persicae* generated in this study were compared between them. The results of our molecular analysis based on the mtCOI region revealed that in term of species identification the use of mtCOI gene enabled rapid and effective identification between the three species. The two species of Aphidinae subfamily (*A. gossypii* and *A. spiraecola*) were identified in a neighbor-joining tree. Their mean interspecific sequences divergence was 6.4% with a range of 5.8% to 7%. Mean nucleotide sequence variations between subfamilies (Aphidinae and Lachnidae) was significantly high (10%) with a range of 9.4% to 10.6%. The phylogenetic tree was made to compare the studied aphids' population shown in Fig 2 demonstrated that the aphid samples used in this study were divided in three distinct and robust clades. *A. gossypii* populations were clustered together with mtCOI partial gene marker of several *A. gossypii* worldwide populations. In the same way *A. spiraecola* samples collected from hibiscus, citrus, zucchini, pepper and potato were grouped in a single cluster with *A. spiraecola* species from several parts of the world. A third cluster grouped sequences of the studied *Pterochloroides persicae* with other populations collected overseas.

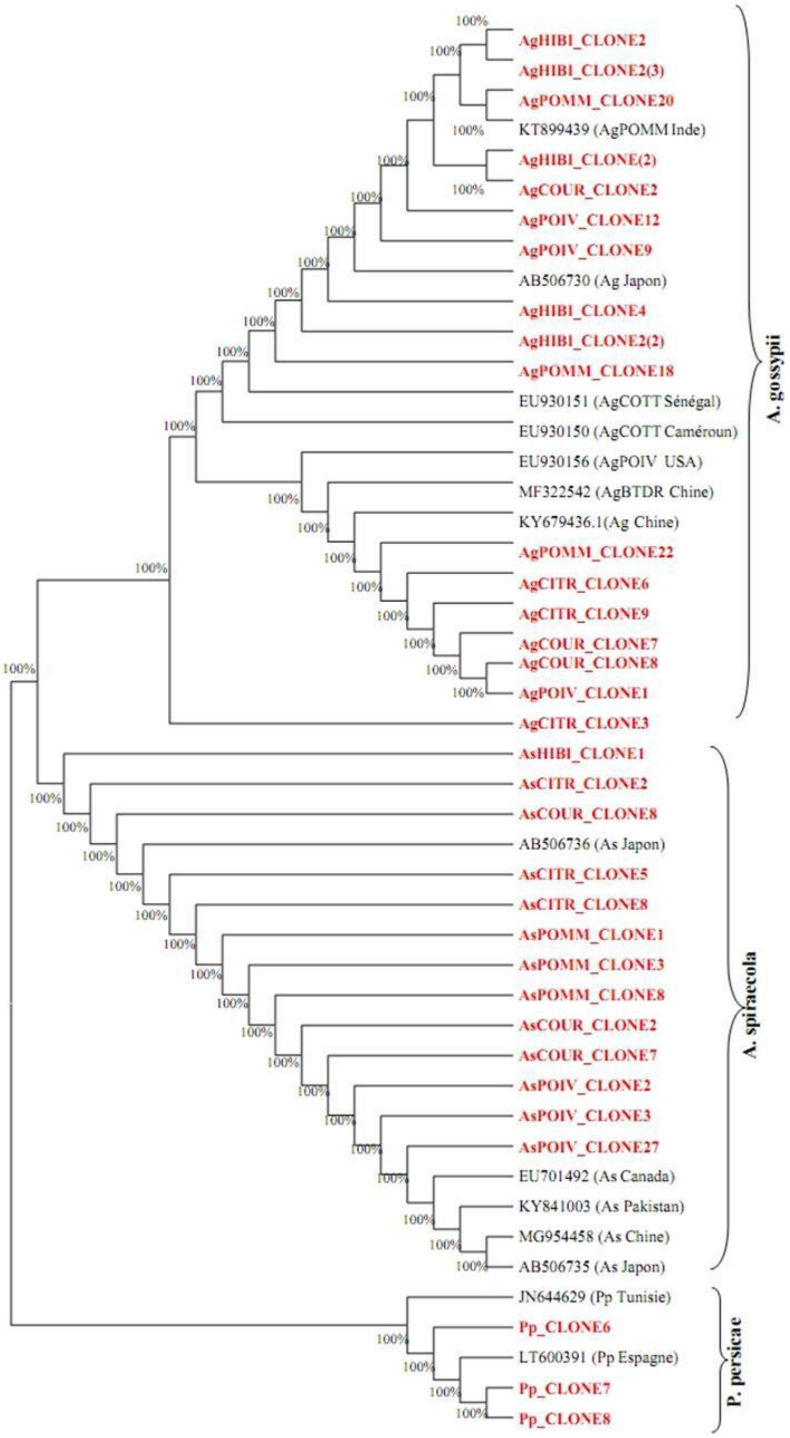

Figure 2. Neighbor-Joining phylogenetic tree depicting evolutionary relationships among various populations of *A gossypii*, *A spiraecola* and P persicae collected at Chlef valley based on analysis of a 709 bp mitochondrial cytochrome c oxidase subunit I – COI DNA fragment.. Evolutionary analyses were conducted in MEGA7

Comparison of the mtCOI sequences from 17 populations of *A. gossypii* collected from five different host plants showed that there were a little nucleotide variation among them, confirming a relatively recent divergence as reported by various researchers (Lee *et al.*, 2015; Lokeshwari *et al.*, 2015; Chen *et al.*, 2013; Komazaki *et al.*, 2010, Carletto *et al.*, 2009). Sequence identity matrix of mtCOI sequences of *A. gossypii* and *A. spiraecola* populations showed a very few nucleotide divergence between the studied population (data not shown).

However intraspecific variations within species was very low, *A gossypii* and *A. spiraecola* populations showed only 00% to 0.3% and 00% to 0.9% of nucleotide variation respectively. We suggest that intraspecific identification requires other molecular tools, either nuclear or mitochondrial markers in addition to biological assays such as host transfer experiments.

Conclusion

In the present study, we have shown the utility of the mitochondrial cytochrome oxidase mtCOI gene as a molecular marker distinguishing different species of aphids. This is the first time in Algeria that this molecular technique has been used for aphid species identification. Although this molecular tool has demonstrated a high reliability for interspecific aphid's identification, it couldn't decipher intraspecific variations between different populations of *A. gossypii* and *A. spiraecola* collected from various host plant. We considered the work as pioneer trial of molecular identification of aphid species. It will help to solve taxonomic ambiguity of *Aphis* genus populations present on crops of the valley leading in one hand to precise determination and thus adequate sustainable management strategy, and in the other hand allows prompt and reliable identification of alien aphis species that can be of great economic importance.

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FUNGAL PATHOGENS OF BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) IN SERBIA

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Abstract

Birdsfoot trefoil (*Lotus corniculatus* L.) is a species adapted to field conditions in the most important livestock production region in the all continents and constitutes a very valuable forage species. It has a high ability to utilize nutrients and has very modest demands on the conditions of its growth. The roots of birdsfoot trefoil are associated with bacteria that fix atmospheric nitrogen and, thusly, its populations increase the availability of nitrogen in the soil. This species often forms dense, fibrous root networks that reduce soil erosion. Isolation of the pathogen was done from the leaves and roots. Black leaf spots were observed on birdsfoot trefoil in field plots in Serbia. These spots were circular to irregular. Single lesions often coalesced to form larger lesions and became dark brown. On the roots systems of a large number of plants birdsfoot trefoil symptoms of light to dark brown necrosis and discoloration of conductive tissues were observed. There has not been a systematic research of birdsfoot trefoil mycoflora in Serbia. This research aims to present the results of preliminary research of mycopopulation of 12 different genotypes of birdsfoot trefoil. Total of 480 plant parts have been examined and 7 genera of fungi were isolated: *Alternaria*, *Fusarium*, *Phythophthora*, *Mucor*, *Sclerotinia*, *Bipolaris* and *Rhizoctonia.* Considering the importance of birdsfoot trefoil as a fodder crop in Serbia, the aim of this study was to identify phytopathogenic fungi as casual agents of diseases in birdsfoot trefoil for a clearer perception of problems (the extinction of plants, reducing yields, deterioration of the quality of feed and others) arising as a result of the presence of those fungi.

Key words: *birdsfoot trefoil, fungi, mycoflora.*

Introduction

Birdsfoot trefoil is a widespread plant throughout the world. It originates from Western Europe and North Africa. Birdsfoot trefoil is a very valuable fodder crop that has an important place in our country. It can be cultivated as a single crop or mixed with other crops and used for grazing or for conservation in the form of hay or silage. In addition to being cultivated as a single crop, birdsfoot trefoil has the most important place in grass-leguminous mixtures in the establishment of planted grasslands (Vučković et al., 2010). Birdsfoot trefoil is a plant that thrives on all types of soil, whether sour, lime, sandy, clay or salty. It grows on low quality soils that are not favorable for alfalfa (Vučković et al., 2010).

There are small requirements for soil acidity (Petrović et al., 1996). Birdsfoot trefoil hay contains an average of 18% of crude protein. Birdsfoot trefoil does not cause bloat in ruminant animals (Vučković et al., 2010). The Birdsfoot trefoil is distinguished by its high digestibility and ability to utilize nutrients and very modest requirements considering the conditions of its growth, especially the soil (Petrović et al., 1996). Due to the developed symbiotic bacteria at the root, significant quantities of nitrogen can be fixed annually, which according to some authors exceed 200 kg/ha (Vučković et al., 2010).

Phytopathogenic causative agents of birdsfoot trefoil disease can be present year after year. They are of different intensity and can cause significant losses in yield and the quality of hay. Diseases can limit persistence of *Lotus* spp. in production systems. Several pathogens are involved in a ''diseases complex''. Nevertheless, little is known about diseases and their impacts, both in the world and ours. Fungal pathogens are the most prevalent organisms and according to the plant tissue they can affect, diseases are classified as follows.
The genus *Epicoccum* was reported in Argentina as a seed pathogen in *Lotus* spp. (Sisterna and Lori, 2005). Also, *Epicoccum nigrum* in Argentina was cited as a causative agent of brown leaf spot of birdsfoot trefoil (Colavolpe et al., 2018).
Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *loti*) is a serious disease of birdsfoot trefoil, reducing yield of forage and seed (Miller-Garvin et al., 2011). Similarly, inducers of diseases such as *Rhizoctonia solani*, *Cercospora medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Verticillium albo-atrum*, as well as species of genus *Phytium*, *Leptosphaerulina*, *Phoma*, *Fusarium* and *Alternaria*, are significant disease agents in birdsfoot trefoil as a fodder crop, spread in all the areas of its production (Villegas-Fernández and Rubiales, 2011; Sillero et al., 2014, Vasić, 2015, Vasić, 2017). If care is not taken, the disease can cause serious damage to birdsfoot trefoil seedlings.
Considering the importance of birdsfoot trefoil as a fodder crop in Serbia, the aim of this study was to identify phytopathogenic fungi as casual agents of diseases in birdsfoot trefoil for a clearer perception of problems (the extinction of plants, reduction of yields, deterioration of the quality of feed and other) arising as a result of the presence of those fungi.

Material and methods

For the mycopopulations study, samples were collected from the experimental plant genotypes of birdsfoot trefoil (*Lotus corniculatus* L.) originating from Serbia, from the Rasina region (Gaglovo 1 and 2, Globoder 1, 2, 3, 4, 5, 6 and 7), Mačvanski region (Svileuva 1 and 2) and Pomoravski region (Gložane). The samples were collected between Mart and June 2016-2017 at the location of the Institute for forage crops in Globoder. Parts of plants are carefully washed under running water. After washing, the parts of stem and roots are cut to piece of 0.5-1 cm in size. Prepared samples of roots and stems were disinfected with 96% ethanol for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 minute and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (roots and tree) were placed in each Petri dish in four replications. They were kept in a thermostat at 25°C in 12 h light / 12 h night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial grow, the peak part of the mycelium was reseeded on PDA again.
Microscopic examination was performed using microscopes Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. Calculated by the frequency of isolation in % according to the formula Vrandečić *et al*. (2011):

$$\text{(\%) Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

In the study of mycopopulations of birdsfoot trefoil genotypes, total of 480 plant parts were analyzed. Fungi were isolated on all plants from birdsfoot trefoil, and there were clear symptoms on stems in the form of spots and necrotic lesions. Fungi from genus *Alternaria*, *Phythophthora*, *Fusarium* and *Rhizoctonia* were isolated from these plants. Also, in small number of plants, there were necroses with white airy mycelium in the lower third of stems and fungi from the genus *Sclerotinia* and *Bipolaris* were isolated from those plants. Also, saprophytic fungi from the genus *Mucor* were isolated (Table 1).

Table 1. *Frequency of fungal isolation on Lotus corniculatus* L.

Genotypes	***Number of samples***		***Fungi species - stem***	***(%) Isolation frequency***	***Fungi species - root***	***(%) Isolation frequency***
	Plant part - leaf	***Plant part - root***				
Globoder 1	20	20	*Alternaria* sp. *Fusarium* sp. *Phythophthora* sp.	15 10 25	*Rhizoctonia* sp. *Fusarium* sp.	40 35
Globoder 2	20	20	*Alternaria* sp.	60	*Rhizoctonia* sp.	75
Globoder 3	20	20	*Phythophthora* sp. *Alternaria* sp. *Mucor* sp.	25 20 40	*Fusarium* sp.	85
Globoder 4	20	20	*Alternaria* sp.	35	*Rhizoctonia* sp.	65
Globoder 5	20	20	*Fusarium* sp. *Rhizoctonia* sp.	25 10	*Fusarium* sp. *Rhizoctonia* sp.	65 20
Globoder 6	20	20	*Phythophthora* sp.	60	*Fusarium* sp. *Rhizoctonia* sp.	10 85
Globoder 7	20	20	*Alternaria* sp. *Mucor* sp.	40 15	*Rhizoctonia* sp.	75
Svileuva 1	20	20	*Rhizoctonia* sp.	35	*Fusarium* sp. *Rhizoctonia* sp.	25 70
Svileuva 2	20	20	*Fusarium* sp.	10	*Fusarium* sp. *Rhizoctonia* sp.	20 65
Gaglovo 1	20	20	*Alternaria* sp.	60	*Fusarium* sp.	75
Gaglovo 2	20	20	*Sclerotinia* sp. *Bipolaris* sp.	30 35	*Fusarium* sp.	85
Gložane	20	20	*Alternaria* sp. *Fusarium* sp.	15 35	*Fusarium* sp.	90

The symptoms of a light to dark brown necrosis on the root system of the plants were observed, and from these plants fungi of the genera *Rhizoctonia* were isolated. Discoloration of the conductive tissues of the root system was observed in a large number of plants, and from these plants, fungus of the genus *Fusarium* was isolated (Table 1).

Isolations were conducted in all the birdsfoot trefoil plants with clearly visible symptoms of the disease.

In these studies, there was a difference in the frequency of isolation of certain genera of phytopathogenic fungi in birdsfoot trefoil genotypes originated from different regions of Serbia. It has been observed that in birdsfoot trefoil genotypes that originated from the Rasina region, fungi of the genera *Alternaria*, *Rhizoctonia* and *Fusarium* were more frequently

isolated. While the genera *Sclerotinia* and *Bipolaris* were isolated in significant percentages on one sample originating in the Rasina district (Table 1). Likewise, genera *Fusarium* and *Fusarium* were more often isolated from genotypes originated from the Pomoravlje region. While in the Mačva region isolated fungi from the genus *Fusarium* and *Alternaria*.
The results indicate that birdsfoot trefoil is vulnerable to the attack of a large number of phytopathogenic fungi that can have a significant impact on reducing its yield and quality.
In all the plants, in which isolations were conducted, there were clearly visible symptoms of the disease present. In these studies, there was difference in frequency of isolation of some genera of phytopathgenic fungi in birdsfoot trefoil genotypes from three regions in Serbia. It was observed that in the genotypes that originated in Serbia, fungi of genus *Rhizoctonia*, *Fusarium*, *Alternaria*, *Phythophthora* and *Sclerotinia* were frequently isolated.
Birdsfoot trefoil are adversely affected by numerous fungal diseases leading to a steady reduction in the cultivated area in many countries. Crown and root diseases considered chronic diseases, causing plant death and losses of 60 to 80%. Typical symptoms are rot and wilt. The main Genus is *Fusarium* (*F. solani*,*F. oxysporum, F. vertillicioides* and *F. equiset*) (Sisterna and Lori, 2005). Stem and foliar diseases onse do not cause directly the plant death. They contribute to the progressive weakness of the plant through the effects on the basic metabolic processes. The reported pathogens are *Phomopsis loti* (blight) *Colletotrichum destructivum* (anthracnose), *Stenphylium loti* (leaf spot) (Vasić et al., 2016, Sisterna and Lori, 2005). Seed diseases they cause decrease in germination and damping off. Fungi genera present in the seed are: *Alternaria, Aspergillus, Bipolaris, Botrytis, Cladosporium, Colletotrichum, Culvaria, Epiccocum, Fusarium, Leptosphaearulina, Penicillium, Phoma, Phomopsis, Stemphylium* and *Verticillium* (Sisterna and Lori, 2005).
The *Fusarium oxysporum* isolates pathogenic to birdsfoot trefoil have a unique host range relative to other pathogenic *Fusarium oxysporum* tested; they cause severe vascular wilt on birdsfoot trefoil but not on alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), dry bean (*Phaseolus vulgaris* L.), or soybean [*Glycine max* (L.) Merr.]. *Fusarium oxysporum* f. sp. *loti* is only known to occur in USA (Miller-Garvin et al., 2011). Altier et al. (2000) showed that one cycle of phenotypic selection in the greenhouse using a composite of Uruguayan *Fusarium oxysporum* isolates increased the level of resistance to Fusarium root and crown rot in birdsfoot trefoil adapted to Uruguay. The Uruguayan *Fusarium oxysporum* isolates cause vascular wilt and root necrosis similar to the disease symptoms caused by *Fusarium oxysporum* f. sp. *loti*, but the wilt symptoms develop more slowly. The Uruguayan isolates of *Fusarium oxysporum* are primarily associated with disease development on mature plants, with disease symptoms and plant mortality becoming most severe in the second and third production years. *Fusarium oxysporum* f. sp. *loti* causes considerable plant mortality at the seedling stage Miller-Garvin et al. (2011).
Al-Jaradi et al. (2018) state the fungus of the genus *Alternaria* spp. causing various types of spots and blights on *Vicia faba, Pisum sativum* and *Vigna unguiculata.* Likewise, Coca-Morante and Mamani-Álvarez (2012) detected fungus from the genus *Alternaria* on *Vicia faba* plants in Bolivia. At three sites in Bolivia, *Alternaria* sp. were found during the vegetative growth of the plants. Problems appeared as isolated spots. Colavolpe et al. (2018) in Argenitina for the first time stated *Epicoccum nigrum* as causing a brown leaf spot of birdsfoot trefoil. On infected plants birdsfoot trefoil, yellow spot and black spots were spotted (Sisterna and Lori, 2005).

Conclusion

This paper presents the preliminary results of mycopopulations research in 12 experimental birdsfoot trefoil genotypes. Birdsfoot trefoil is an important forage crop and its importance as livestock feed is growing within our country. This research is the beginning of a more comprehensive study of phytopatogenic fungi on of birdsfoot trefoil. So far, there were no significant researches in this direction in Serbia, so the future researches related to birdsfoot trefoil will go in the direction of selection of genotypes with increased tolerance to fungal diseases.

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PRESENCE OF VIRUSES IN THE POPULATION OF GRAPEVINE CULTIVAR "PROKUPAC" (*Vitis vinifera L.*) IN RASINA DISTRICT, SERBIA

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Abstract

Grapevine cultivar "Prokupac" is officially recognized as Serbian autochthonous variety, It has a long history, tracing to the Middle Ages. This cultivar is used to be the most widespread variety in this part of Balkans and best-rated domestic Serbian wine at international markets. Survey of virus infection incidence in grapevine nurseries, was conducted at 14 location of Rasina district (Republic of Serbia), during 2018. The survey was conducted by Agricultural Service Krusevac, as authorized institution with a professional capacity included into a national phytosanitary system, for implementation of the system of control of prevention of fruit tree and grapevines pathogens (including export and import). It also controls the presence of pathogens in main nurseries. Total number of 17 samples was tested on presence of four viruses using ELISA: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated viruses 1, 2* and *3* (GLRaV-1, GLRaV-2, GLRaV-3). The presence of GLRaV-1 was confirmed in one tested sample, and the presence of GLRaV-1, GLRaV-2, GLRaV-3 were also confirmed in one tested sample. Conducted investigation indicates deteriorated viral sanitary status of cultivar "Prokupac" and necessity for intensifying the clonal and sanitary selection program.

Key words: *Virus diseases, Prokupac, Autochthonous grapevine cultivar, ELISA*

Introduction

"Prokupac" is an old autochthonous grapevine (*Vitis vinifera L.*) variety originating in Serbia that belongs to *Convar pontica, Convarietas balcanica* (Bešlić et all., 2012). According to the literary data, besides Prokupac and other autochthonous varieties Smederevka, Plovdina and Začinak have been cultivated in this area since Roman Empire (3rd century B.C.) (Jiriček, 1923). In the past it had much greater significance than it does today. It is grown in Serbia, Macedonia and Bulgaria. The great value of this variety is reflected in the fact that it produces a wine of pink or red color. Grapes are also used for blending, but also for the production of lozovača (rakia made from grapes) and vinjak (a brand of brandy). The trend in wine production is changing and is looking for authentic and specific wines of a certain area, where autochthonous varieties play an indispensable role. In the last few years, work on the prevention of genetic resources and clonal selection of this variety has started, with the aim of its preserving and restoring cultivation on farms. A major problem in its spread is the lack of quality planting material, where by viruses play a significant role since they have a very detrimental effect on the growth and development of the vine (Walter and Martelli, 1998), and, among other things, to a considerable extent may affect the reduction of sugar and increase in acids in freshly crushed must (wine juice) (Besse et al., 2009; Borgo and Angelini, 2002), the reduction of photosynthesis (Bertamini et al., 2004) and, in general, the reduction of grape yields, delay in vegetation and ripening, affect the chemical composition and aromatic profile of must, reduced reception of the rootstock and graft stem and poorer rhizogenesis. Their harmfulness is further enhanced by the fact that as obligate biotrophic

parasites, their development and reproduction requires host living cells, and thus cannot be eliminated by conventional chemical methods (Juretic, 2002).
The aim of this study was to determine the health status of the Prokupac variety in terms of infection with economically the most harmful and the most widespread grapevine viruses by using the ELISA method.

Material and Methods

The research included 14 localities in the Rasina District in Serbia. When testing samples were selecting, those with good viticultural characteristics and satisfactory exuberance were selected which did not show any visible signs of viral infections (leaf curl and deformities, purple color on leaf face, etc.). Samples were collected during the 2018 vegetation resting phase. Sampling was carried out on the presence of the following viruses: Grapevine fanleaf virus / GFLV and Grapevine leafroll virus / GLRaV-1,2,3, in native plantations for the production of the stems of vines. For native plantations of the grapevine for the production of the stems in the gantry system, a visual inspection of all the grapevines in the nursery was carried out in accordance with the Rule Book and mandatory sampling of 10% of the native grapevines from which 1% of the collective samples are formed. A group of 10 adjacent vines or from adjacent rows is selected from which every second (to be marked) is sampled and a group pattern is formed. The specimens contain five central parts of the stems 10-15 cm long. The specimens were packed in plastic bags, labeled and refrigerated at 4 ° C until testing and with the official order of the *competent service – Agricultural Service Kruševac* forwarded to an authorized first-stage diagnostic laboratory (Agriculturaal Service Smederevo), according to the procedure described in the Rule Book on crop health inspection and planting material production facilities (Dulić-Marković, 2008).
Commercial ELISA kits were used in their analysis, and all steps of the analyzes were performed in accordance with the test manufacturer's recommendations. Each sample was tested by ELISA for the presence of four viruses:
- Grapevine fanleaf virus, GFLV,
- Grapevine leafroll-associated virus 1, GLRaV-1,
- Grapevine leafroll-associated virus GLRaV-2,
- Grapevine leafroll-associated virus 3, GLRaV-3.

The following antibody double antibody sandwich ELISA, DAS-ELISA for GFLV, GLRaV-1, GLRaV-2, GLRaV-3 was used for virus presence testing. Monoclonal antibodies were used in GLRaV-2, while polyclonal antibodies and serums were used for other viruses.

Results and Discussion

The conducted analyzes determined a low level of infection of the variety "Prokupac" in the area of Rasina District, Republic of Serbia. Of the 14 samples tested, the presence of GLRaV-1 was detected in one sample, while the presence of GLRaV-2, GLRaV-3 and GFLV was not present in the samples tested. It should also be pointed out that monoclonal antibodies were used in the detection of GLRaV-2, so the possibility of this virus is slightly higher than determined by the conducted research. The main vectors of both GLRaV-1 G and LRaV-3 are in the production of vineyards except humans also the use of infected planting material and different varieties of scale insects. Then the possibility that the plantation can be infected by viruses after vines planting cannot be excluded. From mixed infections, the presence of the combination of GLRaV-1 + GLRaV-2 + GLRaV-3 virus in one tested sample was determined.
The determined health status of native grapevines of the autochthonous variety "Prokupac" is similar to the results of the conducted research on other autochthonous varieties in the Rasina District. According to the research results (Miletaković and Jovanović, 2018) related to the

presence of GFLV, GLRaV-1, T GLRaV-2 and GLRaV-3 on a total of 17 samples in the native vineyard of the autochthonous variety "Smederevka", using the ELISA test, the presence of GLRaV-3 was determined in four tested samples (23.53%), while other viruses did not exist in the tested samples or mixed infections. The occurrence of GLRaV-3 was found in the mixed infection in this Study, so it can be concluded that the infection with this virus of the "Prokupac" variety is much smaller than the infection of the "Smederevka" variety. In both Studies, a large percentage of plants was free from all four studied viruses.

Conclusion

The results of this, as well as similar research, indicate the relatively good health of the native vines of our autochthonous varieties grown in nurseries in the Rasina District. In order to eliminate the presence of viruses in native plants of autochthonous vine varieties, a number of measures should be applied which individually cannot provide major effects. In the future, many varieties, including Prokupac, in order to produce certified planting material, it would be necessary to carry out the thermotherapy procedures and meristem culture to produce virus-free plants. Cured plants could show better production characteristics and thus become more interesting to both nurseries and grape and wine producers. All the above mentioned point to the need to intensify the program of clonal and phytosanitary selection for the purpose of production of certified planting material with the aim of preserving and revitalizing this very valuable perspective and autochthonous variety.

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PHYTOCHEMICAL AND BIOLOGICAL SCREENING OF APIACEAE THAPSIA GARGANICA L.

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Abstract

Phytochemical screening of the crude ethanolic extracts of the aerial part (PA) and the subterranean part (PS) of the plant *Thapsia garganica* L. was carried out by TLC and GC/MS. The results of these phytochemical characterizations showed that the two parts (PA and PS) of the plant contain flavonoids, tannins, saponosides and alkaloids in the form of traces. The study showed that the plant did not contain anthocyanins and leucoanthocyanans. PA and PS are poor in coumarins and iridoids. Gallic tannins are present with significant intensity in both parts of the plant. Phytochemical tests showed the presence of glucosides. The CG-MS gave an idea about the composition of the crude extracts of this plant. Eight compounds were identified for the crude extract of PA and 5 compounds for the crude extract of PS. The major constituents for these extracts were: phytole, hexadecanoic acid, octadecanoic acid, hexadecanoic acid, erucylamide and 13-docosenoic acid amide erucamide. In order to highlight the bioinsecticide potential of the extracts of this plant, toxicity tests were carried out on the 5th instar larvae of the migratory locust by testing 5 doses for PS ranging from 100 to 3000 µg/larvae and 4 doses for AP ranging from 300 to 3000 µg/larvae. The results of the toxicity tests revealed an increase in mortality as a function of time. The ethanol extracts tested hadgood insecticidal activity with a dose-response relationship. The 100% mortality was obtained 4 h after treatment for PS and after 24 h for PA at the highest doses tested.

Key words: *phytochemical screening, biological screening, Thapsia gargarina.*

Introduction

Current methods of curative control against gregarious locusts use liquid insecticidal products whose active ingredients belong to organophosphorus, pyrethroid and carbamate family. These synthetic preparations proved to be both very effective on locust pests but also harmful to human and animal health. Insecticides cause a significant accumulation of active ingredient in the treated ecosystems, and contribute to the development of resistant insects (Barbouche et al., 2001). These environmental and health concerns highlight the need for new strategies to protect crops from insect infestation, that are more "environmentally-friendly" to the environment and human health (Nenaah et al. 2011 and Cespedes et al. 2015).
Many plant secondary metabolites have significant biological activity associated with the presence of alkaloids, iridoids, monoterpenes, sesquiterpene, lactones, di-and tri-terpenes, naphthoquinones, anthroquinones, coumarins, phenylpropanoids, flavonoids, and other types of phenolics.These compoundscan act as insecticidal, ovicidal, ovipositional deterrents, feeding deterrents and growth retardants to pests through acute toxicity, enzyme inhibition, and interference with the consumption and/or utilization of food. In insects. These compounds have different sites of action and different molecular targets when they interact with enzymes and metamorphosis processes (Selin-Rani, 2016 and Cespedes et al., 2006). Recent studies proved that essential oils, alkaloids and extract of some plants like *Calceolaria talcana* and *C.*

*integrifolia*are potent neurotoxic and giving symptoms similar to those produced by organophosphates and carbamates insecticides.
Research focusing on the modes of action of phenolic compounds has shown that their insecticidal and insect growth regulators activities are largely due to their anti-feeding and inhibitory effects on enzymes and metabolism as a whole (Muñoz, 2013 and Isman 2006). The extracts of many plants are a mixture of potentially bioactive substances whitch may act synergisticallyby combiningseveral effects: antifeedants, insect growth regulators, and inhibitors of pupation, emergence, malformations and enzymes (Cespedes et al., 2005 and Cespedes et al., 2015a). These mixtures possess an exceptional activity and can build promising sources of bioactive molecules for botanical insecticides (Cespedes et al. 2015b).
The present work is a continuation of our previous research on alternative natural compounds of chemical insecticides. Thus, the objective of the current study was to assess the bioinsecticidal effect of the crude ethanolic extract from the aerial part (AP) and the subterranean part (SP) of the algerian plant *Thapsia garganica* L. on the fifth instar larvae of the migratory locust *Locusta migratoria*. Also, this work, aims to search and identifiate the secondary compounds of this Apiaceae.

Material and methods

Plant material

The perennial Apiaceae, *T. garganica* was harvested on January 2013 in the region of Ain Derias, in the Wilaya of M'Sila-Algeria. This plant has been authenticated by Professor Keddad, phytopathologist at the High National School of Agronomy (ENSA), El-Harrach-Algiers.The harvest was made during the winter outside the flowering period. The sampling concerned the aerial (stems and leaves) and the subterranean (roots) of the plant. The plant, freshly harvested, was washed and dried in the dark at room temperature for about 4 weeks After drying, the spraying of the AP and SP in fine powder was made separately using "Moulinex" electric grinder "Moulinex". The obtained powders were stored in sealed glass dishes.

Insect rearing

Mass rearing of *L. migratoria cinerascens* was carried out in the laboratory according to the method of Pener et al. (1989), using adults collected in the field in the Adrar region, Algeria. Larvae were held in 45 X 50 X 50 cm cages at 30-32 °C, 50-70% RH, and under a 12 h:12 h, L:D photoperiod. Locusts were fed an *Avena sterilis* based diet, complemented with wheat germ.

Preparation of crude ethanolic extract, phytochemical screening and GC–MS analysis

The crude ethanolic extract of the aerials and the subterraneanparts of *T. garganica*was prepared by macerating the powder for 3 days in ethanol, followed by filtration and evaporation at 40°C. The dried extract was kept at 4 °C until further use. The ethanolic extract was tested for plant secondary metabolites, alkaloids, phenolic compounds, flavonoids, saponins, tannins, iridois and coumarins. Phytochemical screening of the extract was carried out according to the standard method of Dohou et al. (2003). Visible color change or precipitate formation was taken into consideration for presence (+) or absence (-) of particular active constituents.
The crude alkaloids extract of *H. tuberculatum* was analyzed for its composition by GC-MS. The GC-MS analysis was carried out using on a Agilent Technologies 7890A gas chromatograph instrument connected with the Agilent 5957C operating in the EI mode at 70 eV, using a DB-5 capillary column (30m×0.25mm, 0.25 µm film thicknesses). The temperature program was 40◦ (1 min) 280 °C at a rate of 5 °C/min. Injector and transfer line temperatures were 280 °C. Helium was used as carrier gas, flow rate of 1mL/min, split ratio, 1:100; injection volume 1µL. The identification of individual constituents was based on the

comparison of their retention indices (retention time's relative) in relation to those to those of authentic samples and matching spectral peaks available with Wiley, NIST and NBS mass spectral libraries.

Insecticidal bioassays against fifth instars larvae

Bioassays for insecticidal activity, for the two extracts (AP and SP), against the fifth instars larvae of *L. migratoria* were carried out using four concentrations for AP extract :
300, 700, 1500 and 3000 μg/larvae. Five concentrations were tested for SP extract : 100, 300, 700, 1500 and 3000 μg/larvae. Appropriate amounts of each of the test compounds were dissolved in ethanol. For treatment, newly emerged larvae fifth instars (0–12 h post emergence) were treated by forced feeding of an insecticide solution (50 μL/larvae) using a micropipette, without anesthesia. Controls were given absolute ethanol only. Thirteen larvae were used for each concentrationand three replicates were done for treated and control larvae for the two tested extract. Percentage mortalities were determined for each treatment. The LD_{50}, the concentration that produces 50% mortality, was determined by Log-probit analysis.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured, data were submitted to a monofactorial ANOVA using XLSTAT 7.5.2. Means were compared using Tukey's HSD test (P< 0.05).

Results and discussion

Preliminary phytochemical screening

The results of the preliminary phytochemical composition of the different parts of *T. garganica*by chemical sceening are shown in table I. It's noticed that both extracts (AP and SP) of *T. garganica* contain flavonoids, tannins, saponins as well as alkaloids. These compounds may play a role in insecticidal activity. The preliminary study shows that the plant does not contain antocyans and leucoantocyans. The two extracts are thus poor in comarins and iridoids.

The tannins are present with a high intensity in both parts of the plant. Their presence is confirmed by a positive reaction with the ferric chloride solution giving a greenish blue color for the aerial part and black blue for the subterranean part, so it is gallic tannin. Phytochemical tests have shown, also, the presence of glucosides in this plant.

Table 1. Qualitative phytochemical screening of the crude ethanolic extract of the aerial and subterranean parts of *T. garganica*.

	Alkaloids	Anthocyans	Coumarins	Tannins	Saponins	Irridoids	Flavonoids	Glucosides
A P	+	-	-	+++	+	-	++	+++
S P	++	-	-	+++	+	+	+	++

GC–MS analysis

The GC-MS analysis spectra of the identified compoundsof the two extracts are represented by the below figures and tables.

The CG-MS gave an idea about the composition of the crude extracts of this plant. Eight compounds were identified for the crude extract of AP (Fig. 1 and Table II) and five compounds (Fig. 2 and table III) for the crude extract of SP. The major constituents for these extracts are: phytole, hexadecanoic acid, octadecanoic acid, hexadecanoic acid, erucylamide and 13-docosenoic acid amide erucamide. In addition to these compounds, other cyclic form constituents (at 4, 5 or 6 carbons) linked to sugars are identified (as : L- arabitol, pentakis (trimethylsilyl, Arabinofuranose, 1,2, 3, 5- tetrakis-O- (trimethylsilyl). They are certainly glycosylated flavonoids which are poorly identified by the database of this GC-MS.

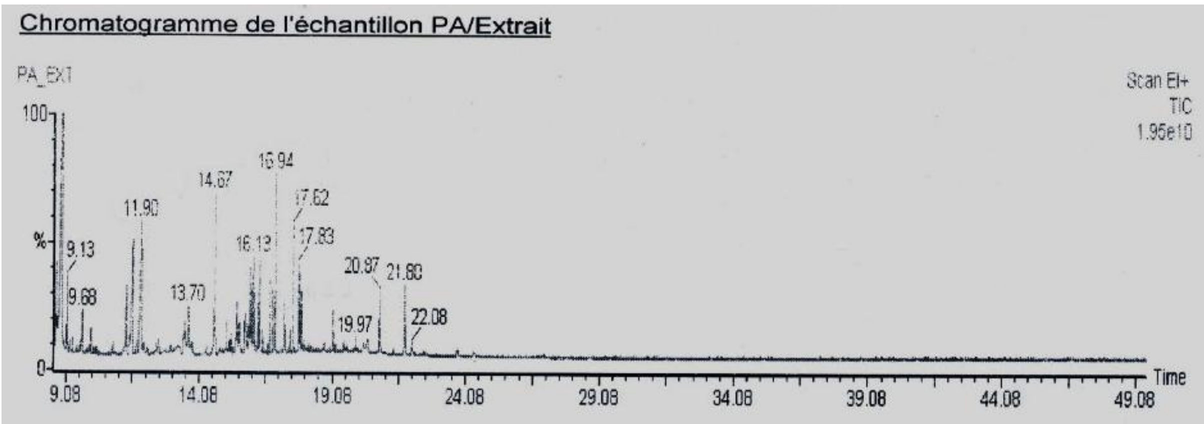

Figure 1: GC-MS chromatogram of the crude extract of aerial partof *T. garganica*

Table 2: Identified compounds by GC-MS for aerial part extract of *T. garganica*

Retention time (mn)	Name of identified compound	Crude chemical formula
8.86	N, O-BIS-(trimethylsilyl) isoleucine	$C_{12}H_{29}O_2NSi_2$
11.90	L-proline, 5 oxo-1- (trimethylsilyl)-, trimethylsilyl ester	$C_{11}H_{23}O_3NSi_2$
14.67	L-(-)- arabitol, pentakis (trimethylsilyl) éther	$C_{20}H_{52}O_5Si_5$
16.13	Beta-D-glucopyranose, 1,2,3,4,6- pentakis-O-(trimethylsilyl)	$C_{21}H_{52}O_6Si_5$
16.94	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$
17.29	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, EPI6(CAS)	$C_{24}H_{60}O_6Si_6$
17.62	Phytol, trimethylsilyl éther	$C_{23}H_{48}OSi$
17.83	9,12- Octadecadienoic acid (Z, Z)-, trimethylsilyl ester	$C_{21}H_{40}O_2Si$

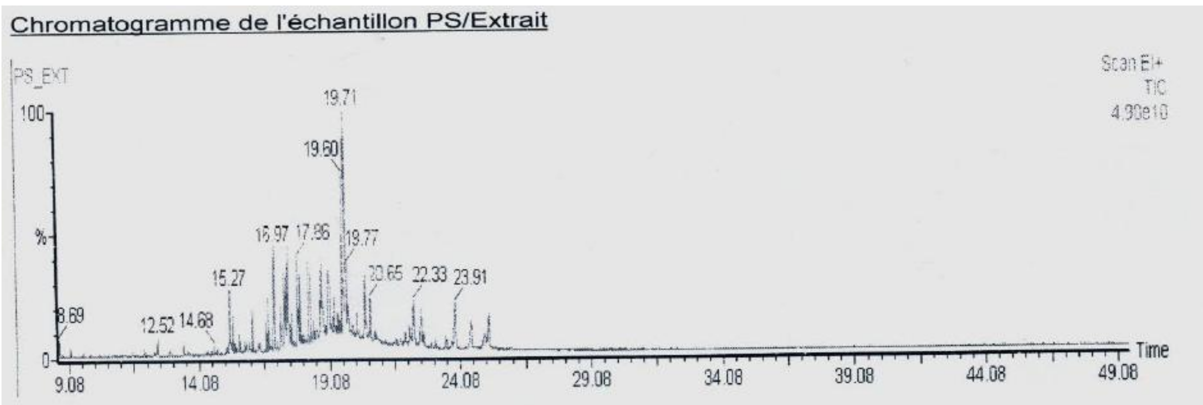

Figure 2: GC-MS chromatogram of the crude extract of subterranean part of *T. garganica*

Table III :Identified compounds by GC-MS for subterranean part extract of *T. garganica*

Retention time (mn)	Name of identified compound	Crude chemical formula
15,27	Arabinofuranose, 1,2, 3, 5- tetrakis-O-(trimethylsilyl)	$C_{17}H_{42}O_5Si_4$
16,97	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$
17,86	2- (Trimethylsilyl) heptanolide- 2-oxocanose, 3-(trimethylsilyl)	$C_{10}H_{20}O_2Si$
19,71	Octadecanoic acid, trimethylsilyl ester	$C_{21}H44O_2Si$
20,45	Erucylamid, 13- docosenoic acid amide - erucamide	$C_{22}H_{43}ON$

Insecticidal activities

From the obtained results (figures 3 and 4), it appears that both parts of the test plant showed larvicidal activity against the five instar larvae of *L. migratoria*. This activity increases with concentration and exposure period. The insecticidal effect of SP was more potent than that of PA.

The results indicate that the cumulative rates mortality on the all treated larvae with extracts from both of the two parts of the plant are significantly higher than those of controls. Regarding the high dose of 3000 µg/larvae (Figs 3 and 4), the 100% of larvae mortality was obtained 4 hours after treatment for SP extract and after 24 hours for PA extract. For the dose of 300 µg/larvae, cumulative mortality rates exceeding 50%, and 70%, respectively for AP and SP, 72 hours after treatment were obtained.

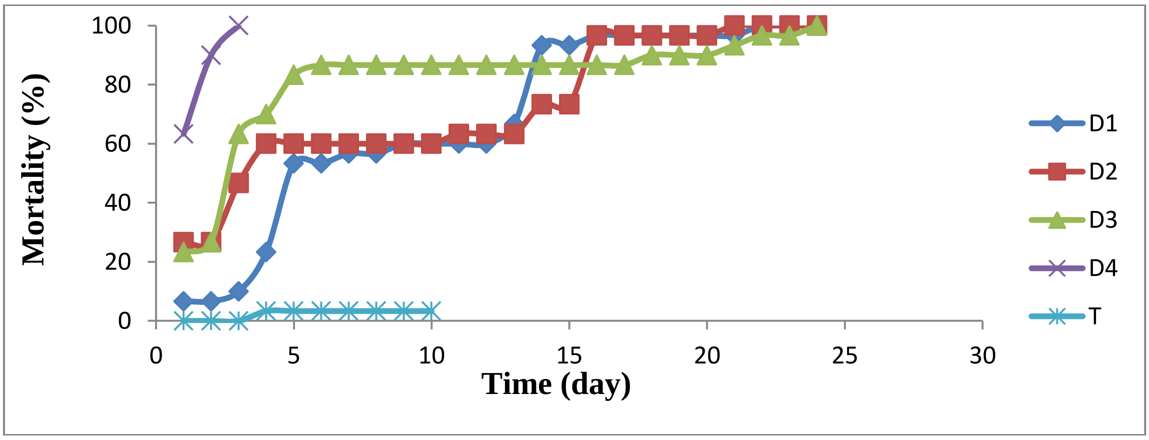

Figure 3: Mean mortality kinetics of *L. migratoria* five instars larvae treated and control with the crude extracts of the aerial part (AP) of *T. garganica*. N=10/replicate

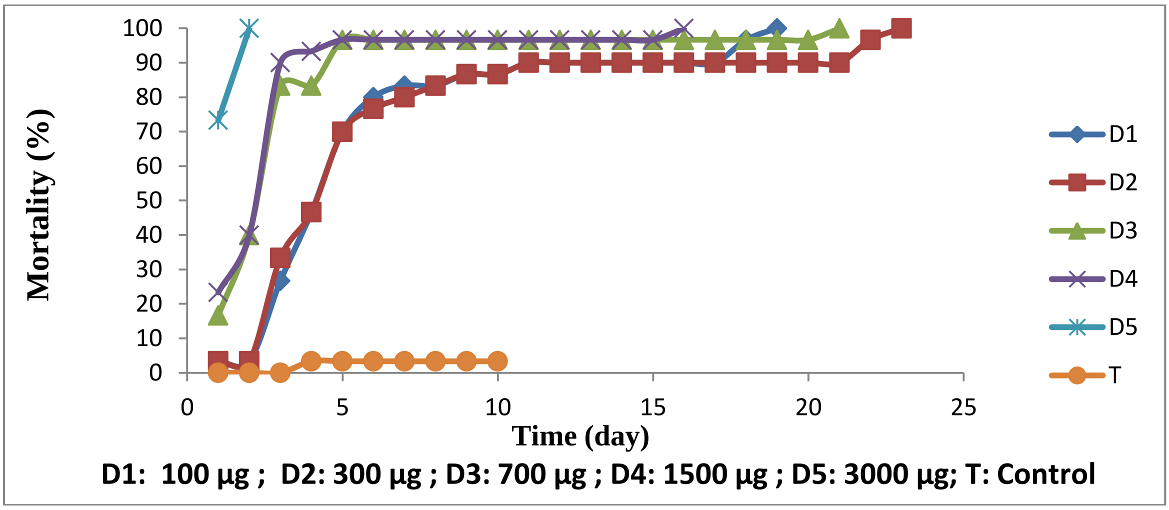

Figure 3: Mean mortality kinetics of *L. migratoria* five instars larvae treated and control with the crude extracts of the subterranean part (SP) of *T. garganica*. N=10/replicate.

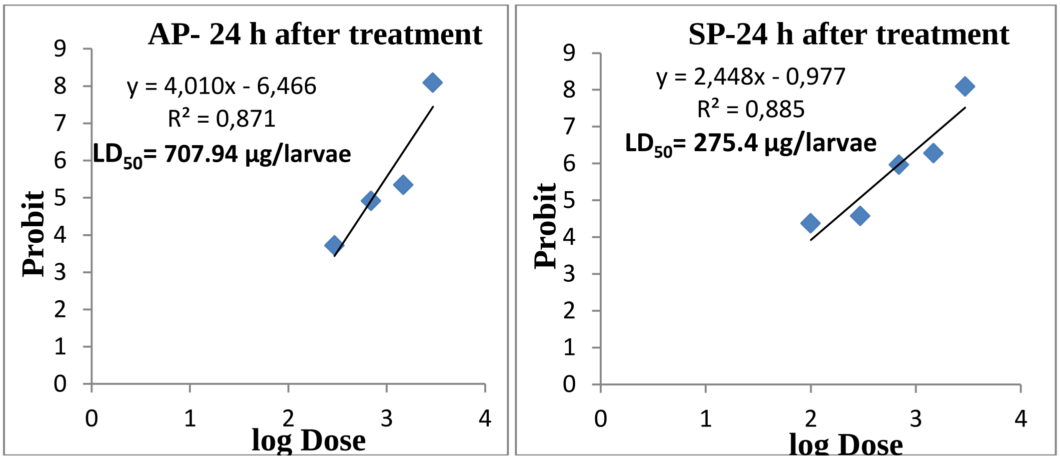

Figure 4. Effect of the crude ethanolic extract of AP and SP of *T. garganica* on the five instars larvae of *L. migratoria* 24 h after treatment, (Mean ± SD). N = 10 insects/replicate.

The LD_{50} calculated 24 h after treatment of the L5 larvae were 708 and 275 µg/larvae respectively for the AP and SP crude ethnolic extract of *T. garganica*(Fig. 4). Therefore, the extract of SP is more toxic than AP.

Effect on behavior

Behavioral observations were carried out from the first hours following treatments until moulting or death of treated larvae.After treatment, the treated larvae with crude extracts of *T.garganica* showed animmediate behavior effect, which resulted in disturbances and incordination of the movements. This state lasts 15 to 30 minutes, followed either by the death of the larvae (high doses) or by athe resumption of activity (low doses). In surviving larvae, a delay in growth and molting was observed. Moreover, these larvae have difficulties to moult and they remain encapsulated in their old exuvia for the two extract (Fig 5 A and B). Others have abnormalities in molting deformations of wings, and legs (Fig 5 C and D).


(A) (B) (C) (D)
Figure 5. Effects of crude extracts of *T. garganica* on growth and molting of the five instar larvae of *L. migratoria*

Plants represent a rich source of bioactive molecules or secondary metabolites that can affect growth, development, behavior, and act as antifeedants, toxins or growth regulators. These natural molecules can replace conventional insecticides and appear to be able to solve the environmental problems caused by synthetic pesticides (Campagne et al., 1992 in Kim et al., 2005). Phytochemical study of the crude ethanolic extract of AP and SP indicate that *T. garganica*contain flavonoids, tannins, saponins as well as alkaloids. Our results are in agreement with fhose obtained by Bouimeja et al., (2018) who report that quantitative colorimetric analysis showed an important amount of polyphenols, flavonoids and condensed tannins. For our plant, major constituents identified by GC-MS, for these extracts are: phytole, hexadecanoic acid, octadecanoic acid, hexadecanoic acid, erucylamide and 13-docosenoic acid amide erucamide. Christensen *et al.*, 1984 and Smitt and Christensen, 1991 report thatthe main constituents in the roots as well as in the ripe fruits are thapsigargin I and thapsigargicin II, whereas nortrilobolid III, thapsivillosin J IV and thapsivillosin I V were found as minor constituents. The crude ethanolic extract of *T. garganica* administered by forced ingestion to the last instar larvae of *L. migratoria* at different doses showed a good insecticidal action of the two tested extracts.Effect of SP extract was more powerful than that of AP extract. Indeed, for SP extract, all the doses showed high % mortality on the first days after.The total death (100 %) of larvae was obtained 2 day after treatment. For the AP extract, the effect of the D5 (3000 µg/larvae)was also potent, the total death of larvae was noticed third day after treatment. Botanical insecticidesare expected to be possible alternatives to the traditional chemicalinsecticides. The neem products were recommended by several researches asalternatives to the currently used harmful pesticides for the control of desert locust (Krall and Wilps, 1994). In their study, Mansour et al.,(2015) indicate that the essential oils extracted from ten different plants belonging to five families tested against the 3rd nymphal instars of the desert locust, S*chistocerca gregaria* (Forskål) (Orthoptera: Acrididae) by topical application, the *Allium cepa*oil proved to be the most toxic followed by the *Petroselinum sativum*. In another study Mansour and Abd El Hamid (2015), Cumin *(Cuminum cyminum)* oil was the highest toxicto the nymphs, while Parsley *(Petroselinum sativum)* was the lowest. They also noticed that Sweet Basil oil decreasedseverly consumption index, efficiency of conversion of digested and ingested food. The rapid effect aof plan extract or phytochemicals against some pests isindicative of a neurotoxic mode of action and there is evidence forinterference with the neuromodulator octopamine or GABA-gatedchloride channels (Priestly et al., 2003; Enan, 2005; Rattan, 2010).
Crude ethanolic extract of SP and AP of *T. garganica* showed, also,molting and growth disturbances at low doses. As our results, Kabir et al., (2013) report that, crude ethanolic seed extractof *Seseli diffusum*, at lower concentration of 100 ppmand 200 ppm, induced a high level of morphological deformities in 4th instar larvae and pupae. These disturbances may be related to a possible interference with hormones governing moulting.

Conclusions

Phytochemical ssreening of the algerian plant T. gatganica revealed the presence of flavonoids, tannins, saponins and glucosides in both parts of the plant as well as the presence of traces of alkaloids. The GC / MS analysis of the of the two parts of our plant allowed to identify eight compounds for the crude extract of the AP and five compounds for the SP extract. The major constituents for the crude extracts are: phytole, hexadecanoic acid, octadecanoic acid, hexadecanoic acid, erucylamide, 13-docosenic acid amide erucamide. Insecticidal activity of the two crude extracts of *T. garganica*se are shown to be active against larvae of the 5^{th} instar of *L. migratoria*. The maximum efficacy (100% of mortality) was observed for the higher dose (3000 µg/larvae) after 24 hours for the AP extract and after 4 hours for the PS extract.These encouraging results suggest the possibility of use of the extracts of this plant in integrated locust control programs. However, further studies on characterization of bioactive compounds, mode of action, formulations, field trials and impact on non-target fauna are recommended.

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AMARANTH GRAIN AS ENRICHING INGREDIENT FOR BAKERY TECHNOLOGY

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Abstract

Specialized and functional food technology development is able to smooth out a problem of essential elements deficit in populations diets. Bakery products is traditional for Russia – majority of people consume it every day, so its suitable product for enriching. For mass products enrichment it's often to use non-traditional, but perspective raw materials, which an amaranth is. Amaranth is rich with protein, minerals, vitamins, dietary fiber, squalene and other nutrients. At the same time, amaranth varieties are significantly differentiated in composition and properties. The purpose of the first stage of the study was comparing the composition of amaranth as a factor determining the indicators of the purpose of its processed products. 8 grades of amaranth grain were analyzed. According to the results of research, it is recommended to use Universal variety amaranth grain as enriching raw ingredients of multifunctional action. However, the adjustment of the food products composition, including bakery products through the introduction of enriching ingredients often leads to a change in traditional consumer properties of products - the shape, condition of porosity, color, taste and other organoleptic indicators. In this connection, as the second stage of research using flour from extruded amaranth was determining of some indicators - autolytic activity and acidity of dough. Results of study showed indicators increasing with makeweight of amaranth flour. 30% of additive non-traditional flour provides abnormal value of dough acidity, therefore that amount is critical.

Keywords: *amaranth, extrudate, enrichment, acidity, autolytic activity.*

Introduction

Currently, the problem of rational nutrition is one of the main factors determining the state of human health, its performance, and resistance to various adverse environmental factors. A decrease of the consumption of essential elements causes an increase in the number of diseases among the population. Therefore, the problem of maintaining public health is closely related to the production of biologically complete and safe food products. Lately, the production and use of functional foods has become widespread, in particular, the enrichment of mass consumption foods with scarce nutrients (fiber, vitamins, protein, etc.) ((Ryabova, 2015)).

Bakery products undoubtedly belong to the traditional and mass - for example, previously, it was established (Derkanosova, 2015) that 65% of Voronezh residents consume bread products daily. Therefore, the adjustment of the recipes of bakery products may affect the nutritional status of the population.

The enrichment of bakery products is often carried out at the expense of rare, but promising in their properties plant sources. One of these plants is amaranth - an annual herbaceous plant of the amaranth family, with small flowers collected in lush panicle inflorescences. Due to the high content of essential amino acids, vitamin E, mono- and polyunsaturated fatty acids, the use of amaranth in food helps stimulate the immune system, detoxifies the body, and has an antimicrobial, anticarcinogenic and fungicidal effect (Saratovskiy, 2014; Tovar-Pérez, 2018; Coelho, 2018).

Amaranth is widely used in gluten-free diets due to the lack of gluten proteins in its composition. So, I.M. Zharkova and L.A. Miroshnichenko proposes the variable use of amaranth flour - in technologies of "gluten-free" bread, muffins, waffles, etc. (Zharkova, 2012; Zharkova, 2014). At the same time, its use is also effective as an ingredient for traditional baking - it is possible to enrich the product due to the high content of valuable substances in amaranth and preserve the organoleptic qualities familiar to the consumer. A number of researchers (Roslyakov, 2016) developed a technology for obtaining a new variety of bakery products - a loaf "Zebra" type with the addition of amaranth flour, with which the finished products acquire dietary properties. Amaranth attracts additional attention due to the possibility of complex use - for example, it is known to develop a method for producing food colors from the leaf mass of Valentine grade in two colors - green and cherry (Gins, 2015).
However, nutrition problems and dissatisfaction with the range of enriched foods leaves the development of technologies for enriching bakery products with amaranth relevant. Using non-traditional raw material in regular technology requires preliminary check of some characteristics. The aim of our work is identification the most optimal amaranth variety for the use in flour technology and determination the effect of its application on the flour mixtures acidity and autolytic activity.

Material and methods

Over 60 species of amaranth of fodder, food, medicinal and decorative purposes are cultivated in different countries (Saratovskiy, 2014), and, accordingly, the composition of amaranth grain differs significantly depending on the grade. Therefore, preliminarily it was analyzed 8 grades of amaranth of the Voronezh selection and selection of the FSBSI "Federal scientific center of vegetable growing": Voronezh-36, Voronezh, Emperator, Rubin, Universal, Gigant, Dobrynya and Valentina. Amaranth was grown in a collection nursery of Voronezh State Agrarian University. By geographic location, the nursery is located in the forest-steppe zone of the Central Chernozem Region. The soil of the experimental plot is leached medium loamy chernozem. The availability of its mobile forms of nitrogen, phosphorus and potassium is medium and high. Humus content is equal to 4.5%, pH is fluctuated from 5.4 to 5.8. The total rainfall for a period with a temperature above +10 ° C is 250-260 mm. The total amount of active temperatures is 2581° C. Sowing was carried out in the second half of May 2018.
The amaranth grain composition was determined by standardized methods at the accredited research center of the All-Russian Scientific Research Veterinary Institute for Pathology of Pharmacology and Therapy: mass fraction of moisture - according to GOST 13586.5-2015, protein - according to GOST 10886-91, fat - according to GOST 29033-91 , fiber - according to GOST 31675-2012, total sugar - according to GOST 15113.6-77, ash - according to GOST 27494-2016, phosphorus content - according to GOST 26657-97, calcium - according to GOST 26570-95, copper, zinc - according to GOST 30692-2000, iron, manganese - according to GOST 32343-2013.
Amaranth grain was extruded. The extrudate was obtained from whole semi-defatted grains of Amaranth variety Universal on a laboratory universal small-sized extruder (EUM-1). The extrusion temperature was 110-120 ° C. Then the extrudate was ground to granulometry 125 μm and less.
For dough analysis 12 mixtures of wheat flour (of highest and first grade) and amaranth flour were prepared. The method for determining the autolytic activity is standardized by GOST 27495-87 "Flour. Method for determining autolytic activity", for acidity - by GOST 27493-87 "Flour and bran. Method for determining acidity by a talker".

Results and Discussion

Comparative grain characteristics of the studied amaranth grades are given in tables 1 and 2:

Table 1. Composition of amaranth grain

Amaranth grade	Fraction content, %					
	moisture	protein	fat	cellulose	common sugar	ash
Voronezh-36	10.14	15.78	6.46	6.9	2.46	2.98
Voronezh	10.53	16.21	6.38	5.7	2.36	3.41
Imperator	10.19	19.57	11.71	7.5	2.36	3.99
Rubin	11.42	20.66	9.79	7.1	2.65	4.75
Universal	9.46	26.47	13.81	8.9	4.10	8.08
Gigant	10.14	19.29	10.29	7.1	2.94	3.03
Dobrynya	10.91	16.79	7.24	16.3	2.36	4.33
Valentina	11.60	16.10	6.94	19.4	2.55	6.04

Table 2. Composition of the mineral substances of amaranth grain

Amaranth grade	Content					
	phosphorus, %	calcium, %	copper, mg /kg	iron, mg /kg	zinc, mg /kg	manganese, mg /kg
Voronezh-36	0.46	0.17	8.62	108.4	32.54	51.89
Voronezh	0.54	0.24	7.23	76.5	35.80	55.14
Imperator	0.45	0.25	7.20	81.8	30.99	50.78
Rubin	0.55	0.17	6.06	73.4	30.82	50.62
Universal	0.63	0.36	12.98	90.0	30.89	44.88
Gigant	0.54	0.20	14.86	76.9	32.66	28.97
Dobrynya	0.50	0.36	8.11	72.4	28.19	46.72
Valentina	0.46	0.48	5.36	75.9	31.33	82.40

Based on the totality of indicators for further research, amaranth Universal was selected - a variety characterized by high productivity, valuable composition and predominant light grain color (Figure 1).

Figure 1 – Amaranth Universal grade

Due to small size of the grain and the presence of a dense hemicellulosic membrane on its surface, it needs an additional processing. As such process extrusion was selected. The big advantage of extrusion as a technological method is an increase in the degree of assimilation of the product. As a result of hydrothermomechanical processing, gelatinization of starch occurs, it is more easily hydrolyzed by enzymes and is better absorbed by the organism. Protein substances contained in raw materials, under the influence of hydrothermal treatment, are first denatured, and then plasticization occurs due to mechanical action. As a result, they

are better attacked by proteolytic enzymes, which increases the rate of digestion of protein substances by the human body (Ostrikov, 2007). Additionally, a decrease in the severity and softening of the aroma of extruded amaranth was noted in comparison with whole-ground grain.Autolytic activity characterizes the activity of flour α-amylase in the accumulation of water-soluble substances in a water-suspended suspension when it is heated in a boiling water bath and is used to determine the activity of α-amylase in flour. Then more active α-amylase of flour, then more water-soluble substances accumulate in it.
The results are shown in figures 2 and 3.

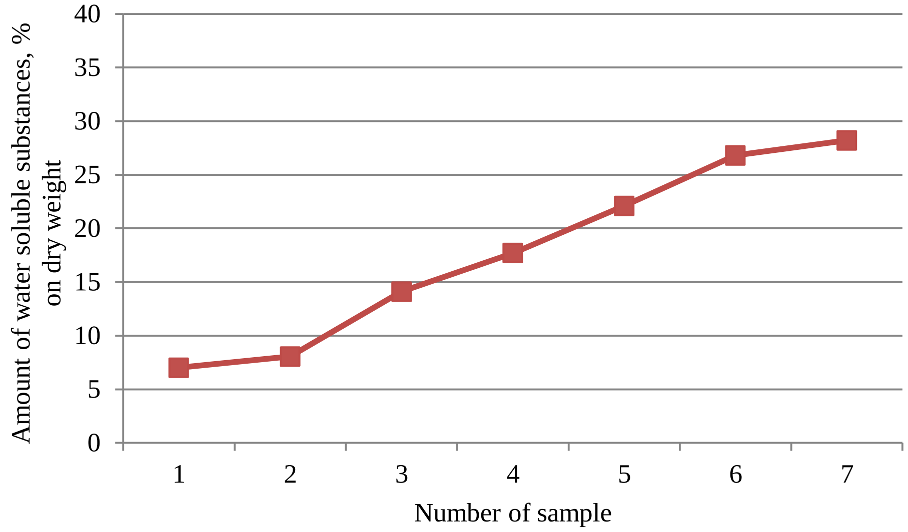

Figure 2 - Autolytic activity change depending on the dosage of extruded amaranth flour mixed with wheat flour of highest grade: no. 1 - without adding amaranth flour, no. 2 - with addition of 5% amaranth flour, no.3 – 10%, no.4 – 15%, no.5 – 20%, no.6 – 25%, no.7 – 30%

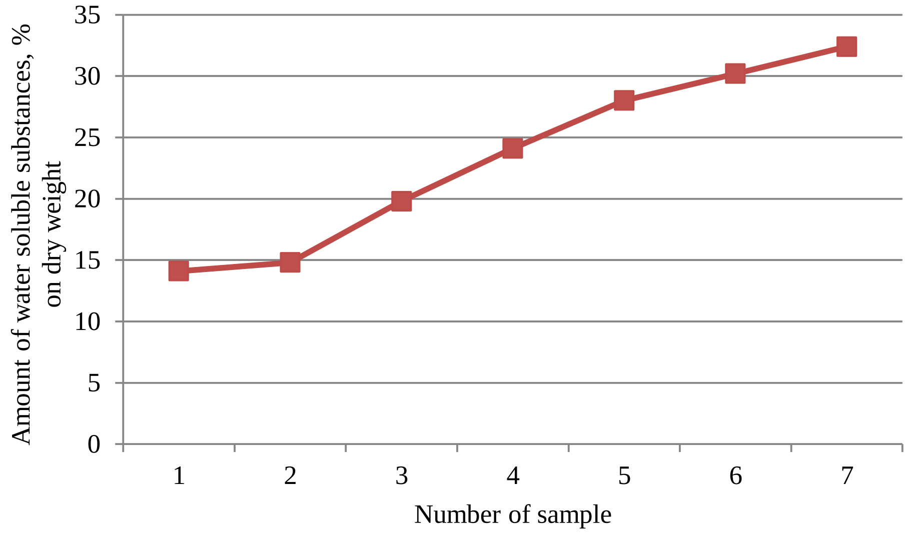

Figure 3 - Autolytic activity change depending on the dosage of extruded amaranth flour mixed with wheat flour of first grade: no. 1 - without adding amaranth flour, no. 2 - with addition of 5% amaranth flour, no.3 – 10%, no.4 – 15%, no.5 – 20%, no.6 – 25%, no.7 – 30%

Studies have shown a higher content of water-soluble substances in mixtures of flour of the first grade compared with wheat flour of the highest grade. An increase in the autolytic activity of flour was noted with an increase in the dosage of amaranth flour in it. However, it is rather difficult to draw an unambiguous conclusion about the increased activity of amylolytic enzymes of flour in the presence of amaranth extrudate. It is likely that the increase in water-soluble substances in model mixtures is associated with their presence in the extrudate. It is known that the extrusion process is accompanied by a partial destruction of biopolymers of grain raw materials with the accumulation of water-soluble substances. The acidity of flour has a serious effect on the quality of bread. The level of acidity of flour depends on its variety and the duration of storage - then lower the grade and then longer the storage period, then higher the acidity. The use of flour with high acidity leads to an increase in the acidity of dough and bread. The increased acidity of the flour leads to a higher initial acidity of the dough and faster accumulation of acids during fermentation. Bread from flour with high acidity is more acidic, with less developed porosity and a lower specific volume. The crumb at the bottom of the product may be compacted. The appearance of the crust worsens: the crust becomes bumpy, the color of the crust is brown. In the baking industry it is recommended to use highest grade of wheat flour with an acidity of 2.5-3.0 degrees; 1 grade - 3.0-3.5 degrees; 2 grades - 4.0-4.5 degrees.
The obtained values are presented in figures 5 and 6.

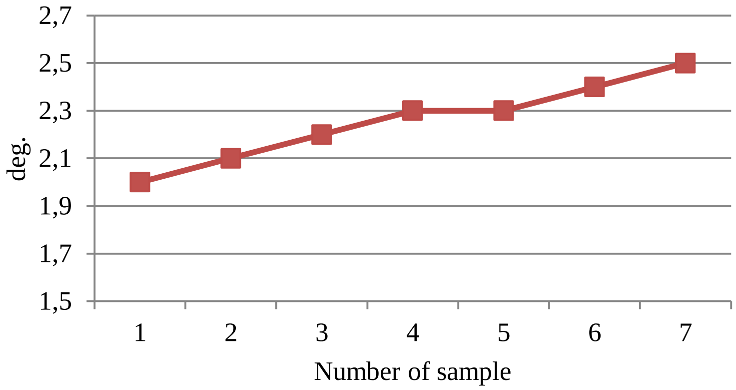

Figure 5 – Acidity change depending on the dosage of extruded amaranth flour mixed with wheat flour of highest grade: no. 1 - without adding amaranth flour, no. 2 - with addition of 5% amaranth flour, no. 3 - 10%, no. 4 - 15%, no. 5 - 20%, no. 6 - 25%, no. 7 - 30%

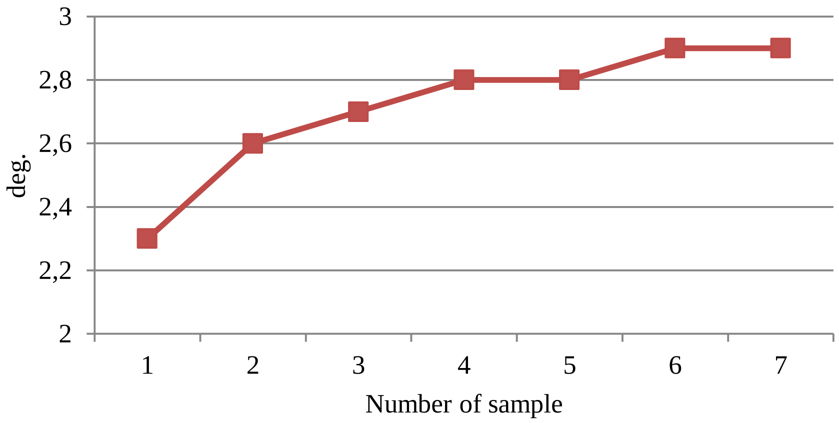

Figure 6 - Autolytic activity change depending on the dosage of extruded amaranth flour mixed with wheat flour of the first grade: no. 1 - without adding amaranth flour, no. 2 - with addition of 5% amaranth flour, no. 3 - 10%, no. 4 - 15%, no. 5 - 20%, no. 6 - 25%, no. 7 - 30%

An increase in acidity was noted with an increase in the dosage of amaranth flour in the mixture in both cases. At the same time, the acidity of flour mixtures with wheat flour of the first grade was naturally above than the highest.

Conclusion

In general, the studies conducted allowed us to draw the following conclusions:

- the addition of extruded amaranth flour of the Universal grade to the composition of bakery mixes affects the baking properties of flour ingredients - the amount of water-soluble substances and acidity increase;
- extruded amaranth flour has an identical effect on the baking properties of flour mixtures based on flour of the first and highest grade;
- increasing the dosage of extruded flour over 30% is impractical due to the achievement of critical values in acid mixtures for acidity and the content of water-soluble substances that affect the quality of bakery products.

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EXAMINATION OF VITAMIN C IN FEED AND MILK

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Abstract

The exact quantification and concentration of vitamin C in fodder is of critical importance for the normal health of dairy cows that are susceptible to oxidative stress, just like humans. Studies have been carried out on vitamin C (ascorbic acid) of feed (alfalfa hay, concentrates for milk cows and wheat straw) from three farms in different regions used for feeding dairy cows and their milk. Based on the performed tests of the chemical composition of the feed, it was concluded that the total feed used was characterized by the best composition. The concentration of vitamin C was examined in extracts of feed and raw milk. Vitamin C was examined through colorimetric method, using a calibration curve of 2,4-dinitrophenylhydrazine and a spectrophotometer (Pharo 300-Merck spectroquant). The highest concentration of vitamin C was found in alfalfa hay, farm B (40.2 μg/ml), while in the raw milk it was mostly present in milk from farm A (2.8 μg/ml). From the above, it can be concluded that regardless of which farms the tested samples of feed and their milk are, higher values for the concentration of vitamin C are obtained in the samples of extracts of alfalfa hay. If we compare the vitamin C concentration in feed and milk, it is concluded that the raw milk values are much lower than the feed and even much lower than the pasteurized milk taken for standard (2.3 μg/ml).

Key words: *feed, alfalfa hay, concentrate, milk cows, vitamin C.*

Introduction

The need of animals for energy, proteins, minerals and vitamins is basically satisfied through basic foods (hay and straw), and feeding stuffs regularly used for eating cows represent a complement to the basic meal with easily digestible and usable nutrients (Andonov *et al.*, 2014). Classification of food was made very long ago (Popov, 1949). According to Morrison (1955), there are: fodder crops for pasture, for green foods, for grain, hay, silage, etc. Another division is made by Pribičević (1976), which divides food into eight groups, including the group of "green food" (free grasses, meadow plants and silage of their green plants), as and a group of "combined foods from the industry" (Egumenovski *et al*., 1998).

Lucerne (alfalfa) as a forage crop is characterized by a high content of raw proteins (18-22%) and raw fibers (25-35%). In addition to raw proteins, raw fiber, minerals and vitamins, alfalfa contain a large quantity of spoons that are not desirable for daily intake of animal nutrition (Animal Nutrition Group 2012, FAO 2000-2010). Lucerne (alfalfa hay) is a crop with high content of vitamins, and pro-vitamin A-carotene is particularly important (1 kg of green alfalfa has 30-1000 mg of carotene). Alfalfa contains high vitamins: B1, B2, C, E, K, H, and PP,investigated from Collins *et al*., (2014) and also on WebMD, LL (2017) found in their study that the chemical composition of different straw types is different.

Concentrates for dairy cows are mainly a source of lightly digested energy and proteins, but usually contain a large amount of minerals and other important nutrients, vitamins that cannot be met through rough beehives - fodder (Ishler *et al*., 2006, 2018). The milk mixtures for milk cows according to the chemical composition contain: water, raw proteins, various types of

carbohydrates, crude fiber, fats, minerals and vitamins (Ivanovski *et al*., 2011). The term vitamin has been introduced by Kazimir Funk (Egumenovski *et al*., 2011).
The vitamins in the diet is very important because the animal organism needs them to perform normal processes all the way.It is found in all green plants and fresh fruits. In fodder, it is found in fresh green food and silage. Vitamin C in the hay and grains, feeds it in small quantities because it during the drying and during the storage of livestock is lost. The physiological function of vitamin C is great because it participates in the synthesis and creation of many hormones, strengthens the immune system of animals, participates in oxidation-reduction processes of the cell, is needed for the metabolism of amino acids and carbohydrates, and for the excretion of toxic substances from the body (Korr, 2017). Importance of vitamin C is particularly important when the animal is in stress. The intake of vitamin C with food can be through feeding stuffs in which it has a vitamin supplement, which meets the daily needs of dairy cows and other categories and types of animals. The needs of vitamin C are quite high and therefore daily needs in all types and categories of animals are expressed in mg / kg of feed. Thus, according to studies carried out at Cornell University (2018) in the paper "Composition of milk," raw cow milk contains: water 87.3% (85.5 - 88.7%); fat 3.9% (2.4-5.5%); proteins 3.25% (2.3-4.4%); casein 2.6% (1.7-3.5%); serum proteins, small proteins and carbohydrates 4.6% (3.8 - 5.3%); organic acids 0,18% (0,13 - 0,22%), such as citric, lactic, formic, acetic, oxalic acid, minerals, enzymes. It also contains vitamins: A, C, D, thiamine, riboflavin; minerals: 0.65% (0.53-0.80%); cations, K, Ca, Mg, K, anions: chlorides, phosphates, citrates, carbonates; enzymes: peroxidase, catalase, phosphatase, lipase; gas: CO_2, N_2, O_2.
Studies of raw milk with thermal treatment have shown that quality is lost. Higher thermal treatment with ultraviolet temperature (UHT), or pasteurization for extended application in combination with increased storage time of these products, caused by some water-soluble vitamins, leads to a reduction in vitamins and minerals. Thiamine is reduced from 0.45 to 0.42 mg/l, vitamin B12 is reduced from 3.0 to 2.7 μg/l, and vitamin C is reduced from 2.0 to 1.8 mg/l (Crlsen *et al*., 2010). According to studies conducted byTomovska, Menkovska, Ahmad (2018), for the content of vitamin C in milk, it was found that its content is the highest in vitaminized milk where it is deliberately added and the highest number of 1.20 mg/dl. From that study concluded even chocolate milk has a significant amount of vitamin C, up to 0.22 mg/dl, most likely derived from cocoa, which is used as a basic supplement for the production of chocolate milk. In skimmed milk, the smallest content of vitamin C is maximal to 0.25 mg/dl, and a minimum of 0.1 mg/d. Full fat milk has a vitamin C content up to 0.35 mg/dL, which is greater than that in chocolate milk, and this is probably due to the higher amount of vitamin C in whole milk. The low content of vitamin C in skim milk is most likely due to decreased processes, which together with ointment, probably also removes the greater amount of this vitamin, which is closely related to vitamin E, which, in turn, is most concentrated in the membranes of fat drops. The content of antioxidants in milk from vitamin C, that are important contributors or neutralizing agents to free radicals is quite low (Clausen et al., 2009). In this context, it is quite important to mention that the possibility of antioxidant activity of proteins and peptides in food is also investigated (EFSA, 2006).

Material and Methods

1. Extraction of feed

For the extraction, feeding stuffs are used, two types of compound mixtures (concentrates) produced by firm "Agroinvest" (2017), feed mixture for dairy cows with the least crude protein (KMK) - 18% and two types of plant materials: alfalfa hay and wheat grains. Dried feeds are firstly sucked with a homogenized in order to obtain a uniform particle size. The solvency ratio was constant as applied (Kothari *et al*., 2014) for all methods. 1 g powdered feed powder was dissolved in a 50 ml solvent, which could be alcohol, ether, ethyl acetate, and the like. For this type of analysis, absolute methanol (Merck KGaA, Frankfurt, Germany) and 50% ethanol (Alkaloid, Skopje) was used. The extraction is made using a mixture of solvents (methanol and ethanol) in a ratio of 1:1 and using the Soxhlet apparatus after 3 hours for each test (in the case of nine samples), maintaining the constant total volume from the extract of 100 ml.

2. Extraction of milk

Samples of cow's raw milk, which cows feeding on the tested feed from three farms (randomly) from three different places on the territory of The North Republic of Macedonia: Kumanovo, Tetovo and Gostivar are extracted with 6% trichloroacetic acid (CCl_3COOH 99%, Sigma Aldrich company).

• Preparation of the filtrate (whey fraction)

In a 50 ml vial, 15 ml of 6% trichloroacetic acid (TCA) is added, add 5 ml of milk and stir with a stalk rod until a fine suspension, leave at room temperature for 5 minutes. The supernatant is then separated by centrifugation at 7550 rpm^{-1} (RCF = 5410g) in the Hettich Universal 320R centrifuge (Andreas Hettich GmbH - Germany) within 10 minutes. The obtained supernatant is filtered through a filter paper Whatman No. 1.

In this study, a colorimetric method with 2,4-dinitrophenylhydrazine, which is widely used to determine the level of ascorbic acid in biological fluids, has been adapted to estimate the total content of vitamin C in the extracts of feed and milk. The procedure depends on the principle of the oxidation of L-ascorbic acid in dehydroascorbic acid and 2,3-diketo-L-gulonic acid, followed by reaction with 2,4-dinitrophenylhydrazine. After treatment with sulfuric acid, a colored product is formed, which is absorbed at 520 nm. Using the standard of ascorbic acid at different concentrations, according to Lambert Beer's law, a 20 mg/dl concentration range was used, which resulted in the following equation of the linear calibration curve: $y = 0.0344x + 0.0519$, $R^2 = 0.9709$. The most critical and decisive step in the precision reading procedure is the preparation of a final and the clear extract of feed and milk. The analysis of the content of vitamin C in selected feeds and milk is made using a spectrophotometric method.

3. The standard curve of ascorbic acid (Burgos et al., 2014).

- Preparation of the basic solution of ascorbic acid (1000 μg/ml). To this end, 100 mg of ascorbic acid are measured in a glass and dissolved in a 50 ml extraction solution (methanol). Transfer the solution into a volumetric flask protected from light and make up to 100 ml with a solution for extraction (methanol). Standard solutions of ascorbic acid (5, 10, 20, 30, 40 and 50 μg/ml) are prepared by taking 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 aliquots ml of the basic solution of ascorbic acid and make up to 50 ml with the extraction solution. Mix 1 ml extraction solution with 9 ml of dissolved 2,4 dinitrophenylhydrazine, (2,4-dinitrophenylhydrazine, DNP, Sigma Aldrich) and read the absorbent (reagent + sample) for 1 minute at 520 nm. The final value of absorption is used to make the standard curve: the actual absorbance (A) versus concentration (μg/ml).

4. Procedure for the determination of vitamin C

With minor alterations to the conditions and reagents applied the method from Al-Ani *et al*. (2007). For one sample, 1 ml of three samples of the clear supernatant, 1 ml of water as a blank and 1 ml of standard solutions in test tubes is pipettes per ml. In the same test tubes, add 1 ml of 6% trichloroacetic acid (TCA) and 0.4 ml of 2,4-dinitrophenylhydrazine (DNP). The tubes are sealed and incubated in a water bath at 37 ° C for 3 hours. Then, the tubes are cooled in a cold bath for 10 minutes. Add 2 ml of cold sulphuric acid (12 mmol) to the cooled solutions, close the tubes and mix to the vortex. The temperature of the mixture should not exceed the ambient temperature due to reading the spectrophotometer. The spectrometer (Spectroquant Pharo 300 - Merck) at 520 nm was zeroed and the absorbance of the standard solutions for compiling the calibration curve was first read, and then the absorbance of the samples were read and applied to the calibration curve from which the concentration values were read.

Results and Discussion

Results from examination of vitamin C (ascorbic acid) in samples of feed taken from 3 farms A, B, C: Concentrate 1 and 2 used on the farm B in Tetovo farm; Concentrate 3 and 4 used in farm A - Kumanovo; Concentrate 5 used on the farm C - Gostivar; Alfalfa hay 6 used in the farm A - Kumanovo; Wheat straw 7 used in the same farm A - Kumanovo; Alfalfa hay 8 used in the B - Tetovo farm; Alfalfa hay 9 used in the farm C – Gostivar were subordinated. Also cow's milk samples from three farms and one commercial milk from sale are examined in the following order: Crude cow milk 1 from Farm A, Kumanovo, Crude cow milk 2 from the farm C, Gostivar, Crude cow 3 from the B farm, Tetovo, Cow's milk 4 pasteurized with 3.2% fat (from sales to a carton). The values for the standard ascorbic acid curve was in the measurement range (0.00 to 40.00 μg/ml).
Measured values for the absorption of samples of feed (concentrates and alfalfa) are shown in Table 1. Absorbance values are transferred to the standard ascorbic acid curve and read analogously to their concentration curves. Figure 1 shows the concentration of vitamin C in the feed samples from 3 farms.

Table 1. Statistical Calculation Absorbance and Concentration of vitamin C in feed

N^{o} measurement	Samples of feed Absorbance /A (λ = 520nm)								
	1	2	3	4	5	6	7	8	9
1	0.623	0.789	0.394	0.960	0.640	1.019	0.698	1.187	0.942
2	0.625	0.780	0.393	0.953	0.636	1.02	0.695	1.184	0.947
3	0.624	0.777	0.394	0.954	0.635	1.018	0.696	1.182	0.943
n = 3									
$\bar{x}$ =	0.624	0.782	0.394	0.956	0.637	1.019	0.735	1.184	0.944
s =	0.001	0.006	0.001	0.004	0.003	0.001	0.002	0.003	0.003
RSD	0.160	0.799	0.147	0.396	0.415	0.098	0.219	0.212	0.280
(c) (μg/ml)	16.0	23.0	5.2	32.5	16.8	32.5	20.8	40.2	30.5

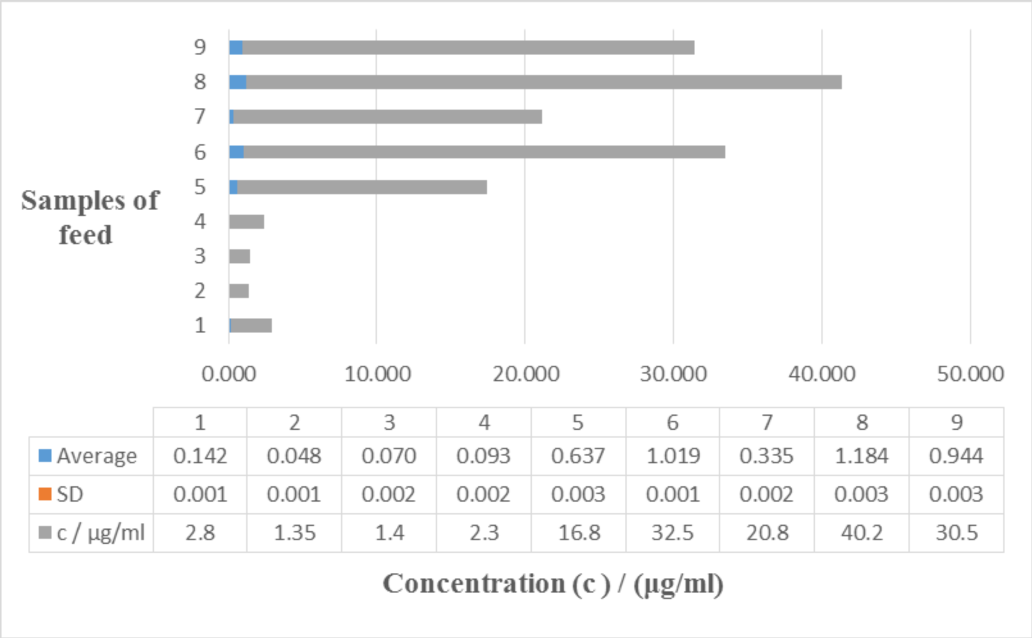

Figure 1. Vitamin C concentration in feed samples (concentrates and alfalfa)

Measured values for absorbance, A of the milk samples are shown in Table 2. Absorbance values are transferred to the standard ascorbic acid curve and read analogously to their concentration curves. Figure 2 shows the concentration of vitamin C in milk samples from the three farms.

Table 2. Statistical Absorbance and Concentration Calculation of vitamin C in milk

N^{o} of measurement	Samples of milk Absorbance /A (λ = 520nm)			
	1	2	3	4
1	0.143	0.049	0.072	0.091
2	0.142	0.047	0.068	0.095
3	0.142	0.047	0.069	0.093
n = 3				
$\bar{x}$ =	0.142	0.048	0.070	0.093
s =	0.001	0.001	0.002	0.002
RSD	0.0040	0.0242	0.0298	0.0215
(c)/ (µg/ml)	2.8	1.35	1.4	2.3

The sampled number of the sample absorbing apparatus (absorbance A) is applied to the calibration curve and reads the concentration values (c) measured in (µg/ml).

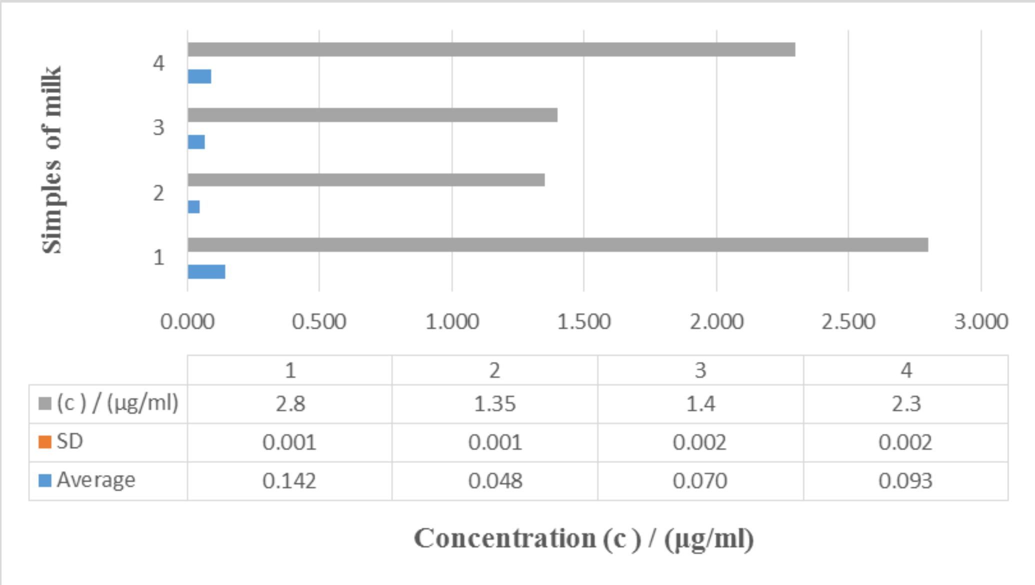

Figure 2. Vitamin C concentration in milk from the three farms

Vitamin C (ascorbic acid), as a strong antioxidant, is most present in feeding stuffs with a concentration of 40.0 µg/ml, in the alfalfa acne from Tetovo. Samples with the same concentrations of 32.5 µg/ml of vitamin C are concentrate 2 and alfalfa from Farm A from Kumanovo. A sample of 30.5 µg/ml is an alfalfa sage from the farm C of Gostivar, which shows that a similar concentration of vitamin C has all the samples of alfalfa, which means that alfalfa is abundant with vitamin C. The presence of vitamin C in milk is shown in Figure 27, which shows that the milk from Farm A from Kumanovo has the highest value of 2.8 µg/ ml, and in pasteurized milk with 3.2% fat has a value of 2.3 µg/ml. The need for vitamin C has been very studied in humans, and there is very little in the domestic animals. Vitamin C can also be added as an additive in feed (Milosavljevic, Pauca, 1978). The presence of vitamin C in milk is low, and this is also shown by this research. This has also been confirmed recently by the investigations of Tomovska *et al*. (2018), for the presence of vitamin C in vitaminized milk species, chocolate milk species and in milk with different concentrations of milk fat.

Conclusions

From the analysis of vitamin C in feed, concentrates and milk, it concludes:
1. The results of the studies of vitamin C in feeds - concentrates and alfalfa hay from the three farms (in the measured range from 0.00 to 40. 00 µg/ml), the highest value is found in the sample of alfalfa hay from farm B of 40.3 µg/ml, and the lowest value on the concentrates (KMK) 1 sample of farm A of 5.2 µg/ml.
2. With respect to the concentration of vitamin C in raw milk, the highest value indicates milk from farm A of 2.8 µg/ml and the lowest value of milk from farm B with a value of 1.4 µg/ml.
The main conclusion is that vitamin C is present in a large amount of alfalfa hay feed, while the amount of vitamin C in the raw milk is low, regardless of the type and diet of the cattle.

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STUDY OF BIOLOGICAL EFFECTIVENESS AND RESIDUE DYNAMICS OF USED NEONICOTINOIDS AND PYRETHROIDS ON CEREALS

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Abstract

Cereals are one of the most important crops grown for food, fodder and technical purposes. Reliable yields and grain quality is one of the most important tasks of agriculture. The purpose of this work is to present results on the biological effectiveness and safety of the use of insecticides for protection against a complex of piercing-sucking pests. Field experiment was conducted for two years, according to. Frequency of assessment was carried out the day before spraying and on the 3rd, 7th and 14th days after spraying. The biological effectiveness of the insecticides was determined by Henderson-Tilton formula. The dynamics of the decomposition of residual amounts of thiamethoxam and alpha-cypermethrin in 2016 and 2017 were conducted. Analysed plant material was sampled on the day of spraying and at 8, 15, 23, and 30 days after.

The analysis of residual quantities of thiamethoxam was performed under laboratory conditions using high performance liquid chromatography, residual quantities of alpha-cypermethrin are analyzed by gas chromatography.

The test pesticide showed high biological efficiency. Based on the results of the analyzes, it was established that the level of thiamethoxam and alpha-cypermethrin content in the crop did not exceed the maximum allowable levels.

Key words: *Cereals, Insecticide, Russian Federation, Neonicotinoids, Pyrethroids, Piercing-sucking pests.*

Introduction

The aim of developing and improving the field crop protection system is to obtain high-quality yields. Representatives of the complex of piercing-sucking pests (aphids, thrips, leafhoppers) have a negative impact on the quantitative and qualitative characteristics of the crop. In addition, they are transmitters' of vector born-diseases of plants (Orlov, 2006).

Expanding the arsenal of registered plant protection products allows the producer of agricultural products to compile an optimal protection scheme that will be effective against pests, and will allow obtaining products safe for the consumer.

Neonicotinoids are a perspective group of insecticides that significantly expand the possibilities of creating integrated schemes for the protection of cereals. Compared to other chemical classes of insecticide compounds, neonicotinoids have fundamentally different mechanism of action on the organism arthropods inhibiting nicotine acetylcholine receptors. Pyrethroids similarly interact with the nervous system of insects by acting on calcium metabolism in synapses on the sodium-potassium channels, disrupting the function of the nervous system. This leads to a significant excessive release of acetylcholine during the passage of a nerve impulse (Ibragimhalilova, 2008). Poisoning manifests itself in the defeat of the motor centers, in a strong excitement.

The use of mixed preparations based on neonicotinoids and pyrethroids on crops provides resistance to most pest species.

The aim of the study was to determine the effective and environmentally safe consumption rates of the test mixture, to prove that the pesticide allows effectively pest population control in the field, and also does not have a negative impact on the safety of consumed products.

Materials and Methods

The field part of the experiment to assess the biological efficiency of the insecticide was carried out in the Russian Federation, in the city of Moscow, in the zone of podzolic and sod-podzolic soils. The humus content in the soil is 1.6-1.8%, pH = 5.9. The trail was conducted on the experimental station field, in the Russian State Agrarian University named after K.A. Timiryazev (RSAU-MTAA) in 2016 and 2017. The experiment was set up on four replicates of 4 model plots measuring 50 m^2 in each of the variants (Guidelines for insecticide testing, 2009). The variety of winter wheat is Galina.

The test preparation contained 150 g L^{-1} thiamethoxam and 150 g L^{-1} alpha-cypermethrin in the form of a suspension concentrate. Two rates of consumption of 0.1 and 0.15 L ha^{-1} were studied.

The insecticide based on 106 g L^{-1} of lambda-cyhalothrin and 141 g L^{-1} of thiamethoxam in the form of a suspension concentrate was used as a reference preparation at a rate of 0.2 L ha^{-1}. The spraying were carried out on 17 June 2016 and 30 June 2017.

Frequency of assessment was carried out the day before spraying and on the 3rd, 7th and 14th days after treatment. The biological effectiveness of the pesticide was determined by reducing the number of pests with the correction for control and calculated according to the Henderson-Tilton formula (Guidelines for insecticide testing, 2009).

Plant material was sampled on the day of spraying and at 8, 15, 23, and 30 days after for analyses of dynamics of decomposition of residual amounts of thiamethoxam and alpha-cypermethrin in 2016 and 2017.

Analysis of residual amounts of thiamethoxam was carried out on an Agilent 1260 Infinity liquid chromatography with a diode array detector (Sychev, 2013), and the determination of the residual amounts of alpha-cypermethrin was carried out using an Agilent 6890 N gas chromatograph with an electron capture detector (Tsarev, 2000).

The lower limits of quantitative detection of thiamethoxam and alpha-cypermethrin were established at the level of 0.025 and 0.01 mg kg^{-1}, respectively. The lower limits of the detection of pesticides in agricultural products are established on the basis of the actual maximum permissible levels (MRLs) for the pesticides under study. In Russia, the following hygienic standards for thiamethoxam MDI in products (mg kg^{-1}) in grain cereals for thiamethoxam and alpha-cypermethrin in cereal grains were established at 0.05 and 2.0 mg kg^{-1}, respectively (HS 1.2.3539 -18, 10/05/2018).

Results and Discussion

In 2016 in the complex of piercing-sucking pests dominated aphids (*Rhopalosiphum padi*), from other pests during the counts were observed thrips (*Thysanoptera* sp.) and cicadas (*Cicadellidae* sp.) - in single amounts and only in the control variant.

The spraying of plants was carried out when pests invade 6 to 10% of plants, with an average of 5.8 to 10.2 aphids per one ear. On day 3 after spraying, the effectiveness of the test pesticide at 0.1 L ha^{-1} is 80%; 0,15 L ha^{-1} - 87%, and the standard - 82%. On the 7th day after spraying, the effect of the test pesticide was reduced at 0.1 L ha^{-1} - 72%; 0,15 L ha^{-1} - 81%, and the standard - 70%. On the 14th day after spraying, further decrease in the effect of the test pesticide was noted when the aphid population was restored - 0.1 L ha^{-1} - 69.5%; 0.15 L ha^{-1} - 78%, and the standard - 70%, with the number of pests in the control variant of 7.5 specimens/ear.

In 2017, against the background of incessant precipitation, the appearance of a complex of

piercing-sucking pests on winter wheat was noted from the first decade of May (aphids) to the beginning of June (thrips and leafhoppers). The number of aphids at the time of the treatment was small and varied from 2 to 4 specimens / ear. The number of thrips did not exceed the threshold values, the individuals of leafhoppers were noted singly on the control plots. The date of application of the pesticide was associated with the appearance of colonies of aphids.
After the application of the test preparation, the biological efficacy in fighting the aphids was not inferior to the standard and amounted to 0.1 L ha^{-1} at the rate of application: 91% for the 3 rd day; 7th day - 62%; Day 14 decreased to 39%; at a rate of 0.15 L ha^{-1} was: 3 days - 100%; 7 days - 80%; Day 14 decreased slightly to 53%. The reference preparation provided biological efficacy: for 3 days - 100%; 7 days more than the experimental variant-85%; 14 days below the experimental variant - 50%.
Based on the results of the analysis of the dynamics of degradation, it was established that in the variant of the experiment in 2016 the residual amounts of thiamethoxam (Guidelines 4.1.1142-02) in samples of green mass of winter wheat on the day of treatment amounted to 0.0946 mg kg^{-1}, and in 2017 - 0.09122 mg kg^{-1}. 8 days after spraying, the content of residual amounts of thiamethoxam in the green mass of winter wheat was reduced and was found in an amount of 0.0513 mg kg^{-1}, and in the experiment of 2017, it was detected below the limit of quantification of the control method used. After 15, 23 and 30 days after spraying, thiamethoxam residues in samples of green mass, grain and straw of winter wheat were not detected within the sensitivity of the monitoring method used in both years.

Table 1. The residual quantities of thiamethoxam in 2016-2017

Year of research	Product type	Date of treatment by pesticides	Date of selection samples	Result, mg kg^{-1}
2016	Green mass	24.06.2016	24.06.2016	0.0946
	Green mass	Control	24.06.2016	Not detected
	Green mass	24.06.2016	02.07.2016	0.0513
	Green mass	Control	02.07.2016	Not detected
	Graine (yield)	24.06.2016	24.07.2016	Not detected
	Graine (yield)	Control	24.07.2016	Not detected
	Straw (yield)	24.06.2016	24.07.2016	Not detected
	Straw (yield)	Control	24.07.2016	Not detected
2017	Green mass	11.08.2017	11.08.2017	0.09122
	Green mass	Control	11.08.2017	Not detected
	Green mass	11.08.2017	19.08.2017	0.005
	Green mass	Control	19.08.2017	Not detected
	Graine (yield)	11.08.2017	10.09.2017	Not detected
	Graine (yield)	Control	10.09.2017	Not detected
	Straw (yield)	11.08.2017	10.09.2017	Not detected
	Straw (yield)	Control	10.09.2017	Not detected

The content of residual amounts of alpha-cypermethrin (Guidelines 4.1.2087-06, No. 4344, 1987) in 2016 in samples of green mass of winter wheat on the day of treatment was 0.8668 mg/kg, and in 2017 0.9458 mg kg^{-1} . In 2016, in samples taken 8 days after spraying, the content of the residues of alpha-cypermethrin decreased to 0.6239 mg kg^{-1}, and after 15, 23 and 30 days after spraying, the residual content of alpha-cypermethrin was not detected. In

2017, 8 and 15 days after spraying, the content of alpha-cypermethrin residues decreased to 0.7986 mg kg^{-1} and 0.6672 mg kg^{-1}, respectively. 23 days after spraying, the content of alpha-cypermethrin residues in the green mass of winter wheat was found to be 0.5523 mg kg^{-1} . 30 days after spraying, the residual content of alpha-cypermethrin in grain and winter wheat straw was not detected within the limits of the quantitative determination of the control method used.

Table 2. The residual quantities of alpha-cypermethrin in 2016-2017

Year of research	Product type	Date of treatment by pesticides	Date of selection samples	Result, mg kg^{-1}
2016	Green mass	24.06.2016	24.06.2016	0.8668
	Green mass	Control	24.06.2016	Not detected
	Green mass	24.06.2016	02.07.2016	0.6239
	Green mass	Control	02.07.2016	Not detected
	Graine (yield)	24.06.2016	24.07.2016	Not detected
	Graine (yield)	Control	24.07.2016	Not detected
	Straw (yield)	24.06.2016	24.07.2016	Not detected
	Straw (yield)	Control	24.07.2016	Not detected
2017	Green mass	11.08.2017	11.08.2017	0.9458
	Green mass	Control	11.08.2017	Not detected
	Green mass	11.08.2017	19.08.2017	0.7986
	Green mass	Control	19.08.2017	Not detected
	Green mass	11.08.2017	26.08.2017	0.6672
	Green mass	Control	26.08.2017	Not detected
	Green mass	11.08.2017	03.09.2017	0.5523
	Green mass	Control	03.09.2017	Not detected
	Graine (yield)	11.08.2017	10.09.2017	Not detected
	Graine (yield)	Control	10.09.2017	Not detected
	Straw (yield)	11.08.2017	10.09.2017	Not detected
	Straw (yield)	Control	10.09.2017	Not detected

Conclusions

The biological efficacy of the pesticide, containing 150 g L^{-1} f thiamethoxam and 150 g L^{-1} of alpha-cypermethrin, against aphids at a rate of 0.1 L ha^{-1} was 62-91% a week after spraying, and decreased to 40-70% in two weeks. At a rate of 0.15 L ha^{-1}, the effectiveness of the pesticide in the week after spraying varied between 80-100%, after two weeks, 53-78%. The effectiveness of the reference pesticide was at the level of the maximum application rate of an experienced pesticide. Obtained results indicate that this insecticides could be highly effective against other species of the complex of piercing-sucking pests on cereals. In the course of two-year field and laboratory experiments it was found that the active substances of the investigated pesticide-thiamethoxam and alpha-cypermethrin are devastate in successively selected samples of winter wheat for 30 days. At the time of harvesting, the residual amounts of thiamethoxam and alpha-cypermethrin in grain and straw of winter wheat were not detected either in 2016 or in 2017.

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BIOCHEMICAL CHARACTERISTICS OF MEDICINAL RAW MATERIALS OF MYRTUS COMMUNIS L. IN CONDITIONS OF THE SOUTHERN COAST OF THE CRIMEA

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Abstract

Myrtle (*Myrtus communis* L.) is an evergreen shrub (Myrtaceae) with a natural habitat in the Mediterranean region. In the Nikitsky Botanical Gardens (the Southern Coast of the Crimea) this culture is studied as a decorative, medicinal and essential oil plant. Myrtle leaves are medicinal raw materials. In the subtropical climate of the Mediterranean type the content of essential oil in myrtle leaves obtained by Clevenger's method (time extraction -1.5 hours) (method of Pharmacopoeia XIII) is 0.82-0.88% on a completely dry mass. Analysis of the component composition of the essential oil, investigated by GC on chromatograph firm Chromatek Crystal 5000 showed that this oil belongs to the 1.8-cineol chemotype: the relative content of 1.8-cineol is 22.05%. Myrtenyl acetate (10.17%), linalyl acetate (5.72%), D-limonene (7.34%), methylevgenol (1.14%) were also identified in the oil. Quantitative analysis of phenolic compounds carried out using HELC method on the chromatograph of Agilent Technologies (model 1100) found that the mass fraction of phenol phenolcarbonic acids (gallic and ellagic) is 3.3%, flavonoids (catechine and its derivatives, quercetine and glycosides of myricetine) - 87.3%. D-catechine, myricetine-3-O-rhamnoside and myricetine-3-O-galactoside are dominate among flavonoids. The mass fraction in the extract of D-catechine is 30.2%; myricetin-3-O-rhamnoside - 26.2%; myricetin-3-O-galactoside - 16.1% The studies have shown that under the conditions of the Southern Coast of the Crimea common myrtle accumulates a significant amount of biologically active compounds, including essential oil of 1.8-cineol chemotype, flavonoids and phenolcarbonic acids, which allows us to talk about the prospects of using this culture as a source of valuable medicinal raw materials.

Keywords: *Myrtus communis* L., *medicinal raw materials, essential oil, 1.8-cineol, flavonoids*

Introduction

Myrtle (*Myrtus communis* L.) is an evergreen shrub (Myrtaceae) with a natural habitat in the Mediterranean region. In the Nikitsky Botanical Gardens (Southern Coast of the Crimea) this culture is studied as a decorative, medicinal and essential oil plant. It is used as a medicinal and food preservative. In Italy, the fruits and leaves are currently used for the production of two well-known liqueurs Mito Rosso and Mirto Bianko. Water-ethanol extracts of myrtle have anti-inflammatory, antidiabetic, anti-mutagenic and anti-atherogenic, anti-ulcerative, insecticidal properties and have proapoptotic effect in cancer cells (Amira et al., 2012; Ogur, 2014; Izgi et al., 2015).

Myrtle leaves contain the essential oil, which contains 1.8-cineol, α-pinene, limonene, linalol, myrtenyl acetate (Mimica-Dukic et al., 2010; Логвиненко, 2017; Bakova et al., 2018). The qualitative composition of the essential oil from different organs of the plant is identical, the differences are observed only in the quantitative ratio of the components (Wannes et al., 2010).

Myrtle essential oil has an inhibitory effect against pathogenic fungi *Rhizoctonia solani* Kuhn. (Curini et al., 2003), *Fusarium subglutinans, F. vertricilioides, F. oxysporum, F. tricinctum, F. sporotrichioides, F. equiseti, F. incarnatum, F. proliferatum* and *Macrophomina phaseolina* (Starovic et al., 2016), against strains of *Escherichia coli*, *Klebsiella pneumoniae, Bacillus subtilis*, *Bacillus licheniformis*, *Candida albicans* (Mosul, Lobe, Slobojan, 2011), against gram-positive bacteria, in particular *Staphylococus aureus* (Cherrat et al. 2013) and can be used as fungicidal agents at Rhizoctonia plant disease. The antifungal and antimicrobial activity of myrtle essential oil has been shown to be significantly higher than that of mint and sage oil (Starovic et al., 2016).

As biologically active components exhibiting a wide range of pharmacological and therapeutic effects, such as antiviral, antibacterial, antifungal effects, the following components of essential oils were identified: 1.8-cineol, α-pinene, myrtenyl acetate, limonene, linalool (Alipour, Dashti, Hosseinzadeh, 2014; Mosul, Dolya, Slobozhan, 2011; Cherrat et al., 2013). The insecticidal and repellent activity of myrtle essential oil is explained by the presence of linalool and α-pinene (Cheraghi et al., 2016)

Antimicrobial, antifungal, antioxidant and insecticidal activity of extracts and essential oil of common myrtle show its prospects of use in pharmaceutical, food and agricultural practice.

The aim of our research was to study the content of biologically active substances (essential oil and phenolic compounds) in the leaves of myrtle in the conditions of introduction in the Nikitsky Botanical Gardens.

Materials and Methods

The Nikitsky Botanical Gardens (NBG) is located on the Southern Coast of the Crimea (SCC) in the zone of dry subtropical climate of Mediterranean type (Plugatar, Korsakova, Ilnitsky, 2015).

Myrtle leaves were collected in the fruiting phase in the 3rd decade of October. The quantitative content of essential oil in fresh and dry myrtle leaves was determined by the Clevenger's method in accordance with the general pharmacopoeia article. 1.5.3.0010.15. of National Pharmacopeia, XIII edition (Pharmacopoeia, 2015). The component composition of the essential oil was determined by gas-liquid chromatography (Tkachev, 2008; Dmitriev et al., 2016) on a chromatograph Crystal 5000.2 with mass spectrometric detector (Chromatek, Russia). Capillary column CR – 5ms, length 30 m, inner diameter 0.25 mm. Phase – 5% phenyl 95% dimethylpolysiloxane, 0.25 microns. The analysis time was 50.25 min. Carrier gas – helium, flow rate 1 ml/min. The temperature of the ion source 200 operating system. The transition line temperature is 250°C. The division of the flow 200. Electronic ionization of 70 eV. The scanning range is 20-450. Duration of scan is 0.2. Identification of the components was carried out on the basis of comparison of the obtained mass spectra with the data of the NIST14 MS Search library. Determination of the ratio of essential oil components was carried out by the method of internal normalization of peaks.

For the study of phenolic compounds, dry leaves were ground to the size of particles passing through a sieve with a hole diameter of 1 mm. The crushed raw material of 1.0 g was boiled in 30 cm^3 of 90% ethyl alcohol containing 0.5% concentrated sulfuric acid in a boiling water bath with a reflux refrigerator for 30 minutes in Soxhlet extractor. The supernatant was poured into a volumetric flask with a capacity of 100 cm^3 and the extraction was repeated again. The combined mixture was cooled, filtered and adjusted to 100 cm^3 by 90% ethanol (Pharmacopoeia, 2015). The total content of phenolic compounds was determined spectrophotometrically by Folin-Ciocalteau technique (Methods ..., 2002).

The study of the phenolic complex was carried out on the chromatograph of Agilent Technologies company (model 1100), equipped with a flowing vacuum degasser G1379A, a 4-channel low-pressure gradient pump G13111A, an automatic injector G1313A, a thermostat of columns G13116A, a diode-matrix detector G1316A. A chromatographic column measuring 2.1 × 150 mm filled with octadecylsilyl sorbent, grain size 3.5 μm, "ZORBAX-SB C-18" was used for the analysis. Identification of phenolic compounds was carried out according to the retention times of standards and spectral characteristics.

Results and discussion

Under the conditions of the SSC common myrtle develops as a perennial evergreen shrub, withstands short-term frosts to 11-12C, and it freezes at a lower temperature. In the seasonal cycle of development of the world there are two periods of growth: spring-summer and summer-autumn. Spring-summer growth of shoots begins in the second half of April – early May, the second one - in August – early September and ends in October. Flowering was observed in July, starting with the third year of life. Flowers of medium size, sepals are short, triangular-ovoid. Petals are white, obovate. At the beginning of August it begins the fruit formation. The period of formation of the fruit under the conditions of the SCC is stretched and is 100-110 days and only by the beginning of October their maturation begins. Fruit is berry, round or ovate-elliptic and has the size of a pea (Logvinenko, 2017).

Myrtle's leaves are medicinal raw materials. The leaves are opposite, leathery, lanceolate, pointed with a length of 30 mm and a width of 5 mm. The yield of raw materials from the plant of three years old is 0.5 kg. Myrtle oil is a transparent, slightly yellowish liquid with a refreshing floral-balsamic odor. The content of essential oil in the leaves obtained by hydrodistillation according to Ginsberg is 0.35% of the wet mass (0.87% of the air-dry mass), in the fruits – 0.007%, in the shoots – trace amounts (Logvinenko, 2017; Bakova et al., 2018).

The period of processing of fresh myrtle leaves for essential oil is limited due to significant fluctuations in air temperature during myrtle fruiting period - the end of October - the second, third decade of November due to a decrease in air temperature, processing of fresh raw materials can last from 3 to 5 days.

One of the criteria for the quality of medicinal raw materials, according to the requirements of National Pharmacopeia, is the content of essential oil obtained by Clevenger's method. The use of this method makes it possible to obtain 35% more essential oil - 0.76-0.82% (from the air-dry mass) compared to Ginsberg's method used (Bakova et al., 2018).

In total, the oil obtained from fresh and dry raw materials is dominated by terpenoids, their content is 86.0-72.6% of the total content of components, and the share of terpene compounds accounts for 4.6-21.6%, the amount of non-terpene compounds is 4.8%. A comparative assessment of the component composition of essential oil from raw and dry raw materials obtained by Clevenger's method showed that the main component of essential oil from raw materials is myrtenyl acetate - its mass fraction was 60%, and in the essential oil from dry raw materials 1.8-cineol (22,0%), linalool (16,6%), α-pinene (13,7%) dominate (table. 1).

The analysis of the literature data on the variability of the component composition of the essential oil showed that it significantly depends on the ecological and geographical place of growth.

Table 1. Main components of myrtle essential oil in different ecological and geographical regions

Compoment	RI	Component content, % of whole oil volume						
		NBG *	NBG** (Bakova et al., 2018)	Serbia** (Alipour et al., 2014)	Tunisia ** (Wannes et al., 2010)	Italy** (Flamini et al., 2004)	Iran ** (Weyerstahl et al., 1994)	France ** (Curini et al., 2003)
Isobutyl - isobutyrate	907	-	2,36	0,4	-	-	0,53	0,64
α-pinene	944	**0,21**	**13,72**	**6,0**	**58,05**	**28,9**	**35,20**	**52,90**
p- cymene	1028	-	0,61	0,2	-	0,8	0,65	-
D- limonene	1035	**3,21**	**7,34**	**-**	**0,11**	**5,2**	**11,00**	**-**
1,8-cineol	1038	**6,51**	**22,05**	**22,0**	**21,67**	**24,2**	**28,50**	**32,92**
Linalool	1094	**6,59**	**16,60**	**35,7**	**2,45**	**11,7**	**8,74**	**4,21**
terpinene -4-ol	1183	1,26	0,21	0,4	0,36	0,6	0,36	0,42
α- terpineole	1196	-	3,19	8,3	0,82	3,6	5,25	2,46
Myrtenol	1200	**-**	**9,26**	**-**	**0,02**	**-**	**-**	**-**
Linalyl acetate	1250	**4,01**	**5,72**	**-**	**0,72**	**2,9**	**2,05**	**-**
Myrcenyl acetate	1326	**60,00**	**10,17**	**0,6**	**0,05**	**-**	**-**	**-**
Terpinyl acetate	1350	5,87	0,90	1,4	0,36	1,6	1,37	0,64
Geranyl acetate	1374	1,77	1,90	2,9	1,35	-	0,46	1,64
Methyleugenol	1394	-	1,14	0,7	0,38	1,1	0,95	-
β-Caryophyllen	1434	1,16	0,10	-	0,06	1,0	0,15	1,33

∗ Raw fresh materials, ∗∗ Dry materials

The main dominant components of essential oil from myrtle dry raw materials when grown in the NBG can be attributed to 1.8-cineol-linalool chemotype; when grown in Serbia – linalool-1.8-cineol; in Tunisia, Italy, Iran and France – pinene-1.8-cineol (table. 1). As you can see, myrtle essential oil is characterized by high variability depending on the zone of growth.

The quality of medicinal raw materials also depends on the content of secondary metabolites, namely phenolic compounds. 3704.09 mg/dm^3 of phenolic compounds were specified in water-ethanol extraction from myrtle leaves. Phenolic substances are represented by phenol carboxylic acids (gallic and ellagic) and flavonoids (catechin and its derivatives, quercetin and glycosides of myricetin. The concentration of phenol carboxylic acids in aqueous-ethanol extraction was 190.0 mg/100 g, and flavonoids – 3142.9 mg/100 g. The main components of the extract are: (+) D-catechin, myricetin-3-O-rhamnoside and myricetin-3-O-galactoside. It is known that the derivatives of catechin, myricetin-3-O-rhamnoside and myricetin-3-O-galactoside are very strong antioxidants and in their activity they exceed vitamins C and E (Hayder, Bouhlel, Skandrani, 2008; Tsao, 2010), so it causes a high antioxidant activity of myrtle extracts.

Table 2. Phenolic compounds in water-ethanol extract from myrtle leaves

Component	Holding time, min.	Content in water-ethanol extraction, mg/dm^3
Gallic acid	4.48	117,43
Ellagic acid	20.57	72,57

(+) D- catechin	**11.32**	**1094,39**
EGCG- Epigallocatechin -3-O- gallate	13.68	62,73
EGC- Epigallocatechin	14.54	303,05
EG- Epicatechin gallate	16.51	13,74
Myricetin -3-O- galactoside	**18.07**	**624,00**
Myricetin -3-O- rhamnoside	**19.14**	**1043,29**
Quercetine	23.21	2,03
Sum of polyphenols by HELC	-	3704,09

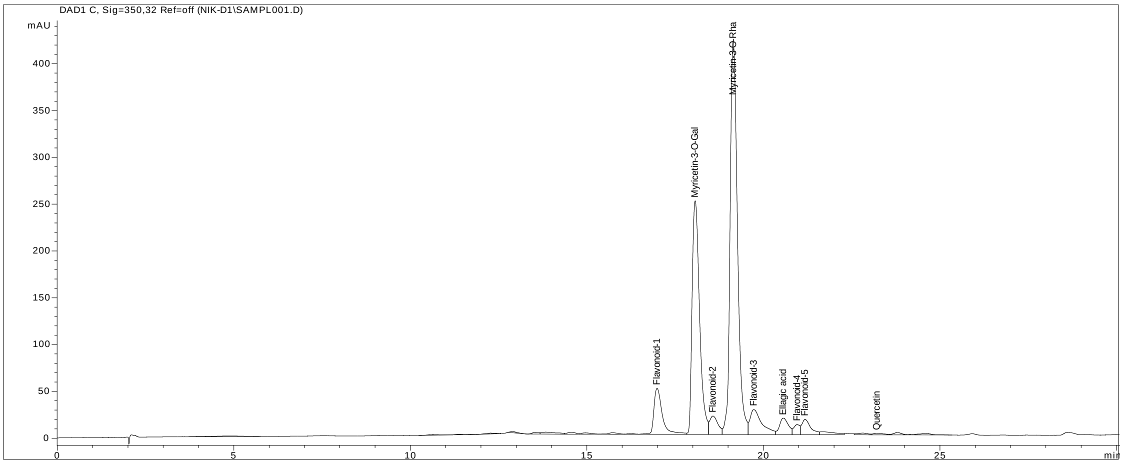

Figure 1. Chromatogram of phenolic compounds of water-ethanol extraction from myrtle dry leaves

Conclusion

The conducted studies have shown that under conditions of the Southern Coast of the Crimea in myrtle dry raw material contains a significant number of biologically active compounds: 0.82% essential oil in absolutely dry mass of 1.8-cineole-linalool chemotypes and 3704.09 mg/dm^3 of phenolic compounds, mostly flavonoids, derivatives of catechin and myricetin. The data obtained allow us to discuss about the prospects of using this culture as a source of valuable medicinal raw materials with antibacterial and antioxidant properties.

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ANALYSIS OF FUNGICIDES BOSCALID AND PYRACLOSTROBIN RESIDUES IN LETTUCE

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Abstract

In terms of importance and occurrence frequency, the most important pathogen of lettuce is fungus causing downy mildew. Considering short vegetation of lettuce and its fresh use in diet, chemical protection must be conducted with caution. Lately, fungicides from the group of carboxamide (boscalid) and strobilurin fungicide pyraclostrobin are used for protection against this pathogen. In this study, the analytical method for the simultaneous determination of boscalid and pyraclostrobin residues in lettuce was developed and validated. The method is based on QuEChERS extraction, followed by HPLC/DAD analysis. The best separation was achieved using Zorbax Eclipse XDB-C18 column, while elution was conducted by acetonitrile and water (75/25) at a flow rate of 0.55 ml/min. The detection wavelength was 210 nm. The retention times for boscalid and pyraclostrobin were 1.492 and 2.211 min, respectively. The accuracy of the QuEChERS method was expressed as recovery at the levels 0.1, 1.0 and 2.5 mg/kg. The recovery values varied at 80–106%. In addition, the RSD obtained was 1.87–13.54%. Precision was evaluated trough repeatability of the peak areas and were 1.59% and 0.31%, while LOQ values were 0.1 mg/kg and 0.05 mg/kg, for boscalid and pyraclostrobin, respectively. The linearity of the detector responses of these compounds within the concentration range 0.1-10 μg/ml, were higher than 0.9995. The results are completely in accordance with SANTE guidelines. The obtained method was applied for the analysis of boscalid and pyraclostrobin residues in lettuce samples, collected on the market in Novi Sad. Obtained results showed that the contents of the analyzed fungicides were below MRLs.

Keywords: *Boscalid, Pyraclostrobin, Lettuce, Residues.*

Introduction

Lettuce (*Lactuca sativa*) is among the most important leafy vegetable. It contains a range of beneficial secondary plant metabolites, including phenolics, ascorbate, α-tocopherol, and lignans. Lettuce production involves intensive agricultural management throughout the year, with short cycles of 30–60 days (Opatovsky et al., 2019). It is commonly cultivated in a greenhouse, under controlled conditions. However, the humidity and temperature, prevailing in these conditions, encourage the development of harmful pests. In terms of importance and occurrence frequency in our agro-ecological conditions, the most important pathogen of lettuce is fungus causing downy mildew. For controlling this pathogen, boscalid and pyraclostrobin based pesticides are used.

In the Republic of Serbia, for the control of downy mildew (*Bremia lactucae*) in lettuce, formulated product based on boscalid and pyraclostrobin are registered. Boscalid, 2-chloro-N-(4′-chlorobiphenyl-2-yl) nicotinamide, a fungicide that contains a carboxamide structure as the functional group (Figure 1). Mode of action is based on inhibition of succinate ubiquinone reductase in the fungal mitochondrial electron transport chain (Hirakawa et al., 2015). Pyraclostrobin is a fungicide belonging to strobilurin family that inhibits mitochondrial respiration by binding at the Quinone Outside site of cytochrome B (Figure 1).

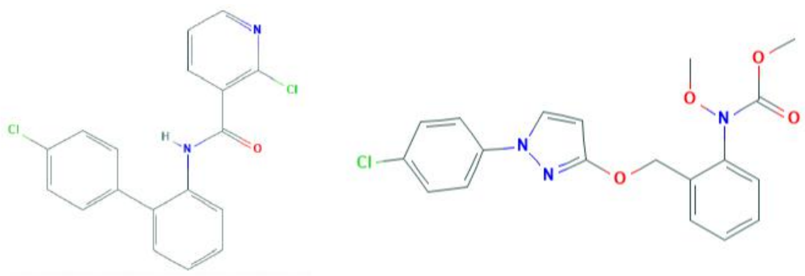

Figure 1. Boscalid and pyraclostrobin

In addition to controlling pests, these substances are often harmful to nontarget organisms. Consumers can be affected by the presence of pesticide residues in agricultural produces, especially when they are consumed fresh. The toxic nature of these compounds makes the monitoring of pesticide residues obligatory in food products in order to assess human exposure to pesticides through foods. Leafy vegetables have been given a lot of attention in monitoring programs since most of them are highly consumed and expected to contain higher pesticide residue levels than other food groups of plant origin (Esturk et al., 2014; Thabet et al., 2016).

Since lettuce is used in fresh, it is important to control the presence of pesticide residues, including boscalid and pyraclostrobin fungicides. Maximum residue levels of fungicides boscalid and praclostrobin in lettuce in the European Union and in the Republic of Serbia are 50 mg/kg and 2 mg/kg, respectively.

This study was conducted in order to obtain enough reliable and sensitive method that could be used for the analysis of boscalid and pyraclostrobin residues in lettuce samples.

Material and methods

Experiments were conducted in the Laboratory for biological research and pesticides, at the Department for Environment and Plant protection, of the Faculty of Agriculture, University of Novi sad, Serbia.

Extraction of boscalid and pyraclostrobin of the lettuce was carried out using QuEChERS methods (Figure 2). Briefly, 10g of the untreated sample was weighed and transferred to a 50 ml polypropylene cuvette. The extraction process was then carried out using acetonitrile; the cuvette was vigorously shaken for 1 minute, then a buffer mixture of salts (4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate) was added, shaken vigorously for 1 minute and centrifuged for 5 minutes at 3000 rpm.

In the clean-up process, a 6 ml aliquot was transferred into a cuvette with 150 mg PSA and 900 mg $MgSO_4$ and carbon shaken vigorously for 1 minute and centrifuged for 5 minutes at 3000 rpm. The purified extract was evaporated to dryness, dissolved in 1 ml of acetonitrile, filtered through a 0.45 μm membrane filter, transferred to a vial and analyzed by HPLC-DAD.

Figure 2. Extraction and clean-up procedure

Simultaneous determination of boscalid and pyraclostrobin was performed using high-performance liquid chromatograph Agilent Technologies 1100 Serie (HPLC-DAD) with Zorbax Eclips column (50 × 4.6 mm, 1.8 µm). As a mobile phase acidified water pH 2.8 with H_3PO_4 and acetonitrile (ACN/pH 2.8 H3PO4, 25:75 v/v) was used; flow rate 0.55 ml/min; injected volume 20 µl; detection wavelength of 210 nm, 230 nm and 254 nm.

Results and discussion

The method for the simultaneous determination of boscalid and pyraclostrobin in lettuce is based on the reverse-phase high-performance liquid chromatography. Figure 3 show the chromatogram of a mixture of boscalid and pyraclostrobin standards in acetonitrile, obtained under the previously mentioned conditions. The satisfactory separation was obtained at all applied wavelength, however, the best response of pyraclostrobin was at 210 nm, and for further analysis this wavelength was used (Figure 4).

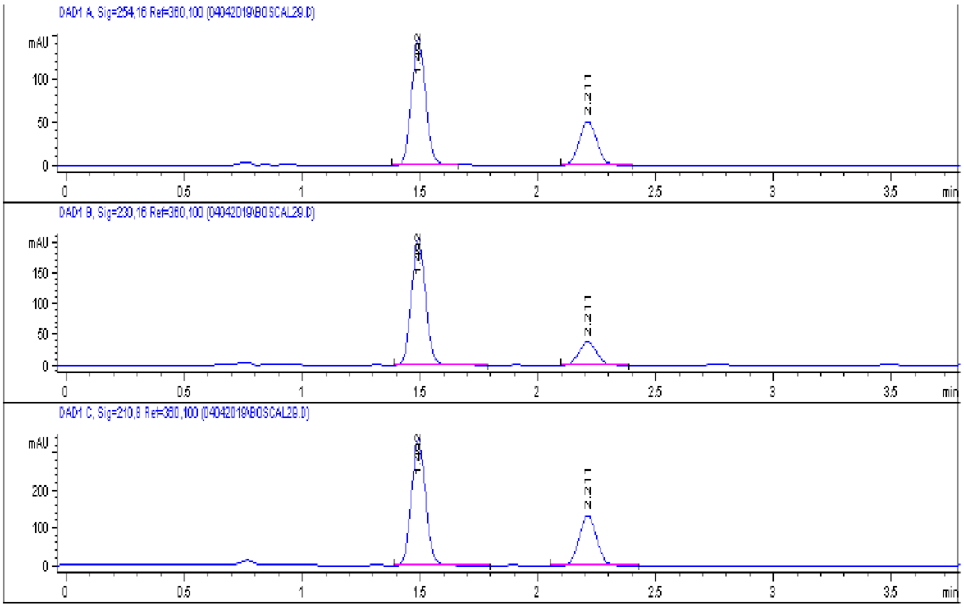

Figure 3. Chromatogram of the mixture of boscalid and pyraclostrobin analytical standards in acetonitrile at 210 nm, 230 nm and 254 nm

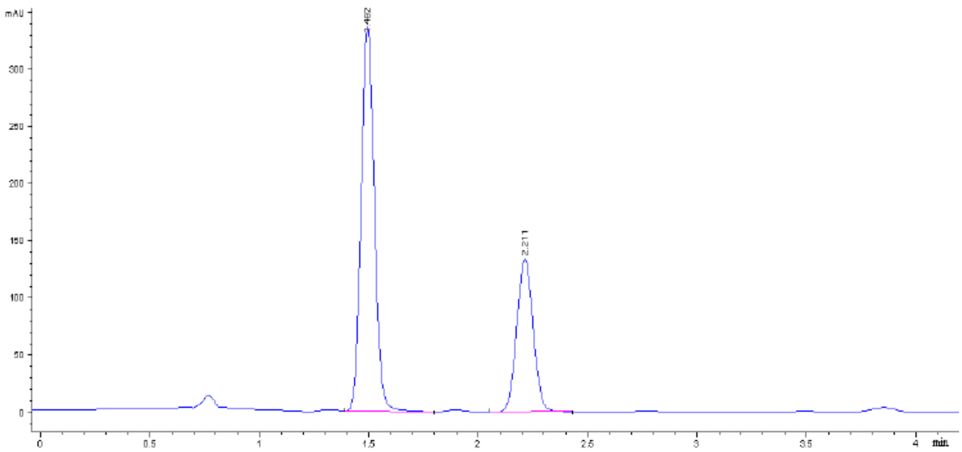

Figure 4. Chromatogram of the mixture of boscalid and pyraclostrobin analytical standards in acetonitrile at 210 nm

In addition to the retention time, for the confirmation of the presence of a compound, overlapped the spectra of the analytical standard in acetonitrile with the spectra of the peak at the same retention time in the test sample, was used (Figure 5).

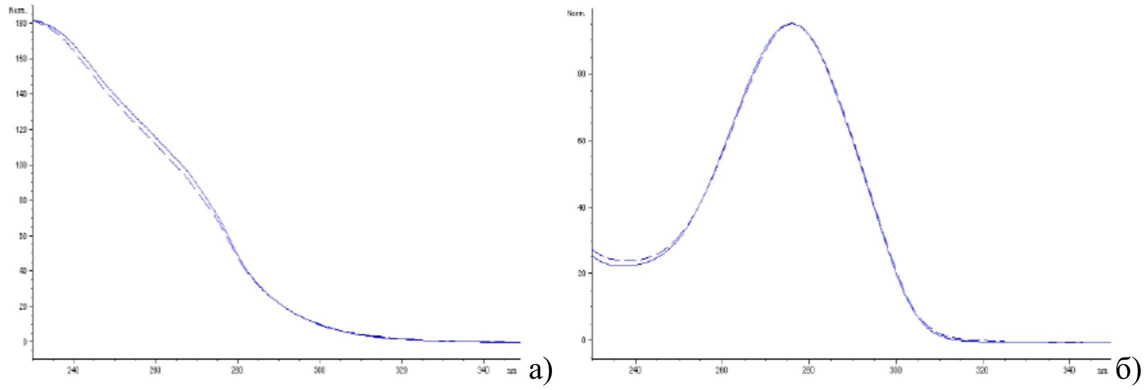

Figure 5. Overlapped spectra of boscalid a) and pyraclostrobin b) in analytical standard and in lettuce matrix

Validation of the method was evaluated through the linearity, reproducibility, recovery and limit od quantification (LOQ). A calibration curve of boscalid and pyraclostrobin exhibited good linearity ranging from 0.1-0.10 μg/ml with the correlation coefficient (r^2) of 0.999 and 0.997, respectively. According to the analysis of standard solutions, 0.1 mg/kg and 0.05 mg/kg were determined as the LOQ, which was satisfactory for the residual analysis of boscalid and pyraclostrobin, respectively. These values are below maximum residue level (MRL) of 50 mg/kg for boscalid and 2 mg/kg for pyraclostrobin in lettuce. For obtaining the recovery rate, the control samples were spiked with a mixture of boscalid and pyraclostrobin standard solution at two three concentrations and analyzed according to described method. Average recovery rates from 80-105% and 81-106% (RSD=1.87–13.54%) for boscalid and pyraclostrobin in lettuce, respectively.

In addition, the effect of lettuce matrix on the determination of boscalid and pyraclostrobin was analyzed. The obtained results indicate the influence of lettuce matrix on the reduction of boscalid and pyraclostrobin signals by only 1.3% and 2%, respectively, and a calibration curve of analytical standards dissolved in acetonitrile is used to determine the content of these fungicides in real lettuce samples. Repeatability was determined by injecting 20 μl of the standard solution of boscalid and pyraclostrobin prepared in the lettuce matrix at a concentration of 1.0 μg/ml six times. A relative standard deviation value (RSD%) of 1.59% and 0.31% indicates that a high reproducibility of the determination of boscalid and pyraclostrobin by the method used has been obtained, since RSD≤20% are acceptable according to SANTE/11813/2017.

The validated method was applied for the analysis of boscalid and pyraclostrobin residues in lettuce samples. Samples were collected at markets in Novi Sad, Serbia. In total, 60% of lettuce samples were with positive findings. Obtained results showed that the contents of the analyzed fungicides were below MRLs.

Conclusions

In this study method for the simultaneous determination of boscalid and pyraclostrobin fungicide residues in lettuce was developed and validated. All parameters are completely in accordance with SANTE/11813/2017 criteria. The reliable and sensitive method is based on the QuEChERS extraction technique, followed with HPLC-DAD. The described method was applied for the control of the presence of boscalid and pyraclostrobin resides in lettuce for the markets in Novi Sad. Obtained results showed that the contents of the analyzed fungicides were below MRLs, thus agricultural producers apply fungicides in accordance with good agricultural production.

Acknowledgement

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INFLUENCE OF BIOTIC STRESS ON TURKEY OAK SEEDLINGS IN ELEVATED CO_2 CONDITIONS

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Abstract

Expected climate changes, especially elevated CO_2 content can significantly disrupt the host-pathogen-herbivore relationship. Previous studies analyzed the influence of increased CO_2 content in relations between herbivores and their host plants, according to scenarios, which predict concentrations between 550 and 750 ppm in the future. This paper presents the results of influence of biotic stress, caused by inoculation with a root pathogen (*Phytophthora plurivora* T. Jung & T. I. Burgess) or gypsy moth (*Lymantria dispar* L.) herbivory, on the properties of Turkey oak (*Quercus cerris* L.) seedlings in elevated CO_2 conditions in extreme scenario, which predicts concentration of up to 1000 ppm in the future. For testing the influence of infection with *P. plurivora*, and feeding of gypsy moth larvae on the seedlings, analysis of covariance (ANCOVA) with plant status (exposure to infestation, feeding on the leaves or no biotic treatment) and environmental conditions (ambient or elevated CO_2 level) as independent variable and leaf mass, shoot mass and plant height as dependent variables, were used. Plants height before treatment was used as covariate. Fisher's least significant difference test was used to examine the differences in mean values of individual treatments. Biotic treatments (infestation and feeding on the leaves) significantly influenced the shoot mass and height of the seedlings, while the CO_2 concentration significantly influenced all the three observed plant properties. Interaction between biotic and environmental conditions showed no statistically significant influence on any of the analyzed properties. Plants height before treatment, used as a covariant in the analysis of variance, significantly influenced all of the analyzed properties.

Keywords: *gypsy moth, Phytophthora spp., Quercus cerris, elevated* CO_2

Introduction

Natural and artificial regeneration of oak stands is threatened by numerous biotic and abiotic factors. Success of this process depends on the characteristics of the species on one side, and from environmental conditions on the other. The most vulnerable are the seedlings during their first few years after afforestation or natural regeneration, when their survival depends on the capability of the species to survive the attack of different organisms (Price, 1991; Chaar *et al*., 1997). Most often, the influence of individual harmful factor like pathogens or harmful insects is studied (Milenković, 2015; Dobrosavljević *et al*., 2018), However, the detailed analysis of interactions of abiotic and biotic factors, when one can reduce the harmful influence of the other, or increase it in the case of synergistic activity, is needed for deeper understanding the decline of particular plant species, e.g. oaks (Thomas *et al*., 2002). Performance of the plants, in addition to pathogens and herbivores, significantly depends on the environmental conditions whose influence can be achieved directly or indirectly (Aldea *et al*., 2006). Environmental conditions influence the plants indirectly, by enhancing or diminishing the effect of the pathogens and herbivores. Expected climate changes, particularly

extreme occurrences like drought or extreme amount of precipitation, can enhance or diminish the effect of harmful organisms. Also, elevated content of CO_2 can significantly influence the relations between the host and the pathogen, as well as between the food plants and herbivorous organisms (Aldea *et al*., 2006).
Results of the research on the influence of biotic stress caused by infestation with a root pathogen (*Phytophthora plurivora* Jung and Burgess) and feeding of the gypsy moth larvae (*Lymantria dispar* L.), on the properties of the Turkey oak seedlings in the conditions of elevated and ambient CO_2 concentrations is presented in this paper.

Material and Methods

Plant material: One-year old Turkey oak (*Quercus cerris* L.) seedlings, grown from acorns that were collected on multiple locations in the vicinity of Belgrade, were used for the experiment.
The seedlings were grown on peat and perlite substrate (70:30 ratio), in PVC bags of 750 cm^3.
Inoculum: 500 cm^3 of fine vermiculite and 40 cm^3 of millet were measured in one liter Erlenmeyer flasks. Then, 350 ml of liquid V8 substrate was added to the mixture. The V8 substrate was prepared with 200 ml/l of V8 juice (Biotta, Swiss), 3 g/l of CaCO3 and 800 ml/l of distilled water (Jung *et al*. 1996). After mixing, the mixture was sterilized in an autoclave for 20 minutes at 120°C. Pure culture of *P. plurivora* was taken from the University of Belgrade-Faculty of Forestry collection, maintained by Milenković I., and developed for 3-5 days on V8-agar media (200 ml/l of V8 juice (Biotta, Swiss), 20 g/l of agar (Torlak, Serbia), 3 g/l of CaCO3 and 800 ml/l of distilled water). After inoculation, the Erlenmeyer flasks were closed with a sterile plug made out of wadding and aluminum foil. Incubation was at room temperature in the dark for 4-6 weeks. Before the inoculation itself, the inoculum was washed in sterile distilled water, so that the potential bacterial contaminants and sugar from the substrate that could be a carrier of bacterial colonies could be removed.
Inoculation: The inoculation was conducted by adding of around 40 cm^3 of inoculum to the plants rhizosphere. After inoculation, the plants were immediately flooded for 72 hours.
Insect material: Gypsy moth egg masses were collected in an oak forest in the vicinity of Bor. They were kept in a freezer at a temperature of 4 °C until the spring 2018. Before the beginning of the experiment they were put at a temperature of 25 °C in order to induce hatching. After hatching, the larvae were fed with artificial food in petri dishes (120 x 15 mm) at a temperature of 23º C, Rh 75% and light regime 15:9 (day: night) until molting to the fourth larval stage.
Experiment design: After germination of the acorns in the laboratory, from January, the seedlings were kept in controlled conditions at a temperature of 20 +/-1 °C. At the beginning of March half of the total number of plants were exposed to the elevated CO_2 concentration, while the rest of the plants were grown in the conditions of ambient CO_2 concentration. At the beginning of April, a part of the plants was subjected to the inoculation with *P. plurivora*, and then to the feeding of the gypsy moth larvae. Thus, 6 experimental groups were formed as such: CA (no biotic treatment, ambient CO_2), CE (no biotic treatment, elevated CO_2), PA (inoculated with *P. plurivora*, ambient CO_2), PE (inoculated with *P. plurivora*, elevated CO_2), LA (feeding of *Lymantria dispar*, ambient CO_2) and LE (feeding of *Lymantria dispar*, elevated CO_2). The influence of biological stress in the conditions of ambient and elevated CO2 levels on Turkey oak seedlings was estimated by comparing the leaf mass, shoot mass and plants height.
Statistical analysis: For testing the influence of inoculation with *P. plurivora*, and feeding of the gypsy moth larvae on the Turkey oak seedlings, analysis of covariance (ANCOVA) with plant status (exposure to larval feeding on the leaves, inoculation or no biological treatment) and environmental conditions (ambient or elevated CO_2 level) as independent variable and

leaf mass, shoot mass and plants height at the end of the experiment as dependent variables, were used. Plants height before treatment was used as covariate. Fisher's least significant difference test was used to examine the differences in mean values of individual treatments.

Results and discussion

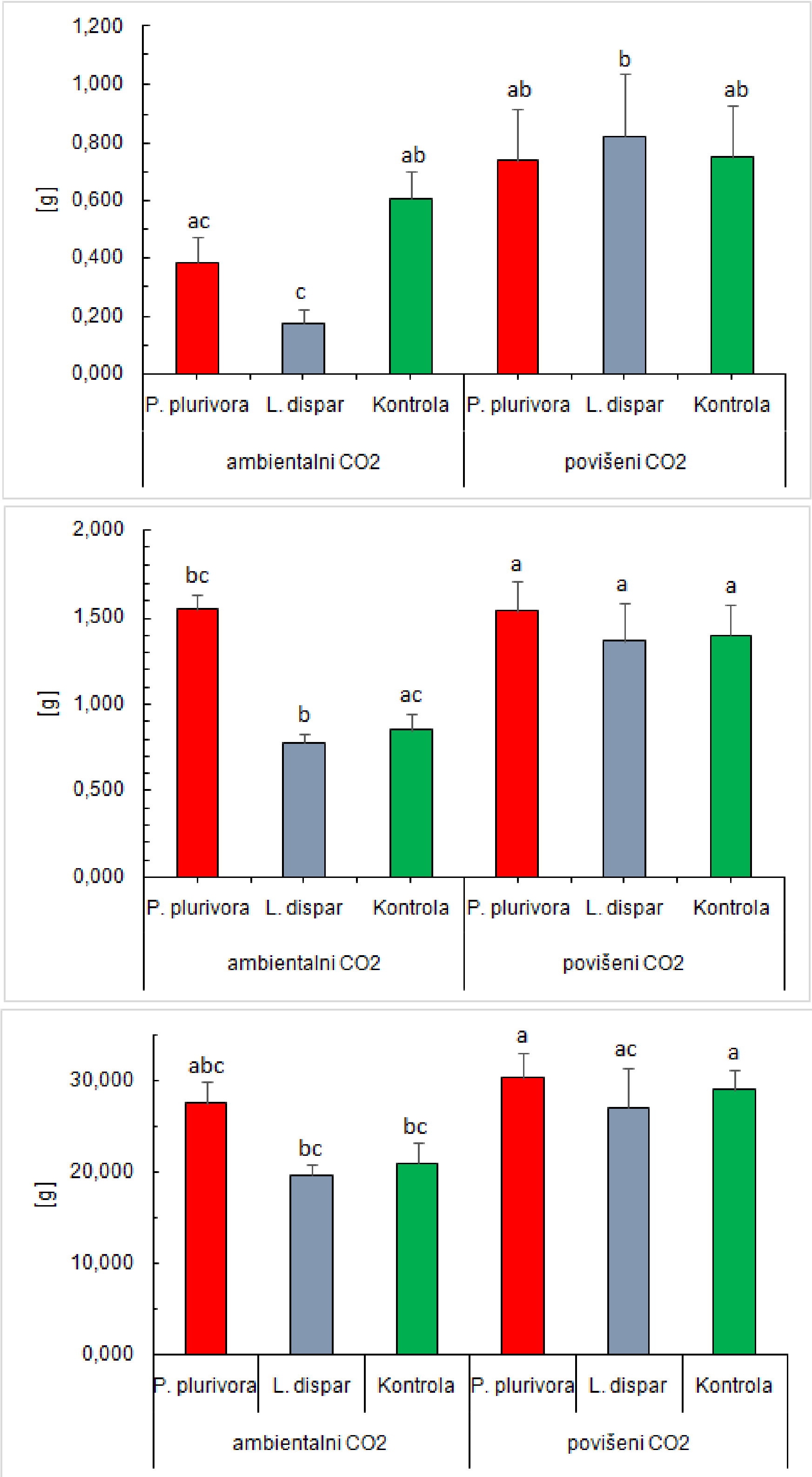
Results of the research on the influence of inoculation with *P. plurivora*, feeding of the gypsy moth larvae as well as elevated CO_2 concentrations on the performance of Turkey oak seedlings are presented in Table 1 and Graph 1. The results of the covariance analysis (ANCOVA) shoved statistically significant influence of the biotic treatment (inoculation and feeding on the leaves) on the shoot mass and height of the Turkey oak plants, while the CO_2 concentration had a significant influence on all of the 3 monitored properties of the plants. Interaction between biotic and environmental conditions showed no statistically significant influence on any of the analyzed properties. However, plants height before the beginning of the experiment, used as a covariant in the analysis of variance, significantly influenced all of the analyzed properties (Table 1).

Plants from the LA group had the lowest average leaf mass, while the plants from the LE group had the greatest average leaf mass (Graph 3a). Statistically significant difference existed between the leaf mass in these two groups, while the other groups except the PA group showed no statistically significant differences (Table 1, Graph 3a). No statistically significant differences in the shoot mass existed between the plants which grew in the conditions of elevated CO_2 concentration, regardless of the biotic treatment, as well as between these three groups and groups of plants of the PA group (Table 1, Graph 3b). However, between this group of seedlings, and the other two which grew in the conditions of ambient CO_2 concentration, statistically significant differences in the average shoot mass existed (Table 1, Graph 3b). The shoot mass was about 50% lower in the CA and the LA group. The same pattern was recorded with the height of the plants, although the differences between the PA group and the other two groups that were grown in the same conditions was about 30% lower (Table 1, Graph 3c)

Table 1. Results of the covariance analysis (ANCOVA) for investigated biotic influences (feeding on the leaves and inoculation) and environmental conditions (ambient and elevated CO_2 content) on the performance of Turkey oak seedlings

Source of variation	d.f.	Leaf mass			Shoot mass			Plants height		
		MS	F	P	MS	F	P	MS	F	P
Biotic treatment	2	0.143	0.72	0.4892	**1.103**	**5.26**	**0.0083**	**119.524**	**3.88**	**0.0270**
CO_2	1	**1.743**	**8.83**	**0.0045**	**1.313**	**6.25**	**0.0156**	**354.713**	**11.50**	**0.0013**
Biotic * CO_2	2	0.188	0.95	0.3924	0.430	2.05	0.1394	42.467	1.38	0.2613
Plants height (covariate)	1	**1.509**	**7.64**	**0.0079**	**7.698**	**36.68**	**0.0000**	**1936.709**	**62.82**	**0.0000**
Error	52	0.197			0.210			30.831		

*Bold values indicates a statistically significant difference



Graph 3. Influence of elevated CO_2 content on (a) leaf mass, (b) shoot mass and (c) plants height

Turkey oak, as well as other oak species are threatened by many pathogens such as fungi-like organisms from the *Phytophtora* genus (Jung *et al*., 1996, 2000; Balci and Halmschlager, 2003 a, b; Balci *et al.*, 2007). Among them significant damage is caused by the species *P. plurivora*, previously known worldwide as *P. citricola* (Balci and Halmschlager, 2003 a, b;

Jung and Burgess, 2009). In the same time, Turkey oak is the most favorable host plant for the development of the gypsy moth larvae (Milanović, 2014). This harmful pest causes the most significant damage in its forests (Mihajlović, 2015). On the other side, we are witnesses to the changes that are happening under the influence of elevated CO_2 concentration, that can also influence the insects. A large number of scientists researched the influence of CO_2 on the feeding and development of insects on different tree species (Traw *et al.*, 1996; Henn and Schopf, 2001; Hättenschwiler and Schafellner, 2004; Wang *et al.*, 2009).
As Turkey oak seedlings are concerned, an interesting fact is that the greatest leaf mass was recorded on the plants which were exposed to the conditions of elevated CO_2 concentration. It was 30% higher compared to the leaf mass of the plants that grew in the ambient CO_2 conditions. Broadmeadow and Jackson (2000) determined that the sessile oak (*Quercus petraea* (Mattuschka) Liebl) leaf mass was 39% greater if the plant were exposed to the elevated CO_2 concentrations. The plants height and shoot mass were also higher if the plants were exposed to the elevated CO2 concentration. The research of Norby *et al*. (1986) shows an increase in growth of white oak (*Quercus alba* L.) seedlings by 85% if the plants are grown in conditions of increased concentration of CO_2 (690 ppm).
According to Jankowiak *et al*. (2014) *P. plurivora* is a common species that occurs in oak forests, and next to *P. quercina* Jung (Jung *et al*., 1996, 1999), *P. cinnamomi* Rands (Jung et al., 2018) it is considered as one of the most aggressive pathogens threatening the oak trees.

Conclusion

The results obtained in this paper show that the inoculation of the root with a pathogen can have a stimulative effect on plants height and shoot mass. As the root of the plant was attacked, the resources were allocated to the unaffected parts of the plants, which eventually led to the increase in growth of the above ground parts.

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MACRO AND MICRO ELEMENT CONTENTS OF SOME HERB AND CONDIMENTS

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Abstract

The study was conducted to investigate the macro and micro elements of several herbs and condiments. Mineral contents of samples were measured by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The macro elements of herbs and condiments were Ca (234-40059 mg/kg), Mg (649-7312 mg/kg), K (5788-37781 mg/kg), P (526-8412 mg/kg), and S (741-8117 mg/kg). The highest Ca, P and S contents were found in urtica, black cumin and ginger, respectively. Basil had the maximum Mg (7312 mg/kg) and K (37781 mg/kg) contents. The lowest Ca (234 mg/kg) and K (5788 mg/kg) amounts were determined in sesame, while the cinnamon, turmeric and sumac have the minimum Mg, P and S contents, respectively. The micro elements of samples were B (6.4-51.1 mg/kg), Cr (0.10-2.62 mg/kg), Cu (5.9-22.8 mg/kg), Fe (36-781 mg/kg), Mn (3.6-624.3 mg/kg), Ni (0.12-5.80 mg/kg) and Zn (5.0-62.1 mg/kg). The highest and lowest Fe contents were found in balm (781 mg/kg) and rose (36 mg/kg) samples, respectively. While Zn contents of samples change between 5.0 (rose) and 62.1 mg/kg (black cumin), Cu contents ranged from 5.9 (turmeric) to 22.8 mg/kg (basil). The Mn content of clove (624.3 mg/kg) was higher than other condiments. Additionally, rosemary and thyme contained higher B content, with the ranges of 48.1 mg/kg and 48.0 mg/kg, respectively. The study revealed that investigated edible condiments are good source of P, K, Ca, Mg and Fe. The urtica, senna and basil condiments are thought to be beneficial for bones and teeth due to their high Ca content. However, the clove, rosemary and thyme contain a high amount of Mn and B elements, so over-consumption of these herbs can create toxic effects.

Key words: *condiments, herbs, mineral, heavy metal, ICP-AES*

Introduction

Herbs and spices, grown wildly in various regions of the world, have been used for several purposes since ancient times. Several uses of these plants are known for culinary purposes (Baytop, 1984; Koedam, 1986). Spices refer to all of the edible parts of a plant used for flavoring or coloring foods, including fruit, seed, root, bark or vegetable substance (Bouba et al. 2012; Susheela, 2000). Some are often used as preservative against the harmful bacteria or prevented their growth (Bitting and Sherman, 1998). Actually the specific uses of spices tend to vary considerably among cultures and countries: medicine, religious rituals, cosmetics, perfumery and foods. As food, they have been shown to play an important role in health partially as sources of nutrients (Brakat et al., 2003; Takruri and Dameh, 1998; Bouba et al., 2012). Plants constitute an important source of active natural products, which differ widely in terms of structure and biological properties. They have played a remarkable role in the traditional medicine of various countries. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of spices, fresh fruits, vegetables, or teas rich in natural antioxidants (Virgili et al., 2001). In terms of structure, biological properties and role in the traditional medicine, plants are an important source of active natural products (Virgili et al., 2001). Spices stimulate the appetite and increase the flow of gastric juice. They also play a role in many of the industries, and are used in perfumery, soaps, incense, as dyes in histology and in various acts (Onyesom and Okoh, 2006).

Macro and micro elements have significant effect on bio-chemical processes in the human body (Arceusz et al., 2010). The proper intake of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine reduces the risk of especially cardiovascular diseases in humans. In addition, the main minerals function as structural components of tissues and show great importance in cellular and basal metabolism and water and acid-base balance (Imelouane et al., 2011). Some micro elements exhibite a significant role as catalysts or parts of prosthetic groups for enzymes, and therefore, element deficiency causes various health problems (Ebrahim et al., 2012). However, excessive consumption of mineral-rich plants causes to various health problems (Jabeen et al., 2010). Herbal plants and spices include not only essential but also toxic elements in different concentrations (Soylak et al., 2012). Accordingly, determination of mineral content of herbal plants and spices have importance and it is necessary to investigate the mineral contents of these plants for quality controls. The objective of present study was to determine the mineral and heavy metal contents of several herb and condiments used for several purposes in Turkey.

Material and Methods

Materials

Spice samples were purchased from local market in Turkey (Konya). Samples were dried at 70 ^{o}C in oven, and than the dried materials were then ground in a mortar and the ground material sieved in 10 mesh diemater, and sealed in bottles for storage until analysis. The common, scientific and family names of the spices are given in Table 1.

Determination of mineral contents

Methods

Provided condiment samples were dried at 70 ºC in a drying cabinet with air-circulation until they reached constant weight. Later, about 0.5 g dried and ground samples were digested by using 5ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a closed microwave system (Cem-MARS Xpress). The volumes of the digested samples were completed to 20 ml with ultra-deionized water, and mineral contents were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP AES) (Varian-Vista, Australia). Measurements of mineral concentrations were checked using the certified values of related minerals in the reference samples received from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) (Skujins, 1998).

Working conditions of ICP-AES:

Instrument	:ICP-AES (Varian-Vista)
RF Power	: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar)	: 10.5-15 L/min. (radial) 15 " (Axial)
Auxilary gas flow rate (Ar)	:1.5 "
Viewing height	: 5-12 mm
Copy and reading time	:1-5 s (max.60 s)
Copy time	: 3 s (max. 100 s)

Statistical analyses

Results of the research were analysed for statistical significance by analysis of variance (Püskülcü and İkiz, 1989).

Table 1. Plants used in this study

General Name	Botanical name	Family	Used parts
Sage	*Salvia officinalis* L.	Labiatae	Leave+Flower
Ginger	*Zingiber officinale*	Zingiberacae	Root
Rose	*Rosa canina* L.	Rosaceae	seed
Clove	*Syzygium aromaticum*	Myrtaceae	Flower bud
Turmeric	*Curcuma longa*	Zingiberaceae	Root
Marshmallow	*Althaea officinalis*	Malvaceae	Leaves + Flower
Fennel (sweet)	*Foeniculum vulgare* subsp. *dulce*	Umbelliferae	Fruit
Thyme	*Thymbra spicata* L.	Labiatae	Leave+Flower
Tilia	*Tilia* sp. L.)	Tiliaceae	Leaves + Flower
Black cumin	*Nigella sativa* L.	Ranunculaceae	seed
Paprica	*Capsicum annuum* L.	Solanaceae	Fruit
Mint	*Mentha piperita* L.	Labiatae	Leave
Cumin	*Cuminum cyminum* L.	Umbelliferae	Fruit
Pepper (black)	*Piper nigrum* L.	Piperaceae	Fruit
Rosemary	*Rosmarinus officinalis* L.	Labiatae	Leave+Flower
Sumac	*Rhus coriaria* L.	Anacardiaceae	Pericarp
Cinnamon	*Cinnamon zeylanicum*	Lauraceae	Bark
Sesame	*Sesamum indicum* L.	Pedaliaceae	seed
Basil	*Ocimum basilicum* L.	Labiatae	Leave+Flower
Coriander	Coriandum sativum	Umbelliferae	Fruit
Muğla Sage	Salvia fruticosa	Labiatae	Leave +Flower
Scotch thistle	*Onopordum acanthium* L.)	Compositae	seed
Olive	*Olea europaea* L.	Oleaceae	Leave
Urtica	*Urtica dioica* L.	Urticaceae	Leave
Senna	*Cassia spp.*	Fabaceae	Leave
Anise	*Pimpinella anisium* L.	Umbelliferae	Fruit
Parsley	*Petroselinum crispum* L.	Umbelliferae	Leave
Fenugreek	*Trigonella foenumgraecum* L.	Fabaceae	seed
Balm	*Melissa officinalis* L.	Labiatae	Leave
Fennel (bitter)	*Foeniculum vulgare* subsp. *piperitum*	Labiatae	Fruit

Results and Discussion

The macro element compositions of several condiments and spices are given in Table 2. Mineral contents changed in regard to condiment types. According to results, Ca, Mg, K, P and S contents were very high in all the condiments. Cr, Cu, Mn and Ni contents of condiments were found low an important level (Table 3) ($p<0.05$). The level of potassium of all samples in this study was found to be higher than other elements. K contents of samples ranged from 7567 mg/kg (Cinnamon) to 37781 mg/kg (Basil). Also, Ca contents were found between 549 mg/kg (turmeric) and 40059 mg/kg (urtica). While Mg contents of condiments change between 649 mg/kg (cinnamon) and 7312 mg/kg (basil), P contents ranged from 526 mg/kg (turmeric) to 8412 mg/kg (black cumin). In addition, S contents of condiments changed between 741 mg/kg (sumac) and 8117 mg/kg (ginger). Micro element and heavy metal contents of condiments are shown in Table 3. While the highest Fe content is found in balm (781 mg/kg), the lowest Fe was determined in rose (36 mg/kg). Zn contents of all condiments were found between 5.0 mg/kg (rose) and 62.1 mg/kg (black cumin). Cu contents of samples ranged from 5.9 mg/kg (turmeric) to 22.8 mg/kg (basil).

Özcan (2004) reported that sahlep (*Orchis* spp.) and dill (*Anethum graveolens* L.) contained 2384 ppm and 35723 ppm potassium, respectively. According to the same researcher, while iron content change between 46.7 ppm caraway (*Carum carvi* L.) and 1229 ppm (*Lavandula officinalis* L.), selenium content varied from 0.15 ppm in savory (*Satureja hortensis* L.) to 5.03 ppm in mustard (*Sinapis alba* L.). Besides, zinc contents of spices were found between 5.54 ppm sumac (*Rhus coriaria* L.) and 49.7 ppm in black cumin (*Nigella sativa* L.) (Özcan, 2004). The result shows that the aniseed contained phosphorous (2027.10 mg/kg), iron (5.40 mg/kg), magnesium (270.10 mg/kg), calcium (602.8 mg/kg), sodium (365.10 mg/kg) and potassium (887.80 mg/kg). The poppy seeds had phosphorous (3980.0 mg/kg), iron (5.475 mg/kg), magnesium (287.20 mg/kg), calcium (690.50 mg/kg), sodium (81.16 mg/ kg) and potassium (746.70 mg/kg) contents. The Fenugreek seeds contained phosphorous (2950.0 mg/kg) and potassium (124.82 mg/kg) (Kumaravel and Alagusundaram, 2014). In other study, K content was found very low in *T. spicata* (1200.99 ppm), *R. canina* (5316.84 ppm) and *C. angustifolia* (6728.64 ppm), but was very high in *M. chamomilla* (27497.81 ppm), *C. casia* (29167.53 ppm) and green tea (28890.93 ppm). In addition, *C. casia* (11733.96 ppm), *F. vulgare* (10145.48 ppm) and green tea (12698.05 ppm) were rich in P (Özcan et al., 2008). Our results shown partly differences with literature values conducted on different spice and herbs. These differences might be due to growth conditions, geographical variations and analytical procedures.

The most of elements which may contribute to biological processes, but which have not been established as essential, are garium, copper, crom and nickel (Macrae et al., 1993). Decreasing of these toxic element contents is an advantage. This study supports to contribute to knowledge of the nutritional properties of these plants. In addition, knowledge of the mineral contents, as condiments is of great interest.

Table 2. Macro element contents of condiments (mg/kg) (dw basis)

Condiment	Ca	Mg	K	P	S
Sage	15259 ± 33	3525 ± 58	14734 ± 15	935 ± 27	1358 ± 54
Ginger	1549 ± 30	3242 ± 16	30259 ± 44	3101 ± 87	8117 ± 20
Rose	8533 ± 18	2288 ± 65	12758 ± 41	1422 ± 24	894 ± 50
Clove	9375 ± 23	3581 ± 52	20049 ± 35	1251 ± 25	1665 ± 29
Turmeric	549 ± 22	1740 ± 18	15195 ± 55	526 ± 18	1238 ± 98
Marsh Mallow	19228 ± 26	5143 ± 38	27798 ± 15	4158 ± 35	4258 ± 11
Fennel (sweet)	11373 ± 59	5147 ± 16	25222 ± 27	6734 ± 15	3324 ± 41
Thyme	23116 ± 33	2638 ± 63	23385 ± 50	1384 ± 51	2375 ± 62
Tilia	11568 ± 47	2640 ± 65	20528 ± 59	2580 ± 10	1343 ± 93
Black cumin	7406 ± 31	3201 ± 10	11447 ± 30	8412 ± 10	3190 ± 15
Paprica	1238 ± 57	1751 ± 81	26965 ± 18	4098 ± 97	2219 ± 18
Mint	16121 ± 17	4928 ± 45	33513 ± 25	5305 ± 60	5544 ± 24
Cumin	15483 ± 15	4359 ± 53	25756 ± 26	4423 ± 10	3625 ± 11
Pepper (black)	6270 ± 81	2684 ± 80	22537 ± 62	2100 ± 10	1459 ± 47
Rosemary	24123 ± 85	4490 ± 36	13698 ± 60	1776 ± 58	2630 ± 51
Sumac	4666 ± 86	715 ± 23	16195 ± 22	1005 ± 19	741 ± 25
Cinnamon	15341 ± 20	649 ± 18	7567 ± 85	798 ± 14	1852 ± 13
Sesame	234 ± 13	4125 ± 15	5788 ± 31	8145 ± 93	3775 ± 20
Basil	32525 ± 58	7312 ± 35	37781 ± 17	5852 ± 48	3310 ± 15
Coriander	8878 ± 16	3791 ± 11	24578 ± 82	5747 ± 17	2678 ± 19
Muğla Sage	12757 ± 34	2751 ± 87	24062 ± 23	983 ± 19	2527 ± 14
Scotch thistle	10856 ± 21	3821 ± 95	11906 ± 20	5418 ± 17	2034 ± 53
Olive	28737 ± 17	2871 ± 52	17047 ± 55	1268 ± 30	1738 ± 79
Urtica	40059 ± 94	4548 ± 69	35041 ± 43	7426 ± 16	6557 ± 16
Senna	34678 ± 46	4568 ± 26	16176 ± 22	1647 ± 17	2675 ± 19
Anise	10250 ± 74	4212 ± 89	26187 ± 78	6910 ± 19	4427 ± 74
Parsley	12098 ± 21	5286 ± 52	20816 ± 63	4661 ± 85	3725 ± 95
Fenugreek	1211 ± 60	2123 ± 10	16550 ± 34	5457 ± 23	2572 ± 13
Balm	23133 ± 16	5234 ± 20	25692 ± 70	3249 ± 57	3280 ± 21
Fennel (bitter)	17428 ± 55	3848 ± 38	23678 ± 55	3126 ± 34	3414 ± 16

*mean±standard deviation

Table 3. Micro element contents of condiments (mg/kg) (dw basis)

Condiments	B	Cr	Cu	Fe	Mn	Ni	Zn
Sage	39.2 ± 0.8	0.38 ± 0.07	7.2 ± 0.1	112 ± 2	6.8 ± 0.4	0.99 ± 0.07	14.9 ± 0.4
Ginger	8.4 ± 0.5	1.67 ± 0.03	9.6 ± 0.4	436 ± 14	413.1 ± 7.4	2.12 ± 0.22	16.1 ± 0.2
Rose	18.4 ± 1.3	0.16 ± 0.03	6.4 ± 0.3	36 ± 2	30.3 ± 0.5	1.18 ± 0.16	5.0 ± 0.2
Clove	27.3 ± 2.2	0.23 ± 0.04	11.9 ± 0.7	72 ± 4	624.3 ± 12.6	0.12 ± 0.01	9.6 ± 0.2
Turmeric	6.4 ± 0.4	0.67 ± 0.05	5.9 ± 0.2	182 ± 6	201.7 ± 3.9	1.77 ± 0.10	11.4 ± 1.2
Marshmallow	38.1 ± 1.1	1.07 ± 0.13	10.0 ± 0.3	235 ± 4	29.8 ± 0.1	4.00 ± 0.34	23.7 ± 0.7
Fennel (sweet)	34.3 ± 1.7	0.71 ± 0.05	15.2 ± 0.7	87 ± 3	47.2 ± 2.0	2.76 ± 0.10	26.1 ± 2.3
Thyme	48.0 ± 2.6	0.35 ± 0.04	11.2 ± 0.2	111 ± 3	79.3 ± 3.1	0.50 ± 0.09	19.6 ± 0.8
Tilia	23.3 ± 1.0	0.13 ± 0.03	11.8 ± 0.5	59 ± 4	29.9 ± 0.5	0.20 ± 0.03	18.1 ± 1.4
Black cumin	23.2 ± 2.1	0.24 ± 0.04	14.9 ± 1.0	119 ± 2	25.9 ± 1.8	1.81 ± 0.04	62.1 ± 2.8
Paprica	12.0 ± 1.2	0.18 ± 0.03	9.8 ± 0.8	58 ± 2	3.6 ± 0.3	0.26 ± 0.03	16.2 ± 1.0
Mint	29.7 ± 0.6	0.76 ± 0.04	14.0 ± 0.2	190 ± 4	62.7 ± 1.0	1.52 ± 0.09	26.4 ± 0.8
Cumin	30.2 ± 2.3	2.62 ± 0.25	14.5 ± 0.5	337 ± 3	34.1 ± 0.9	5.80 ± 0.58	36.6 ± 1.8
Pepper (black)	14.7 ± 0.3	0.99 ± 0.18	17.7 ± 1.1	290 ± 3	65.0 ± 1.3	2.83 ± 0.37	17.5 ± 1.0
Rosemary	48.1 ± 2.5	0.46 ± 0.05	6.4 ± 0.4	136 ± 4	56.2 ± 0.4	2.10 ± 0.22	19.7 ± 1.2
Sumac	24.8 ± 0.3	0.77 ± 0.06	8.3 ±0.5	381 ± 6	4.0 ± 0.3	1.39 ± 0.24	8.4 ± 0.5
Cinnamon	15.8 ± 0.3	0.10 ± 0.02	7.4 ± 0.6	74 ± 2	193.1 ± 6.4	0.68 ± 0.06	17.0 ± 1.1
Sesame	13.7 ± 1.2	0.26 ± 0.03	19.5 ± 0.6	73 ± 4	13.4 ± 0.8	1.58 ± 0.32	50.9 ± 2.3
Basil	34.1 ± 1.4	0.38 ± 0.05	22.8 ± 1.2	119 ± 1	78.8 ± 1.5	2.43 ± 0.29	21.4 ± 1.0
Coriander	24.0 ± 1.7	0.31 ± 0.06	17.3 ± 1.0	83 ± 4	23.4 ± 1.4	0.88 ± 0.06	39.2 ± 2.2
Muğla Sage	35.6 ± 1.3	1.64 ± 0.26	9.0 ± 0.2	527 ± 11	42.1 ± 2.1	2.26 ± 0.11	39.2 ± 0.6
Scotch thistle	18.8 ± 1.3	0.19 ± 0.03	19.4 ± 0.6	63 ± 2	15.9 ± 0.4	0.97 ± 0.14	39.9 ± 2.6
Olive	22.2 ± 2.0	0.81 ± 0.07	7.8 ± 0.5	173 ± 4	50.1 ± 1.5	2.45 ± 0.45	14.9 ± 0.9
Urtica	40.4 ± 2.0	0.59 ± 0.05	12.0 ± 0.7	245 ± 8	40.4 ± 1.0	0.57 ± 0.05	29.9 ± 0.1
Senna	51.1 ± 2.0	0.92 ± 0.20	7.9 ± 0.3	313 ± 9	35.0 ± 0.4	0.54 ± 0.11	16.5 ± 1.7
Anise	24.8 ± 1.8	0.56 ± 0.04	10.7 ± 0.7	171 ± 5	20.6 ± 1.0	2.24 ± 0.37	36.9 ± 1.6
Parsley	29.3 ± 1.4	0.37 ± 0.07	20.8 ± 0.2	188 ± 9	38.7 ± 0.8	2.99 ± 0.39	50.9 ± 2.4
Fenugreek	16.0 ± 0.5	0.16 ± 0.02	15.8 ± 0.7	89 ±5	12.6 ± 1.2	2.31 ± 0.13	32.5 ± 1.8
Balm	26.7 ± 1.2	1.68 ± 0.17	15.4 ± 0.4	781 ± 29	46.0 ± 4.1	2.04 ± 0.14	29.0 ± 1.3
Fennel (bitter)	26.2 ± 3.8	0.25 ± 0.02	12.1 ± 0.2	82 ± 6	27.1 ± 0.8	0.83 ± 0.11	24.6 ± 1.0

*mean±standard deviation

Conclusion

These spices have the potential of contributing tothe nutritional and health needs of their consumers. The results show that spice powder is a good source of calcium, sodium, potassium, magnesium, selenium and phosphorous and spice was a very important human nutrient since their consumption has increased in recent years (Kumaravel and Alagusundaram, 2014). As a result, minerals are important in the diet because they serve as cofactors for many physiologic and metabolic functions. K, P and Mg were detected at significant levels in condiment and herbs. A significant levels of Ca, Fe, Mn and Zn were also present in the condiments. The study revealed that investigated edible condiments are good source of P, K, Ca, Mg and Fe. Heavy metals in spice are a problem for human consumption and hence a detailed study of minerals in spices is needed.

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EFFECT OF SALICYLIC ACID APPLICATION ON PHENYLALANINE AMMONIA-LYASE ACTIVITY AND TOTAL PHENOLIC CONTENT IN WHEAT (*Triticum aestivum* L.) AND BUCKWHEAT (*Fagopyrum esculentum* MOENCH) PLANTS UNDER CADMIUM STRESS

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Abstract

Salicylic acid (SA) is considered as one of the most important signaling molecule involved in both abiotic and biotic stress tolerance. Application of optimal concentrations (0,05 mM) of SA enhances plants tolerance to cadmium stress by modulating levels of several metabolites including components of antioxidative defense, osmolytes, secondary metabolites, and metal-chelating compounds. When SA and cadmium (Cd) were applied simultaneously, the damage was less pronounced than without SA. Salicylic acid treatment itself also caused the oxidative stress, but decreased the phenylalanine ammonia-lyase activity, regulating phenolic synthesis. Thys, when the SA treatment was used prior the Cd stress, it prevented the damaging heavy metal effect.

Keyworlds: *Phenylalanine ammonia-lyase (PAL), phenolic compounds, salicylic acid, cadmium chloride.*

Introduction

Wheat is a major staple agricultural crop in Ukraine and other countries. Buckwheat (*Fagopyrum esculentum* Moench) belongs to crops of secondary importance in many countries. The main producers of buckwheat are China, Ukraine, Kazakhstan and Russian Federation, but generally it is consumed or traded locally. A heavy metal contamination issues have been of increasing in Ukraine and worldwide, with many documented cases of metal toxicity in a mining industries areas, foundries, smelters, coal-burning power plants and agriculture. The heavy metals accumulation in soils is of concern in agricultural production due to the adverse effects on food safety and marketability, crop yields and environmental health of soil organisms. Cadmium toxicity has a high impact on plants and consequently it affects an ecosystem, where plants form an integral component (Pinto *et al*., 2004; Bessonova *et al*., 2005; Topchiy, 2010; Versieren *et al*, 2017). On the other hand salicylic acid (SA) plays a key role in a plant disease resistance and responses to abiotic stressors. There are report that Cd exposure increased the free SA contents and increased total phenolic content in wheat and buckwheat plants.

Current research data support the possible mediatory role of the salicylic acid (SA) in protecting physiological and biochemical processes under the cadmium toxicity. Seed pretreatment with SA alleviated the negative effect of Cd on plant growth parameters. The same tendency was observed for the chlorophyll level. The positive effect of SA on salt-resistance for different plant species reported Who?. SA belongs to the class of phenolic compounds (Kohli, 2017). SA pretreatment of 0,05 mM also decreased the extent of negative Cd effect on wheat plants, as revealed by the decline in the level of phenolic content (Kolupaev, 2010; Szalai, 2013).

It was shown that SA activated and regulated phenylalanine ammonia-lyase (PAL), the key enzyme of phenolic compounds biosynthesis, in roots of wheat and buckwheat plants. This assumption is supported by the data on significant decline in Cd accumulation in SA-pretreated plants, especially in the shoots. We have studied the influence of the SA pretreatment of wheat and buckwheat seedlings on plant resistance to subsequent treatment with cadmium chloride. The aim of this work was to investigate the influence of exogenously applied SA on the content of phenolic compounds and PAL activity under the cadmium stress.

Materials and Methods

Wheat plants (*Triticum aestivum* L.) Podolianka variety and buckwheat (*Fagopyrum esculentum* Moench.) Rubra variety were used in the laboratory experiments. SA was applied for the seeds pretreatment. Previously sterilized how? seeds were soaked for 5 h in the SA (0,05mM) solution or distilled water (control). Then the seeds were germinated on a filter paper in Petri dishes at 23 °C, for three days. After that uniform seedlings were transfferred to the pots with a sterile sand and grown under the controlled conditions (16-h photoperiod). Cadmium chloride was applied in the dose 25 mg/kg to the which one ? part of plants. For our research we used plants grown without Cd and SA treatments – control, plants grown from seeds, treated with SA+Cd in substrate, and plants seeds treated only with SA, without $CdCl_2$. The content of phenolic compounds and phenylalanine ammonia-lyase activity were determined on the 7, 14 and 21 days of plant growth.

Phenylalanine ammonia-lyase activity assay. The PAL activity was determined spectrophotometrically by modified Zucker method (Zucker, 1965). For enzyme analysis 0,5 g of plant tissue were homogenized manually in 2 ml 25 mM borate buffer (pH 8,8) containing 23 μL mercaptoethanol. The homogenates were centrifuged for 20 min at 8000 g. The supernatant was used for enzymatic assay. The PAL assay system contained of 1 ml of the supernatant, 1 ml of buffer, 1 ml of 12 mM L-phenylalanine. The resulting mixture was incubated at 37 °C for 1 hour, the reaction was stopped by 15% trichloroacetic acid. Absorbance of the mixture was measured using spectrophotometer (ULAB 101). PAL activity was expressed in mM of cinnamic acid per gram of protein. Protein was determined by of Bradford method (Bradford, 1976).

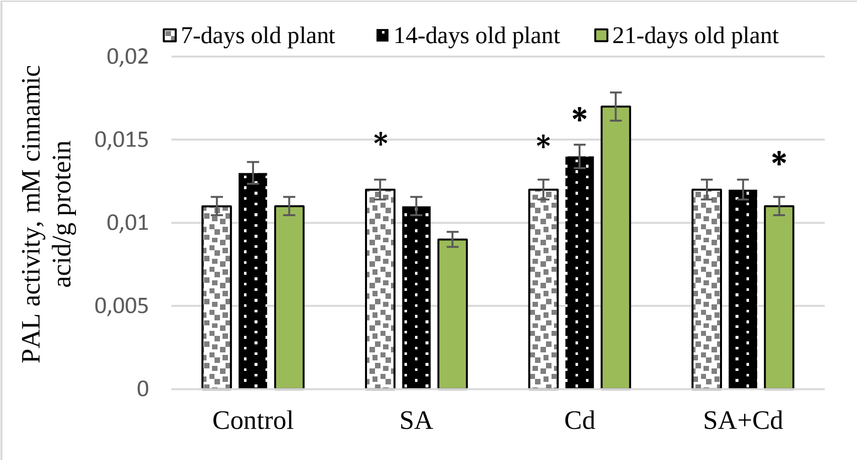
The content of phenolic compounds in the leaf and root extracts were determined by the Folin-Denise method. Absorbance readings were performed at 725-730 nm with ULAB 101 Spectrophotometer (Zaprometov, 1971).

Statistical analysis. Each experiment was performed in five replications. The means and standart deviations were calculated by the JMP Pro and Microsoft Office Excel. Statistical significance of difference was evaluated with Student's t-test ($P<0.05$).

Results and Discussion

PAL a key enzyme in the phenylpropanoid pathway, it is involved in the defense responses of plant cells (Apel, 2004). Synthesis of phenolic compounds and their accumulation under the stress conditions may be associated with an increased activity of PAL (MacDonald, 2007). Therefore, PAL has been generally recognized as a marker of environmental stress in different plant species (Korobkova, 2009). The PAL activity show significant changes for a short period of time in plant tissues (Smirnov, 2012). There are data whose? that PAL salt stress increased dynamic changes of the PAL activity in the 7-, 14-, and 21-days plants.

In our experiments under the SA (0,05 mM) treatment we observed significant 5 fold increasment of PAL activity in both wheat and buckwheat plants on the 7-day of experiment (Figure 1). But it decreased in 14- and 21-days old plants. Moreover, wheat plants had significantly higher PAL activity in roots tissue, than in shoots tissue. The obtained results indicate that roots are the main place of synthesis and localization of the phenolics. $CdCl_2$ increased the PAL activity by 3 times in the 7-days old plants. But, on the next time points $CdCl_2$ decreased enzymatic activity. PAL activity was lower under cadmium stress with SA (Figure1).



* P<0.05

Figure 1. Effect of $CdCl_2$ and SA on phenylalanine ammonia-lyase activity in wheat roots,

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In shoots we observed linear change of enzyme activity, with a slight increase under cadmium stress. Salicylic acid has positive effect on the PAL activity in the wheat (Fig.2). The obtained data confirm data about early changes in PAL activity under the stress condition.

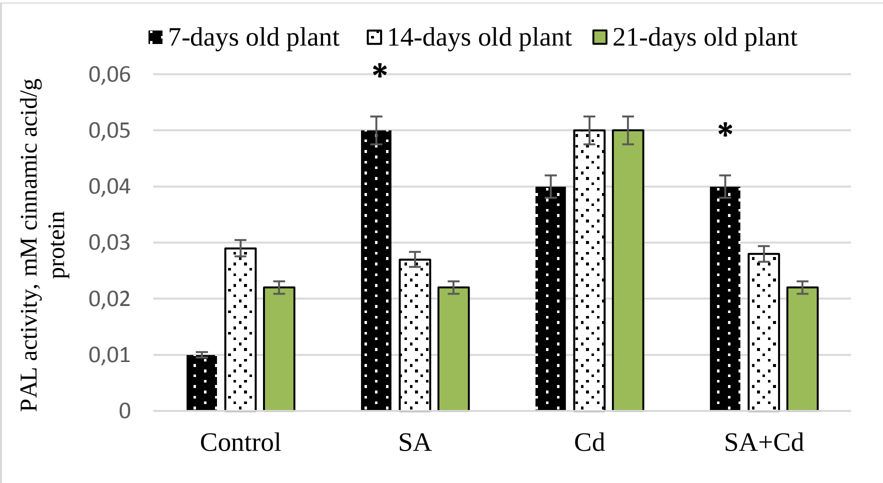

Fig.2 Effect of $CdCl_2$ and SA on phenylalanine ammonia-lyase activity in wheat shoots,
* – P< 0,05

Buckwheat plants in control have been characterized by a comparatively high level of the PAL activity in the roots. Under the $CdCl_2$ impact the enzyme activity increased 2-fold. Prolonged influence of cadmium caused a higher enzyme activity (Fig.3).

An increase of the enzyme activity have been shown in the shoots of the 7-days old plants under the cadmium stress, and decrease of this activity by 3 times in the 21 days-old plants. SA led to increase of the PAL activity, suppose, salicylic acid is phenolic compound and has a common way of synthesis with phenols (Fig.3, 4).

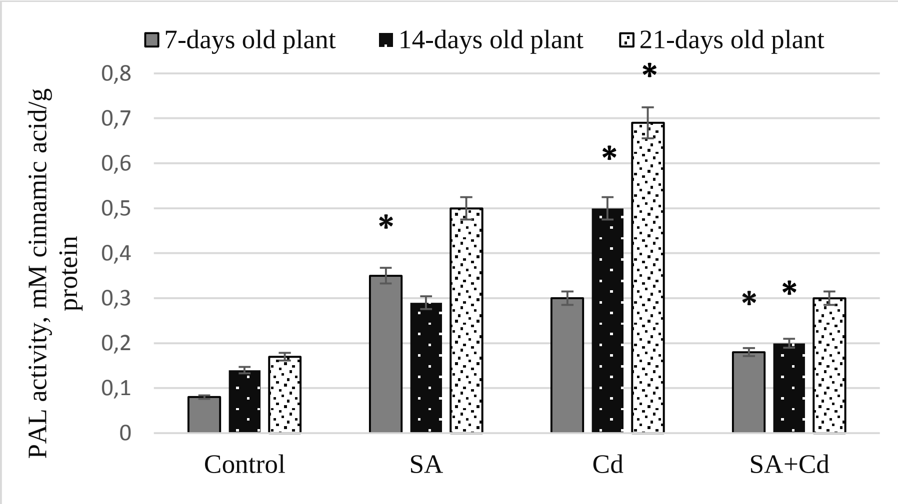

Fig.3 Effect of $CdCl_2$ and SA on phenylalanine ammonia-lyase activity in buckwheat roots, * – P<0,05

Comparing results with literature data, plants tissue usually have early PAL activity. The short-term increase in the activity of PAL and peroxidase enzymes, which activate the protective mechanisms of plants, was shown by (Evtushenko, 2008). Also changes in PAL activity caused by the action of biogenic factors - chitosan and salicylic acid (Szalai, 2013). Our results showed changes in the enzymes activity, without time or age dependence. Whereas, it was revealed that the enzyme activity depends on the cadmium chloride and SA. The higher PAL activity in buckwheat plants was detected (Fig. 3,4).

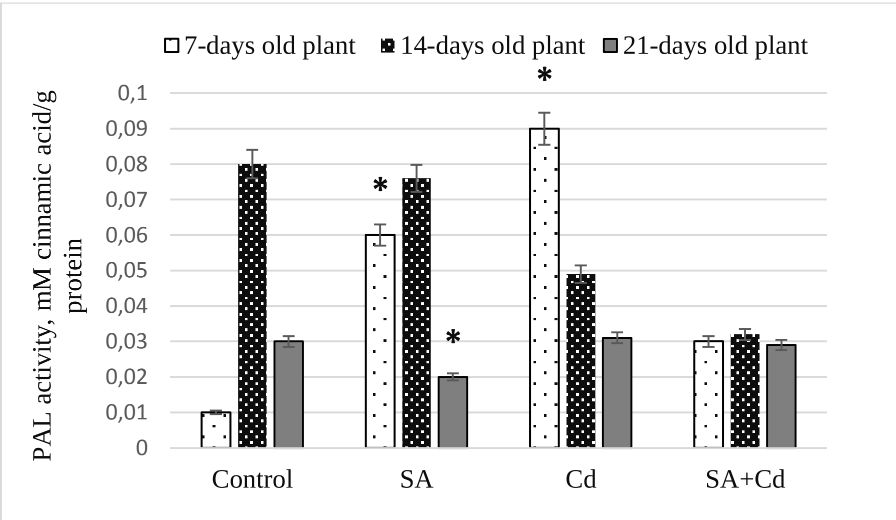

Fig.4 Effect of $CdCl_2$ and SA on phenylalanine ammonia-lyase activity in buckwheat shoots, * – P<0,05

The changes of PAL activity led to an increase or decrease in the content of total phenolic compounds, that are involved in a major processes, such as: cell wall formation, photosynthesis, respiration, plant-plant allelopathic interactions, protection plants against pathogens and herbivores. They are produced by plants in response to biotic or abiotic stresses (Pinto, 2004).

Determination of the total phenolic compounds in the wheat showed a differential accumulation predominantly in the roots tissues, and its decrease in the shoots of 14 and 21-days old plants (fig.5 A,B).

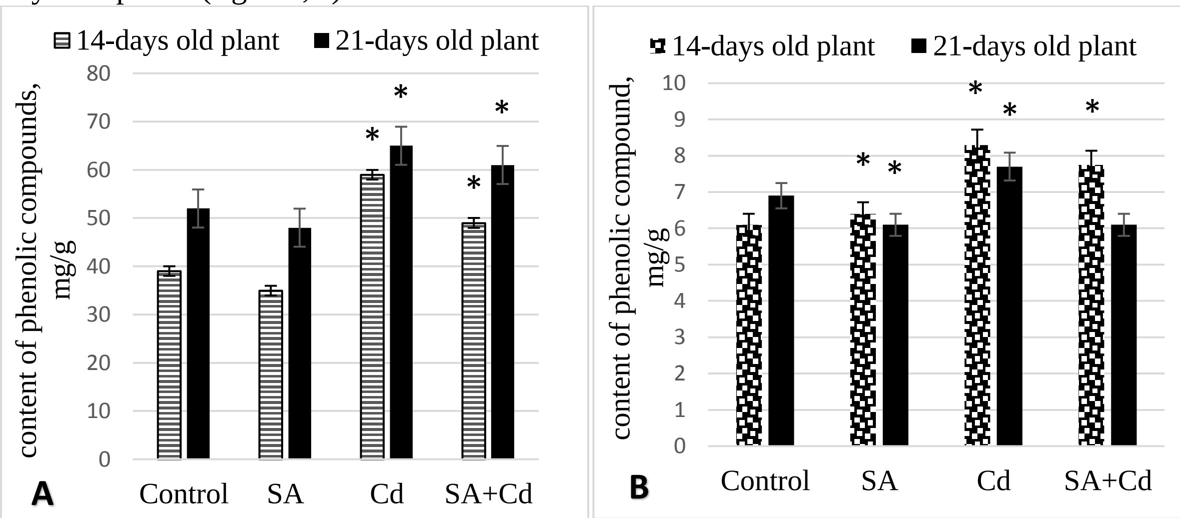

Fig.5 Effect of $CdCl_2$ and SA on phenolic contents in wheat A-roots, B-shoots, * – P< 0,05

The obtained results are consistent with the literature data, where in most cases the root tissue is the place of synthesis and localization of phenolic compounds. Significant accumulation of phenolic compounds is determined under the heavy metal stress. Cd induced an increase in the phenolics in the wheat roots and shoots. Under the prolonged action of cadmium chloride we noted the increase of the phenolic compounds in the roots, which is consistent with their protective functions and the ability to form chelate complexes with heavy metals (Clemens, 2006). Roots are first exposed and are the main source of a heavy metals in the plant organism. The high content of the phenolic compounds in roots and its relatively low content in shoots serve as a protective mechanism and a logical redistribution of phenolic compounds under the action of the stressor. The SA induced decrease in the content of phenolic compounds in both the roots and shoots of wheat plants.

In the buckwheat plants - the content of total phenolic compounds was high. Cadmium led to the increase of phenolic compounds by 1,5 time at the both time point – 14-, and 21 days-old plants (Fig.7).

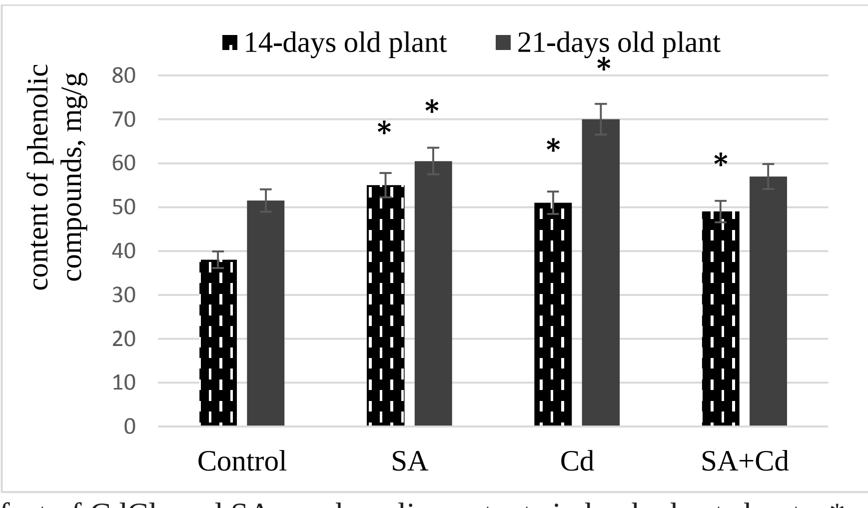

Fig.7 Effect of $CdCl_2$ and SA on phenolic contents in buckwheat shoots, * – P< 0,05

SA alone also increase their accumulation, but in the presence of cadmium chloride the concentration of phenolics was lower than in variants with Cd or SA. These results confirm the involment of SA in the stress responces under the cadmium impact.

Conclusions

In this research the effects of cadmium stress and salicylic acid on wheat and buckwheat plants in laboratory experiments were studied. SA can be recommended for the pretreatment of wheat and buckwheat seeds to alleviate cadmium toxicity. The protective role of salicylic acid in cadmium-stressed plants is unambiguous and can be related to inhibited antioxidant process, enhanced PAL activity or decreasing in content of total phenolic compound in leaves tissues and increasing in roots tissue.

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CORNERSTONE OF STRATEGY AIMED AT CREATION OF RESISTANT VARIANTS OF CARROT (DAUCUS CAROTA L.) TO WHITE AND GRAY ROT PATHOGENS AT THE FEDERAL SCIENTIFIC VEGETABLE CENTER (FGBNU FNCO, RUSSIA)

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Abstract

The storage period of carrots (garden carrots) in Russia lasts 210-250 days, and commercial product losses exceed 25-40%. In this period, roots may be damaged by microorganisms representing 75% of all pathogens of this economically valuable crop. Composition and proportion of pathogens are variable and depend on year, carrot variety and place of growing. This impacts objectiveness of assessment of resistance to both individual and multiple pathogens and effectiveness of search for resistance sources for selection for immunity. In Moscow region, the most damage of carrots is caused by storage rot, especially by low temperature sclerotial pathogens *Sclerotinia nivalis* and *S. sclerotiorum* (white rot) and *Botrytis cinerea* (gray rot) and more rarely *Typhula ishikariensis* (Typhula blight). Major challenge of collaborative studies of FNCO phytopathologists, geneticists and crop breeders is search for resistance sources and creation of resistant forms for carrot protection. Samples of different origin are annually put into operation. Wild species of *Daucus L.* are engaged, inter- and intraspecific hybridization, inbreeding are used. The resistance of initial samples and resultant progeny is evaluated both at natural infection background (field, storage facilities) and *in vitro* with artificial inoculation with pure cultures of pathogens. Selection for resistant variants is based on population and individual multistep evaluation during the vegetation period, after placement for storage, before the mother roots planting. This resulted in carrot varieties and hybrids with highly resistant roots (Margosha, Rif F_1, Nadezhda F_1). The Institute is heavily involved in variety-improving selection of the key economic varieties for resistance to the most aggressive races of white and gray rot agents.

Keywords: *Daucus carota* L., varieties, resistance, *Sclerotinia*, *Botrytis.*

Introduction

Carrot is one of the most widespread and essential vegetable crops in Central Russia (Stepanov V., 2018; Zaltsman V.,2018). To resolve the problem of year-round supply of fresh carrot to the population, it is necessary to have cultivars that maintain high commercial quality for the storage period of 220-250 days (Borisov V., 2010; Yanchenko E.,2009). About 75% of the representatives of all pathogenic microorganisms inhabiting roots of this economically important crop were detected within this period. Phytosanitary situation deteriorates due to the fact that, along with increasing aggressiveness of local races of pathogens, introduction of new races with imported commercial produce of foreign cultivars takes place in recent years (Pidoplichko N., 1977).Current assortment comprises carrot cultivars and hybrids of Russian and foreign breeding, but foreign ones are often nonresistant to local races of phytopathogens, what leads to a need for using additional plant protection agents and to a rise in net cost.

Low-temperature-tolerant sclerotial fungi and fungal-like organisms that cause pit-storage rot do the most damage to carrot during storage. Causative agents belong to different taxonomical groups and have a common trait – they are adapted to cool environmental conditions. In relation to temperature, low-temperature-tolerant fungi comprise two types (Morita R., 1976).

They are obligate psychrophiles, with the optimum temperature for development about 0°C, and psychrotrophs having the optimum temperature about 20°C, but being able to maintain growth at a temperature 0°C or less. They include: fungal-like low-temperature-tolerant organisms from the kingdom Chromista, genera *Pythium* and *Phoma* (order Peronosporales); true fungi (*Mycota*, or *Fungi*) - anamorphic hyphomycetes of the genus *Fusarium*, several sac fungi (division Ascomycota) - *Sclerotinia* spp. and *Botrytis cinerea* of the order Helotiales, and a basidiomycete *Typhula* spp. There are psychrotrophic fungi of the agonomycete genus *Rhizoctonia* (Tkachenko O.,2017). At present, selection of resistant carrot cultivars and hybrids remains the most efficient method of storage diseases control. Therefore, one of the fundamental requirements of modern selection for immunity is the search for sources of resistance not only against a single agent, but against a complex of agents.

Researches of the Laboratory of Immunity at FGBNU FNCO have the following priority directions:

- Annual monitoring of storage diseases distribution; isolation, identification, and evaluation of aggressiveness of isolates of economically important carrot pathogens;
- Search for sources of resistance to pit-storage rot agents to obtain source material as a basis of a strategy of protection of carrot against these diseases.

The work is carried out in close collaboration with leading plant breeders of the center, which use obtained material for creation of new cultivars and hybrids of carrot.

Material and Methods

Studies were carried out in 2009-2019. The material for the studies includes carrot samples of different provenance and genetic origin from collection and breeding pool of the Laboratory of Edible Roots Selection and Seed Production and the Laboratory of Genetics and Cytology at Federal State Budgetary Scientific Institution "Federal Scientific Vegetable Center" (FGBNU FNCO). To broaden genetic diversity, our center uses inbreeding, intra- and interspecific hybridization, with involvement of wild species of *Daucus* L. as sources of resistance.

Roots were stored in a vegetable store cellar at a temperature of 1-2^{o}C and 90-92% humidity for six months (from the last ten days of September through the second ten days of April). Examination during storage period, sampling of affected roots in spring for analysis, evaluation of the degree of damage and resistance level of cultivar samples, identification of species composition of pathogens were conducted using appropriate methods and identification keys (Gannibal F., 2011; Gagkaeva T., 2006; Levkina L., 2013; Nelson P., 1983; .Pidoplichko N., 1977; Simmons E., 2002). Resistance of the collection and breeding carrot samples during storage period was evaluated using conventional indices: prevalence (%), affect index (mean score), disease progression degree (%).

Immunological appraisal of carrot sample resistance was done using the method of artificial inoculation of root discs under the conditions of dampening chamber *in vitro* by means of placing of an agar block of corresponding isolate of ten-days-old culture of the agent (Czapek medium) in the center (Fig. 1). The most aggressive isolates of main phytopathogens were used for inoculation. Cuvettes with inoculated discs were placed in the refrigerated heating circulator and incubated in accordance with biological characteristics of a phytopathogen at corresponding temperature (2-4°C, 10-12°C, 18-20°C, 24-26°C). The number of replicates was five. On the 7th and 14th days after inoculation, the diameter and depth of affected zone were measured, macro- and micromorphological characteristics of development of phytopathogens at discs were described (color, mycelium growth intensity, presence of conidial fructification, exudate, etc.).

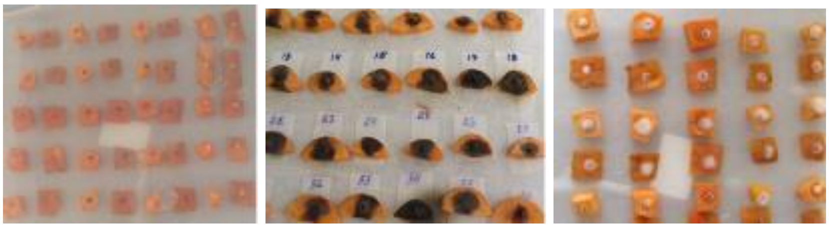

Figure 1. Inoculation of carrot root disks with agar blocks and isolates of different agents.

Samples were classified in respect of resistance against each agent according to the magnitude of total damage volume within an individual variant to five groups: resistant (I), tolerant (II), medium susceptible (III), susceptible (IV), and highly susceptible (V). Evaluation of samples for group resistance against examined groups of pathogens was conducted on the basis of analysis of all data obtained in natural conditions *in vivo* and in laboratory experiments *in vitro*. Promising sources of resistance were distinguished on the basis of the complex of all estimates.

Results and Discussion

Selection of adequate preventive and well-timed protective measures shall be based on understanding of composition of the most deleterious agents in vegetable crops with due account for ecological and geographical peculiarities of agricultural zones. Recently, the following factors further changes of phytosanitary situation: rise of aggressiveness of local pathogen races; introduction of new races with imported seeds; insufficient knowledge about species composition of micromycetes; and increasing of abiotic stress level in agrocoenosis of vegetable crops. All these factors lead to significant alteration of geographical distribution and genetic composition of populations of phytopathogens and influence appraisal of host plant resistance/susceptibility, what is especially important to take into account when searching for sources and donors of resistance in breeding.

Monitoring of carrot cultivation within last 40 years demonstrates structural changes of pathocomplex, replacement of dominant species, increase of virulence and aggressiveness of microbial groups being of little pathogenicity earlier. The causes of these population shifts are related mainly with ecological factors influencing interactions in pathogen-plant system. The most important factor in this system is thermal balance of agro-ecological niche in Moscow region –observations demonstrate sharp rise of mean daily atmospheric temperature in the last decade as compared with 1970-1980s (Fig. 2).

Under the conditions of Moscow region, white rot caused by fungi of the genus *Sclerotinia* inflicted the most damage to carrots within the

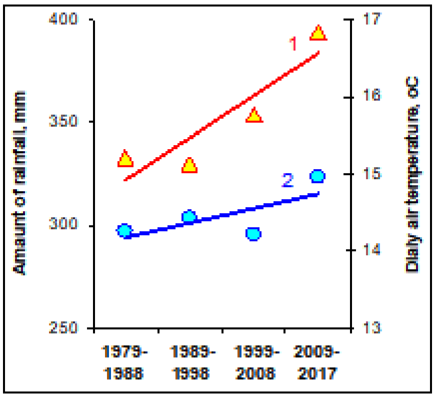

Figure 2. Mean daily atmospheric temperature (1) and total precipitation (2) per vegetation at different times (1979-2017).

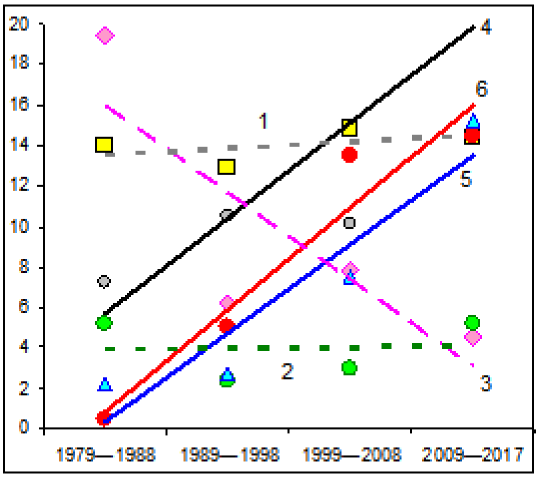

Figure 3. Динамика распространения вредоносных болезней корнеплодов моркови столовой при хранении в разные интервалы времени (1979-2017 годы): 1 - Sclerotinia spp.; 2 - Phoma spp.; 3 - Botrytis spp.; 4 - Alternaria spp.; 5 - Fusarium spp; 6 - Pectobacterium carotovora

whole observation period (Figure 3-1). In the last decade, apart of previously widespread *Sclerotinia sclerotiorum* (Fig.4C), increase of aggressiveness of the fungi *S. nivalis* (Fig. 4B) and less often *Typhula ishikariensis* (typhulosis) is detected.
For the first time, staff of the Laboratory of Immunity at FGBNU FNCO detected *S. nivalis* on carrot roots in storage in 2009, and *T. ishikariensis* – in 2011 (Timina L., 2010). It is difficult enough to obtain a perfect stage of *S. nivales*, hence, to identify this species, polyacrylamide gel electrophoresis of globulins that allows distinguishing *S. nivales* among systematically closely related fungi was used (Tkachenko O., 2017). The local population of this pathogen is heterogeneous and consists of strains with various aggressiveness (Tkachenko O., 2010). Losses in carrots in storage caused by white rot amounted to 10-60% depending on the conditions and cultivars.

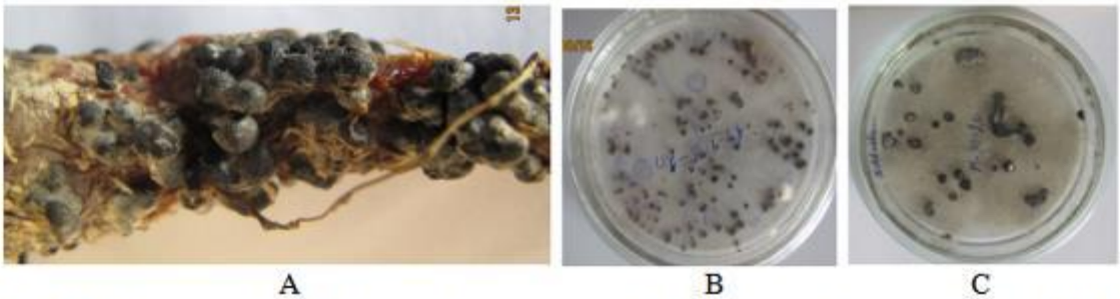

Figure 4. Симптомы поражения корнеплода моркови столовой белой гнилью (A); склероции гриба *S. nivalis* (B); склероции гриба *S. sclerotiorum* (C) на КМА.

Starting from 2000s, along with the rise of mean multi-year temperatures, decrease of harmfulness of grey rot (caused by *Botrytis cinerea*) (Fig. 3-3) and expanding of distribution and increase of aggressiveness of bacteriosis (caused by *Pectobacterium carotovora*), fusariosis and alternariosis occur (Fig. 3-6).
The assemblage of pathogens involved in Alternaria rot or black rot includes alternarioid hyphomycetes of the genera *Alternaria*, *Stemphilium*, *Ulocladium*, *Embilisia*. The fungi of the genus *Alternaria* - *A. radicina* (Fig.5A), *A. cheiranthi* (Fig.5B), *A. corotiincultae* (Fig. 5C), *A. cinerariae* (Fig.5D) are the most harmful; in some years, their expansion reaches 34%.
According to the data of long-term monitoring, species composition of micromycetes of the genus *Fusarium* in the Moscow region has expanded. Among the fungi of this genus being the agents of Fusarium rots, the following species were isolated and identified: *F. oxysporum*, *F. avenacium*, *F. nivale*, *F. chlamidosporum*, *F. solani*, *F. culmorum*, *F. semitectum*. Occurrence of new species is explained by extreme plasticity of majority of them. Having high adaptivity and prompt modifiability, the fungi of the genus *Fusarium* are highly persistent (Seredin T., 2018). In recent years, *F. solani*, *F. semitectum*, and *F. oxysporum*

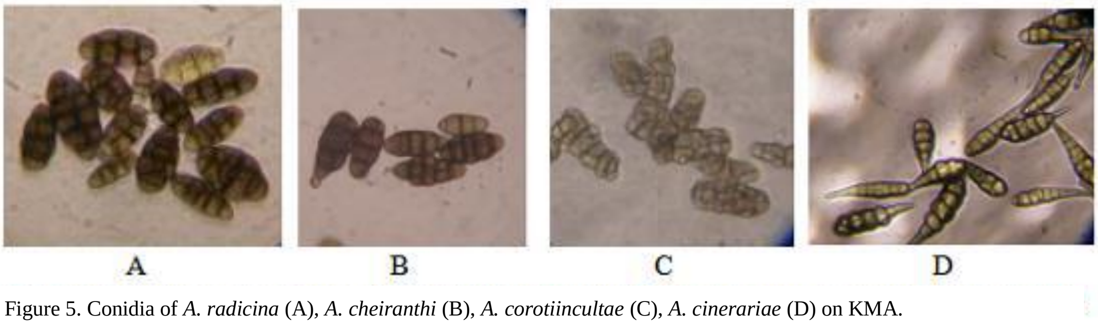

Figure 5. Conidia of *A. radicina* (A), *A. cheiranthi* (B), *A. corotiincultae* (C), *A. cinerariae* (D) on KMA.

dominate (Fig. 6 -A,B).

Recently, we isolated and identified on damaged carrot roots a thermophilous fungus *Trichotecium roseum* causing soft pink rot of roots in storage (Fig.6-C). In 2013 and 2015, roots of susceptible cultivars were damaged by 23 and 55%, respectively (Timina L., 2015). One of the causes of significant post-harvest carrot losses in storage may be Gliocladium rot, caused by a fungus *Gliocladium roseum* (Fig. 6-D). It is poorly known in carrots, but detected previously in the Far East causing wilting in kidney bean, pea, soybean, as well as soft watery rot on tomatoes. In Germany, Gliocladium rot of potatoes was described (Timina L., 2015). According to our data, this agent may occur in carrot roots both in mixed dry rots and separately.

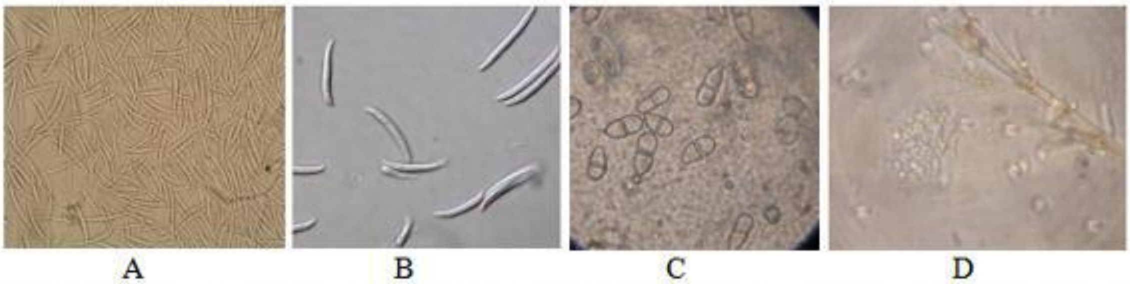

Figure 6. Conidia of *F. oxysporum* (A), *F. solani* (B), *Trichotecium roseum* (C), *Gliocladium roseum* (D) on KMA.

In the pathogenesis of pit-storage rot, assemblages of microorganisms may include the fungi of the genera *Pithium* (Pithium rot), *Cylidrocarpon* (cavity spot), *Verticillium* (verticillosis), *Aspergillus* and *Penicillium* (storage molds) along with aforementioned pathogens.

As noted above, harmfulness of some pathogens is of epiphytotic nature, while others demonstrate focal occasional character as yet. Therefore, the Center conducts proactive breeding continuously, because cultivars resistant against some pathogens are in many cases nonresistant against others. Moreover, the requirements of contemporary breeding for immunity determine the necessity of search for sources of resistance both against single agents and against a complex of pathogens. To make reliable estimate of carrot cultivar types resistance against main agents of storage diseases, the Laboratory of Immunity at FGBNU FNCO conducts laboratory immunological examination by the method of artificial inoculation annually. The most virulent fungal isolates of the genera *Sclerotinia*, *Botrytis*, *Alternaria*, *Stemphilium*, and *Fusarium* from the Laboratory's collection are used for inoculation. In accordance with the results obtained in recent years, cultivar types Margosha, Nadezhda F_1, Rif F_1, Chantenay 2461 have group resistance against fungal storage diseases, therefore, they may be of interest as a valuable source material for carrot breeding for resistance (Fig. 7).

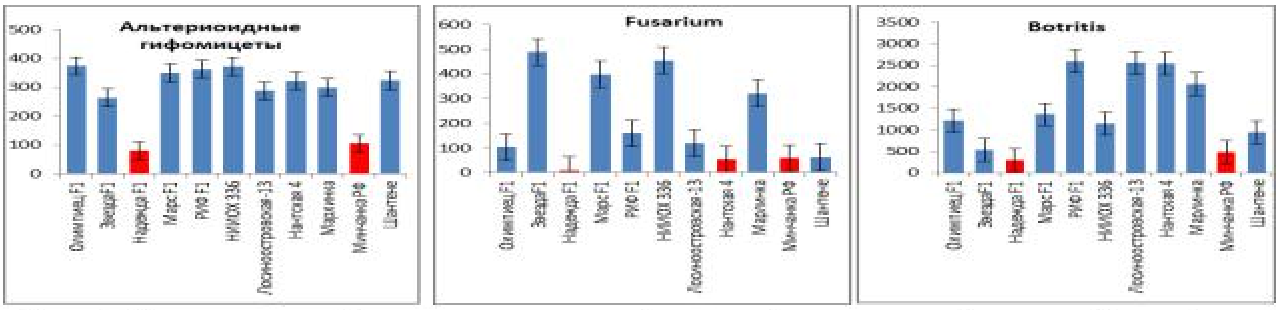

Figure 7. Comparison of carrot cultivar types in respect of resistance against pathogens in conditions of artificial inoculation of root discs. The vertical line sets mean size of damage zone of discs in mm^3.

On frequent occasions, economically valuable cultivar types lose eventually their resistance against storage diseases agents. Damage level varies to a large extent within populations of some cultivars. This gives us an opportunity to conduct intrapopulation selection of the most resistant forms as a part of strain-improving breeding. The Laboratory carries out artificial

inoculation and individual evaluation of resistance of genotypes against the complex of pathogens; groups of roots with different levels of resistance against a complex of pathogens are formed in populations, and they are passed to breeders for obtaining seed progeny in group isolators on isolated plots. The most valuable group is the group comprising genotypes with high resistance against most agents. In the majority of cases, their ratio does not exceed 10% of all analyzed roots. The material obtained as a result of immunological examination is passed to breeders, who include it into breeding process of creation of new cultivars and hybrids of carrot.
The degree of resistance of roots of a particular cultivar/hybrid to storage diseases eventually influences preservation and output of carrot roots following storage, and high crop yield not always ensures low costs of commercial produce at the time of selling (see Table).

Table – Comparative characteristics of Russian and foreign cultivars of carrot on the basis of resistance against storage diseases (2018-2019)

Group	Sample	Crop yield, t/ha	Losses due to storage diseases		Cost of commercial products after storage, rub/kg
			%	t/ha	
I	Marlinka (Russia)	46	11,7	5,4	8,71
	Narman	42	17,2	7,2	9,52
	Spedo	42	22,2	9,3	9,60
	Nantes 2 Tito	42	56,5	23,7	10,71
II	Nadezhda F_1 (Russia)	55	2,6	1,4	7,35
	Chantenay 2461 (Russia)	69	6,1	4,2	6,10
	Baltimore F_1	60	37,5	22,5	7,45
	Kuroda 5 Sun	65	16,7	10,8	6,56
	Nerac F_1	61	18,2	11,1	6,94
	Kuroda Power	70	40,0	28,0	7,69
III	Rif F_1 (Russia)	86	4,9	4,2	5,09
	Margosha (Russia)	99	5,9	5,8	4,56
	Purple Haze F_1	75	7,7	5,8	5,72
	Morelia F_1	73	17,6	12,9	6,99
	Mello Yello F_1	103	7,1	8,2	5,19

According to the results of production testing of Russian (FGBNU FNCO) and foreign cultivars of carrot, four groups according to crop yield was formed: group I - 40-50 t/ha, group II - 50-70 t/ha, group III - >70 t/ha. Within each group, samples were graded according to the level of damage to roots due to pit-storage rot at the end of storage period (April). The overall percent of damaged roots for all analyzed samples was from 3% to 56% and varied considerably within each group. It should be noted that the in-storage losses of commercial roots within each crop-yield-ranked group were lowest in case of Russian cultivars and hybrids (1,4-5,4 t/ha), and though several foreign hybrids were superior in respect of crop yield, profitability of growing Russian cultivars was in general higher or similar in comparison with foreign ones taking into account long-term storage.

Conclusions

Created by joint efforts of breeders, geneticist and phytopathologists, productive Russian cultivars and hybrids of carrot having high resistance against local populations of phytopathogens should be considered not only the basis of the strategy of integral crop protection against diseases, but also supporting the interests of domestic agricultural producers within the framework of national policy of import substitution and ensuring food security of the country. Taking into account increasing variability of the pathocomplex and aggressiveness of pathogens, immunological and molecular investigations aimed at the creation of donors of complex resistance against storage diseases of different ethiology should be now a priority direction in the framework of targeted selection along with continuous phytomonitoring and detection of new economically deleterious carrot diseases.

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3. ORGANIC AGRICULTURE

DEPENDENCE OF GRAIN YIELD OF SPELT ON LOCALITIES AND DIFFERENT TYPES OF FERTILIZER

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Abstract

Investigations of organic technology for spelt in the different regions were carried out by placing the field experiment using the random block system method, in three repetitions, during 2010/11 and 2011/12 year, at the "Radmilovac" experimental school of the Faculty of Agriculture in Belgrade, in the village Jasenica near the town of Valjevo and on Zlatar, Radijevici village. As a material was used late varieties of spelt (Nirvana).
Commercial organic fertilizer was used in the form of granules under the trade name "Biohumus Royal offert". The second treatment was the use of zeolite and hydrogel soil conditioners, which is also ploughed down in autumn, with the aim of keeping moisture, preventing nutrient losses and, consequently, positively affecting useful microorganisms, as well as stabilizing heavy metals in the soil. The third variant was the combined application of organic fertilizer and soil conditioner. Also, it was used microbiological fertilizers "Uniker" and "Slavol" and their combination with organic fertilizer and soil conditioners.
Statistical analysis of data for grain yield was performed using analysis of variance for one factorial experiment and for individual comparisons; we used the least significant difference (LSD test). The grain yield of spelt cultivated in the organic production system in the different regions of Serbia was statistically varied depending on the fertilizer variant. The average yield of grain of spelt was 4954 in first, 3093 in second and 2241 kg ha^{-1} in third locality. The best result was achieved by combined use of zeolite and biohumus, where the average two-year yield of spelt grain was 3550 kg ha^{-1}. It is interesting to note that yields (4377, 2756 and 2216 kg ha^{-1}) were obtained on the control treatment without the use of fertilizers, is not much lower than the yield in fertilizing variants, because crop rotation effect in first and the soil has not been used for agriculture production for a long time in other localities. This fact is very important because in Serbia there are a lot of those lands, which can be used for organic production, especially low-input crops, such as a spelt.

Key words: *growing technology, organic fertilizer, soil conditioner, yield*

Introduction

Organic agriculture as a comprehensive food production management system is based on ecological practice, a high degree of biodiversity and the conservation of natural resources. Although the organic production area in Serbia records constant growth (Simić, 2017), the lack of organic fertilizer, due to the insufficiently represented organic livestock breeding, calls into question the sustainability of the entire system, especially when it comes to maintaining the natural fertility of the soil. Manufacturers are forced to use commercial certified fertilizers, which significantly increase production and undermine its economic justification. There are many types of cereals, "alternative", which can be successfully grown in different regions of our country. One of them is the Spelt wheat (*Triticum spelta* L.), that belongs to the hexaploid group of cultivated *Triticum wheat* with fragile spikes and hulled kernels (Glamoclija *et al*., 2015). Spelt is an old subspecies of soft wheat whose use is constantly growing in the world because it is considered healthier and more natural compared to modern varieties of common wheat (Schober *et al*., 2006), but it is very poorly represented in production and nutrition in Republic of Serbia. It can be cultivated at altitudes higher than 800-1100 m, but certainly its place can occupy in crop rotations at lower altitudes. Grain yields are lower than for common wheat, but are more stable (Roljević Nikolić *et al*., 2018). In Serbia, spelt is grown on about 300 ha, which represents 6.3 % of organic areas under cereals (4607 ha). Investigations by Kovačević *et al*. (2007) showed that the spelt responds very positively to the application of organic fertilizers. The studies that were concerned with the study of productivity of spelt in different locality of cultivation (Dolijanović *et al*., 2014; Ugrenović *et al*., 2018) showed that there is no single solution for maximizing the genetic potential of the spelt, and the cultural practices must be adapted to the conditions of production in different regions.

Establishment and maintenance of soil fertility in organic farming systems is based on improving the physical, chemical and biological properties of the soil by increasing the content of organic matter and the activity of microorganisms. Optimum amounts of nutrients in organic production are provided by the most commonly applied alternative methods, which include the addition of organic and natural mineral fertilizers, plant residues, use of leguminous plants, green manure, as well as stimulating the activity of useful soil microorganisms using microbiological fertilizers. The application of organic fertilizers influences the correction of the physical and chemical properties of soil rhizosphere (Tobiašová, 2011), as well as the increase in biomass and activity of microorganisms in relation to the application of mineral fertilizers (Chang *et al*., 2007). Also, there are results on the very positive effect of the use of organic and microbiological fertilizers on the yield of crops in organic production (Ebrahimpour *et al*., 2011; Jablonskytė-Raščė *et al*., 2013). Zeolites, as soil conditioners, can alleviate the rinsing of ammonia by taking ions from sources, such as manure and fertilizers, which are then released and made available to plants (Karličić *et al*., 2017). Also, zeolites improve the water-air and physical properties of heavy soils, but also bind to toxic elements (Stojiljković *et al*., 2002), reducing their bioavailability. Ćupina *et al*. (2017) deals with the topics of annual cover crops and nitrogen management, and more research is available with various commercial certified organic fertilizers and soil conditioners (Oljača *et al*., 2012; Dozet *et al*., 2017).

For these reasons, the main goal of this research was to examine the influence of locality (L) and types of fertilizer (Y) on the grain yield of spelt wheat produced in Serbia.

Material and methods

Adaptation of organic technology for spelt in different regions was carried out by setting field trials using the random block system, in three repetitions, during 2010/11 and 2011/12 years. The study in the plain area was carried out at the "Radmilovac" experimental school of the Faculty of Agriculture in Belgrade (44^{o}45'N, 20^{o}35'E), 130 meters above sea level, in a hilly area in the village Jasenica near the town of Valjevo (44° 19'0.6 "N , 19° 57'12.6 "E), 300 meters above sea level, while the investigation in the mountainous region was carried out on Zlatar, on the territory of Nova Varos municipality, Radijevici village (43° 23'52" N, 19° 52'33 " E), 1065 meters above sea level.

At the first locality, the soil is chernozem luvic soil type with the following characteristics: pH (in H_2O) 8.04, total nitrogen content 0.13%, P_2O_5 22.18 mg, K_2O 19.10 mg, humus content in the ploughing layer is 2.45 %. The area under examination was in the organic production system since 1992, in the area of four-crop rotation, which implies the following rotation of crops: maize - winter wheat – spring barley spring barley + red clover - red clover. The surface of the elementary plot was 6 m^2. Sowing was carried out manually, October 21 in first and October 07 in second Year with the amount of seed of 500 seedlings m^{-2}. To maintain and increase the potential fertility of the soil, the following varieties have been applied:

- T_1 - fertilization only with microbiological fertilizer in top-dressing (5 l ha^{-1});
- T_2 - fertilization with biohumus (3000 kg ha^{-1}) and microbiological fertilizer in top-dressing (5 l ha^{-1});
- T_0 - control - no fertilizer application.

Harvest was done on July 5, 2011 and June 30, 2012 years.

In the second locality, the soil is of the type of eutric cambisol, the following characteristics: acidic chemical reactions (pH in KCL = 5.3-5.5), with about 3% humus, high reserve of mineral nitrogen (over 200 kg N ha^{-1} for the whole investigated layer), poorly provided with avialable phosphorus (2.4-2.6 mg per 100 g of soil) and well secured with potassium (34-37 mg per 100 g of soil). The surface of the elementary plot was 12 m^2. Sowing was carried out manually, October, 15 2010 and October 05, 2011 with the amount of seed of 550 seedlings m^{-2}. The experiment included the following variants:

– T1 - organic commercial fertilizer (3000 kg ha^{-1}),
- T2 - zeolite (2670 kg ha^{-1}),
– T3 - combined application of biohumus (3000 kg ha^{-1}) and zeolite (2670 kg ha^{-1})
- T4 - control variant without fertilizer application.

Harvest was done on July 10, 2011 and July 04, 2012 years.

In the third locality, the soil is of the type pseudoqley that exhibits unfavorable chemical and physical properties, as well as the scarce content of nutrients, primarily phosphorus. Phosphorus is considered to be a weakly moving element in the soil, and in pseudogley the additional problem is the chemical mobilization of phosphorus Al, Fe and Mn due to high acidity. The areas under examination were certified for organic production and no agricultural crops were cultivated in the previous seven years. The surface of the elemental plot was 12 m^2. Sowing was done by hand, November 01, 2010 and October 20, 2011 with the amount of seed of 600 seedlings m^{-2}. The following variants have been applied:

– T1 - soil conditioner zeolite (2670 kg ha^{-1})
– T2 - microbiological fertilizer "Uniker" (10 l ha^{-1})
– T3 - microbiological fertilizer "Uniker" + zeolite
– T4 - microbiological fertilizer "Uniker" + hydrogel (20 kg ha^{-1})
– T5 – control

Harvest was done in August 2011 and 2012.

The different number of treatments at the investigation localities is the result of differences in soil use (crop rotation), soil characteristics and meteorological conditions (multi-year average). As a material, late varieties of spelt (Nirvana) were used, selected at the Institute of Field and Vegetable Crops in Novi Sad. The best results are achieved on moderately fertile soil, it is resistant to winter, but it is sensitive to intensive nitrogen fertilization because it has a high stem and can be laid down. The yield potential of spelt is over 4000 kg ha^{-1}. "Biohumus Royal offert" is ploughed down in autumn. Characteristics of fertilizers are as follows: pH 8.63, N 2.2%; P_2O_5 4.8% and K_2O 2.8%. Zeolite (manufacturer "AquaVitadi Natura" doo) was also ploughed down with basic tillage treatment in autumn, and top dressing application with microbiological fertilizer (trade name "Slavol") was performed at the beginning of the tillering phenofase (BBCH 31-33) in the amount of 5 l ha^{-1}.

Statistical analysis of data for grain of yield was performed using analysis of variance for factorial experiments and for individual comparisons, we used the least significant difference (LSD test).

Results and discussion

The grain yield of spelt cultivated in the organic production system in different agricultural regions of Serbia were significantly different (Table 1). Lower yields are specific for marginal, mountainous areas, while higher yields are achieved in better cultivation conditions. The highest average grain yield of 4954 kg ha^{-1} was obtained in the plain area, on the chernozemian soil, with optimal physical and chemical characteristics. Significantly lower grain yields of 3093 kg ha^{-1} was obtained by cultivating the spelt in a hilly area, on eutric cambisol soil, acid reactions and low content of light-chain phosphorus. In the hilly-mountainous region, at an altitude above 1000 meters above sea level, the average grain yield of spelt was 2241 kg ha^{-1}, which is statistically significantly lower than yields in the flatland and hilly agricultural area.
In the first and second locality, all treatments of fertilizer application compared to the control variant showed statistically significant influence on the grain yield of spelt. In the third locality, the lowest grain yields were obtained, both on the control variant and on the fertilizer variants applied. What should be emphasized is the fact that the application of individual fertilizers did not lead to an increase in grain yield compared to the control variant. Only the combined application of the investigated fertilizers led to an increase in the grain yield of spelt (Table 1). This can be explained by the fact that the soil was not used for a long time in agricultural production and that it was of high potential fertility and rich in useful species of microorganisms.

Table 1. Grain yield of spelt in different regions in organic growing system

Locality	Treatment	Grain yield (kg ha^{-1})
Locality 1 (Radmilovac, Belgrade)	T1 - microbiological fertilizer	4883
	T2 - biohumus + microbiological fertilizer	5601
	T3 - Control	4377
	Average	4954
Locality 2 (Jasenica, Valjevo)	T1 – biohumus	3139
	T2 – zeolite	2925
	T3 – biohumus + zeolite	3550
	T4 - Control	2756
	Average	3093
Locality 3 (Radijevići, Nova Varoš)	T1 – zeolit	2193
	T2 - mikrobiološko đubrivo	2190
	T3 - microbiological fertilizer + zeolite	2303
	T4 - microbiological fertilizer + hidrogel	2301
	T5 - Control	2216
	Average	2241

When individual fertilizers were concerned, each area of cultivation or agro-ecological conditions had their own specificities. In the plain and hilly region, good grain yield results were achieved by combined application of organic and microbiological fertilizers/soil conditioners, and in the mountainous region the best combination were soil conditioners and microbiological fertilizers. In experiments with winter rye, hulles oats, buckwheat and spelt, it has also been found that different microbiological fertilizers combined with soil conditioners give maximum results (Oljača *et al.*, 2010). With the independent application of microbiological fertilizer (T1) in the plain region, grain yield of spelt was 4883 kg ha^{-1}, which is significantly higher than the control (T3)-4377 kg ha^{-1}. Spelt recharged with microbial formulation (Slavol) gave higher yields compared to the variant where was used only the basic fertilization (Dolijanović *et al.*, 2013). Kovačević *et al.* (2011) cited that they achieved significantly higher yields of different alternative types of winter wheat (*Triticum spelta*, T. *durum*, T. *aestivum* ssp. *compactum*) in treatments with top dressing of microbiological fertilizers. Having used ecological fertilizer (Ekoplant), spelt grain yield increased by 7%, and having used it in combination with bio-activator (Biokal 01) the yield increase amounted to 8% (Jablonskytė-Raščė *et al*., 2013).

Table 2. Analysis of variance of grain yield of spelt

	df Effect	MS Effect	df Error	MS Error	F	p-level	$LSD_{0.05}$
Locality 1	2	19.00	6	0.67	28.50	0.0009	120.00
Locality 2	3	28.08	8	3.33	8.43	0.0074	100.15
Locality 3	4	42.73	10	3.40	12.57	0.0007	25.03

Conclusion

By applying adequate and balanced fertilizer formulations, the grain yield can be significantly influenced in all three production locality. In the flatland area, the highest grain yield of 5601 kg ha^{-1} was obtained by combined application of organic and microbiological fertilizer (T2). On the hilly region, the best result is achieved by the combined application of zeolite and biohumus, where the average grain yield of spelt was 3550 kg ha^{-1} (T3). In the mountainous region where the soil is pliable and poorer physico-chemical characteristics compared to the flatland and hilly regions, and meteorological conditions are sharper, the best result achieved by ploughing zeolite and microbiological fertilizer, when the spelt had a yield of 2303 kg ha^{-1} (T3).

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INFLUENCE OF SEEDING RATE ON COMMON VETCH-WHEAT FODDER CROP MIXTURE PERFORMANCE UNDER ORGANIC MANAGEMENT

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Abstract

Common vetch (*Vicia sativa* L.) originates from southern Europe and is also widespread in the Mediterranean region of Croatia. Due to the economic and ecological dimensions of growth, it is valuable to ascertain whether the application of seed inoculants or growth bio stimulators can enable ecological management of this crop in mixture with wheat (*Triticum aestivum* L.). Research was conducted through field experiment set up on agricultural area near city of Zadar, Dalmatia, during the vegetative seasons 2016/17 and 2017/18. The effect of different common vetch *cv*. Poppelsdorf sowing rates (40, 60, 80, 100 grains/m²) in mixture with wheat *cv*. Valerius (200 grains/m²), on fodder yield was evaluated through 3 treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bc *Viciae* 1001 and Bio-algeen growth bio stimulator application). The highest dry matter yield (DMY) determined on control was achieved in both years with 100 grains/m², 7,98 t ha^{-1} in 2016/17 and 8,82 t ha^{-1} in 2017/18, ($P<0.05$). The highest DMY determined with growth stimulator application was in both years significantly highest ($P<0,05$) with 100 grains/m², 9,24 t ha^{-1} in 2016/17 and 10,29 t ha^{-1} in 2017/18, respectively. The DMY determined with *Rhizobium leguminosarum* bc *Viciae* 1001 seed inoculation treatment in both years was not significantly different between 80 and 100 grains/m², although those 2 higher rates had significantly higher yield ($P<0,05$), compared to 2 lower seeding rates (40 and 60 grains/m²). The highest DMY and the biggest potential for ecological management in both years was shown by applying seed inoculation with *Rhizobium leguminosarum* bc *Viciae* 1001 (11,34 t ha^{-1} in 2016/17 and 10,71 t ha^{-1} in 2017/18).

Keywords: *Vicia sativa* L.**,** *legume cereal mixture***,** *seed inoculation, Mediterranean*

Introduction

Common vetch (*Vicia sativa* L.) originates from southern Europe and is also widespread in the Adriatic part of Croatia and other Mediterranean countries. It provides good quality palatable forage biomass, and it is usually sown mixed with a cereal companion for animal feeding (Lithourgidis *et al*. 2006, Uher *et al* 2019). Oat, barley, wheat and triticale are added to provide a climbing frame for the legumes and to increase the bulk of feed produced (Tuna and Orak, 2007). Due to its high ability to fix nitrogen and to produce quality fodder, it could serve as one of the richest, yet potentially the cheapest protein source for profitable ecological management. The price of common vetch seeds is high, so it is important to optimize the sowing rate. It is documented in previous research that mixture of legumes and small grain cereal has some advantages over pure stands, as crops within the mixtures use the environmental resources efficiently and produce more yield than their pure stands (Atis *et al*., 2012, Roberts *et al*. 1989, Štafa *et al*. 1998, Uher *et al*., 2009). Intercropping of legumes and cereals has produced higher yields than sole cereal crops primarily on soils with no N-fertiliser (Jensen, 1996; Hauggaard-Nielsen *et al*., 2001a, Lauk and Lauk, 2005). The above-ground plant material of common vetch may contain more than 100 kg N ha^{-1} originating from N_2-fixation (Papastylianou 1999, Mueller and Thorup-Kristensen, 2001). Cereal crops use the nitrogen fixed by the legume crops and that fact could influence not only quantity of fodder, but quality. Uher, et al., 2007 reported that the highest forage pea crude protein yield was obtained in mixture with wheat compared to other cereals, and all cereals in mixtures with forage pea significantly better yielded in late boot vegetative stage compared to early head. Protein and carbohydrate rates of feed originated from mixtures are balanced and the feed from mixture has higher feeding value compared to the feed from pure sowings of mixture components (Atis *et al*., 2012, Caballero *et al*., 1995, Lithourgidis *et al*., 2006, Karadag and Buyukburc, 2003, Tuna and Orak, 2007, Mariotti *et al*., 2009, Uher, *et al*., 2007). Feeding vetch in mixtures with small grain cereals is reported to increase milk yields of cows and growth performance of beef cattle (Štafa *et al*., 2001). The choice of a legume species and compatible plant densities are very important for high forage yields and quality in intercrops with cereals (Altinok *et al*., 1997). Furthermore, it is valuable to ascertain whether the application of seed inoculants or growth bio-stimulators can enable ecological management and successful fodder production in the Mediterranean region of Croatia.

Material and Methods

Research was conducted through field experiment set up on agricultural area near city of Zadar, Dalmatia, during the vegetative seasons 2016/17 and 2017/18. The effect of different common vetch *cv*. Poppelsdorf sowing rates (40, 60, 80, 100 grains/m²) in mixture with wheat *cv*. Valerius (200 grains/m²), on fodder yield was evaluated through 3 treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bc *Viciae* 1001 and Bio-algeen growth bio stimulator application). Four common vetch seeding rates (40, 60, 80, 100 grains/m²) in mixture with wheat *cv*. Valerius (200 grains/m²), were grown through three treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bc *Viciae* 1001 and Bio-algeen growth bio stimulator application), in all possible combinations in a randomized, completed block experimental design with 3 replications. The dates of sowing were on 10th of October 2016 and 12th of October 2017. Common vetch and wheat seeds were mixed prior to sowing and were sown in the same row using a special plot drill. The area of the test plots was 30 square meters. The previous crop was wheat in both years of the study. A 5 m^2 section was harvested for green herbage and samples of 1 kg were dried at 60 °C for 48 h, to determine dry matter yield. The experiment was established on an anthropogenic carbonate, moderately dry soil having a slightly sandy-clayey texture. The soil characteristics were as follows: PH_{KCl} of the ploughed layer was 4.65, organic matter content 2.45 %. An analysis of variance was carried out using SAS 9.3 (SAS Institute Inc. 2011). The statistical significance of the treatments was determined at the $P<0.05$ probability levels using the F-test.

Results and Discussion

The excellent stand establishments in both years were obtained due to suitable soil moisture and temperature conditions for germination and emergence of crops. As expected, increasing the seeding rate resulted in increased plant density. Therefore, the plots sown at high common vetch seeding rates (80 and 100 grains/m²) covered the ground faster in early spring. The green mass yield (GMY) is shown on Table 1.

Table 1. Green mass yield of the mixtures seeding rate as affected by the treatment (t ha^{-1}).

Seeding rate / seed no. /m^2	Treatment/Year					
	Control		Growth bio stimulator		*Rhizobium leguminosarum bv. viciae* 1001	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
40 vetch + 200 wheat	20,0^c	21,0^d	23,0^c	22,0^d	28,0^c	29,0^c
60 vetch + 200 wheat	24,0^b	28,0^c	27,0^{bc}	28,0^c	39,0^b	43,0^b
80 vetch + 200 wheat	35,0^a	34,0^b	33,0^b	32,0^b	54,0^a	51,0^a
100 vetch + 200 wheat	38,0^a	42,0^a	44,0^a	49,0^a	54,0^a	50,0^{ab}

Values with the different small letters in column in a year are significantly different according to the LSD test at P <0.05.

The highest green mass yield (GMY) determined on control was achieved in both years with 100 grains/m², 38,0 t ha^{-1} in 2016/17 and 42,0 t ha^{-1} in 2017/18, (P<0,05), respectively. The highest GMY determined with growth stimulator application was in both years significantly highest (P<0.05) with 100 grains/m², 44,0 t ha^{-1} in 2016/17 and 49,0 t ha^{-1} in 2017/18, respectively. The GMY determined with *Rhizobium leguminosarum* bc *Viciae* 1001 seed inoculation treatment in both years was not significantly different between 80 and 100 grains/m², although those 2 higher rates had significantly higher yield (P<0.05), compared to 2 lower seeding rates (40 and 60 grains/m²). Furthermore, The GMY determined with *Rhizobium leguminosarum* bc *Viciae* 1001 seed inoculation treatment was in both years higher, compared to control and bio stimulator treatments at the same seeding rate (between columns).

The dry matter yield (DMY) is shown on Table 2.

Table 2. Dry matter yield of the mixtures seeding rate as affected by the treatment (t ha^{-1}).

Seeding rate / seed no. /m^2	Treatment/Year					
	Control		Growth bio stimulator		*Rhizobium leguminosarum bv. viciae* 1001	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
40 vetch + 200 wheat	4,20^b	4,41^c	4,83^c	4,62^c	6,44^b	6,74^b
60 vetch + 200 wheat	5,04^b	5,88^{bc}	5,67^{bc}	5,88^{bc}	8,19^b	9,03^{ab}
80 vetch + 200 wheat	7,89^a	6,92^b	6,93^b	6,72^b	11,34^a	10,71^a
100 vetch + 200 wheat	7,98^a	8,82^a	9,24^a	10,29^a	11,34^a	10,50^a

Values with the different small letters in column in a year are significantly different according to the LSD test at P <0.05.

The highest dry matter yield (DMY) determined on control was achieved in both years with 100 grains/m², 7,98 t ha^{-1} in 2016/17 and 8,82 t ha^{-1} in 2017/18, (P<0,05), respectively. The highest DMY determined with growth stimulator application was in both years significantly highest (P<0,05) with 100 grains/m², 9,24 t ha^{-1} in 2016/17 and 10,29 t ha^{-1} in 2017/18, respectively. The DMY determined with *Rhizobium leguminosarum* bc *Viciae* 1001 seed inoculation treatment in both years was not significantly different between 80 and 100 grains/m², although those 2 higher rates had significantly higher yield ($P<0,05$), compared to 2 lower seeding rates (40 and 60 grains/m²). The highest DMY and the biggest potential for ecological management in both years was shown by applying seed inoculation with *Rhizobium leguminosarum* bc *Viciae* 1001 (11,34 t ha^{-1} in 2016/17 and 10,71 t ha^{-1} in 2017/18). Seeding rate treatments showed significant effects on fodder yield as well as higher seeding rates of common vetch in mixture with wheat.

Conclusions

Our study clearly shows that common vetch-wheat fodder crop mixture could be grown under organic management for purpose of animal feeding in the Mediterranean-type climate of Croatia. High seeding rates provided greater dry matter yield. A seeding rate of 80 seeds of common vetch inoculated with *Rhizobium leguminosarum* bc *Viciae* 1001 in mixture with 200 seeds of wheat per 1 m^2 proved to be the optimum, and there is no need to apply higher seeding rates.

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ORGANIC AGRICULTURE IN CENTER REGION OF PORTUGAL: THE BEIRA LITORAL AND LIS VALLEY DISTRICT CASE STUDY

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Abstract

In Portugal, the expansion of organic agriculture has become crucial within national agriculture context The methodology included interviews with farmers and field visits to confirm the regional database. The first region to be analyzed was Beira Litoral and then, based on the results obtained, surveys were carried in the Lis Valley for a more delimited analysis and with the objective of evaluating the predisposition for agricultural system change. This work revealed elements that have enabled researchers to develop organic agriculture, to overcome the obstacles faced by farmers, to help boosting chain elements' confidence and reducing confusion between different farming practices. In the Lis Valley the improvement of the agricultural system was considered to allow more efficient production, while saving resources; also, the improvement of the agri-environmental system would allow the conversion to organic agriculture. The conversion is not achieved solely by means of a holistic approach, and it is necessary to use both personal communication and social groups in each region to show farmers the importance and the benefits that may result from this change. According to the evidence available, providing farmers with credible information is essential to increase their confidence in organic farming. Trust in organic farming also depends on farmers' confidence. It is necessary to work on creating adequate training for their needs and to provide accurate and real information on the difficulties and benefits of organic agriculture.

Keywords: *Organic farmer, Portuguese Center Region, Lis Valley irrigation district*

Introduction

The organic farming movement in Europe emerged in the early 20th century as a result of a set of ideas, beliefs and philosophies. According to FAO/WHO (1998), organic agriculture is a holistic production management system that promotes and improves the reality of the agroecosystem, including biodiversity, biological cycles and soil biological activity. IFOAM (2019) defines organic farming as a "… production system that promotes the health of soils, ecosystems and people". It is based on ecological processes, biodiversity and adapted cycles. Organic agriculture plays a dual role, satisfying consumers' demand for organic products and, on the other hand, offering public goods. The consumer's understanding of the word "organic" is complex and several studies show that this understanding changes between different countries and cultures. Vega-Zamora et al. (2014) show that "organic" plays an important role as a heuristic attribute that allows the consumer to infer product quality. The concept of quality has two dimensions: an objective dimension linked to the technical product and a subjective dimension, i.e the consumer's perception. Some authors refer two dimensions of quality perception: a horizontal dimension of quality perception (before and after purchase), and a vertical dimension that includes a set of intrinsic or extrinsic characteristics and the way consumers perceive these attributes as desirable and interconnected with the variable of human behaviour; the attribute of promoting health and the environment is perceived by consumers as one of the most important ones (Steenkamp, 1990; Grunert, 2005; Magistris and Gracia, 2008). The consumer of products cannot evaluate their quality at the time of purchase so, they can only evaluate this quality at the light of the idea they have about the product, and by using information and the perception of this information. Considering information, it is undeniable that the label is a fundamental tool for the differentiation of the organic product (Van Loo *et al*., 2011). The objectives of this study are to analyze the structure of agriculture and carry out the socioeconomic characterization of organic agriculture in the Central Region of Portugal and assess the willingness to change to organic farming.

Materials and methods

This study presents two case studies in two subregions of the Center region. In the first case study, interviews were conducted with farmers of organic agriculture in Beira Litoral (BL) and the second case study, carried out the in Lis Valley (LV) interviews were conducted with a stratified sample. In BL 86 interviews were carried out (38% of the organic farmers and 69% of the organic area in BL. In BL, for the socioeconomic organic producer analysis only the replies of the owners of the holding were considered. The interviews took place in 2018. In the LV, interviews were conducted with all farmers and not only with organic farmers; these interviews were supposed to evaluate the predisposition to change to organic agriculture. The study area is managed by the Lis Valley Water User Association (LVWUA), which covers approximately 2000 hectares and has a high heterogeneity in relation to the areas of holdings. Stratification was performed in order to obtain the sample, according to Gauss's Law and resulting in three samples: 20 owners with the largest area (> 8.5 ha), called "Large"; 20 owners with a very small area (area ≤ 25 m2), called "Small" and 21 owners with areas between 0.5 and 2 ha, called "Medium". This stratification was required because small area owners are predominant, but owners of large farms are the ones with the highest sales and those with the greatest participation and influence in the LVWUA decisions. From March to April 2019, 57 inquiries were carried out. The data were analysed using SPSS 25 software. The correlation coefficient of Pearson (r) and Spearman's correlation coefficient (ró) was used to measure the strength of the association between the variables.

Results and discussion

Organic Agriculture in Portugal

Portugal has had several Rural Development Program (RDP) programs to develop organic agriculture, namely the PRODER (Rural Development Program, 2007-2013) through Action 2.2.1 (Modification of agricultural production modes) and the PDR2020 (RDP 2014-2020) through Action 7.1 (Organic farming), that includes two Operation (OP): OP. 7.1.1- Conversion to Organic Agriculture and OP. 7.1.2- Maintenance in Organic Agriculture. These agricultural policy programs allowed the development of Portuguese organic agriculture, as well as the expansion of the organic area, which reaching 7% of the Portuguese Utilized Agricultural Area (UAA) and representing in 2017 about 2% of UAA organic area in European Union (EU). The Portuguese organic area recorded a growth rate between 2012 and 2017, similar to that of the EU-28. From 2012 to 2017, the total area converted to organic agriculture in Portugal increased by 26%, a growth above the EU-28 average of 25%. (Eurostat, 2019). It was found that in 2010 most of the organic area was supported by PRODER (114 thousand hectares). Between 2010 and 2014 there was a progressive reduction of the organic area supported by PRODER, reaching a minimum of approximately 72 thousand hectares in 2014. The adoption of the new Community framework (RDP2020) allowed a significant increase in the area supported by the RDP, in OP. 7.1.1-Conversion to Organic Agriculture, reaching approximately 80 and 50 thousand hectares in 2016 and 2017, respectively. Action 7.1 supported 222 and 225 thousand hectares in 2016 and 2017 respectively. In 2015 and 2016, the area supported for conversion to organic production was systematically lower than the area receiving maintenance support, ie, there was not an increase of new entries in relation to the previous periods, but there was a switch from OP. 7.1.1. to OP. 7.1.2. The behaviour of the Center region is similar to the evolution Portuguese mainland, only differing in OP. 7.1.1 where there is an increase in new areas for conversion in the Center region. The budgeting of Common Agricultural Policy (CAP) is consistent with the areas supported. In 2011, PRODER attributed a maximum value of 15025 thousand € to organic farming in Portuguese mainland, but between 2011 and 2014 there was a progressive reduction of subsidies granted via PRODER. PDR2020 allowed a significant increase in the allocation of organic farming to the conversion of 21,008 thousand € in 2016. It should be noted that PRODER did not separate conversion to organic agriculture from maintenance. PDR2020 shows this separation and, considering the two supports in 2016, the total reached 51,672 thousand €. In 2017 the financing of both operations was reduced to 26102 thousand € in total. In 2015-2017 the Center region received 22% from RDP financial support for conversion aid (OP.7.1.1) and 24% from national support for organic farm maintenance (OP 7.1.2). The highest value for both operations was reached in 2016, with 12191 thousand €. There was both a potential growth and a linear growth of the organic area and of the number of organic producers, respectively (Fig.1). It should be noted that between 1994-1998 (five-year average) in Portuguese mainland, the average area *per* holding (ha/hold) in organic farming was 39 ha/hold, and grows to 72 ha/hold in 2013-2017. This value is much higher than the average value for Portuguese agriculture, which in 2016 was 14.9 ha/ hold (INE, 2017)

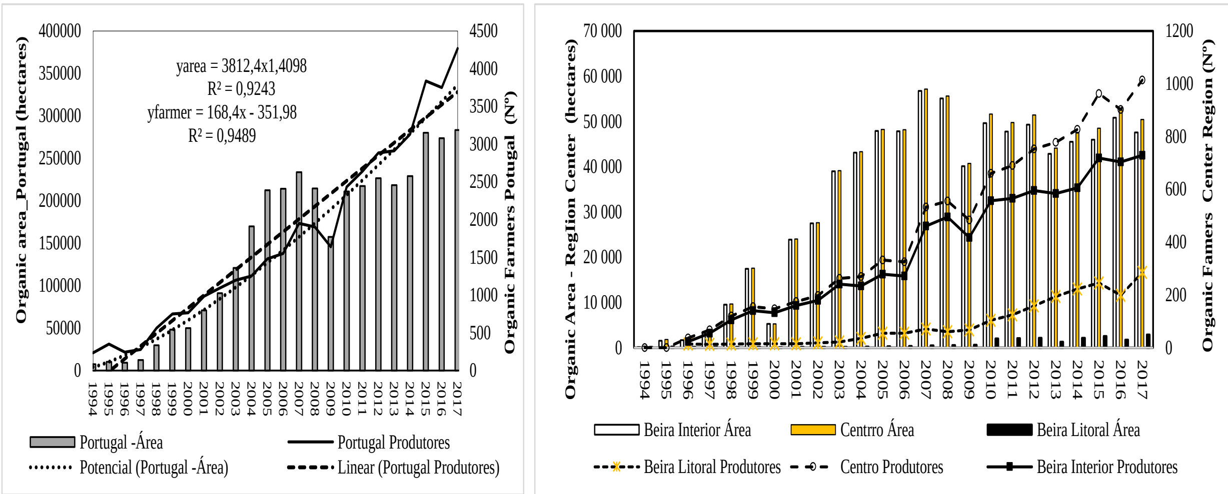

Figure 1, Evolution of the organic area and of the number of producers in Portugal
Source: DGADR (2010), accessed on 21/05/2019

The allocation of the organic area supported by crop type remains similar in both PRODER and PDR2020 programs. Permanent pasture and biodiverse permanent pasture accounted for about 60% of the total area supported by PDR. In 2017, permanent pastures and pastures accounted for 61%, other temporary crops 22%, olive groves and nuts represented about 13% of the area supported by PDR2020 through OP 7.11 and OP. 7.1.2. Between 2008 and 2017, pastures accounted for around 67% of organic crops, followed by forage (9%), with an increase of this crop in relative and absolute terms (growth of 128% between 2011 and 2017), followed by olive grove that represents 9% of organic crops (growth rate of 16% between 2011 and 2017). The most significant growth rate was observed in nuts (451%) and horticulture (253%). Between 2013-2017, the Center Region represented 19% of the organic area and 25% of organic farmers in Portuguese mainland. There was an increase in the number of producers in relation to the national figures and a share reduction of the area by increasing surfaces in other regions. However, it is important to emphasize the importance of the Center region plays a pioneering role in the development of organic agriculture. The differences between the two sub-regions, Beira Litoral (BL) and Beira Interior (BI) between 1994 and 2017 are clear, as regards the areas and the number of producers in the Center region. Between 2013-2017 the BL region contributed with 4% (five-year average) to the organic area in the Center region and represented 25% of organic farmers. Between 2013-2018, BI represented 96% of the organic area and 75% of the organic agriculture of the Center region. Between 2013-2017, in the Center region, the average area was 55 ha *per* holding (BL: 9 ha/hold., BI: 70 ha/hold.) (DGADR, 2019). The allocation of crops in mainland Portugal and the Center region is similar. In the subregions, there is a difference in terms of area and crops. It is relevant to refer that in 2017 about 51% of the aromatic herbs grown in the Center region were found in the BL subregion. However, the weight of these crops is negligible.

Survey Results

The first case study was conducted with organic farmers in BL by means of interviews; 65% of the respondent are land owners and 12% work the land as tenants. The owner is usually male (72%); 62% of them are between 30-49 years old, 36% are between 40-49 years old and 50% of farmers have higher education. Only about 25% of the farmers have training in agriculture, but 55% of them depend on agriculture as their main activity. These figures are higher than the average for Portuguese agriculture, where only 5.8% of farmers have higher education, only 6% of farmers depend exclusively on agricultural income and 12.8% have agriculture as their main income activity. (INE, 2017). The main motivation to take up farming was the profitability of the land (64.5%). Family experience in agriculture and professional discontent were important factors. About 62% of respondents indicated that they adopted organic farming as a philosophy of life which is also economically viable, due to the highest market price in relation to products resulting from other cultivation systems. The versatility of organic farming products to be marketed as produced under another mode of cultivation was reported by 22% of respondents. Most farmers (61.4%) financed their farming activity only with their own resources. Producers stated that technical support comes from internal agricultural expertise or from consultancy and/or commercialization of agricultural products and from peer associations. In what concerns marketing orientation, 52% of the producers sell to wholesalers, 34% sell their production directly to the final consumer and 33% sell to small retailers (short circuit). However, around 33 producers (40%) sell directly for export. The sale price of the production is mostly established by customer (51%). and 53% of the producers do their own marketing. Some farmers wanted to increase farm areas, but it was difficult to find land either to buy or to rent. Owners prefer, regardless of the proposed lease or purchase values, to keep abandoned land. In the interviews, criticism about the lack of mandatory training initiatives, both in terms of dispersion throughout the territory and in terms of frequency of performance and publicity, was revealed. Questions and problems related to the quality of training were mentioned, considering that the contents of the programs are superficial and out of touch with the reality of agriculture. Criticisms have been submitted to certifying bodies and agricultural advisory/consultancy. Regarding consumer perception, it was mentioned that the consumer of organic products values certification, but the general public does not recognize the difference between organic agriculture and integrated production agriculture. Other names such as "natural production" or "traditional production" are also sources of misunderstanding. The different designations generate confusion and make it difficult to distinguish between different agricultural systems. In the second study case, in the Lis Valley, the results of the survey conducted with farmers showed that about 76% of the respondents in the LV belong to the age group > 50 years old, 35% are over 65 years old, and 84% are male. The education level is lower than the one of the previous case study: 54% only attended primary school. This may be related to the fact that in this study all respondents were landowner farmers, according to the stratification, but their production is done under different agricultural systems, whereas in the previous case study only organic farmers were interviewed. At the same time, it is related to the higher age group of respondents. Agricultural activity plays a secondary role as income source for 58% of the respondents. The majority of owners work their own land (78%). The owners who rent land intend to continue to do so, but do not intend to sell the land. The income obtained allows adding extra income to the one they get from their main activity. This confirms the importance of family farming in Portugal and of self-consumption of the agricultural structure (DR, 2018). There was a weak positive correlation which is significant at 5% level of significance (*p-value*= 0.05) between the relevance of the agricultural activity and the size of farms (r = 0.301; ró = 0.328). As the size of farms decreases, farming becomes less important as a main activity and becomes a secondary source of income. The motivation for the practice of agriculture is related to the profitability of the land (43%) and 30% of the farmers are in the business due to the fact they want to keep the family activity. About 44% of the respondents sell all their production and 33% only produce for self-consumption. However, there is a weak positive correlation (*p-value*= 0,05), between these farm sizes and marketing orientation (r = 0.282; ró = 0.314), i.e. small farms tend to produce for self-consumption. There is also a positive correlation (r = 0.433 and ró = 0.432; *p-value*= 0.05) between the farmer age and the destination of the production, which means that farmers within a higher age group tend to produce for self-consumption. Only one farmer practises organic agriculture and the remaining indicated that they apply integrated production. Concerning the predisposition to change, the majority (84%) of the respondents replied that they were not interested in changing their production system to organic agriculture. Out of the 45 responses obtained for the predisposition to change, 32 responses indicated that there is no public support for changing the production system to organic farming. However, the surveyed respondents who were available highlighted two fundamental supports for helping the conversion/change of decision, ensuring both the sales outflow and higher production prices. There is a significant negative correlation at *p-value*= 0.05, between literacy and the predisposition to change (r = -0.37 and ró = -0.34). If we correlate this with age, this correlation is negative, significant at 1% level of significance (r = -0.49; ró = -0.45). The higher the education level is, and the younger the farmer is, the greater the predisposition to change is. Farmers converted to organic farming as well as non-converts have adequate knowledge of the benefits of this production system and acknowledge the need for more sustainable agriculture in order to enhance the economic sustainability of farms. The fact that in the case of BL the respondents were farmers converted to organic farming and that in the Lis Valley interviewed farmers are producing under integrated production system, allowed to conclude that some of the difficulties are common even when different production systems are adopted or when farmers' socio-economic characteristics are different.

Conclusion

The results obtained point several obstacles to the conversion of the productive systems and to the maintenance of those already engaged in this mode of production**.** The two case studies in the Center Region allowed characterizing the organic farmer and assessing the predisposition to change in two geographically nearby areas. The age factor, land ownership and size for the development of organic agriculture should be noted. It is important to emphasize the importance of consumer's recognition of the differentiation of organic farming products through price. The two areas present different forms of commercialization, but the short circuit is an issue to be developed. The responsibility of Public Institutions in the promotion of organic agriculture is evident, through the clarification of the various production systems and also of the way of commercialization; however, the development of organic agriculture should result from consumption by the valorization of the product in terms of price and sales development. The increase of farm plots and the clarification of production costs, mechanization, support to young farmers and generational renewal are important issues and will be factors to be taken into account, when developing policies for this type of agriculture. Great responsiveness to expand knowledge to support decision-making, through dissemination actions or participation in demonstrative actions has also been highlighted.

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CORRELLATION BETWEEN MULCHING, MYCORRHIZA FUNGI, AND OTHER PARAMETERS IN LETTUCE IN TWO FARMING SYSTEMS

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Abstract

The most important soilborne fungi are mycorrhizal fungi, including Arbuscular mycorrhiza. Their importance is that they create a symbiotic relationship with the roots, therefore helping the plant to absorb nutrients and water. Recently, more research has been done on their use in agriculture, with more promising results. Our experiment took place parallel in Szent István University, experimental fields of conventional and organic farming system, Budapest, Hungary. Control, alfalfa and rye straw mulch treatments were prepared. We investigated the correlation between mulching, mycorrhizal colonization, morphological and inner content parameters of lettuce. In addition, the effect of the two kind of cultivation methods on mycorrhizal colonization was Lettuce is one of the leaf vegetables grown worldwide in the largest area, and the importance of an environmentally conscious lifestyle is becoming more and more important, and as a result the role of organic farming has become more valuable. Alternative methods are applied to the occurring problems (pests, diseases) in organic farming. One of these methods is mulching, which has positive effects, such as: weed control, regulation of soil water balance, fertilizing effect and soil life stimulation. studied. Results showed several correlations between the different parameters. Mulching and farming system affected the quality of lettuce and mycorrhizal colonization of roots. Also, colonization affected the phosphorus uptake of the plants. However, due to the method of conventional cultivation, these differences were less remarkable. Altogether, more environmentally friendly cultivation has a positive effect on the amount of mycorrhizal fungi.

Keywords: *mycorrhiza, lettuce, inner content, organic farming, conventional farming.*

Introduction

Lettuce is one of the most important leaf vegetables. Due to low energy content, easy digestibility and high fiber and mineral content makes it easy to integrate it into modern diets. The spread of salad mixes and eating trends, occured an increasing interest in different salad specialties. As a result of environmentally conscious lifestyle the role of organic farming has become more valuable (Terbe, 2000). Alternative methods are applied to the occurring problems (pests, diseases) in organic farming. One of these methods is mulching, which has positive effects, such as: weed control, regulation of soil water balance, fertilizing effect and soil life stimulation (Grünefeld, 2010). Mycorrhiza fungi is a symbiont fungus which infects the plant roots and obtains photosynthetic products from the plant, in return, contributes to the supply of nourishing water (Brundrett, 1991; Ishii, 2016). In vegetable growing systems the most common mycorrhiza fungi is arbuscular mycorrhiza (AM). It is a type of endomycorhizza which goes into the root cell. The name is derived to a characteristic structure which occur within the cortical cell. It is a tree-like structure which enlarge the nutrient uptake surface (Smith and Read, 2008). Plant inoculation with arbuscular mycorrhiza (AM) can be a sustainable technique for the improvement of yield and plant resistance to biotic and abiotic stresses (Gosling et al., 2006; Guillermo et al., 2009).

The experiment was carried out to investigate the effect of alfalfa and rye straw mulch on the morphological -, inner content parameters and mycorrhizal colonization of lettuce, and to find out if there is any correlation between mycorrhizal colonization and morphological and inner content parameters.

Materials and methods

The experiment was carried out at Soroksár Experimental farm of Szent István University (SZIU) parallel in Conventional and Organic systems in 2018. Soil analysis was made for both systems. Important results of the analysis marked in the Table 1. The places are close to river Danube, and they are categorized by sandy casting that has the physical properties of sandy soil.

Table 1. Most important parameters of soils

	Organic system	**Conventional system**
pH (KCl)	7,45	7,10
NO2+NO3-N (mg/kg)	71,6	33,6
P2O5 (mg/kg)	577	1000
K2O (mg/kg)	166	99

"Voltron" Batavia lettuce from Rijk Zwaan was used. Seeds were sown 11th April in styrofoam trays, the media was Latagro KB2 type peat moss. The transplants were planted in 3 twin-row with 3 repetition with borders after and between treatments on 24th May in both places. The treatments were: *Control*, *Alfalfa mulch* and *Rye straw mulch*. Abbreviations of treatments marked in the Table 2. There were two sampling time: 26th June and 4th July. Morphological (stem and head diameter, weight), inner content parameters (dry matter, nitrogen, phosphorous, potassium), and SPAD were measured, furthermore roots were collected for mycorrhizal colonization measurements. Roots were painted with arbuscular-mycorrhiza painting method based on Phillips and Hayman et al. (1970). According to Giovanetti and Mosse (1980) colonization was measured with gridline intersect method with an Olympus Szx7 stereomicroscope. The partial results were calculated further with MycoCalc software. From these head diameter, nitrogen, potassium, SPAD and root colonization measurements will be presented.

Table 2. Applied abbreviations

Treatments	**Organic system**	**Conventional system**
Control	O-S1	Z-S1
Alfalfa mulch	O-S2	Z-S2
Rye straw mulch	O-S3	Z-S3

Results and discussion

<u>Morphological parameters: Head diameter</u>

At the first sampling time (26.06.2018.), in the Conventional system the head diameters did not show significant differences. On average, the diameters from all the three treatments were around 29 cm. The largest head diameter was measured in case alfalfa mulch (Z-S2) treatment with 37 cm, while the smallest, 23 cm, was measured in the rye straw mulch treatment (Z-S3). There were major differences in the Organic system. Here, the size of the control (O-S1) and

alfalfa mulched (O-S2) areas was similar in size, with an average of 21-22 cm heads, while the average diameter from the rye straw mulched (O-S3) parcels was 15 cm.
At the second sampling time (04.07.2018.) in both systems the controls (S1) were the largest with around 36 cm (the largest measured head was 46 cm) and rye straw mulched treatments (S3) were the smallest with around 32 cm. In the Organic system the lettuce heads taken from the control (O-S1) were ca. 17 cm, the highest measured was 23 cm. The rye straw-treated (O-S3) lettuces were around 14 cm. The ANOVA-probe proved significant difference between the two systems (Figure 1.).

Figure 1. Lettuce heads at 2nd sampling time at Conventional system (photo: author)

<u>Inner content of lettuce:</u>
<u>Nitrogen and SPAD measurement</u>
In the Conventional system all the three treatments showed an almost balanced nitrogen content of about 40 mg/g. In the Organic system, the highest values, average 23mg/g, were measured on alfalfa mulched (O-S2) plants. In rye straw-treated (O-S3) samples, the average was about 19 mg/g, while in control (O-S1) plants it was 17 mg/g.
In the Conventional system the SPAD values correlated with the nitrogen content. In all three treatments values were around 21. There were differences in the Organic system. The lowest chlorophyll content was found on rye straw-treated (O-S3) plants with values around 12. The results of alfalfa-treated (O-S2) and control (O-S1) were similar, with 17-18 SPAD value. Thus, the correlation between nitrogen and chlorophyll content was less visible in this place (Figure 2.).

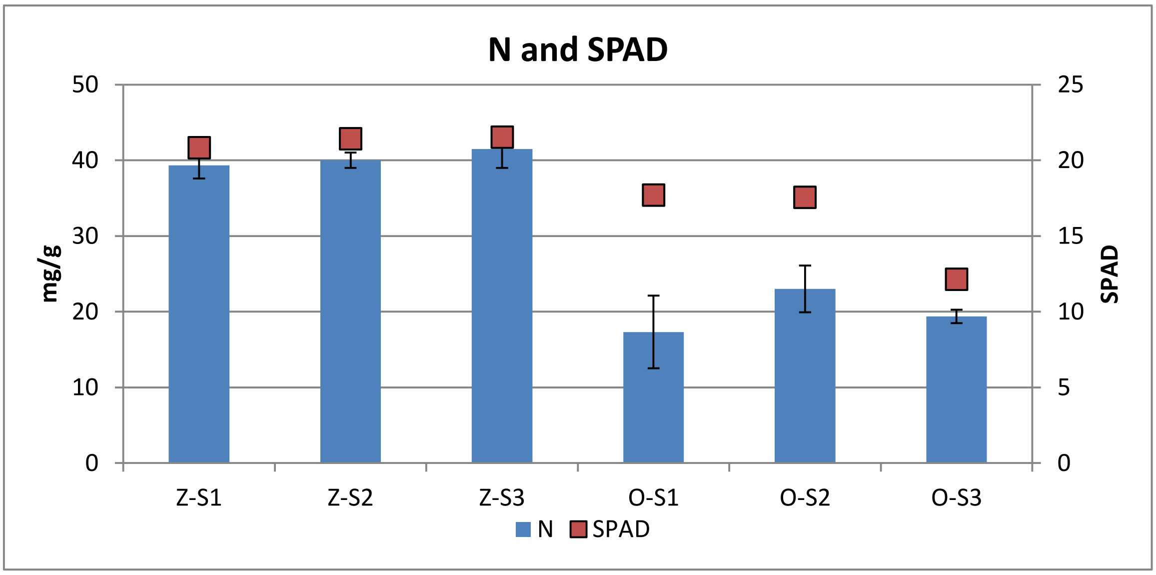

Figure 2. Nitrogen and SPAD values of lettuce samples

<u>Phosphorous content and mycorrhiza colonization</u>

Colonization was higher in the Organic System for both sampling times. After the first sampling, the highest colonization, 68%, was found on the control (O-S1) plants. Plants treated with alfalfa (O-S2) and straw (O-S3) had 52% and 50% colonization. However, the increase in colonization in the two treated areas was observed in the second harvest, but a decrease occured in control plants. Fungus samples on O-S1 plants decreased to 55.5% and increased to 62% and 65% in O-S2 and O-S3 treatments.

Of the samples taken at both times in the Conventional system, the Z-S2 plot showed the highest colonization ratio. First time 28,5% second time 40%. Samples of Z-S3 were first measured at 25% and second at 30%. The amount of mycorrhiza was 22% and 36% on the control plants. Thus, all treatments showed an increase in the degree of colonization. ANOVA-probe showed significant differences in the colonization both times.

We also examined the arbuscules, but the results were not sufficient for definite conclusions. There is a definite relationship between P content and root colonization. In the Organic system, the P content was significantly lower than the Conventional system. For the first sampling time, the highest value of 2,6 mg/g was measured in O-S1 plants, the lowest, 2,15 mg/g on O-S3, and 2,53 mg/g on O-S2. Mycorrhization on these plants followed the same trend. However, there was a change at the time of the second sampling. The average P content decreased to 1,55 mg/g for O-S1, 2,28 mg/g for O-S2, and 1,36 mg/g for O-S3. Mycorrhization in control areas decreased proportionally, while in treated areas, in contrast to phosphorus, increased.

The correlation between the two measured parameters in the Conventional system was observed on both times. At the first measurement, the average P content in Z-S1 were 5,77 mg/g, 6,54 mg/g in the Z-S3 and 6,67 mg/g in the Z-S2. The percentage of colonization showed the same order. For the second measurement, the P content of the control plants increased to an average of 6 mg/g, while the alfalfa and rye straw treated lettuces decreased to 6,26 mg/g and 5,81 mg/g. Although mycorrhization increased in all three treatments, Z-S3 treatment showed the slightest increase, with the highest decrease in P content. The P content in Z-S1 areas increased the most, and colonization also showed the biggest growth (Figure 3.). The difference in phosphorus content in different treatments was noticeable at the level of root colonization. In most cases, the highest phosphorus content was measured in alfalfa mulched plants and colonization on these plots was also slightly higher.

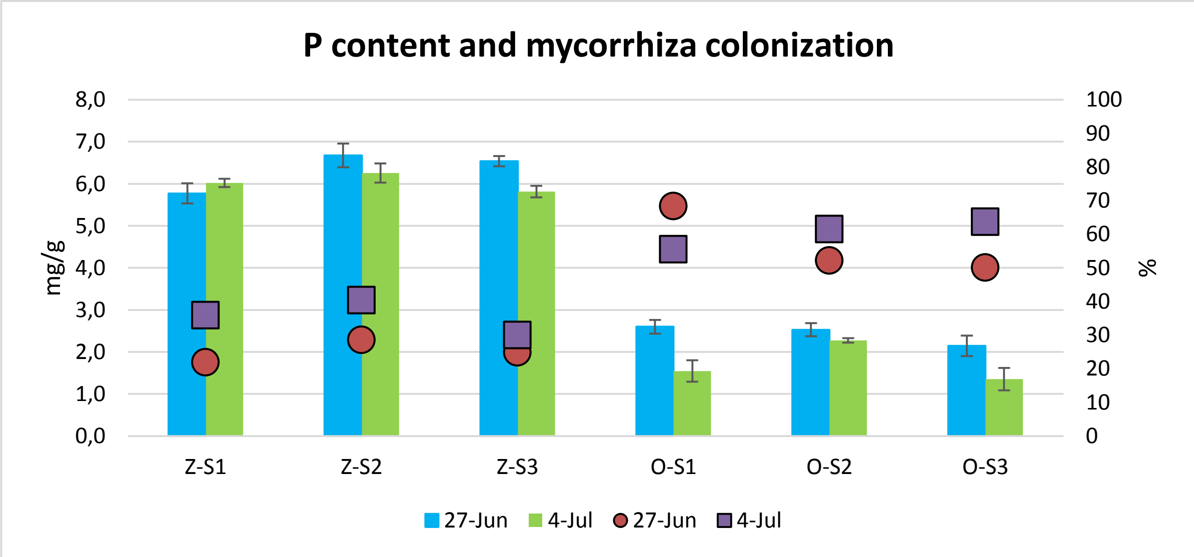

Figure 3. Correlation between phosphorous content and mycorrhizal colonization at both times

Conclusion

For head diameter in the Conventional system, in most cases, the highest values were measured on the control (Z-S1) and alfalfa (Z-S2) mulched plants, the rye straw mulched (Z-S3) plants showed the lowest results. In the Organic system the lowest results were also measured on rye straw mulched plants (O-S3), but in this system, alfalfa treatment (O-S2) showed the highest values. Based on these, may the allelopathic effect of rye straw was present in both areas (Jabran, 2017). A kind of 'good fertilizer effect' of alfalfa mulch was well observed in the Organic system, but this effect was not observed in the Conventional system (Hatwig and Ammon, 2002). This could be the effect of fertilizers used in conventional cultivation, which resulted a better nutrient supply in unmulched areas.

In the Conventional system there were no significant difference between treatments neither in the nitrogen content nor in the chlorophyll content. Due to small differences, no definite conclusions can be drawn. In the Organic system, the highest values for both N and SPAD were measured on treatment O-S2, which can be deduced from the positive effect of alfalfa mulch. Data from the other two treatments showed no correlation between nitrogen and chlorophyll content, however in other cases we verified the results of Sharaf-Eldin et al (2015). Mycorrhiza fungi were more present in the Organic system than in the Conventional system. The ratio of colonization can be associated with the phosphorus supply of plants. The results of the phosphorus measurement in the Conventional system showed the same tendency that we experienced with mycorrhization. In this system, the phosphorus content of the soil was almost twice as the value measured in Organic system (1. table). There was as high amount of phosphorus available in the soil, that the plants did not need the help of mycorrhizas. This also could contribute to the lower colonization ratio, because if the soil phosphorus content is high or increases, a decrease in colonization occurs (Guttay et al., 1989). Comparing the different plots, it was observed that the two measured parameters were related. Although at the time of the second sampling, in most cases the phosphorus content was reduced, while the number of mycorrhizas increased, the differences between the plots showed similar differences in both measured parameters. Mosse et al. (1973) studies proved there is correlation between phosphorus content of soil and mycorrhiza colonization.

Altogether, mulching has an impact on the morphological and inner content parameters of lettuce and on root colonization. Also, mycorrhiza fungi influence phosphorus uptake. However, due to the nature of conventional cultivation, these differences were less pronounced. It can be stated that cultivation method, which is less burdensome to the environment, has a positive effect on the mycorrhizal ratio. Although several correlations can be observed between the measured parameters in present experiment, for more definite conclusions further experiments should have been carried out.

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KNOWLEDGE OF THE ECOMOMIC EFFECT OF BIOLOGICAL METHODS APPLICATION IN AGRICULTURE

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Abstract

During the past few decades investigations have been conducted on the impact of different biological methods on yields, production quality and early maturity in agriculture. The benefits of their application have been established. From a theoretical point of view, the isolated established economic effect of a factor would be confirmed if there is no a deficit of resources. The effect of the application of biologically active substances may be economically beneficial for one agricultural holding and proven as useless for another farm. The economic efficiency of the various factors must be assessed in the context of the whole set of conditions under which an agricultural enterprise operates. The aim of this study is to test the mathematical model for assessment of the economic effect of biological methods application in optimizing the structure of production. The elaboration of the production structure is one of the most important and complex agro-economic tasks in the management of the farm. The assessment of the economic effectiveness of the different biological methods will be done through an optimization model to improve the farm production structure. The results of the preliminary studies show that in assessing the impact of biological substances it is necessary to take into account not only the increase in profitability and the profit per unit area of agricultural crops, but also whether the profit of the holding has increased as a whole.

Keywords: *knowledge, agriculture, economic effect, biological methods, optimizing of production structure.*

Introduction

The growth of the planet's population in line with the objectives of sustainable governance and environmental protection, required the introduction of innovations in agriculture. Besides the way of selection and mechanization in plant production, there is also a relatively new direction in the field of agricultural science. These are the biological production methods - growth regulators (Belcheva, S., 1989; Ankita, M., Debasish, P., 2017) or biologically active substances (BAS) (Brown P. and Saa S., 2015). The natural growth regulates include auxins, gibberellins (Rademacher, W., 2015, 2018), cytokinins, defoliants, femininizers, retardins (Izumi, K., Yamaguchi, I., Wada, A., Oshio, H. & Takahashi, N, 1984), abscissic acid, ethylene, etc. All of these are natural or synthetic substances and some of them are even extracted from the plants themselves (leaves, stems, fruits), i.e. they are completely harmless to human and animal health when applied in a certain ratio. They regulate plant growth, reveal and increase their biological potential (Looney, N. & Jackson, D., 2011; Fridlender M., Kapulnik Y. and Koltai H., 2015).

However, there is still a shortage of knowledge and research in Bulgaria for the economic benefits of their application. It is known that they contribute to increased yields in plant growing, early maturity and production quality. They reduce the fall of the flowers, seeds, and fruits, and they can regulate the maturation time of the fruits. The growth regulators are applicable to different crops such as wheat, legumes, vegetables, orchards, etc.

To determine the practical effectiveness of different growth regulators, all costs (not just the price of regulators) and absolutely all positive effects must be taken into account. At this stage, this has not been done by the BAS's developers and distributors. Normally, when analyzing the economic effects of a given method, the complex set of factors, interrelationships and interdependencies among all factors of production are not taken into account.

Typically, the same economic effect from the application of a factor (when identified in isolation from the specifics of the conditions in which the farm operates) may be beneficial to one enterprise and otherwise proven to be useless. From a theoretical point of view, the isolated established economic effect of a factor would be confirmed if there was no deficit of resources. The effect of the application of the biologically active substance may be economically beneficial to one agricultural holding and proven to be useless for another farm. The economic efficiency of the various factors must be assessed in the context of the whole set of conditions under which an agricultural enterprise operates. Therefore, knowledge about the economic impact of BAS is needed. In other words, the economic efficiency of a factor must be judged not only on whether the profit per unit area of the crop in question is significant, but rather on whether it increases the profits of the enterprise in general.

The usefulness of the factors of production should be established by the elaboration of the farm business plan. The basis of the business plan of an enterprise is the production structure. Therefore, I will evaluate the economic efficiency of different BASs by optimizing the farm structure by using linear programming.

The aim of this study is to test the mathematical model for the assessment of the economic effect of the biological methods in optimizing the structure of production.

Material and Methods

The necessary information was gathered with the assistance of specialists from the studied farm. The farm is a legal entity registered under the Cooperative Law as agricultural production cooperative. Its activities are in a flat and hilly terrains of Veliko Tarnovo region, Bulgaria. Climate conditions create prerequisites for growing the following crops of wheat, barley, maize, silage maize, sunflower, Burley tobacco, vineyards, fruit trees (apples, peaches, cherries), vegetables (tomato, pepper), lucerne (for seed and hay) and dairy cows. The agricultural cooperative cultivated land area: 8059 decares[1]

A detailed description of the linear programming is available in Dantzig, (1987); Nikolov, N., Ivanov, G., Stefanov, L., (1994); Vanderbei, (2015); Piryonesi, & Tavakolan, (2017), etc.

For the creation of the model, the study is based on Nikolov, N., Ivanov, G., Stefanov, L., (1994). Solving a particular economic problem with mathematical methods means creating an economically-mathematical task. Economic modeling is a mathematical task, which reflects with satisfactory precision the most important relations and characteristics of the economic problem. These tasks are constructed in a system of linear dependencies. They should reflect the conditions to be taken into account when solving the task. The objective function expresses the optimality criteria (min, max):

$$
\begin{array}{l}
A_{11}X_1 + A_{12}X_2 + \cdots + A_{1n}X_n \leq B_1 \\
A_{21}X_1 + A_{22}X_2 + \cdots + A_{2n}X_n \geq B_2 \\
. \\
\\
A_{m1}X_1 + A_{m2}X_2 + \cdots + A_{mn}X_n = B_1 \\
\hline
F = C_1X_1 + C_2X_2 + \cdots + C_nX_n \rightarrow \max\ (min),
\end{array}
\qquad (1)
$$

Where:

- X_j – indicates the size (magnitude) of the activities or metrics,
- A_{ij} and Cj – indicates the activities that will be done,

[1] 1 ha=10 decares

- B_i – means the amount of available resources or the amount of activities (restrictions).
- The objective function F gives the optimality criteria.

Objective function:

$$F_{max} \text{ (gross margin/profit)} = \sum_{j \in T}^{n} C_j X_j, \qquad (2)$$

where:

- C_j is (gross margin/profit) from j-th unknowns;
- T is the aggregate of the indices of the unknowns from which the gross margin / profit is obtained.

The solution of the model will answer the following questions:

1. Establishing the optimum production structure according to the constrains and the optimality criteria;
2. Establishing the impact of the BAS on efficiency and production structure, depending on the chosen optimal criteria;

In order to establish the production structure of the selected agricultural holding, it is necessary to determine: a crops area; a number of animals; a feed balance, labor resources and other activities. When developing the model, the optimality criteria will be max gross margin. Additionally, we will set another criterion for optimality - max profit.
The argument for choosing this agricultural cooperative is based on the fact that experts and researchers in agri-economic science find that farms in this areas have a mixed specialization - plant growing and livestock breeding. In order to determine the production structure of the selected cooperative, it is necessary to determine the area of the crops, the number of animals and other activities to achieve maximum economic impact. During the developing of the model, the criterion is to achieve the maximum gross margin with the inclusion of BAS and without applying them. The solution of the problem will also answer the questions regarding the most cost-effective production processes for crop production (for feed and commodities) and for livestock breeding (cows, feeding for 4 000 l milk, ration). On the other hand, the model provides the opportunity to quickly and easily develop different options for optimizing the production structure in case of a change of production or some of the limiting conditions. Additionally, the decision will present the impact of BAS on the production structure and, accordingly, on the economic outcome. The production structure in the case at hand depends on the specifics of natural conditions and limiting factors. On the chosen farm, the organizational conditions are as follows:

1. The quantity and quality of the land, irrigated conditions.
2. The amount of grazing meadows.
3. The quantity and quality of the main productions (breeds of selected animals, milking, presence of cowshed, warehouses, etc.).
4. Labor resources (number of permanently employed workers, plant breeders, livestock-breeders, mechanics, zootechnics, agronomist.
5. Agrotechnical crop rotation requirements (minimum / maximum limits in which they may vary, green / dry weight ratio).
6. Zootechnical conditions regarding the feeding of animals according to the milking (ration, green, dry, concentrated, fodder).
7. Contracts to buy milk, sale of commodity crops.
8. Ability to purchase concentrated fodder.
9. Prices of marketed production and means of production.

For all activities with and without application of BAS develop technical and economic norms of 1 decar or 1 t. production - for an average yield, selling price, incoming, material costs, labor costs, production costs, income, profit, business days, etc. I use the Solver application in MS Excel, for the purpose of optimization, the objective function, Solver is an application that can be used to find an optimal solution (minimum or maximum) of an equation that is subject to various constraints. The optimal solution should be located in one cell and to be the result of the mathematical calculation of the variables of the objective function. The article presents one case from which no conclusions can be drawn about the effects of the biological methods under farms. This is due to the fact that the more criteria from different groups (land, plant, livestock, labor) are included in the optimization, the more the model strikes for a balance between the created organizational conditions, which is often a prerequisite for compromise solutions to the task.

Results and Discussion

The results make it possible to assess to what extent and how the resources in the cooperative are used and, accordingly, the economic effect. Linear optimization allows to take into account changes in the production structure under the influence of a BAS, taking into account the whole complex of factors and their relationships.

Table 1 show the solution of the task of optimizing the production structure of the agricultural cooperative under different criteria of optimality: maximum income and maximum profit respectively with / without use of growth regulators (BAS). In the criterion of optimality of maximum income, using wheat and barley BAS occupies 41.9% of the arable land and the maize - 40.3%. Without BAS, wheat and barley occupy 50% and maize is reduced to 27.3%. Attention is paid to the fact that the pepper is inefficient production and does not enter the production structure when using BAS. The same applies to tobacco, which however disappears from the farm's production.

Table 1. Results of the optimization model, decares/n.

Products, decares	F max gross margin with biological activity substance	F max gross margin without biological activity substance	F max profit with biological activity substance	F max profit without biological activity substance
Wheat	991	991	991	1239
Barley	2179	2665	2116	2614
Maize	3046	1980	3047	793
Maize silage	104	125	104	125
Sunflower	97	111	97	111
Tobacco	-	-	-	-
Tomato	567	313	567	407
Pepper	-	459	-	345
Vineyards	91	91	91	91
Apples	60	60	60	60
Peaches	72	72	72	72
Cherries	16	16	16	16
Lucerne, seed	270	270	270	270
Barley feed	63	75	126	126
Dairy cows, n	20	20	20	20
Leased land	503	831	502	1790
Total decares	8059	8059	8059	8059

Source: Author's elaboration based on survey result.

Table 2 show the economic efficiency again at maximum income and maximum profit, respectively with / without using growth regulators (BAS).
The results show that the application of BAS is economically efficient. In the optimum criterion in the maximum income function, the optimal use of BAS gives 47% more incoming. At the same time, the production costs with BAS increased by 9.9%. This has an impact on income, which as a result of the application of BAS has increased by 52% and the profit by over 91%.
The results change with optimal maximal profit criterion, i.e. incoming with application of BAS gives an increase - 50%; production costs - 13%, income - 57% and profit 94.4%.

Table 2. Results of economics effect[2][3], leva[4].

Indicators	F max gross margin with biological activity substance	F max gross margin without biological activity substance	F max profit with biological activity substance	F max profit without biological activity substance
1. Incomings -1 da	1 863 309.6 246.6	1 211 412.8 167.6	1 862 044.8 246.4	1 123 100.8 163.2
2. Material costs -1 da	555 366 73.5	492 949.6 68.2	553 172.4 73.2	402 469.8 64.2
3. Labor costs -1 da	201 745.2 26.7	165 521.2 22.9	201 016.2 26.6	147 948.4 23.6
4.Production costs (2+3) -1 da	757 111.2 100.2	658 470.8 91.1	754 188.6 99.8	550 418.2 87.8
5. Income (1-3) -1 da	1 661 564.4 219.9	1 045 891.6 144.7	1 661 028.6 219.8	975 152.4 139.6
6. Profit (1-4) -1 da	1 106 198.4 146.4	552 942 76.5	1 107 856.2 146.6	572 682.6 75.4

Source: Author's elaboration based on survey result.

More than 200 biological methods for regulating the physiological processes of plants are currently used worldwide with the help of BAS. Production and sales of growth regulators on international markets are steadily increasing. In Bulgaria, the application of growth regulators is still limited mainly due to the lack of knowledge and information on the positive economic effect. The main reasons for this lag are the insufficient scientific potential, the poor relationship with agribusiness and the lack of marketing.

Conclusions

Increasing plant productivity can be achieved on the one hand by genetics and selection, as well as by managing physiological and biochemical processes through the use of growth regulators, on the other hand. Great hopes have been addressed to growth regulators to increase the absorption of solar energy from plants. New groups of natural BAS will be discovered in the near future. They will refine existing growth regulators to achieve high physiological activity combined with highly selective action and safety for human health and the environment. At the same time, there is a need for more knowledge about the economic efficiency of applied biological methods in agriculture.

[2] Not included CAP subsidies
[3] Not included fixed costs
[4] 1 lev= Euro 1,95583 (2019).

The assessment of the economic efficiency of different biological methods was done through an optimization model for the structure of agricultural production. The results showed that when assessing the impact of biological substances, it is necessary to take into account not only the increase in the profitability and the profit per unit area of the crops, but also whether the profit of the holding is increasing in general.

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SOME ASPECTS OF THE ORGANIC FOOD MARKET IN SERBIA

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Abstract

The paper aims at analyzing organic food market in Serbia with a particular focus on structural characteristics on production units and organic food distribution channels. Secondary data were collected mainly from Serbia Organica's database and includes 102 registered domestic producers/processors. Our research showed that the organic market in Serbia was still immature, as it was not well-organized and the products were not readily available on the market. The overall market share for organic food was extremely low as well as share of utilized area under organic production in total agricultural land. Research results indicate that the majority of farms are small, with fruit growing being the most prevalent activity. Majority of producers sold their products to wholesalers and to processing companies. The size of farm is not the main obstacle for further development of the organic food market since there is no statistically significant correlation between distribution channels and the utilized land for organic production. Despite that, the sector showed positive tendencies - domestic growers were increasingly turning to certified organic farming systems; large retails chains upped their offer of organic imported products; a large companies getting involved in primary agricultural production on larger land areas; and promotion of organic products was intensified in the past years. Taking into consideration that the majority of organic producers in Serbia are small farmers, in order to increase the supply, an improved framework for business development should be applied. In order to develop the local organic market, a complex measures must be adopted.

Key words: *Distribution channels, organic agriculture, organic market, Serbia*.

Introduction

The year 2017 was a record year for global organic agriculture: 69.8 million hectares or 1.4% of the world's agricultural land were dedicated to organic production (including in-conversion areas); about 2.9 million farmers from 181 countries/territories were engaged in this production; 93 countries had adopted organic legislation; global sales of organic products reached 97 billion US dollars with an average per capita spending of 12.8 billion US dollars (Willer *et al*., 2019). Thanks to a shift from natural shops to mass retail sales the organic agro-food system has been transformed from a local network to a globalized system. As a result of this process that lasts more than three decades over half of organic sales is done through supermarkets (Sahota, 2016). Many mergers, acquisition, and investments were announced over the past years. Among other, the world's largest organic food retailer Whole Foods Market was bought by Amazon, who is now actively promoting organic food on its online platform (Sahota, 2019).

Despite the overall positive outlook there are a few potential threats that may hinder the future growth of the organic sector: rising number of standards, supply shortfalls, competing labels, trade agreements, and demand concentration (Sahota, 2019). Regarding demand concentration it is important to emphasize that although approximately 87% of organic producers and a quarter of organic agricultural land were in developing countries (Willer *et al*., 2019), North America and Europe comprised almost 90% of global revenues (Sahota, 2019). The absolute leader - the US - worth US $45.2 billion, while the Europe worth US $39.6 billion in 2017. The highest per capita consumption in 2017, with more than 300 US dollars were found in

Switzerland and Denmark (Willer *et al*., 2019). In the same time, in India - home to the highest number of organic producers in the world (835,000)- *per capita* spending on organic packaged food and beverages was just about 0.04 US dollars (Organic Trade Association, 2019). Similar problem can be found in many other Asian as well as African and Latin American countries. In addition, internal market of organic food in Central & Eastern (CEE) countries, including Serbia is also slowly developing. Also, there is *a lack of* reliable data on the *organic market* in Serbia. Several articles describe the general tendencies of the sector (*e.g.* Brankov *et al*., 2014; Grubor and Djokic, 2016; Lukic, 2011).
That's why the objective of the present paper is to provide an overview of organic food market in Serbia with a particular focus on the structure of producers and processors in relation to activity, and the distribution channels.

Material and Methods

The paper is based on an extended review of secondary data provided by International Federation of Organic Agriculture Movements (IFOAM) and the National association for development of organic production *Serbia Organica.*
In 2015, there were 334 organic producers and they had a certificate issued by internationally recognized organizations, as well as around 2.000 producers-cooperants whose production is subjected to group certification (Serbia Organica, 2017). With the support of the USAID Project for Competitive economy, in 2019 Serbia Organica placed under its website, unified producers and processors with data on the types of primary and processed organic products, their quantities, the standards for which they are certified, and other important information. So far, 102 producers/processors out of 334 have been registered. The sampling frame for this study included those producers registered in the database. After the introduction, the paper gives an overview of organic agriculture in Serbia. In the next part of the paper structural characteristics of production units are described. Finally, organic food distribution channels are described. For the purpose of the analysis, the distribution channels of organic food in Serbia have been classified into: (1) *direct*: on-farm sales, farmers markets and door-to-door-sales*;* (2) *indirect:* specialized shops and retail chains. Furthermore, some suggestions for further development of the organic food market in Serbia are highlighted.

Results and Discussion

Organic agriculture in Serbia

Organic production in Serbia concerning the area of utilized land significantly increased in the period 2012-2015: from 6.340 ha to 15.298 ha. Total utilized land under organic production includes areas in conversion and those with organic status as well as meadows and pastures. However, the share of organic production in overall arable land remains extremely law - just 0,44% in overall arable land (Serbia Organica, 2017). A significant increase is noted also in number of heads of animals included in this production. But, in the context of the total number of heads of animals, participation of organic production is very infirm. For example, the share of organic sheep is accounted for 0.27% of the entire population.
Consequently, domestic market of organic products is still underdeveloped despite significant positive changes in the recent years. The major cities in Serbia – Belgrade and Novi Sad – are the major markets for organic food. It is quite expected and correlated with purchasing power of the citizens. Generally speaking, organic market in Serbia is still immature: first specialized market of organic products started to work in mid-2011 in New Belgrade; the first organic meat appeared in 2015; promotion of organic products has been intensified in the past years, but national logo of organic products is still not enough promoted (Serbia Organica, 2017). Regardless, there are some improvements: imported organic products are more present on the market, especially processed fruits with lower prices than domestic one; online shopping

becomes available; retail chains showed interest and demanded bigger quantities of organic food (Serbia Organica, 2017). Unfortunately, domestic producers are not capable to provide continuity and quantity to increase supply and organic products are not enough visible on domestic market.

Structural characteristics of production units

According to their status, the majority of farms are organic (n=84), while 18 of them are in conversion. Organic food producers from almost all Districts in Serbia, except Sumadijski and Borski District were included in the research. The number of organic food producers who participated in research is as follows: Grad Beograd (n=14), Severno-Backi (n=8), Srednje-Banatski (n=6), Severno-Banatski (n=2), Juzno-Banatski (n=5), Zapadno-Backi (n=2), Juzno-Backi (n=13), Sremski (n=7), Macvanski (n=1), Podunavski (n=1), Kolubarski (n=3), Branicevski (n=2), Pomoravski (n=1), Zajecarski (n=2), Zlatiborski (n=11), Moravicki (n=3), Raski (n=1), Rasinski (n=8), Nisavski (n=5), Toplicki (n=1), Pirotski (n=4), Jablanicki (n=1), and Pcinjski (n=1). According to the type of the registered subject[5], a majority of the producers (n=96, or 94.1% out of 102 who are registered in the Serbia Organica database) are registered as producers- family farm, trade or limited liability company, sole proprietorship and cooperative. As mixed units for production and processing, 4.9% producers are registered, while 5.9% producers are a registered processors. Majority of producers have up to 5 ha of agricultural land (60.5%), and 12.7% of these less than 1 ha. These farms are followed by those managing 2 to 5 ha (26.5%). Larger farms are few; those exceeding 50 or 100 ha are only 11, 4 of which account from 100 to 200 ha and three exceeding 2,000 ha. 38.7% of producers are controlled and certified by Organic Control System, 32.1% by Ecocert Balkan, 14.1% by TM CEE, 10.4% by Centar za ispitivanje namirnica and 4.7% by Ecovivendi. Serbian National Organic logo was awarded to 91.2% of all producers, EU logo to 50% of all producers, and BioSuisse logo to 2.9% of producers.

Fruit growing is the most dominant activity in which 52.9% of producers are engaged, whereas the least represented activities are mushroom growing and bee keeping. However, not all producers engage in only one activity, e. g. besides fruit growing they also grow vegetables. Table 1shows the structure of producers in relation to the activity.

Table 1. Structure of producers and processors in relation to activity

Activity	n	%
Producers		
crop production	23	22.5
vegetables growing	27	26.5
fruit growing	54	52.9
wine growing	7	6.8
medicinal, aromatic and spicy herbs – growing and wild collection	15	14.7
mushroom growing and wild collection	2	2.0
livestock breeding	5	4.9
bee keeping	1	1.0
Processors		
forest products	7	6.9
plant products	3	2.9
animal products	2	1.6

Source: Authors' calculation based on Serbia Organica's database

[5]A– registered producer; family farm, trade, limited liability company; sole proprietorship, cooperative; B – other type of registered units; C - registered processor; ABC – mixed unit - production/processing.

Organic food is exported by 24.5% of producers registered in Serbia Organica's database and mainly to the following countries: the US, Switzerland, and the EU countries.

Distribution channels of organic food produced by domestic producers

According to Renko (2010) retail is an important distribution channel for organic food market supply in Serbia and the *objective of the research was to check the hypothesis that retail is a significant distribution channel for domestic producers.*The structure and frequency of organic food sales are shown in Table 2.

Table 2.Th**e** structure and frequency of organic food sales through intermediaries

Organic food retail	n	%
specialized shops	6	5.9
retail chains (supermarkets, hypermarkets)	7	6.9
retail chains (supermarkets, hypermarkets) + specialized shops	6	5.9
total	19	**18.6**
Direct distribution channels		
on-farm sales	2	2.0
farmers markets	2	2.0
door-to-door-sales + farmers markets	5	4.9
total	9	**8.8**
Direct sales and sales through intermediaries	4	**3.9**

Source: Authors' calculation based on Serbia Organica's database

As could be seen from the Table 2, organic retail sales is practiced by 18.6% of producers, while direct sales e.g. on-farms sales, on green markets or door-to-door sales is practiced by less than 9% of the individual farmers. In addition, 3.9% of producers use both distribution channels (direct and through intermediaries). Most probably, the rest (68.7%) of producers sold their products to wholesalers and to processing companies. This is in line with finding that "the mark up in price they obtain for their organic produce is very moderate (with 10-20% on the average) and confirms that added value is not generated on the farm level" (Serbia Organica, 2017).

Considering that majority of organic producers are small producers (have up to 5 ha of agricultural land) *the second objective of the research was to determine whether small producers are in the position to reach certain distribution channels.* In order to examine the statistically significant correlation between distribution channels and the size of the area under organic production, chi-square ($\chi 2$) test was calculated (Table 3). The obtained results ($\chi 2$=23.870; $p>0.05$) show that there is no statistically significant correlation between distribution channels and the area of agricultural land under organic production.

Table 3. Cross tabulation of utilized land (in ha) under organic production and the distribution channels in Serbia

Area under organic production		Distribution channels through which the organic food is marketed			
		direct sales	sales through intermediaries (wholesale and/or retail)	direct sales and sales through intermediaries	Total
less than 5 ha	n; % of total	8; 25.0%	8; 25%	2; 6.2%	18; 56.2%
5 to 10 ha	n; % of total	0; 0.0%	5; 15.6%	0; 0.0%	5; 15.6%
10 to 20 ha	n; % of total	0; 0.0%	0; 0.0%	2; 6.2%	2; 6.2%
20 to 50 ha	n;	1;	1;	0;	2;

	% of total	3.1%	3.1%	0.0%	6.2%
50 to 100 ha	n; % of total	0; 0.0%	2; 6.2%	0; 0.0%	2; 6.2%
more than 100 ha	n; % of total	0; 0.0%	3; 9.4%	0; 0.0%	3; 9.4%
Total	n; % of total	9; 28.1%	19; 59.4%	4; 12.5%	32; 100%

Source: Authors' calculation based on Serbia Organica's database

$\chi 2$=23.870; p>0.05
Source*: Authors' research*

Producers operating in *less* than 5 *hectares use both distribution channels equally. The products are sold directly to consumers (n=8) and through intermediaries (n=8). 2 of small-scale producers claim they have sold their products through both channels. Producers* with *20-50ha also use both distribution channels equally, while producers with 5 to 10 ha, and more than 50 ha sales through intermediaries.* This finding was confirmed by the chi-square test, which showed that there was no statistically significant correlation between small and big producers regarding the distribution channels.

Conclusions

This paper provides a short overview of organic production and marketing in Serbia. It investigates distribution channels of organic food by domestic producers based on the analyses of the available literature and presents the results of the research conducted on the Serbia Organica's sample of domestic organic food producers. Research results indicate that the majority of farms are small (60.5%), with fruit growing being the most prevalent activity (52.9%). The aim of the research was to assess the role of retail chains in organic food distribution and to determine whether small producers distribute their organic food exclusively through direct channels of distribution. The importance of retail sales cannot be denied since 18.6% of producers sold their products through this distribution channel. However, majority of producers sold their products to wholesalers and to processing companies. This is one of the most important reasons for the "invisibility" of organic products on the market. Considering that a statistically significant correlation between distribution channels and the utilized land for organic production has not been determined, it can be concluded that the size of farm is not the main obstacle for further development of the organic food market. For further development of the local organic market, a wide range of *measures must be adopted.* A stable *government support* is crucial for boosting growth and improving the sector of *organic* agriculture. *In addition, it is necessary to* improve the political and economic framework in the country; to facilitate access to finance and or operation of special credit lines, to implement strong and intensive campaigns with clear communication strategy etc.

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AGRICULTURAL UTILISATION OF BIOWASTES AND BIODEGRADABLE WASTES

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Abstract

Due to rapid urbanisation and an increasing global population huge amounts of diverse wastes are produced. In this group a particularly important role is played by biowastes and biodegradable wastes. Presently in most European countries landfill disposal of these substances is banned, which makes them a potentially very attractive alternative for organic agriculture, after appropriate preparation such as stabilisation and/or composting. It is well known that the chemical composition of individual waste groups varies and consequently it will influence their agricultural quality. Therefore this study is focused on the verification of the above assumption. For this purpose 3 different composts (prepared from sewage sludge, garden and park wastes and a mixture of kitchen and garden wastes) and 2 municipal sewage sludge samples originating from one small and one bigger municipal wastewater treatment plant were evaluated in terms of their chemical properties (contents of macro- and micronutrients, the quality and quantity of humic compounds). Additionally, phytotoxicity of the composts and sewage sludges was analysed using phytotests. The study clearly demonstrated that the sewage sludges, independently of their origin, were more abundant in most macronutrients (e.g. C, N, P, S, Mg and Na) and micronutrients (Fe, Zn, Cu, Ni) in comparison to composts. At the same time it should be underlined that regardless of compost compositions they were richer in organic matter and Ca, K and Mn contents. Moreover, the composts were characterised by more favourable properties of humic substances and their influence on plants manifested in phytotests was more advantageous than that of the sewage sludges. The obtained showed both the analysed composts and sewage sludge to be promising organic substances as potential substitutes of mineral fertilisers according to the principles of sustainable agriculture, which becomes the predominant trend in agronomy.

Keywords: *Compost, Biowaste, Sewage sludge, Nutrients, Humic compounds.*

Introduction

Modern farming practices, such as intensive cropping, tillage and removal of crop residues, contribute to the depletion of soil organic matter reserves and low nutrient availability in arable land. At the same time the decline of soil organic matter has been identified as one of the most important threats to soil quality, as underlined in the document named Thematic Strategy for Soil Protection (2006) passed by the European Commission. Thus, to enhance productivity and restore degraded soils, organic fertiliser application is often required. Moreover, nowadays application of organic amendments has been employed as a fundamental principle of sustainable agriculture. Simultaneously, in view of the decreasing population of farm animals and the related limited availability of manure and slurry, a highly promising alternative may be provided by biowastes (garden and park wastes, food and kitchen wastes from households and restaurants) and biodegradable wastes (wood, paper, cardboard, sewage sludge, forestry and agricultural residue) utilised for agricultural purposes. It is very to implement thanks to an increasing generation of organic waste as a result of rapid urbanisation at the simultaneous trend to follow circular economy principles. Among the huge group of various wastes those above-mentioned constitute a very important and significant part. Presently according to the European Council Directive (1999) as well as Polish legal regulations landfilling of these types of wastes is forbidden. At the same time, biowastes and biodegradable wastes represent a valuable resource thanks to their chemical composition. For this reason, organic fertilisation using these wastes could be an alternative method substituting inorganic fertilisers, improving general soil fertility and maintaining soil macro- and micronutrients at appropriate levels (Jakubus, 2013, Sharma *et al.*, 2017). Obviously, first of all the applied wastes must be properly prepared and stabilised to meet criteria of environmental safety imposed by legal regulations. Composting is one of the methods of biowaste and biodegradable waste preparation. This process has been presented as an environmentally friendly alternative applied to manage and recycle organic waste with the aim to produce products used as amendments in agriculture (Gu *et al.*, 2011, Deepesh *et al.*, 2016).

Therefore, the main aim of this study was to evaluate applicability of various organic wastes such as sewage sludge and composts in agriculture.

Material and Methods

Chemical analysis of composts and sewage sludges

The composts were commercially prepared from a mixture of green plant residues and kitchen wastes (C1), a mixture of sewage sludge, green plants and straw (C2), and a mixture of green plant residues, chopped wood and park wastes (C3). The sewage sludges (SS1 and SS2) originated from municipal wastewater treatment plants located in the Wielkopolska region, SS1 from a small, local installation and SS2 from a big sewage treatment plant, respectively. The samples of the composts and sewage sludges were collected at the end of autumn and then they were undergone to analyses.

The chemical analyses were conducted on dried samples. Total organic carbon (TOC), nitrogen (N) and sulphur (S) contents were determined using Vario Max CNS. The composts and sewage sludges were ground and ashed in a furnace at 450^oC for 6 h. The ash was dissolved in 5 mL of 6 mol· dm^3 HCl (Ostrowska *et al.*, 1991) and diluted to a constant volume with distilled water. The obtained extracts were analysed to determine the levels of macronutrients (K, Ca, Mg, Na), micronutrients (Fe, Mn, Zn, Cu, Ni) and heavy metals (Pb, Cd) using atomic absorption spectrophotometry in a Varian Spectra AA 220 FS apparatus. Total phosphorus (P) content was measured colorimetrically by the vanadium–molybdenum method. All the assays determining the amounts of individual elements in the tested samples were performed in three replications and the presented results are their mean values.

Humus fractionation was determined according to the method proposed by Kononova and Bielczikova (Dziadowiec and Gonet, 1999), in which humic substances were determined in a mixture of 0.1 mol·dm^{-3} $Na_4P_2O_7$ + 0.1 mol·dm^{-3} NaOH solution. The fulvic acid fraction (FA) was separated after precipitation of humic acids at pH 1.5 (HA). Carbon in the obtained fractions (C_{HS} and C_{FA}) was oxidised by 0.1 mol·dm^{-3} $KMnO_4$ in the H_2SO_4 medium. Humic acid carbon (C_{HA}) was calculated by subtracting C_{FA} from C_{HS}. Optical density ($Q_{4/6}$) of the obtained fractions was determined at 465 nm and 665 nm.

The germination index (GI) and the Phytotoxkit (seed germination and early growth test) were used to determine phytotoxicity of composts. The tests were performed using seeds of cress (*Lepidium sativumm* L.). The germination index (GI) was calculated according to the formula given by Miaomaio et al. (2009), while seed germination and early growth tests followed the Phytotoxkit procedure (ISO 11269-1).

Statistical analysis

Analyses of mean samples were carried out in three replicates. The obtained results were subjected to formal evaluation by the analysis of variance using the F test at the significance level $p \leq 0.95$. The least significant differences were calculated using the Tukey test at the significance level $\alpha \leq 0.05$ and then uniform groups within the factor level were established. Simple correlation coefficients were computed to show the relationship between evaluated parameters of compost maturity and stability.

Results and Discussion

Differences in the chemical composition of tested organic amendments were noticeable and statistically confirmed (Tables 1 and 2). The data in Table 1 clearly show that sewage sludges contain considerably greater amounts of N, P, S and Na than the composts. The same disparity between sewage sludge and compost was indicated by Alvarenga *et al.* (2015). The composts were characterized by higher Ca contents. The Mg amounts determined for both composts and sewage sludges were very similar, although they differed statistically. Levels of potassium, an essential nutrient for plants, were highly variable, with the highest value recorded for C3 (4.03 $g \cdot kg^{-1}$) and the lowest for C1 (0.85 $g \cdot kg^{-1}$). Simultaneously the amounts determined in C1 and SS2 were comparable, similarly as for C2 and SS1 (Table 1). Generally compost C2 prepared from sewage sludge, green plants and straw was far more abundant in N, P, Mg, S and Na, whereas C1 was very poor in all the analysed macronutrients except for Ca. Similar levels of macronutrients in composts produced from municipal waste were presented by Becher *et al.* (2018).

Table 1 The mean content of macronutrients in analysed organic amendments ($g \cdot kg^{-1}$)

Amendment	N	P	K	Ca	Mg	S	Na
C1	5.46 e	1.63 d	0.85 c	7.49 a	1.0 c	0.40 d	0.17 d
C2	14.29 c	4.24 b	2.91 b	6.24 b	1.18 ab	0.77 c	0.54 c
C3	13.22 d	2.56 c	4.03 a	7.03 a	1.15 ab	0.75 c	0.50 c
SS1	88.33 a	9.14 a	3.02 b	1.85 d	1.10 b	2.13 b	1.31 a
SS2	52.62 b	9.90 a	1.02 c	3.45 c	1.20 a	2.86 a	0.98 b

The sewage sludge from the small installation (SS1) was more abundant in N, K and Na, whereas SS2 from the big sewage treatment plant contained significantly greater amounts of Ca. Simultaneously, the discussed sewage sludge was very rich in microelements (Fe, Mn, Cu, Ni). Also this waste showed a 9-fold higher amount of Pb than SS1, while Cd amounts were comparable for these sewage sludges, ranging from 1.47 $mg \cdot kg^{-1}$ to 1.91 $mg \cdot kg^{-1}$ (Table 2). It should be underlined that neither SS1 nor SS2 exceeded the thresholds of heavy metal contents indicated in the Polish regulations for sewage sludge recommended for application as natural fertilisers.

Table 2 The mean content of metals in analysed organic amendments ($mg \cdot kg^{-1}$)

Amendment	Fe	Mn	Zn	Cu	Ni	Pb	Cd
C1	3801.5 c	175.3b	41.5 d	24.8 d a	2.5 c	14.3 c	0.07 d
C2	5059.1 b	196.4a	308.0 b	160.7 c	21.6 b	19.2 c	0.31 c
C3	3760.1 c	198.3a	243.6 c	22.1 d	3.4 c	64.1 b	0.08 d
SS1	998.2 d	134.6c	312.8 b	375.3 b	7.4 b	10.8 cd	1.91 a
SS2	16886.6 a	176.6b	430.6 a	421.2 a	54.4 a	101.3 a	1.47 b

The sewage sludge originating from the big wastewater treatment plant was characterised by higher contents of metals than sewage sludge from the small treatment plant. Comparing obtained data with literature sources (Turek *et al.*, 2019) it may be noted that amounts of Cu, Ni and Pb were higher, while those for Zn and Cd were lower. Similarly as it was presented above, among the tested composts C2 was richest in all the analysed micronutrients, while levels of micronutrients determined for C1 and C3 were very comparable and significantly lower in relation to the values presented for C2 (Table 2). Generally the sewage sludges were richer in microelements than composts. Irrespective of compost mixture composition the amounts of nutrients increase as follows N> Ca> P > K > Mg > S > Na for macronutrients and Fe > Zn > Mn > Cu > Ni for micronutrients. Regardless of the size of the wastewater treatment plant the macronutrient contents in sewage sludge increased in the following order: N> P > Ca > K > S > Mg > Na, while for micronutrients it was Fe > Zn > Cu > Mn > Ni.

Taking under consideration the potential agricultural utilisation of both sewage sludges and composts, the organic matter and organic carbon contents are especially important. The data given in Figure 1 show that composts, particularly C1 and C2, are highly abundant in organic matter, which amount ranged from 920 to 940 $g \cdot kg^{-1}$. Organic carbon contents in composts were determined between 256.2 $g \cdot kg^{-1}$ to 424.2 $g \cdot kg^{-1}$ at the percentage share in organic matter of 33 – 46%. In turn, the sewage sludges were characterized by huge organic carbon contents ranging from 540 $g \cdot kg^{-1}$ to 780 $g \cdot kg^{-1}$ and relatively smaller amounts of organic matter (653 $g \cdot kg^{-1}$ and 780 $g \cdot kg^{-1}$) in relation to the data shown for the composts (Figure 1). Regardless of the above, the percentage share of organic carbon in organic matter was 82%. One of the most commonly used methods of stabilising sludge consists in the addition of lime. Calcium oxide promotes both immobilisation of metals and reduction of organic matter content, so that explains its small amounts and it is consistent with literature data (Turek *et al.*, 2019).

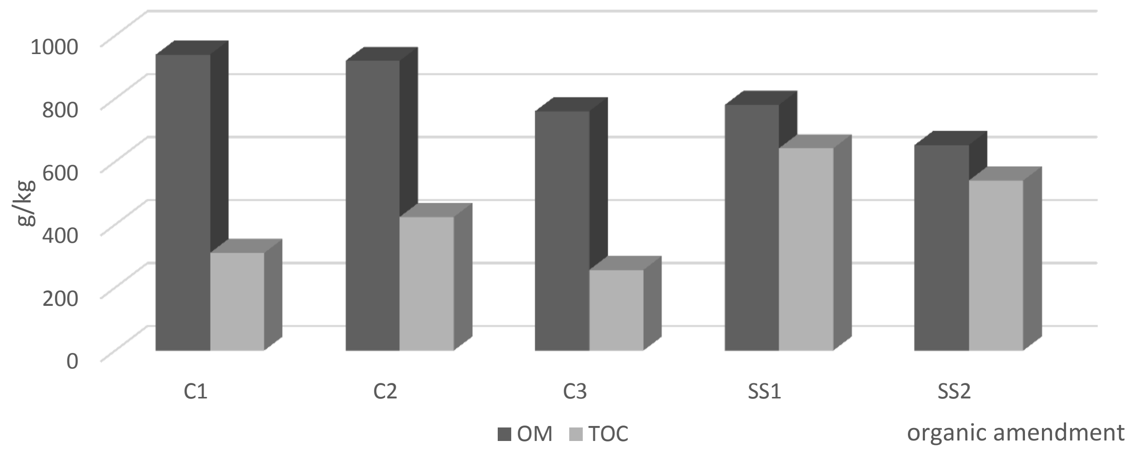

Fig. 1 The organic matter and organic carbon amounts in analysed organic amendments

Contents of nitrogen, organic carbon and organic matter determined for compost prepared from sewage sludge are very similar to those cited by Jakubus (2013).

Not only the quantity, but also quality of organic matter is very important, especially when we consider soil fertility improvement in the term of increasing soil humus levels. The sewage sludges contained significantly bigger amounts of humic substances (59 – 120 $g \cdot kg^{-1}$) in relation to composts (30.8 – 48.6 $g \cdot kg^{-1}$) (Figure 2). Regardless of the above, the quality of sewage sludge humic substances was lower because they were mainly composed of the fulvic acid fraction (45 – 90 $g \cdot kg^{-1}$). In contrast, the compost humic substances consisted primarily of humic acids (24 – 42 $g \cdot kg^{-1}$), i.e. more valuable and more polymerised compounds. These differences were also reflected in the values of $Q_{4/6}$ presented in Table 3. The values of optical density for the mixture of FA and HA as well as for FA were significantly higher in relation to the data obtained for the composts. The lower values of $Q_{4/6}$ indicate greater maturity of humic substances, their more advanced humification stage and aromatisation, which is more desirable. The noticeable differences in the composition of humic substances between sewage sludges and composts are related to the composting process, which accelerated the humification transformation of organic compounds into humic acids. This phenomenon is well documented in literature (Li *et al.*, 2017, Becher *et al.*, 2018).

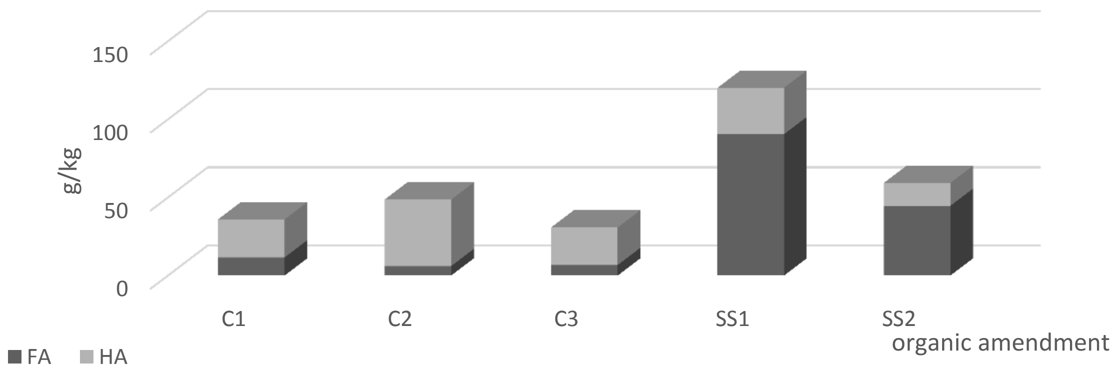

Fig. 2 The content of organic carbon of humic acid (HA) and fulvic acid (FA) in analysed organic amendments

Table 3 The values of $Q_{4/6}$ obtained for humic substances of analysed organic amendments

Amendment	Mixture of FA and HA	FA
C1	5.62	4.34
C2	6.06	5.27
C3	6.15	2.69
SS1	17.28	8.34
SS2	13.88	5.08

Application of the composts or sewage sludges not only influences soil properties, but also vigor of seed germination, therefore analysis of such an effect is fundamental. The organic amendments analysed in this study were subjected to this type of assessment using the obligatory biotests such as the germination index and the phytotoxkit and the final results are presented in Table 4. The application of bioassays is considered a sophisticated method to evaluate environmental threats, supplementing chemical methods. Generally the best conditions for germination of cress seeds were observed in the case of C2 and SS1, because the values of GI were the highest (78.2% for C2 and 87.1% for SS1). According to Jakubus and Bakinowska (2018), the range of GIs between 80 and 160% reliably indicates a low phytotoxic effect of the tested organic amendments. Moreover, inhibition of both seed germination and root elongation was the lowest in the case of C2 and SS1, amounting to 3.6 % and 0%, respectively (for seed germination inhibition) and 22.1 % and 9.3 % (for root elongation inhibition) (Table 4).

Table 4 The values of GI, inhibition of seed germination and root elongation (%)

Amendment	GI	inhibition of seed germination	inhibition of root elongation
C1	68.2	25	36.6
C2	78.2	3.6	-22.1
C3	64.4	28.6	51.2
SS1	87.1	0	9.3
SS2	70.2	14.3	30.2

Conclusions

The presented study confirmed that sewage sludge is a highly valuable organic amendment, which can serve as a source of both organic matter and nutrients essential for plants. Additionally, various composts apart from nutrients provide significant amounts of quality organic matter. Generally it should be underlined that regardless of the organic amendments tested in this study, their chemical composition shows considerable abundance in macro- and micronutrients as well as organic matter exceeding the typical composition of manure. Thanks to such favourable properties both composts and sewage sludges fit perfectly into the concept of circular economy and provide a promising alternative to commercial fertilisers in modern agriculture.

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THE USE OF FUNGI OF THE GENUS TRICHODERMA TO REDUCE THE INFECTIOUS BACKGROUND OF THE SOIL

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Abstract

Fungi of the genus *Trichoderma* and preparations based on them have long been used as effective antagonists to suppress a wide spectrum of phytopathogenic microflora and to induce soil improvement. The effect of seven strains of fungus of the genus *Trichoderma*, isolated from the soils of different climate zones, were studied for 1) their ability to inhibit the growth and activity of pathogens, 2) their effect on the biodiversity of the soil microbiota and the increase in associative nitrogen fixation, and 3) their stimulation of growth and development of *Brassica oleracea*. It has been shown that *Trichoderma* strains isolated from the soil of agrophytocenoses, where organic fertilizers have been used for a long time, have the greatest impact in suppressing the growth and development of pathogenic microflora. These strains differ from those that are isolated from badlands due to a steady nitrogen-fixing effect and the high destruction rate of plant residues. They are characterized by a low growth rate and insignificant sporulation when compared to other strains of the same kind. However, a positive impact has been noted in the increase of the number of non-pathogenic soil microflora, and the approximation of this indicator on fertile soils. This would suggest that the search and selection of products for the development of biological preparation, potentially effective for organic farming and bio- vegetable production, should be carried out in resistant agrophytocenosis soils, as they accumulate a genetic memory for maintaining the sustainability of the soil ecosystem and for the conservation of biodiversity.

Keywords: *Trichoderma, soil fertility, organic agriculture*

Introduction

Preparations based on the fungi of the genus *Trichoderma* are known as effective biofungicides and are used to reduce the infection of vegetable crops (Gromovs *et al*., 2010; Sadykova *et al*., 2007; Sternshis *et al*., 2007). Currently, the choice of biological products that can replace toxic chemical fungicides is quite extensive, their potential is not limited to suppressing the growth of phytopathogenic fungi and bacteria. The use of *Trichoderma*-based biologics for improving fertility and natural nitrogen fixation of soils is known (Inuwa, 2013; Triveni, 2012; Sternshis *et al*., 2007).

We have created our own collection of potential biofungicides, including representatives of the genus *Trichoderma*, isolated from the soil of agrocenoses of different natural zones of Russia, and we assessed the activity of strains in relation to the most common pathogens of diseases of vegetable crops found in the Nonchernozem zone of Russia. These include numerous *Fusarium*, bacteriosis , *Alternaria*, *Cladosporium.*

Material and Methods

Collection strains of fungi of the genus *Trichoderma*:

The *Trichoderma* TS, TT, K-15, AV strains were isolated from the soils of the experimental field of the FSBSI FSVC (Nonchernozem Zone of Russia, Moscow Region);

Strains 9B and 19B were isolated from soils of many years of experience using organic fertilizers in Western Siberia (West Siberian Station, Barnaul);

Strain U-9 isolated from natural soils of the north of the Komi Republic (Usinsk district).
Collectible strains of pathogenic fungi of the genus *Fuzarium*, provided by the Laboratory of Immunity and Plant Protection of the FSBSI FSVC, were isolated from the surface of diseased carrot and tomato plants. In addition, strains of *Fuzarium, Alternaria, Cladosporium* genera and *Xanthomonas* genus bacteria isolated from the soil after the cultivation of tomato and cabbage were used. All pathogenic strains were highly aggressive towards vegetable crops.
Traditionally, chemical fungicides, such as Ridomil, have been used to reduce disease. Ridomil is known as a potent fungicide against fungal diseases, manufacturer Syngenta Crop Protection, Switzerland. The active ingredients of the preparation dithiocarbamate and phenylamide mefenoxam. The preparation is toxic to humans, warm-blooded animals, insects, including bees, can accumulate in the soil and vegetables (https://udobreniya.info). In experiments, we considered the use of strains based on fungi of the genus *Trichoderma* as a possible alternative to chemical methods of plant protection.
The strains of *Trichoderma* and pathogens of fungal diseases were grown on the Czapek agar medium; for experiments with vegetable cultures, the biomass of fungi was produced under conditions of intensive aeration on a sterile grain medium containing water (1000 ml); grain of wheat or oats (15 g); $NaNO_3$ (3 g); K_2SO_4 (1 g); K_2HPO_4 (1.5 g). After 5 days of production, the preparations were left at rest for 7 days, then they were filtered through sterile gauze and soil was infused at the rate of 10 ml of preparations per 100 g of soil. Concentrate preparations based on fungi of the genus *Trichoderma* contained spores in the amount of 250-350 million / ml. This concentrate was diluted 100 times before the experiment.
Bacteria of the genus *Xanthomonas*, causing vascular bacteriosis on cabbage cultures were grown on the liquid medium of Czapek. The titer of the preparation after 3-4 days under the conditions of liquid phase fermentation was 1.0-1.5 billion cells / ml. For the experiments, the concentrate of the preparation was diluted 100 times and inoculated the soil at the rate of 10 ml per 100 g of soil. As a test culture was taken the *Brassica oleracea* variety "Severyanka" collection of the FSBSI FSVC. As a test soil was taken soil for growing seedlings "Agrobalt" on the basis of peat. pH (H_2O) – 5.5-6.6; pH (KCl) – 5.0-6.2; N 180 mg/l; P 210 mg/l; K – 360 mg/l; Mg – 40 mg/l; Ca – 120-160 mg/l.

Results and Discussion

The evaluation of the fungicidal potential of the studied *Trichoderma* strains was carried out in experiment with pure pathogen cultures. Express experiment was carried out in Petri dishes. The seeding of the tested *Trichoderma* strains was carried out on the edges of the cup on the Czapek agar medium, fixing the spores of fungi from a tube with a pure culture at the tip of the dissecting needle. Pathogens were sown in the center of the cup with a piece of agar medium from a tube with a pure culture of the pathogen. As the fungi grew, a description was made of the intensity of the growth of pathogens depending on the presence of different *Trichoderma* strains. The results of the observations are given in table 1. Against the tested *Fusarium* strains, the most effective were *Trichoderma* TT, K-15, 9B and 19B strains.

Table 1. Effect of different strains of *Trichoderma* on the growth and development of pathogenic microflora.

	The growth pattern of pathogens in the presence of *Trichoderma* strains					
Pathogen strain	Tr TC	Tr TT	Tr K-15	Tr 9B	Tr 19B	Tr У-6
Fusarium 1M	±	±	±	±	±	±
Fusarium 2M	-	+	+	+	+	±
Fusarium 3M	+	±	±	±	±	±
Fusarium 4M	±	±	±	+	+	+
Fusarium 5M	-	±	±	±	±	±
Fusarium 6M	-	±	±	±	±	±
Fusarium 7M	+	+	+	++	++	+
Fusarium 8M	±	+	±	±	±	±
Fusarium 9M	±	±	±	+	+	±
Fusarium K6-2	++	+	++	++	+	+
Alternaria K6-1	+	++	++	++	++	+
Cladosporium K15-2	+	+	+	+	++	+
Xanthomonas K10	++	+	++	±	±	+

- does not prevent the development of the pathogen
+ competes with the pathogen, a clear border is visible in the development of mycelium
+ inhibits pathogen growth
++ destroys the pathogen

Fusarium differ in significant diversity and are capable of infecting almost all vegetable crops. In this regard, it is necessary to evaluate the potential of biofungicides for further development of biological preparation based on them, taking into account the characteristics of each specific disease and type of vegetable crops. From our experience, we selected strains 9B and 19B for the next phase of testing in vegetable crops.
All tested strains of biofungicides were effective against *Alternaria* K6-1 and *Cladosporium* K15-2, the suppression of the growth and development of *Xanthomonas* K10 was most pronounced in the presence of strains TC and K-15. This was also taken into account by us later in the development of biofungicides of directional action.
The effect of biofungicides on the reduction of the infectious background was evaluated when growing *Brassica oleracea* seedlings. *Brassica oleracea* often suffers from vascular bacteriosis caused by bacteria of the genus *Xanthomonas.* The disease can reduce the yield by 20-50%, and in adverse years (significant temperature drops, cold and rainy summer) yield losses can reach 100% (Ignatov, 2006). *Fusarium* on *Brassica oleracea* can lead to crop loss up to 25%, the risk of damage increases in hot and dry years (https://agroflora.ru/fuzarioznoe-uvyadanie-kapusty). Diseases can infect plants through the soil, so it is necessary to carry out timely treatment to prevent infections.
The growing season for growing *Brassica oleracea* seedlings was carried out in stationary conditions, the soil was infected with inocula of pathogens *Fusarium* 5M and *Fusarium* 9M,

Alternaria K6-1, *Cladosporium* K15-2, *Xanthomonas* K10. The control was non-inoculated soil. The treatment with biofungicides from *Trichoderma* 9B and 19B strains was carried out in two ways: soaking *Brassica oleracea* seeds for 1 hour in a working solution of biofungicide and tilling the soil before planting seeds. The character of seed germination, the intensity of plant development and the quality of seedlings were tracked. The duration of the experience - 1 month. The biometric characteristics of seedlings at the end of the experiment are shown in Table 2.

Table 2. Biometric characteristics of seedlings a month after planting seeds into the soil.

Variant	Pathogen	Seed germination on the fifth day, %	Plant height, cm	Sheet width, cm	The average weight of the plant, g
control	-	66.7	13.2	3.07	1.12
Treatment with no biofungicides					
1- I	*Fusarium* 5M	46.7	12.5	2.56	0.76
1- II	*Fusarium* 9M	50.0	11.2	2.45	0.98
1-III	*Alternaria* K6-1	41.7	12	2.35	0.99
1- IV	*Cladosporium* K15-2	56.7	9.12	2.14	0.7
1-V	*Xanthomonas* K10	33.3	10.3	3.1	0.95
Soil treatment with biofungicide 19B					
1- I Г1	*Fusarium* 5M	66.7	15.8	3.2	1.22
1- II Г1	*Fusarium* 9M	58.3	13.7	2.83	1.09
1-III Г1	*Alternaria* K6-1	58.3	13.7	3.17	1.23
1- IV Г1	*Cladosporium* K15-2	60.0	14.3	3.17	1.14
1-V Г1	*Xanthomonas* K10	75.0	12.8	3	1.11
1-Г1.0	-	81.7	16.5	3.33	1.33
Soaking seeds in a solution of bifungicide 19B					
2- I Г1	*Fusarium* 5M	70.0	14.7	3	1.05
2- II Г1	*Fusarium* 9M	76.7	14.7	3.17	1.23
2-III Г1	*Alternaria* K6-1	76.7	14.2	3.1	1.22
2- IV Г1	*Cladosporium* K15-2	68.3	12.7	3.23	1.03
2-V Г1	*Xanthomonas* K10	70.0	12.5	3.33	0.98
2-Г1.0	-	81.7	16.3	3.4	1.04
Soil treatment with biofungicide 9B					
1- I Г2	*Fusarium* 5M	80.0	14.8	4	1.05
1- II Г2	*Fusarium* 9M	60.0	15.2	3.17	1.11
1-III Г2	*Alternaria* K6-1	91.7	14	2.83	1.12
1- IV Г2	*Cladosporium* K15-2	68.3	16.3	3.2	1.22
1-V Г2	*Xanthomonas* K10	68.3	13.3	3.57	1.17

1- Г2.0	-	80.0	15	3.07	1.24
Soaking seeds in a solution of bifungicide 9B					
2- I Г2	*Fusarium* 5M	73.3	16.3	3.6	1.29
2- II Г2	*Fusarium* 9M	68.3	15	3.17	1.00
2-III Г2	*Alternaria* K6-1	81.7	14.3	3.27	1.47
2- IV Г2	*Cladosporium* K15-2	93.3	16	3.67	1.90
2-V Г2	*Xanthomonas* K10	80.0	14.5	3.07	1.28
2- Г2.0	-	86.7	14.5	3.17	1.33

As experience has shown, the tested pathogenic microorganisms reduce seed germination and seedling survival, slow down the growth and development of plants, and are capable of causing mutations in the form of leaf deformation. Ultimately, the seedlings become weak and poor quality. Seed and soil treatment with biofungicides enhances germination energy and increases survival in the presence of various pathogens. Of the strains tested, biofungicide 9B was the best. The seedlings prepared during the treatment of seeds and soil with this biofungicide were distinguished by the highest biomass and survival. Seed treatment had a slightly better effect than tillage.

The effect of fungicides on the biodiversity of soil microflora is the most important criterion for the safety and preservation of the natural balance of agrobiocenoses. The balance of soil microbiological processes reflects the resistance of the ecosystem to adverse factors and will largely determine the immune potential of vegetable crops (MSU, 1989). It can be disturbed by changing substrate conditions, such as acidity, organic matter content, excess or deficiency of basic nutrients, fluctuations of humidity and temperature, as well as the introduction of agrochemicals, pesticides and fungicides. In technogenic soils, the balance is disturbed, and the ecosystem seeks to eliminate the cause of the disturbance and mobilizes the microbial potential. The microbiocenosis is rebuilt in a short time, the ratio of the number of main groups of soil microorganisms changes (MSU, 1989). The introduction of biofungicides into the soil can also disrupt the microbial balance of the soil and lead to the dominance of invasive species, which is highly undesirable. We evaluated the soil microbial state after treatment with the studied *Trichoderma* strains and established the following.

In the presence of all the studied strains in the soil, the total number of pathogenic fungi of the genus *Fusarium* decreased. At the same time, a month after tillage with biofungicides 9B and 19B, representatives of the genus *Fusarium* were found in the soil in insignificant amounts. These strains did not affect the overall microbial pattern of the soil, the ratio of the number of bacteria and micromycetes determined on the Czapek medium and bacteria on the MPA was close to the soil without biofungicide treatment. At the same time, an increase in the number of bacteria was observed on the Ashby nitrogen-free medium. The studied strains also grew on the Ashby medium, which indicates that they belong to the group of nitrogen fixers, while they did not inhibit the growth of nitrogen fixation bacteria. *Trichoderma* 9B and 19B strains were isolated from soil experience with long-term use of organic fertilizers and, obviously, genetic information on the sustainable functioning of the ecosystem and the preservation of biodiversity was accumulated in the microbiocenoses of such soils. These strains can be considered as significant for reducing the infectious background and as a bio-fertilizer with a high natural nitrogen-fixing potential.

The strains of Trichoderma 9B and K-15 also reduced the infectious background, but in addition they had a pronounced cellulolytic effect. They were isolated from natural soils, and, naturally adapted to the destruction of plant residues, therefore, may be promising in the development of biological products intended to increase soil fertility.

The TT and TS strains significantly suppressed the total number of soil micromycetes and significantly reduced the biodiversity of bacterial microflora. Sowing from the soil treated with these strains was distinguished by the uniformity of the colonies represented by small non-pigmented mucous forms. At the same time, on a high initial infectious background, these strains gave a significant effect of its reduction.

This suggests that the search and selection of products for the development of biological preparation of directional action and potentially effective for organic farming and biological vegetable growing should be carried out taking into account the peculiarities of the soils from which the strains are isolated, and the properties of the soils on which the biopreparations will be used in the future.

Conclusions

Biological preparations based on *Trichoderma* strains are distinguished by a high fungicidal effect and inhibit the development of *Fusarium*, vascular bacteriosis, *Alternaria*, *Cladosporium*. The use of biofungicides based on *Trichoderma* strains in the form of seed and soil treatment before planting seedlings is an effective prevention of diseases of *Brassica oleracea* and reduces the infectious background of the soil. Selection of optimal combinations of *Trichoderma* strains depends on the characteristics of the soil from which the products are isolated, and the soil for which they will be used. Biofungicides have been and remain the most important element of organic farming, because with proper selection they allow to get ecologically pure vegetable products and preserve soil fertility.

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ISOLATION, IDENTIFICATION AND PERFORMANCE OF PHOSPHATE-SOLUBILIZING BACTERIA FROM WARM-WATER FISH POND IN IRAN

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Abstract

As a critical nutrient, phosphorus plays an important role in controlling primary productivity in warm-water fishponds. Concentration of water-soluble phosphate is often very low, since a large portion of soluble phosphate in fish ponds often bonds and forms insoluble complexes with calcium (Ca^{2+}) and magnesium (Mg^{2+}) in calcareous soils and with iron (Fe^{3+}) and aluminum (Al^{3+}) in acidic soils. Application of phosphate solubilizing bacteria (PSB) is one of the most important new approaches for increasing water-soluble phosphate that are consistent with the principles of sustainable aquaculture. For this purpose, sediment sampling from 30 warm-water fishponds in Mazandaran province was carried out. A total of 64 PSB strains were isolated using National Botanical Research Institute medium (NBRIP) that contained tricalcium phosphate (TCP). In this study, 11 stronger PSBs, identified by 16s rRNA gene sequence, showed good ability in solubilizing TCP, including *Acinetobacter lactucae* (Persian$_{11}$), *Pseudomonas frederiksbergensis* (Persian$_7$, Persian$_8$ and Persian$_9$), *Pseudomonas deceptionensis* (Persian$_9$), *Pseudomonas kilonensis* (Persian$_6$), *Pseudomonas putida* (Persian$_3$), *Pseudomonas taiwanensis* (Persian$_1$ and Persian$_2$) and *Pseudomonas umsongensis* (Persian$_4$ and Persian$_5$). Based on the ability to dissolve TCP in liquid medium as well as growth in the range of environmental parameters of warm-water fishponds (temperature, pH and salinity), the Persian$_{10}$ was selected as the strongest PSB strain, with an ability to release 80.52 mg/l of phosphorus. According to the results of this study, the Persian$_{10}$ strain can be used as an effective bio-fertilizer in warm-water fishponds.

Keywords: *Phosphate solubilizing Bacteria, Bio-fertilizer, Fishpond, Sustainable aquaculture, Sediment*

Introduction

Phosphorus (P) is an essential macronutrient for phytoplankton that plays a pivotal role in biological productivity of earthen ponds for freshwater fish culture, especially filter-feeding carp species. Organic and chemical fertilizers are commonly used in fish farms to increase the level of phosphorus in the water. However, phosphorus tends to bind with bivalent cations such as calcium, iron, and aluminum forming insoluble complexes in sediment (Mahanty *et al*., 2017). It also remains bound as a part of organic matter, remains biologically non-available and may even be refractory. It is estimated that in freshwater sediment about 8–82% of total P remains mineral-bound, only part of which is easily exchangeable and biologically available in short- and long-term (Maitra *et al*., 2015). Jana (2007) also reported that about 10% of the fertilizer applied caused phosphorus increase in the water phase, whereas the rest is rapidly precipitated and settled at the bottom and converted into insoluble compounds. Phosphorus deficiency is also more critical in fishponds due to the alkaline conditions and the effects of liming. Under such conditions, phosphatic fertilizers are extensively used to augment fish production in most of the ponds (Sahu and Jana, 2000). These chemicals adversely affect soil in terms of depletion of water holding capacity, soil fertility, trophic state, biodiversity, increased salinity, and disparity in soil nutrients (Maitra *et al*., 2015; Wang *et al*., 2016). Global assessments showed that non-renewable resources of high quality phosphates are rapidly depleted and expected to be exhausted in less than 100 years (Cooper *et al*., 2011).

Nowadays, the demand for more environmentally friendly alternatives is higher than ever. Exploitation of microbes as bio-fertilizers is considered to some extent an alternative to chemical fertilizers in agricultural sector due to their extensive potentiality in enhancing crop production (Mahanty *et al*., 2017). Increasing the bioavailability of immobilized phosphorus (P) in soil by phosphate solubilizing bacteria (PSB) is an effective strategy for sustainable agronomic use of P and for mitigating the P crisis (Maitra *et al*., 2015). PSB can increase soil available P through the release of organic acids and enzymes (Behera *et al*., 2014; Zhang *et al*., 2014). Unlike in agricultural system where PSB have been intensively studied and are commercially exploited, few works (Jana, 2007; Hu *et al*., 2010) have been conducted regarding P release potential of PSB in freshwater aquaculture systems.

The aims of this study were, therefore, to isolate and identify endemic PSB strains from fishpond, and evaluate their performance in culture media.

Material and Methods

Sediment samples were collected in aquaculture earthen ponds (30 warm-water fishponds) using a Van-Veen grab sampler in Mazandaran province, Iran. The samples were stored at 4°C in the sterile containers until further analysis. For each soil sample, several sub-samples were taken, homogenized in sterile MilliQ water containing 0.85% NaCl (wt/vol) and serially diluted. Aliquots of each dilution were spread on NBRIP (National botanical research institute's phosphate growth medium) medium containing D-glucose (10 g l^{-1}), $Ca_3(PO_4)_2$ (5 g l^{-1}), $MgCl_2.6H_2O$ (5 g l^{-1}), $MgSO_4.7H_2O$ (0.25 g l^{-1}), KCl (0.2 g $l^{-})^{1}$, $(NH_4)2SO_4$ (0.1 g l^{-1}), with 15 g agar and pH 6.8 to 7.0 (Nautiyal, 1999). The plates were incubated at 28-30°C for 7 days. Colonies were selected from the plates based on the appearance of a solubilization zone. The colonies were purified on nutrient agar for further study.

In order to screen an efficient PSB, the phosphate solubilization was tested in plate assay using NBRIP medium. Briefly, bacteria were inoculated onto solidified NBRIP medium, incubated at 28-30°C for 10 days and examined for any visible bacterial growth with surrounding clear zones. The experiment was repeated three times and results are expressed as solubilization efficiency = (solubilization diameter/colony diameter) × 100 (Nguyen *et al*. 1992).

Quantitative estimation of phosphate solubilization was performed using NBRIP liquid medium carried out (Mehta and Nautiyal, 2001). Briefly, Erlenmeyer flasks (250 ml) containing 80 ml of freshly prepared NBRIP medium containing 5g l^{-1} TCP were prepared. After 48h, broth culture grown in NBRIP medium was inoculated into Erlenmeyer flasks in triplicate. Autoclaved uninoculated medium served as control. The flasks were incubated for one week at 26-28°C in a shaking incubator at 100 rpm. The cultures were harvested by centrifugation at 10,000 rpm for 10 min. Soluble phosphate in culture supernatant was determined by the molybdenum-blue method (Murphy and Riley, 1962).

Based on culture conditions on northern Iran, the effect of environmental effect on strains were carried out in three cluster. The first group of study was examined the tolerance of strains to different temperatures (4, 18, 26, 34°C) by use of shaking incubator at 150 rpm. The second study was to evaluate tolerance to different pH (6.4, 7.4, 8.4, and 9.4) with HCl-tris and acid acetic buffer-tris and the third dealt of experiment was carried out to examine the ability of strains to growth in different salinities (0, 1.5, 3, 5, 10 ppt). In all the experiments, Erlenmeyer flasks (250 ml) containing 100 ml of the freshly prepared Nutrient broth (NB) medium were inoculated in triplicate with a 1% (v/v) 48 hours culture inoculum. Bacterial population was estimated using OD600 by spectrophotometry technique at 24h of incubation (Koch, 1970).

In this study, strains were identified by 16s rRNA gene sequence. Bacterial DNA was extracted using using CinnaPure DNA kit (SinaClon CO., LTD). Amplification of 16S rDNA was performed in a 50 microliter final volume containing 2 µl of total DNA, 1 µl of 27F primer (AGAGTTTGATCMTGGCTCAG), 1 µl of 1492R primer (5′-GGTTACCTTGTTACGACTT-3′), 4 µl of each dNTP, 5 µl of $MgCl_2$, and 1 µl of Taq DNA polymerase (Weisburg *et al*. 1991). The reaction conditions were as follows: 95°C for 1 minute (min) followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 1 min and primer extension at 72°C for 2 min; followed by a final extension at 72°C for 7 min. The reaction products were separated by running 4 µl of the PCR reaction mixture in 1.0% (w/v) agarose gel and staining the bands with simplySafe (EURx® LTD). Sequences data were aligned and similarities were analyzed using available standard sequences of GenBank database for of bacterial lineage and BLAST-in software at http://www.ncbi.nih.gov/. A phylogenetic tree was constructed by the neighbor-joining method using the software MEGA 6.00. Statistical analysis of the data was carried out using ANOVA, SPSS 13.0 software.

Results and Discussion

About 64 isolates were selected from the soil samples. However, based on solubility index in solid media, only 11 strains were able to highly solubilize actively phosphate in vitro, which showed persistence of this capability after five or more subcultures (Table 1, Fig 1). The phylogenetic tree of 11 strains showed that the strains belonged to the genus of *Pseudomonas* and *Acinetobacter* (Fig 2). The development of molecular biology makes the identification of microorganisms had an enhancement of research ideas. 16S rDNA sequencing analysis as an important research method is widely used (Weisburg *et al*. 1991). In present study, strains were identified using 16S rDNA sequencing analysis.

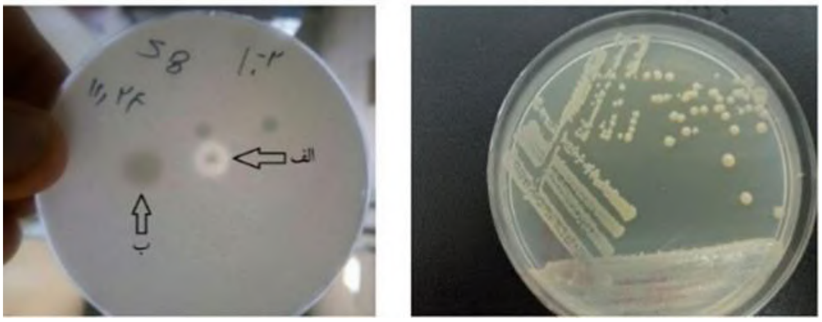

Fig 1. Purified Colony in Neutrite Agar Culture (Right Image) - Colony with Halo and without Halo (left image)

Table 1. Solubility index (halo diameter / colony diameter) and concentration of phosphate of efficient strains in NBRIP solid and liquid media

Strains	Similar species	Solubility index in solid medium	Dissolved phosphorus in liquid medium (mgl-)
Control		-	26.12 ± 0.10
Persian 1	*Pseudomonas taiwanensis*	2.10 ± 0.05	86.78 ± 0.72
Persian 2	*Pseudomonas taiwanensis*	1.86 ± 0.04	21.97 ± 0.10
Persian 3	*Pseudomonas putida*	1.64 ± 0.03	81.90 ± 1.90
Persian 4	*Pseudomonas umsongensis*	2.87 ± 0.03	22.15 ± 0.07
Persian 5	*Pseudomonas umsongensis*	3.60 ± 0.01	92.11 ± 0.42
Persian 6	*Pseudomonas kilonensis*	2.75 ± 0.02	79.26 ± 0.28
Persian 7	*Pseudomonas frederiksbergensis*	2.80 ± 0.01	21.94 ± 0.06
Persian 8	*Pseudomonas frederiksbergensis*	4.00 ± 0.01	90.27 ± 0.28
Persian 9	*Pseudomonas frederiksbergensis*	4.66 ± 0.08	25.87 ± 0.84
Persian 10	*Pseudomonas deceptionensis*	3.51 ± 0.01	80.52 ± 0.70
Persian 11	*Acinetobacter lactucae*	2.87 ± 0.05	142.20 ± 0.14

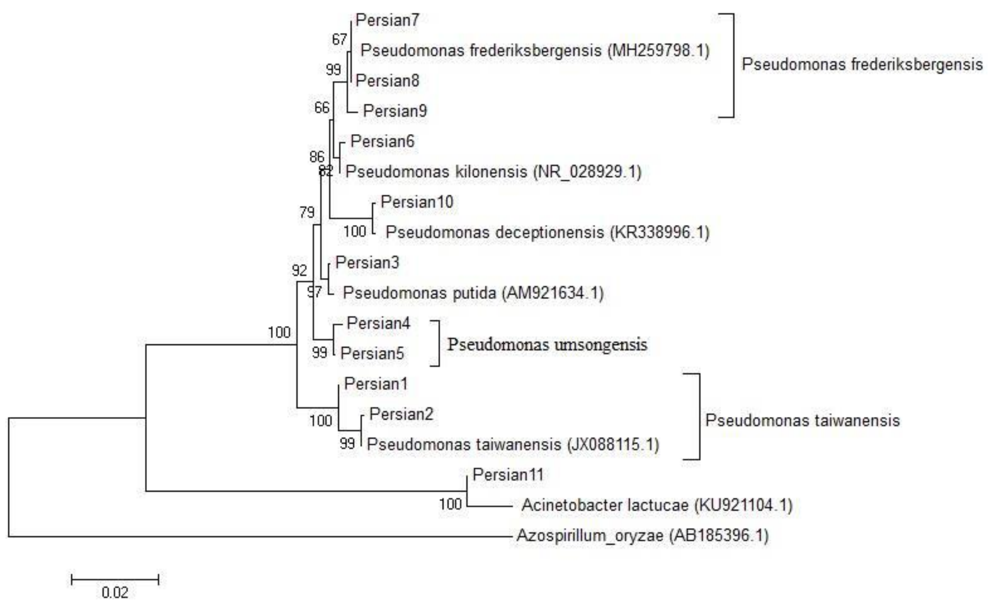

Fig 2. Phylogenetic tree based on 16s rRNA gene sequence showing the evolutionary position and relationship of strain with other bacteria

The range of phosphorus release from strains was between 20.93 and 142.20 mg l-1 in liquid medium (Table 1). It was approximately similar to the results of Maitra *et al*. (2015). Many considered that contrary to indirect measurement of phosphate solubilization by plate assay, the direct measurement of phosphate solubilization in NBRIP broth assay always resulted in reliable results (Hu *et al*., 2010).

In the temperature resistance test, growth and proliferation of Persian1, Persian 2, Persian10 and Persian11 strains were higher than others. In addition, only the Persian10 strain has the ability to grow at 4°C (Fig 3.) Results of pH effect showed that at pH 8.4, Persian3, Persian8, Persian10 and Persian11 strains had higher ability to grow and proliferate. However, at pH 4.9 only the Persian11 and Persian10 strains showed favorable growth (Fig 4.). The results of salinity effect showed that all strains were able to grow and reproduce at 0-10 ppt except

Persian 5 strain, which decreased at 10 ppt salinity (Fig 5.). Among the water quality factors, temperatures, salinity, and pH are the key ones in choosing more efficient strains, which affect the growth of bacteria. Many researchers in agriculture pay much attention to these factors (Malboobi *et al*., 2009; Hu *et al*., 2010).

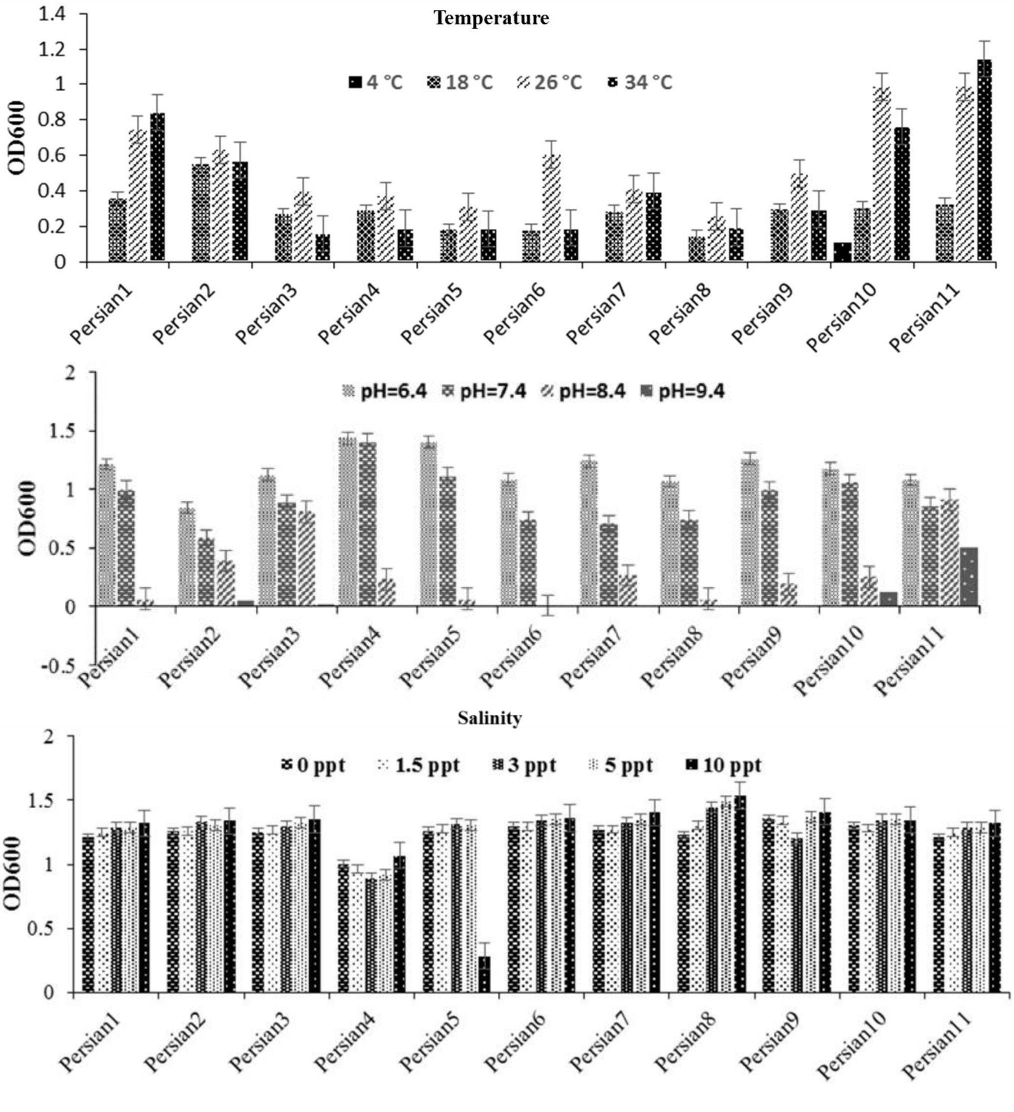

Fig. 3. Growth of strains under different temperatures, pH and salinity

Conclusions

In this study, considering all criteria (solubility, growth and proliferation ability in different environmental parameters, Persian 10 strain was selected as the most efficient strain. This strain was selected as the most efficient strain for the following reasons: Optimum phosphorus solubility, optimum growth at different temperatures especially at 4°C, optimum growth at pH above 8. Optimum growth at 4°C increases the assurance of winter survival of this strain as well as stabilization and effectiveness of bio-fertilizers in the fishpond throughout the year. In addition, Optimum growth at pH above 8 increase the probability of phosphorus release under alkaline conditions.

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ORGANIC BEEKEEPING DEVELOPMENT IN LATVIA

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Abstract

Organic farming as the more sustainable agriculture system shows considerable growth on the global and EU level, including Latvia. The paper discusses the achievements of organic beekeeping, as well as perspectives and some threats. Organic beekeeping is an important sector, which produce more than 27% from total honey production in 2017. The paper discusses achievements of organic beekeeping, as well as perspectives and some threats. The aim of the paper is twofold: 1) to assess the role of organic beekeeping (i.e., production of bee products and related services), as well as the latest development trends and issues of sector; 2) to evaluate the role and impact of CAP or RDP 2017-2020 measures on the further development of the sector. Beekeeping is an important organic sector, which produce more than 27% of honey from total national production in 2017. There is growing trend to diversify of or bee products and related services (i.e., heritage or agritourism, apitherapy etc.). Various activities, cooperation and collaboration forms support the distribution channels of organic bee products and services, and boost the added value and beekeepers' income. The main threat of further development of organic beekeeping is as follows: reduction of biodiversity (i.e., agrobiodiversity), increasing monoculture (i.e., rape) and usage of pesticides.

Keywords: *Organic beekeeping, bee products, Latvia.*

Introduction

There are many indications that pollination by a variety of species ensures optimal crop development and increased yield (Melece & Shena, 2018a). Diversified agriculture with a large number of crop types and small field sizes promote pollinator diversity, community stability and pollination success of crops and wild plants (Kovacs-Hostyanszki et al., 2017).

Intensification was aspect of agriculture modernisation, but it had the unforeseen side effect of increasing pressure on the environment. Recently biodiversity, especially in Central and Eastern European farmlands has decreased sharply, due to rapid economic transformations that are changing the agricultural landscape (Melece & Shena, 2018b). Nowadays agriculture faced with contradictory groups of requirements that could be fulfilled to provide a number of ecosystem services. So called provisioning services are aimed to supply a wide range of agricultural products as goods that requires intensifying production effectively, e.g., to increase application of mineral fertilizers, to replace less productive traditional breeds (i.e., decrease biodiversity), etc. (Melece & Shena, 2018b). However, supporting and regulating services (i.e., agro-ecosystem services) are provided interaction between humans and nature; and often are not monetary valued. One of the main regulating ecosystem service—pollination—provides direct and indirect benefits to nature and human well-being.

The economic value supplied by bees involves pollination and the production of honey and other bee products, as well as apitourism that also has great importance (EP, 2018).

It is expected that organic beekeeping will continue to grow worldwide thanks to the increasing demand for organic honey and bee products (Willer & Lernoud, 2019), particularly for medical and pharmacological purposes. The medical use of honey bee and bee products—apitherapy—such pollen, propolis, royal jelly and bee venom against disease and disorders (Ghosh & Satpute, 2016) became more important. For instance, propolis possesses a wide range of pharmacological potentials including antibacterial, antifungal, antiprotozoal,

hepatoprotective, antioxidant, anti-inflammatory, antiviral, anticancer and antitumor properties (Anjum et al., 2018). Kieliszek et al. (2018) argue that each bee product is pharmacologically active. Since the target audience include very vulnerable people or patients, also children (i.e., parental), seriously ill people (i.e., cancer, immunodeficiency), the bee products should be free of any contaminants, in particular residues of agro-chemicals (i.e., pesticides). Therefore, organic bee products is best source of medical and pharmacological preparations—drugs, dietary supplements and medicated cosmetics.
The aim of the paper is twofold: 1) to assess the role of organic beekeeping, as well as the latest development trends and issues of sector; 2) to evaluate the role and impact of CAP or RDP 2017-2020 measures on the further development of the sector.

Material and Methods

The principal materials used for the studies are as follows: different sources of literature, e.g., scholars' articles, research papers and the reports of institutions. Data have been obtained from various online databases: Eurostat, CSB, LDC – Agricultural Data Centre, and PVD – Food Veterinary Service. For comparison of organic beekeeping (i.e., beehives, honey production) development trends among neighbouring countries, the data of Baltic States were evaluated. Organic beehives' number was compared among the EU member states—Baltic Sea Region countries. The mixed methods, combining suitable qualitative and quantitative (correlation-regression analysis) research methods have been used. Significance levels are as follows ** - α<0.05; *** - α<0.01.

Results and Discussion

Comparing the organic beehives' number among the Baltic Sea countries, results show that Latvia has second highest number after Germany, but other countries has considerable less numbers (Fig. 1).

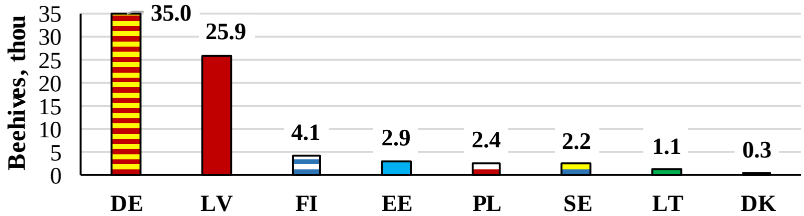

Figure 1. Number of organic beehives in the Baltic Sea countries, 2017
Source: authors' elaboration based on Willer & Lernoud, 2019

The volume of organic honey and number of organic beehives, and its trends among the Baltic States (Fig. 2 and 3) show that beehives have significantly increase in all countries, but production of honey has increased in Estonia and Latvia, but not in Lithuania.

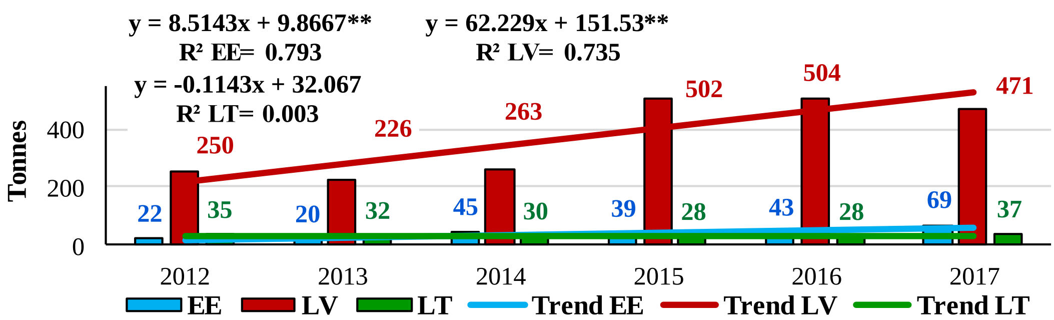

Figure 2. Organic honey production and it trends in the Baltic States, 2012-2017
Source: authors' calculation based on Eurostat (2019) data

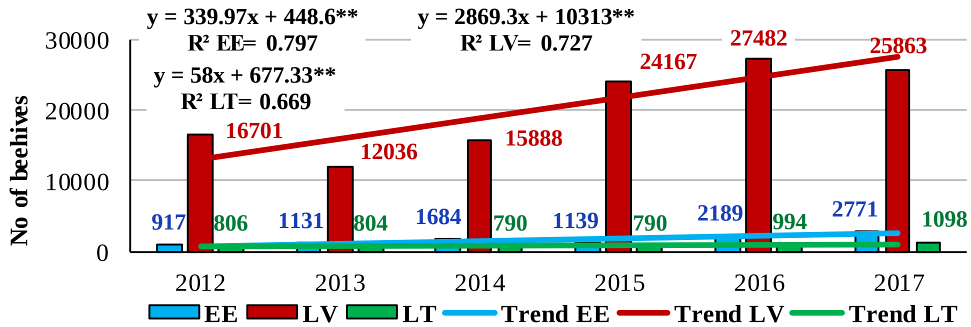

Figure 3. Number of organic beehives and it trends in the Baltic States, 2012-2017
Source: authors' calculation based on Eurostat (2019) data

In Latvia both number of conventional and organic beehives' number has significantly increased from 2012 to 2017, and the share of organic ones is 27% in 2017 (Fig. 4).

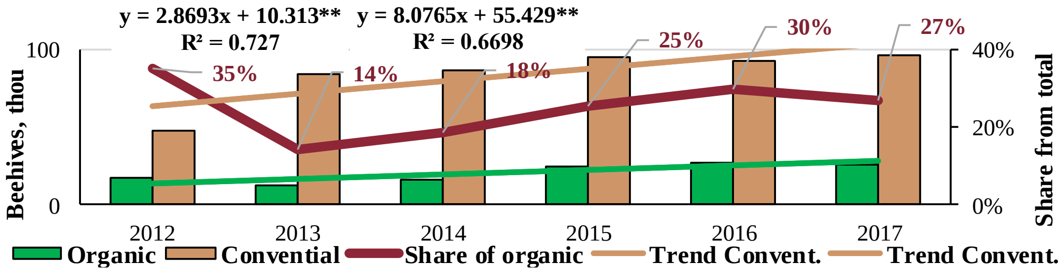

Figure 4. Organic and conventional beehives' number, and the share of organic beehives from total in Latvia, 2012-2017
Source: authors' calculation based on LDC (2019) data

Diversified agriculture with a large number of crop types and small field sizes promote pollinator (i.e., honey bee) community stability and pollination success of crops and wild plants (Kovacs-Hostyanszki et al., 2017). Controversially, in Latvia diversification of agricultural crops (esp. related to beekeeping), show decreasing tendency (Table 1).

Table 1. The changes of some crops' area from 2005 to 2017

Crop	Sown area, thou ha		Changes 2017/2005, %	
	2005	2017		
Wheat (winter & spring)	129.1	331.2	154%	↑
Rye	45.1	34.0	-25%	↓
Spring barley	145.9	78.2	-46%	↓
Oat	58.0	70.9	22%	↑
Buckwheat	10.4	30.9	**197%**	↑
Mixed cereals and pulses	8.1	5.3	**-35%**	↓
Field beans	0.4	42.5	**10525%**	↑
Winter rape	22.9	90.0	**293%**	↑
Potatoes	45.1	22.7	-50%	↓
Open field vegetables	12.9	8.0	-38%	↓
Forage crops	372.2	297.6	-20%	↓
Perennial grass	360.6	270.3	**-25%**	↓
Nectar crops	2.1	1.9	**-10%**	↓

Source: authors' calculations based on CSB (2019) data

Requier et al. (2015) argue that honey bees' demand very wide plants diversity for pollen needs over season; furthermore, the pollen resource diversity enhances resistance to fungal diseases, tolerance to pesticides, and immunity in honey bees to parasites, diseases, and pathogens. Oilseed rape, wheat and maize (for green mass) in Latvia is cultivated as energy crops in intensive agriculture, including several pesticide treatments. Crops' treatment also can spread to wild flowers around fields, and thus can cause the residues of pesticides in honey and bee products. The use of insecticides directly affect bees, but other pesticides has an indirect effect. Use of pesticides (active substances, kg) on 1 ha of several crops' sown area show that the highest doses of pesticides are used for field beans, winter wheat and rape (Fig. 5).

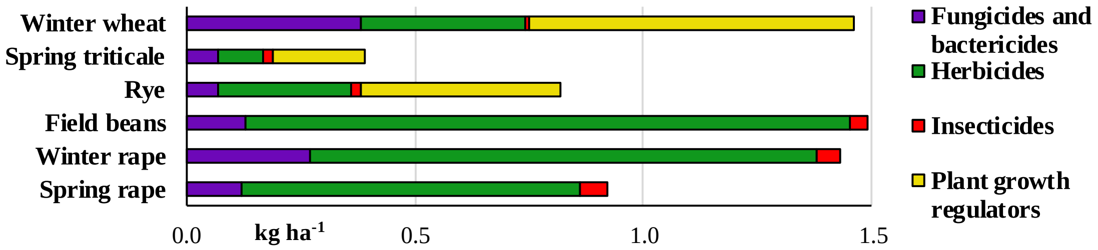

Figure 5. Use of pesticides (kg ha^{-1}) on crops, where insecticides were used in Latvia, 2017
Source: authors' elaboration based on CSB (2019) data

New organic EU Regulation—2018/848 was adopted, which will come into force in 2021 (European Parliament and…, 2018). For certification as organic apiary, its location must be within a radius of 3 km from the nectar and pollen sources (i.e., organic crops or spontaneous vegetation, or crops treated by low environmental impact methods). Since agricultural intensification (i.e., monocultures, mineral N fertilizers and pesticide use) is growing, this could be a main threat for further preservation and development of organic beekeeping.
Summary of some adverse agricultural practices and threats for pollination and honey bees, as well as beneficial agricultural practices influenced pollination and honey bees are reported in Table 2 and 3. Despite that practices mainly are aimed for beekeeping in general, they should be useful for organic beekeeping also, since the literature sources for it are very limited.

Table 2. Some adverse agricultural practices and threats for pollination and honey bees

Practice	Impact	Reference
Use high level of inorganic nitrogen fertilizers	decreased diversity of wild plant; significantly reduced worker bee longevity	IPBES, 2017; Melece & Shena, 2018b
Loss of biodiversity	destruction of habitat & lack of forage due to monocultures, and reduction of wild flora	IPBES, 2017; Kovacs-Hostyanszki et al., 2017; EP, 2018; Melece & Shena, 2018a
Changes in land-use and management intensity	agricultural intensification, i.e., land cover and spatial configurations	Kremen et al, 2002; Potts et al., 2016; IPBES, 2017; Melece & Shena, 2018b
Use of pesticides, esp. insecticides	have of lethal & sublethal effects on bees; destroy wild flowers near crop fields	Potts et al., 2016; IPBES, 2017; Kovacs-Hostyanszki et al., 2017; EP, 2018

Table 3. Some beneficial agricultural practices influenced pollination and honey bees

Practice	Impact	Reference
Organic or ecological farming	high biodiversity without any application of chemical pesticides or fertilisers	IPBES, 2017
Diversified farming systems & Diverse agricultural landscapes	organic, multifunctional, sustainable, and agroecological management approaches	Kremen & Miles, 2012; IPBES, 2017; Melece & Shena, 2018a
	crop rotations, cover cropping, fallowing; field margins, buffer strips, field edges, grass strips & hedgerows	
	semi-natural habitats, small landscapes & pastures	
	wild flowers left in crop fields can facilitate honey bee movement and therefore crop productivity	

There is strong evidence that the current CAP is inefficient regarding its environmental objectives. Pe'er et al. (2017) indicate that support is the highest for greening measures (Table 4), for agri-environment measures (i.e., organic farming) it is more than three times less, but payment for Natura 2000 territories is absolutely negligible.

Table 4. Area and potential average payment per ha under RDP 2014-2020 measures in EU

RDP measure	Area, mill. ha	EUR ha^{-1}
Greening: Ecological Focus Area	8	790
Agri-environment measures (i.e., area of organic farming)	13	247
Natura 2000 territories	12	25

Source: authors' elaboration based on Pe'er et al., 2017

In Latvia only support for organic beekeeping is EUR 40 per beehive in case if beekeeper has more than 21 beehives (LAD, 2019).

EP (2018) underlines that the financing of beekeeping for food production and therapeutic purposes must be structured in a more targeted and effective way, and appropriately increased in a future agricultural policy (expected from 2021).

For increase the income organic beekeepers via value added products and services, they are involved in several rural tourism activities, such as follows: Heritage Agritourism farms and Countryside goodies (Lauku ceļotājs…). Heritage Agritourism farms inform visitors about products or skills that relate to farming that has been inherited from at least one generation, and continues to exist and is developing in modern way. Owners offer tours, tastings of products, demonstrations of processes, a chance to take part in some of the work on the farm. For instance, organic beekeeping farm 'Lapegles farm' has 150 beehives, which are carefully tended by beekeepers. The farm also produces nectar plants such as buckwheat, clover and phacelia. Visitors can learn all about beekeeping, as well as to taste and purchase various bee products. Another organic farm 'Turaidas' is one of the smallest organic farms in Latvia, with 2.4 ha of land, produces and sale herbal teas, also has beehives to produce honey. Farm products are sold on website. For visitors among others owner offer an apiary tour with a demonstration of the beekeeper's work, tasting and sale of different sorts of honey. Countryside goodies—Bee— the tour may involve beehives, where visitors will learn about the structure of hives, about the lives and hierarchy of the bees, etc.; to taste various types of honey; to purchase the honey which they like the most; and some farms sell not just honey, but also other bee products. Besides, visitors are encouraged to take part in various processes, among which the most popular is the production of wax candles.

Conclusions

Further preservation and development of organic beekeeping mainly are threatened by intensification of agriculture (e.g., use of mineral nitrogen fertilizer, pesticides, reduction of

both agro-biodiversity and wild), as well as new EU requirements for certification (3 km radius claim).
There is necessary expanding supporting measures capabilities to organic beekeeping, especially in Latvia. For instance, to support cooperation initiatives, to cover certification payments, as well as expenses of honey and bee products analyses. Besides, to support short supply chains (i.e., direct sale) development, and include organic honey in green public procurement.

Acknowledgements

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4. ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

ESTIMATION OF CLIMATE CHANGE IMPACTS ON EVAPOTRANSPIRATION BY USING DIFFERENT METHODS

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Abstract

In recent years, environmental issues have come to the fore with their effects. Two of these important environmental issues are climate change and the water problems. Especially, the impact of climate change on water resources has been increasingly attracting attention as an important environmental issue. Therefore, the effects of climate change on water resources are needed to be known to manage water-related issues in the future. In this context, with this study, the impacts of climate change on evapotranspiration, which is one of the important components of water resources as a loss, were analyzed by using 18 different evapotranspiration equations (temperature-based, radiation-based, and mass transfer-based) for the three cities of Thrace part (Edirne, Kırklareli, Tekirdağ) of Turkey. The past situation was investigated by using actual data obtained from meteorological stations for the period of 1975-2010. The future projection was estimated for the period of 2015-2040 by using ECHAM-5 model data in the framework of the A1B scenario. As a result of the study, in the framework of 18 methods, it was estimated that evapotranspiration would increase in the Thrace part of Turkey from 13 to 17% as an average in the future.

Key words: *Climate change effects, evapotranspiration, evaporation, water resources.*

Introduction

Changing precipitation regime and increasing temperature are two of the main climate change effects, which have been predicted by many researches and are now being encountered worldwide. This situation will increase the importance of water even more in the future. Depending on temperature increase, it is undeniable fact that evaporation may increase especially for the open surface waters (Şaylan *et al*., 2011). In parallel to this, evapotranspiration (ET), which expresses transpiration from plants and evaporation from soil and open water surface, will also be affected significantly by climate change.

Evapotranspiration is an important component of the hydrological cycle and the knowledge of water loss from ET within the hydrological cycle is an important issue for the management and planning of water resources. Especially for the countries with an agriculture-based economy in which the majority of water demand arises from the agricultural sector, water management and planning is of great importance (Azlak, 2015). Therefore, in order to improve planning and to support the efficient use of water resources, accurate estimation of ET in irrigated lands is necessary (Tabari *et al*., 2011).

In practice, ET can be estimated either by using available climatic data or from the pan observation multiplied by a conversion factor (K_{pan}) (Xing *et al.* 2008). In the past half century, in order to estimate ET, numerous empirical and/or physically based equations, classified as temperature-based, radiation-based, pan evaporation-based, mass transfer-based and combination-type, have been developed for different climatic regimes (Alexandris *et al.* 2006). Although performances of the methods vary in different environments (Gocic and Trajkovic 2010), most of the study related to the planning and efficient use of water resources is based on these equations for the places where the actual measurement of ET is non-exist.

On the other hand, The Penman-Monteith FAO 56 (PM) equation has been reported to be able to provide consistent ET values in many regions and climates (Allen *et al.* 2005, 2006) within all these methods. Hence, it is accepted as a well-developed worldwide ET estimator (Cai *et al*. 2007) and is recommended by FAO.
In recent years, there has been a significant increase in the number of studies related to ET. In general, recent studies have focused on researches comparing ET methods and determining the effects of climate change on ET. In this scope, the study made by Şaylan *et al.* (2011) by using 6 methods for Edirne provinces and the study made by Kişi (2013) by using 8 methods for Mediterranean part of Turkey are two of the ET comparing study made for Turkey. Beyond this, the study made by Tabari *et al.* (2011) by using 31 methods and the study made by Bormann (2010) by using 18 methods are the other studies, which made ET comparison by using a large number of ET equations. In this study, in line with this concept, we focused on the comparison of 18 different ET methods. Using climate change model data for the 2015-2040 period, we estimated the possible ET changes that will occur in the future compared to the reference period 1975-2010.

Material and Methods

The study was conducted in three cities (Kırklareli, Edirne, Tekirdağ) locate in the Thrace part of Turkey. The Thrace region is located in the vicinity of Istanbul, one of the largest metropolitans in the world. Intensive agriculture is one of the main sources of water demand in the Thrace region. Most of the territory of the region is used as agricultural land.
In this study, the daily climatic data (1975-2010) of three weather stations, Edirne station (41°40' N, 26° 33' E, 51 msl), Kırklareli station (41°44' N, 27°13' E, 232 msl), and Tekirdağ station (40°59' N, 27°29' E, 4 msl) operated by the Turkish Meteorological Organization (TMO) in Turkey were used. The study was carried on by using the daily global solar radiation, wind speed, relative humidity, maximum and minimum temperatures and average temperature data of the stations.
For the period of 2015-2040, the model data obtained from the grids, which contain the coordinates of the selected meteorological stations, were used. The model data were obtained by running the results of ECHAM5 global climate model with the RegCm4 regional climate model within the framework of the A1B scenario. A1B scenario is similar to the second mid-level route RCP6 (RCP; Representative Concentration Routes) scenario, which is one of the new approaches in climate change studies and the new scenarios to be used in these studies within the framework of the decisions taken at the IPCC experts meeting (Moss *et al.*, 2008) in 2007 (Rogelj *et al.*, 2012). The data gap in the actual data was filled by using data obtained from the climate model.
In this study, the following 18 different evapotranspiration equations were used to estimate ET (Table 1).

Table 3. Equations used for the calculation of evapotranspiration

Category*	Abbreviation / Method	Equation**
MT	DA / Dalton (1802)	$ET = (0.3648 + 0.07223u)(e_s - e_a)$
RB	HA / Hargreaves (1975)	$ET = 0.0135(T + 17.8)R_s$
MT	MH / Mahringer (1970)	$ET = 0.15072\sqrt{3.6u}(e_s - e_a)$
RB	MK / Makkink (1957) (from Xu and Singh, 2000)	$ET = 0.61\frac{\Delta}{\Delta+\gamma}\frac{R_s}{2.45} - 0.012$ $\Delta = 33.8639[0.05904(0.00738T + 0.8072)^7 - 0.0000342]$ $\gamma\left(\frac{\text{mbar}}{°\text{C}}\right) = \frac{C_p P}{0.622\lambda}$ $\lambda\left(\frac{cal}{g}\right) = 595 - 0.51T$

		$P = 1013 - 0.1055EL$ *(EL:Elevation (m))*
RB	MB / McGuinness and Bordne (1972) (from Xu and Singh, 2000)	$ET = \left[(0.0082T - 0.19)\left(\frac{R_s}{1500}\right)\right] 2.54$
MT	MY / Meyer (1926)	$ET = (0.375 + 0.05026u)(e_s - e_a)$
RB	PT / Priestley and Taylor (1972)	$ET = \alpha \frac{\Delta}{\Delta + \gamma} \frac{R_n}{\lambda}$
TB	TW / Thornthwaite (1948)	$ET=16L_d.(T/I)^a$ $\sum_{n=1}^{12} (0.2T_{ma})^{1,514}$ $a = 6.75.10^{-7}I^3 - 7.71.10^{-5}I^2 + 1.7912.10^{-2}I + 0.49239$
RB	TU / Turc (1961)	$ET = 0.013 \frac{T}{T+15}(R_s + 50)$; RH ≥50 $ET = 0.013 \frac{T}{T+15}(R_s + 50)\left(1 + \frac{50-RH}{70}\right)$; RH <50
MT	WMO / WMO (1966)	$ET = (0.1298 + 0.0934u)(e_s - e_a)$
RB	dB / de Bruin and Keijman (1979)	$ET = 10 \frac{\Delta}{0.85\Delta + 0.63\gamma} \frac{R_n}{\lambda}$
TB	HS / Hargreaves and Samani (1982 and 1985)	$ET = 0.0023\ 0.408R_a\left(\frac{T_{max} + T_{min}}{2} + 17.8\right)(T_{max} - T_{min})^{0.5}$
RB	IR / Irmak (2003)	$ET = -0.611 + 0.149R_s + 0.079T$
RB	JH / Jensen and Haise (1963)	$ET = C_T \frac{(T - T_x)\ R_s}{\lambda}$ *C_T (temperature constant) = 0.025, T_x = -3*
RB	JR / Jones and Ritchie (1990)	$ET = \alpha[3.87x10^{-3}R_s(0.6T_{max} + 0.4T_{min} + 29)]$ *5< T_{max} <35 °C α =1.1* *T_{max} ≥35 °C α = 1.1 +0.05(T_{max} – 35)* *T_{max} ≤5 °C α = 0.1 exp [0.18(T_{max} + 20)]*
CM	PM / FAO Penman–Monteith (Allen *et al.*, 1998)	$ET = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2(e_s - e_a)}{\Delta + \gamma\ (1 + 0.34\ u_2)}$
TB	SC / Schendel (1967)	$ET = 16 \frac{T}{RH}$
MT	TR / Trabert (1896)	$ET = 0.3075\sqrt{u}(e_s - e_a)$

*MT: Mass transfer, RB: Radiation-based, TB: Temperature-based, CM: Combination method.
**ET: Evapotranspirasyon in mm day^{-1}, in cm day^{-1} for MB; e_s: saturation vapour pressure (hPa), (kPa) for PM; e_a: actual vapour pressure (hPa); (kPa) for PM; RH: relative humidity (%); u: wind speed (m/s), α: empirical constant (−; 1.26 for humid conditions); λ: latent heat of vaporisation (MJ/kg), γ: psychrometer constant (kPa°C^{-1}), (mbar°C^{-1}) for MK; Δ: rate of change of vapour pressure with temperature (kPa°C^{-1}), (mbar°C^{-1}) for MK; R_n: net radiation ($MJm^{-2}day^{-1}$); R_s: global radiation ($MJm^{-2}day^{-1}$); (cal $cm^{-2}day^{-1}$) for TU and MB; R_a: external radiation ($MJm^{-2}day^{-1}$); T_{max}: daily maximum temperature (°C); T_{min}: daily minimum temperature (°C); T: daily average temperature (°C), (°F) for MB; T_{ma}: monthly average temperature (°C); G: soil heat flux ($MJm^{-2}day^{-1}$).

For this study, the PM method was used as a reference method for comparison. In this context, first, ET was calculated with the PM for three stations, and then the results of the other approaches were assessed according to the results of the PM method.

Results and Discussion

In the study conducted with 18 different ET equations, first, the long-term average of annual total ET values for the period of 1975-2010 were calculated. By using selected methods, ET values were estimated between 654 and 1703 mm for Edirne, 644 and 1702 mm for Kırklareli and 623 and 1708 mm for Tekirdağ. For Edirne province, the study has given similar results with the study made by Şaylan *et al*. (2011). The highest ET values for all stations were calculated by the dB method, whereas the lowest ET values were estimated by the MB (Table 2).

Table 4. Long-term average of annual total ET values (mm) for the period 1975-2010 (actual) and 2015-2040 (model)

	Edirne		**Kırklareli**		**Tekirdağ**	
	1975-2010	**2015-2040**	**1975-2010**	**2015-2040**	**1975-2010**	**2015-2040**
DA	1679.8	**2058.2**	1509.7	**1903.7**	1344.6	1667.3
HR	946.5	994.7	939.0	978.9	912.4	1024.3
MH	1263.0	1710.0	1056.8	1584.6	1095.1	1417.6
MK	776.3	807.4	779.1	804.0	753.2	832.8
MB	**654.4**	**698.0**	**644.5**	**682.5**	**623.3**	**720.0**
MY	1583.4	1862.9	1435.0	1718.3	1228.6	1473.7
PT	875.3	901.0	878.8	908.2	866.7	964.2
TW	783.8	823.7	765.7	802.1	761.9	826.5
TU	876.7	963.7	870.3	945.3	853.1	966.0
WMO	1001.4	1443.9	866.2	1349.0	910.9	1269.5
dB	**1703.1**	1725.3	**1702.8**	1729.4	**1708.8**	**1847.4**
HS	1146.5	1131.3	1060.5	1100.9	912.3	941.2
IR	906.2	942.9	900.6	931.1	894.7	988.3
JH	1023.8	1096.4	1006.4	1070.2	972.1	1130.9
JR	1081.0	1113.4	1089.2	1096.0	1007.1	1085.9
PM	1077.4	1277.9	1002.5	1236.8	1040.2	1211.1
SC	1328.8	1663.5	1285.7	1655.3	1110.3	1419.1
TR	1358.1	1838.8	1136.4	1703.9	1177.5	1524.3

Comparing the long-term annual total ET results of the methods with the PM reference method showed that the DA, MH, MY, MY, dB, SC, and TR methods gave higher ET values than the PM method, whereas the MK, MB, PT, TW, TU, and IR methods gave lower ET values. These calculations gave different results when compared with the study, which made by Şaylan *et al.* (2011) for Edirne province and which the PT, HS, JH, TU, MK, and PM equations have been used in. This is because of the used time period differences between the studies. In this study, the calculation has been made for a long period (35 years), whereas it has been made for a year period in the other study.

Figure 1 shows the long-term averages of monthly total ET values of all methods. While the divergence between the methods, which have the same type (temperature-based, radiation-based, etc.), is lesser, a high level of divergence has seen between different method types mostly. As shown in the figure, the divergences between the methods increase during the summer period. On the other hand, the PM method and the average of the 18 methods gave close results to each other.

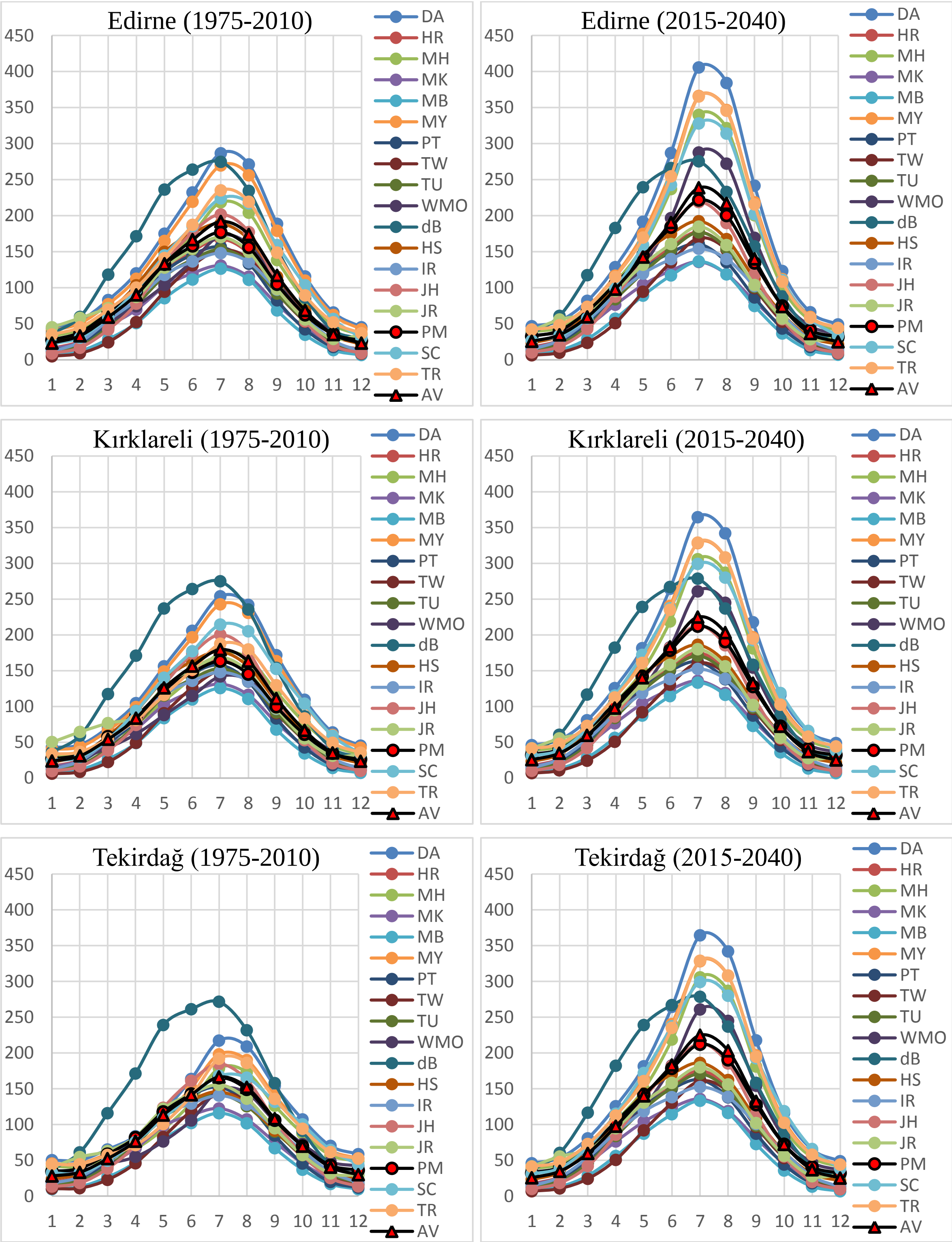

Figure 3. Long-term average of monthly total ET values for the period 1975-2010 (actual) and 2015-2040 (model)

As a next step, scatter plots diagram between the PM reference method and the other methods were drawn. Although the MB method gave the best determination coefficient for the stations as general (0.98 for Edirne, 0.96 for Kırklareli, and 0.98 for Tekirdağ), good determination coefficients, which are higher than 0.9, were obtained almost for all methods.

As the final step, in order to estimate the effect of climate change on ET, calculated results of long-term annual total ET values of all equations for the 1975-2010 reference period was compared with the calculated results for the period 2015-2040. The changes were presented as a percentage in Table 4. When the table is examined, it can be seen that the WMO method estimated the maximum changes for all provinces with the value that higher than 39%, which is more than twice the average of all methods. The minimum changes were estimated with the HS for Edirne and Tekirdağ, while estimated with the JR method for Kırklareli. Beyond this, as an interesting result, while all methods estimated increase in ET value for all provinces, the HS has come to the fore as the only method, which estimated the decrease in ET for Edirne province. As general, according to the average value of the 18 selected methods, it is estimated that ET would increases 13.7% for Edirne, 16.6% for Kırklareli, and 16,9% for Tekirdağ province in the future.

Table 5. Average ET changes (%) in the future (2015-2040) according to reference years (1975-2010)

Method	Method Type*	Edirne	Kırklareli	Tekirdağ
DA	MT	22.5	26.1	24.0
HR	RB	5.1	4.2	12.3
MH	MT	35.4	49.9	29.4
MK	RB	4.0	3.2	10.6
MB	RB	6.7	5.9	15.5
MY	MT	17.7	19.7	20.0
PT	RB	2.9	3.3	11.3
TW	TB	5.1	4.8	8.5
TU	RB	9.9	8.6	13.2
WMO	MT	44.2	55.7	39.4
dB	RB	1.3	1.6	8.1
HS	TB	-1.3	3.8	3.2
IR	RB	4.1	3.4	10.5
JH	RB	7.1	6.3	16.3
JR	RB	3.0	0.6	7.8
PM	CM	18.6	23.4	16.4
SC	TB	25.2	28.7	27.8
TR	MT	35.4	49.9	29.4
AVERAGE		13.7	16.6	16.9

*MT: Mass transfer, RB: Radiation-based, TB: Temperature-based, CM: Combination method;

Conclusions

Within this comparison study of ET methods, which use different inputs and constants, calculated ET values have been analysed and compared with each other for the three provinces of Thrace region. As a reference method, the PM method was used to make comparison and assessments have been presented according to the PM method. Although the calculated ET amounts are different depending on equations, high determination coefficients were obtained between the methods and the PM method. Beyond this, by using model data for the period 2015-2040, the impact of climate change on ET has been estimated in the Thrace region. According to the analysis made, although the increase rates vary from method to method, it is seen that ET would increase in the future as a common result of all methods. The methods selected in the scope of the study depend on four different approaches as temperature-based, radiation-based, mass transfer, and combination. From these four approaches, methods, which depend on mass transfer approach, estimated the highest ET values and the highest percentage increase for the future. As average, it is estimated that ET would increase as 13.7% for Edirne, 16.6% for Kırklareli, and 16.9% for Tekirdağ. On the other hand, without using the results of methods, which depends on the mass transfer approach, these averages were calculated as 7% for Edirne, 7.5% for Kırklareli, and 12.4% for Tekirdağ. However, it should be remembered that climate change models continue to be developed and updated to include new approaches. In addition, as can also be seen from the ET methods used in the study, the methods give very different ET values as a result of calculations. Therefore, first, by comparing with actual measurements, the best methods must be determined to estimate ET accurately for the region. Then, by using data obtained from the new climate change scenarios, climate change effect on ET must be determined. In this way, the effect of climate change on ET can be estimated as more accurately and depending on accurate results water resources planning can be made better.

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PHOSPHORUS, POTASSIUM AND NITROGEN ABUNDANCE IN SOIL OF DOLJEVAC MUNICIPALITY

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Abstract

Phosphorus, potassium and nitrogen are major macronutrients for plants which means that plants are using them in large amounts for their growth and survival. Due to their importance, those three are classified as necessary macroelements. The aim of this paper is to present soil condition of Doljevac municipality in Serbia regarding abundance of those macronutrients. The collecting of samples and testing was carried out in 2016. Precisely 116 samples were taken from 10 different villages of this municipality. The contents of readily available potassium and phosphorus were tested using the Egner-Riehm method, AL-method. The content of total amount of nitrogen was analysed using Kjeldahl method. The data processing was carried out in the IBM SPSS Statistics – Version 20 (trial version). It was found that 42,2% of the phosphorus samples belong to a class of soil badly supplied with readily available phosphorus while only 22,4% of samples were in class of well supplied or rich soil. On the other hand, the majority of samples (79,3%) were classified as well supplied and only 1,7% as badly supplied soil. Regarding nitrogen, almost all samples were classified as moderate supplied soil (>0,20% N) with 91,4% of total amount of samples.

Keywords: *phosphorus, potassium, nitrogen, Doljevac municipality, soil.*

Introduction

The availability of nutrients from the soil to plants is limited, and those nutrients can be lost from the soil by yield, erosion, rinsing, etc. (Varga, 2015). Soil nitrogen (N), phosphorus (P), and potassium (K) are important macronutrients (Tang et al., 2016) which can limit or co-limit plant growth and developement (Li et al., 2016; Tripler et al., 2006). Due to their importance, those three are classified as necessary macroelements along with other six elements: carbon (C), oxygen (O), hydrogen (H), calcium (Ca), magnesium (Mg) and sulfur (S) (Kastori & Maksimović, 2008). Nitrogen is essential for carbohydrate use within plants. A good supply of nitrogen stimulates root growth and development, as well as the uptake of other nutrients. Nitrogenous fertilizers play vital roles in different plant's growth and developmental processes. But, excessive use of nitrogen is no more beneficial to plants. Only 30 to 50% nitrogen use efficiency is recorded in plants, the remaining nitrogen is used by soil microbes, leached down in soil profile or volatilized (Haroon et al., 2019). The accessibility of phosphorous by plants from the soil depends on the pH of the soil and on the form in which the phosphorus is found. The poor availability of phosphorus in the soil is one of the limiting factors of growth and development of plants. Phosphorus is involved in the construction of structural components and the regulation of energy economy of the plant and can be found in soil in organic and inorganic form (Jurišić, 2017). Unlike nitrogen and potassium, phosphorus is found in small amount. In the earth's crust, it has about 0.28%, of which 0,02 - 0.15% is available (Komljenović and Kondić, 2011). Potassium is the only element that occurs in the plant exclusively in ionic form. Potassium is not embedded in organic compounds and has the highest content in metabolic tissues where cell division takes place (Ilić, 2016; Kastori et al. 2013). Problems related to the use of chemical fertilizers are long-term adverse effects on fertility and productivity of the soil, and environmental safety. That's why scientists look for alternative sources of fertilizers such as biological fertilizers or biofertilizers. Biofertilizers are microbiological preparations that contain living cells of bacteria, blue-green algae or fungi that effectively fix atmospheric nitrogen (*Azotobacter chroococcum*), improve the accessibility of phosphorus (*Bacillus polymyxa, B. circulens, ...*) and potassium (*B. mucilaginosus*) in soil (Dimitrijević, 2018). Aim of this paper was to determine abundance level of nitrogen, potassium and phosphorus in soil of Doljevac municipality in order to take appropriate measures of soil improvement if necessary.

Materials and methods

Doljevac is a municipality that borders in the north with the municipality of Merošina and the city municipality of Palilula of the city of Niš. With municipality of Gadžin Han in the east, the town of Leskovac in the south and the municipality of Žitorađa in the west. Municipality of Doljevac occupies an area of 121 km², of which 9161 ha are on the agricultural soil. Total number of collected samples was 116. Different amount of samples was taken from different villages which belong to Doljevac municipality (Table 1). The collecting of samples and testing was carried out in 2016. Average samples were taken by combining 20 to 25 individual samples (Gudžić, 2011) and sent to labaratory for chemical analysis.

Table 1. Number of samples per town / village in Doljevac municipality

Town / Village	**Number of samples**	**Percent (%)**
Doljevac	9	7,8
Pukovac	52	44,8
Kočane	10	8,6
Čečina	5	4,3
Orljane	14	12,1
Mekiš	3	2,6
Šainovac	6	5,2
Malošište	3	2,6
Belotinac	10	8,6
Šarlince	4	3,4
Total	**116**	**100**

The concentration of available forms of phosphorus and potassium in the soil is expressed in mg P_2O_5 or K_2O $100g^{-1}$ of the soil and in percentages for total amount of nitrogen (N). Available forms of phosporus and potassium were measured by using Egner-Reihrn method (AL-method). Potassium is determined by flame photometry way. Phosphorus is determined colorimetrically by spectrophotometer (Grčak et al., 2018). Nitrogen determination was performed by using Kjeldahl method (Bogdanović et al., 1966). The data processing was carried out in the IBM SPSS Statistics – Version 20 (trial version) and results are shown in the following chapters. The aim of this paper is to present soil condition of Doljevac municipality regarding abundance of macronutrients such as phosphorus, potassium and nitrogen.

Results and discussion

In 2016 samples were analized regarding the amount of available phosphorus (Table 2), available potassium (Table 3) and total amount of nitrogen in the soil (Table 4). Chemical analyses were conducted in a laboratory of the Agricultural Advisory Professional Service in Nis and the results of the given analyses are shown in following tables.

Table 2. Soil classification regarding the readily available phosphorus supply (classification by Jelić, 2012)

Types of soil	Content P_2O_5 (mg $100g^{-1}$soil)	Percent (%) of samples P_2O_5
Badly supplied - poor	< 10	42,2
Moderate supplied soil	10 - 20	35,3
Well supplied soil – rich	> 20	22,4
Total		100.0

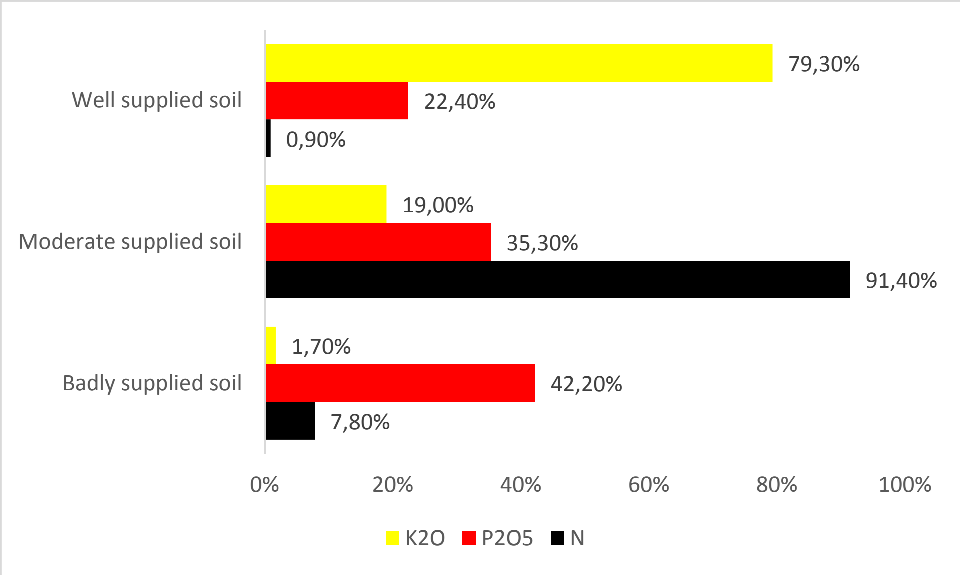
Table 3. Soil classification regarding the readily available potassium supply (classification by Jelić, 2012)

Types of soil	Content K_2O (mg $100g^{-1}$soil)	Percent (%) of samples K_2O
Badly supplied - poor	< 10	1,7
Moderate supplied soil	10 - 20	19,0
Well supplied soil – rich	> 20	79,3
Total		100.0

Table 4. Soil classification regarding the total amount of nitrogen supply (classification by Jelić, 2012)

Types of soil	Content N (N%)	Percent (%) of samples N
Badly supplied - poor	< 0,10	7,8
Moderate supplied soil	0,10 – 0,20	91,4
Well supplied soil – rich	> 0,20	0,9
Total		100.0

A total number of 116 samples were taken from agricultural soil of Doljevac municipality. Different number of samples was taken from different villages. The highest number of samples was taken in Pukovac, 52 samples, which is 44,8% of total amount of samples. The lowest number of samples was taken in Mekiš, 3 samples, which is 2,6% of total amount of samples. Samples were dried and analysed in labaratory and after that data was examined in IBM SPSS Statistics – Version 20 (trial version). Samples were divided acording soil supply level (Jelić, 2012) and data was imported into tables and graphic (Graph 1) for easier understanding.



Graph. 1. The percentage of the soil supply with K_2O, P_2O_5 and N

Based on sample analysis regarding phosphorus it was found that 42,2% of the analyzed samples of agricultural soil belonged to a group of badly supplied soil or ''poor soil'' in which the amount of available phosphorus was lower then 10 mg P_2O_5 $100g^{-1}$soil. Moderate supplied soil or soil with medium abundance (10-20 mg P_2O_5 $100g^{-1}$) had 35,3% of samples while 22,4% of the analyzed samples belonged to the class of well supplied soil (rich soil) in readily available phosphorus (>20 mg P_2O_5 $100g^{-1}$).

In the analysis of potassium results show that only 1,7% of samples can be treated as badly supplied soil namely soil with less than 10 mg K_2O $100g^{-1}$. Moderate suplied soil (10-20 mg K_2O $100g^{-1}$) owned 19% of total amount of samples while 79,3% of samples were for well supplied soil (>20 mg K_2O $100g^{-1}$). These results show us that soil in Doljevac municipality doesn't have problems regarding level of readily available potassium. On the other side, abundance of readily available phosphorus has majority of its samples marked as badly supplied.

Results based on total amount of nitrogen in soil point out that 7,8% of all samples represent badly supplied soil (<0,10% N). The largest amount of samples was classified as moderate supplied soil with 91,4% of total amount of samples. Only 0,9% of samples can be classified as well supplied or rich. Agricultural soil of Doljevac municipality has optimal amount of nitrogen so there is no need for excessive fertilization.

Conclusion

This paper presents the contents of plant available nitrogen (N), potassium (K_2O) and phosphorus (P_2O5) contents in the soil of Doljevac municipality. Based on our results it can be concluded that 79.30% of examined soil samples were well supplied with potassium, while only 1,70% of soil ware badly supplied with it. In case of nitrogen 91,40% was moderately supplied soil and only 0.90% was estimated to be well supplied soil. 42,20% of soil samples were estimated to be badly supplied with phosphorus and 20,40% were well supplied. Based on this, it can be concluded that soil of Doljevac municipality does not have problem with deficit of potassium and nitrogen but phosphorus level can be improved in order to have optimal level of all this macronutrients.

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ABUNDANCE OF POTASSIUM AND PHOSPHORUS IN AGRICULTURAL SOIL OF THE MUNICIPALITIES AND THE CITY OF NIŠ

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Abstract

Biogenic elements constitute a group of elements necessary for all living organisms. Among them, phosphorus and potassium take an extremely important place. This paper presents the results of readily available potassium (K_2O) and phosphorus (P_2O_5) contents in the soil of all five municipalities of the city of Niš (Serbia) in 2015. Samples were taken from the following municipalities of the town of Niš: Crveni Krst (181.5 km^2), Medijana (16 km^2) Pantelej (141 km^2) Palilula (117.37 km^2) and Niška Banja (141.1 km^2). The contents of readily available potassium and phosphorus were tested using the Egner-Riehm method, AL-method. The obtained data were processed in a IBM SPSS Statistics – version 20. The concentration of available forms of potassium and phosphorus was expressed in P_2O_5 or K_2O 100 g^{-1}. It was found that 63% of the potassium samples belonged to a class of soil abundant in potassium, while only 1.6% of the samples belong to a class of soil poor in potassium. The phosphorus content in the soil of Niš showed that 37.7% of the samples belong to a class of soil with medium abundance in phosphorus, 27.8% belonged to the soil abundant in phosphorus, and 34.5% belonged to the soil poor in phosphorus. The average pH in H_2O of the examined territory was 6.48.

Keywords: *available, potassium, phosphorus, soil, Niš.*

Introduction

Of all the cations, potassium is adopted by plants in the largest quantity. Potassium is not included in the organic compound of the plants, but it does affect a number of vital processes of plants (Kastori et al., 2013). Young plant organs that are growing contain the largest quantities of potassium. Of the major and secondary nutrient elements, potassium is usually the most abundant in soils. However, the range of total potassium contents which occur in soils is enormous (Reitemeier, 1951). It can vary from 0.2-3.0% which for plowing part of soil deep 20cm means 6 to 90t ha^{-1}. Potassium is the most abundant in the primary minerals of the feldspar and lichen group. Sandy soils contain small amounts of total potassium since they have minerals that are highly resistant to decomposition and some of them do not even contain potassium. Of the total amount in the soil, only a small part is available for plant nutrition. Potassium fertilizing is therefore necessary (Jelić, 2012).

Phosphorus occurs in nature almost exclusively as phosphate, in all known minerals more specifically as orthophosphate with an ionic form of PO_4^{3-}. The distribution of the different species of orthophosphate is pH-dependent (Holtan et al., 1988). Phosphorus in the soil originates from the parent rocks, mostly from apatite and its content in the lithosphere is highly variable (0.02-0.15%) since it is a constituent of many different soluble minerals, and it is also bound in the organic matter of the soil. Most agricultural soils contain between 60% - 80% inorganic phosphorus and 20% to 40% organically bound phosphorus (Jelić, 2012).

In examining the contents of N, P and K in pear seedlings, in the bark and wood during winter Šebek et al., (2007) have found that the level of phosphorus in one-year old seedlings of indigenous varieties affects the resistance of pear seedlings to low temperatures. It has been

found that certain pear seedlings had a higher level of potassium in the bark and wood in relation to other seedlings examined, even more than wild pear seedlings, which leads to the conclusion that potassium has an important influence on the resistance to low temperatures.
Apart from the influence of phosphorus and potassium to plants, these elements are also necessary for animals, which receive these two elements through feed. Over 70% of the phosphorus contained in animal feeds of plant origin is in the form of phytic acid and its salts, phytates. Phytic acid is a storage form of phosphorus and energy in many plant species (cereals, legumes, seeds and nuts). However, phytic acid cannot be utilized by monogastric animals (horses, pigs, poultry...), since they create a very small amount of phytase enzymes, so it has to be added to their feed. There is research, according to which, phytic acid is "antinutrient," i.e. it prevents the body from absorbing minerals. This acid is linked to the minerals: zinc (Zn), iron (Fe), manganese (Mn) and calcium (Ca), in this way reducing the amount that is available to the body (Sakač et al., 2007). However, this absorption interference is only temporary, which means feeds rich in phytic acid and its salts should not be avoided. Studies have shown that phytic acid acts as an antioxidant, that it is anti-inflammatory and lowers cholesterol... (Gibson et al. 2010; Schlemmer et al. 2009).
Phytase is an enzyme that improves the digestibility of phosphorus from phytic acid (inositol-6-phosphate). The paper of Grčak T. D. (2015) examines the influence of premixes (vitamin and mineral supplements) that, in addition to other necessary nutrients for egg-laying hens, also contain phytase. It was found that the premix had a positive effect on the quality of table eggs. Testing the application of phytase in broiler chickens (a breed of chickens for fattening), with the aim to increase the efficiency and reduce the excretion of phosphorus in the environment (Živkov-Baloš et al., 2007) it was found that there was an increase in utilization of P and Ca (calcium) from feed, i.e. the amount of Ca and P in excretions was reduced. Due to a lack of phytase, phosphorus passes through the digestive system unchanged and is excreted through manure. In this way, it pollutes the environment with non-digestible organic matter in the manure.

Material and Methods

Analysis of the phosphorus and potassium contents in the soil covered the following municipalities of the town of Niš: Crveni Krst (181.5 km^2), Medijana (16 km^2) Pantelej (141 km^2) Palilula (117.37 km^2) and Niška Banja (141.1 km^2) (Figure 1). The total area, from which 284 phosphorus samples and 307 potassium test samples were collected, was 597 km^2 (0.7% of the territory of Serbia). The collection and analysis of samples was carried out in 2015.

Figure 1. Municipalities of the town of Niš

The concentration of available forms of phosphorus and potassium in the soil is expressed in mg P_2O_5 or K_2O $100g^{-1}$ of the soil. The measuring of available phosphorus and potassium in the soil was carried out using the Egner-Riehm method, AL-method (Grčak et al., 2018). Potassium is determined by flame photometer. Phosphorus is determined colorimetrically, by spectrophotometer.
The Egner-Riehm method, AL-method, is considered to be more suitable than other methods, because both readily available phosphorus and potassium are determined from the same extract. It is also suitable for the determination of readily available phosphorus in soils with a wide range of pH (Predić, 2011). The obtained data was processed in a IBM SPSS Statistics – version 20

Results and Discussion

In study, 284 samples were analysed regarding the amount of available phosphorus and 307 samples regarding the amount of available potassium in the soil. Chemical analyses were conducted in a laboratory of the Agricultural Advisory Professional Service in Niš and the results of the given analyses are shown in Table 1 (Jelić, 2012) and Figure 1.

Table 1. Soil classification regarding the readily available potassium and phosphorus supply (Jelić, 2012).

Types of soil	**Content P_2O_5 (mg $100g^{-1}$soil)**	**Content K_2O (mg $100g^{-1}$soil)**
Badly supplied - poor	< 10	< 10
Moderate supplied soil	10 - 20	10 - 20
Well supplied soil – rich	> 20	> 20

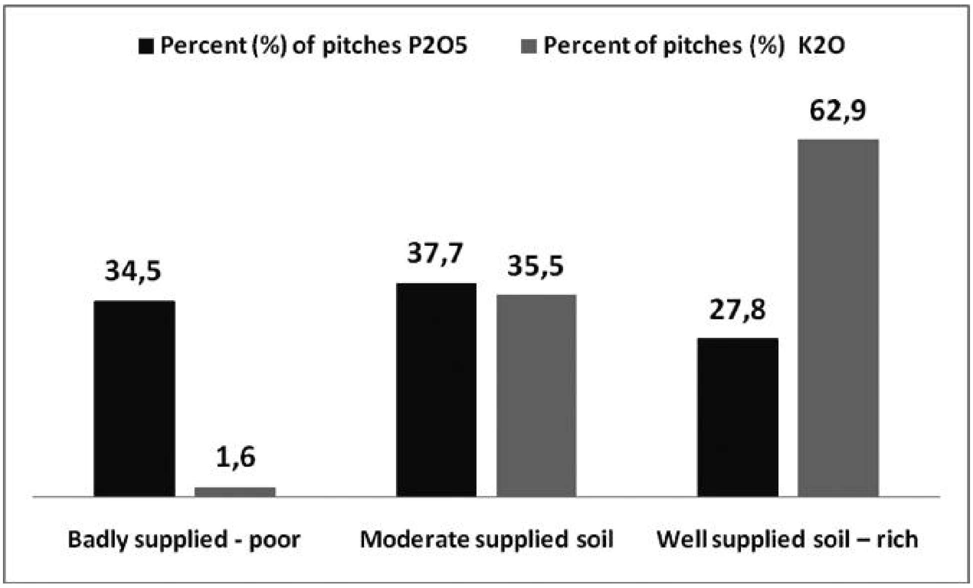

Figure 1. The percentage of the soil supply with P_2O_5 and K_2O

Based on 284 samples of the soil of the municipalities and the town of Niš that were processed for phosphorus, it was found that 34.5% of the analyzed samples belonged to a group of soil poor in available phosphorus (< 10 mg P_2O_5 $100g^{-1}$), while 37.7% of the analyzed samples of agricultural land belonged to the class of soil with medium abundance (10-20 mg P_2O_5 $100g^{-1}$) in available phosphorus.

Based on a large number of studies, our conclusion is that the content of phosphorus in the soil is around 0.02-0.15%. It is the most abundant in a non-organic form of different solubility, and this is why it is necessary to determine the abundance of readily available form of phosphorus P_2O_5 by analyzing the soil. After the end of the plant life cycle, it returns to the soil through organic matter. Acidic soils are poor, while neutral and alkaline soils are rich in readily available phosphorus. An excess of phosphorus in the soil causes the symptoms of Zn, Mn or Fe deficiency, since phosphorus binds these elements. Phosphorus deficiency causes reduced plant growth, where leaves are smaller and fall off prematurely. Phosphorus deficiency causes the synthesis of anthocyanins, where leaves turn purple at the margins and the lower side (on the back). Microorganisms adopt phosphorus to build their bodies, and after their death, phosphorus becomes available to plants. Phosphorus is accumulated in plant fruits. The amount of phosphorus that has left the soil should be returned through fertilizers. The acidic reaction of soil and an insufficient abundance of readily available phosphorus negatively affect the number of microorganisms in the soil. Microbial activity has a strong influence on soil fertility (Bjelić et al., 2015).

The total number of test samples for readily available potassium in the soil of the municipalities of Niš amounted to 307 samples. In terms of percentage, around 62.9% of the samples belonged to the class of soil abundant in readily available potassium (over 20 mg K_2O $100g^{-1}$), i.e. 193 samples of the total number of samples (307).

Around 35% of the samples (109 samples) belonged to the class of soil with moderate abundance (10-20 mg K_2O $100g^{-1}$), while we classified only around 1.6% of the tested samples (5 samples) as soils insufficiently abundant in potassium.

The soil samples taken were also analyzed for pH values. The average pH was 6.48. The minimum registered value of pH was 4.20 and the maximum was 7.17. Variability was calculated by means of the standard deviation was ± 0.39, indicating that there were no major differences between the pH values.

Based on many studies, it was found that the amount of potassium in the soil was around 2%, of which a small portion was readily available. Nitrogen (N) is the only element that plants adopt from the soil faster than potassium. These elements are followed by phosphorus, calcium, magnesium... Soils of a heavier texture, with more clay, have more potassium than sandy soils. Potassium affects photosynthesis, synthesis of proteins, transport, construction of carbohydrates, plant resistance to diseases and low temperatures, and the like. On the other hand, potassium deficiency can first be observed on leaves; the tops and margins of leaves turn yellow-green in color and the plant is more vulnerable to diseases.

An uneven application of fertilizers in non-agricultural soil results in a state of excessive content of readily available phosphorus and potassium (Tintor et al., 2015). A high content of phosphorus in soil affects the adoption of zinc (Zn) by plants, due to the antagonism of phosphorus and zinc (Bogdanović and Čabilovski, 2007). The same authors state that soil that has 40-60 mg K_2O $100g^{-1}$, where vegetables are grown, is considered to have sufficient content of potassium.

Chemical properties of soil are very important for the adoption of chemical elements by plants, and the soil reaction (pH) is particularly pointed out. The optimal pH of the environment for the adoption of macroelements is in the range of pH 6-7.5, while the acidic environment of pH 4.5-5.5 suits microelements, with the exception of molybdenum, to which neutral and alkaline environments suit. Different plant species have different requirements for soil pH (Bogdanović et al., 2004).

Humus is a very important factor of soil fertility. Humus soils contain large amounts of biogenic elements. Humic substances bind cations and anions in a form that is easily accessible to plants.

The analysis of soil from the municipality of Aleksinac found that over 55% of the soil belonged to a class of soil abundant in humic substances, having a content of humus from 3% to 5% (Grčak M.D, 2015).
A low phosphorus content in soil is usually solved by applying phosphatization, which is one of meliorative ways to introduce phosphorous fertilizers into the soil. The excess of phosphorus in soil can cause Zn, Fe, Ca, B and Mn deficiencies. It is recommended to omit phosphorus fertilization, and apply deep tillage, since phosphorus is generally immobile in soil, so it is accumulated in topsoil.

Conclusion

From results obtained by examining the contents of available phosphorus and potassium in the agricultural soil in the municipalities and the town of Niš, the following conclusions can be drawn:
When it comes to phosphorus, 37.7% of the analyzed samples were moderately supplied. There were 34.5% badly supplied samples. Finally, 27.8% of the analyzed samples belonged to the class well supplied soil.
As for the content of potassium in the soil, the largest number of samples, 62.9%, belonged to the group of well supplied soil with readily available potassium. 35.5% of the samples were moderately supplied and only 1.6% of the examined samples belonged to the class of soil poor in potassium.

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FOOD WASTE IN PORTUGAL– A PUBLIC POLICY WITH THE COMMITMENT OF ALL SOCIETY

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Abstract

It is estimated that 30% of the world's useful agricultural area, equivalent to 300 kilos of food lost per inhabitant and corresponding to an economic cost of 750,000 million dollars, is wasted annually. On the other hand, in Europe there is an unacceptable direct relationship between economic development and the level of food waste where 89 million tons of food end up in the trash, in a scenario shared by different developed countries outside the European Union. In this context it is important to define and maximize strategies for reducing food waste, which is a global, social, civic, economic, technological, scientific and human commitment. Any strategy to be implemented will have to include different variables. In a reflected proposal, 5 intervention axes are to be considered: to carry out studies to know how much, how, where and why of food waste; disseminate and promote good practices and awareness raising actions; analyse and review normative aspects; encourage the design and development of new technologies; collaborate with the agents and organizations involved in this field. Considering these variables, in Portugal, in the past year of 2014, through a formal commitment "Combating Food Waste, a commitment of all", a common strategy was defined for all agents of the food chain who were actively involved in an organized, structured and coordinated way, committing to change attitude, working procedures and management systems.

Key words: *Portugal, food waste, strategy, good practices*

Introduction

It is estimated that 30% of the world's useful agricultural area, equivalent to 300 kilos of food lost per inhabitant and corresponding to an economic cost of 750,000 million dollars, is annually wasted. At the same time, the carbon footprint of food loss and waste (FLW) is estimated to be up to 3.49 gigatons of carbon dioxide equivalent (gtCO2e), representing up to 6–10% of total anthropogenic greenhouse gas (GHG) emissions (HLPE 2014) (Gromko and Abdurasulova, 2019). Food is produced for the purpose of being consumed, nevertheless, 33% of all produced food is wasted throughout the value chain, with some regions reaching 40% (FAO,2019). Those foods, that do not serve the purpose for which they are produced, constitute what is meant by "food waste", becoming a problem across the entire food chain, from production to consumption. In addition, an unacceptable and direct relationship between economic development and the level of food waste is observed. In Europe, projections show that between 30% and 50% of edible food will be wasted every year through the whole food chain until it reaches the consumer. This equates, in the 28 member states of the European Union (EU-28), to 173 kilograms of food waste per person. Considering the total amount of food produced in EU in 2011, around 865 kg/person, this represents a waste of 20 % of the total food produced and also an annual food waste around 89 million tonnes in the EU, distributed by households (42%), industry (39%), catering (14%) and distribution (5%). These figures allow us to predict that EU food waste by 2020 will be around 126 million tonnes, which will represent almost 20% increase since 2016 (Stenmarck *et al.*, 2016).

Within this framework, the reduction of food waste is both particularly relevant and also a moral obligation in a world where about one-sixth of the population is hungry and where 870 million people are in a state of malnutrition. In Portugal, considering the sum of food loss and waste that occur along the different stages of the supply chain, it was estimated that food waste represents 17% of the annual food production, approximately 1 million tonnes (Baptista *et al*., 2012). This scenario implies to adopt in civil society, public administration and government strategies, policies and behaviours that prevent and reduce the economic, social and environmental costs inherent to food waste. It is determinant the compromise of all stakeholders, including consumers' and the Government, in order to commit themselves in enhancing the efficiency of the food chain, with the short-term goal of a significant reduction of food waste. It should be present that re-using, redistributing and recycling the products is fundamental for food waste reduction, and that is positively influenced by food safety, food quality and other good practices in all stages in the food chain. This can never be neglected. The objective of the present paper is to deliver and to describe a set of policies and actions based on the commitment assumed in Portugal to reduce food waste by all partners involved in the food chain, consumers and civil society, public administration and government.

Materials and methods

In Portugal, considering the real lack of knowledge about the impact of food waste in the different sectors of the food chain and the non-existence of credible studies to support the implementation of reduction policies and strategies, it was essential the implementation of a set of targeted actions that alerted and committed the community for what was political consider a national purpose.

The different entities, organizations and associations, both public and private, that were directly related to this object were identified and grouped in:

1. Government (Ministry of Economy, Ministry of Agriculture and Ministry of Health), public administration (Directorates-General and Food and Economic Security Authority) and international organizations (FAO);
2. Confederation and associations of productive (agriculture), industrial (agri-food), trade (distribution) sectors, catering and consumers;
3. Teaching and research entities (universities and polytechnics, research institutes) and professional organizations';
4. Civil society (Food Bank Against Hunger and other NGOs).

On the basis of a common understanding, the document "*Food Waste - a commitment of all*!", was prepared. This document aimed to obtain at a precise characterization of the national situation and, at the same time, intended to institute a national strategy to combat food waste. Specific groups and thematic areas were established, in order to define actions, develop activities and disseminate policies and results to all the different focus targets.

Results and Discussion

In Portugal, in the past year of 2014, the formal commitment: "C*ombating Food Waste, a commitment of all*", was defined as the common strategy by all food chain agents. All stakeholders were actively involved in an organized, structured and coordinated way, assuming the importance and the will to change attitude and develop waste free procedures and management systems.

An inclusive approach was defined which required a compromise that united all partners in the adoption of a set of recommendations. This public commitment, which begins in production stage and ends in the final consumer, was based in the following common and crosscutting principles which are determinant "towards zero waste" objectives:

• Promotion of cooperation between all partners in a combination of efforts to fight food waste;
• Development of a waste reduction strategy and monitoring methodology definition;
• Awareness the society, in general, for food waste along the food chain;
• Development and disseminate best practices in the management of food losses;
• Promote the recovery of food products that are not in conditions to be consumed, as well as intermediate products and packaging material;
• Developing alternative solutions for potential food waste (re-use);
• Definition and acceptance of unconventional redistribution chains;
Any strategy to be implemented has to include different variables and domains. This planning foresees 5 intervention axes:
1. Primary production and transformation:
The food supply chain begins on primary production, one of the most wasteful stages. It is estimated that the initial and final stages of the chain area the ones where almost all losses occur. To limit waste as much as possible, the following measures are listed:

- Carrying out adequate and rigorous planning of food production;
- Minimizing losses, using natural resources efficiently and sustainably;
- Limiting food waste on farms by increasing efficiency in the harvesting process;
- Adapting the installed capacity of agro-industry for the estimated production;
- Managing, handling and storing properly the agricultural products for agro-industry;
- Innovating in packaging technology, creating new food preservation solutions;
- Optimizing production capacity to commercial storage and disposal capacity;
- Promoting better information to the consumer, on the contents of the labels, giving priority to the conditions of conservation, dates of minimum durability and consumption deadline;
- Working with stakeholders, in order to stablish different solutions for ingredients or intermediate products and foods that are not suitable for quality reasons but meet all legal requirements for food hygiene and food safety.

Within this axe, the articulation of the defined measures was implemented as in the structure presented in the figure 1related to the management of agricultural surpluses:

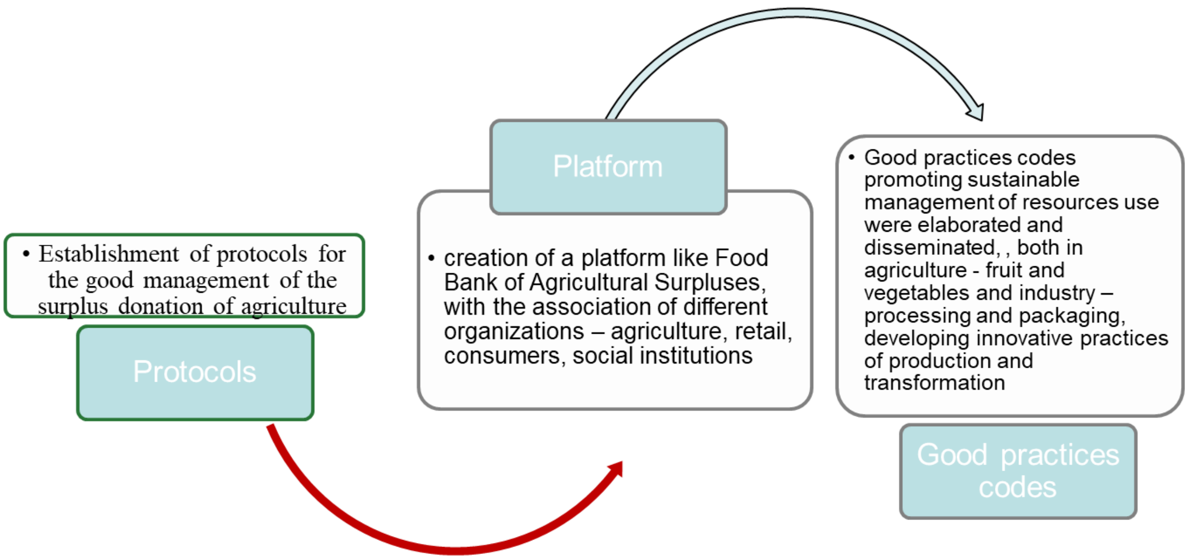

Figure 1 - Primary production and transformation measures

2. Logistic Distribution and Retail Market

Logistic distribution is an important link in the food chain, bringing goods closer to the population and transporting them from the origin and production zones to the places of commercialization and consumption. In the logistics and retail distribution sector, the following good practices were planned and implemented in order to reduce food waste:

- Valuing local production and domestic production;
- Defining strategies of consumption of local/national products, ensuring that Public Institutions (schools, universities, hospitals) prefer national production;
- Schedule logistics and transportation;
- Managing and storing the stocks properly: "first-in, first-out";
- Promoting the rotation of the products in the shelves, guaranteeing that products with nearer validity periods are placed ahead of the rest;
- Promoting the quick sale of products approaching the expiration date;
- Handling products properly in order to avoid packaging deterioration;
- Re-sizing of the packages in face of the needs of the households and consumption patterns;
- Promoting the sale in bulk in an appropriate manner.

The distribution sector already had an enormous concern to the problem of food waste and had already implemented a set of measures and actions towards reducing it. Good practice guides have been developed and disseminated to the distribution professionals and to the consumers, with training in specific activities and in which the concern to ensure food security is constant and a priority.

At the same time, new practices were introduced in product marketing, particularly in packaging, and food surpluses donation to charities was encouraged. Likewise, in the catering activities, actions of sensitization were promoted and started programs of collection of meals for reutilization, in an approach of support to the most deprived ones.

The measurements / results of this axis are summary present in Figure 2 below.

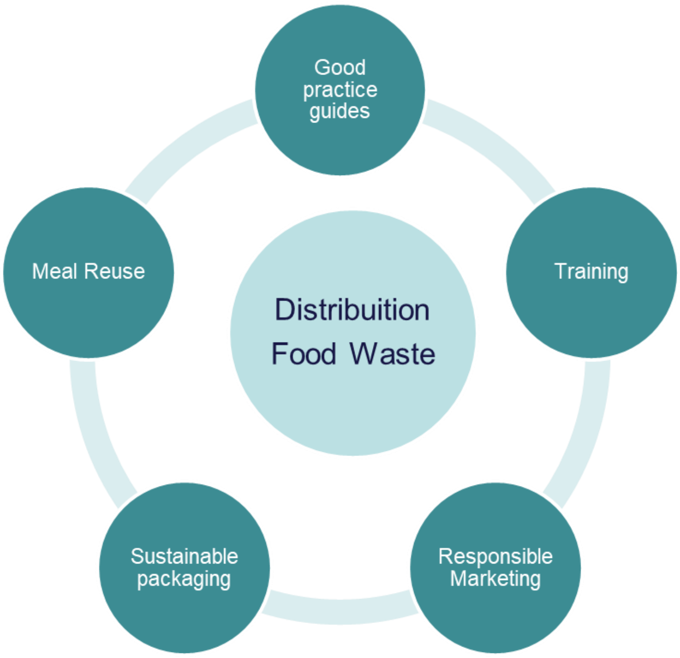

Figure 2 - Logistic Distribution and Retail Market measures

3.Education and Communication

The prevention of food waste is fundamental at the educational level as part of one communication strategy. Reducing food waste in the final stage (consumer) implies changing

behaviours, habits and even the routines of individuals, families and all society. Sensitization and media communication are important key factors for the success of this goal.
A set of recommendations are included to contribute to the reduction of food waste:
Introducing awareness in the school programs to prevent food waste;

- Application of the School Fruit Scheme in as many municipalities as possible;
- Dissemination of good domestic and purchasing practices;
- Raising awareness of the differential treatment of perishable and non-perishable products;
- Promotion of initiatives on food consumption indications and disseminating rules for good conservation practices;
- Motivation for more frequent buying in local markets;
- Encouraging the "shopping list" taking in account the size of the families;
- Promotion and encouraging the consumption of seasonal products;
- Developing culinary skills that prevent food waste and producing safe and healthy meals.

The receptivity of society and the academy to the problem of food waste was very high and very intense its mobilization. Numerous actions have been developed within the different strategic social segments that were translated in the following figure 3:

Figure 3 - Education and Communication measures

4. Awareness and Accountability

The general commitment against food waste implies a great social awareness, including the articulation and partnership with charity institutions. In this context, a group of recommendations were settled in order to boost the reduction of food waste:

- Awareness for the adoption of responsible consumer practices;
- Creation the notion of "*food footprint*" to enable families to calculate their level of loss;
- Food donation programs involving food donors, redistribution organizations and recipients;
- Promotion of agreements to redirect, for food donation institutions, the primary products: fruits and vegetables and other products that do not enter in the agro-industrial sector;
- Introducing the concept of "*healthy food*" into all procedures related to food donation or food support for vulnerable populations;
- Helping the donors to separate food products not eligible for sale, although they meet all legal hygiene and food safety rules and requirements;
- Making easy the exchange of good practices on the prevention, redirection and use of products and surpluses.

Within this axis, one of the most significant realizations was the launch of award "*PRA-TØ - Recognition of Food Waste Prevention Practices",* an initiative of the Government and Civil Society, under the high patronage of His Excellency, the President of the Republic of Portugal. The objective of this award was to distinguish the implementation of policies and models of good management in food waste reduction.

Figure 4 – 2015 Award Ceremony - Seal of Recognition *PRA-TØ*

The first edition distinguished entities that took responsibility for the impacts of their decisions and activities, created value for stakeholders and contributed to sustainable development, involved the Portuguese business community, and society as a whole, in the area of Food Waste and Social Responsibility and to highlighted the work already done in this field by different entities. This award was a success with 37 competitors in 5 categories.

5. Regulation, Implementation and Recognition

The mission of the Government is to regulate when necessary in the prevention of food waste, therefore, tools and studies that, directly or indirectly, promote food waste reduction can be created, as the National Platform of Knowledge on Food Waste. Some of the administrative proposes in order to reduce and prevent food waste were:

- Monitoring the reduction of food waste and improve the food chain sustainability;
- Creation of local markets and promoting local short commercialization circuits;
- Valorisation, at the level of public contracts, companies with social responsibility and good anti-waste practices implemented;
- Evaluate the possibility of acquiring food products from production and local markets by making public procurement rules more flexible, taking into account food security;
- Evaluate the possibility of implementing a fiscal and/or economic benefit aimed at food donation;
- Advertise or distinguish the distribution agents that have, by product range, levels of national products commercialization over 2/3;
- Create a competition to identify best practices in the "Prevention of Food Waste" throughout all phases of the process;
- Advertise good practices related food waste prevention with a "*seal*" that can be identified and valued by the consumers and stakeholders.

The evaluation and segmentation study of food waste in Portugal, with suggestion of proposals and lines of future action, was presented with its first conclusions. This information allowed decision makers and stakeholders to gain a more detailed knowledge of the reality, thus defining lines of action for their prevention, bringing the waste of reuse closer. Another result within this axis was the decree-law published on production local markets.

More recently, following the strategy initiated in 2014 and emphasizing that the fight against waste do not stop with the change of political cycles, the National Commission to Combat Food Waste was instituted (DR, 2016), which resumed the National Strategy to Combat Food Waste.

Last, Portugal was a pioneer in the development and elaboration of Codes of Good Practices that facilitate food donation. These guiding documents are currently undergoing review and adaptation, and these adjustments stemmed from the experience gained by donors and recipients (Food Bank, Re-food, among others), during the implementation of the policies defined in the National Strategy.

Conclusions

The strategy started in 2014 to trigger a more effective and articulated fight against food waste had the capacity to promote the discussion on the subject and served to place at the same table and with the same objective all stakeholders in the agri-food sector.

As an example, it is important to highlight that the aggregating strategy defined in 2014 by civil society, the public sector, the municipalities and NGOs currently has significant results. Observing the 2018 data of the Zero Waste Movement, the measures and attitudes implemented allowed the results presented in Figure 5, reveals:

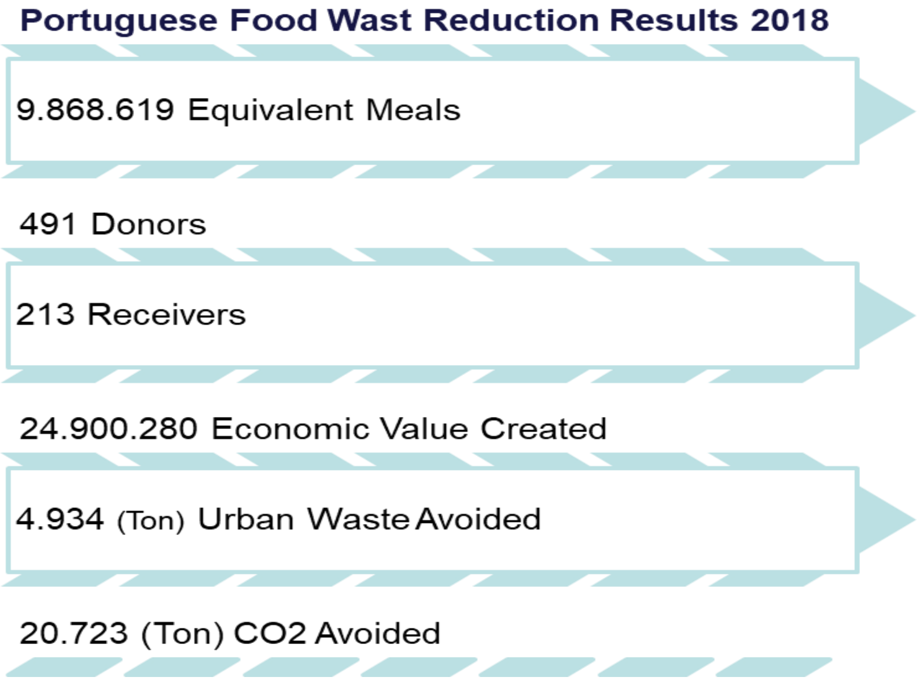

Figure 5 - Portuguese Food Wast Reduction Results 2018 – Zero Waste Movement indicators

Also, it is relevant to notice the Re-food evolution. This NGO, which aims to prevent food waste and distributing leftover meals from canteens, restaurants and caterings to needy people in 5 years achived to the results present in figure 6.

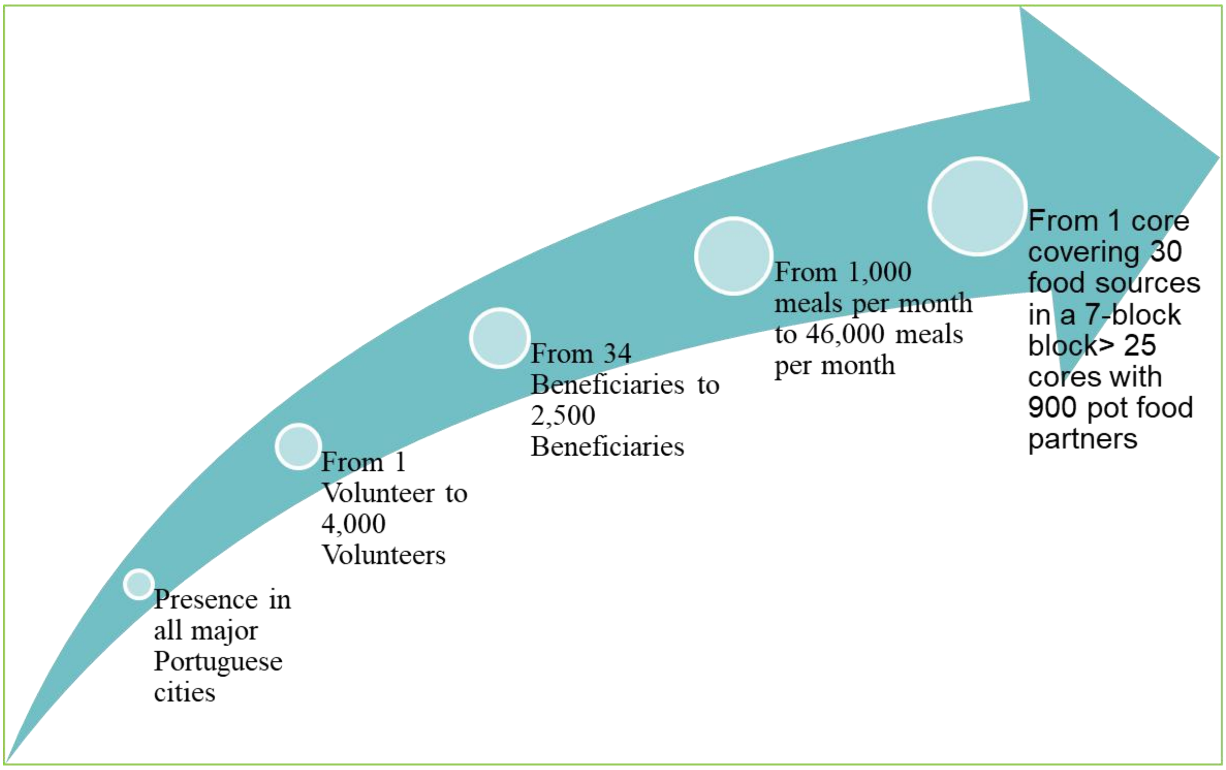

Figure 5 – Re-Food ONG 5 years results 2014-2019

Since then, all the strategy measures were aligned to the sustainable goals defined in the 2030 Agenda. Portugal and the Portuguese food sector undertake the 2030 Agenda, as a commitment to eradicate poverty and achieve sustainable development by 2030 worldwide, ensuring that no one is left behind.

The 17 Sustainable Development Goals (SDGs) and their 169 associated targets are global in nature, universally applicable and interlinked with the 2014 food waste reduction national agenda. All countries, developed and developing, have a shared responsibility to achieve the SDGs, integrating, in a balanced manner, the three dimensions of sustainable development - economic, social and environmental - and reflects for the first time an international consensus that peace, security, justice for all, and social inclusion are not only to be pursued on their own but that they reinforce each other (UN, 2015).

Portugal, as a project based on fundamental values and fairness, embraces the UN 2030 Agenda for Sustainable Development as a unique opportunity for a better future, also in food waste and food access matters. To preserve the future, the right policy choices have to be made today with a strong commitment between all the stakeholders.

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HARMONIZATION OF RELATIONS BETWEEN URBAN AREAS, AGROCOMPLEX AND FOREST BIOCENOSIS IN THE MANAGEMENT FUNCTION

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Abstract

Existent trends in the use of renewable natural resources call into question the self-regulatory abilities of biocenoses to adapt to dramatic climatic and other changes. Activities on the development of rural economy through diversification and development of economic activities in rural areas, intensifying the process of urbanization, generate phenomena that directly threaten not only ambient values, but also nature as a whole. Opportunities and needs that were compatible a hundred years ago are now often in contrast, which is particularly reflected in the relationship between the needs of urban complexes for food, drinking water and air. By harmonizing the relationship between urban units, agrocomplex and forest biocenoses, it is possible to provide development that does not endanger the future generations of meeting needs, and meeting the needs of the present generations. Protecting and improving the state of forests, using existing potentials and their functions, such as raising new forests in order to achieve optimal forestry, spatial distribution and structure of the forest fund is a legal obligation. The law provides a basis for the sustainability of forest management and sustainable forest management. In this regard, from the point of view of area management, the imperative task is to harmonize ecosystems in the boundary areas (ecotons) that are located between different ecosystems. The equilibrium between the two ecosystems is unstable. Small changes in one ecosystem can significantly affect the survival of the second ecosystem, especially when it comes to forest ecosystems in relation to urban areas and agricultural complexes. The aim of this paper is to point out the possible negative consequences and possible solutions on the example of a tourist place based on the principles that the economic interest is equal to the ecological interest.

Keywords: *Renewable natural resources, Forests, Area management, Harmonization.*

Introduction

Existing trends in the use of renewable natural resources are under question because it is about autoregulating the ability of ecological biocenoses to adapt to climate and other changes. Numerous economic and economic activities in rural and urban areas have created phenomena that directly threaten not only ambient values, but also nature as a whole. In this way, there has been a discrepancy between opportunities and needs, which is particularly reflected in the relationship between people's need for food, drinking water and air, and the ability to provide it. Harmonization of relations between urban and rural units, agrocomplex and forest biocenoses can provide sustainable development that will not compromise future generations to undermine their living needs, and at the same time provide a realistic opportunity to meet these needs for the current generation of people. According to the Law on

Forests of the Republic of Serbia (2015) preservation, protection and improvement of the state of forest using all its potentials and functions, then raising new forests in order to achieve optimal forestry, spatial distribution and structure of the forest fund is a legal obligation of our country. This law has a foothold in two basic principles, which are permanent forest management and sustainable forest management. In this regard, from the point of view of area management, the imperative task is to harmonize ecosystem relations in the border areas (ecoton) located between different ecosystems. The equilibrium between the two ecosystems is unstable. Small changes in one ecosystem can significantly affect the survival of the second ecosystem, especially when it comes to forest ecosystems in relation to urban areas and agricultural complexes. The aim of this paper is to point out, in the case of the tourist place Vrnjačka Banja, the possible negative consequences of violation of relations in different ecosystems and possible solutions based on the principles that the economic interest is equal to the ecological interest.

Material and Methods

In this paper, data from the Republic Statistical Office of the Republic of Serbia for forests and agricultural lands from 2017 and 2018 were used, data from the GeoSrbija web portal - National Geospatial Data Infrastructure and the data of the program EU Copernicus Land Monitoring Service (2012), in which they are collected data from Earth observation satellites that are combined with observation data from sensor networks on the surface of the earth. To make the conclusions in this paper the logical method of generalization and generalization was used.

Results and Discussion

Municipality of Vrnjacka Banja is one of the most important tourist destinations in Serbia, and its significance is based on the rich mineral water and surrounding parks and forests, as the greatest treasure of this area. In this area there is a branched hydrographic network of watercourses belonging to the basin of the West Morava River, the Ibar River and Rasina. All watercourses on the territory of this municipality are characterized by large oscillations in the amount of water, which is a disadvantage from the aspect of water supply, but also flood risks. The touristic significance of this municipality rests on the existence of four types of mineral and thermo-mineral waters: Snežnik, Slatina, Topla voda and lake. Municipality Vrnjačka Banja covers an area of 23,900 ha, of which agricultural land occupies about 40%, then forests about 50% and populated areas about 10% of the total area. The importance of the forest ecosystem consisting of forests and forest park is of great importance not only in the landscape of this municipality, but also in the ecological and medical aspect, because the forests and parks have the primary goal of improving the psychophysical condition of the sick people who visit this tourist place (Strategy of Agriculture and Rural Development of the Municipality of Vrnjačka Banja 2014-2024).

The methodology of strategic planning is based on three basic points, the first being based on the assumption that the plan of the field and the sectoral plan and as such should have a binding role in the development of all strategic plans (Vider V. 1996). According to the second approach, the landscape should not be viewed as a specific aspect of sectoral plans, but as part of a whole, especially when it comes to planning and management of forest ecosystems. The third paragraph, which starts from the assumption that the inclusion of the concept of landscapes into the spatial planning system and practice is the establishment of regional diversification of precession units and different regional approaches to landscape planning, is objectively most realistic for the harmonization of urban units, agrocomplex and forest biocenoses (Maksin Mićić M. 2008).

Table and chart 1. Structure of the area of Vrnjacka Banja municipality

Structure	Hectares, ha
Vineyard	91
Infertile land	828
Orchard	1.574
Field and garden	3.292
Meadow and pasture	4.488
Forest and forest land	**12.090**
Town and village	**1.537**
TOTAL	**23.900**

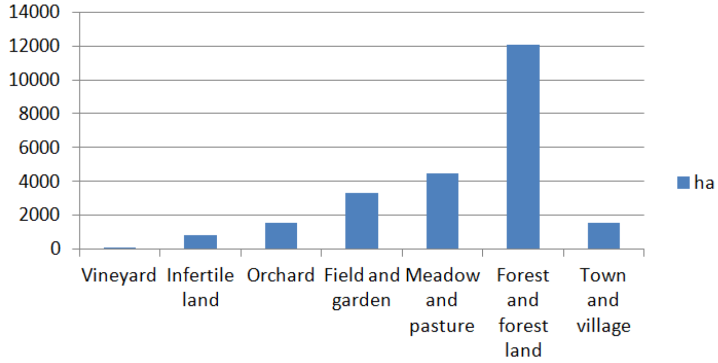
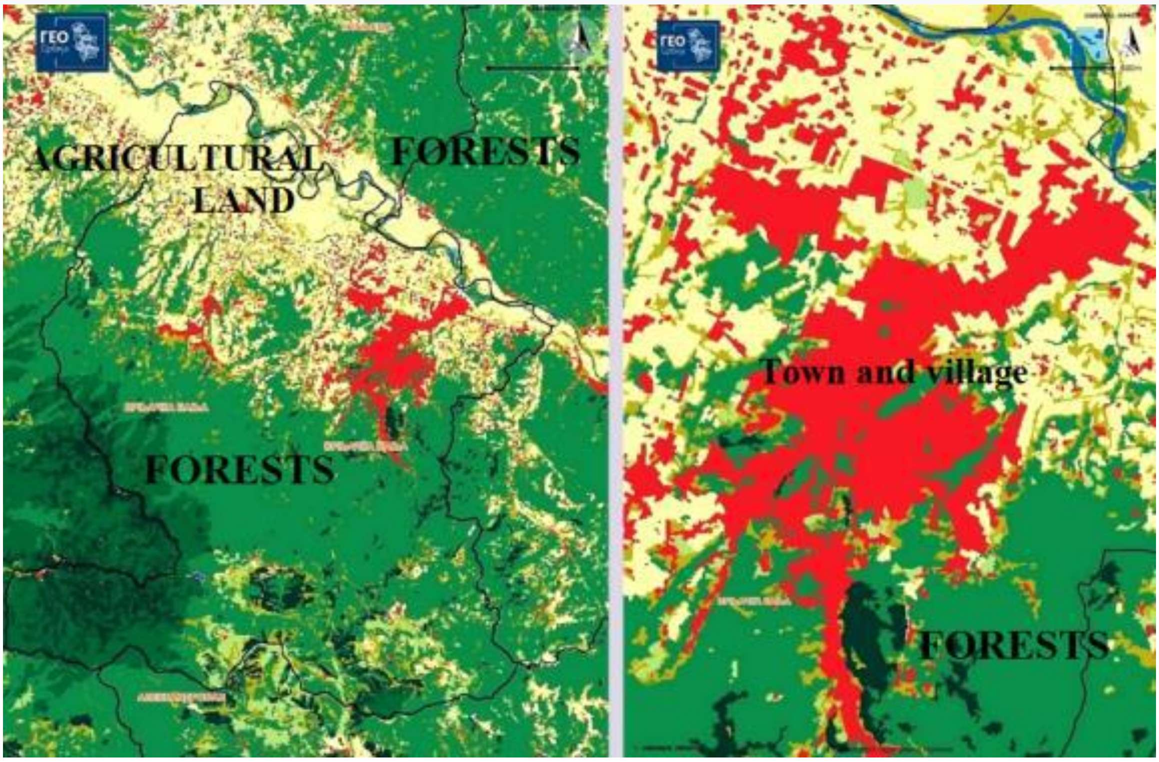


Figure 1. Scheduling of vegetation in the municipality and the urban settlement Vrnjačka Banja



Starting from the methodology of the third strategic planning approach based on sustainable forest management in order to preserve and improve them, it is necessary to preserve the values of this area in order to maximize the use of ecological potentials as well as favorable geostructure. Figure 1 shows the forest areas, agricultural areas and urban settlement of this municipality. Border regions are important in terms of microclimatic processes of regulation, as well as protection against torrential flows, but due to the development of urban settlements and agricultural land, the marginal areas that have the status of forest park have been endangered. Reduction of forest vegetation can result in significant disturbance of ecological values that are treated as economic and tourist values in the current plans of this municipality (Jović et al., 1996, Šarčević, 2012).
According to the European Convention on Areas of 2000, a set of measures defining components of diversity of certain areas, regional and urban planning, which can have a direct

and indirect impact on some landscape, has been defined. If these measures are not properly implemented, the natural values of the Vrnjačka Banja area (forests, parks, water and mineral resources) will soon become a potential problem, since environmental interests are not in line with economic interests. This is particularly contributed by the current policy of renting natural values that become the source of profit, because they lose their original ecological values. The forest ecosystem of the Goc Mountains, as part of the tourist offer of Vrnjacka Banja due to the accelerated urbanization of the settlements, is very endangered. Such an approach is incompatible with the achieved uniformly sustainable development, and we can expect that the edge urban settlements will be increasingly developed, which is why the survival of marginal ecosystems (forests and agricultural land) will be jeopardized.
Future program tasks in the area management function can be articulated through the support of natural forms of bicenosis, avoiding the correct geometric shapes that disturb the dynamic shift of vegetation. Biological and landscape architectural measures need to protect the health and recreational value of the area, protecting the area from biological contamination by supporting diversification elements in the existing space. Only the use of forests does not exclude the principles of sustainable development, but these jobs must be consistent with maintaining functional durability and strengthening general useful forest functions. In this way, the excessive burden of space and its civilization deformation would be avoided, thus protecting the spatial plan from non-compliance with the natural environment. It is also necessary to intensify the development of rural tourism and domestic work, as an integral part of the economic development of this area. This implies the production of various textile and leather items, then items from ceramics and clay, to the collection of medicinal herbs and forest fruits, as well as the development of service activities related to rural tourism.
Harmonization of relations between urban units, agrocomplex and forest biocenoses as part of the management of the area is possible only through the process of optimal use of natural resources (agricultural land, water and forest), as well as their modernization and revitalization. In this respect, from the point of view of the area management, the imperative task is to harmonize ecosystems in the border areas (ecotons) located between different ecosystems, especially this refers to forest ecosystems, forests of parks, agricultural areas and human settlements. A marketing-commercial approach is essential, but within the functions of preserving resources and protecting the environment, by putting all program tasks in interconnection, with a concrete analysis of existing resources and optimizing all processes. Organizational and financial problems could be overcome by the scientific methodological, organizational and multidisciplinary approach, which would enable the harmonization of relations between ecosystems and settlements to gain the dimension of ecological thinking with the necessary financial results.

Conclusions

The harmonization of relations between urban units, agrocomplex and forest biocenoses as part of the management of the area is possible through the process of optimal use of natural resources, as well as the procedures for their renewal and modernization. Special emphasis should be placed on the ecosystem boundary areas, where there is always a risk of disturbing harmonic relations between the two border ecosystems. For the sustainable development of the municipality of Vrnjačka Banja, diversification of this area is necessary, where apart from health and spa tourism, activities in the field of Jaws tourism, sports tourism, as well as scientific and educational activities through the form of congress tourism will be included, which would contribute to the establishment of a complete tourist offer of this municipalities.

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THE CONCENTRATIONS OF HEAVY METALS IN THE LIVER AND MUSCLE TISSUE OF THREE KINDS OF FISH DURING FIVE YEAR PERIOD

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Abstract

Fish, as the top of the trophic pyramid of aquatic ecosystems, are one of the most sensitive bioindicators for the presence of heavy metals in the aquatic ecosystems that inhabit them. Concentrations of cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) were determined in the muscle tissue and liver of selected fish species from the Zapadna Morava River during 2013 and 2018, respectively. The aim of this paper was to obtain a more complete insight into the level of accumulation of heavy metals in the organism of fish, especially fish meat as an edible part that should satisfy the health safety for human consumption. Investigations of the concentration of the presence of heavy metals from *Carassius auratus*, *Abramis brama* and *Squalius cephalus* have revealed various bioaccumulation of heavy metals. Among the examined fish, the highest accumulation of heavy metals was found in *Abramis brama*, *Carassius auratus* and *Squalius cephalus*, respectively. In all fish for all four investigated heavy metals, higher concentrations were detected in the liver and less in fish muscle. Bioaccumulation of heavy metals had the following trend: Zn > Pb > Cd > Hg.The determined concentrations of the analyzed heavy metals in fish muscle were not within the permitted limits of the MAC national legislation of the Republic of Serbia. Meat of investigated fish species is not health-safe and hygienically correct for use in human nutrition (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018). The content of tested heavy metals in fish meat indicates that in this aquatic ecosystem during the five year period there was no significant pollution with these heavy metals.

Keywords: *Heavy metals, Bioaccumulation, Freshwater fish, Zapadna Morava River.*

Introduction

Fish is a highly valued animal food in human nutrition. In addition to the nutritional value from the consumer's point of view, its hygienic or safety correctness is also significant. The majority of freshwater ecosystems are due to urbanization, industrialization and other forms of human activity, permanently exposed to the influence of various forms of hydrosalgia. For these reasons, many hydrobionts in them, and in particular the fish populations, suffer negative consequences, and hence the man as their consumer.

Fish come into contact with harmful pollutants through the skin, gill breathing (benthic organisms and plankton), and predator fish species bring metals into the body through other fish they feed. In this way, the metals directly reach the bloodstream in the liver, kidneys and muscle tissue, and accumulate in them more or less (Mansour *et al*., 2002; Erdoğrul *et al.*, 2006; Has-Schön *et al*. 2006; Yilmaz *et al*., 2007). Fish belong to the most sensitive organisms in the presence of increased concentrations of heavy metals, and the level of accumulation of certain metals in tissues and fish bodies depends on the age, size and trophic status of fish, as well as from the concentration of metal in water (Alibabić *et al*. 2007; Ural *et al*. 2012). The main goal of this research is to determine the level of accumulation of heavy metals (lead, cadmium, mercury and zinc) in the muscle tissue and liver of different species of fish that inhabit the river Moravia. In this way, a comparison of the results obtained in 2018 with the results from 2013 will be made, as well as an assessment of the health safety of their meat for human nutrition. At the same time, information on the concentrations of metal in the liver and muscles of the fish would indicate the degree of anthropogenic water pollution in ecosystem of the Zapadna Morava, as well as the assessment of the health safety of fish fodder for human consumption. According to available literature data, the fish populations in ecosystem of the Zapadna Morava River are largely affected by numerous pollutants, especially those of anthropogenic origin such as industrial, communal water and rural wastewater (Veljović *et al*. l992; Lazić *et al*. 2003; Marković *et al*. 2007).

Material and Methods

A field study of the sample collection in the western Morava River was conducted in June 2013 at the Stančići locality (coordinates: N $43^0$52'15,3" i E $20^0$26'17,1") and during July 2018 at the Parmenac locality (coordinates: N $43^0$53'44,5" i E $20^0$18'15,9"). Fish sampling was carried out using stackable mesh of 20 to 60 mm diameter fenders and a battery electro-picking device (AGK Kronawitter, IG200-2, battery power 12V, 20Ah). The species of fish are determined according to Simonović (Simonović, 2006), and for each fish sample, total body length and weight were measured. After fish dissection, samples of muscle tissue and liver were frozen for analysis. In the laboratory, composite samples for each fish species were prepared separately. The concentration of heavy metals in the muscular tissue and liver of the fish species of silver carp (*Carassius auratus gibelio*), as allochthonous species, bream (*Abramis brama*) and chub (*Squalius cephalus*) as autochthonous species in the ecosystem Zapadna Morava River. As for the selection of heavy metals whose content was analyzed in musculature and liver fish, we defined for cadmium and lead as very toxic metals, zinc due to the competitive relationship with cadmium and a highly toxic animal whose toxic effect on fish is present at very low concentrations in water from the 0,003 mg/l. The analysis were carried out according to the standard methodology for the analysis of heavy metals, using the necessary instrumental techniques. By the method of atomic absorption spectrometry (FAAS flame techniques), the content of cadmium, lead and zinc were determined, while the content of mercury in the tissues and organ was determined by the HGAAS atomic absorption spectrometry method, hydride technique.

Results and Discussion

The concentrations obtained for all four metals in the muscles and liver of the three species of fish from different trophic levels are shown in Table 1. During this study, 15 specimens of silver carp, 9 bream and 7 fish chub were analyzed. The highest concentrations of all four tested heavy metals were found in the liver and the lowest in fish muscle. Metal accumulation had an identical trend in all three types of fish: Zn > Pb > Cd > Hg, and the total content of all four metal fish had the following trend bream > chub > silver carp. In order to determine the health correctness of the meat of the examined fish, the comparison of the obtained values of heavy metals in samples of muscle tissue of fish with the maximum allowed concentrations (MAC) established by the European Union (Commission regulation (EC) No 1881/2006) and national legislation of the Republic of Serbia (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018*)*. According to EU regulations (European Commission Regulation, 2006), the maximum allowable amount of Cd, Hg, Pb is 0.05, 0.50 and 0.30 $mg \cdot kg^{-1}$ per unit of fresh weight. According to the norms of national legislation (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018) MAC za Cd, Hg, Pb is 0.05, 0.50 and 0.30 $mg \cdot kg^{-1}$, while according to the same legal regulation, the maximum content of Zn in fish products in cans is up to 100 mg·kg^{-1}.

Table 1. Average values (± SD) content of heavy metals in the samples of fresh fish (mg·kg^{-1})

2013 year, location Stančić					
Fish species	**Sample**	**Cd**	**Pb**	**Hg**	**Zn**
Silver carp n = 11	muscle tissue	0,004±0,04	0,324±0,15	0,135±0,21	17,392±0,05
	liver	0,084±0,20	0,372±0,03	0,152±0,05	18,190±0,26
Bream n = 8	muscle tissue	0,005±0,08	0,193±0,05	0,098±0,12	19,286±0,05
	liver	0,012±0,05	0,231±0,10	0,112±0,23	32,152±0,06
	∑	0,105±0,37	1,120±0,33	0,497±0,61	87,020±0,42
2018 year, location Parmenac					
Silver carp n = 5	muscle tissue	nd	0,150±0,10	0,110±0,28	19,560±0,06
	liver	0,025±0,31	0,186±0,08	0,150±0,05	21,250±0,14
Chub n = 7	muscle tissue	0,002±0,09	0,090±0,12	0,117±0,12	18,133±0,09
	liver	0,040±0,03	0,156±0,06	0,141±0,07	22,450±0,36
	∑	0,067±0,43	0,582±0,36	0,518±0,52	81,393±0,65

Based on the presented tabular results, it can be concluded that during 2013, the concentration of heavy metals in the musculature of the fish studied was significantly lower than their liver content. The lowest values of metal in the muscle tissue were established for cadmium and varied in the range of 0.004 mg·kg^{-1} in silver carp to 0.005 mg·kg^{-1} in bream. These are the permissible concentrations within the MDC regulations of the EU and national regulations of the Republic of Serbia. The reference concentrations prescribed by the national and EU legal provisions of the EU are determined in terms of the content of other analyzed metals in muscle tissue of the fish. The average lead value was from 0.193 mg·kg^{-1} in the muscle of the bream to 0.324 mg·kg^{-1} in silver carp (maximum of 0,3 mg·kg^{-1} is allowed Serbian Regulation). The zinc content was also in the legal framework for fish products in cans and varied from 17.392 mg·kg^{-1} in muscular weight of silver carp to 19.286 mg·kg^{-1} in muscle tissue.

Similar results, but with a somewhat reduced concentration of metal in fish muscle, were also found during the research in 2018. The concentration of Cd was up to 0.002 mg·kg^{-1} in chub, while Pb concentrations ranged from 0.090 mg·kg^{-1} in chub muscles to 0.150 mg·kg^{-1} in silver carp muscularity. The smallest Hg value was 0.110 mgkg^{-1} in silver carp, while slightly higher values of 0.117 mg·kg^{-1} were recorded in chub. The smallest Zn concentration of 18,133 mg·kg^{-1} was determined in the cluster musculature and the highest of 19,560 mg·kg^{-1} in silver carp. The metal concentrations in muscle analyzed fish did not exceed the permitted values prescribed by national and EU regulations.

By comparing the obtained average values of the content of the tested metals in the muscle tissue of the studied fish from 2018 with the data from 2013 year, it can be concluded that the accumulation of metals in fish from the Zapadna Morava River is significantly lower than the previous period. The total concentration of cadmium in musculature and liver in all types of fish in 2018 compared to 2013 was lower by 36%, lead by 48%, zinc by 6.4%, while the content of mercury was by 4.2% higher. The metal concentrations in the fish organism depend on their type, age, physiological state, type of tissue and feeding (Storelli *et al.* 2006; Szarek-Gwiazda *et al.* 2006). According to Oloy *et al.* (2005) due to the biodegradability and ability to accumulate along the food chain, heavy metals are considered to be one of the primary contaminants of the internal organs of fish and the entire aquatic ecosystem.

The obtained results of the concentration of heavy metals in the musculature and liver of the analyzed fish species clearly show that the aquatic ecosystem of the Zapadna Morava River is not encircled by pollutants from industrial waters, although this ecosystem is their basic recipient. The reason for this is the reduced level of industrialization and the cessation of work of a large number of factories. Determined concentrations of heavy metals in the liver all three types of fish varied within the limits of normal physiological concentrations for all three chemical elements and were below the level of human food tolerance (Biro *et al*., 1991). Lead (Pb), content in silver carp in 2013.year of 0,324 and 0,372 mgkg-1 is above the permitted values.This means that the Zapadna Morava River waterway on the studied profiles can be considered relatively unpolluted in terms of the loads of the tested heavy metals. The results of the research indicate the necessity of continuous sampling and analysis of this water course in order to prevent accidental situations and maintain the health correctness of the meat of fish that inhabit this ecosystem. In order to be able to make a final assessment of the hygienic quality of fish meat for human consumption and the ecological status of this ecosystem, it is necessary to carry out a complex analysis, which would include the determination of the content of heavy metals in water, sediment and commercially important fish species from the ihtiofauna composition. Other authors also point to the necessity of this kind of research (Đukić *et al*., 1998; Farkas *et al.*, 2002).

Conclusions

Taking into account that fish are usually located at the top of the food chain in the aquatic environment, they often accumulate large quantities of certain heavy metals, which is why they are considered one of the most sensitive aquatic organisms for the presence of toxic substances. Since fish meat is one of the basic components of human nutrition, excessive accumulation of heavy metals in fish muscle makes this food unsafe for human consumption. The content of heavy metals (Cd, Hg and Zn) in the muscle tissue of three types of fish silver carp, bream and chub from the Zapadna Morava River was within the boundaries of the maximum perm fish chub issible concentration MAC and in accordance with the prescribed national and EU legal acts on the content of metals and other poisonous substances that can be found in freshwater fish. Lead (Pb), content in silver carp in 2013.year of 0,324 and 0,372 mgkg-1 is above the permitted values. Based on the conducted surveys during 2013 and 2018, it can be concluded that ecosystem of the Zapadna Morava River shows a trend of improving the quality in terms of anthropogenic pollution, because the registered quantities of stored heavy metals in the musculature and liver of the investigated fish varied within normal limits for maximum permissible concentrations.

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DIVERSITY AND DISTRIBUTION OF THE SPECIES OF GENUS *AMARANTHUS* L. 1753 IN VOJVODINA (SERBIA)

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Abstract

The subject of research was diversity and distribution of the species of genus *Amaranthus* in Vojvodina. Genus *Amaranthus* is represented in Europe by 12 introduced and naturalized species, whereas *A. caudatus* is planted as ornamental and it sometimes occurs spontaneously. Other species are weeds or ruderal and common on territory of the northern and central Europe (Aellen and Akeroyd, 1993). In the Serbian flora this genus includes nine species of which all originated from America (Slavnić, 1972). Because of its foreign origin, the absence of predators and adaptive strategies, species of genus *Amaranthus* can easily expand their range in Vojvodina. Based on data from literature and herbarium collections ten species, two subspecies and one hybrid were recorded in Vojvodina. The nomenclature data collected from the literature and herbarium were systematized and harmonized with data provided by Flora Europaea (Aellen and Akeroyd, 1993). Data on general distribution and distribution in Serbia are given according to Flora of Serbia (Slavnic, 1972) and List of invasive species in Vojvodina (IASV, 2017). Data of distributuion in Europe are given by Flora Europaea (Aellen and Akeroyd, 1993) and List invasive species in Vojvodina (IASV, 2019). Floral elements are given to Gajic i Soó (Gajic, 1980; Soó, 1970). Analyzed data covered the period of 80 years, and obtained localities are grouped by regions: Backa, Banat and Srem. Ranges of taxa are shown in UTM maps (Universal Transverse Mercator) of Vojvodina, extent of 10x10 km (Walter and Straka, 1970).

Key words: ruderal flora, weeds, distribution, *Amaranthus*.

Introduction

Weeds and ruderal flora and vegetation of Serbia is very rich and diverse. This is a consequence not only of the impact of anthropogenic factors, but also of geographical location, climatic, geological, geomorphological, hydrological, historical and other circumstances. The area of Serbia is abundant in different categories of weed habitats and is an excellent model for the study of weed vegetation (Nestorović, 2005). Ruderal habitats are open, unstable and subject to rapid changes in floral composition and are found in the living and working environment of man. Anthropogenic impact has a decisive importance for the formation, maintenance, distribution and dynamics of ruderal flora and vegetation. Knowledge of ruderal vegetation is of particular importance for plant production, since many weed plants penetrate from ruderal habitat to arable land and act as strong competitors in crops (Stankovic-Kalezic et al., 2009). The genus *Amaranthu*s comprises about 70 species that are widespread in tropical, subtropical, moderately warm zones, as well as in temperate zones. Genus *Amaranthus* is divided into three subgenus: *Amaranthus* L., *Acinida* L., *Albresia* Kunth (Mosyakin and Robertson, 1996). The species of genus *Amaranthus* forms hybrids. Some species are cosmopolitan and these are mostly introduced naturalized weeds (Mosyakin and Robertson, 2004). The species of this genus were brought to Europe. According to the Flora of Europe, 12 species were naturalized. The species of genus *Amaranthus* in autonomus provinceVojvodina (Serbia), which were examined in this paper, are mostly ruderal and weed plants. The species of this genus are also used in the nutrition of humans and cattle, as medicinal plants, and some species are cultivated as ornamental plants. The aim of this paper is to present diversity of the species of genus Amaranthus in Vojvodina.

Material and methods

Data about distribution of genus *Amaranthus* in Vojvodina province were collected from literature and herbarium collections: Herbarium Department of Biology and Ecology at the Faculty of Natural Sciences of the University of Novi Sad (BUNS), Herbarium of Institute of Botany and Botanical Garden "Jevremovac" of the Faculty of Biology of the University of Belgrade (BEOU), Herbararium of the Natural History Museum in Belgrade (BEO), Herbal collections of the Serbian Nature Conservation Institute, Department of Novi Sad (HIPNS). The nomenclature follows Flora Europaea (Aellen and Akeroyd, 1993). Floral elements are given to Gajic i Soó (Gajic, 1980; Soó, 1970). Data on general distribution and distribution in Serbia are given according to Flora of Serbia (Slavnic, 1972) and according to the list of Invasive species in Vojvodina (IASV, 2019). Data on distribution on Europe are given by Flora Europaea (Aellen and Akeroyd, 1993) List of invasive species in Vojvodina (IASV, 2019). Analyzed data covered the period of 95 years, and obtained localities are grouped by regions: Backa, Banat and Srem. Ranges of taxa are shown in UTM maps (Universal Transverse Mercator) of Vojvodina, extent of 10x10 km (Walter and Straka, 1970). The data is grouped chronologically, and the timeframes are presented on maps: until 1925 the red circle, from 1925 to 1950 the blue circle, from 1950 to 1975 the green circle and from 1975 to 2017 the black circle.

Results and Discussion

Based on data from literature and herbarium collections ten species (*A. albus* L., *A.blitoides* S.Watson, *A. blitum* L., *A. caudatus* L., *A. crispus* (Lesp. & Thév) N. Terracc., *A.deflexus* L., *A. graecizans* L., *A. hybridus* L., *A. retroflexus* L., *A. viridis* L.), two subspecies (*A. hybridus* L. subsp. *cruentus* (L.) Thell., *A. hybridus* L. subsp. *hypochondriacus* (L.) (Thell.) and one hybrid (A. x budensis Prisztez) were recorded in Vojvodina. Of the total number of analyzed species of the genus, as very frequent can be distinguished taxa: *A. albus*, *A. blitoides*, *A. caudatus*, *A. crispus*, *A. deflexus*, *A. hybridus* and A. *retroflexus*. According to the List of invasive species in Vojvodina (IASV, 2019), this species have invasive character in Vojvodina.

Amaranthus albus L. 1759.

Floral element: adventive (Gajic, 1980); northamerican (Soó,1970)
General distribution: Originating in North America, today is adventive in warmer parts of Eurasia, Africa and South America (Slavnic, 1972)
Distribution in Europe: naturalized in almost all of Europe (Aellen and Akeroyd, 1993)
Distribution in Serbia: Vojvodina, eastern, southeast, and western Sumadija (Slavnic, 1972)
Distribution in Vojvodina (figure 1)

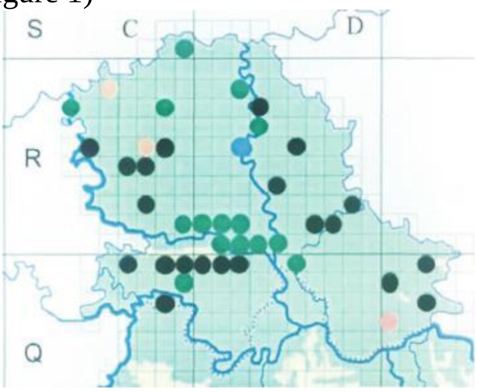

Figure. 1. Distribution of the *Amaranthus albus*, in Vojvodina

Amaranthus blitoides S. Watson 1877.

Floral element: adventive (Gajic, 1980); northamerican – midamerican (Soó,1970)

General distribution: introduced from North America, this species is acclimatized in all the hot areas of Europa and Central Asia (Slavnic, 1972)

Distribution in Europe: naturalized in Central and Southern Europe (Aellen and Akeroyd, 1993)

Distribution in Serbia: Vojvodina, northern, eastern, southern and western Serbia (Slavnic, 1972)

Distribution in Vojvodina (figure 2)

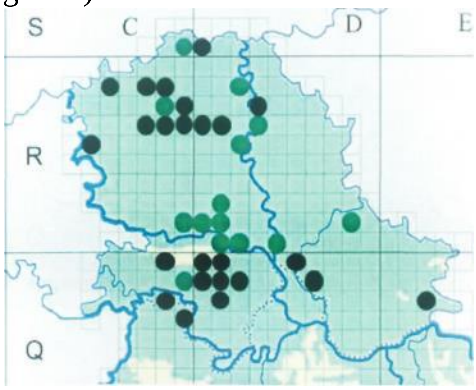

Figure. 2. Distribution of the *Amaranthus blitoides*, in Vojvodina

Amaranthus caudatus L. 1753.

Floral element: adventive (Gajic, 1980)

General distribution: northern, mid, southern America, in warm and moderate zone, eastern Africa and eastern Asia in warm and moderate zone, Australia and Oceania (Slavnic, 1972)

Distribution in Europe: eastern, southern and central Europe (Aellen and Akeroyd, 1993)

Distribution in Serbia: around Belgrade (Slavnic, 1972)

Distribution in Vojvodina (figure 3)

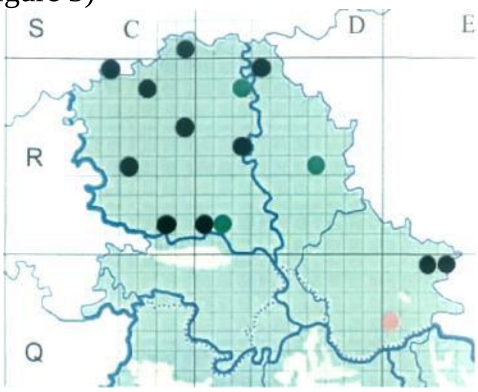

Figure. 3. Distribution of the *Amaranthus caudatus*, in Vojvodina

Amaranthus crispus (Lesp. Et Thév.) N. Teracc. 1890.

Floral element: adventive (Gajic, 1980), southamerican (Soó, 1970)

General distribution: Western Mediterranean and Pannonian basin in other parts of Europe is in expansion phase (Slavnic, 1972)

Distribution in Europe: naturalized in Central and Southern Europe, spread across the whole of Europe (Aellen and Akeroyd, 1993)

Distribution in Serbia: eastern and western Serbia (Slavnic, 1972)

Distribution in Vojvodina (figure 4)

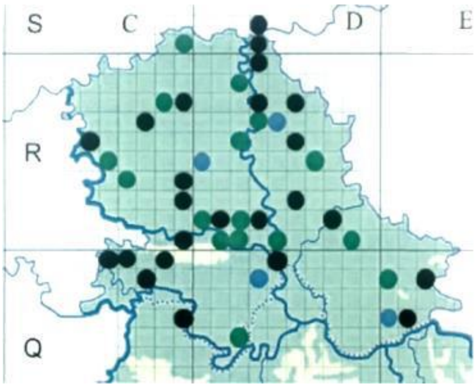

Figure. 4. Distribution of the *Amaranthus crispus,* in Vojvodina

Amaranthus deflexus L. 1771.

Floral element: adventive (Gajic, 1980), southamerican (Soó, 1970)

General distribution: South American plant naturalized in the warmer parts of Europe, North America and Africa (Slavnic, 1972)

Distribution in Europe: naturalized in southern Europe, widespread throughout Europe (Aellen and Akeroyd, 1993)

Distribution in Serbia: Vojvodina, south Serbia (Vranje) (Slavnic, 1972)

Distribution in Vojvodina (figure 5)

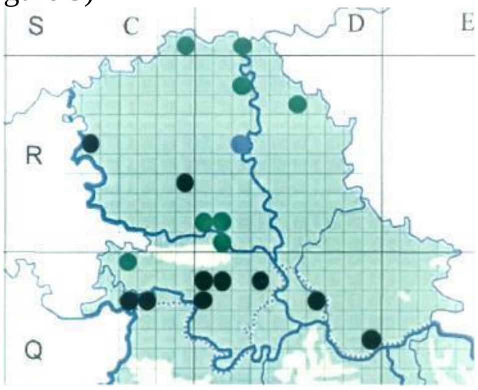

Figure. 5. Distribution of the *Amaranthus deflexus,* in Vojvodina

Amaranthus hybridus L. 1753.

Floral element: adventive (Gajic, 1980), originated from tropical parts of America (Soó, 1970)

General distribution: originating in South America, widespread in North America, North and South Africa and Central and Eastern Europe (Slavnic, 1972)

Distribution in Europe: almost all over Europe (Aellen and Akeroyd, 1993), eastern, southern, central and western Europe (Boza, 2011e)

Distribution in Serbia: Northern (Beograd), western (Sabac, Koviljaca), eastern (Ramski pesak) Serbia, Vojvodina (Slavnic, 1972)

Distribution in Vojvodina (figure 6)

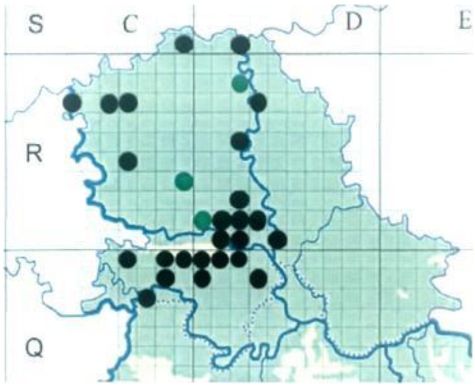

Figure. 6. Distribution of the *Amaranthus hybridus*, in Vojvodina

Amaranthus retroflexus L. 1753.

Floral element: adventive (Gajic, 1980), (northamerican) now cosmopolitan (Soó, 1970)
General distribution: almost cosmopolitan adventive plant, originating in northern america (Slavnic, 1972)
Distribution in Europe: almost all over Europe (Aellen and Akeroyd, 1993)
Distribution in Serbia: very widespread in Serbia (Slavnic, 1972)
Distribution in Vojvodina (figure 7)

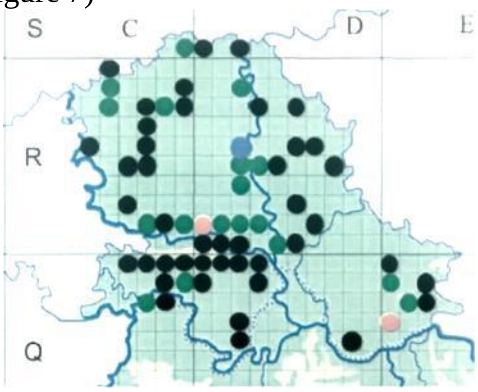

Figure. 7. Distribution of the *Amaranthus retroflexus*, in Vojvodina

All analyzed species are adventive weeds or ruderal plants largely originating from North America, Central America and South America. Genus *Amaranthus* is represented in Europe by 12 introduced and naturalized species, whereas *A. caudatus* is planted as ornamental and it sometimes occurs spontaneously. Other species are weeds or ruderal and common on territory of the northern and central Europe (Aellen and Akeroyd, 1993). In the Serbian flora this genus includes nine species of which all originated from America (Slavnić, 1972). In Vojvodina they inhabit nitrophilic, thermophilic, open anthropogenic dependent habitats. This kind of habitat corresponds to their invasive character in the area of Vojvodina. The species of genus *Amaranthus* today represent significant portion of the Pannonian part of Serbian flora. A significant number of species that were moved to Vojvodina, became naturalized and integrated as a part of the flora, points out enough about the potential of this genus. Because of its foreign origin, the absence of predators and adaptive strategies, species of genus *Amaranthus* can easily expand their range in Vojvodina which is covered with 76% of arable land (Bokic et al., 2012). Analized taxa are potentially marked as the most invasive (because in addition to habitation of the typical artificial, ruderal, arable and disturbed habitats, they also populate natural and salty habitats because of their vastness, high light intensity and small species number. These natural habitats are extreme concerning high salt content and negative water regime, which indicates high adaptability of taxa. The spreading of many alien species is closely related to human activities. Regarding this, these taxa are highlighted, *A. albus*, *A. blitoides* and *A. caudatus*, which appear on arable lands, orchards and ruderal places, where human and his activities predominantly affect the expansion (Bokic et al., 2012).

Although global chronological data is very valuable in the presentation of the total area, data from individual regions contribute to knowing the structure of the area and its more precise boundaries. Invaluable is the importance of such approaches to the analysis of the areals in those species that show the strategy of spreading the areal. The obtained information is of importance in the prediction of areals of alien plants, in particular in the characterization of the invasive character of certain taxa.

Conclusion

Based on data from literature and herbarium collections of ten species, two subspecies and one hybrid were recorded in Vojvodina. Seven taxa are potentially marked as the most invasive (because in addition to habitation of the typical artificial, ruderal, arable and disturbed habitats, they also populate natural and salty habitats because of their vastness, high light intensity and small species number. Ranges of that taxa are shown in UTM maps. The obtained information is of importance in the prediction of areals of alien plants, in particular in the characterization of the invasive character of certain taxa.

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ROOT DISTRIBUTION OF DRIP IRRIGATED CITRUS TREES PLANTED ON RIDGE

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Abstract

This study investigated the effects of drip irrigation on citrus root distribution that were planted on ridge system. The study was conducted in a farm in Zagarlı village of Adana in Turkey. The trees were planted in 3 m intrarow and 7 m row. The orchard size was 364 daa and the trees were 6 years old. Root samples selected randomly from trees representing the orchard by the lateral side (L50, L100) and non-lateral side (Ln50, Ln100) of the tree from 4 different points and four different depths. According to the research findings Nova (*Citrus reticulate Blanco*), Okitsu (*Citrus unshiu Marc*), Valencia (*Citrus X sinensis*), the density of plant roots was found to be decreasing as the horizon deepens and away from the tree trunk. Furthermore, the root distribution on the lateral side was observed to be more uniformed and ampled than the non-lateral lines. It was observed that the root density on the lateral side was higher and uniformed more than on the non-lateral side.

Keywords: *Root distribution, orchard, planting on ridge.*

Introduction

Citrus cultivation is one of the major agricultural production activities which has an important place in fruit cultivation of Turkey. Citrus cultivation is developing at an increasing rate and contributing greatly to Turkish economy. Annual water requirement of citrus varies between 900-1200 mm depending on soil, climate and physiological condition of the citrus tree (Doorenboss and Kassam, 1979). Ridge planting systems were improved especially on citrus planting orchards in Turkey as locally in the Mediterranean part of the country. Inadequate Drainage, extreme winter rainfall and excessive surface irrigation causes fungal disease (Phytophthora citrophthora), damage the tree root and trunk while the ridge planting systems is successfully applied to avoid the negative effects. Citrus orchards are generally established as conventional planting in Turkey. Various researches about plant root distribution in these conventional orchards, which were established in the form of conventional planting and irrigated by drip irrigation. However, the effect of drip irrigation on the root distribution of the plant has not been studied well in the ridge cultivation systems. When there is enough water in the root area to meet plant water requirements, the development of root and stem system is at the highest level. Water, root volume and yield relationships in clayey soils are; a) Insufficient water and large root volume cause less yield b) Sufficient water and mid root volume cause high yield, c) excesive water and low root volume cause low yield (Hagan *et al*., 1967) In this context, the aim of this study is to investigate the effect of drip irrigation on the plant root distribution in citrus trees as planted on ridge cultivation systems.

Materials and Methods

The research was carried out in Turunçgil A.Ş., located in Zağarlı Village of Yüreğir District of Adana Province (Turkey) in summer 2005 as consturucted at 364 da 3x7 m intervals parcels. In citrus orchard, drip irrigation system were used on Nova, Okitsu and Valencia varieties with ridge cultivation system.

The ridges where citrus were planted created before planting during the establishment of the orchard. Ridges are trapezoidal and 80 cm high. The upper width of the ridge is 1.60 m, the base width is 6 m, the ridge edges are 2.3 m and the flat part between the two ridges is 1 m wide (Figure 1).

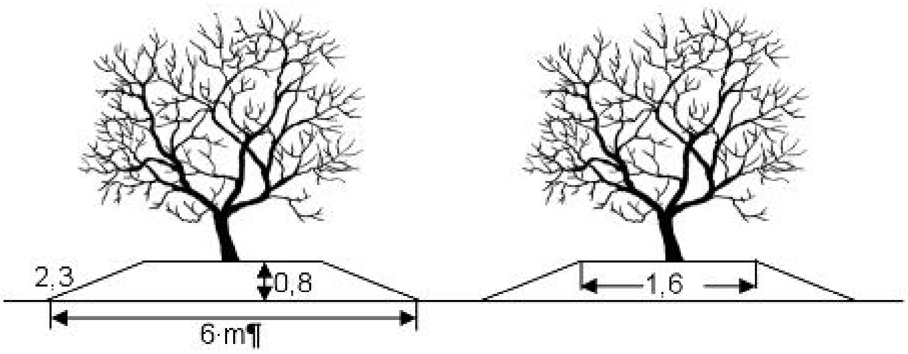

Figure 1. Cross section of ridges in the trial area

The research garden soils are AC horizon soils developed on alluvial deposits of aged river terraces called as Mursel series. In this series of soil pH values varies between 7.1-8.0. The average cation exchange capacity is 23 me/100 gr. The amount of lime varies between 15-30% and the clay content varies between 25% and 42% (Dinc *et al.*, 1990).

Table 1. Some physical and chemical properties of the soil of the trial area

Soil Depth (cm)	Texture (%)			Structure	Salinity dS/m	Lime (%)	pH	Field Capacity (%)	Wilting point (%)	Volumetric Weight. gr/cm^3
	Sand	Silt	Clay							
0-30	15.21	44.87	39.92	Clay-loam	0.50	15.80	7.6	33.58	16.25	1.23
30-60	24.41	42.33	33.26	Clay-loam	0.42	18.33	7.6	33.74	17.83	1.35
60-90	20.25	44.41	35.34	Clay-loam	0.39	18.33	7.6	33.61	18.62	1.41
90-120	43.53	29.64	26.83	Sandy-Clay-loam	0.28	22.12	7.7	33.41	17.54	1.33

Table 1 reflects that the soil in the trial area field capacity is 33.58-33.74%, wilting point 16.25-18.62%, volume weight 1.23-1.41 gr/cm^3, lime content was found to vary between 15.80-18.33 and pH value was 7.6. The parcels tested have a clayey loam structure and no problems were detected in terms of salinity.

Irrigation water is supplied from a 170 m deep well in the citrus orchard where the survey was conducted. Water samples were taken from the inlet and outlet of the irrigation system and some physical and chemical properties were analyzed in the laboratory according to USSL (1954), and the analysis results are given in Table 2. Irrigation water is classified as C2S1.

Table 2. Some physical and chemical properties of irrigation water

pH	Salinity dS/m	Cation me/l					Anion me/l					SAR
		Ca	Mg	Na	K	Tot.	CO_3	HCO_3	Cl	SO_4	Tot.	
6.8	0.476	0.98	2.09	1.96	0.06	5.09	-	2.50	1.63	0.94	5.09	1.59

Three trees were chosen by random sampling method. For each of the selected sample trees, samples were taken up to a depth of 50 cm (L50) and 100 cm (L100) at a distance of 120 cm from the tree trunk to the canopy area in the lateral direction (Figure 2). Similarly, a total of 16 samples from 50 cm (Ln50) and 100 cm (Ln100) distances from the non-lateral direction were taken after irrigation in July 2006 with the aid of a Viehmeyer Pristine soil sampling device (Yeşilsoy, 1976). The inner wall of the tube is lubricated to allow the Viehmeyer tube to advance more easily in the soil and to take out from the block-shaped soil. The tractor power was used to remove the Veihmeyer tube.

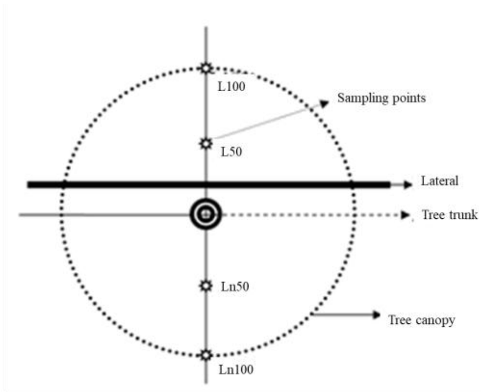

Figure 2. Schematic representation of root sampling points and root sampling with Viehmeyer intact soil sampling tool

Root samples were removed from the 120 cm profile in blocks with the help of Viehmeyer pristine soil sampling device and then cut into 30 cm layers. The samples taken in the field were labeled and packaged and brought to the laboratory.
The samples were taken into plastic containers and 0.1N NaOH solution was added and left to stand for 24 hours. A solution of 0.1 N NaOH (prepared by adding 1 liter of pure water over 4 grams of Na OH) lead to the heavy soil for dispersion. The samples were then taken into larger containers one by one, diluted with water, passed through 8.0, 6.3, 4.0 and 1.0 mm meshed filters to separate the roots.
Statistical analysis; SPSS in statistical analysis of collected data on trial subjects package program was used. LSD method for comparison of means was done.

Results and Discussion

Samples taken at 4 different depths at 50 cm and 100 cm distances from the both side (lateral side, non-lateral side) of the tree trunk for the distribution of the extracted roots determination as weight percent.
Root samples taken from a distance of 50 cm in the direction of drip irrigation lateral of the tree trunk were examined and the calculated root densities are given in Figure 3. When the root densities of all 3 cultivars are examined, Valencia and Okitsu varieties have a similar root distribution in the roots located 50 cm away from the stem, while it is seen that 30% of the roots are located in 90 cm in Nova cultivar.

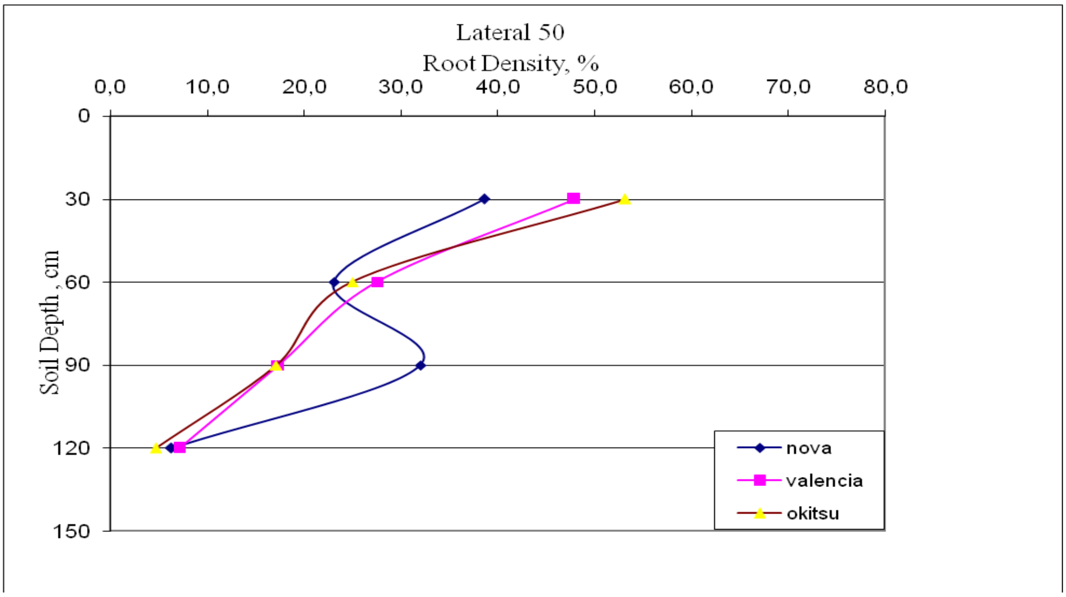

Figure 3. Root distribution according to depths at a distance of 50 cm in the direction of lateral side.

It is seen that the effect of water on the root distribution decreases as it moves away from the trunk and the lateral. When the Figure 4 is examined, it is seen that the roots are collected at the first 30 cm and the density of the roots decreases as the horizon deepens. It is seen that the root distribution of Nova cultivar is different from the other two varieties and the roots are not dense at surface but more uniformly distributed.

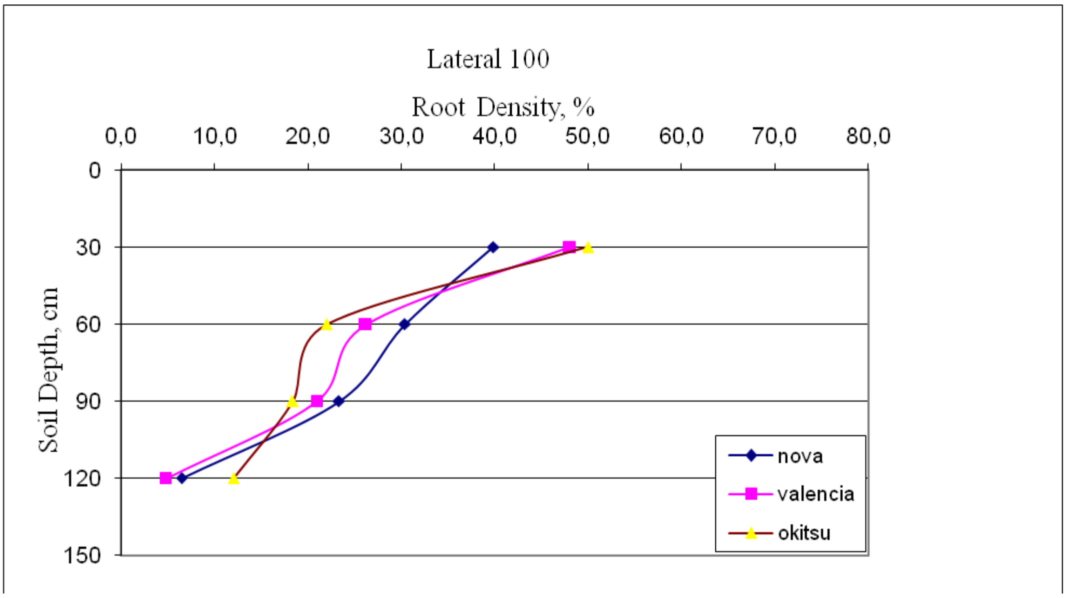

Figure 4. Root distribution according to depths at a distance of 100 cm in the direction that lateral side.

As the non lateral side away from the trunk, the roots showed a more homogeneous distribution. When the Figure 5 is examined, it can be seen that a large part of the roots were located near the surface for all three varieties.

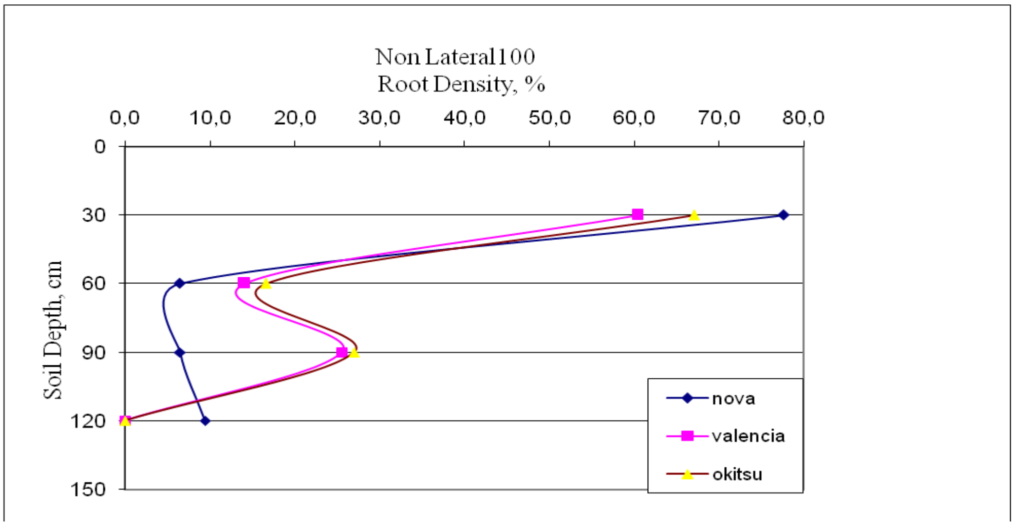

Figure 5. Root distribution relative to depths at a distance of 100 cm in the direction that the non-lateral side.

Juan (1977) stated that, citrus relative root density was decreased under the first 60 cm depth away from the tree trunk. Kanber *et al*. (1996) stated that the root density values obtained in the study decreased as the citrus root density moved away from the tree trunk and deeper in the soil horizon. Berkman (1996) was found that citrus, which is an outgrowth root structure, decreases as the roots move away from the tree trunk and deeper in the soil horizon, and differences in effective root depths occur depending on the water consumption of the grafted species in Karataş region of Adana. Kanber *et al*. (1996) reflect that the use of a single lateral drip irrigation systems and the wetting rate according to the percentage of the cover stated that the insufficient irrigation water is given. Root samples taken from a distance of 50 cm in the direction not exceeding the drip irrigation lateral of the tree trunk were examined and calculated root densities are given in Figure 6. When the root densities of all 3 cultivars in the experiment were examined, a similar root distribution was observed in the varieties of valencia and okitsu in the roots located 50 cm away from the tree trunk, while 70 % of the Nova cultivars were concentrated in the first 30 cm and had a root structure.

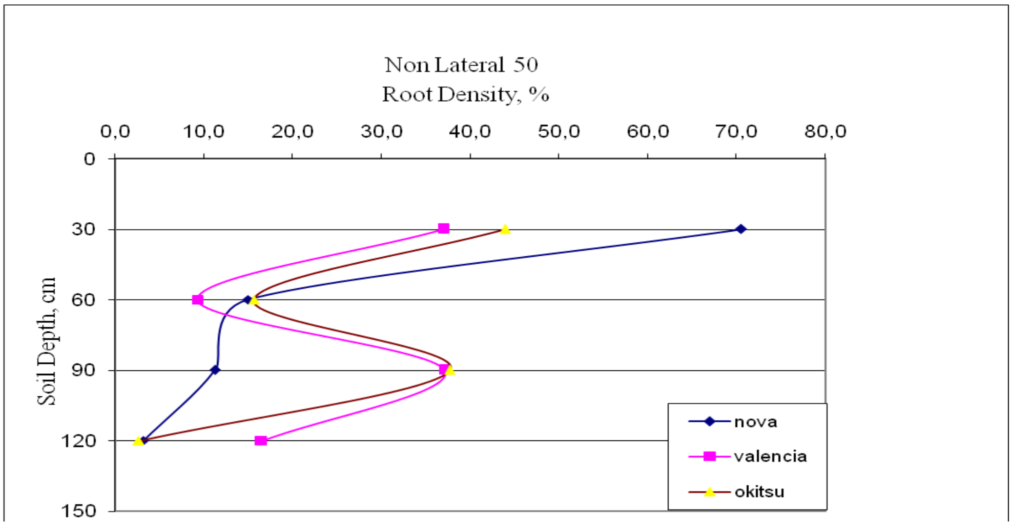

Figure 6 Root distribution relative to depths at a distance of 50 cm in the direction that the non-lateral side.

As the non lateral distance from the tree trunk, the roots showed a closer distribution to the soil surface. When examined in Figure 6, it can be seen that most of the roots of all three varieties are collected near the surface.
According to the results of the analysis of variance to determine the effects of lateral side and variety on plant root density (Table 3) separately, the direction of drip irrigation laterals side on the plant root densities and the varieties had significant effects at 1% significance level.
The results of this variance analysis reflects that the root development has significant differences between the lateral side and non-lateral sides. It also shows that the varieties gave different reactions to root development (Table 4).

Table 3. Root Density of Cultivars (gr/m^3)

		Cultivars			
	Reiteration	Nova	Valencia	Okitsu	**Average**
Lateral	1	2012.7	1159.2	2093.4	**1758.1** a**
	2	2059.4	1244.2	2617.0	
	3	1753.7	1027.6	1855.6	
Non-Lateral	1	845.0	233.5	530.8	**611.4** b**
	2	1273.1	106.2	836.5	
	3	743.1	144.4	789.8	
Ort.		**1447.8** a**	**652.5** b**	**1453.8** a**	

LSD: 345.3

Table 4. Variance analysis results related to the amount of root

Variation	Degree of Freedom	Mean error square	Mean square	F
Reiteration	2	290366.674	145183.337	5.0127
Cultivar	1	5917258.266	5917258.266	204.3023 **
Eror	2	57926.510	28963.255	
Lateral	2	2549399.756	1274699.878	40.1164 **
Cultivar X Lateral	2	234653.331	117326.666	3.6924
Eror	8	254200.233	31775.029	
Total	17	9303804.769		

The aim of this study is to determine the root distribution in citrus orchard planted on the irrigated ridge by drip irrigation method. At the end of the studies, the following results were obtained. Root samples taken 50 cm away from the trunk were examined on the lateral side of the tree trunk. When the root densities of all 3 cultivars in the experiment were examined, a similar root distribution was observed in the varieties of valencia and okitsu in the roots located 50 cm away from the tree trunk , while it was observed that 30% of the roots were located at 90 cm. As a justification for this situation, it can be said that the plots where Nova varieties are used have more frequent irrigation application and this affects root development. When the root densities of all 3 varieties in the experiment in the non lateral side, a similar root distribution was observed in the Valencia and Okitsu varieties in the roots located 50 cm away from the tree trunk while 70% of the Nova cultivars were concentrated in the first 30 cm.
In the study, the root density was found to be higher in all three cultivars in the lateral side. Analysis of variance revealed that the root development showed significant differences between the lateral and non-lateral sides. This can be explained by the fact that the roots are oriented towards the side where they can easily find and use water. When the varieties were examined separately, it was seen that Valencia and Okitsu varieties on the same ridge showed

very similar root distribution. In the same parcel with the lemon variety meyer Nova has been found to have more root distribution. The reason for this is that the water demand of the citrus varieties varies and accordingly the irrigation in this parcel is done according to the lemon variety which has higher Evapotranspiration ET value. Because of this situation, the roots of the Nova variety found in the same parcel as lemon found the water they needed easily in the upper layers of the soil, and showed a more rooted distribution than the other varieties tested. And deep root development can be said to be less compared to Okitsu and Valencia.

Conclusions

It would be a more appropriate approach for irrigation programming and root development if the producers use the varieties with similar water consumption in the same parcels.

Considering that root development is more intense on the lateral side, the producers' use of systems or lateral designs that can supply water to both sides of the tree will provide a more uniform root distribution and the trees will be less affected by the physical damage caused by strong winds due to the developing stronger root structure.

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SUSTAINABILITY OF RICE PRODUCTION SYSTEMS: AGRO-ECONOMIC ANALYSIS OF *BAIXO MONDEGO* AND *LIS* IRRIGATION DISTRICTS, PORTUGAL

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Abstract

Rice crop has an important economic and social value in Portugal, being cultivated under continuous flooding irrigation. Despite the technological advances achieved in the last decades in Portugal, such as the common use of laser precise land leveling, the problems of water management are yet frequent. To cope with the global changes and the raising social pressure to reduce water consumption and the environmental negative impacts, there is an emergent consensus to improve water saving methods and technologies, for water use efficiency and the safeguard environmental quality. A research project into solving this problem is underway. It deals with the evaluation of water saving solutions, and the assessment of agro-economic sustainability. The case studies here reported refer to Lower Mondego Valley and *Lis* Valley Irrigation District, located on Center of Portugal. These perimeters have a Mediterranean Temperate climate, with critical issues such as water scarcity, soil salinization risks, and economic sustainability. The applied methodologies consider: I) Monitoring of the water irrigation use of on-farm systems, both the traditional practices and the newly water saving techniques; II) Evaluation the effect of water saving technologies on cropping system operation; III) Development of performance indicators, to allow the comparison with other regions. The evaluation of the sustainability of rice production will use several data sources with *stakeholders* support, including data collection in field parcels, irrigation district records of crop performance, farmers' questionnaires and interviews, social acceptability, and environmental issues. This communication will present the preliminary results of referred research project, particularly farmers' socioeconomic data.

Keywords: *Rice sustainability, Rice continuous flooding, Rice water saving, Lower Mondego Valley, Lis Valley.*

Introduction

Rice is the most cultivated and the most consumed cereal in the world. Rice crops, together with Wheat and Corn, occupy more than half of the world's agricultural land and, even though rice is neither a staple food nor an important crop in Europe, it has important socio-cultural significance and environmental importance in several European Mediterranean countries. The total area of rice cultivation in the 27 member countries of the European Union (EU) is about 472 thousand hectares, the average annual rice production is about 3.1 million tons and the annual average import of rice is 1,1 million tons. In 2015, the rice-growing cultivated area of Italy and Spain together comprised around 75 % of the total area, *i.e.* around 360 thousand hectares. (Kraehmer et al., 2017). Greece and Portugal account for around 12% of Europe's rice production (Pinto, 2015). The world's demand for milled rice can be expected to rise

from 439 million tons in 2010 to 496 million tons in 2020 (World Atlas, 2019). The annual *per capita* consumption of milled rice ranges from 3.5 to 5.5 kg in non-growing countries of northern Europe to 6-18 kg in southern Europe. Portugal has the highest European *per capita* consumption of rice, which is 17 kg *per* year. This figure, no more than one tenth of the Asian average, far exceeds consumption in other European countries, including Italy (Ricepedia, 2019). To improve rice cultivation and water management, with the aim of improving their competitiveness and environmental sustainability, some Mediterranean countries have joined together in a Prima Program research project, named MEDWATERICE. It deals with the quantification of water use efficiency and environmental, economical, and social sustainability of innovative water saving irrigation options, compared to the traditional flooding irrigation practice. Alternative irrigation techniques to be tested will be selected taking considering the specific characteristics of rice production, on the basis of available data and *stakeholders'* (SHPs) knowledge. A multi-scale – multi-disciplinary and multi-actor – approach is used in the project, which goes beyond the state-of-the-art in five dimensions.
The first ground-breaking objective is methodological: the introduction of participatory action research as innovation strategy in the Mediterranean rice sector. MEDWATERICE will use participatory action research to test non-conventional irrigation-efficient methods tailored to local conditions. Secondly, the project will develop a comprehensive multidisciplinary indicators-based tool to assess the overall sustainability of rice systems at the on-farm and district scales. Production with sustainable methods have the added advantage of being people and planet-friendly and of having greater chance of success and higher prices in the market. Thirdly, MEDWATERICE will build a novel framework for computing effective water efficiency and productivity by up-scaling farm efficiency data to irrigation district scale, in order to prevent fake or rebound effects of water saving measurements. Given that constraints on rice irrigation improvements are often connected to the difficulty to monitor and control irrigation inflows and outflows, the fourth action of the project will use state-of-the-art hydraulics and sensors to monitor and support water saving management practices.
Additionally, this will be a contribution to the implementation of the EU Water Framework Directive whose requirements for all water uses measurements is clearly unfulfilled in the rice sector. Finally, the set of water-saving techniques, proven in the field and ready to be adopted, which the project will produce, is unique in the Mediterranean basin; firstly because of the innovative nature of some of the techniques and secondly because of the methodology and approach to transnational research that will give them unprecedented strength and robustness. To the best of our knowledge, none of these five dimensions have been explored exclusively in Mediterranean rice agroecosystems in order to improve irrigation efficiency and overall sustainability. The development of water management practices as an alternative to continuous flooding is imperative to enhance water use efficiency and safeguard environmental quality in Mediterranean rice agroecosystems. (Masseroni *et al.,* 2017). The agroeconomics study considers the development of a set of indicators, which in turn will allow the comparison of water saving technologies, to assess the evaluation of rice production sustainability. Data collected through farmer's surveys and interviews is analysed and processed. This project meets the recent innovation system PROAKIS (Labarthe, et al, 2014) which stipulates that innovation is set up by stakeholders, including farmers, researchers, advisers, and companies operating in the agricultural and agri-food sector.

Materials and methods

Description of case studies: Lis: Valley Irrigation District and Lower Mondego Valley
The Lis Valley Irrigation District (LVID) is a state initiative, with a total area of about 2000 ha, it is an agricultural perimeter of the Centre of Portugal, located in the municipalities of Leiria and Marinha Grande, whose irrigation and drainage system dates from to 1957. The

main problems of water management in collective irrigation and drainage networks, as well as the field level, are due to the scarcity and poor water quality in the summer, flood risk and poor drainage, and incipient source of hydraulic and hydrological information to support the Lis Valley Water Users Association (ARBVL) planning and operating the network so that optimization of water productivity is achieved, and farmers' income is improved. Under the rural development program, modernization works are planned to transform water distribution networks (in sub-perimeter I, downstream), which will stimulate agricultural development. The dominant LVID soils are modern alluvial soils of high agricultural quality, some subject to poor drainage. The works present defence objectives of the fields through slope collectors, valley drainage and irrigation carried out with the application of several reservoirs from the Lis River and its tributaries (Fig. 1). The Perimeter is structured in seven hydraulic blocks designed according to the logic of the drainage network. The irrigation network comprises 17 dams and 15 pumping stations (PS), collecting water from the Lis, tributaries and drainage ditches, running by water through a gravity network with channel water conduction. The length of the primary irrigation network is 44,5 km; the secondary irrigation network consists of 180 km of small lined or earthen channels. In terms of water distribution, the most important problems are water shortage and poor water quality in the dry summer period, and the absence of automation mechanisms to control water levels in the network channels, leading to malfunctions and high labour load. The Baixo Mondego Irrigation Scheme (Lower Mondego Valley) has an area around 12,000 hectares and is located along the Mondego River valley, from Coimbra to Figueira da Foz. It is 5 km long and 4 km wide. Only 6,538 hectares of the potential irrigated lands are equipped: the secondary Ega and Arunca valleys and Pranto and Cernache streams, on the left bank, as well as river Foja and ribeira de Ançã, on the right bank, still lack infrastructures. The water source is a dam located in Coimbra, out of which a multi-purpose canal was built (for the irrigation of the right bank). The left bank is served by a water main installed along the left stripe irrigation fields. The irrigation scheme is divided into 18 irrigation blocks and, except for São Martinho and São João block, covering 696 hectares near Coimbra, irrigation infrastructures were designed and built for gravity irrigation. The predominant crops are maize and rice, with an area of more than 90%. In the areas of the secondary valleys, these are also the dominant crops, but with greater emphasis on rice. The total number of irrigation plots is around 16,600 and the number of farmers around 2,000. At this stage an inquiry was made, attempting to cover the different typologies of agricultural enterprise This approach allows obtained indicators that are "based on real farm conditions and represent the current agricultural management practices" and capture significant information on impacts on agricultural systems and simplify complex concepts such as sustainability (Dantsis *et al*. 2010)

The survey was conducted in person and at the site of the farms. The survey presented four sets of questions that allow (i) socio-economic characterization of the farmer; ii) characterization of production factors use; iii) optimization of production costs and lastly the characterization of production according to its environmental sustainability, especially in relation fertilizers and pesticides use. The questions were closed, single choice or multiple choice in as many as two possible choices. It attempted to evaluate the degree of satisfaction with the options taken in relation to certification mechanisms and commercialization channels. The concern with agrienvironmental issues was also evaluated. Some results are present in this paper.

Results and discussion

The survey was carried out in June 2019 in the *Mondego* Valley with a total of 34 surveys (20% of rice farmers in the Mondego Valley). These farmers hold 767 hectares (42% of the total rice area in the Mondego Valley). SPSS 25 software was used to analyse the data. The correlation between the coefficient of Pearson (r) and Spearman's coefficient (ró) was used to measure the strength of the association between the variables. According to the production area four farmer/producer classes may be considered. (Table 1)

Table 1 – Categorization of sample and farmers population in relation to production areas

Hectares	Farmers Survey		Rice Farmer		representative sample
	Number	%	Number	%	%
≤6,30	5	14.7	103	14.7	4.9
≥6.31 and ≤ 22,60	16	47.1	52	47.1	30,8
≥22,61 –and ≤ 38,80	5	23.5	8	23.5	62.5
≥38.81	5	14.7	11	14.7	45.5
Total	34	100	174	100	19.5

The study carried out with rice producers, has showed 82.4% of the interviewees are full-time farmers, 2.9% are part-time farmers and 14.7% are retirees from farming or other economic activities. Around 32.4% of the respondents are between 20 and 40 years old and 26,6% are over 60 years old; 62% had access up to the ninth year of schooling, while 26,5% have a higher education degree All farmers apply integrated production methods. When compared to national results, these values are better in terms of both farmers' age and at educational level. National average show 71.4% of the farmers hold basic education and only 5.8% possess higher education; in terms of age at national level, 54.6% are ≥65 years old (INE, 2017). There was a weak correlation which is significant at 5% or 1% of level of significance between the relevance of the agricultural activity and farmers educational level and age. For younger farmers, agriculture becomes more important as a main activity; they have a higher education level, but there is no correlation between the characteristics of the farmer and the size of the farm. Access to land is an important issue for generational turnover (Table 2).

Table 2 – Socioeconomic correlation between economic variables

	Education Level		Age Class		Size famer (há)	
Variables	Spearman (rô)	Pearson (r)	Spearman (rô)	Pearson (r)	Spearman (rô)	Pearson (r)
Farmer relevance activity	-,364*	-,350*	,517**	,569**	-0,013	0,036
Sig. Level	*0,034*	*0,043*	*0,002*	*0,000*	*0,942*	*0,840*
Education Level			-,748**	-,720**	0,088	0,067
Sig. Level			*0,000*	*0,000*	0,622	0,705
Age Class	-0,748	-0,720			0,027	0,076
Sig. Level	*0,000*	*0,000*			*0,880*	*0,669*
Size famer (ha)	0,088	0,067	0,027	0,076		
Sig. Level	*0,622*	*0,705*	*0,880*	*0,669*		

* significance level of 5% (p=0,05); ** significance level of 1% (p=0,01

About 77% of the surveyed area is occupied by Carolino rice (Oryza L, subspecies Japónica), Ariete cultivar. Although the European Commission recognized the name "Arroz Carolino do Baixo Mondego" as a Protected Geographical Indication (PGI) in 2015, none of the respondents stated having the PGI certification. All respondents applied for agricultural support (within the Rural Development Program), over the past ten years. There were three measures respondents did not apply to. Applicants degree of satisfaction concerning the results of the applications and their development is high and they feel satisfied or more than satisfied with the progress of the measures on their farms (Table 3)

Table 3 – Application for RDP measures over the past ten years and satisfaction degree

Measures	Advice Service	Modernization	Greening	Organic farm	Integrate d farm	Diversification	Marketing and processing	Young Farmer	Basic payment include rice grant
Application	33	7	34	0	31	0	0	8	33
% in total survey	97%	21%	100%	0%	91%	0%	0%	24%	97%
	Satisfaction degree in percentage for each application support (% in total of applications)								
Partly Satisfied	9,1								3,0
Satisfied	66,7	28,6	38,2		32,3			12,5	42,4
More than Satisfied	21,2	71,4	44,1		48,4			75,0	42,4
Very Satisfied	3,0		17,6		19,4			12,5	12,1

All respondents want to continue farming in the next two years. Respondents could choose at most two main reasons for continuing agricultural activity. Economic viability and family income complement represented 24.3% of the responses, respectively, but 21.6% answered that agricultural activity would continue because of the lack of economic alternatives. Concerning rice cultivation, only one respondent (2.7%) answered he does not intend to keep producing rice because of the lack of economic viability of the crop. About 89,2% want to continue producing rice, and 45.5% said they keep growing this crop because alternative uses for their land are not available. About 24.2% consider that knowledge of the crop characteristics was an important factor to continue growing rice and 21.2% consider that rice economic viability justifies the option to go on growing rice. Considering marketing channels used by farmers, only one farmer sells 80% of his products to retail, under his own brand name, and the remaining 20% are sold to industry. About 59% of the farmers sell their yield directly to industry, and about 29% sell their production to the Montemor-o-Velho Cooperative. Only three producers use two marketing channels simultaneously. Thirty-two respondents (94%) answered the question concerning the commercialization channel which allowed the choice of two options, having provided 55 responses. About 22% of the respondents have chosen a marketing channel due to "production flow ease", and 20% have done it because they have no processing capability. About 16% have chosen the option "guarantee production output", and 15% have chosen "price stability". The options "best price" and "fastest payment" have been chosen by 13% and 11% of the respondents, respectively. Only two interviewees (4%) have chosen the channel because of the support given to production. These preliminary results of the survey demonstrate that rice cultivation is done by farmers with both knowledge in the area and skills to use community and national support measures. More data have been obtained to allow the development of socio-economic and quantifiable indicators of the environmental sustainability of agriculture (Reytar *et al.*, 2014). Studies are being conducted in different areas of the world to study alternatives to rice production systems with the aim of increasing productivity, net income and at the same time reduce environmental impacts (Sita and Ponnarasi, 2009**)**. The sixth task, fundamental to deal with knowledge transfer and innovation, is being carried out.

Conclusions

Among the numerous problems Mediterranean rice agroecosystems are currently facing, sustainability is of utmost importance and a great challenge. Due to drivers such as climate change, population growth, increasing concern of civil society towards environmental impacts of human activities, there is pressure on the rice production sector to reduce water consumption and minimise environmental impacts (OECD, 1993). At the same time, rice is a strategic production for food security, a nutritional alternative to wheat, and an important economic exchange-earning agricultural product. Therefore, there is great pressure to increase rice production within the Mediterranean basin, but these pressures have not changed much the state of rice production yet; continuous flooding is still predominant. So far, results have shown a dynamic sector in need of options to deal with new challenges, such as consumer perception of water use in agriculture and profitability increase generated by inputs efficiency boosting. The interest of this project is observable in the support farmers and Water Users Association have provided to the development of the work, which is a key indicator of the need for applied research to the business sector in which it operates.

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POSSIBILITY AND EFFECTIVENESS OF MICRO-ORGANISMS IN PURIFICATION OF WASTE WATER, LAGUNA AND WATERWAY

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Abstract

The quality of life on Earth in the future will largely depend on the amount of safe water. Any use of water from numerous sources for different purposes, leads to negative changes in its physical, chemical and biological characteristics, resulting in the formation of wastewater in many forms, as industrial, communal, agricultural or rain. This type of water should be collected, and purified using efficient and economical cost-effective technology, or otherwise pose a serious environmental problem. In order to stop this trend, a large number of countries are undertaking extensive measures and invests large resources in order to stop water pollution and return pure, unpolluted water to nature. In this paper, possible biological procedures for purification of the Ada Huja Lagoon Belgrade, using microorganisms from the genus *Bacillus*, are presented. The Laguna Ada Huja is the recipient of all the listed types of wastewater and as such represents a serious environmental problem. The experiment was performed in laboratory controlled conditions using two different doses of microorganisms. Measurements of essential parameters (pH value, Ammonium ion, Nitrites, Nitrates, Consumption of $KMnO_4$, Chemical consumption of oxygen, Biochemical consumption of oxygen (BPK), Total organic carbon (TOC)) were performed for 1, 15 and 60 days from wastewater treatment. It has been found that proposed technology, a mixture of heterotrophic, aerobic and optional anaerobic microorganisms produces extracellular enzymes for increased degradation of organic compounds in aforementioned environmental conditions. The use of these microorganisms leads to a decrease of the wastewater BOD/COD content, in addition, it reduces the amount of waste suspensions, reduces the filamentous bacterial reduction of the sludge population, and improves the characteristic of deposits, and therefore wastewater meets the regulated boundaries of the outflows and discharges into the watercourses.

Keywords: *wastewater treatment, lagoons, channels, environmental protection, microorganisms.*

Introduction

Municipal wastewater from industry is a major source of pollution in all environments. Municipal wastewater untreated by any technology (mechanical, chemical, biological), directly discharged into watercourses causes pollution of surface and groundwater as well as soil. The biological treatment of industrial and domestic wastew-ater is an important biotechnological application, constituting amultibillion-dollar global industry. When microbial populationsare enhanced in wastewater treatment systems, their benefi-cial activities result in the efficient removal of organic matter,toxic substances, nutrients and pathogens (Wang *et al.*, 2012). Despite the economic and environmental importance of these processes, understanding of the microbial communities existing in wastewater treatment plants and their potential for continued use is still limited, especially in developing countries. Bacteria are important contributors to the transformation of complex organic compounds in

wastewatertreatment systems and are essential for the optimal operation andpreservation of biological treatment systems (Moura *et al.*, 2009).
Special interest has been placed treat wastew-aters with high levels of organic pollutants in the form of fats, oilsand greases (FOG), since the removal of these substances repre-sents an important wastewater treatment challenge at a globallevel (Facchin *et al.*, 2013).
Several techniques involving microorganisms have been complemented in complementary treatment systems to processwastewater with high FOG content. These techniques have used enzyme extracts of pure cultures obtained by solid state fermentation of agro-industrial residues (Leal *et al.*, 2002) or active consortia grown in the wastewater to be treated (Fadile *et al.*, 2011).
Previously, Bacillus sp. has been described as a novel strain with a good performance of nitrogen removal. For example, Bacillus sp. LY was isolated from the membrane bioreactor system in which the efficiency of TN removal was up to 80%. After 24-day incubation, the removal efficiency of COD by Bacillus sp. LY was 71.7%. It also can denitrify nitrate while nitrifying (Lin *et al.*, 2007). The Bacillus sp. strain YX-6 is highly effective in removing nitrite. It could degrade the nitrite nitrogen (nitrite-N) from 10 mg/L to zero in 14 h and the nitrite-N degradation rate was approximately to 100% at the DO concentration of 5.2–5.8 mg/L (Song *et al.*, 2011.
Nitrogen has become a key factor leading to the eutrophication of receiving waters. Accordingly, in order to reduce its discharge, increasingly stringent environmental regulations are carried out, under which circumstance it is urgent to create a novel technological solution to improve nitrogen removal. As a fact, many solutions such as physical, chemical and biological methods have been applied widely, among which biological methods seem to be more promising for some advantages. It is well known that conventional nitrogen removal is composed of two steps: (1) nitrification by autotrophs under aerobic conditions and (2) denitrification by heterotrophs under anaerobic conditions. As such, this technology usually requires extra facilities and land, resulting in high costs of treatment. Thus, it is especially necessary to explore more cost-efficient and less land-occupied technology to remove nitrogen from wastewater. Simultaneous nitrification and denitrification (SND) might be an alternative, and a variety of microorganisms have an ability of SND like Acinetobacter calcoaceticus (Zhao *et al.*, 2010), Pseudomonas stutzeri (Zhang *et al.*, 2011), Halomonas campisalis (Guo et al., 2013).
This study aimed to determine whether bioaggregation with the strain Bacillus sp. and enzymes could successfully improve the removal of nitrogen, organic matter, under laboratory conditions. Attention was focused on the stability and efficacy of microorganisms in such an environment and on detecting the possible path of transformation of the present nitrogen compounds based on the measured data (pH, Ammonium ion, Nitrites, Nitrates, Consumption of KMnO4, Chemical oxygen consumption, Biochemical oxygen consumption (BOD 5), Total organic carbon (TOC). This study is of great importance to investigate the potential application of strains of microorganisms and enzymes and to establish an effective wastewater treatment system in lagoons and lakes.

Material and Methods

The Danube lagoon "ADA HUJA", Belgrade represents a water habitat of about 30 ha, which in the past few years has been the wastewater collector of the Mirjevski Potok. For many years, the lagoon was transformed into a fecal recipient. Samples of 50 l wastewater were taken from the most contaminated part of the lagoon (X 44o 49 "10.41" "Y 20o 31" 14.75 "" Z 69 m) for laboratory investigation. Homogenization (obtaining a representative sample) of the waste water samples "NULTI-Control sample" was performed under laboratory conditions. Experiments are performed in plastic buckets with a volume of 10 liters, with the following variants: 1. Control (untreated sample), 2. Variants 1 - treated wastewater sample with lower dose, optimal condition, 3. Variants 2 - treated wastewater sample with higher dose. Samples were left for two days under laboratory conditions to stabilize the optimum wastewater temperature for microorganisms (Bacillus sp) and enzymes: 22-25 ^{o}C. Wastewater quality was controled and monitored by measuring pH value, ammonium ion content, nitrite content, nitrate content, potassium permanganate consumption, chemical oxygen demand (HPK), biochemical oxygen consumption (BOD 5), total organic carbon (TOC). Controling was done at two time intervals of 15 and 60 days from biological treatment. Catalysts were added to maintain the optimal C: N: P ratio.

Results and Discussion

The chemical and biochemical changes of the untreated wastewater sample (Control) as well as the changes that occurred after the treatment of the wastewater with two different dose-variants are shown in Table 1.

According to results, it can be concluded that the Ada Huja is seriously endangered area and due to it cannot be used in the purpose as an Eco Zone for sports, recreation, rehabilitation and business.

The wastewater pH in the Control sample showed increased value and increases from 7.6 to 7.9 after 60 days. In the treated samples the pH value decreases from 7.7 to 7.6 in the first 15 days and from 7.1 to 7.2 after 60 days.

The average concentration of ammonia nitrogen in the control sample is 32.36 mg N / l which is significantly higher than legal values for V class water (1.5 mg N / l). After biological treatment of wastewater, there was an increase in the content of ammonia nitrogen due to both: bio-enzymatic activity and partially added catalysts, nitrogen-phosphate compounds for biological activation of microorganisms.

The concentration of mineral forms of nitrogen is low in the control sample of wastewater, so that it can be classify to the first class. In the first 15 days of biological treatment the NO_2 and NO_3 content decreases and after 60 days significantly increases.

The chemical oxygen consumption values of HPK-Kubel ($KMnO_4$ consumption) in the control sample are very high. The maximum measured value in the sample "Control 60" was 1286.5 mg / l, which belongs to the V class (Legislation 50/12), ie. corresponds to "very poor ecological quality" and that kind of waters cannot be used for any purpose. After biological treatment of wastewater, after 15 days, there was a significant decrease in chemical oxygen consumption by 6.3-7 times depending on the treatment procedure.

A high concentration of fresh organic matter in wastewater causes high HPC values. The average HPK value in the control sample of the Ada Huja lagoon is significantly higher than the HPK value defined by Class V wastewater (> 125 mg / l), indicating a significant degree of pollution of the lagoon. Concentrations of organic matter, expressed through the chemical oxygen cosumption, are very high for this type of surface water. Based on the classification (Legislation 50/12), water from the Ada Huja lagoon corresponds to a very poor ecological status and cannot be used for irrigation and industrial use as well as for its proposed purpose. After biological treatment of wastewater, there was a significant decrease in HPC values by 6.1 - 7.8 times depending on the treatment variant. HPK value decreases from 1760 mg / l to 226-289 mg / l after 15 days of treatment in Variants 1/15 and 2/15 and after 60 days of biological treatment there is significant decreasing of HPK value.

Tabela 1. Wastewater characteristic before treatment, and 15 i 30 days after treatment

Sample	pH value	Amonijum jon (NH_4)-N (mg/l)	NitritsNO_2^- -N (mg/l)	Nitrats NO_3^- -N (mg/l)	Cosumption $KMnO_4$ (mg/l)	Chemical oxigen cosumption $(K_2Cr_2O_7)$ O_2	Biochemical oxigen cosumption BPK_5 (mg/l)	Total organic carbonTOC (mg O_2 /l)
Control	7,7	32,36	0,024	0,23	1111,6	1760	1376,8	64,6
Variant1/15	7,6	40,2	0,011	0,23	158,1	226	11,2	28,8
Variant2/15	7,6	37,8	0,031	0,19	176,7	289	22,3	34,1
Control (60)	7,9	1,19	0,025	0,48	1286,5	1430	221,9	22,57
Variant 1/60	7,1	37,04	0,38	1,6	179,8	220	27,4	15,9
Variant2/60	7,2	37,34	0,27	1,68	148,8	239	42,8	21,25

The content of organic matter expressed through BOD-5 are very high (1376.8 mg O2 / l) for surface water in the control sample, indicating a high content of organic matter and pollution. The average value of BOD-5 in water from the Ada Huja lagoon is much higher than the value defined by the legislation for Class V. Water with an organic matter content of BOD-5 greater than 25 mg O_2 / L cannot be used for bathing and recreation and "cannot be used for any purpose" (Legislation 50/12). After biological treatment of wastewater, there was a significant decrease in BOD-5 depending of the treatment variant ie. from 1376.8 mg O_2 / l to 11.2-22.3 mg O2 / l after 15 days in Variants 1/15 and 2/15. Also, a decrease in BOD-5 was observed in the experimental variants (Variant 1/60, Variant 2/60) after 60 days.

The presence of biodegradable substances in wastewater is manifested by higher levels of COD relative to BOD. Biodegradable organic substances include: cellulose, coal dust, lignin, tannin and other synthetic organic compounds. HPC is always greater than or equal to BOD in lagoons, lakes and rivers. The HPK / BOD5 ratio is a characteristic of individual wastewater and their quality can be defined by this ratio.

The total TOC in the wastewater control sample is higher than defined value for the V surface water class (> 50 mg / l), ie. is 64.6 mg / l. After biological treatment of the wastewater from the Ada Huja lagoon, the organic carbon content was reduced to 28.8 mg / l in 1/15 variant and to 34.1 mg / l in 2/15 variant after 15 days. After 60 days in treatment variants 1/60 and 2/60, there was also a decrease in TOC content relative to control sample 60.

Additionally, organoleptic characterization (color, odor) was carried out. Based on this it was concluded that the effect of microorganisms usage was achieved, Figure 1.

Figure 1. Wastewater samples: ADA HUJA, 1-Variant 2/60, 2-Variant 1/60, 3-Control 60.

Conclusions

The applied technology represents application of unique bacterial *Bacillus* strains that increase the degradation of complex organic compounds into simpler compounds by the hydrolysis process. Used *Bacillus sp*. for verification of technology is formulated with four major enzymes that are important for the degradation of complex organic matter: Amylase, Protease, Cellulase, Lipase. Catalysts are defined as substances, food for microorganisms, which will accelerate or change the speed of reactions. Used *Bacillus sp*. is an advanced mixture of heterotrophic, aerobic and facultative anaerobic microorganisms known for production of extracellular enzymes for increased degradation of organic compounds. Purified bacterial strains and enzymes result in non-pathogenic, non-toxic and non-corrosive formulations.

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HE HOUSEHOLDS BIO-WASTE MANAGEMENT ON THE EXAMPLE OF THE MALOPOLSKA REGION

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Abstract

An aim of this article is to assess the process of biological waste management from households in Małopolska voivodship. Households are a source of waste biomass, which can be used, inter alia, as a raw material for energy and for agricultural purposes. The use of this biomass is part of the implementation of sustainable development goals, which tools are the bioeconomy and the circular economy. Households are a source of two types of waste biomass: food waste and sewage. Food waste is a particular problem, as 9 mln tons of food is being wasted in Poland and about third part is being wasted by the consumers. To examine Malopolska inhabitants regarding their attitude towards the food waste and waste segregation we run a survey. The results of this research revealed that Malopolska did not differ from the consumers of other developed countries. As many as 57% of respondents declared that they were throwing away bread; 54.5% vegetables and fruit. There were noted some differences of consumers behaviour, depending on the population groups of women and men as well as residents of villages and cities. Regarding the segregation of a waste, only 21.6% of respondents confirmed that they always segregated the waste. Among conscientiously segregating, there were more women than men and more inhabitants of rural areas then small towns. The second source of biomass generated by households is a sewage. The processing of the sewage and its purification by the Wastewater Treatment Plants is a source sewage bio-sludge. It is a problematic material, but can be potentially valuable due to the high phosphors content. However, in Małopolska, the infrastructure for using the biomass is developing, but not fast enough in a context of constantly growing amount of waste and not in a direction of achieving maximum value from the biomass.

Keywords: *food waste, bioeconomy, biowaste, Malopolska Voivodship*

Introduction

The purpose of the paper is to assess the process of management of biological waste from households in the Małopolska region. In 2016, the European Union generated 482 kg of waste per person, of which an average of 47% was in a subject of a circular economy, and 25% went to landfills. In Poland, these values were 307, 44% and 37%, respectively, which is a very poor result in comparison with countries such as Denmark, Sweden, Belgium, Germany or the Netherlands, where only 1% of waste is landfilled. The increase in the amount of waste is a result of the exploitation of raw materials, and it is in close relation with the growth of the world's population, the enrichment of societies and related changes in the consumption model (Kowalska *et al*., 2016), and has become a serious environmental threat enforcing a question about the efficiency of resource usage. Despite some initiatives related to the transformation concerning circular economy, in a global perspective, the dominant model is still linear economy, fed by a huge mass of raw materials to satisfy the ever-growing consumption (McDowall *et al.,* 2017). It is also worrying that 70% of the world's waste generated is not recovered or recycled in any way, by being directed to landfills (Tisserant *et al.*, 2017), what is not compatible with the policy promoted by the European Commission on circular economy and product life extension. This approach leads to a number of environmental problems and a significant burden on consumers' budgets. An important fraction of municipal waste is biodegradable waste. This fraction is a heterogeneous group, which includes kitchen waste, food remains, as well as waste from parks and gardens. Food waste offers a large reuse potential, especially in the paradigm of bioeconomy (Priefer *et al.,* 2016), has a significant share in this group. The second group of bio-waste is municipal sewage sludge (MSS).

Materials and Methods

The article uses primary data from surveys and data from biological waste management databases. The analysis apples to the area of the Małopolskie Voivodeship. A survey questionnaire (n=384) was used to investigate the behaviour and attitudes of the inhabitants of Małopolska in terms of food waste and waste segregation. The survey was carried out in 2018. The questionnaire consisted of 17 closed questions. The results of the questionnaires were analysed by gender and place of residence. The survey covered the frequency of food disposal by product category and the respondents' attitude to waste segregation. An analysis of documentation concerning the procedure of MMS handling and the organic fraction of municipal waste was also carried out.

Results and Discussion

The level of food waste in Poland is one of the highest in Europe. 42% of Poles admit to throwing away food, most often bread (49%), fruit (46%), cold meats (45%), vegetables (37%) (Food Banks, 2018). Among the respondents, the most wasted food products were bread (57%), and fruit and vegetables (54.5%). The least wasted articles were dairy products and eggs, which coincided with the conclusions of the national research. The analysis taking into account the gender of respondents shown that women more often threw away outdated and spoiled foods in all the categories considered. This can be linked to the still existing division of social roles of women and men in households, where women are primarily responsible for decisions related to the nutrition of household members.

Table 1. Food wastage (in %) by gender.

Details	Women		Men		In total	
	YES	NO	YES	NO	YES	NO
Meat and sausages	48,1	51,9	38,3	61,7	45,1	54,9
Dairy products and eggs	40,4	59,6	33,9	66,1	38,4	61,6
Bread	61,5	38,5	47,8	52,2	57,3	42,7
Fruits and/or vegetables	56,9	43,1	48,7	51,3	54,4	45,6
Other products	49,2	50,8	47,8	52,2	48,8	51,2

Source: Own development, on the basis of research conducted.

Similar conclusions are presented by Visschers *et al*., (2016). Other studies (Koivupuro *et al*., 2012**;** Silvennoinen *et al*., 2014) show a correlation between women's responsibility for food shopping and higher levels of food wastage. However, Secondi *et al*., (2015) presents a conclusion to the contrary, showing that at the level of EU farms, it is men who waste food more often.The origin of respondents had some importance in terms of the category of foods disposed of. Among Małopolska residents from rural areas, slightly less dairy products, eggs, bread, fruit and vegetables were wasted (Table 2). This may be related to different consumer preferences, satisfying nutrition needs with own produce, or feeding farm animals with waste food.

Table 2. Food waste (expressed in %) by origin of respondents.

Details	Large city		Medium city		Small city		Village	
	YES	NO	YES	NO	YES	NO	YES	NO
Meat and sausages	52,7	47,3	34,8	65,2	46,0	54,0	41,3	58,7
Dairy products and eggs	42,6	57,4	41,3	58,7	38,0	62,0	34,0	66,0
Bread	56,6	43,4	71,7	28,3	56,0	44,0	54,0	46,0
Fruits and/or vegetables	58,1	41,9	63,0	37,0	52,0	48,0	49,3	50,7
Other products	49,6	50,4	43,5	56,5	50,0	50,0	49,3	50,7

Source: Own development on the basis of research conducted.

The respondents were also asked about waste segregation (Table 3). As regards the segregation of municipal waste, only 21.6% of respondents confirmed that they always segregate waste (Table 4). Among those who conscientiously segregated waste, women outnumbered men quite considerably (77.7% vs. 60.9%). The largest number of respondents segregated waste in villages and medium-sized towns, while the smallest number of respondents segregated in small towns.

Table 3. Segregation and recycling of municipal waste in households (expressed in %).

Details	Women	Men	In total
Definitely yes	23,5	17,4	21,6
Rather yes	44,2	43,5	45,3
Rather no	22,3	27,0	23,7
Not at all	10,0	12,1	9,4
IN TOTAL	100,0	100,0	100,0

Source: Own development on the basis of research conducted

Village residents were more likely to segregate waste than residents in cities with a population exceeding 100,000. This can be attributed to a functioning system of segregated municipal waste collection (especially paper, glass, plastics and metals), which has been solved quite well with respect to single-family housing, as opposed to multi-family housing estates in large cities. In Małopolska, in 2014, there were 115 PSCMW (Points of Selective Collection of Municipal Waste), in the region in 2017 – there were 122 points where selectively collected municipal waste is accepted (SP, 2017).

Table 4. Segregation and recycling of municipal waste in households (expressed in %) by the origin of respondents.

Details	Large city	Medium city	Small city	Village
Definitely yes	17,1	23,9	20,0	25,4
Rather yes	45,7	37,0	40,0	49,3
Rather no	26,3	30,4	34,0	16,0
Not at all	10,9	8,7	6,0	9,3
IN TOTAL	100,0	100,0	100,0	100,0

Source: Own development on the basis of research conducted

The research indicates that the model of behaviour of the Małopolska population in terms of food waste and waste segregation does not differ significantly from the behaviour of inhabitants of other regions of Poland. The results obtained are consistent with the conclusions of the KPGO (2016).

Until recently, most of the waste produced on farms was sent to landfills. Only in 2016, almost 1 million tons of municipal waste was collected from the Małopolskie Voivodeship. The direction of waste management generated by inhabitants from Małopolska region, which is developing dynamically, is the thermal recovery of energy (369 thousand tons). In addition to incineration plants, there are a number of installations for processing organic fraction waste in the region. According to data from SP (2017), the following operated in Małopolskie Voivodeship: 16 MBP installations (mechanical and biological process), where waste is processed mechanically; 17 composting plants, with an annual capacity estimated at 118.000 tonnes; 11 installations for the storage of waste generated in the MBP process, and residues from the sorting of municipal waste – 213.800 tonnes in total; and the rest was recycled (295.900 tonnes). Apart from municipal waste increase, the mass of municipal sewage sludge is constantly growing, and its proper management becomes a challenge not only in the Małopolskie Voivodeship. This is related to the dynamic growth of the population's access to the sewage network. In 2017, over 2.8 million inhabitants of the region used sewage systems (SP, 2017). Similarly, the length of the sewage network for the years of 2012-2017 increased by 39% (from just over 18.000 km to 20.500 km), while the population of Małopolska increased by only 3.2% at the same time. Consequently, only in 2017, 113435 dm^3 of municipal wastewater was discharged. As a result of their treatment, more than 51.000 tonnes of sewage sludge were generated. The largest sewage treatment plant operating in the Voivodship (Kraków Płaszów) operates a mono-combustion plant for sewage sludge with a nominal capacity of 23.000 tonnes of dry matter (d.m.) per year. To a certain extent, it guarantees the management of this fraction of organic waste generated from urban wastewater from Kraków. The remainder of sludge from the Voivodship was stored (2.1 thousand tons), used in agriculture, and to cultivate composting plants (11.5 thousand tons), and over 20 thousand tons was managed in a different, unrecorded manner.

Conclusions

The research confirmed that the residents of Małopolska are in line with the global trend of developed economies and the waste food level is high. At the same time, consumers are not ready to segregate waste, which means that some valuable raw materials, including biomass, are not fully segregated, and end up in mixed municipal waste. Significant amount of organic waste does not fulfil its potential in the circular economy model, going to landfills or incineration plants. Low environmental awareness is a barrier to the implementation of bio-management and circular economy solutions. Legislation which tries to force separate collection of organic fraction and its proper management is equally important. At the same time the existing landfill system fees does not motivate the commune and its subordinate entities responsible for municipal waste management to use other methods than landfilling. There are also infrastructural obstacles - in terms of municipal waste, there is still not enough infrastructure to manage it. From almost 1 million tonnes of waste in Małopolska Voivodeship, 213.000 were disposed of in landfills (over 20%). At the same time, over 369 000 tonnes were subjected to thermal transformation. Thus, about 50% of municipal waste is incinerated and stored.

Thanks to the development of water and sewage infrastructure in Małopolska, the amount of treated sewage and the municipal sewage sludge (MMS) volume is growing. Thermal transformation (mono-incineration or co-incineration) is becoming an increasingly important method of its management. The operation of sludge incineration plants, apart from numerous MBP installations or composting plants, allows for partial management of this type of waste generated by the inhabitants of cities and villages of the Małopolska. It should be added, that not all water and sewage companies, especially those with a small number of population equivalent, are able to finance the operation of the MMS management system on their own. Moreover, the data quoted above show that still, a disturbingly large volumes of sludge are landfilled.

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CONTENT OF NITROGEN IN AGROCULTURAL SOIL ON THE TERRITORY OF THE MUNICIPALITIES AND THE TOWN OF NIŠ (SERBIA)

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Abstract

Soil nitrogen (N) is important macronutrient for plant growth and productivity. The aim of this paper was to examine the overload of the soil with easily available nitrogen on the territory of the Nis city (Serbia) in 2015 and 2016. Totally 6523 samples from 1219 farms were collected (4669 samples from 862 farms in 2015 and 1854 samples from 357 farms in 2016). The analysis showed that the soil of this area is medium provided with nitrogen. In 2015: 82% of samples belonged to the class of medium supplied and 13.8% were class of rich soil with nitrogen, while in 2016: 89.1% samples belonged to the class of medium supplied and 9.1% were class of rich soil with nitrogen. The percentage of nitrogen was determined from the percentage of humus, while the humus content was determined using the Tyurin method. Soil samples were taken from a depth of 0-30 cm in field crops and vegetables, and 0-60 cm in orchards and vineyards. According to the results of the research, the soil on the territory of the city of Nis is moderate-supplied by nitrogen. Certainly, it is highly recommendable to analyze the soil in order to be informed about the content of nitrogen which is necessary to all producers. Research data processing was performed by using a mathematical-statistical method in the computer program SPSS – Trial version (Statistical Package for the Social Sciences).

Keywords: *Nitrogen, Soil, Serbia.*

Introduction

Nitrogen (N), of all macronutrients, mostly affects the yield and quality of plant products. Despite the fact that nitrogen is mostly present in the air (N_2=78%), plants draw (assimilate) nitrogen from the soil. Nitrogen in the soil is found in the organic form (humus) in the amount of over 97% and in the inorganic (mineral) form (ammonia and nitrate), which is accessible to plants (2-3%). In nature, there is the nitrogen cycle, where Earth's atmosphere is the source, the transformation into soil nitrogen is performed by microorganisms in the process of nitrogen fixation. Nitrogen is introduced into the soil by fertilizing, as well.

Nitrogen originates from Earth's atmosphere (N_2), but is assimilated in the mineral form, therefore it belongs to mineral elements. In order to transform the molecular form of nitrogen (N_2) into ammonia (NH_3) and nitrate ($NO^-{}_3$), in which form plants assimilate it, energy is needed. In contrast, nitrogen is very easily returned to the molecular state, in which it is most stable, so it is easily lost from the soil. Nitrogen can be in the organic form and in the inorganic form. The organic form of nitrogen in the soil is humus and partially decomposed plant and animal remains. The mineral (inorganic) part of nitrogen in the soil is NH_4^+ (ammonium ion) and NO_3^- (nitrate ion), which is completely available for plant assimilation, only a small part (2-10%) of total nitrogen is in the soil. This quantity is generally insufficient for good nutrition of agricultural plant species (Jelić, 2012; Gudžić, 2015; Predić, 2011).

Soil microorganisms play a key role in the creation and maintenance of soil fertility. Microorganisms are indispensable members of all life functions; they are key to maintaining

the circulation of matter on Earth. In addition to azotofixators, which return nitrogen from the atmosphere to the soil, microorganisms mobilize phosphorus, potassium, iron and sulfur, making them available to plants (Ilić, 2016).
This study presents the results of the nitrogen content in the soil in the territory (567 km²) of the municipality of Niš (Republic of Serbia); coordinates of the municipality of Niš are 43°19′29″N and 21°54′11″E.

Materials and Methods

The research was carried out during 2015 and 2016. Humus (%) from the soil was determined by the Kotzmann method, and nitrogen (%) was calculated from the humus. The humus calculation method was: % N = % humus x 0.05. The mean pH of the soil in the research area was 6.48. The objective of this paper was to examine the content of nitrogen in the research area, which was the municipality of Niš, as well as to point out the importance of the optimal nitrogen content in the soil for plant production and the ecosystem. The mean annual temperature in the territory of the municipality of Niš in 2015 and 2016 was over 12°C, and the annual precipitation in both years was around 630 mm (Republic Hydrometeorological Service of Serbia, 2016, Republic Hydrometeorological Service of Serbia, 2017). By quantifying the impact of climate changes on plant production, making long-term plans in agricultural production is facilitated (Jančić, 2015). There are several methods for the determination of humus, and what all the methods have in common is the action of strong oxidants on organic matter, which leads to the oxidation of carbon (C) from humus (Grčak *et al*, 201).The research was carried out as a long-term stationary field experiment of the Institute of Field and Vegetable Crops in Srbobran, Serbia, in the period from 1996 to 1999, on the soil type carbonate chernozem. The paper examines the influence of increasing nitrogen doses (control group: 40 kg N ha^{-1}; 80 kg N ha^{-1} and 120 kg N ha^{-1}) on the yield of six inbred corn lines belonging to different FAO groups of ripening (L1-FAO 300, L2-FAO 400, L3-FAO 400, L4-FAO 600, L5-FAO 600, L6-FAO 600). Nitrogen fertilization showed a significant positive effect on the crop yield of corn lines. The crop yield depended on the line and year of research. On average, for all four years of research and all lines, the yield increase in the variant where 40 kg N ha^{-1} was applied amounted to 13.7% compared to the control group, in the variant where 80 kg N ha^{-1} was applied 18.7 %, and in the variant where 120 kg N ha^{-1} was applied 23.6%. The yield decrease in the non-fertilized variant also depended on the plant species, e.g. the yield decrease in barley was 49%, while there was only 10% of reduced yield in maize. Theoretically, the maximum yield of 4.91 t ha^{-1} is achieved at a quantity of 114 kg N ha^{-1} (Marinković et al., 2010). Assimilation of nitrogen also depends on the content of heavy metals in the soil (Župunski, 2017). In the turnover of nitrogen in plants, the release of nitrogenous compounds into the environment has an important role. One of the types of nitrogen release is volatilization of reduced and oxidized forms of nitrogen through the above-ground organs. Depending on plant species, genotypes and ecological conditions, through volatilization, plants can release, during vegetation, a significant part of the assimilated amount of nitrogen, and up to 80 kg of ammonia/ha. Plants, in addition to releasing ammonia into the atmosphere, simultaneously assimilate it from the atmosphere, and partly compensate for the loss caused by volatilization. The loss of nitrogen caused by volatization can be reduced by breeding. There are attempts to reduce the volatization of nitrogen compounds from above-ground plant organs by using physiologically active substances (Kastori, 2004).The effect of long-term harvesting of crop residues in combination with growing nitrogen (N) doses on the yield of two varieties of winter wheat in a four-year period has shown a significant increase in wheat yields. Long-term harvesting of crop residues has the same or similar results on the yield level and the improvement of soil properties, as well as the use of manure (Jaćimović et al., 2017).

Nitrogen is available to plants in the form of nitrate (NO_3) and the form of ammonia (NH_4). The nitrate form of nitrogen stimulates the accumulation of potassium (K), calcium (Ca) and magnesium (Mg) in plants. Nitrogen positively affects the resistance of plants to low and high temperatures and diseases. Not all plants have the same need for nitrogen; nitrogen deficiency or excess in the soil is not recommended. Nitrogen deficiency or a lack of nitrogen significantly reduces plant growth, thus branches are thinner. In winter grains, nitrogen deficiency is manifested through weaker tillering, plants have one stem and short spikes, and leaf growth is reduced. Nitrogen in plants is well mobile and moves from older organs to younger ones, so its deficiency is first spotted on the oldest leaves. Surplus/excess nitrogen negatively affects the productivity of plants; it is difficult to spot it on plants. Excess nitrogen leads to the excessive development of vegetative organs (roots, leaves and trunk), and such excessive development causes plant lodging. In winter grains (wheat, rye, barley, oats), it increases tillering, which results in uneven ripening. Nitrogen excess also affects the plant's roots, which become shorter and thicker, and become more susceptible to soil drought. Excess nitrogen prolongs vegetation and slows down the plant's ripening. The negative impact of excess nitrogen also has an ecological aspect, as it can lead to pollution of the environment by nitrates. Soil contamination by chemicalization from agriculture is increasing every year. The amount of nitrogen and phosphate fertilizers used to increase yields is growing. Therefore, attention should be paid to the possibility of pollution control (Zlatković D. and Zlatković J., 2017).

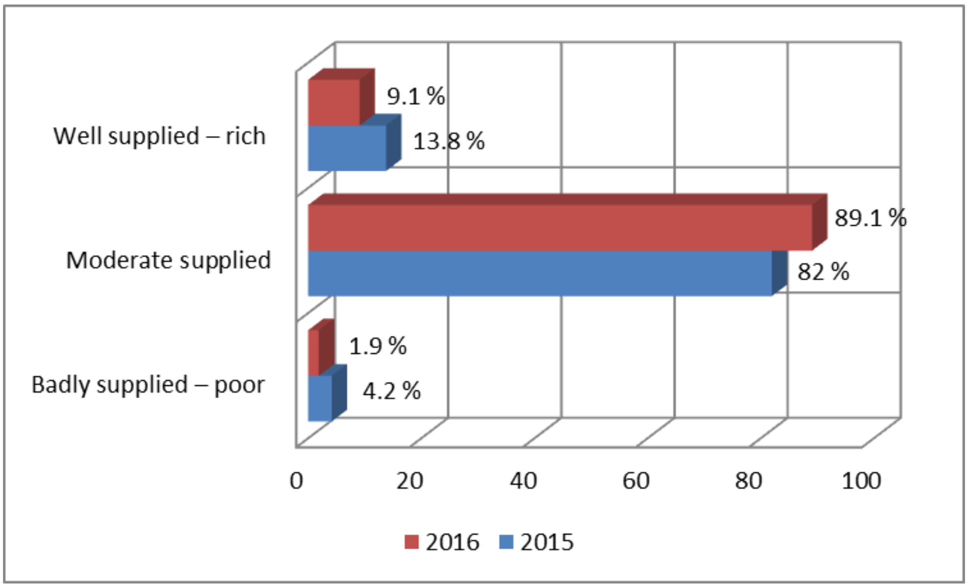
Results and discussion

The extraction of nitrogen from the soil also depends on plant species that is cultivated. Of the studied plant species, the highest amount of nitrogen has been found in triticale, and then in winter feed mixture, while the lowest amount of nitrogen has been found in vetch (Radanović, 2017). Due to growing ecological problems in developed countries, the so-called quotas for the use of mineral fertilizers (maximum quantities of mineral fertilizers per hectare are determined for each farm) have been introduced, especially in order to apply nitrogen in a controlled manner. The healthy and quality soil is a key component of healthy agricultural production. The main soil pollutants are: mineral fertilizers (especially nitrogen fertilizers), organic fertilizers, nitrates in plants, heavy metals, sewage sludge, municipal waste, plant protection products (herbicides, fungicides, insecticides, rodenticides), etc. (Aksentijević et al., 2017; Trikić, 2014). Plant species can be indicators of soil characteristics, based on the presence or absence of some herbs. If some "weeds" appear, for example nettle (*Urtica dioica*), it implies that the soil is rich in humus (Obratov-Petković et al., 2006).

Table 1: The sample share of particular classes based on the content of the available nitrogen (the categorization by Jelic, 2012) according to the analysis of 6523 pieces of land on the territory of Nis in 2015 and 2016

Classes	**Number of plots**		**Percentage (%)**	
	2015	**2016**	**2015**	**2016**
Badly supplied – poor (0-0,9 %)	194	35	4.2	1.9
Moderate supplied (0,1-0,19%)	3830	1651	82.0	89.1
Well supplied – rich (>0,2)	645	168	13.8	9.1
Total	**4669**	**1854**	**100.0**	**100.0**

On the basis of the processing of the data from 6523 pieces of land on the farms in the Nis municipality, it was concluded that 4.2% of the analyzed samples were the parts badly supplied by the available nitrogen (under 0,10%) in 2015 and 1.9% in next year. The part with moderate supply, with the nitrogen from 0.10 to 0.20% had the share of 82% in 2015 and 89.1 % in year of 2016 of the analyzed agriculture areas. The class of the soil well-supplied by the easily available nitrogen where the N was 0.20% or more had the share of just 13.8% and 9.1% (Table 1).



Graph 1. The sample share of particular classes according to the supply of the available nitrogen of the air-dry soil (%) in the Nis municipality by years (6523 samples)

Based on a classification (Graph 1), the samples with the nitrogen content ranging from 0.1 % to 0.19 % (moderate supplied) are far dominant, whereas the soils with the nitrogen content ranging from 0% to 0.9% and class where nitrogen content were more than 0.2% were less represented. In Serbia and in other parts of the world, experiments that have been carried out show that plowing harvest residues increases the yield and its quality, and it also increases the content of total nitrogen (N) and carbon (C) and organic matter - humus (Powlson *et al.*, 1987). Nitrate accumulation in nature, apart from causing ecological problems, directly endangers human and animal health (Kovačević et al., 2011).

Conclusion

Regular soil testing for the nitrogen content can provide an insight into the amount of nitrogen in the soil, so that the quantities of fertilizers applied are optimal and have no harmful effects on plants. It is very important to know the plant's need for nitrogen, so that nitrogen doses for certain plants are adequate and have no harmful consequences for both plants and the environment. In order to preserve soil fertility in terms of the yield and quality, it is necessary to re-enter the most important macroelements, such as nitrogen, phosphorus and potassium, into the soil every year. Often, intensive fertilization is carried out without previously conducted chemical soil testing, which can cause a contamination of the soil, plants and water; thereby, higher quantities of nitrogen and phosphorus fertilizers have shown the most unfavorable effects. Fertilizers that have not been utilized by crops reach the environment through the soil, and go to underground and surface waters, where they cause the greatest damage.

Based on the results of the analysis 6523 soil samples from the surface..., that is... *number of the households*, in the area of the municipality and city of Niš (Serbia), it can be concluded that the soil abundance in nitrogen is variable as a result of different types of soil, fertilization, and different exploitation of agricultural land. It is important that the soil analysis is carried out for every plot separately, at least once in five years, so that in this way, we obtain adequate recommendations for fertilization for the next four years, i.e. so that fertilizers are applied in accordance with their needs in order to avoid pollution and reduce excess costs.
According to the results of the research, the soil on the territory of the city of Nis is moderate-supplied by nitrogen. Certainly, it is highly recommendable to analyze the soil in order to be informed about the content of nitrogen which is necessary to all producers.

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YIELD OF GRASSLAND BIOMASS IN SECOND SWATH AND IMPACT OF APPLIED MEASURES ON CHEMICAL REACTION OF REKULTISOL

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Abstract

The study of biological phases of soil reclamation by seeding the grassland has been conducted on the Deposol at internal disposal area for overburden from Raskovac open pit in Stanari coal mine (Republic of Srpska, Bosnia and Herzegovina). The aim of this study refers to implementation of biological phase of reclamation on yield biomass (green mass) in second swath and improvement of technogenic soil fertility, type Rekultisol. The survey task refers to measurement of impact of fertilization and liming on yield of biomass grassland and chemical properties of forming Rekultisol. The study was expended through three-year period (2011–2013). Three grass-leguminous mixtures and one grass mixture were studied; altogether four treatments of various doses of mineral fertilizer and lime. The research covered the selection of treatment with the most productive green mass and impact of chemical reaction of Rekultisol. Statistical analysis of measuring quantitative properties of vegetable mass was conducted by method of ANOVA, 3×4×4. The sandy-loam Deposol at the beginning of research had unfavorable physical and chemical properties. The applied treatments and interactions born impact on measuring quantitative properties of researched mixtures and chemical properties of Rekultisol. The TDS-1 mixture had the biggest green mass production (3.6 t ha^{-1}) in 2011. The TDS-3 mixture had the biggest green mass production in 2012 (6.9 t ha^{-1}) and 2013 (7,2 t ha^{-1}). The application of liming increased the pH of Rekultisol. The biological reclamation in researched agro-ecological conditions was successfully conducted by seeding grasslands, raising selected species in grass-leguminous mixtures and application of optimal agromeliorative measures.

Key words: *open pit, soil reclamation, grass-leguminous mixture, Stanari*.

Introduction

Main objective of the reclamation of degraded areas, caused by open pit mine exploitation of the ore reserves, is to establish the management functions on these newly created technogenic soils (technosols). The main task of all these man-built terrestrial ecosystems is to stabilize and revive production and ecological functions of a technogenic soil, ie Deposol (the surface layer of the disposal area for overburden).

The fertility of the Deposol and most other types of technogenic soils (e.g. mine soils) is usually low. The concentration of the Deposol with the basic biogenic elements (N, P and K) is within or below the minimum concentrations (Dvurechensiy and Seredina, 2015; Pivić et al., 2011; Sheoran et al., 2010; Coppin and Bradshaw, 1982; Antonović et al., 1978). In addition to the deficit of nutrients, technogenic soils have low content of pedobi'os and organic matter, and poorly developed adsorptive complex (Rasulić et al., 2005). Shukla et al. (2004) states the following disorders in technogenic soil: loss of aggregate and soil structure, decrease in soil C concentration, increase in volume, and decrease in porosity.

The results of the past physical and chemical analyzes of Deposol at disposal area for overburden at the Stanari mine found that they have favorable physical - mechanical but unfavorable chemical properties (Malić et al., 2017a; Malić, 2015; Malić i Marković, 2012).

The same authors state that, based on the content of organic matter, the researched Deposol belongs to the class of low and medium content, while there is no pure humus and nitrogen. According to the content of P_2O_5 and K_2O in the Deposol, they are classified as very poorly secured by these elements. The Deposol is characterized by a non-carbonate substrate, a strong unsaturation with base cations, a medium and highly acidic chemical reaction. The fact that the content of organic matter is very low and acidic chemical reaction make are the biggest problems in Stanari Deposols.
A significant part of the agricultural reclamation refers to the establishment of artificial grassland. Studies on the methods of grassland establishment in the reclamation process and potential yields have started in 2008 at the Deposols of Raškovac open pit external disposal area in Stanari (Malić and Lakić, 2011).
Pioneer species in agricultural reclamation include species from the families of Poaceae and Fabaceae. Seeding grasslands establishment through seeding grass-leguminous mixtures and pure cultures of certain grass species is significantly present at mine in Bosnia and Herzegovina, Serbia and abroad. Since the earliest reclamation works in the USA, a vast expanse of recultivated areas has been under seeding meadows and pastures (Thorne, 2010, Skousen and Zipper, 2010, Lyle, 1987, Vogel and Berg, 1968). Normally, most commonly sown species during reclamation process are grasses (family Poaceae) because they are producing a large amount of biomass and quickly adapt to specific and harsh environmental conditions. For biological reclamation most commonly used grass species are from the following genus: *Poa*, *Festuca*, *Lolium*, *Panicum*, *Agrostis*, *Phleum*, *Dactylis* (Malić and Lakić, 2011, Smith et al., 2002). Participation of leguminous in mixtures depends on the type and characteristics of Deposols used for reclamation. In addition to the potential yields of forage and hay, multiple significance of all types of grasslands is reflected in the changes of basic physical, chemical and biological properties of Deposol by increasing its fertility. The aim of this survey refers to implementation of biological phase of reclamation and improvement of technogenic soil fertility.

Material and method

The coal basin Stanari is located between 44°40' and 44°50' N and 17°45' and 18°00' E, in the northern part of the Republic of Srpska / Bosnia and Herzegovina. Research on biological reclamation of a direct type was carried out at the experimental field (GPS Coordinates: y = 6.486.822.33; x = 4.957.645.63; altitude 220 m a.s.l) within internal disposal area for overburden of the excavation from the open pit Raškovac in the lignite coal basin Stanari: ''EFT - Mine and Thermal Power Plant Stanari''. The survey was conducted in a three-year period (2011–2013). Part of the disposal area for overburden where the experimental field is located was formed during 2010.
The three-factorial experiment was set by the random block method in four repetitions. The experiment plots area is 10 m^2 (5×2 m). The distance between the plots is 50 and 80 cm, and between the blocks is 1 m. Plots are without inclination.
The first factor of research refers to the year (factor A), with three treatments. The second factor refers to various grass and leguminous species in mixtures (factor B), with four treatments. The third factor is agromeliorative measures (fertilization, liming and mulch, factor C), with four treatments (Table 1).
Statistical analysis of the measured results has been conducted with the green mass yield by the ANOVA method, 3×4×4. Establishment of the seeding grassland was carried out during the spring sowing period in 2011. Seeding rate amounted to 45 kg/ha. When the crops reached their maximum growth, mowing – lawn mulcher was performed. The measurements of green mass yield were carried out on: 31 August 2011, 20 July 2012 and 05 August 2013).

Table 1. Treatments three-factorial experiment

<table>
<tr><th>Factors</th><th colspan="4">Treatments</th></tr>
<tr><td>Factor A (three years)</td><td>a_1 (2011)</td><td>a_2 (2012)</td><td colspan="2">a_3 (2013)</td></tr>
<tr><td>Factor B (four mixtures)</td><td colspan="4">b_1 (TDS-1):
Festuca arundinacea Schreb. 25%, *Festuca rubra* L. 20%, *Dactylis glomerata* L. 10%, *Phleum pratense* L. 10%, *Trifolium repens* L. 10%, *Trifolium pratense* L. 10%, *Medicago sativa* L. 10%, *Poa pratensis* L. 5%
b_2 (TS-2):
Festuca arundinacea Schreb. 40%, *Festuca rubra* L. 20%, *Poa pratensis* L. 20%, *Dactylis glomerata* L. 10%, *Phleum pratense* L. 10%
b_3 (TDS-3):
Festuca rubra L. 50%, *Poa pratensis* L. 30%, *Lotus corniculatus* L. 10%, *Trifolium repens* L. 10%
b_4 (TDS-4):
Dactylis glomerata L. 30%, *Phleum pratense* L. 30%, *Lotus corniculatus* L. 20%, *Arrhenatherum elatius* (L.) Mert. et Koch. 20%</td></tr>
<tr><td>Factor C (four agromeliorative measures)</td><td>c_1: $N_{90+54+54}P_{90}K_{90}$</td><td>c_2: $N_{60+54+54}P_{90}K_{90}$+ 8 t ha^{-1} $CaCO_3$</td><td>c_3: $N_{60+40+40}P_{60}K_{60}$</td><td>c_4: $N_{90+40+40}P_{90}K_{90}$ + wheat straw mulch</td></tr>
</table>

The third factor (C) represents dosages of mineral fertilizer (pure nutrients) applied during sowing period (doses: $N_{90}P_{90}K_{90}$, $N_{60}P_{90}K_{90}$ and $N_{60}P_{60}K_{60}$) and spring supplementation with nitrogen fertilizer (doses: N_{54+54} and N_{40+40}). The mineral fertilizer NPK 15:15:15 was used before sowing, and KAN (27% N) during supplementation phase. Limestone was added during the primary tillage of the Deposol (treatment c_2). After the sowing, wheat straw in the form of mulch was used on treatment c_4.

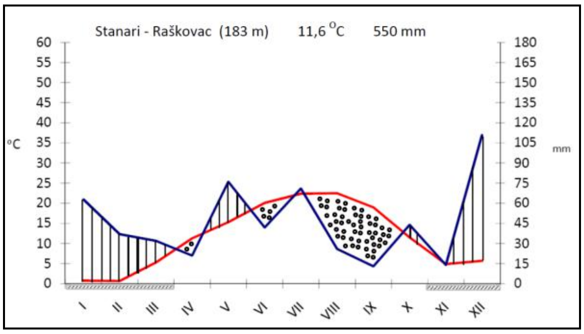
The determination of the studied types of technogenic soil was carried out according to Resulović and Čustović (2007), and the WRB classification (2014). According to the soil classification in Bosnia and Herzegovina, the newly discovered soils mine belong to the class of technogenic soils (types Deposol and Rekultisol). Deposol represents the type of surface layer of disposal area for overburden prior to biological phase of the reclamation. Rekultisol is a layer of soil where reclamation measures have been carried out and the initial processes of humification and mineralization begin. The researched Deposol and formed geogenic Rekultisol are of a silicate subtype. According to the WRB classification (World Reference Base for Soil Resources, 2014), these soils are determined as technosols (Epiarenic and silicit material).

For the purposes of laboratory pedological research, the average samples of the Deposol were taken before the study at the beginning of 2011, and the samples of Rekultisol in the final phase of vegetation in 2013. The samples were taken from a depth of 0-20 cm. The analysis of the Deposol included the examination of the following parameters (Table 2): content of organic matter (dry burning method at 550 ^{o}C), humus (Tjurin method), soil reaction (pH) in H_2O and 1M KCl (electrometrically combined electrode in pH-metre), total N (semimycro Kjeldahl method), plant available P and K (AL-method). At the end of the research in 2013, the pH value was analysed one more time. The classification of Deposol and Rekultisol based on the chemical reaction was carried out according to Živković (1991) for pH in H_2O, and according to Šefer-Šahtašabel for pH in KCl.

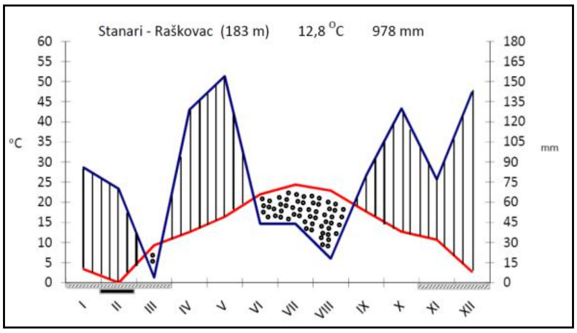
Table 2. Results of the analysis of chemical properties of Deposol

No. of sample	pH		Organic matter	Humus	Total N	AL - P_2O_5	AL - K_2O
	H_2O	KCl	%			mg/100g soil	
1	6.2	4.9	2.1	0.0	0.0	0.0	1.3
2	5.8	4.5	1.2	0.0	0.0	0.5	2.2
3	5.2	4.0	1.6	0.0	0.0	0.6	2.7

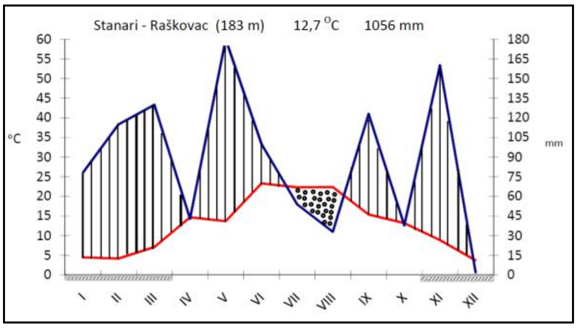
The basic climate indicators (precipitation and air temperature) in the studied three-year period are shown in the following graphs (climate diagram to Walter and Lieth).



Graph 1. Climate diagram in 2011



Graph 2. Climate diagram in 2011



Graph 3. Climate diagram in 2013

Results and discussion

Three-year research and application of the intensive agrotechnical measurements in the implementation of the biological phase of reclamation (by the dominance of the anthropogenic factor) resulted in formation of the Rekultisol as the thin surface layer of the technogenic soil. The researched Rekultisol is formed in the process of the direct reclamation while the lawn forming.

The crops were mulched so the dry mass stayed on the Deposol surface in the process of reclamation. Table 5 shows the average values of the green mass in the second swath, the results of the variance analysis, and the Lsd test. The identified interaction effect is given in Graph 4.

Table 5. Average yield (t ha^{-1}) of green mass in the second swath

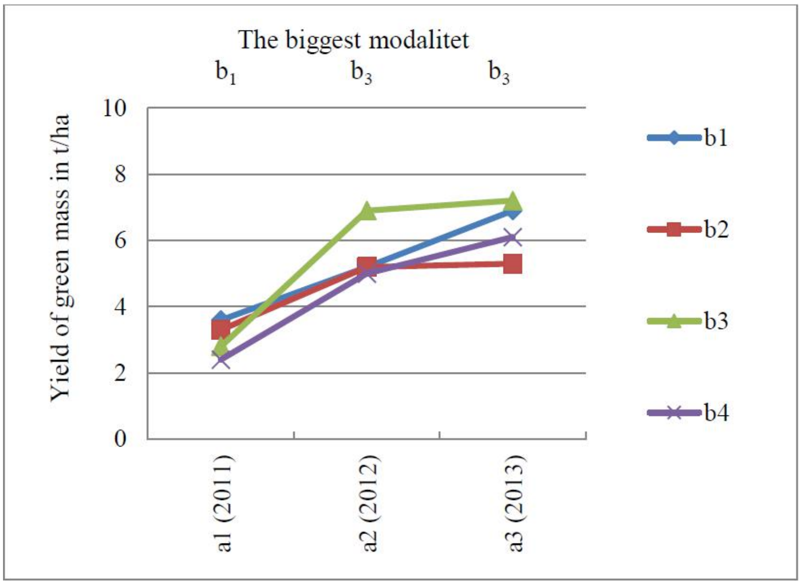
Treatments basic factors				Factor B (mixtures) b1	b2	b3	b4	$\bar{x}_C$
Factor A (years)	a_1 (2011)	Factor C (agromeliorative measures)	c_1	5.2	5.4	2.2	1.8	3.6
			c_2	3.05	2.8	2.7	2.3	2.7
			c_3	2.5	2.05	3.0	2.6	2.5
			c_4	3.6	2.9	3.2	3,05	3.2
			$\bar{x}_B$	3.6	3.3	2.8	2.4	3.0
	a_2 (2012)	Factor C (agromeliorative measures)	c_1	4.3	4.8	7.8	3.6	5.1
			c_2	3.9	4.7	5.4	7.3	5.3
			c_3	6.7	5.0	8.3	4.5	6.1
			c_4	6.1	6.2	6.0	4.6	5.7
			$\bar{x}_B$	5.2	5.2	6.9	5.0	5.6
	a_3 (2013)	Factor C (agromeliorative measures)	c_1	5.0	5.1	5.3	6.0	5.6
			c_2	7.8	5.3	7.8	7.0	7.0
			c_3	6.7	5.7	9.03	6.3	6.9
			c_4	7.2	5.04	6.6	5,3	6.03
			$\bar{x}_B$	6.9	5.3	7.2	6.1	6.4
		$\bar{x}_{BC}$		5.23	4.6	5.63	4.5	5.0

ANOVA $F_{calc.}$	A	B	C	A×B	A×C	B×C	A×B×C
	61.03**	4.17**	0.41⁻	2.29*	2.09⁻	1.53⁻	1.47⁻

Lsd		A	B	C		
	0.05	0.61	0.71	1.23	-	-
	0.01	0.81	0.94	1.62	-	-

With the researched mixtures, yields during the research were variable in some of the treatments. The results in Table 5 indicate the increase of the average yield of the green matter during the research. The average value of the green mass yield in the first year was 3.0 t ha^{-1}, in the second 5.6 t ha^{-1} and in the third 6.4 t ha^{-1} of green mass. The analysis of the variance shows the statistically significant influence of the basic factors of the year (A) and different mixtures (factor B), while the interaction of factor A and B (year × agromeliorative measurements) is significant. The factor of a year with three treatments had a statisticly significant impact on the green mass yield in 2012 and 2013 from other 2011.

The analysis of the interaction effect of the years × mixtures (A × B) on the average values of green mass yield in the second swath shows the variations between the individual mixtures in the second and third year compared to the first year of the study. Differences in the yield of the green mass between the modalities of the factor B are not significant in 2011. Change in these tendencies was recorded in 2012, when the mean value of treatment b_3 showed a statistical significant influence comparing it to other treatments (b_1, b_2 and b_4). The differences between these three treatments are random. In the third year of the study there are very significant differences only between the treatment b_3 (the highest mean value) and treatment b_2 (yield with the lowest mean value).



Graph 4. Interactive effects of research year (factor A) and mixtures (factor B) on the yield of green mass in t ha^{-1}

According to Malić et al. (2017b) mixture TDS-3 (treatment b_3) had the biggest green mass production in three-year study in the first swath (4.8 t ha^{-1} in 2911, 16.7 t ha^{-1} in 2012 and 25.8 t ha^{-1} in 2013. The same authors reports the maximum average yield of green matter in the second two years of the survey (17.3 t ha^{-1} in 2012 and 32.1 t ha^{-1} in 2013) was measured in the combination of the b_3c_3 treatment. The minimum variation of the yield is evident for the TS-2 mixture on all treatments of factor C, which is the result of seedling of grass species only. The measured values of green mass yield match the results of previous research with the type of *Festuca arundinacea* Schreb on the Deposol of the outlying landfill of Raškovac, where Malić and Lakić (2011) state the average yield of 7.9 t ha^{-1} of green mass in the first swath and 5.0 t ha^{-1} in the second swath at the dose of fertilizer $N_{35+80}P_{100}K_{150}$.
Lower values of the green mass of grass-leguminous mixtures are also reported by Grandt and Lang (1958), who achieved an average yield of *Medicago sativa* L., with more grass species of 8.4 t ha^{-1}, in three years of direct reclamation of Deposols in Illinois. The same authors report the yield of the green mass of the mixture *Lotus corniculatus* L. and grass of 8.1 t ha^{-1}, with a mixture of other legumes and grasses, the yield of 5.4 t ha^{-1} was measured. Moreover, with the mixture of *Lespedeza bicolor* Turcz. and grass, the yield of 4.6 t ha^{-1} was measured. Measured production of green mass of the mixture *Trifolium repens* L. and grass is 7.1 t ha^{-1} and *Trifolium pratense* L. and grass 5.1 t ha^{-1}. The measured yields of green mass in direct reclamation are significantly lower than the potential of these species in the production of green fodder (Malić et al. 2017; Malić, 2015). The main reason for this is the difference in the fertility of natural soils and Deposols.
The extent and intensity of the applied agro-technical measures are interrelated with the time of the reclamation, which together affects the properties of the re-cultivated land. Beginning of the pedogenesis of the Deposol at the investigated site overlaps with the completion of the discharge of the discovery on this part of the internal landfill site Raškovac, which was carried out in late 2010. The results of the analysis of the chemical reaction of the Rekultisol at the end of the study are shown in Table 6.

Table 6. Analyses of pH of Rekultisol in late 2013

No. of sample	Treatments	pH	
		H_2O	KCl
1	b_1c_1 (TDS-1, $N_{90+54+54}P_{90}K_{90}$)	5.3	4.2
2	b_1c_2 (TDS-1, $N_{60+54+54}P_{60}K_{60}$ + 8 t ha^{-1} $CaCO_3$)	7.5	6.5
3	b_1c_3 (TDS-1, $N_{60+40+40}P_{60}K_{60}$)	6.3	5.0
4	b_1c_4 (TDS-1, $N_{90+40+40}P_{90}K_{90}$ + mulch)	5.9	4.8
5	b_2c_1 (TS-2, $N_{90+54+54}P_{90}K_{90}$)	5.6	4.3
6	b_2c_2 (TS-2, $N_{60+54+54}P_{60}K_{60}$ + 8 t ha^{-1} $CaCO_3$)	7.5	6.5
7	b_2c_3 (TS-2, $N_{60+40+40}P_{60}K_{60}$)	6.0	4.6
8	b_2c_4 (TS-2, $N_{90+40+40}P_{90}K_{90}$ + mulch)	5.7	4.8
9	b_3c_1 (TDS-3, $N_{90+54+54}P_{90}K_{90}$)	6.2	6.9
10	b_3c_2 (TDS-3, $N_{60+54+54}P_{60}K_{60}$ + 8 t ha^{-1} $CaCO_3$)	7.5	6.6
11	b_3c_3 (TDS-3, $N_{60+40+40}P_{60}K_{60}$)	5.8	4.9
12	b_3c_4 (TDS-3, $N_{90+40+40}P_{90}K_{90}$ + mulch)	6.2	5.1
13	b_4c_1 (TDS-4, $N_{90+54+54}P_{90}K_{90}$)	5.9	4.8
14	b_4c_2 (TDS-4, $N_{60+54+54}P_{60}K_{60}$ + 8 t ha^{-1} $CaCO_3$)	7.2	6.2
15	b_4c_3 (TDS-4, $N_{60+40+40}P_{60}K_{60}$)	6.1	5.0
16	b_4c_4 (TDS-4, $N_{90+40+40}P_{90}K_{90}$ + mulch)	5.9	4.9

Based on the value of the results of the analyzed Rekultisol chemical properties of all the average samples given in Table 6, an improvement in the pH value was observed, comparing to the values of the results in the testing of the Deposol before the start of the biological reclamation (Table 2).
The values of the active reaction (pH in H_2O) of all the samples in Table 6 enter the interval from strongly acid to weak alkaline chemical reaction of the soil. The value of the pH of the sample 1 represents the soil of a highly acidic reaction. According to the sample values no. 4, 5, 7, 8, 11, 13 and 16 in these combination treatments, the Rekultisol is determined in the category of moderate or moderate acid reaction. Examined samples include all treatments with grass-leguminous mixtures (TDS-1, TDS-3 and TDS-4). As far as factor C is concerned in these samples, samples with treatments c_1, c_3 and c_4 are present. Rekultisol with the values of the pH within samples 3, 9, 12 and 15 is classified as low acidity. In this case, there was no significant change in pH compared to the applied treatments in the study. Neutral and weak alkaline reaction was determined in samples 2, 6, 10 and 14, or on plots under treatment c_2 where calcification was performed at a dose of 8 t ha^{-1} of $CaCO_3$.
The values of the substitution reaction of Rekultisol (pH in KCl) in Table 6 assign samples 1, 4, 5, 7, 8, 11, 13 and 16 to the category of very acidic reaction. Samples 3, 12 and 15 are the categories of moderately acidic reaction. The values of pH in the sample 2, 6, 9, 10 and 14 categorize the Rekultisol into the soil of a slightly acidic reaction. Comparing the results of this division and the division of the pH into H_2O, the samples 2, 6, 10 and 14, in which the acidity is the lowest (ie, the highest pH), are the same treatment of factor C. In these

treatments, calcification is applied, and the pH value in the KCl moved from 6.2 to 6.6, and belong to the category of poorly acidic reactions.
According to Malić and Marković (2012), the partial increase of the pH value of the Deposol in the reclamation process at the Raškovac mine was also achieved in previous researches of agricultural reclamation, where calcification was applied. An increase of pH in KCl from 4.12 to 6.46 with the previous application of 15 t ha^{-1} of hydrated limestone is indicated by Kovačević et al. (2011), which corresponds the results on treatments where calcification is applied. The same authors note the decrease of pH in KCl in the case of meliorative fertilization with phosphorus and potassium, without the use of calcification. In a study of the pedological changes on the marl substrate during the thirty-two-year period, Mujačić (2013) states that the pH in H_2O had an insignificant decrease of value, which is 7.7. The possibility of successful calcification in the reclamation process of Deposol using limestone materials is cited by Sheoran et al. (2010).

Conclusion

The establishment of a seeded grassland with the sowing of grass-leguminous and grass mixtures, with the application of agromeliorative measures, concludes the following:

- The chemical properties of the researched Deposol are characterized by the non-carbonate and non-humus substrate, the strong unsaturation of the base cations, the medium and highly acidic chemical reaction, the low content of organic matter and basic biogenic elements.
- The ecological conditions during the research and applied agro-technical measures have had an impact on the growth and development of grass and legumes, as well as on the changes in the surface layer of the researched technogenic soil.
- The highest average yield of green mass in the second swath was measured with the TDS-1 mixture (3.6 t ha^{-1}) in 2011 and TDS-3 mixture in 2012 and 2013 year (6.9 t ha^{-1} and 7.2 t ha^{-1}).
- Under the influence of intensive implemented measures of biological reclamation on the Deposol, the condition of the reclamation soil was conditioned as well as the type of Rekultisol, which on the basis of the analysis indicates the increase in the pH value.

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AGRICULTURE AND LAND USE: CONTEXT ANALYSIS AND FUTURE PERSPECTIVES FROM THE MEDITERRANEAN TO THE APULIA REGION

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Abstract

Land use patterns have, over the centuries, reflected the continuous interaction between human activities and natural environment, and have resulted in significant environmental impacts and its consequences, both positive and negative on the well-being of human groups. The scarcity of resources, land and water, represents a great social challenge, especially in a context of climate change and food insecurity. These challenges are particularly significant for the Mediterranean, a region characterized by dynamic population and high density, with strong constraints on land and water resources. The Mediterranean has a long history of land use and represents one of the richest areas in terms of biodiversity; because of its cultural and environmental characteristics and its long history, the Mediterranean basin hosts a variety of land use systems of varying intensity and levels, ranging from intensive systems to traditional "mosaic" ones. These traditional mosaic systems are associated with high biodiversity values and landscapes particularly vulnerable to global changes, which see their survival threatened not only in terms of food supply, but also with respect to a number of ecosystem services linked to the multifunctional dimension of agriculture. In this context, starting from the report on land use in Italy, the contribution aims to provide a broad and precise picture of its current patterns at different levels, up to the Apulian regional scale. Agriculture itself, to which the delicate issue of land use is necessarily linked, today represents a fundamental strategic sector for the Mediterranean countries, whose centrality in Euro-Mediterranean regional cooperation requires an in depth reflection and analysis.

Keywords: *Land use, Mediterranean, Multifunctional agriculture, Ecosystem services, Biodiversity.*

Introduction

Nowadays, as to the term "land use", it is difficult to refer to an unambiguous definition; Pileri (2009) defines it, generally speaking, as the anthropogenic process that involves the gradual transformation of natural or agricultural surfaces through the construction of buildings and infrastructures; it is assumed that the restoration of the pre-existing environmental state is very difficult, if not impossible, due to the nature of the earth matrix. This definition encompasses a negative perspective as long as the issue of the subtraction of natural or agricultural surfaces is perceived as a serious problem, considering the finiteness of the earth's surface; as a consequence, it could be more correct to define or talk about land or soil transformations, rather than mere consumption. Land use, as a matter of fact, is a practice inherent in the anthropogenic activity, since all the human communities have abandoned nomadic life, built, transformed and consumed land according to their needs through time. On the one hand, the most extremist exponents of ecology argue, for example, that even the transformation of forests for agricultural purposes represents a phenomenon to be limited; this is particularly true with reference to tropical countries, due to the presence of rainforests, while it can be considered a "non-European" problem, inasmuch as almost all European forests have disappeared.

On the other hand, the issue at stake has gained attention within the scientific community as a result of what can be observed in the last 20-30 years: the really important aspect is that the consumption and transformation of land does not constitute a problem in itself, but becomes so when it is consumed without a specific and real need. It is vital and appropriate to specify that, as well as a quantitative question, the consumption of land is also a matter of distribution: it is often possible to observe, outside the built-up areas, construction works that erode agricultural or natural territory, which is fundamental for guaranteeing food production and to regulate the climate and the water cycle. It is vital to analyse the reasons why land use and consumption has to be considered as an issue and a problem. Following the profound changes that the processes of industrialization have brought to the geomorphology of the earth in the last century, a strong interest in ecology issues has been affirmed both in the scientific community and in civil society, supported, since the 1970s, by environmental movements. This is true with particular reference to, on the one hand, the more or less stable presence of environmentalist political parties in the parliaments of most developed nations (e.g. in Italy: Verdi and SEL, Sinistra-Ecologia-Libertà) and, on the other hand, the fact that it is possible to observe an increasing interest from political parties and political coalitions in organizing their own programs with numerous references to the environmental component. The European Union strongly supports States and Regions for the promotion and development of programs and policies aimed at enhancing the safeguard of the environment, in general, as well as in the specific field of land use. Starting from the definition of land use and consumption, the present contribution aims at carrying on a sound analysis of legislation concerning the mentioned subject and, as a context analysis, of the case of the Alta Murgia National Park in Apulia region. Conclusions will be drawn regarding the importance of investigating the link between land use and sustainable agriculture.

Materials and Methods

Analysis of land use causes

It is fundamental to carry on an in depth analysis of the underlying causes of the phenomenon of land use. Firstly, industrialization[6] and its expansion in many countries has been perhaps the greatest change in the history of mankind. It is a process that is based on the intensive exploitation of the main resources made available by nature (i.e. air, water, earth and fire, energy). After centuries of technological development and economic growth, most countries have begun to become aware of the negative effects of this development model, while other countries, those of the so-called "South of the World", have in turn taken the industrial model as a guide to well-being, with results, however, at least questionable in many respects. As a consequence, it is fundamental to consider how the beginning of the new millennium presents itself as a challenge to face: reconciling the growing demands of democracy and well-being of all peoples with the needs of survival of the earth. Sociologists and economists repeatedly try to find an academic and theoretical answer to this question (Pallante, 2011), whose solution needs to take into account a simple but vital concept, i.e. the awareness that land use and consumption is a real and serious issue, whose origin lies in the fact that natural resources are not infinite, and that land and soil are part of these same resources. Nevertheless, as above mentioned, it is important to emphasize that nowadays, with regard to the "North of the World", it would be more correct to speak of "waste" of land; this circumstance derives from the expansion of cities on order to meet specific needs of society, the satisfaction of main needs, such as housing and services. This is certainly true up to a certain limit, beyond which land use becomes a speculative phenomenon, unjustified by the mentioned needs. Starting from a basic ecological point of view, it is vital to cease land waste, as this represents a unique and irreproducible resource, and has vital and strong relationships with the quality of air, water, and energy consumption. As an example, in addition to occupying the soil and

[6]The term industrialization refers to the process of transforming a community (society) from a type of rural economy to a type of life with an industrial economy.

limited ground, a built-up area interferes with the infiltration of rainwater into the subsoil, partially or totally blocking the recharge of the aquifers; the population that inhabits it consumes energy resources, contributing to the use of fossil fuels, such as methane for the operation of boilers, coal or oil or nuclear energy used for the production of electricity that serves the functioning of the technological components of homes and public lighting equipment[7]. Moreover, the need linked to mobility of the inhabitants is satisfied with the use of means of transport (whether public or private) that obviously use other fossil fuels (petroleum derivatives or methane) that release emissions into the atmosphere. The air of urbanized areas is, for these reasons, partly polluted, causing damage to the health of the inhabitants and, in general, to the ecosystem; in addition, rainwater carries pollutants into the soil and underground, creating further environmental damage. The construction of free soils not only involves the concreting of a specific piece of land, but also includes the consumption of other soils, i.e. those used for the extraction of materials to produce cement, or quarries, which represent further land consumption. The issue of hydrogeological instability needs also to be taken into consideration, as building works are often carried on in areas that, although they appear to comply with norms, prove to be extremely dangerous, causing severe damages and accidents with victims. As clearly stated in the European Commission Document entitled "Framework for the protection of soil" highlighted the importance of land as a vital and non-renewable resource (European Commission, 2006. N.232), land embeds multiple functions, being a strategic resource for a country and its citizens, as well as a common good. Free soil is an irreplaceable potential for food production, and an ecological and environmental multifunctional resource, as it preserves carbon, regulates the hydrological cycles, governs humidity, provides shelter to many animal species, and represents a habitat for other species, supports vegetation and its functions (primarily oxygen production and the subtraction of CO_2) and so on. Free soil is a fundamental condition for the quality of the landscape, as it guarantees the indispensable presence of open spaces, crucial for urban well-being and the health of citizens. As a consequence, the future development of cities, indicated by illustrious scholars and urban planners (Arcidiacono et al., 2014), in the context of the National Urban Planning Institute (official website of INU), needs to take into account the reuse of already compromised land.

Results and Discussion

Legislation and focus on Apulia region

As above mentioned, with regard to the legislation on land consumption, there is a lack of organic unity. The mentioned European Commission Document entitled "Framework for the protection of soil" highlighted the importance of land as a vital and non-renewable resource (European Commission, 2006. N.232). This concept was reiterated by the Commission itself in 2011 with the "Roadmap to a Resource Efficient Europe" (European Commission, 2011. N.571), linked to the 2020 Strategy, with the goal of a zero increase in net employment of land to reach in Europe by 2050. This Objective was reaffirmed with the approval of the Seventh Environmental Action Program, called "Living well, within the limits of our planet" (European Parliament and Council, 2013. N.1386).

In Italy a series of government initiatives, dating back to 2012, embed several expectations related to the need to safeguard natural and agricultural soils as well as relaunch the building

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[7]Although research on photovoltaic and solar thermal technologies are attempting to gradually replace the use of these fuels for civilian use, they still contribute in a minority to the total production of electricity; nuclear energy, on the other hand, beyond the possible and well-known incidents that can occur in power stations (Cernobyl, 1986; Fukushima, 2011), provides for the disposal of radioactive waste in special landfills placed hundreds of meters underground, which in turn, they could have (and have) negative repercussions on the entire ecosystem since the radioactivity of uranium or plutonium is extinguished in tens of thousands of years.

activity towards the regeneration of urban fabric, aimed at improving the quality of life of citizens, to improve the environment and urban and suburban landscape and to recover eco-systemic functions. It is possible to observe, nevertheless, a lack of an organic law for the protection of the environment, the territory and the landscape, with a specific focus on sustainable development and on increasing the resilience of urban areas in the face of old and new challenges, due to both the fragility of Italian territory, and to the need to adapt to ongoing climate change. Many regions have specific land use regulations, while others set targets in the area of land governance laws, although there are significant derogations or exceptions related to envisaged types of interventions and land transformations that, as long as they are not included in the calculation (and, therefore, in the limitation), in reality represents a further cause of land consumption. (ISPRA, SNPA, 2018; ANCE, 2016). As far as the Italian territory is concerned, land consumption continues to grow at a rate of 15 hectares per day, thus strengthening the progressive artificialization of the same territory that continues to cover natural and agricultural areas with asphalt and cement, buildings, roads and other infrastructures, commercial, productive and service settlements, and through the expansion of urban areas. Given the geomorphological structure of the Italian territory, it is vital to analyse the coastal strip where the phenomenon shows itself in all its dramatic force. In fact, at a national level, almost a quarter of the range within 300 meters from the seaside is consumed; Liguria and Marche, with almost 50% of land consumed, represent the most serious data, while Abruzzo, Campania, Emilia Romagna and Lazio show values between 30 and 40%. Between 300 and 1,000 meters from the seaside, Abruzzo, Emilia-Romagna, Campania, Liguria and Marche show values equal to or greater than 30% of consumption. In the range between 1 and 10 kilometers, it is possible to observe the greatest percentage increase (ISPRA, 2017) and Campania stands out with 16.4% of land consumed. It is fundamental to note how, although the increase is less evident in the bands adjacent to the seaside, i.e. in the range below 300 meters, data show an increase in land consumed equal to 0.10% (ISPRA, 2017; ISPRA, 2018).
Another important focus, as regard the Italian territory, concerns protected areas, such as national and regional parks, natural areas and reserves. According to the ISPRA report (2017) almost 84 hectares (+ 0.11%) with the most dramatic situation in the Monti Sibillini National Park (over 24 hectares) and in the Gran Sasso and Monti della Laga National Park (over 24 hectares as well). The highest percentages of land used can be observed in the national parks of Vesuvius, the Archipelago of La Maddalena, and Circeo (ISPRA, SNPA, 2017). At a national scale, protected areas are still less consumed than the remaining areas, with a percentage of soil consumption within protected areas equal to 2,4%. In total, almost 6% of the bound territory, compared to 7.6% registered throughout the national territory, is consumed. The regions with the highest percentage of bound territory consumed are Campania (11%), Veneto (9%), Apulia (9%), Emilia Romagna (8.5%) and Lombardy (8%). Land use, in these mentioned cases, is due to construction sites and areas devoted to infrastructures, buildings or other permanent coverings over the next few years, as well as yards on agricultural or natural soil, linear infrastructures and new extractive areas.

Apulia Region

In particular, in Apulia region, the regulation on land use stems from the Regional Law 13/2008 which states that the territorial government tools must contain the necessary indications to promote the sustainability objectives of territorial and urban transformations, including the reduction of the consumption of new territory, avoiding the occupation of soils of high agricultural and/or naturalistic value, favoring the rehabilitation and recovery of degraded areas. Regional Law 15/2017 (which modified Regional Law 26/2014) defines land use as the reduction of agricultural areas due to interventions that determine its waterproofing, urbanization, construction and overbuilding, of areas used de facto for agricultural purposes, regardless of their urban destination and those areas, free from buildings and infrastructures, susceptible to be used for agricultural purposes. Finally, Regional Law 12/2018 (which amended Regional Law 24/2015) aims at favoring a planning of the territory in compliance with the criteria of sustainability and reduction in land use. More in detail, protected areas in Apulia extend for a total of approximately 245,154 hectares, of which 75.8% is represented by national parks (Gargano National Park and Alta Murgia National Park) and 8.3% by regional natural parks and reserves. It is vital to consider the ICSANP indicator (Intensity of Soil Consumption in Protected Natural Areas. http://www.paesaggiopuglia.it/aree-protette-in-puglia-footer.html) that represents the increase/decrease in land use over time within protected natural areas in Apulia, calculated as a percentage resulting from the relationship between change in land consumed in a given time period (in the specific case 2016-2017) and land consumed at the initial time (T0 = 2016) (Arpa Puglia, Environmental indicators of Puglia, 2018). This shows the percentage of land consumption within protected natural areas which, although limited to relatively low values (around 3%), showing on average an increased propensity to naturalness of these territories, proves values different from zero and therefore not in line with the objectives of maximum protection of these areas (ISPRA, 2017; ISPRA 2018).

Figure 1: Location of the Alta Murgia National Park in Apulia region

Source: our elaboration from Google Maps.

Conclusions

The Alta Murgia National Park

In the context of Apulia region and its natural territory, the Alta Murgia National Park, founded in 1998 and officially proclaimed a National Park in 2004, covers a total area of almost 68,000 hectares and is entirely included in the Natura 2000 Site SIC / ZPS IT9120007 "Murgia Alta" of about 125,000 hectares. The municipalities that are part of the same Park are: Altamura, Andria, Bitonto, Cassano delle Murge, Corato, Gravina in Puglia, Grumo

Appula, Minervino Murge, Poggiorsini, Ruvo di Puglia, Santeramo in Colle, Spinazzola and Toritto (Table 1). The territory represents a typical Mediterranean karst landscape, with the presence of medium deep or shallow karstic valleys (Fracchiolla et al., 2017; Ricchetti et al., 1988). Overall, the Park territory is composed of agricultural areas (33,399 hectares), forests and semi-natural areas (33,421 hectares), artificial-man-made surfaces (1,211 hectares) and water bodies (8 hectares) (official website of Alta Murgia National Park. Figure 1).

Table 1: Municipalities in the Alta Murgia area

Alta Murgia	Surface in the area	Surface in the area/Total surface (%)
Total surface	1.992,73	---
Province:	---	---
Bari	1.489	39%
Barletta Andria Trani	381,85	25%
Taranto	121,89	5%
Comuni:	---	---
Acquaviva delle Fonti	42,21	32%
Altamura	427,70	100%
Andria	136,52	34%
Bitonto	19,86	11%
Cassano delle Murge	53,26	60%
Castellaneta	58,42	24%
Corato	65,58	39%
Gioia del Colle	176,94	86%
Gravina di Puglia	380,82	100%
Grumo Appula	6,86	9%
Laterza	63,47	40%
Minervino Murge	121,15	47%
Poggiorsini	43,01	100%
Ruvo di Puglia	109,78	49%
Santeramo in Colle	143,18	100%
Spinazzola	124,18	68%
Toritto	19,81	27%

Source: our elaboration from Puglia Region

In the last decades of the twentieth century, thanks to the use of modern technology and machinery, and favored by a questionable European Community subsidy policy (Giordano, 2017), the removal of stone was carried out with a high intensity. Large parts of the territory have been affected by changes in land use, resulting in the destruction of karst systems (and karst ecosystems therein) through the removal of stones, even large ones, in order to create fields where to plant crops such as wheat or vineyards. A definite loss of the natural karst landscape (Caldara and Ciaranfi, 1988; Parise 2011) has therefore been recorded in vast areas of the Murge. Furthermore, the removal of the original soil has had the direct consequence of an increase in erosion processes during adverse atmospheric phenomena, even in those sites characterized by low or very low gradient. The long history of occupation of this territory has led to the loss of most of the ancient remains of the original karst landscape; this is particularly true with reference to the last decades, when an often uncontrolled urban expansion characterized large areas of Apulia region in general, and Murge as well, and many traces of historical heritage were irretrievably destroyed (Perrino, 2013). In other cases, land forms such as lakes, or small depressions and karst caves, have become sites of frequent discharge of solid and liquid waste, with serious consequences for the natural environment, the karst ecosystem and quality of groundwater. Pollution and degradation events are continuously observed and recorded, with more or less serious consequences on soil quality and consumption. According to the Regional Territorial Landscape Plan and with regard to the Alta Murgia, it is fundamental to note how land and landscape are under threat because of the different types of anthropic occupation (e.g. homes, plants, service areas) that contribute to fragmenting the natural morphological continuity of the landscape, and to increasing hydrogeological risk. Further research in this field should be carried out aimed at analysing the vital connection between sustainable agriculture and land safeguard.

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CHRONOSEQUENCE OF NATURAL REGENERATION IN ABANDONED MINING SITES IN THE AMAZON RAINFOREST OF MADRE DE DIOS, PERU

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Abstract

Gold extraction via small scale mining in the Amazon rainforest of Peru has become one of the greatest threats to deforestation and land degradation in the Amazon, especially in the Madre de Dios region which is one of the last biggest remnants of continuous tropical rainforest in the world. Restoration of these degraded ecosystems have become a priority in the last decade but without concrete actions, however, few research has been conducted in response to these restoration activities nor natural regeneration. The significance of this research was to study a chronosequence of natural regeneration in two active gold-mining sites in Madre de Dios-Peru (Paolita-PA; Santa Rita-ST) and how the nearby remnant forest contribute to natural regeneration. Sites were chosen depending on its management and the proximity to nearby remnant forest. Floristic composition of natural regeneration following abandonment of mining activities was studied by establishing a total of 12 plots (20x50m each), 6 with an abandonment period of 2 to 16 years and 6 were considered as reference forest. A total of 753 individuals from 44 families and 144 species were identified. To analyze biodiversity and similarity composition, Shannon and Jaccard indexes were used, respectively. The results showed that the abundance of species (Shannon) was higher in Paolita than in the Santa Rita mining site. From Jaccard's similarity index each mining site was analyzed in clusters finding that in Paolita, nearby remnant forest might not have a great influence over natural regeneration when compared with Santa Rita site which showed similarity between remnant forest, but instead the time of abandonment, availability of nutrients and forest fragmentation might be the cause of the recovery of degraded forest.

Keywords: *Chronosequence, Natural regeneration, Gold mining, Amazon rainforest, Peru.*

Introduction

The tropical rainforest is considered as one of the most biodiverse sites in the world (Connell, 1978) and the efforts of preserving these valuable ecosystems are highly important for the sake of humanity (Venter, et al., 2016). In the attempt of preserving the biodiversity, Peruvian amazon rainforest hosts various protected areas designate not only to preserve nature itself but also human populations. According to an official list of protected areas in Peru published by SERNANP (2019), only in the Region of Madre de Dios 3 National Parks, 1 National Reserve, and 2 Communal Reserves are part of the National Administration , and 25 Private Conservation Areas aim to preserve this great biodiverse ecosystem (Vuohelainen, et al. 2012). But despite these efforts, the region has face severe deforestation caused by agriculture, logging, cattle raising and gold mining (Román - Dañobeyta , et al., 2015) . A recent study (Caballero Espejo, et al., 2018) showed that from the years of 2010 to 2015, mining took the lead as cause of deforestation in the region and in a laps of 7 years (2010 – 2017) 64584ha were lost to gold mining. Given the current situation of deforestation and consecutively loss of biodiversity due mining, this study aimed to 1) conduct a chronosequence of secondary successional forest in active gold mining sites in the Region of Madre de Dios to analyze the response of forest after abandonment taking a close look into the floristic composition and structure, and 2) to analyze how remnant forest influence natural regeneration of nearby degraded lands. Thus, to accomplish our objectives, satellite images and aerial photographs were used for the site selection. To address biodiversity, Shannon's diversity Index was used (Kennard, 2016) and to analyze similarity between successional stages, Jaccard's similarity index was used.

Materials and methods

Study area and site selection

The region of Madre de Dios is one of the 24 first-level administrative subdivisions of Peru. This region, part of the tropical rainforest, is located in the southeast side of the country neighboring Bolivia and Brazil, with an area of 85,182.63 km² and, divided into 3 provinces (Tambopata, Manu and Tahuamanu), is the third biggest region in Peru (Instituto Nacional de Estadistica e Informatica [INEI], 2017) According Holdridge (1966), 14 life zones are found and, notwithstanding the richness in biodiversity that this region holds (Gentry, 1988), it has been suffering the constant pressure of human activities such as cattle raising, agriculture, logging and mining (Venter, et al., 2016). Gold mining has increased rapidly in the region (Asner, et al. 2013, et al**)** and has become the main source of income for local and outer residents. This activity is mostly realized in a small scale, but it has led to a severe loss of forest cover, 6145ha/year (Román - Dañobeyta , et al., 2015).
To conduct this study the sites and plots were chosen based on two criteria: 1) managed by the same individual or organization and 2) early successional plots must had a considerable distance to remnant forest. Thus, following the first criteria, 2 sites were chosen (Figure1). The first was Paolita Mining Site, following the Madre de Dios River 30 minutes away from the town Laberinto which is located 45 minutes away from the capital of the region (Puerto Maldonado), and Santa Rita mining site, located in the buffer zone of the Tambopata National Reserve, adjacent to the Interoceanic Highway. Satellite images and aerial photos where used to stablish the plots.
The study area lies within two Landsat scenes (path-rows 02-69 and 3-69). A collection of all Landsat Thematic Mapper (TM)/Enhanced Thematic Mapper Plus (ETM+)/Operational Land Imager (OLI) images with less than 40% cloud cover from archives of the United States

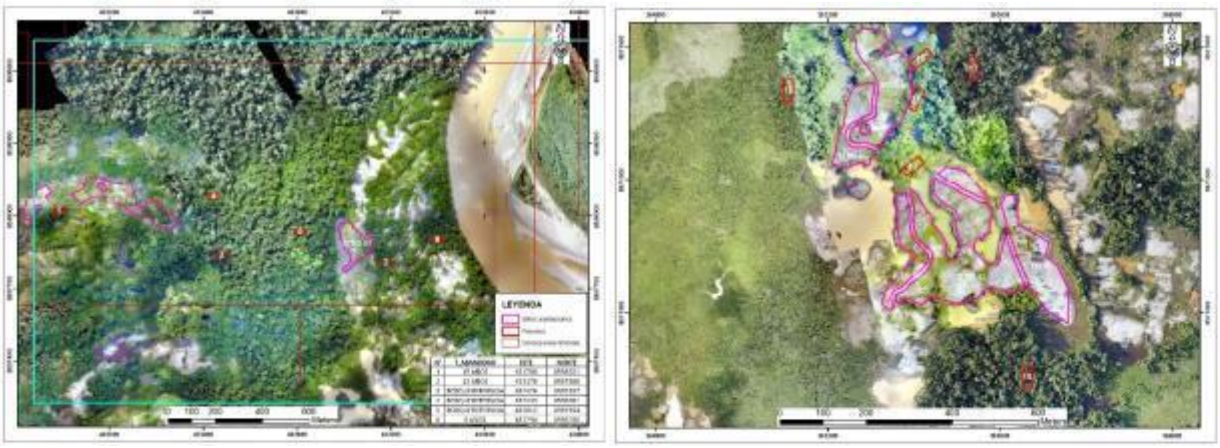


Figure 4: Aerial photos of Paolita mining site (left) and Santa Rita mining site (right).

Geological Survey (USGS) were used. This resulted in a collection of 68 Landsat images spanning 34 years from 1985 to 2017. With this results 6 plots, each one measuring 20mx50m

(0.1ha), were randomly stablished at each site, giving a total of 12 plots (6 plots with an early stage of succession and 6 plots that served as reference forest) with an age after abandonment that goes from 2 to 18 years.

Data analysis

Each plot was given a name depending on the mining site. For Paolita mining site plots were named after the letter P and numbered from 1 to 6 been early stages of succession plots P1, P2 and P6, and old growth forest P3, P4 and P5. In Santa Rita mining site plots were named after ST and number from 13 to 18, where early successional plots were ST13, ST 14 and ST15, and old growth forest ST16, ST17 and ST18. For each plot, the total number of individuals was recorded as well as recognized to the maximum level classification (specie). Nomenclature were valeted and cross-checked using the Taxonomic Name Resolution Service v4.0 (Boyle, et al., 2013) and the website www.tropicos.org for double rectification. Diameter at breast height (DBH) of all seedlings equal or greater than 1cm was measured. For multi-stemmed individuals, each steam with a DBH ≥ 1cm was considered as one individual. Basal area of all species were computed. Diversity and similarity were measured with quantitative and qualitative indices (Kalacska, et al., 2004; Mora, et al., 2014). Thus, Shannon diversity index (H') and its respective evenness (E) was estimated (Kalacska, et al., 2004) and, for similarity analysis Jaccard's similarity clustering was computed (Kennard, 2016).

Results and discussion

Species composition

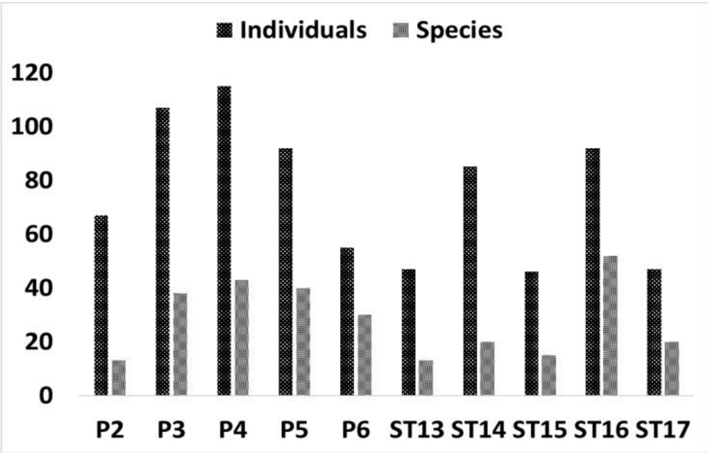
A total of 753 individuals were described in both sites, representing 144 species scattered in 44 families. The total number of individuals and species on each mining site were significantly different. At Paolita 436 individuals and 164 species were identified whereas at Santa Rita 317 individuals and 120 were found. The results showed a variation of species and individuals along the successional stage gradient as well as the dominance of some species. Number of individuals per plot and species composition was variable. It was expected (Wilkinson, 1999) that the number of seedlings of all species will decrease and become even as the stage of regeneration increases due its light demanding or shade tolerance. As other studies found (Peterson & Heemsker, 2001 and Liebsch , et al., 2008, et al.), it was confirmed that seedlings in old stages of regeneration were small in number compared with early stages. Early successional stages had a higher quantity of pioneer species and, although some pioneer species were found in remnant forest (P4&ST16) suggesting that at some point old growth forest were perturbated, results showed that there are some families exclusive to early and old stages (URTICACEAE and SAPOTASEA respectively) while others are present in all stages (MORACEAE). See Table 1. Stem density also changes along the years after abandonment together with dominance. For example, in Santa Rita mining site, plots with 8 years after abandonment(ST13&ST14), *Hieronyma alchorneoides* (Allemão) and *Guatteria megalophylla* (Diels) were the most representative species in early stages with a Relative Abundance (RA) equal to 20.83% and 25% respectively but, in the old grow forest the RA for both species changed significantly. Only one individual of *Guatteria megalophylla* (Diels) was found in both reference forest, and no natural regeneration of *Hieronyma alchorneoides* (Allemão) was found in old stages. In Paolita mining site, *Ochroma pyramidale* (Cav. ex Lam.) Urb. and *Cecropia pachystachya* Trécul were two of the most abundant species in plot P2 (2 years after abandonment) with a RA equal to 41.79% and 17.91% respectively but, as the forest matures in age (P6 = 6 years) the RA of both species changed dramatically (1.8% and 3.64% respectively) and these two were not found in old growth forest. Presence or absence of some species generally suggest that the area has been perturbed at some point of its successional growth. Pioneer species, such as *Hieronyma alchorneoides, Ochroma pyramidale* and *Cecropia pachystachya* are early successional species or pioneer species with rapid growth, low nutrient requirement but highly light requirement (Kennard, 2016). Also, basal area (Relative Dominance) will change across successional stages as seen in the results. Early stages in Santa Rita's plot ST13&ST15 had a Relative Dominance (RD) of 33.26% and 23.25% for *Cecropia pachystachya* Trécul and *Ocotea obovata* (Ruiz & Pav.) Mez respectively while plots in Paolita showed a RD of 49.99% and 19.81% for *Muntingia calabura* L. and *Zanthoxylum rhoifolium* Lam. respectively.

Table 6: Structure composition of families found in Paolita mining site (P) and Santa Rita mining site (ST). Plots are arranged from early successional stages (upper row) to old growth stages (downer row). Only families with a composition major or equal to 6% are represented in this table

	Family	**Composition (%)**	**N◦ of species**		**Family**	**Composition (%)**	**N◦ of species**
ST 13	MELASTOMATACEAE	27.08	1	**P2**	MALVACEAE	49.25	2
	PHYLLANTHACEAE	20.83	1		URTICACEAE	17.91	1
	URTICACEAE	18.75	1		MORACEAE	14.93	1
	MALVACEAE	12.50	2				
	EUPHORBIACEAE	8.33	1				
ST 14	ANNONACEAE	25.00	1	**P6**	FABACEAE	25.45	5
	PHYLLANTHACEAE	21.43	1		ANNONACEAE	16.36	2
	MELASTOMATACEAE	16.67	2		EUPHORBIACEAE	9.09	2
	APOCYNACEAE	11.90	1		MORACEAE	7.27	3
	EUPHORBIACEAE	7.14	3		URTICACEAE	7.27	3
ST 15	MELASTOMATACEAE	26.09	2	**P3**	MORACEAE	17.76	4
	ANNONACEAE	19.57	1		MYRISTICACEAE	14.95	2
	MALVACEAE	13.04	1		FABACEAE	11.21	5
	FABACEAE	10.87	4		ANNONACEAE	9.35	3
	PHYLLANTHACEAE	10.87	1		SAPOTACEAE	7.48	5
	CLUSIACEAE	8.70	1				
ST 16	FABACEAE	14.13	7	**P4**	MYRISTICACEAE	14.78	3
	ANNONACEAE	8.70	4		ANNONACEAE	13.91	3
	MELASTOMATACEAE	8.70	2		MORACEAE	12.17	6
	SAPOTACEAE	7.61	5		SAPOTACEAE	10.43	4
	MELIACEAE	6.52	2		MALVACEAE	7.83	2
					MELIACEAE	6.96	3
ST 17	MORACEAE	21.28	4	**P5**	MORACEAE	25.00	6
	MELASTOMATACEAE	19.15	1		ANNONACEAE	9.78	3
	POLYGONACEAE	17.02	1		MALVACEAE	9.78	3
	APOCYNACEAE	6.38	1		MYRISTICACEAE	8.70	3
	FABACEAE	6.38	3		SAPOTACEAE	8.70	3

Diversity and similarity measurements

Wilkinson (1999)proposed that immediately after the disturbance of an ecosystems, low levels of diversity is expected and as the successional stage increases, diversity will increase proportionally (Intermediate Disturbance Hypothesis). Not only our results agree with this hypothesis but also the results from several studies (Kalacska, et al., 2004; Mora, et al., 2014; Peterson,et al., 2001).



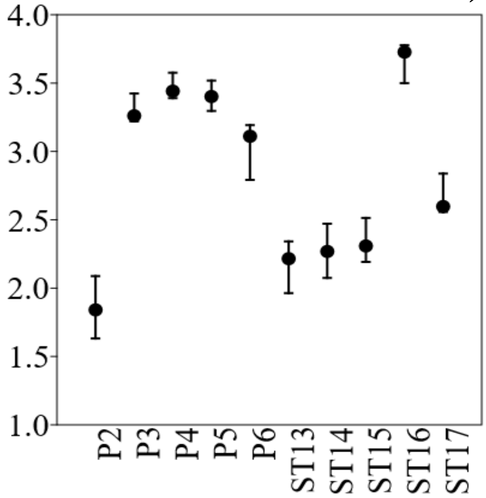


Figure 5: Paolita and Santa Rita's individuals and species composition (left). Shannon's diversity values of all plots (right)

As seen in Figure 2 and Table 2, Low levels of biodiversity, species and individuals were found in early stages and showed progressive increase over time. A significant difference in diversity was found not only within plots but also between mining sites (Paolita had higher diversity than Santa Rita mining site). In Paolita, the most resent disturbed plot (P2) showed the lowest level of diversity among all study plots, consecutively plot P6 (6 years successional stage) showed a remarkable diversity, almost similar of the remnant forest.

Table 7: Detailed summary of data from Paolita and Santa Rita's plots indicate that as the successional stage of regeneration increases, levels of biodiversity increases in a proportional way.

Plots	SHANNON (H')	Evenness (J')	Individuals	Species	Years after abandonment
P2	1.84	0.72	67	13	**2**
P6	3.11	0.91	55	30	**5**
P3	3.26	0.90	107	38	**RF**
P4	3.44	0.92	115	43	**RF**
P5	3.40	0.92	92	40	**RF**
ST13	2.22	0.86	47	13	**8**
ST14	2.27	0.76	85	20	**8**
ST15	2.31	0.85	46	15	**15**
ST16	3.73	0.94	92	52	**RF**
ST17	2.60	0.87	47	20	**RF**

Remnant forest showed in Paolita mining site showed an oscillation of diversity values as well as in its structural composition and, compared with Santa Rita mining site, early stages

also presented low but increasing levels of diversity. When the data was computed, plot ST16 and ST17 (both old growth forest) presented a significant difference in diversity (ST16 = 3.73 and ST17 = 2.60). This significantly difference between these two old growth forest's diversity and number of individuals made us suggest that 1) although plots in Santa Rita are older than the stablished in Paolita and despite the fact that Santa Rita is located in the Tambopata National Reserve's buffer zone, the pressure over the land its proved to be higher (Asner et al. 2013)since its proximity to the Interoceanic Highway (Caballero Espejo, et al., 2018), 2) soil condition varies between sites and plots. Moran, et al. (2000) inicates that soil fertility greatly influences seccondary succession and that nutrients are stock in the vegetation rather than the soil itself. Thus, with a simple visual identification of soil properties, we found that soil in Santa Rita where secondary forest was present was sandy (Podzol)
indicating that leaching of nutrients was hiegher. Therefore, having sandy soils and low nutrients avobe-ground gives as results less individuals and diversity with a slow recovery.

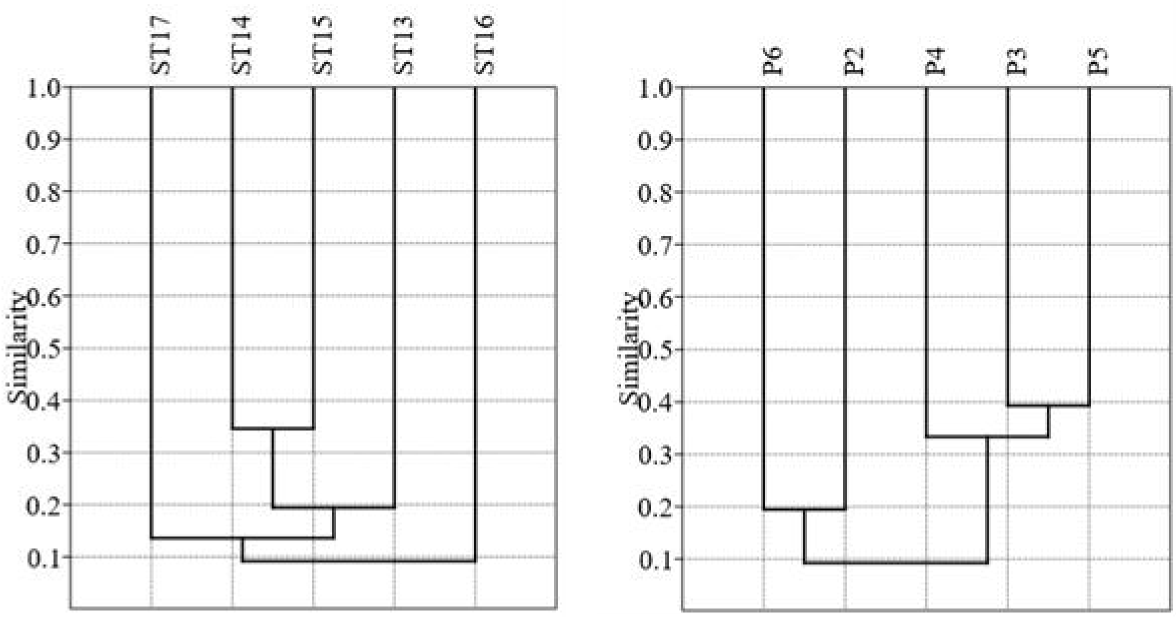


Figure 6: Jaccard's similarity clustering computed for both mining sites suggested that nearby remnant forest in Santa Rita has effect over natural regeneration over secondary succession.

Paolita, in the other hand, presented a different type of soil, where alluvial rocks were predominant, its proximity to the Madre de Dios river makes the soil much richer in nutrients which increases favorable conditions for the forest recovery (Jordan, 1985) even when disturbed. Plot P6 is an example of how fast the area could recover giving a great expectancy over P2 (2 years after abandonment) to follow the same trent (Liebsch, et al. 2008). Finally, 3) although both sites presented extreme pioneers, we proposed that nearby remnant forest accelerates recovery of disturbed areas and that the isolation of remnant forest and how far are located from the early stages could suggest lower or higher levels of diversity affecting recovery after disturbance. Thus, to address this hypothesis we conducted a Jaccard's similarity clustering (Figure 3) . This index was used to find floristic similarity between successional forest and old grown forest (Kalacska, et al., 2004). In the clustering graphic the similarity distance goes from 0 (0% of similarity) and 1 (100% similarity). Thus, clustering analysis showed that the closest similarity between early successional stage (P6) and old grown forest (P4) was 18%, both plots 713m away from each other, and the less similarity was found between P2 (early successional stage) &P3 (old growth forest), these two plots were 100% dissimilar and both plots 616m away from each other. For Santa Rita the closes similarity was found between plot ST17&ST15 (old growth forest and early stage respectively) with a similarity equal to 18%, both plots 124m away from each other, and the lest similarity was found between plots ST13&ST16 with a similarity equal to 2%, both plots 544m away from each other. From these results we could corelate diversity and similarity between plots at each site. Even though in both sites, the closest similarities between early stages and old growth forest is the same (18%) and considering the distance between plots, suggest that there is no significant influence of remnant forest over species composition and recovery in Paolita since these plots (P6&P4) are considerably far from each. This observation has relation with the study presented by (Tabarelli, Mantovani, & Peres, 1999) on how fragmentation affects diversity. Another factor for boosting the recovery observed in Paolita might be caused by the Madre de Dios river itself which could serve as a natural corridor for seed dispersal (Tewksbury, et al., 2002) and, following the same statement, in Santa Rita mining site, similarity between plots ST17&ST15 suggest that remnant forest has influenced over the recovery of early stages of regeneration due its proximity from each other but, considering that ST17 has a low diversity level and the forest fragmentation is considerably higher, it also caused a low-diversity regeneration present in early stages, even when plots in this area are older than those stablished in Paolita.

Conclusion

The impact produced by gold mining over forest lost has been studied previously by Caballero Espejo, et al., (2018) and several reforestation trials have been conducted in the last decade (Román - Dañobeyta , et al., 2015). Between 1987 and 2017 nearly 100,000ha of forest were lost and, as seen during the process of making this paper, it is more likely that this number has already been surpassed. Ecological restoration is far to be accomplished due the severity of the degradation not only above-ground but soil itself. Results proved that forest regeneration could be achieve in at least 6 years after abandonment as seen in Paolita (Table2) but we must consider that there are several factors that might and might not boost natural regeneration although nature has a high resilience over disturbances; severe deforestation, forest fragmentation and soil condition directly affects natural regeneration. We might concluded that nearby remnant forest might not have a direct influence over regeneration as results from Paolita showed (Figure 3), but instead several factors influence the recovery of degraded tropical forest. For a deeply understanding of the recovery of degraded forest, the addition of some other factors (seed bank, proximity of remnant forest to the degraded area, etc.) is required. This study focused on the passive ecological restoration of the tropical rainforest ecosystem. There are some studies that proposed different approaches of restoration or reforestation (Attiwill, 1994; Romell, et al., 2008, Pereira, et al., 2008; et al.) but given the scale of deforestation seen in Madre de Dios, a holistic approach that include stakeholders and other actors as part of the restoration of this valuable ecosystem is needed.

Acknowledgment

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DISPERSION AND BIOACCUMULATION OF HEAVY METALS IN PLANTS AT THE BASIN OF WEST MACEDONIA LIGNITE CENTER, GREECE

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Abstract

Lignite used in thermoelectric factories contains a number of trace elements which are dispersed in the environment during coal combustion. In this scientific research a comparative investigation has been carried out concerning the bioaccumulation of seven heavy metals (Cr, Mn, Fe, Ni, Cu, Cd and Pb) in trees, mosses and lichens from the northwest part of Greece, where the facilities of West Macedonia Lignite Center (WMLC) are located. In that direction, 71 soil samples and 92 samples of plant organisms were collected and utilized. In essence, the heavy metals concentrations were determined in all samples and then the calculation of the soil to plant Transfer Factors was conducted. The results revealed a discernible loading in some tree species, which however, did not exceed the legally established limits. On the contrary, mosses and lichens displayed much higher transfer factors, confirming their ability to bio accumulate air pollutants. Another conclusion is that chromium, manganese, iron, nickel, cadmium and lead compared to unpolluted areas are systematically increasing in tree leaves in the vicinity of WMLC. This fact cannot be attributed to soil uptake, given that soil parameters in the study area immobilize most of the heavy metals. The most reliable explanation for their origin is the flying ash of CPPs, which is rich in more of the metals in question.

Keywords: *Fly ash of lignite, Heavy metals, Bioaccumulation, Lichens and Mosses.*

Introduction

Low quality lignite is the main fuel for electricity production since 1950 years in Greece (Ganatsios *et al*., 2001). West Macedonia Lignite Center (WMLC), located in Northwest Greece has four lignite power plants, which release significant amounts of fly ash. The particulate matter of ash, which contains heavy metals (Adamidou *et al*., 2007), is the main pollutant of the WMLC basin (Triantafyllou *et al*., 2006). This paper investigates the uptake and accumulation of pollutants in plants, which may follow two different paths, i.e. through the root system or through the leaves or other aerial parts. From this point of view it is interesting to investigate simultaneously two different kinds of plants. The first one includes mosses and lichens, which have neither real roots nor vascular system. They are well known to scientists as ideal pollution bio-indicators, especially for airborne particles containing metals and radionuclides, because they appear high values of accumulation rates (Sawidis *et al*., 1993), (Sawidis *et al*., 1995). The second category contains two types of trees, evergreen and deciduous, which are not the best indicators, however they are the major plant types found around the WMLC stations.

The ability of plant organisms to absorb and accumulate pollutants is better described by numerical parameters such as Transfer Factor (TF), defined as the ratio of the concentration of a given element in the investigated terrestrial organism to that found in the soil (Papastefanou *et al*., 1999).

In this paper the concentrations of seven heavy metals were determined in samples of mosses, lichens and trees, collected from the WMLC basin and an investigation was performed towards the relationship of the metal concentrations in the plants with the respective ones in the soil. Furthermore, the TF values were calculated using data for the soil concentrations based on previous work (Tsikritzis *et al*., 2002).

Material and Methods

The samples were collected from 13 sampling points, i.e. 11 for trees and 2 for mosses and lichens (Fig. 1) following the North-Northwest to South-Southwest direction of the wind, which is the dominant one at the WMLC basin (Triantafyllou *et al*., 2006). Sample point No 11 was chosen as a reference.

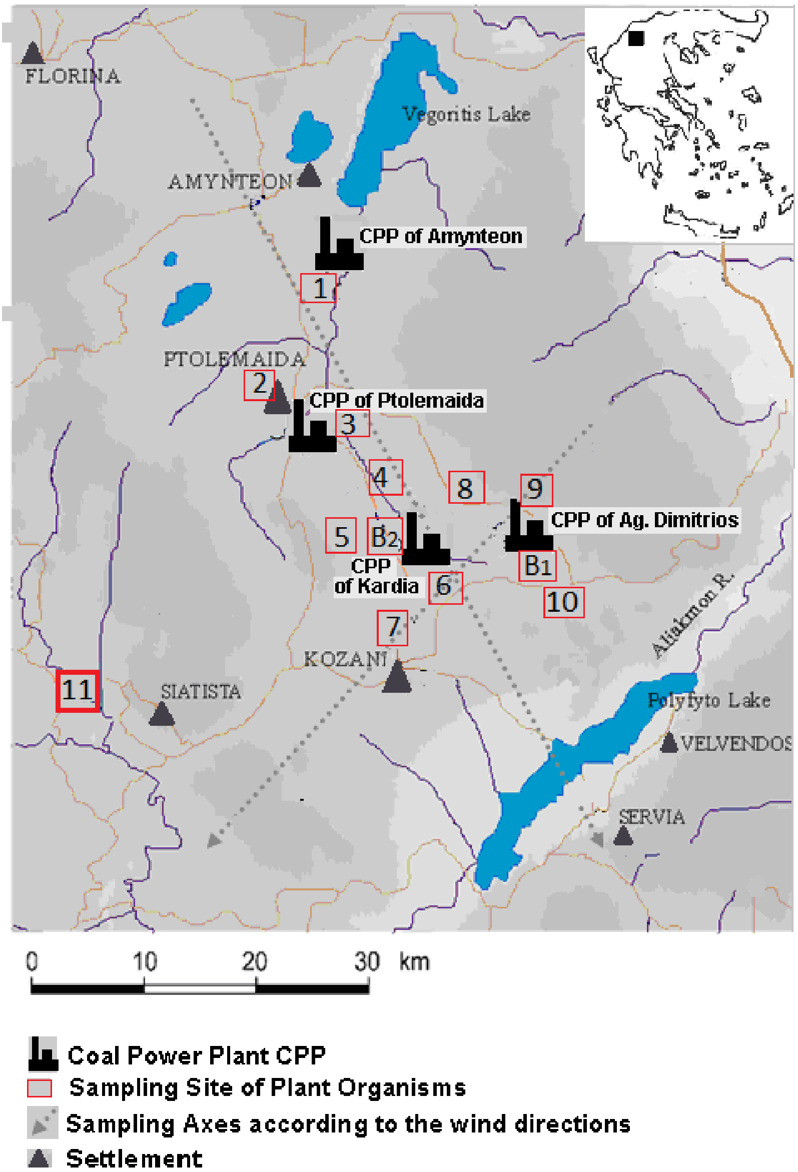

Figure 1. The general map of WMLC with the sampling points. (B1) and (B2): the sampling points of both mosses and lichens. (1) – (11): the sampling points of tree leaves.

The following species of plant organisms were investigated:

a) Six moss species: *Grimmia laevigata, Rhocomitrium canescens, Tortula ruralis, Eucalypta strteptocarpa, Homolotecium aureum, Tortella tortuosa.*
b) Five lichen species: *Cladonia rangiformis*, *Parmelia sulcata*, *Pertusia sp.*, *Cladonia convoluta*, *Xanthoria parietina* and

c) Leaves of four tree species: *Pinus nigra, Pyracantha coccinea, Prunus amygdalus* and *Populus nigra.*

After being collected all samples were washed, air dried, grind and sieved with a 2 mm mesh. Instrumentation: The metals Cr, Mn, Fe, Ni, Cu, Cd and Pb were determined using a graphite furnace Perkin-Elmer AAnalyst 800 complete with AA Winlab software. For this purpose 0.5 g of dry samples were digested with 4 ml of HNO_3 (Merck pro analysis) 65%, and 1 ml of HCl (Merck pro analysis) 37 % for 20 min in a Paar Physica microwave oven.

Results and Discussion

The concentrations of the investigated 7 heavy metals in plants are shown in Table 1 for each plant category. Table 2 contains the TF average values for each metal excluding Fe, that had not been measured in soil and therefore its TF value was not calculated.

Table 1. Average values (± SD) of the heavy metals determined in lichens, mosses and tree leaves (in mg/kg).

Type of plant	Cr	Mn	Fe	Ni	Cu	Cd	Pb
Lichens (23 samples)	53.3 ± 9.2	75.8 ± 45.0	5935 ± 2693	42.2 ± 1.7	12.4 ± 2.0	0.48 ± 0.15	19.53 ± 6.08
Mosses (25 samples)	68.93 ± 43.7	313.2 ± 93.6	11982 ± 9335	46.2 ± 23.7	23.7 ± 8.4	0.95 ± 0.81	28.44 ± 10.20
Tree leaves (44 samples)	2.07 ± 1.44	73.3 ± 46.5	382.1 ± 173.9	4.87 ± 4.47	7.31 ± 2.61	0.18 ± 0.35	1.45 ± 0.70
Toxicity range*	5 to 30	400 to 1000		10 to 100	20 to 100	5 to 30	30 to 300

*Kabata-Pendias A. and Pendias H. (1992)

Table 2. Average values of the TF of the metals determined in lichens, mosses and tree leaves

Type of plant	TF of Cr	TF of Mn	TF of Ni	TF of Cu	TF of Cd	TF of Pb
Lichens	0.172	0.151	0.127	0.324	1.824	1.207
Mosses	0.291	0.406	0.152	0.584	3.015	1.712
Tree leaves	0.010	0.140	0.032	0.233	0.737	0.134

The following conclusions could be extracted with regard to the dispersion and bio-accumulation of the determined metals in the plant organisms through the air and the soil:

In mosses and lichens the metal values are classified according to the following descending order Fe > Mn > Cr > Ni > Pb > Cu > Cd. These results are similar to those reported earlier (Sawidis *et al*., 1993), (Sawidis *et al*., 1995), (Sawidis *et al*., 2001), for the same region. However, the metals Fe, Mn, Cr, Ni and Cu revealed higher concentrations than those found in other polluted regions (Rodrigo A. *et al.*, 1999), (Genoni P. *et al*., 2000), (Bargagli R. *et al*., 2002).

The TF values are less than one (<1) for all investigated metals, except Cd and Pb, which show TF values more than one (>1) for both mosses and lichens.

Mosses display systematically higher transfer factors (TF) than lichens and therefore a higher bioaccumulation level. *Grimmia loevigata* is the best Cr and Fe indicator of all mosses, whereas the species *Cladonia convoluta* is the best Cr indicator of all lichens.

Concerning the tree leaves, the concentrations of the investigated metals are classified according to the following descending order Fe > Mn > Cu > Ni > Cr > Pb > Cd. In comparison with the respective classification of metals in mosses and lichens the Cr has been seen to recede from the third to the fifth place in tree leaves, which is mainly due to the almost zero uptake of Cr^{3+} through the tree roots, as explained in detail at the end of this chapter.

The bioaccumulation capacity in tree leaves is much smaller than in mosses and lichens and the TF values of trees is always <1. This profound "superiority" of mosses and lichens is attributed to the fact that they have a long cycle of life during which they continuously accumulate heavy metals from the environment and thus are more suitable subjects for long term pollution investigation. On the contrary, trees, evergreen or deciduous, are more suitable for monitoring of short term pollution processes.

It should also be mentioned that for the same category of plants, the concentrations of the metals in question are slightly influenced by the type of each species.

The final and perhaps most important conclusion is that the concentrations of most metals (Cr, Mn, Fe, Ni, Cd and Pb) in the leaves of all tree species are systematically higher than those of the reference (unpolluted) station. This cannot be attributed to their exclusive uptake through the soil, because: 1) The concentrations of these metals in the soil are very low. 2) The characteristics of the most soils in the study area, such as the alkaline pH values and the high concentrations of calcium, drastically reduce the mobility of most metals and result in low Transfer Factors to the plants.

Therefore, taking into consideration that the possibility of metal uptake from the soil is quite low, the relatively high concentrations of metals in the leaves of most tree species must be attributed to the uptake through the atmosphere and mainly through the deposition of the suspended particles of the lignite fly ash, which is rich in most of the investigated metals. More specifically, the main source of metals Cu, Cd, Ni, Pb and Cr is the fly ash particles, whereas the presence of Fe and Mn is basically attributed to their uptake from the soil. Figure 2 shows a Multidimensional Scaling (MDS) diagram, where Fe – Mn determined in tree leaves form and belong to a separate cluster (I) from the other metals (II). This division of metals into "soil group" (I) and "fly ash group" (II) does not apply to mosses and lichens, which is quite reasonable, since the nutrients and other ingredients of these organisms are almost exclusively uptaken from the atmosphere.

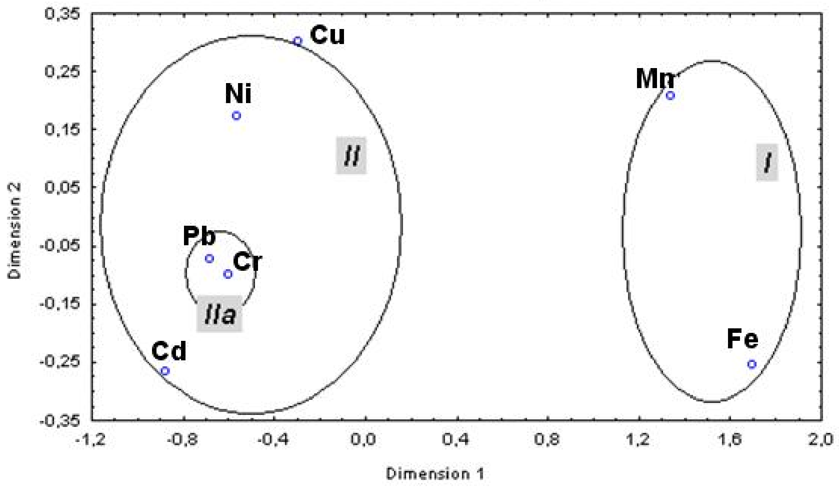

Figure 2. Results of MDS analysis applied on the concentrations of the heavy metals determined in the tree leaves

Furthermore, the spatial distribution of the sampling points shows that the trees around the coal power plants (especially the Ptolemais one) are more polluted, without great differences though.
Finally, special consideration should be given to Chromium. Cr values for all species are systematically higher than those of the reference (unpolluted) station. Particularly in *Populus Nigra* they approach the bibliography toxicity or excess limits, that is 5-30 ppm (Kabata-Pendias A. and Pendias H., 1992)
One would expect that the high concentrations of Cr in the soil of some areas of WMLC basin (central–eastern and southwestern) (Tsikritzis *et al*., 2002) should be accompanied by similar increase in the concentrations of Cr in tree leaves collected in the above mentioned areas (No 7,8,9,10). But in reality this is not the case, or more correctly it is less common than expected, because in these soils Cr appears mostly as Cr^{3+} ions which are insoluble at high pH values as those ones of the study areas, pH 7- 8,5 (IGME, 2001).
Since the likelihood of the leaf Cr being uptaken from the soil has been almost eliminated, the relatively high concentrations of Cr in the tree leaves should be attributed to the air pollutants, i.e to the deposition of the flying ash of CPPs, which is rich in Cr.

Conclusions

The comparative investigation of three different categories of plants with respect to the distribution and bioaccumulation of seven heavy metals (Cr, Mn, Fe, Ni, Cu, Cd and Pb) has shown that the highest capacity to absorb and accumulate these elements belongs to mosses and lichens and less to tree leaves.
In comparison with unpolluted areas, the concentrations of Cr, Mn, Fe, Ni, Cd and Pb are systematically increased in vascular plant leaves in the vicinity of all CPPs. This fact cannot be attributed to soil uptake, given that soil parameters in the study area such as high pH and calcium values, immobilize most of the heavy metals. The most reliable explanation for their origin is the flying ash of CPPs, which is rich in more of the metals in question.

Abbreviations

TF. Transfer Factor
WMLC. West Macedonia Lignite Center ()
CPP. Coal fired Power Plant
MDS. Multidimensional Scaling

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BROWNFIELDS AS ENVIRONMENTAL QUALITY INDICATOR: CASE OF LATVIA

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Abstract

The background of investigation is the growing importance of the global economy, which shows that one of the basic natural resources - intensity of land use - is increasing. Often it is the cause of land degradation processes. Expressions of brownfields are diverse, and their elimination and gradual prevention is the first prerequisite for the sustainable use of land resources and development of each territory. Earlier studies in Latvia show that degraded land does not have one significant feature which would allow to determinate it as a "typical" brownfield. Sustainable land management is a key factor in the rational use of land resources, including the reduction of land degradation and putting in order of degraded territories. Improvement, maximal and efficient engagement of brownfields in economic activity is one of the key challenges for sustainable resource use that makes significant contribution to regional development. Determination of brownfields is essential part of territorial and land use planning which is strongly related to the implementation of sustainable development programmes and planning of further actions in all municipalities. The reuse of brownfields has significant impact on sustainable development as meets all objectives: improving the economy, improving social cohesion and the environment. The objective of investigation is on the basis of special literature to develop scientifically based proposals for possible solutions in regeneration of brownfields and its benefits for development and sustainable use of resources, transformation of brownfields into recreational areas, as well as further use of brownfields in cities and rural areas.

Keywords: *Degraded territory, brownfield, land resources, regeneration, sustainable development*

Introduction

It is very important to ensure conservation and sustainable use of the land because the land is limited, non-renewable resource. In circumstances of growing of global economy, intensity of land use is increasing as well. Because of different economic activities, including industrial activities, and influence of natural conditions, land and soil degradation process has occurred leading to the formation of brownfields. Reduction of land degradation and arrangement of brownfields is one of the factors in rational use of land resources (Akhtar-Schuster M. et al., 2016).

One of the aims of sustainable environment resolutions adopted by the United Nations General Assembly in 2015 was "to restore degraded land and to aim to achieve having a world neutral to land degradation" (UN General Assembly, 2015). This is the first worldwide document where universal and comprehensive measures regarding land degradation are provided. In recent years have been accepted a lot of high-level decisions in regard to land and soil degradation and its prevention. These documents set goals for sustainable development that includes an economic, social and environmental dimension. One of objectives of environmental dimension is regeneration of brownfields.

The process of formation of brownfields in Latvia has been similar to other Eastern European countries and partly to Western Europe. Most amount of brownfields appeared after collapse of the Soviet system, their development was influenced by transition to market economy and changes in industry. Brownfields mostly are industrial sites, their infrastructure, abandoned military bases and sites where construction started many years ago but still is not completed. Total area of brownfields in Latvia is considerably small compared to some Eastern European countries. Ministry of Environmental Protection and Regional Development has discovered that about 6 thousand ha of municipal and private land is degraded (Gerhards, 2018).

At the end of 2014, Land Management law in Latvia was adopted. The law defines the term of land degradation and obliges local authorities to display degraded territories on territorial planning documents, while land owners are obliged to carry out prevention measures of land degradation. Land degradation is defined as reduction or disappearance of the economic or ecological value of land and associated to land resources, as a result of natural processes or human activity, or inaction. In general, degraded area is explained as an area with damaged surface of the earth, or abandoned build-up, mining, economic or military activity territory.

The general objective of brownfields revitalization is to promote the sustainable development of urban and rural area: maximal elimination of brownfields thus contributing to environmental regeneration. This includes solving of environmental problems and increasing the economic efficiency of territory use, improving visual and structural functional quality of environment, as well as humanization elements of the social environment. Specific circumstances in each region determine which aspects should be more observed in the context of real situation.

Latvian scientists have shared results of their scientific investigations and experience in evaluation of brownfields (Parsova *et al*., 2017; Platonova *et al*., 2017; Klavins *et al*., 2008). Systematic approach to land degradation issues has been demonstrated in investigations of Western European scientists (Ferber, 2006), etc. Significant contribution to this problem has been made by Central and Eastern European scientists (Kunc *et al*., 2014; Frantal *et al*., 2015; Simion, 2016; Juozapaviciute, 2016)

Material and Methods

The **purpose** of this article is to evaluate Latvian and foreign experience in brownfield regeneration on the basis of scientific literature and other information, as well as analyse its benefits. To achieve this goal **tasks** were performed as follows:

- to gather and analyse the sources of foreign and Latvian scientific literature on brownfields and their regeneration;
- to develop possible solutions in regeneration of brownfields;
- to analyse benefits of brownfield regeneration for development and sustainable use of the land.

To achieve the aim of study, information about types and features of present land and soil degradation had to be summarized. The authors have evaluated the information about land degradation in regulations of Latvia. They also examined international experience and had discussions and interviews with competent specialists in public institutions of Latvia (Ministry of Agriculture, Ministry of Environmental Protection and Regional Development, State Plant Protection Service, etc.). The study was conducted as a part of municipal and state institutions competent specialists' survey to find out the opinion of respondents on land degradation types, identification and prioritization of degraded land areas and information about the maintenance of degraded territories.

Knowledge and impressions obtained during planned study trip in Poland and Germany on regeneration of brownfields have been used as well. There general scientific methods of theoretical research: analysis and synthesis, induction and deduction, as well as monographic method have been used.

The **object** of research is degraded build-up area. Processes of land degradation can express in different ways. At the moment in Latvia does not exist accepted on governmental level

classification of brownfields. Public discussions on proposals of classification of types of land degradation and methods for its evaluation have been opened by Cabinet of ministers in 2019 (Table 1).
Table 1 shows that degraded build-up territories are one of the types of land degradation and they can be divided into three sub-types: degraded residential or public build-up, industrial and military territories. In all cases there are located abandoned buildings, constructions or other objects that are not managed and endanger human health or life due to their physical wear and tear or harm the environment.

Table 1

Classification of types of land degradation
(draft, developed by Cabinet of Ministers of Latvia)

Type of land degradation	Sub-type of land degradation
Degraded build-up area	Degraded residential or public build-up area
	Degraded industrial area
	Degraded military territory
Degraded mineral extraction site	Non-recultivated mineral extraction site
Waste disposal at non-designated for this purpose sites	Waste disposal, preservation and storage in non-designated for this purpose sites
Land pollution	Pollution of the territory by hazardous substances
Spread of invasive plants	Territory infested with invasive plants

To determine degraded build-up area it is necessary to define characteristic features (table 2).

Table 2

Features characterising degraded build-up areas

Sub-type of land degradation	Feature of land degradation
Degraded residential or public build-up area	Territory on which are located abandoned or incompletely used residential buildings or public buildings which do not fulfil their intended function, or which are not managed, or endanger human health or life due to their physical wear and tear, or harm the environment
Degraded industrial area	Territory on which are located abandoned or incompletely used production facilities where economic activity has been suspended, or they do not fulfil its intended function, or they are no longer managed, or endanger human health or life due to their physical wear and tear, or harm the environment
Degraded military territory	Territory on which is located abandoned military object, which do not fulfil its intended function, or is no longer managed and endanger human health or life due to physical wear and tear, or harm the environment

Results and Discussion

It can be concluded that in the world, especially in Europe, the issue of brownfield regeneration began to play a prominent role in development programs in the end of last century. Already almost for 50 years solutions with international approach has taken place and theory and experience of brownfields regeneration has been developed. They are linked to wide range of issues of sustainable urban and regional development. Regeneration of brownfields strengthens the vitality and efficiency of urban area.

It can be also concluded that brownfields are rapidly developing in process of social and economic changes. Symptoms of chronic unemployment and social stratification of society always have very negative economic and physical impact on the territory. In Eastern Europe in post-industrial period brownfields developed due to transformation of industrial environment. In post-industrial society much less space is needed for production, but much more for services, consumption and entertainment (Juozapaviciute, 2016). Examples of post-industrial transformations can be found in Latvia, too.

Reuse of brownfields has significant impact on sustainable development because it meets all three of its objectives: improvement of economy, social cohesion and environment. When economic activities return to degraded areas, these areas have been significantly improved and therefore greenfield areas are preserved. New activities taking place on former brownfields create new opportunities for public, increase employment, income, etc. Remediation of brownfields can also improve social cohesion, prevent risks to the environment, protect cultural and historical values and improve quality of life. The contrast between modern and historic, creating new tower of views from which a view of the historic castle is open, is showed on fig.1.

Fig.1. Park for recreation and entertainment near Bauska castle

Renovation of brownfields has positive impact on real estate prices. Further savings are achieved through the opportunities offered by existing resources and infrastructure (buildings, energy, sewerage networks, etc.) and transport options. Planning taking into account public needs can maximize investment in brownfield redevelopment also for builders and landowners. Extraordinary residential complex "Gypsum Factory" is located on the bank of Daugava River and has wonderful panorama to Old Riga (fig.2).

Fig.2. Arrangement of residential and office spaces in old abandoned factory in Riga

One of the main drivers of brownfield regeneration is the economic revitalization of the urban area and potential profits. The role of brownfields in supporting economic development and competitiveness in Europe has become increasingly important. Most often this is case in traditional former industrial areas. Revitalization of brownfields affects different market areas: land market, real estate market, labour market, capital market, financial market, resources market; infrastructure market; innovation market. Analysing foreign experience, it can be concluded that aim is to develop sustainable localities with higher quality of life. One of the key elements of high-quality urban development is accessibility and good connection with open space (fig.3).

Fig.3. Arrangement of park for recreation and entertainment near railway station in Rezekne

Many of regenerated sites once have been an "engines" that facilitated the industrial revolution - coal mines, quarries and edges of channels. With the decline of heavy industry, often these territories became abandoned, sometimes dangerous.

There are many examples in the world with a long history and relevant experience, where public and commercial centres, museums and exhibition galleries, relax and recreation areas, amusement parks, sports centres etc. cultural objects for local public and visitors have been created. Often they change the habits of the city and people. The development of urban areas takes place on the basis of more efficient use of existing built-up land and with infrastructure for new construction, thus preventing new investments in the construction and maintenance of transport and other infrastructure (Kunc, 2014; Simion, 2016).

Getting acquainted with activities of several Eastern European countries in the regeneration of brownfields can be recognized several similar features. Major cities are undergoing major changes in functions. New activities take place in the premises of former industrial companies and in areas where military objects and railway lines were predominant. As a result, rehabilitation of brownfields became one of the first issues of territorial development along with changes in property rights after breaking off of industrial activity and real estate market reorganization after 1990.

More exposed to reconstruction are former production sites in industrial areas with good location, where it was easier and cheaper to reconstruct or completely demolish the buildings. The changes are also characteristic for less favoured areas of industry, where typical phenomenon is continuous preservation of previous buildings, simply replacing their earlier functions with commercial, warehouse and logistics functions. In many abandoned industrial and infrastructure territories the preserving industrial buildings and structures as monuments of the era and their use for cultural, educational and tourism purposes have been observed (Juozapaviciute, 2016; Platonova, 2017).

Summarizing and analysing scientific publications on regeneration of brownfield sites and evaluating benefits from it, several regularities and variants of brownfield regeneration can be distinguished:

- arrangement and construction of shopping centers;
- arrangement of science and technology parks;
- preservation of industrial heritage;

- development of cultural, educational and business centres;
- arrangement of residential and office spaces in old abandoned industrial buildings;
- arrangement of parks for recreation and entertainment, amusement and sports activities, etc.

Conclusions

In large cities of Europe are undergoing major functional changes. In post-socialist countries these changes have taken place in shorter period of time than in Western Europe. For reconstruction more exposed have been former manufacturing sites with good location.

Arrangement and construction of shopping centres is one of widely used forms of abandoned brownfields in Eastern Europe. New science and technology parks promote innovations, research and development, and support start-ups. Protection of historic buildings and monuments is a case of transformation and replacement of architecture heritage, because existence of an industrial culture has been the driving force of the economy since the Industrial Revolution.

Reuse of brownfields has the significant impact on sustainable development – improvement of economy, social cohesion and environment.

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HABITAT TYPES OF EUROPEAN IMPORTANCE ON THE JAHORINA MOUNTAIN (BOSNIA AND HERZEGOVINA)

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Abstract

The paper presents types of habitats of Jahorina Mountain which are of European importance. The review is made on the basis of studies of flora and vegetation and the Guide of the types of habitats according to the EU Habitats Directive. Research was carried out in the period of 2015-2016. Taking plant material and making phytocoenological recordings were done at different habitats. Identification of species was based on floristic literature. Phytocoenologically recordings were made by the method Braun-Blanquet. During the research the following habitats have been isolated: 4060 - Alpine and Boreal heaths, 6230 - Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe), 6410 - Molinia meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*), 6430 - Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels, 6510 - Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*), 6520 - Mountain hay meadows, 7110 - Active raised bogs, 7120 - Degraded raised bogs still capable of natural regeneration, 7220 - Petrifying springs with tufa formation (Cratoneurion), 9140 - Medio-European subalpine beech woods with Acer and *Rumex arifolius*, 9410 - Acidophilous Picea forests of the montane to alpine levels (*Vaccinio-Piceetea*). The aim of the paper is to present a habitat in Jahorina that is significant for Bosnia and Herzegovina and the European Union.

Key words: *habitat, Natura 2000, Jahorina*

Introduction

The Jahorina Mountain is located in the central part of Bosnia and Herzegovina, southeast of Sarajevo. The Jahorina Mountain range is determined with coordinates 43˚ 39´ to 43˚ 47´ north latitude and 18˚ 31´ to 18˚ 43´ east longitude. It belongs to continental Dinarides with the direction of the main ridge from northwest to southeast. Jahorina massif is long about 30 km, with the highest peak Ogorjelica (1916 meters above sea level). In geological terms Jahorina is composed of a variety of rocks of Paleozoic, Mesozoic and Permkarbon Triassic age. The base consists of Carbon and Permian sandstones and shales, and above them there are Mesozoic Triassic limestones. From the land, on carbonate surface, the following have been developed: lithosols, organogenic and organomineral calcomelanosol, calcocambisol, luvisols, and on a silicate substrate: brown podzols, podzols, swampy gley peat and ranker. The lowest altitude position is in the climate which is temperate continental, in a central part of Jahorina is subalpine climate, and in the highest mountain areas is alpine continental climate (Petronic et al., 2009).

Because of the negative anthropogenic impact, natural habitats are destroyed on a daily basis, and many species die out. Connecting protected areas of Bosnia and Herzegovina in the European network of protected areas Natura 2000 is aimed to prevent the loss of biodiversity, preserve the habitat of endangered species and ensure their long term survival. For Bosnia and Herzegovina, WWF MedPO started a project to support the implementation of the European ecological network Natura 2000 in 2007. Based on the literature data, the research of authors, supported by The European Union, made the Habitat Types Guide of BiH according to the EU Habitats Directive (Milanovic et al., 2015). The aim of this paper is to present types of habitats in the area of Jahorina Mountain, which are significant for Bosnia and Herzegovina and the European Union.

Materials and methods

Floristic and vegetation research on areas are carried out from 2015. to 2016. Taking plant material and making phytocoenological recordings were done at different habitats. Identification of species was based on floristic literature (Javorka et Csapody, 1979; Beck, 1903 and 1927; Josifovic ed. 1970-1977). Phytocoenologically recordings were made by the method Braun-Blanquet (1965). During allocations European significant habitats on the Jahorina Mountain used data research of flora and vegetation and Guide to the types of habitats according to the EU Habitats Directive (Milanovic et al., 2015).

Results and Discussion

Based on explored flora, vegetation and habitats, according to the Review of the Habitats Directive of the European Union, the following habitats were isolated (table 1).

Table 1. Overview of habitat areas of Jahorina

Code	Name of habitats
4060	Alpine and Boreal heaths
6230	Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe)
6410	Molinia meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*)
6430	Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels
6510	Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*)
6520	Mountain hay meadows
7110	Active raised bogs
7120	Degraded raised bogs still capable of natural regeneration
7220	Petrifying springs with tufa formation (Cratoneurion)
9140	Medio-European subalpine beech woods with Acer and *Rumex arifolius*
9410	Acidophilous Picea forests of the montane to alpine levels (*Vaccinio-Piceetea*)

4060 - Alpine and Boreal heaths

Alpine and Boreal heaths represent the stands of low, bushy and patchy shrubs of subalpine and alpine belt of the Eurasian Mountains. The communties of mountain pine, nowdays are limited on narrow belt of the highest dinaric mountains (Redzic at al., 2008). They are characterized with a very dense cover of characteristic species, usually up to 50 cm high. They usually occur as a result of the cessation of grazing on the subalpine grasslands and represent successive transitions to the bushes of mountain pine or vegetation of subalpine forests (Milanovic, 2015). Geological foundation are either carbonate or silicate rocks. Soil belongs manly in the class of humus-acumulative ones, which is black earth, rendsine and ranker (Redzic at al., 2008).

The degradation of forest vegetation of the highest parts of the Jahorina, from 1600 m to the peaks of the mountain, occurred in the ancient past, and this place was named Gola Jahorina (figure 1). Alpine and Boreal heaths on Jahorina were developed in the zone of mountain pine (*Pinus mugo*). The vegetation of mountain pine was retained on a small number of sites (Trijeska, Lokvansko brdo and Stjeniste) in extremely small areas. Surfaces under mountain pine have been cut down in the past and turned into spacious pastures (Bjelcic, 1966).

In deep and acidic soils of these natural successions dominate *Vaccinium myrtillus* in community *Hyperico maculati-Vaccinietum myrtilli* (Lakušić et al. 1979) Surina 2013, *Hyperico grisebachii-Vaccinietum myrtilli* (Lakušić et al. 1966) Surina 2013, while on the shallow soil on the carbonates there are comon Juniper communities (*Juniperus communis*) *(Arctostaphylo-Juniperetum nanae-intermediae* Stefanovic 1964 and *Sempervivo schlechani-Juniperetum alpinae* Bjelcic 1966) and *Arctostaphylos uva-ursi (Arctostaphyletum uvae-ursi Lakušić* et al. 1979). On wet recess there are community with *Salix silesiaca* (Bjelcic, 1966).

Figure 1. Gola Jahorina

6230 - Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe)

Species-rich Nardus grasslands are dry to mesophilic. They develop on acidic soils of Atlantic, subatlantic and boreal lowlands, hills and mountains, but in the southern part of Europe only occur on the mountains. In these grasslands is dominated *Nardus stricta* and other acidophilic species. They are typical for silicate massifs, but also occur on carbonates, only on flatlands and valleys, where the soil is deeper and more acidic, and the influence of the substrate is isolated or limited. They are mainly formed as a result of the shrinkage of subalpine forest or shrub vegetation, because the cattle are glad to graze and have plenty of food, but they are often so impoverished that they become almost monotypical. These monotypic grasslands can not be included in this type of habitat (Milanovic, 2015).
Species-rich Nardus grasslands on Jahorina belong to association: *Aurantiaco-Nardetum strictae* Ht 1960, alliance: *Jasionion orbiculatae* Lakušić 1966, order: *Seslerietalia comosae* (Sim.) Lakušić 1966 and class: *Caricetea curvulae* Br.-B. 1948.

6410 - Molinia meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*)

The habitat includes meadows of *Molinia caerulea* from the lowland to the mountainous belt, which developed on wet, basic, neutral to acidic soils, mainly poor nitrates (figure 2). They usually occur by natural or artificial drying of peat bogs and by cutting of forest vegetation on acidic soils and slightly sloping slopes. Fertility reaches into the late summer and maintained by regular mowing or annual burning.
Purple moor meadows are very heterogeneous and should be observed in the proper interpretation of the habitat. Purple moor stands out as a dominant species in several types of lawn, and only some of them can be included in this habitat. In addition, the genus *Molinia* encompasses more similar species, which make ecologically and physiologically different

lawns. This habitat type includes Central European meadows of alliance *Molinion caeruleae* W. Koch 1926, which are rarely found in our conditions and are found mainly in the mountain range of silicate massifs. These are secondary constructions created by the cutting of forest vegetation on the gentle slopes with very deep, in springs extremely humid, acidic soils. These lawns are mowing once in the late summer and dont give good food for cattle, because they are abandoned and left to heal or held by burning before the end of the vegetation season (Milanovic, 2015). In the area of Saracevo polje, on wet meadows, there are few stands where dominated purple moor (Petronic i Pavlovic, 2012).

Figure 2. Molinia meadows on Jahorina

6430 - Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels

Hydrophilous tall herb fringe communities belonging to the class *Betulo-Adenostylete* Braun-Blanquet 1948, which develop from montane to alpine levels, are commonly observed on humus and humid soils. They follow the edges of forests, forest cleanings, water flows, etc. (Muratovic, 2015).

On Jahorina they are classified in 4 order (*Atropetalia belladonnae* Vlieg 1937, *Betuletalia* Lakušić et al 1978, *Adenostyletalia* Br. 1931 and *Pteridetalia* Lakušić et al., 1978) and 6 alliance (*Atropion belladonnae* Br.-Bl. 1930, *Sambuco-Capreae capsules* Tüxen & Neumann ex Oberdorfer 1957, *Betulion verrucosae* Lakušić et al 1978, *Adenostylion alliariae* Br. 1925, *Petasition* Lakušić et al. 1978 and *Pteridion aquilini* Lakušić et al. 1978) and 8 associations (*Telekietum peciosae* Treg. 1945, *Salicetum incanae* Jov. 1963, *Capreeto-Populetum tremulae* Glišić (1950) 1975, *Adenostylo-Doronicetum* Ht 1956, *Deschampsietum subalpinum* Ht 1956, *Petasitetum albi* Ht 1956, *Pteridietum aquilini* Lakušić 1975, *Aegopodio-Petasitetum hybrids* R. Tx.) (Petronic et al., 2009).

The habitat is characterized by the nitrophilic ruderal vegetation of the order *Glechometalia hederaceae* R. Tüxen in Brun-Hool et R. Tüxen 1975 and *Convolvuletalia sepii* Tüxen 1950, and excludes communities in which prevails alpine dock (*Rumex alpinus*) (Muratovic, 2015).

6510 - Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*)

Lowland hay meadows are maintained by a constant anthropogenic influence. In Bosnia and Herzegovina, they develop in the valley, upland and mountain areas and belong to alliance of *Arrhenatherion* Koch 1926, *Cynosurion* Tüxen 1947 and *Calthion* Tüxen 1937 (Muratovic, 2015).

In the area of Jahorina there is a vegetation of alliance *Arrhenatherion* and *Cynosurion*. From the vegetation of alliance *Arrhenatherion elatioris* the most common community is *Arrhenatheretum elatioris* Tx. 1937, which occupies the lowest habitats on flat surfaces (Petronic and Pavlovic, 2012).
Vegetation of alliance *Cynosurion* involves the mesophilic meadows in the lower areas caused by degradation of oak forests. On Jahorina there is an association *Bromo-Cynosuretum cristati* H-ić 1930 which differentiates into subassociations of *holcetosum lanati* and *brometosum racemose* (Petronic and Pavlovic, 2012).

6520 - Mountain hay meadows

Mountain hay meadows are covered by alliance of *Polygono-Trisetion* Braun-Blanquet et Tüxen ex Marschall 1947 n. inv. and *Poion alpinae* Oberdorfer 1950, and on the territory of Bosnia and Herzegovina there is endemic alliance *Pancicion* Lakušić 1966 (Muratovic, 2015). The vegetation of mountain hay meadows on Jahorina belongs to class of *Arhenatheretea* Br.-Bl. 1947, order *Arrhenatheretalia* Pawl. 1928 and endemic alliance *Pancicion* Lakušić 1966 which differentiates on two endemic associations *Pancicion-Lilietum bosniacae* Bjelcic et Lakušić 1969 and *Alchemillo-Crepidetum bosniacae* Bjelcic in 1967. These communities build a large number of mesophilic species among which a significant number are endems (Petronic and Pavlovic, 2012).
On Jahorina association *Alchemillo-Crepidetum* bosniacae occupies fewer areas. It is developed in the lower part of the subalpian belt. It is mainly used as a pasture because it contains a lot of valuable species in terms of feeding cattle (Petronic and Pavlovic, 2012).

7110 - Active raised bogs

The raised bogs in B&H occupy small areas but in their specificities they represent a unique but little known value. Since they were formed during the glacial period, they are categorized as glacial relics. Relative to the worldwide distribution of raised bogs, they are located at the southern border of their areal, indicating their high isolation and vulnerability (Barudanovic et al., 2017).
Natural or semi-natural majority ombrotrophic elevated peat complexes on peat substrate. Often the raised surface is developed, or dome, with levels of water that is considerably higher than the groundwater level in the environment, which receives water exclusively from atmospheric precipitation. This raised bogs complex is characterized by the dominance of moss from the genus *Sphagnum* spp., and includes all zones lying within retention zone of water. The necessary prerequisite for the development of bogs formations is the high amount of rainfall (active bogs) (Djug and Milanovic, 2015).
In the syntaxonomic view vegetation belongs to class *Oxycocco-Sphagnete* Br.-Bl. et Tx. 1943 order *Sphagnetalia fusci* Tx. In 1955. On Jahorina, in the area of Dugo polje, there is alliance of *Sphagnion fusci* Br.-Bl. 1920, which includes the associations of *Sphagnetum fusci* Luq. 1926 and *Sphagnetum medii* Käsn. et al. 1933. Active raised bogs are very rare and poorly explored in BiH, and often have a transitional character.

7120 - Degraded raised bogs still capable of natural regeneration

These habitats include degraded raised bogs which are under negative influence (on the hydrological regime or exploited) but still capable of natural regeneration. Stages of their degeneration are recognized by colonization of species *Molinia caerulea* and other species that make changes to the trophic regime from oligo-distrophic conditions to mezotrophic and eutrophic conditions. Regeneration ability means that it is possible to restore the hydrological regime, after which the native vegetation can be expected to recover for about 30 years. Plant species that are typical for raised bogs such as moss (*Sphagnum* spp.) represent a significant part of vegetation with significant presence of species characteristic of the degradation stages (*Molinia caerulea* and other) (Djug and Milanovic, 2015).

7220 - Petrifying springs with tufa formation (Cratoneurion)

Petrifying water sources that are rich in potassium bicarbonate and where the sediment sits right next to the spring in the forest or open areas. There are frequent mosses which belong to alliance of *Cratoneurion commutatum* W. Koch 1928. This type of habitat includes the main streams where the tuff is formed and is characterized by the presence of tufa formations. It is necessary to include all zones that are directly related to the source as well as the zones inhabited by relevant vegetation (Cratoneurion), even where it extends from sources to the upper streams of the watercourse (Đug, 2015).The spring of the Paljanska Miljacka (figure 3) is located on the sedimentary deposits, represents the hydrological value. Downstream from the source, about 200 m, there is a waterfall on Paljanska Miljacka. The sedentary area at Paljanska Miljacka is about 400 m downstream from the spring and is active. Rich populations of the genus *Cratoneurion* have been developed on it. This type of habitat is distinguished by the presence of a yellow-orange blanket which is made of moss *Cratoneuron commutatum* and *Cratoneuron filicinum* (Petronic and Pavlovic, 2012).

Figure 3. Petrifying springs with tufa formation on Jahorina

9140 - Medio-European subalpine beech woods with *Acer* and *Rumex arifolius*

Subalpine forests with *Acer pseudoplatanus* are a permanent climaregional stage in western Dinarides in BiH. The mild slopes of the subalpine belt are matured, and happiness is a little lower, on flat terrain and deep plains of obstructed glacial lakes (lakes), where the secular succession gradually shifts to the climaregional community of the mountain forest. The dominant species is mainly *Acer pseudoplatanus*, and there are more beech and spruce trees on the floor of the trees. In eastern Dinarides, there are similar subalpine forests with *Acer heldreichii* ssp. *visianii*, *Cicerbita pancicii* (Brujic, 2015).

In Jahorina they are subalpine mountain forests of beech and mountain maple *Aceri heldreichii-Fagetum* Jov. 1957 (*Aceri visiani-Fagetum* Fuk. And Stef. 1958) - subalpine beech forest with Greek maple. In the Jahorina area in mixed deciduous-coniferous forests above 1300 m there is an endemic species of maple *Acer heldreichii* subsp. *visianii*, known as the mountain maple. It is significantly and prominently represented along the streams and the road of Pale - Jahorina, the sites Mali and Veliki javor. In the highest part of the forest zone, have been developed subalpine beech forests with maple trees (*Aceri-Fagetum subalpinum* Fuk. Et Stef. 1958 and *Aceri visianii-Fagetum Fuk* 1969. They are not a continuous belt, they are often degraded with individual but numerous old trees of maple (*Acer heldreichii* subsp. *visianii* and *Acer pseudoplatanus*) (Petronic and Pavlovic, 2012).

9410 - Acidophilous Picea forests of the montane to alpine levels (*Vaccinio-Piceetea*)

Subalpine spruce forests
In the area of Jahorina, *Aceri visiani-Piceetum subalpinum* Stef. 1970., this habitat occupies a cooler and colder position under the limestone ridge where deeper clayey soil formed, such as pseudogley or sour brown soil (distric cambisol). This community is conditioned by orographic (microclimate) - edaphic and does not make the altitude belt here, it is already included in the belt of the subalpine beech at an altitude of 1600-1750 m (Petronic and Pavlovic, 2012).
Frosty spruce forests
Piceetum montanum illyricum Horv. et al. 1974 - the frosty forest of spruce, often azonal and extrasonal, orogens. This frosty type of spruce forest is represented on several sites with smaller areas on Ravna planina (Careve vode), best developed in the area of the Dvorista. It is distinguished by specific relief, curvature and depression, with sour brown and brown subsoil that is formed on verphic (quartz) pedestrians (Petronic and Pavlovic, 2012).
Secondary spruce forests
Abieti-Piceetum silicicolum Stef. 1960 in the Jahorina (figure 4,5) is a widespread forest of fir and spruce. It was developed mainly in the severely northern exposures, mainly on the verphic peaks of sour brown and brown subsoil, with the appearance of raw humus. There are also on the verphic clays, in deeper illimerized or poorly clad soil. The larger areas are located in the area of Vrhpraca, less on Ravna planina and above Pale towards Begovina. It occupies a wide altitude range of 1000-1600 m above sea level (Petronic and Pavlovic, 2012). The leading environmental pressures in B&H are inadequate and uncontrolled deforestation (especially coniferous forests). Total logging creates imbalances in the ecosystem and landscape zones, landslides and soil displacements, floods, aerial pollution, loss of resources, forest timber, fertile humus, etc., which leads to loss of diversity (Barudanovic et al., 2015).

Figure 4,5. Conifer woods on Jahorina

Conclusions

Connecting protected areas of Bosnia and Herzegovina in the European network of protected areas Natura 2000 is aimed to prevent the loss of biodiversity, preserve the habitat of endangered species and ensure their long term survival. For Bosnia and Herzegovina, WWF MedPO started a project to support the implementation of the European ecological network Natura 2000 in 2007. The aim of this paper is to present types of habitats in the area of Jahorina Mountain, which are significant for Bosnia and Herzegovina and the European Union. During the research the following habitats have been isolated: 4060 - Alpine and Boreal heaths, 6230 - Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe), 6410 - Molinia meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*), 6430 - Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels, 6510 - Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*), 6520 - Mountain hay meadows, 7110 - Active raised bogs, 7120 - Degraded raised bogs still capable of natural regeneration, 7220 - Petrifying springs with tufa formation (Cratoneurion), 9140 - Medio-European subalpine beech woods with Acer and *Rumex arifolius*, 9410 - Acidophilous Picea forests of the montane to alpine levels (*Vaccinio-Piceetea*).

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ANALYSIS OF DROUGHT IN NEGOTIN LOWLAND FROM THE ASPECT OF PLANT PRODUCTION

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Abstract

The research covered drought analysis in Negotin lowland from plant production issues point of view. Monthly data for the period 1961-2010 from the weather station Negotin were used for the study. Data were analyzed in two sections; first thirty and the last twenty years. Mean air temperature, precipitation, potential evapotranspiration, number of ice days and number of tropical days were processed. For the drought indices, the Standardized Precipitation Index (SPI-6) for September 30, the Walter diagram, the De Martonne index, the Climatic precipitation deficiency and the Aridity index were used. In comparison of the second with the first period, an increase in mean air temperature (1°C for annual temperature and 1.2°C for growing season) and decrease in precipitation (4% for both annual and growing season) were observed. The average climatic precipitation deficit was 375mm, without any significant difference between two periods of research. The average number of tropical days was significantly higher (>50%) in the second study period compared to the first, while the average number of ice days did not show a significant difference. SPI-6 for 30th September indicate 6 dry growing season during the first thirty and 4 in the other twenty years of research period. Aridity index showed a higher frequency of occurrence of semi-arid growing season in the second period of research (every other year) than in the first period (every third year). The Walter diagram and the De Martonne-index pointed to the emergence of drought and the need for irrigation during July, August and September. The forecast indicated further increase in air temperature and decrease in precipitation for the analyzed area. The study conclusion is that in order to secure agricultural production, efforts must be made to find solutions for increasing of the irrigated surfaces in this area.

Keywords: *drought, drought indices, lowland, Negotin, Serbia.*

Introduction

The Negotin lowland is characterized by a very specific climate, the most continental in the whole of Eastern Serbia, with the most pronounced average annual amplitudes and the most pronounced absolute amplitudes of air temperature (Rakićević, 1976). The summers are fierce, with temperatures above 40°C, and cold winters (up to -30°C). The highest precipitation (over 50% of the total annual sum) fall during the summer half of the year (Rakićević, 1976). However, despite this statement, August and September are the least rainfall (RHMZ). The unfavorable rainfall and high summer temperatures have influenced the fact that Negotin lowland is classified as the driest areas in Serbia (Radić, 2017). Climate projections for the future in this area are also not favorable for crop production. An increase in air temperature and decrease of precipitation are expected (Vuković et al., 2018). The frequency of drought, duration and their intensity are the parameters on which the degree of vulnerability of agricultural production depends. In the Negotin lowland area, agricultural production occurs mainly in rainfed conditions, which means that is directly dependent on climate conditions. This production is of great importance for the population of the Negotin lowland, to which agriculture is often the only way of generating income. The aim of this paper is that with use of different Drought indices to analyze the phenomenon of drought in Negotin lowland in the last fifty years, to determine the eventual trend of an increase or decrease in individual indicators of drought and thereby determine their impact on plant production and the need for irrigation.

Materials and methods

The Negotin lowland is located in the eastern part of Serbia, on the triple border of Serbia, Romania and Bulgaria. It is surrounded by mountain ranges (Miroč, Crni Vrh and Deli Jovan) from the north and west.

Monthly climate data for a period of 50 years (from 1961/62 to 2010/11) were taken from the National Hydrometeorological Service of Serbia, from the Negotin weather station. Drought analysis was performed based on the following parameters: mean annual air temperatures and mean air temperatures in the growing season (April-September); annual precipitation and precipitation in the growing season; number of tropical days (Tmax> 30^{o}C) and number of ice days (Tmax <0^{o}C). The annual data discussed in this paper reflect the hydrological year, from October to September of the next year. Drought Indices are determined on an annual basis and/or for growing season. The Standardized Precipitation Index (SPI-6) for September 30 (McKee et al., 1993; Zarch et al., 2015; WMO, 2016), Walter diagram (Walter et al., 1975; Stricevic, 2007), De Martonne Index (De Martonne, 1926; Stričević, 2007; Zambakas, 1992), Climatic precipitation deficiency (Spasov, 2003) and Aridity Index were calculated (UNEP, 1992). Potential evapotranspiration calculated using the FAO method Penman Monteith (Allen et al., 1998) was used to determine the Climatic precipitation deficiency, and Thornthwaite method (Thornthwaite, 1948; Kafle and Bruins, 2009) was used for the Aridity Index.

Results and discussion

The fifty-year analysis (1961/62-2010/11) shows that mean daily air temperature (Tmean) in the Negotin area is 11.5^{o}C during the year, and 18.8^{o}C during the growing season (Tab.1), with an average of 37 tropical days. The average annual precipitation is 635mm, of which 323mm are on average during the growing season. In the last two decades, annual precipitation was lower by 4% than in the previous period. Ruml et al., 2016, analyzing the climate of Serbia in the period 1961-2010, came to the conclusion that Negotin is one of the four meteorological stations in which the greatest decreasing trend in precipitation in Serbia is registered, and is <10mm per decade. Growing season precipitation (323mm) as the income of water and the needs of the reference plant species for water (ETo = 698mm), make the average climate precipitation deficit of 375mm. The division of the investigated period in the

Tab. 1. Mean air temperatures (Tmean), precipitation (P), potential evapotranspiration (ETo), number of ice days, tropical days and average climatic precipitation deficit (P-ETo) in growing season (April-September) at Negotin in period 1961/62-2010/11.

Period	Tmean (^{o}C)		P (mm)		ETo (mm)		Ice days (days)	Tropic.days (days)	P-ETo (mm)
	Hydr. year	Grow. season	Hydr. year	Grow. season	Hydr. year	Grow. season			
1961/62-1990/91	11.1	18.3	646	328	908	703	20	31	-374
1991/92-2010/11	12.1	19.5	619	315	882	691	19	47	-376
1961/62-2010/11	**11.5**	**18.8**	**635**	**323**	**898**	**698**	**20**	**37**	**-375**

first thirty and in the second twenty years shows that in the last 20 years (1991-2010) there was an increase in the average daily temperature compared to the previous period, by 1.0^{o}C per year, ie by 1.2^{o}C during the growing season (Tab.1). This result is complemented by the results of the analysis of extreme air temperatures in Serbia which were got by Ruml et al., 2016. Namely, for the same period of research, the authors noted a statistically significant trend of increasing both the minimum and maximum temperatures in Negotin. This trend of temperature increase has also been reflected in the increase in the length of the growing season, which is statistically significant in the period 1980-2010, and is 22days (Ruml et al., 2017). The rise in mean air temperature during the growing season, in Negotin is shown by the normalized deviation of mean air temperature (NDTmean) (Figure 1).

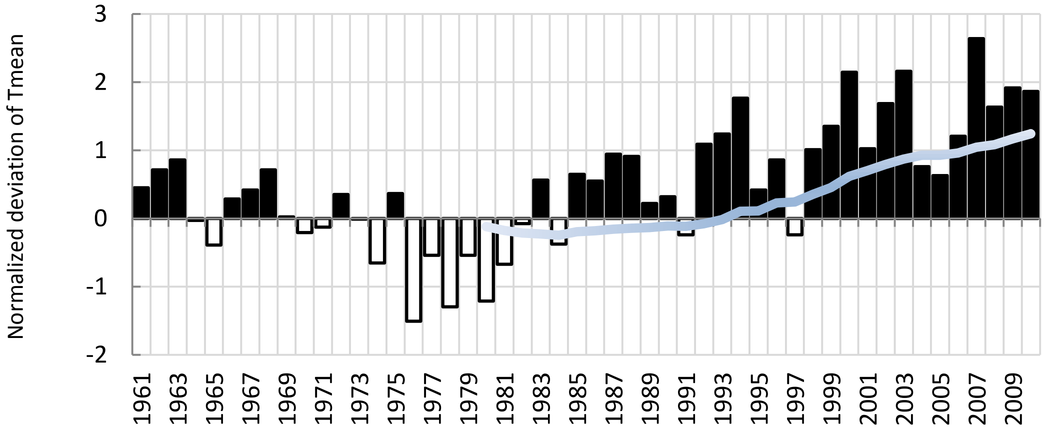


Figure 1. Normalized mean air temperature deviation (NDTmean) during the growing season (April-September) with a mowing average in Negotin lowland

The average number of tropical days is also significantly higher (> 50%) in the second study period compared to the first (Tab.1), while the average number of ice days does not show a significant difference.

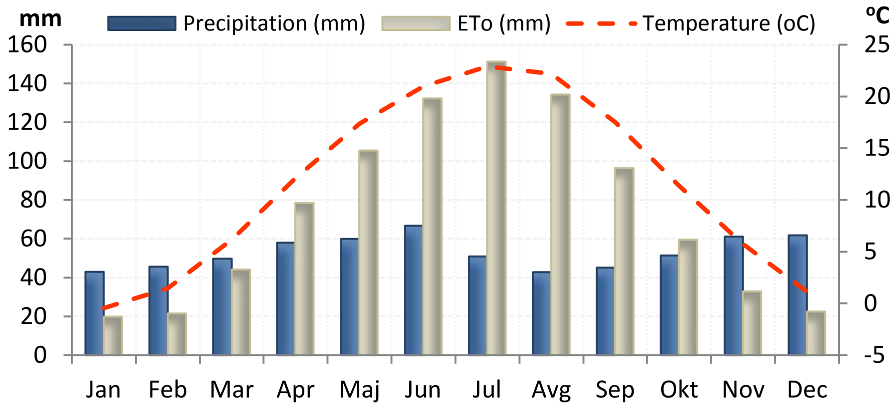
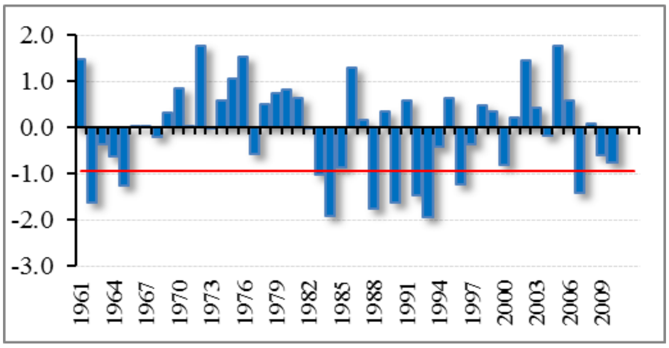


Figure 2. Mean monthly (1961-2010) air temperatures, precipitation and potential evapotranspiration in the Negotin lowland

Monthly distribution shows that June is the most rainy month, with an average precipitation of 67 mm (Figure 2). Maximum precipitations during this month were recorded in 1969 and amounted to 197 mm. The minimum rainfall in August is on average 43 mm. Six times during the fifty-year period, August was characterized by less than 5 mm precipitation. A small amount of precipitation during the summer months is at a disagreement with the high demand of plants for the water during that period. ETo, as an indicator of the potential water expenditure, ie the need for water of a reference plant species, is 375mm for the entire growing season. The highest values of ETo were in July (151mm), so the climatological deficit water of that period was 100mm in average and in August 91mm (Figure 2). Climatological deficit greater than 350mm was recorded in Serbia next to the Timocka Krajina (to which it belonging to the Negotin lowland) and in Banat, as well as the extreme south of Serbia (Spasov et al., 2003).

Moisture conditions in Negotin lowland, shown by the Standardized Precipitation Index (SPI-6) for 30th September, indicate 6 dry growing season during the first thirty and 4 in the other twenty years of research period (Figure 3) .



Moisture conditions	**SPI Value**
Exceptional drought	≤ -2.326
Extreme drought	-2.326 to -1.645
Severe drought	-1.645 to -1.282
Moderate drought	-1.282 to -0.935
Minor drought	-0.935 to -0.524
Near normal	-0.524 to +0.524
Slightly increased moisture	+0.524 to +0.935
Moderately increased moisture	+0.935 to +1.282
Considerably increased moisture	+1.282 to +1.645
Extremely wet	+1.645 to +2.326
Exceptionally wet	≥ +2.326

Figure 3. Moisture conditions during the growing season (April-September) for the period 1961-2010 in Negotin lowland, based on the Standardized Precipitation Index (SPI-6) for 30^{th}September

The Walter diagram (Figure 4) drawn on the basis of average 50-year values (1961-2010) points to droughts in July, August and September, with the most pronounced in August.

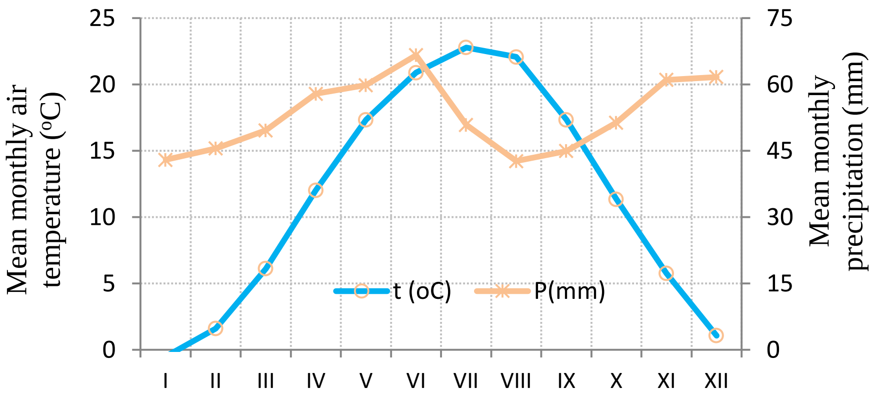


Figure 4. Walter diagram for Negotin lowland (1961/62-2010/11)

The De Martonne index calculated on an annual basis shows that the Negotin area (Ia = 29.5) belongs to the humid condition. However, the values of this index at the monthly level (Tab. 2) indicate the need for irrigation (Im < 20) during July, August and September. As well, in Leskovac lowland, using the same index, the need for irrigation during July and August was determined (Idrizović et al., 2018).

Tab. 2. De Martonne index calculated on the 1961-2010 database for Negotin lowland

1961-2010	Jan	Feb	Mar	Apr	Maj	Jun	Jul	Avg	Sep	Okt	Nov	Dec
Im	54.2	47.1	36.9	31.5	26.3	25.9	18.6	16.0	19.7	28.9	46.4	66.9

The aridity index (at annual level) is 0.71, which, like the Walter index, classifies the climate as humid (AI>0.65). However, AI for the period of the growing season (average 1961-2010) is 0.46, which indicates semi-arid conditions. A higher incidence of semi-arid growing season (AI=0.2-0.5) was observed in the last two decades of research than in the previous period (Figure 5).

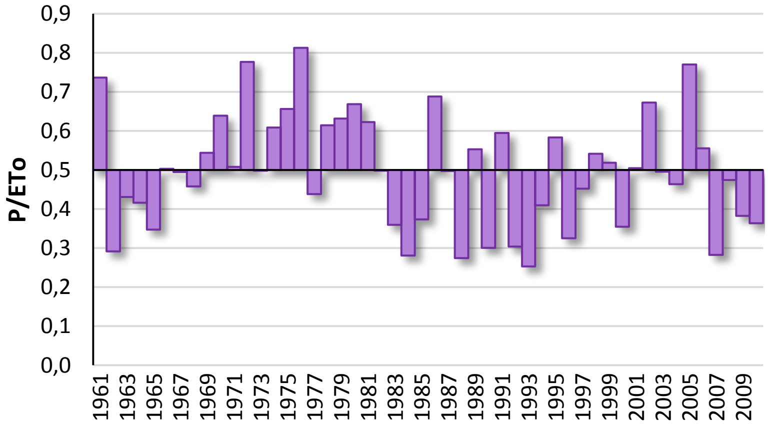


Figure 5. Aridity index (P/ETo) calculated for the growing season (April-September) in Negotin lowland

Conclusions

If the fifty-year research (1961/62-2010/11) was divided into the first (1961/62-1990/91) and the second (1991/92-2010/11) period, the analysis of the climate parameters of the Negotin lowland shows the following:

- The mean air temperature increase in the second period is recorded in relation to the first. Annual T are higher by 1°C, and growing season T is 1.2°C.
- The average number of tropical days is significantly higher (> 50%) in the second study period compared to the first one, while the average number of ice days does not show a significant difference.
- Reduction of annual and growing season precipitation is recorded in the second period compared to the first one, by 4%
- Climatological deficit of precipitation occurs in the growing season. The average is 375mm, with no significant difference between the two research periods. The highest climatic precipitation deficit is in July (100mm) and August (91mm).
- SPI-6 for 30th September indicate 6 dry growing season during the first thirty and 4 in the other twenty years of research period.
- Aridity index shows a higher frequency of occurrence of semi-arid growing season in the second than in the first period of the research.
- The Walter diagram indicates the occurrence of drought in July, August and September.
- De Martonne-index points to the need for irrigation during July, August and September.
- The results show that climatic conditions for plant production are worse in the last twenty years than in the previous thirty. Projections of climatic conditions in the future show further increase of air temperature and decrease of precipitation in this area, so conditions are expected in which agricultural production will be even more threatened. Therefore, the need for irrigation will be even greater.

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INTENSITY OF OOGENESIS AND SPERMATOGENESIS IN RAINBOW TROUT (*Oncorhynchus mykiss Wabaum, 1792)* IN VARIOUS MICROENVIRONMENTAL CONDITIONS

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Abstract

Aquaculture in the world and in Bosnia and Herzegovina is the fastest growing food production sector. The high nutritional value of fish meat, which is also rich in proteins, high-value fats, minerals and vitamins, is also a challenge in respect to a more intensive production of salmonid species in Bosnia and Herzegovina and in the Balkans. As the production is closely tied to the reproductive abilities of fish and animal population as a whole, our research studies compared the intensity of oogenesis and spermatogenesis in rainbow trout in various rearing environments, with a special emphasis on water temperature and oxygen saturation at the time of fishing. The research is one of the guidelines of how to intensify the production of rainbow trout while providing the most favorable living conditions and a faster growth of fish population, contributing to the faster production and market placement without undermining the fundamental postulates of the natural environment.

Key words: *rainbow trout*, *oogenesis*, *spermatogenesis*, *microenvironment conditions.*

Introduction

Hydro resources of Bosnia and Herzegovina belong to the basins of the Adriatic and Black Sea. They cover 20,000 km of rivers and tributaries, 400 ha of lakes and accumulations and 1,400 m of the sea coastline. The special potentials of aquaculture in Bosnia and Herzegovina include a high-quality and clean water, non-infected fish species in the controlled farming conditions, open waters, a high-quality base stock of fish, hatcheries and other fish farming objects. As an agricultural branch, the aquaculture in our country can produce high amounts of various species and categories of fish. If Bosnia and Herzegovina is to keep up with the rest of the world in terms of industrial economic growth, it needs to increase significantly the fish diversity, quality and total production. The most important fish species reared on the trout farms across the country is the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Besides the fact that farming of this fish stock is attractive to a large number of producers, and given its high-yield possibility, another trait is its tolerance to the varying microclimatic conditions of environment. The rainbow trout is reared in concrete or cage systems and can yield between 100,000 and 500,000 kg of trout per hectare of the pond (Hamzić, 1993).
Making a right selection of the base material under the intensive farming secures high production parameters of the offspring (Korjenić, 2010). The intensive production of the rainbow trout (*Oncorhynchus mykiss*) requires meeting several factors to allow for the most favourable conditions for the life and fast growth of fish population, i.e., to contribute to a faster achievement of the planned production and market-placement of the final product. Producers must provide the highest-

quality genetic potential of fish, high-quality feed in line with the age categories, health and veterinary control and physical and chemical parameters of water quality, with a sufficient amount of water supplied to fish-farm, water temperature, concentration of dissolved oxygen in water and pH of water, as the most important ones (Aganović, 1979; Treer *et al*., 2001; Ćuk *et al*., 2006; Katavić, 2009). Minor changes in the keeping and feeding disturb the exploitation of the biological potential of fish, and in case of exceeding certain limits, they even endanger the physiological functions, i.e., they lead to health disorders (Jeremić *et al*., 2004). Our studies tried to examine and bring closer the optimal microenvironment conditions favouring a more intensive oogenesis, i.e., spermatogenesis in the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792).

Material and methods

The material for our research was sampled from three fish farms in Bosnia and Herzegovina:

- Royal-fish farm located on the Jablanica lake (Donja Jablanica), hydro-accumulation on the river Neretva;
- Magazin MAPRIM located on the Ramički creek (Pazarić-Resnik) that flows into the river Bosna, and,
- Eko-projekt located on the river Krupica (Jeleć-Miljevina), which together with Govza flows into the river Bistrica and subsequently into the river Drina.

The research was conducted during the spawning period, in November and December. The male and female samples of the rainbow trout were sexually mature and between 2 and 5 years of age. Due to the relatively large samples and for the purpose of an easier manipulation of the fish samples and duration of measurement, the fish were anaesthetized using Benzoak solution. A total of 40 specimens (26 females and 16 males) from the three fish farms were used in the morphological and histological studies of the gonads. The gonads were separated into plastic bottles and then fixated using 10% formaldehyde. They were transported to the laboratory for further analysis. Prior to moulding, the samples were dehydrated by immersing them in a series of increasing concentrations of alcohol. First, the samples were placed in alcohol solution with concentration of 70% for two days, after which they were transferred into alcohol solution with concentration of 96% concentration for one day and, in the end, they were placed in 100% alcohol for one day. Following this procedure, the samples were transferred into a mixture of 100% alcohol and toluol for two hours, and after that only in toloul for another four hours. The samples thus prepared were left in paraffin I for five hours, and then in paraffin II for twelve hours. After this, the moulding with paraffin blocks was completed. The sample processing from fixation to moulding with paraffin, was carried out on a rotational tissue processor (MICROM model STP 120). After the moulding, the samples were cut using digital microtome LEICA RM 2145, several serial cuts from 0.5 to 1.5 micrometre thick. A total of 400 preparations of 80 samples (40 left and 40 right gonads) were prepared. The cuts were placed on glass slides and deparaffinized by immersing them in a series of decreasing concentration of alcohol. After that, the cuts were stained using haematoxylin eosin, covered with a coverslip and fastened with Canada balsam. The examination of the histological preparations was done using a light microscope equipped with camera MOTIC TYPE 102M, under magnification of 100, 200 and 400x. The analyses of histological structures were carried out using a special program Motic Images Plus 2.0 ML. The microscopic examination included analysis of the gonads in females and males during the spawning period, monitoring the developmental phases and degree of gamete maturation at various fish farms. Water temperature (°C) was measured by the same instrument with an inbuilt thermometer. Oxygen solubility (mg/l) in water was measured by an oximeter Multi 340i/SET (Germany) according to the reference method ISO 5814:1990. A probe was immersed into the water rendering the values of the dissolved oxygen.

Results and discussion

Temperature and photoperiod play a big role in the control of the reproductive cycle (Taranger & Hansen 1993; Pankhurst *et al*., 1996; Davies & Bromage 2002). The uniformity of the water temperature allows for a proper diet, normal metabolism of substances and thus achievement of the desired production results. Farmed rainbow trout receives feed most intensively at the water temperature of around 10 °C, but the best degree of the feed utilisation is achieved at 9 °C (Korjenić, 2010). Ideal water temperatures in salmonid farming (regardless of the production orientation) should be between 9 and 12 °C, with smaller deviations, and the best growth in the fish between 6 months of age and its table size is reached at the water temperatures between 10 and 16 (Mulabdić, 2007). As such water temperatures are usual for the springs of mountain streams and some shorter streams, it becomes a fundamental requirement when selecting the location and projecting the salmonid economy to build such economies in the spring areas of the streams, mostly 500 m to 1000 m downstream. The spring and well waters have constant temperatures throughout the year (between 9 and 12 °C); they are perfectly clean; they are not susceptible to pollution and they are most suitable for supplying the salmonid economy. But, river waters are significantly richer in dissolved oxygen; they contain higher amounts of dissolved salts and they almost never contain a surplus of gasses for example, carbonic acid, nitrogen compounds, etc. (Korjenić, 2010). The optimal temperature in the cage system is between 15 °C and 20 °C, whereby its further growth during summer periods is not desirable for longer periods and can lead to death of fish Pillay, 1996; Mulabdić, 2007). The optimal concentrations of the available oxygen for fish is one of the basic factors that affects and defines the capacities of production (Mulabdić, 2007; Arnautović 2007; Ćirić, 2013). The lack of the sufficient amounts of oxygen causes impairments and imposes various limitations on the living and production conditions. Salmonid species require certain amounts of oxygen dissolved in water, which is closely tied with the temperature of water in which fish live. Needs for oxygen are smaller at lower temperatures than at higher temperatures. The need for certain amount of oxygen depends on the fish species, sexual maturity and its activity. Disruptions in the production on salmonid fish farms can be caused by a decline in the concentrations of dissolved oxygen below 6 mg/l, while the desirable values for a normal functioning of the fish farm are between 8 and 12 mg/l (Ržaničanin *et al.* 1982; Treer *et al*., 1995; Boyd & Tucker, 1998; Jeremić, 2003; Ćuk *et al.*, 2006). The morphological and histological description of the gonads included examination of 40 pieces of fish between two, three and four years of age, due to a more credible monitoring of the developmental stages and degree of maturation of the gametes at various fish farms.

The histological studies of the ovaries involved sexually mature specimens between two and five years of age, during the spawning time. The studies show that, given the different farming environment, there are certain discrepancies in the histological structure of the ovaries. The histological analysis of the ovaries of the specimen from the Royal fish farm (location Donja Jablanica) determined that there was a large number of the primary yolk oocytes (Image 1). This developmental form of the oocytes shows a clear homogenous basophilia of the cytoplasm-ooplasm, and the nucleus is surrounded by several nucleolus (even up to 20) that are varying in size and that are located at the edge of the nucleus membrane, while the entire form is surrounded by zona radiata composed of the granulosa and theca cells. Given the presence of the yolk granules (rich in protein), we can determine that it is a previtellogenic phase of the development. Besides these developmental stages of the oocytes, we observed that the same ovaries had a small number of the secondary yolk oocytes (Image 2), preovulatory follicles (Image 3) as well as postovulatory follicles (Image 4).

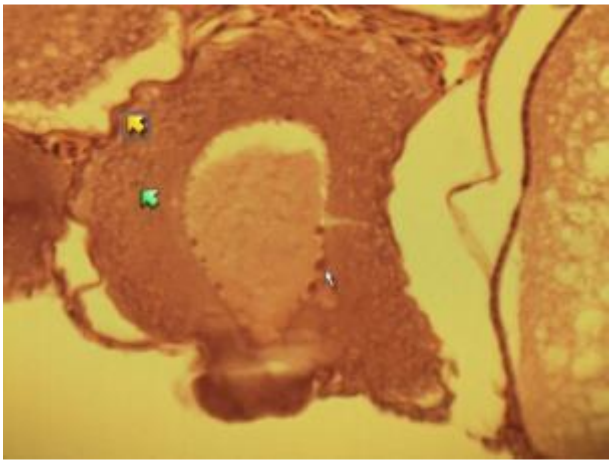

Image 1. Primary yolk oocyte: the green arrow indicates the yolk granules; the white arrow indicates the nucleoli; the yellow arrow indicates the zona radiata (HE X 400)

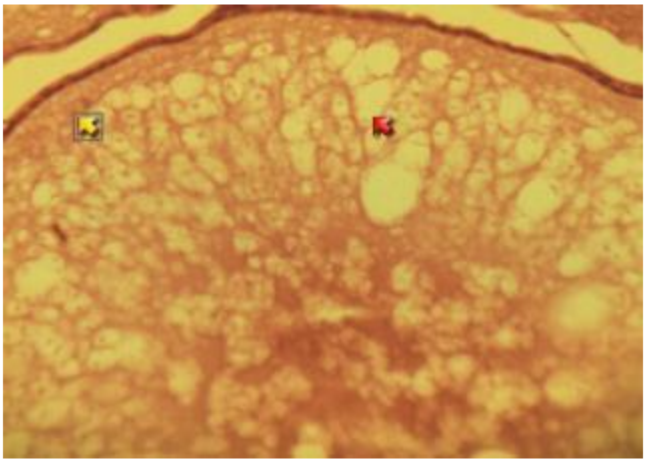

Image 2. Secondary yolk oocyte: the red arrow indicates the vacuoles; the yellow arrow indicates the yolk granules (HE X 400)

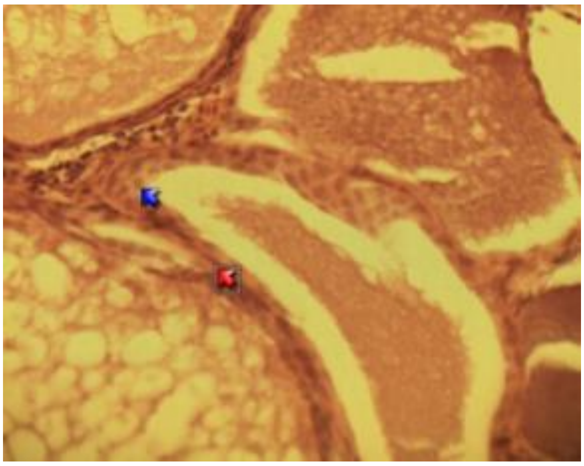

Image 3. Preovulatory follicle: the blue arrow indicates granulosa cells; the red arrow indicates theca cells (HE X 400)

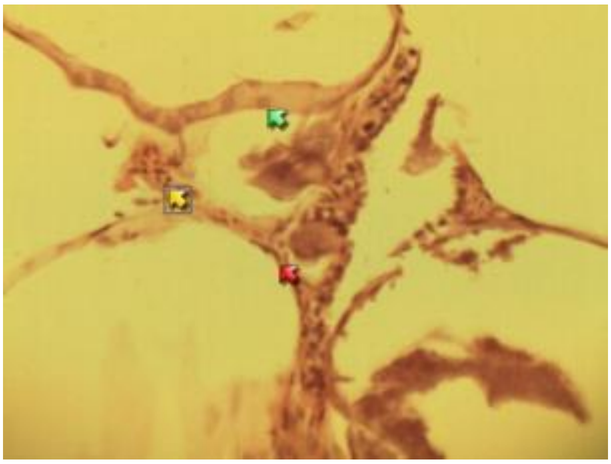

Image 4. Postovulatory follicle: the green arrow indicates granulosa cells; the red arrow indicates theca cells; the yellow cells indicates blood vessels with visible erythrocytes

The presence of secondary follicles indicates that the oocytes are in the vitellogenesis phase. Almost entire ooplasm is filled with yolk granules and fat vacuoles. The oocytes are surrounded by zona radiata and follicular epithelium (theca follicles) in the form of low-prismatic cells. Preovulatory follicles are characterised by migration of the nucleus towards the periphery, which occurs immediately before maturation. The yolk granuloses are clearly visible (fusion occurs) and very basophilic; the cytoplasm of the granulosa cells is lighter and have the ball-shaped nuclei, while the nuclei of the theca cells are elongated, darker and have a darker cytoplasm. The studied histological preparations of the ovaries of the specimen taken from the Royal fish farm show a visible presence of postovulatory follicles, however, to a lesser extent than in the ovaries of the specimen from other fish farms. The postovulatory follicles show the presence of granulosa, theca lutein cells, degraded follicles and blood capillaries with free and elongated erythrocytes of the clearly visible basophilic nuclei.
The conditions at the Royal fish farm (Donja Jablanica) were as follows: water temperature was 8^0 C; the water pH value was 8.15; and oxygen solubility was 8.27 mg/l. The fish were reared in the cage systems. We observed that the nuclei on some histological preparations migrated towards the periphery and that there was fusion of the yolk granules that appeared as one compactness and a distinctive basophilia, which indicated the immediate maturation – pre-maturation (Image 5 and 6).

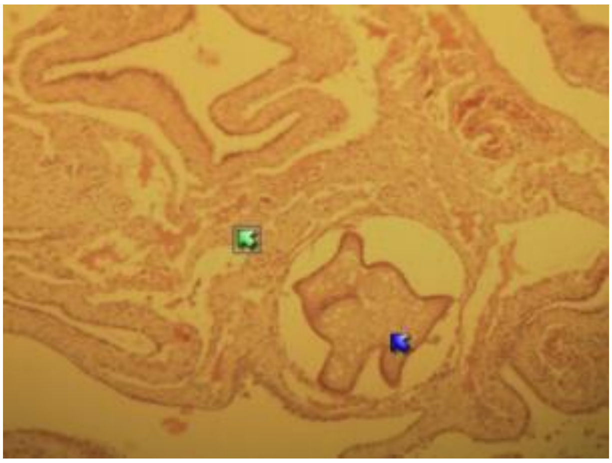

Image 5. Pre-maturation and ovulation; the blue arrow indicates the pre-maturation oocytes; the green arrow indicates the postovulatory follicle (HE X 200)

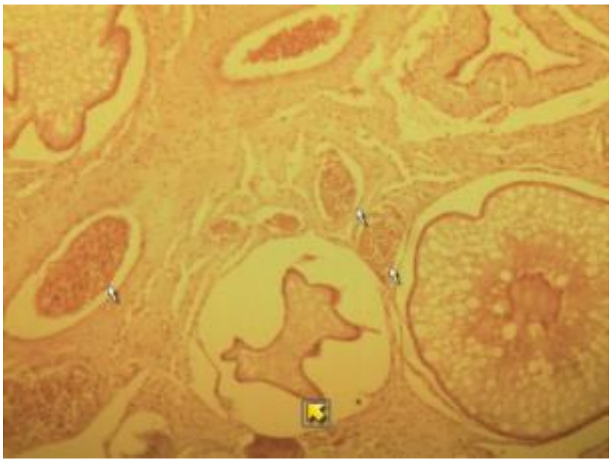

Image 6. Oocytes immediately before maturation-the yellow arrow; the white arrows indicate blood vessels in the places of degraded follicles (HE X 200)

However, the largest number of the histological preparations of the ovaries from the studied location, showed the presence of postovulatory follicles and clearly visible large granulosa cells of the ball-shaped nuclei, somewhat darker theca cells, and abundance of the sections of blood vessels – capillaries, oval lumens filled with erythrocytes.

The water temperature at the time of sampling was 7^0 C; oxygen solubility was 10.5 mg/l, the water pH value was 7.8; the Eko-projekt fish farm is located at the latitude of 760 m, which probably contributed to this picture of the oogenesis intensity. We can conclude that the oogenesis of the specimen from this fish farm, when compared to other two farms, at the same period of the year, during the spawning, in November, was at the very peak, given a significant presence of the mature oocytes. The fish from the Eko-projekt farm (at the Jeleć-Miljevina site) were farmed in the spring water concrete ponds with a raceway system. The studied samples showed a small number of oocytes in the pre-maturation phase (Image4). The oocytes are clearly visible on the histological preparations, considering that they were extremely large and surrounded by the granulosa and theca cells. They are fully filled with yolk granules, while a smaller number of vacuoles are visible on the periphery. A very small number of mature oocytes is what draws attention while studying the samples of the ovaries in the specimen taken from this fish farm. The conditions at the Magazin MAPRIM farm (Ramički potok-Pazarić) located at the latitude of 653 m were as follows: the water temperature was 11° C; the water pH value was 8,3 and solubility of oxygen was 10 mg/l. The fish at the Magazin MAPRIM farm were reared in the concrete ponds. We can conclude that the histological picture of the ovaries of the specimen taken from the Magazin MAPRIM farm (Ramički potok -Pazarić) is characterised by all phases of oogenesis (Image 7).

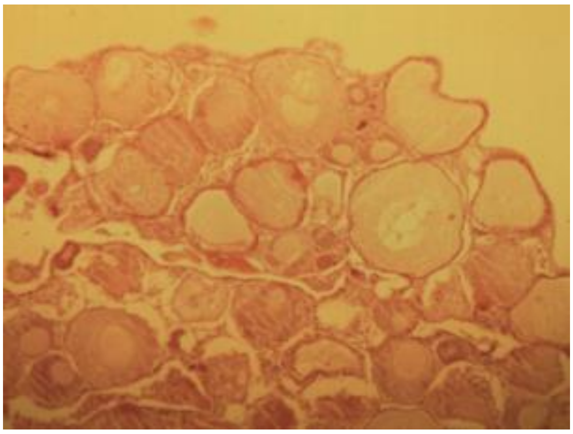

Image 7. General image of the ovaries of the specimen taken from the Magazin MAPRIM farm at the time of spawning (HE X 200)

The histological analysis of the ovaries of the rainbow trout sampled from different fish farms characterised by different microenvironment conditions showed that the fish from the Eko-projekt farm (Jeleć-Miljevina site) had the oogenesis of the highest intensity. The conditions at this site were optimal for oogenesis (pond farming with a raceway system (spring water), located at the latitude of 760 m, the water temperature was 7° C, the water pH value was 7.8 and oxygen saturation was 10.5 ppm). Having studied the histological structure of the testicles of the rainbow trout from different locations during the sexual season we can conclude that the microstructure is more or less equal.By comparing the results of the histological studies of the testicles of the rainbow trout from three fish farms in BiH located at different watercourses and with different microclimatic farming conditions, we can freely conclude that there are certain discrepancies in the intensity of spermatogenesis. Considering that the studied testicles were sampled during the spawning time, we expected that the overall histological picture would be uniform. However, it is evident that, in addition to other factors, the water temperature of 7° C at the Eko-projekt farm and 11° C at the Magazin MAPRIM farm contributed significantly to the intensity of spermatogenesis. The histological studies of the testicles of the fish sampled from the Eko-projekt farm, which had the lowest temperature during the sampling, and the water pH value of 7.8, showed an intensive spermatogenesis and presence of all cells of the developmental phases, especially spermatocytes, spermatids and spermatozoa, and a clearly noticeable lumen of the seminal walls which contained some spermatozoids (Image 8). Interstitium, i.e., the wall of the lobes on the analysed samples, was quite thin and discontinuous in certain places.

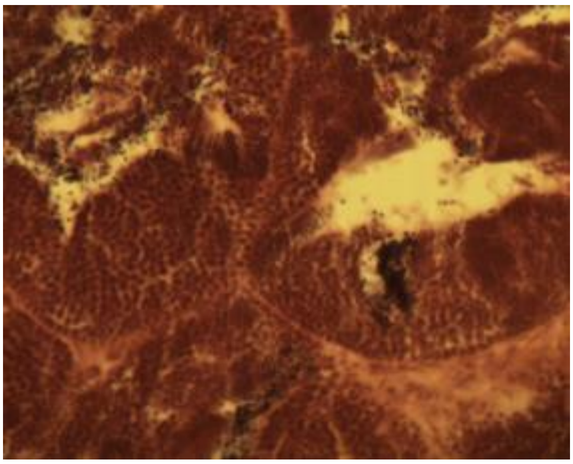

Image 8. Spermatogenesis: Eko-projekt fish farm (HE X 400)

The testicles of the rainbow trout fished from the Royal-fish farm, where the water temperature at the time of sampling was 8° C and the water pH value was 8.15, showed a discrepancy in the histological structure relative to the other two fish farms. Parenchyma of the seminal ducts contained abundance of spermatozoa, while the lumen of the seminal ducts was not notable. Spermatids were observed at certain places along the side of the seminal ducts, however in smaller numbers relative to spermatozoa and other cells of the spermatogenesis. The wall of the seminal ducts was thin and well-preserved, with a clearly noticeable lobularity (Image 9).

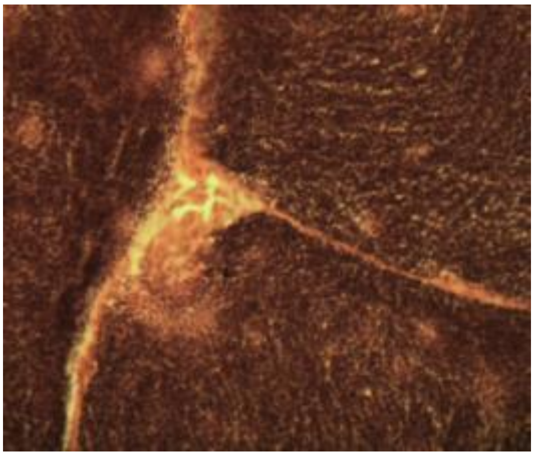

Image 9. Spermatogenesis: Royal-fish farm, (HE X 400)

The testicles collected from the specimen from the Magazin MAPRIM farm showed a higher intensity of spermatogenesis relative to the specimen collected from the Royal-fish farm, and a lower intensity of spermatogenesis relative to the specimen taken from the Eko-projekt fish farm. The water temperature at the time of sampling was 11° C, the highest water pH value was 8.3 and solvability of oxygen was 10 mg/l. Spermatogonia A and B, spermatocytes, spermatids and for the most part spermatozoa are present along the walls of the seminal ducts. The wall of the seminal ducts is continuous and well-preserved with visible sections of blood vessel.

Based on our histological research and microscope elaboration of the organs, we can determine with certainty that the most intensive spermatogenesis was at the Eko-projekt farm, given the presence of cells of all developmental phases, which was likely due to the water temperature of 7° C and pH value of 7,8 and solubility of oxygen in the water, which was 10.5 mg/l. The samples were taken from the pool environment with a flow-through spring water.

Conclusion

Considering the growing trend of the rainbow trout production in our country, it is necessary to typify the histological structure of the reproductive organs, to assess differences regarding the changed living conditions in order to provide useful information about the biological aspects of reproduction and to improve certain conditions to achieve a more productive spawning and farming. It is expected that the results of the study will have an applicative significance and that they will serve to improve the rainbow trout production both in the concrete and cage ponds.

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Fe, Cu AND Zn PRESENCE IN THE SOIL AT DIFFERENT LOCATIONS IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract
The aim of this research was to determine the quality of the soil and Fe, Cu and Zn content by the help of physico-chemical analysis at different areas of Herzegovina region (Bosnia and Herzegovina). The increased presence of the investigated elements has negative influence and the accumulation of it in the cultivated cultures is directly brought into the chain of nutrition, which results in their accumulation in the human organism as the final consumer. The research was carried out at three sites of Mostar, and from each of them, samples were taken from two different depths, from 0 to 30 cm and from 30 to 60 cm. The highest presence of Fe was found at the Salakovac site (42.447,11 mg/kg), where an increase of 37% was observed at the first depth, up to 42% at the second depth of testing, compared to MAC. Cu was above the limit values only at the site of Vrapcici, while Zn was measured in the highest values at the Salakovac site, at the first depth of the test (177,36 mg/kg). It can be concluded by comparing localities that Dubrava site is unloaded with the content of the examined elements, and it is considered acceptable for the cultivation of various herbal crops without fear for the health of final users. On location Salakovac recorded increased values of Fe in both depth testing, as well as increased concentration Zn on the first depth. This can cause long-term negative effects on the plants, but also human health. The Soil at the Vrapcici site contained higher concentrations above the permissible values of all tested elements which is the most polluted of these three explored sites. It can be said that represent great risk to human and animal health that consume the cultures cultivated on this land.

Keywords: *iron, copper, zinc, soil, MAC*

Introduction
The land, as a loose surface of the Earth's crust, is the basis for plant production, and it is necessary to examine its quality. Agricultural land is particularly vulnerable to degradation, given the numerous, often radical, technical-technological interventions in plant cultivation and thus become a potential source of environmental contamination. Rational use of these surfaces can reduce the risk of both the production of health defective food and groundwater contamination.

Most of the pollutants that are considered to be contaminants are the natural components of soil and groundwater, although they are often found only in traces. Determination of the total content of heavy metals in the soil is a basic indicator for the determination of the degree of pollution, although long-term risk assessments and direct impacts of pollution are based on their bio sensitivity and/or mobility (Salomons, 1995).

Natural and synthetic organic matter in the land is mainly degraded by microbiological and chemical, light photo chemically. If the intake of substances, even hardly degradable, is greater than their exposure by flushing, disintegrating or digging, the deposition occurs in the soil. Dangerous loads may increase to such an extent that they can permanently endanger natural soil flow.

According to Maxymiec (2007), heavy metal toxicity is one of the major forms of abiotic stress, which results in different impacts on animal and plant health, and therefore on humans.

The effect on plants, as the author believes, may be the result of their direct adverse effect on the membranes and the photosynthesis apparatus, leading to inhibition of organic matter synthesis and plant aging stimulation, which is the most exogenous external indicator of plant stress.
The term heavy metal is in principle used for metals and semi-metals that are related to environmental pollution and toxicity phenomena, but also to some elements that are essential to low concentrations in cells (Gratão et al., 2005). According to Dučić and Polle (2005), Cu is a major contaminant that occurs in the environment due to anthropogenic activity, especially due to the long-term use of Cu-based fungicides (Diaz et al., 2001).
In the research on adsorption of Cu, Cd, Ni, and Zn in individual and multi-metal solutions of agricultural and forest land (Sprynskyy et al., 2011), significant differences in the absorption capacity of the examined soil were demonstrated. The largest absorption of the investigated elements is in agricultural land, followed by surface forest land, and deep forest land. Agricultural and deep soil adsorb Cu, Zn, Ni, and Cd, while for surface woodland adsorption is Cu, Ni, Zn, then Cd.
Heavy metals such as cadmium, mercury, nickel, lead, uranium, copper, iron, manganese, cobalt, zinc, and similar metals are toxic substances that are included in the nutrition chain that pose a high risk for long-term biodiversity. The risk may have different forms: worsening of human, animal or plant health, damage to structures or contamination of ground or surface waters that are in contact with soil. The effect of toxic metals depends on the form in which the contaminant is found, further affecting its behavior in the soil. The mobility of contaminants depends on the pH value and the type of soil. For instance, As and Se are more mobile in alkaline pH, while Hg, Pb, Cd, and Zn are more mobile in acid pH soil (Adriano, 1986, Alloway, 1990).
The literature suggests a very wide variety of biological functions of zinc as a biogenic element and its participation in various biochemical processes (Deighton and Goodman, 1995; Chaney, 1992). Zinc and its compounds are irritant to the skin and mucous membranes of the respiratory and digestive tract, damage the liver, kidneys, myocardial, lymphocytic and neutrophil granulocytes, central and peripheral nervous system, pancreas, and carbohydrate metabolism, protein, and fat.

Material and Methods

The subject of this study is to determine the concentration of investigated metals in the land at three sites in the wider area of Mostar municipality. The research methodology was carried out in the following phases: selection of sampling sites, taking representative soil samples, preparing samples for analysis, physical-chemical soil quality analysis, laboratory analysis of Fe, Cu and Zn content.
The soil was sampled using standard methods using a chromium-plated probe, by taking five individual samples per parcel diagonal, which were collected in an average sample, an average mass of about 2 kg. The soil was sampled with two different depths of 0-30 cm and 30-60 cm. Six soil samples were prepared, analyzed on basic soil quality indicators, especially on the metal content of the study: Fe, Cu, and Zn. Sampling was carried out at the time of vegetation in 2012. The sampled soil was air dried for a month without the presence of direct light. After that, each individual sample was triturated in an auspicious advance and sifted through a 2 mm plastic drill bit, squeezed into paper bags and sent to the laboratory for further analysis.
Laboratory soil analyzes included the following parameters: pH in H_2O and n-KCl, hygroscopic humidity, humus content, carbonate content, physiologically available K_2O, and P_2O_5, determination of Fe, Cu and Zn content.

The pH values in H_2O and n-KCl were determined electrochemically. Electrometric determination of soil pH is based on measuring the potential difference between two electrodes immersed in a soil suspension. By the methodology, it is customary to determine pH in the soil suspension supernatant.
Determination of total nitrogen was made using the Kjeldahl method. The method is based on the destruction of the soil sample with sulphuric (H_2SO_4) acid, in the presence of a catalyst and at elevated temperature. Concentrated sulfuric acid destroys organic compounds wherein the organic compounds of nitrogen are reduced to ammonia.
Hygroscopic humidity was determined by weighing the soil sample (about 2 g) measured in the cauldron before and after drying. Humidity is determined by placing a certain amount of soil in a pot and heating to 105 ° C and drying until all the water has evaporated. The content of hummus was determined by a colorimetric method. The method is based on the wet burning of soil organic matter, using concentrated H_2SO_4 and potassium dichromate as a strong oxidizing agent. The content of carbonate in the soil was determined by a calcimeter, volumetric by Scheibler. The carbonates are destroyed by hydrochloric acid with the release of carbon dioxide, the volume of which is measured by a calcimeter at the current temperature and atmospheric pressure. The content of metals in soil samples was made by the Atomic Absorption Spectrophotometer (AAS) method using the flame technique or graphite excitation technique. This method is one of the most commonly used methods for determining the number of elements in a sample. When the characteristic wavelength of light passes through the sample, there is some absorption by the free atoms of the element whose content is determined. A hollow cathode lamp emitting intense monochromatic radiation serves as the radiation source.

Results and Discussion

The results obtained are shown in the tables. Table 1 shows the results of soil analysis on basic physicochemical parameters, while Table 2 shows the content of investigated elements (Fe, Cu, and Zn) in the explored soil.

Table 1. Physical-chemical analysis of the soil

Locality	Depth (cm)	pH-value in (±0,01)		% Nitrogen content	% Phosphorus content /100g land	% Potassium content/100g land	% Humus content	% Hygroscopic humidity
		H_2O	n-KCl					
Dubrave	0 - 30	7.50	6.70	0.21	6.50	10.4	1.24	2.38
	30 - 60	8.12	7.20	0.23	9.15	14.8	1.20	2.21
Vrapčići	0 – 30	7.55	6.65	0.22	6.15	14.1	4.31	4.98
	30 – 60	7.72	6.90	0.17	5.15	12.6	1.63	4.74
Salakovac	0 - 30	7.21	6.40	0.21	4.80	9.50	2.85	4.45
	30 - 60	7.75	6.95	1.15	9.70	13.8	3.79	5.33

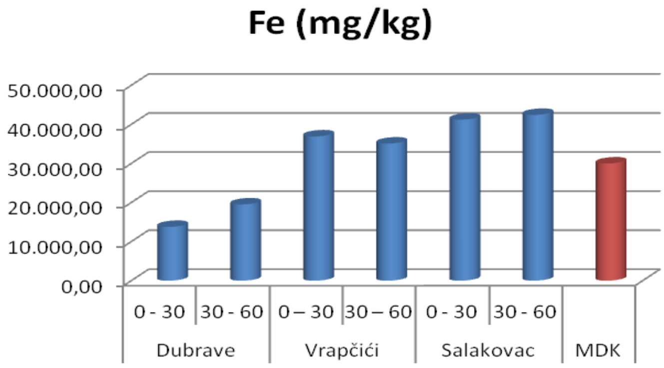
According to pH values in water as shown in the table 1, it is slightly alkaline soil and the values range from 7.21 to 8.12.
The values in n-KCl ranged from 6.40 in Salakovac, while the highest values were found at Dubrave's land, at the depth of 7.20. According to the values of pH in n-KCl, it can be concluded that the investigated land at the sites Vrapčići and Salakovac is classified as a weak acid and the plot from the location of Dubrava to neutral.

The content of nitrogen is quite balanced, and the largest quantity is at Salakovac at a different depth and is 1.15.
According to the nutrient content, all three sites can be classified as unfruitful land.

Table 2. The content of investigated elements

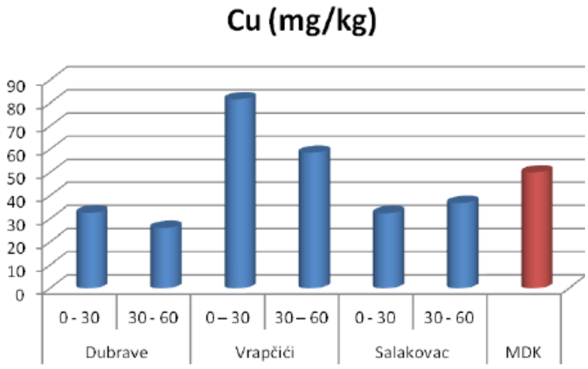
Locality	Depth (cm)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Dubrave	0 - 30	13.774.85	32.56	90.20
	30 - 60	19.529.71	25.97	78.71
Vrapčići	0 – 30	36.934.41	81.58	159.67
	30 – 60	35.103.81	58.43	165.52
Salakovac	0 - 30	41.225.29	32.36	177.36
	30 - 60	42.447.11	36.75	99.22
MDK		30.000.00	50.00	100.00

According to the results of the content of the investigated metals (Table 2), it can be concluded that the Dubrava site is not polluted. At the site of Vrapčići, all the samples showed an increase of the investigated elements irrespective of the depth from which the sample was taken. The locality of Salakovac had increased values in three out of six samples in comporison to MAC.
The amounts of iron present were above the permitted limit values in a total of four samples at the locality of Vrapčići and Salakovac, the copper content was increased in two samples in locality Vrapčići, while the amount of zinc was above the maximum permissible concentration in three samples in locality Vrapčići and locality Salakovac.

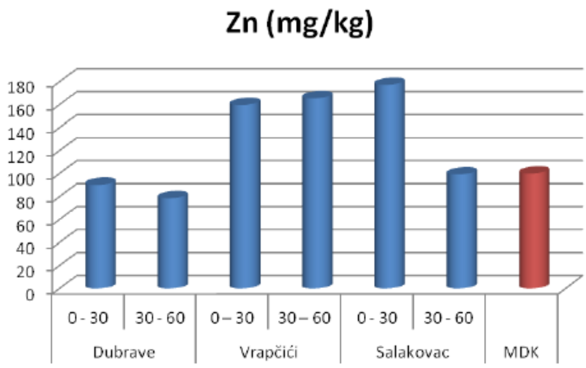


Graph 1. Content Fe in the explored soil

Iron has the highest values in the Salakovac site and its volumes are as high as 37% at the first depth and 42% at a second depth greater than the maximum permissible concentrations. Not negligible values of this element were found in the locality of Vrapčići, where an increase above MAC of 23%, at the first depth and 17%, was also noted at the second depth of the test.



Graph 2. Content Cu in the explored soil



Graph 3. Content Zn in the explored soil

The content of the copper found only at the Vrapčići location was above the limit values, and an increase of 64%, at the first depth and 17% at the other depth was noted.
The largest presence of zinc was recorded at the Salakovac site, where concentrations of this element were increased by 77% above allowed at the first depth, while its contents at the second depth were very close to the limit values. At the Vrapčići site, the zinc content was above MDK at both depths of the study. At the first depth, the presence was increased by 60%, while at the other depth was increased by 66%.
The presence of iron on two sites was larger in the deeper layers of the soil, the copper content was also in two locations somewhat larger in the surface layer of the soil, while the zinc content was also present at two locations somewhat in the first depth of the test.

Conclusions

According to the results of physicochemical analysis based on the parameters of soil quality, the following can be noted:
- all explored soils at pH in the H_2O are slightly alkaline,
- the explored soils are rich in nitrogen,
- the content of phosphorus and potassium is quite low so that the land can be classified as poorly supplied by these elements.

According to the content of the investigated elements in the investigated sites, the following observations have been made:
- the Dubrave site is unburdened with the contents of the explored elements so it is considered acceptable for the cultivation of different herbal cultures without fear of life and health of end consumers,
- In the Salakovac site, 50% of the samples had values greater than allowed, Fe content at both depths and Zn content at the first test depth, which could have a negative effect on plants, but also on human health,
- the Vrapčići site contains large quantities of all the investigated elements, which is the most polluted by the three localities and can be said to pose a high risk to human and animal health that consume crops grown on this soil,
- the amounts of the iron present are quite high and in four samples are found above MAC. The highest values were obtained at the Salakovac site with as much as 37%, at the first depth, and 42%, at a second depth, higher than the maximum permissible concentrations,
- the copper is only at one location above the limit values, that is on the Vrapčići location it was above the limit values, where the 64% increase at the first depth and 17% at the second depth was noted,
- at the Salakovac site, the concentrations zinc were increased by 77% above the permissible value at the first depth, while its contents at the second depth were very close to the limit values. The Zn content at the Vrapčići site was above MAC at both depths, at the first depth

the presence was increased by 60% and at the other depth the increase by 66%. Such land is considered to be highly risky for growing plants.

By comparing the sites with each other in all analyzes it can be stated that according to the pollution can be compared:

- Vrapčići the worst soli quality all three investigated elements above MAC were found at both depths, iron by 23% and 17%, copper of 64% and 17% and zinc by 60% and 66%.
- Salakovac is a plot on which the Fe concentration above MAC was detected at both depths, Zn at the first depth of about 77% above MAC and at a second depth at the MAC limit itself, while amounts of Cu were present below MAC
- Dubrave at which site Fe, Cu, and Zn were present below MAC for both investigated depths.

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AGRICULTURE AND CLIMATE CHANGE IN THE REPUBLIC OF SERBIA

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Abstract

Climate change is one of the greatest global challenges of our time. The consequences of this change are potentially so far-reaching and serious that every country must do its part to help stabilise our planet's climate. In the area of agricultural production, which accounts for about 10% of the total gross domestic product of the Republic of Serbia, the entire national economy is very sensitive to all factors that affect agriculture. There is no doubt that climate change will affect the quality and quantity of yields of major crops in Serbia, as well as the variability of yield which will be more pronounced each year. According to climate change scenarios, which predict a further rise in greenhouse gas (GHG) concentrations, we can expect more frequent occurrences of extreme weather conditions, drought and then reduction of the amount of summer precipitation in particular, the increased number of dry days and days with extreme temperatures in the individual sub-periods of vegetation (high spring and summer temperatures), warmer winters with a lower number of frostless days. Therefore, intensive studies are conducted and introduced in preventive and alternative ways in the production process to combat extreme weather conditions. Through customed agricultural techniques and the application of complex agro-technical measures, it is possible to mitigate, though not completely exclude the negative impacts of climate change on the yield of cultivated plants.

Keywords: *climate change, GHG, agriculture, adaptation measure, Serbia*

Introduction

Climate change projections estimate the increase in frequency and intensity of natural hazards, such as floods and droughts as well as in terms of scope and duration. A substantial number of studies predict increase in intensity and frequency of flooding, particularly in the winter season. Climate change is expected to affect water resources. An assessment on the effects on water resources indicates a general decrease of water flow at the national level of 3 percent per 10 years, caused by a decrease in annual precipitation (Djurdjevic, 2015). However, at the same time the number of extreme weather events, including heavy and excessive rainfall is expected to increase due to climate change. According to the INFORM Risk Index of 2019, Serbia is highly exposed and vulnerable to natural hazards (www.inform-index.org, 2019). The major natural hazards to which the country is exposed to include, among others, (flash and river) floods, storms, drought, landslides and earthquakes. As well as epidemic livestock diseases and the emergence of pests, which may cause significant damages and losses to people and animals.

Agriculture is a highly important economic sector for Serbia, as it accounts for about 8.2 of the total Gross Domestic Product (GDP) (Worldbank, 2019). Agricultural products account for over 20 percent of national exports. Its main export products include, among others, maize, wheat, apples and frozen raspberries.

In some regions of Serbia, agriculture is a core economic activity and most of the residents depend on it for basic livelihoods, for instance the southern and southeastern regions of the country are among the poorest, while the Vojvodina region in the north is relatively more endowned as it is largely based on the developed food and agriculture industry as it is rich in fertile loamy loess soil.

The natural potential for development of agricultural production is significant. Climatic conditions are favourable and Serbia is endowned with considerable water resources for further development of its agriculture production. More than half of the country's territory is classified as agricultural land (4.867.000 ha) with the area of utilized agricultural land to be 3.438.130 hectares (Statistical Office of the Republic of Serbia, 2018).
With regard to resources, food production, technological development and the environment, a lack of adequate responses to the effects of climate change is seen as well as a lack of systemic solutions. Among the key principles to achieve this vision is 'sustainable agriculture', which is viewed as one of the main principles for implementing this agricultural policy as agriculture is still one of the most important economic sectors in the rural areas. Disaster risk reduction is not included among the strategic goals, rather 'sustainable resources management environmental protection' and in particular the sustainable usage of available natural resources through e.g. responding to climate change, protecting the agricultural land, lowering greenhouse gas emissins, enhancing biodiversity and rural areas protection and so on (Ministry of Agriculture, Forestry and Water Management, 2014).
Climate change related measures are included, such as educational and public awareness raising programmes on the issue and effects of climate change, the establishment of greenhouse gas (GHG) inventories for agriculture and forestry, plans that contain emission reduction and climate change adapation measures, improvement of manure and organic waste management, more efficient use of nitrogen fertilizers, promotion of organic farming, and research and development promotion on new crop varieties and livestock breeding.

Expected climate change - climate change scenarios in the Republic of Serbia

During the period 1960-2012, significant increase of daily mean temperature, as well as in daily maximum and daily minimum temperatures was observed in Serbia with an average trend of 0.3°C increase per decade and on annual level. Eight out of ten hottest years ever recorded were observed after 2000.
Climate scenarios predict a plausible temperature rise in the near future. Under A1B scenario for 2011-2040 and 2041-2070 it is likely that the temperature will rise by 0.5-0.9 °C and 1.8-2.0 °C respectively. According to A2 scenario the expected temperature rise is 0.3-0.7 °C in 2011-2040 and 1.6-2.0 °C in 2041-2070. By the end of the century (2071-2100) the predicted change in temperature obtained by A2 scenario is 3.6-4.0 °C, and according to A1B scenario it will be 3.2-3.6 °C. It is expected that warming will be most pronounced in the summer and fall season, with the temperature rise over 4.0 °C by the end of the century. For the period 2011-2040 both scenarios predict precipitation changes that are positive compared to the base period, and that will be decreasing toward negative values by the end of the century. Under A1B scenario, projected precipitation changes for the end of the century range from +5% to -20%, and from +20% to -20% according to A2 scenario. The negative trend is most expected during the summer season. By the end of the century, the number of frost days will have reduced to the level when it can be considered a rare event. The number of summer days will have increased by 20 – 30 days (A2 scenario). The number of days with tropical nights, will have increased by over 20 days by the end of the century. During the second half of the century the length of the growing season will have extended by over a month. Longer periods of drought are to be expected, lasting for over one month, according to the both scenarios (Ministry of Environmental Protection, 2017).

Agriculture and climate change in the Republic of Serbia

In recent years, agriculture in Serbia has suffered significant losses due to adverse weather conditions and distinctive climatic anomalies. The most important include the damage arising from the effects of drought, spring frosts, hale, storms and floods. Since 2000, Serbia went through several episodes of severe drought, which resulted in significant losses. Extreme droughts were recorded in the years 2000, 2003, 2007, 2011 and 2012 (Republic Hydrometeorological Service of Serbia, 2019). Although rarely characterized as drought, lack of precipitation in the period from October to February may also adversely affect the yield of winter crops, such as sugar beet, rapeseed and others. Furthermore, during the vegetation period high temperatures and the higher intensity of solar radiation also have significant adverse impacts. They cause damage to the fruits and leaves, but also to the trunk and the bark of trees. These changes were observed especially during 2007 and 2008.

On the other hand, the emergence of a very warm period during the late winter and early spring, followed by freezing temperatures and frosts, is especially dangerous for the early varieties of fruits as well as vine and some agricultural crops due to early start of the growing season, which becomes interrupted by low temperatures.

Impact of climate change on plant growth dynamics (crop phenology)

Due to warmer winters and fewer frost days in February and March, flowering should occur earlier, as should maturity, owing to higher temperatures in April-May-June. This shift is nearly negligible to 2030, but may bring the entire vegetation cycle forward by about 20 days to 2100.

Detailed analysis of the impact of such temperature changes on physiological and biochemical processes (that determine plant growth dynamics) have shown that climate change will bring the crop growth season forward by about 20 days to 2100. These changes in phenology can affect yields and the timing of farming operations, but may also produce a second harvest.

Impact of climate change on yields and year-on-year yield fluctuations

Extreme weather events, especially drought and greater numbers of dry days, as well as days with extreme temperatures, will exert a decisive influenceon the yields of key crops under climate change conditions.There is no doubt that climate change will influence mean crop yield levels and year-on-year yield fluctuations. In other words, all of Serbia will be affected by changes to agro-climatic conditions, in particular rising temperatures and lower summer precipitation levels, which will reduce crop yields in the absence of adaptation measures.

Impact of climate change on the appearance of pests and diseases

Climate change increases the complexity of the integrated plant management system by promoting uncertainty in the plant-pest-environment triangle. Studies have shown that, in our region,thermophile insects are expected to shift to higher altitudes and increase their number of generations. A movement of these species' ranges northwards is also to be expected. Estimates to 2055 indicate northward shifts of between 3ºin the case of Ostrinianubilalis (European corn borer) to 11ºfor Lobesiabotrana (European grapevine moth) (UNDP, 2015). All crops (both winter and summer ones) will be affected by changing patterns of pests and disease driven by increasing temperatures and changing precipitation regimes, which will particularly increase vulnerability to pests (thermophile insects). Vulnerability of each particular crop and region depends primarily on the relevant crop acreage in each region, crop management, and crop rotation (e.g. these changes will particularly affect maize in Vojvodina and Mačva, sugar beet in Vojvodina, and orchards in the Kruševac region, Vojvodina, and elsewhere in Serbia, excepting mountainous regions).

Adaptation measures

Observed climate changes lead to changes in the environmental, social and economic indicators, i.e. to changes in the overall conditions in which contemporary society lives across the globe and also in Serbia. It is believed that if further changes remain within the limits which have been determined as the goal, the mitigation of negative consequences will be possible through the appropriate and timely measures of adaptation to changing climatic conditions. On the other hand, in the case of continued uncontrolled rise in global temperatures and changes in other elements of the climate system, the consequences will be on a significantly higher scale, while adaptation will require additional efforts and additional financial investments, which can significantly slow down and hamper the progressive development of society. The aim of adaptation is to reduce the potential negative effects of climate change via planned change in the natural and socio-economic systems and to maintain the functioning of the system, or if possible, to improve efforts where such potential exists (Ministry of Agriculture and Environmental Protection, 2015).

There is no doubt that climate change will influence the quantity and quality of yields of staple crops in Serbia, both in terms of mean crop yield levels and year-on-year yield fluctuations. All of the above bear out the urgent need for adaptation. In the Second National Communication of the Republic of Serbia, a series of adaptation measures to changing climatic conditions was listed for the agriculture sector.

Extreme droughts in recent years in the territory of Serbia has been very common (Stricevic *et al.*, 2011; Stricevic and Djurovic, 2013), so that irrigation is imposed as a measure to mitigate losses, in addition to the introduction of new hybrids and varieties tolerant to drought or by using other methodologies which help achieve a more efficient use of water. Irrigation in the climatic conditions prevailing in our country is an optional measure, because crop production can take place relatively successfully with the available natural supply of water, especially when it comes to agricultural crops. Users are not obliged to use any or to pay expenses related to irrigation, even though they have that option. That problem can be twofold: the monetization of bringing water to the plot and the profitability of irrigation on the land if there are not enough interested farmers for irrigation. Previous studies (www.water.worldbank.org, 2019) have shown that irrigation can be profitable if at least 60% of the total number of owners/users use the irrigation system in the irrigated area.

Drainage systems (dewatering) may prove to be especially important if in future climatic conditions there is an increase in shortterm episodes of intense precipitation which is indicated in the future climate change scenarios. Even though the analysis indicates that the climate will be warmer and drier, drainage channels should be maintained regularly, because in drier climates more intense precipitation can be expected with increased risk of flooding.

Adaptation to climate change in agriculture shall primarily include the following priority actions: Changes of plant species and assortments in the sowing structure of sowing in relation to the expected changes in the phenology of plants, Introduction and use of species and varieties/hybrids more tolerant to stressful conditions, Work on the selection, breeding and creation of tolerant genotypes, Increase the representation of growing winter crops, Adapting the basic treatment systems, Adequate rational fertilization, Change and improvement of the treatment system with the aim of improved moisture conservation (application of reduced treatment systems), Changes in time and sowing density, Monitoring and adapting to the new invasive thermophilic invasive pests and diseases (adapted to drought conditions).

Conclusions

The Republic of Serbia has established an important component of the institutional and legal framework for fighting climate change. At the same time, there is still a need for

improvement, as well as capacity building and expanding the knowledge of responsible and competent institutions, both at the national and local level, and among the general public.
The multi-sectoral nature of climate change and the current level of knowledge and awareness certainly indicate the need for systematic and continuous activities to raise awareness of this issue among the general public. As a result of the joint work of the Ministry of Agriculture, Forestry and Water Management of Republic of Serbia and the Food and Agriculture Organization of the United Nations (FAO), brochures "Good agricultural practices and technologies for mitigating the effects of natural disasters in corn production in Serbia" and "Good agricultural practices and technologies for mitigating the effects of natural disasters in soybean production in Serbia" (www.minpolj.gov.rs). The brochures are aimed at agricultural advisory bodies, those employed in the sector farmers, and the broadest interested public.
There is no doubt that climate change will influence the quantity and quality of yields of staple crops in Serbia, both in terms of mean crop yield levels and year-on-year yield fluctuations. All of the above bear out the urgent need for adaptation.

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EFFECTS OF DRAINAGE AND RUNOFF ON THE PRODUCTIVITY OF THE LAGOON OF GHAR El MELH (TUNISIA)

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Abstract

The Ghar El Melh lagoon, Ramsar site and Ramsar City (Tunisia), is a coastal quaternary lagoon edified by the flood and sediments discharge of Mejerdah River and the marine swell. This lagoon maintains hydrological exchanges with the inland waters issues through river and channels, and the marine water across an evolving inlet. The aim of this work focused on how the inland water arrival, strongly linked to the agricultural activities and rainfall regime, may affect the fisheries productivity of the lagoon. In fact from the inland side, 2 rivers and 2 channels discharged into the lagoon the drainage water of more than 2280 ha of irrigated areas. The lagoon communicates with the sea through a naturel outlet of 70 m large and around 0.8 m depth. The yearly balance of water exchange revealed that the lagoon received 4.5 mm^3 of runoff water and 5.7 mm^3 of agriculture drainage waters. The daily tidal exchanges amounted to 3.1 mm^3, ensuring a water renewal time of 8.2 days. These inputs varied strongly with the rain season and the tidal range. The fisheries of the lagoon were directly regulated according to this hydrodynamic characteristics and balance: fish recruitment, trophic and genetic migrations etc. Thus the annual production of fish, dominated by European eels and mullets, seemed to fluctuate according to the years: 64 ± 22 tons per year during the period 2008-2017, the eels production was 8 times greater with runoff and drainage spill (24.10 ± 15.19 ton/year) (Kruskal-Wallis test: $p < 0.001$). However, that was not the case for mullet. The organic and mineral load of these waters may be directly implicated in the irregularity of catches, knowing that the total nitrogen can reach 2.67g/m^3 in the drainage waters.

Keywords: *coastal lagoon, rainfall, water exchanges, fisheries, Tunisia*.

Introduction

Ghar El Melh lagoon is located at the north-western part of the Tunisia (37°08'N 10°10'E), this coastal first-line lagoon cover an approximate area of 2600 hectares, separated from the sea by a sandy coastal strip and communicate throw an intermittent naturel channel of 70m as maximum large. The average depth of the lagoon is 1 meter, water salinity ranges from 25.0 to 46.0 psu and surface water temperature varies between 18.5 and 27.4 °C (Romdhane et al., 2019). A considerable supply of freshwater in this lagoon is observed during the rainy season (September-April) by the flood throw River and channels which overflows. The lagoon was often enriched with nutrients, which provided by the drain of an active agricultural surrounding area. In fact, the main activity linked to this lagoon are based on traditional fisheries coupled with multi agriculture uses from extensive to intensive systems. The fishing yields seem to be widely linked to the rainy or drought cycles and water issues and qualities. In this work we try to explain how the inland water arrival, linked to rainfall regime and agricultural activities affect the global fisheries productivity and the specific catches in the lagoon.

Materials and methods

The analysis is based first on hydrological parameters of rivers and canals that flow into the lagoon, hence we consider the volumes and quality of the drainage and runoff. Secondly we couple the meteorological data series covering the last 5 years, the data mainly cover the average monthly rainfall during the period 2013 to 2017 (INM 2019) and the total and specific fishing data with a complete time series of statistical data obtained over the last ten years for the period from 2008 to 2017 (DGPA2018). The data were structured to obtain mean yearly landings, by dry (April to August) and wet season (September to March), and by major fishing species in the region (particularly European eel and mullets productions). On the basis of these collections, descriptive statistical analyzes were carried out to connect the hydraulic variables (rainfall, inlet and outlet waters) to lagoon production in order to establish possible relationships between them. Principal component analysis (PCA) was applied to classify major fish species according to their relative fishing production during the study period. The non-parametric Kruskal-Wallis test (Kruskal and Wallis, 1952) was used to compare mean fishing productions over time and seasons.

Results and Discussion

The lagoon receipt in fact from the inland side, 2 rivers (Kherba and Nechma) and 2 channels (Mabtouh and Charchara) discharged into the lagoon the drainage water of more than 2280 ha of irrigated areas. The yearly balance of water exchange reveals that the lagoon receives around 4.5 Mm^3 of runoff water with 4.0 Mm^3 during the wet seasons and 0.5 Mm^3 during the dry season. The agriculture drainage release yearly 5.7 Mm^3 of wastewater, about 3.5 Mm^3 during the wet season and 2.2 Mm^3 during the dry season with respectively 2.3 and 7.25 mg l^{-1} of suspended matter charge . The effects of the irregularities of dry and wet seasons are clearly observed in this lagoon (Moussa et al., 2005). Sea side, the lagoon communicates with the sea through a naturel outlet of 70 m large and around 0.8 m depth. The daily tidal exchanges is ranged to 3.1 Mm^3, ensuring a water renewal time of 8.2 days. These inputs vary strongly with the rain season and the tidal range, so we considers yearly the succession of a dry and a wet season (Table 1).

Table 1. Mean hydrological data and fish yields during the last decade (2008-2017)

Parameters	Dry season	Wet season
Rainfall (mm)	47.9	477.5
Runoff (Mm^3)	0.5	4.0
Drainage (Mm^3)	2.2	3.5
Suspended matter (tons)	5.06	25.38
Tidal exchanges (Mm^3)	465	651
Nitrogen input (tons)	5.8	3.1
Total captures (tons)	16.9	47.1
Eels (tons)	3.4	24.1
Mullets (tons)	0.9	0.9
Mullets juvenile (tons)	4.8	6.6

The fishery of the lagoon is directly regulated by the hydrodynamic characteristics and balance: fish recruitment is enhanced by the water flow and water level at the outlet, best recruitment is observed up to 0.5 ms^{-1} current speed and 0.9m of water level, high turbidity disturb larval recruitment. the trophic and genetic migrations are also conditioned respectively by the nutriments levels who enhance the primary production and the physiological cycle of each species. Finally the fishing effort act as regulatory factor of the productivity of the lagoon. Thus the annual production of fish, dominated by European eels and mullets (adult and juveniles), seems to fluctuate according to the years around 64 ± 22 tons per year, mean value during the period 2008-2017. Such effects are widely observed in the coastal areas and lagoons (Garali et al., 2009). The organic and mineral load of these waters may be directly implicated in the irregularity of catches, knowing that the total nitrogen can reach 2.67 g m^{-3} in the drainage waters (Figure 1).

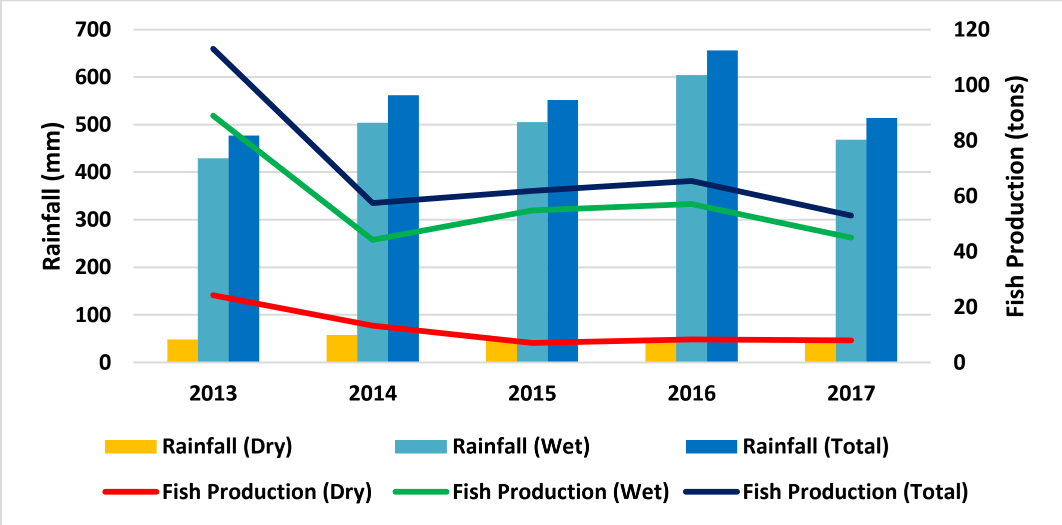

Figure 1. Evolution of the hydrological parameters and the fishery characteristics.

To better characterize the landings of total fish production and by major species (European eels and mullets) in Ghar El Melh lagoon, we carried out a PCA taking into account the year of landings of each fish category as variables and wet and dry seasons as factors (Figure 2). According to the PCA results, it appears that European eel follows closely the distribution of total fish production. However, an agglomerative distribution was observed in mullets totally independent of the seasons.

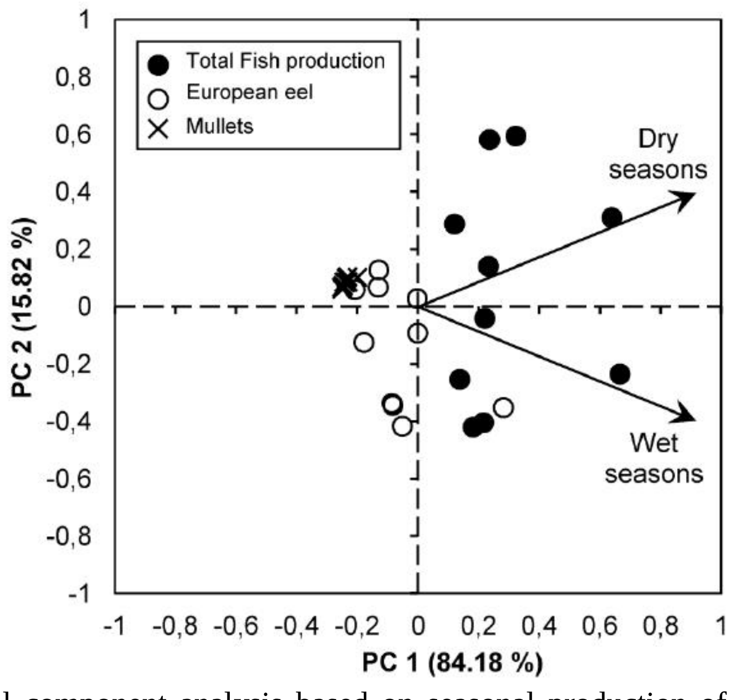

Figure 2. Principal component analysis based on seasonal production of total fish species, European eel and mullets in Ghar El Melh lagoon during the period 2007-2017.

When we compare fishery production for each season separately during the period from 2007 to 2017 in Ghar El Melh lagoon, similar tendency was observed as PCA output. The landing quantities of the total fish production, European eel and mullets in Ghar El Melh lagoon according to wet seasons and dry seasons are shown in Figure 3.

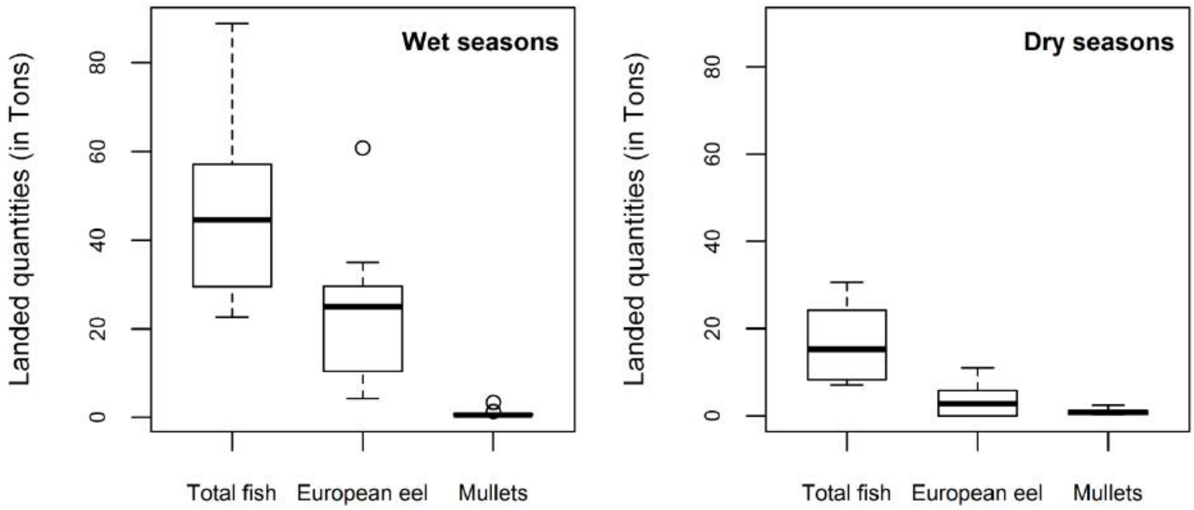

Figure 3. Box-plots indicating the landing of total fish production, European eel and mullets according to the seasons Wet in the right and dry in the left (period 2007-2017). The box portion of the plot represents the interquartile range from the first to the third quartile and the line drawn through the box represents the median. The vertical whiskers extend to the highest and lowest values in the dataset, excluding outliers, which are marked as open circles.

Hydraulic variables, mainly runoff, water exchange and seawater, are important abiotic factors that influence the production of European eels in the Ghar El Melh lagoon (Table 2). This production is 8 times greater in the wet season (24.10 ± 15.19 ton/year) than in the dry season (3.44 ± 3.62 ton/year) (Kruskal-Wallis test: $p < 0.001$). However, it seems that these factors have no effect on the stability of mullet production over the year (mean production during wet season: 0.93 ± 0.87 ton/year; mean production during dry season: 0.93 ± 0.60 ton/year; Kruskal-Wallis test: $p = 0.762$).

Table 2. Kruskal-Wallis test results comparing annual landing quantities (mean ± standard error) for total fish production, European eel and mullets between wet and dry seasons in Ghar El Melh lagoon during the period 2007-2017.

	Mean annual landing quantities in wet seasons (in tons)	Mean annual landing quantities in dry seasons (in tons)	Statistics of Kruskal-Wallis	*p*-value	significance
Total Fish	47.082 ± 19.566	16.857 ± 7.746	10.566	0,0011	**
European eel	24.100 ± 15.627	3.441 ± 3.623	11.147	0,0008	***
Mullets	0.930 ± 0.866	0.932 ± 0.695	0.091	0,7623	ns

From the nutrient point of view, it seems that there is a gradation of nutrient loading that increases from the natural watershed to the urbanized watershed and through the watersheds occupied by intensive agriculture (Deslons-Paoli, 1996). Till nowadays the nutrient inputs play a major role to maintain the lagoon productivity, hope that, phytosanitary products and uses and spill still under control.

Conclusion

The yearly total fisheries landing in the lagoon, is widely related to the hydrological balance of lagoon environments expressed by direct rainfall inputs, hydrological inputs, runoff, infiltration, drainage and evaporation is inevitably under seasonal influence. The dominant fish species (eels and mullets) may be used as indicators of the regularity of the water balance. In fact the large liquid and solid flows from the catchment area to the lagoon are accompanied by a transfer of significant quantities of suspended solids and nutrients, in their particulate and dissolved form, which can influence the productivity of the highly exploited lagoon and consequently the socio-economic situation of the region.

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MERCURY CONTENT IN AGRICULTURAL SOILS AND FIELD CROPS OF CENTRAL SERBIA

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Abstract

Mercury (Hg) is a heavy metal, designated as a pollutant in the environment, due to its harmful effects on biota. Mercury pollution is a significant global concern, not only due to its increased levels in the environment, but also due to its toxic effect on human health across the food chain. The aim of this study was to determine the content of Hg in agricultural soils and main field crops. Total number of 84 bulked soil samples were taken (0-30 cm depth) from agricultural land. At the same plots, 84 plant crops (11 species – used as food and feed) were taken during the vegetation season 2018. The samples were analyzed for total Hg content using Direct Mercury Analyzer DMA 80 Milestone. The obtained results of Hg content in soil were within interval 0.003-0.37 mg kg^{-1}. The average concentration of Hg was 0.08, with median 0.06±0.06 mg kg^{-1}. Obtained values of Hg in soils were below maximum allowable concentration (MAC) and this interval is complied with the most cited range in soils up to 1 mg kg^{-1} without known nearby contamination sources. It was found that Hg content is positively correlated with pH value, slit soil fraction, and $CaCO_3$. The obtained results of Hg content in plant – field crops, were within interval 0.0001-0.9087 μg kg^{-1}. Obtained maximum value is still far lower than MACs for feed and food. According to the average Hg content in plant species, obtained results were classified from highest to lowest, respectively: bean, alfalfa, maize, soybean, rapeseed, sunflower, barley, pepper, wheat, rye and tomato.

Keywords: *Mercury, Hg, Soil, Heavy metals, Field crops.*

Introduction

Mercury (Hg) has been listed as a high priority pollutant by many international organizations due to its mobility and persistence in the environments and high toxicity to organisms (Jiang et al., 2006). A global response to solving this problem has been finalized by the adoption of the Minamata Convention on Mercury. The Minamata Convention on Mercury was approved and signed in Geneva in 2013 and entered into force in 2017. It is an international treaty designed to protect human health and the environment from the harmful effects of mercury (UNEP, 2019). Control and monitoring of anthropogenically introduced Hg throughout its life cycle is the key factor in fulfilling the obligations set by the Convention, and the Republic of Serbia is a signatory country to the treaty.

Mercury is a metal that easily changes the aggregate state, it is volatile at 20^0C, and migrates easily through the environment and builds compounds of different toxicity to biota. Natural sources of mercury released to the atmosphere include volcanoes, evaporation from soil and water surfaces, degradation of minerals and forest fires (Ottesen et al., 2013). The burning of fossil fuels, metal mining and industrial activities, such as ore processing and cement production, particularly coal and oil combustion and gold production, are the major anthropogenic sources of Hg release. Over the past several centuries, mining has been the dominant anthropogenic source of Hg (Amos et al., 2015). Soil contamination with mercury due to mining was studied worldwide (Odumo et al., 2014). More directly, soil contamination can be the result of liquid or solid Hg sources disposal, such as accidental spilling, mine tailings, landfills, polluted sewage sludge, etc. (Leterme and Jacques, 2015).

Hg in soil occurs in several ionic species and it is susceptible to transformations through the various processes. Mercury could be introduced and accumulated in soil through various pathways. On the other hand, Hg from soil could be released into the atmosphere, thus soil becomes an important source of Hg (Shi et al., 2013). Methylmercury (MeHg) is bioavailable and can be bio-accumulated within food webs. Many studies have confirmed Hg biomagnification in the food chain (Krasinska and Falandysz, 2015).
The aim of this study was to determine the content of Hg in agricultural soils of central Serbia region and main field crops.

Material and Methods

Study was conducted as part of the Project: "Global assessment of the tolerance of field crops on dangerous and harmful substance in agricultural soil and irrigation water", which is supported by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia in 2018.

Sample collection and processing

Field activities were carried out during the second half of 2018. The locations of collected samples of agricultural land are shown in Figure 1 and belong to 6 statistical districts of the Republic of Serbia: East, Bor, South, West, Belgrade and Central District. Total of 84 bulked soil samples were taken from topsoil 0-30 cm using a probe drill. One composite soil sample represented 15-25 subsamples from production plots (up to 5 ha area).
During field activities, total of 84 samples of plant material were taken also. Field and industrial crops were sampled after harvest as an average sample of seed from observed plot. Plots with forage crops were sampled immediately after cutting, as an average sample of aboveground part of alfalfa. Vegetable crops were collected as fresh samples of fruit (tomato and pepper), while bean was collected at the stage of mature seed as part of crops consumed in human diet. Samples of pepper and tomato were transported to the Laboratory in refrigerator cars.

Laboratory analysis

The pH value in 1:5 (V/V) suspension of soil in 1 mol/L KCl and water was determined using glass electrode according to ISO 10390 (2010). The carbonate content ($CaCO_3$ content) was determined according to ISO 10693 (1995) volumetric method. The organic matter content was measured by oxidation using the sulphochromic oxidation method by ISO 14235 (1998). Particle size distribution was determined in the <2 mm fraction by the pipette method (Van Reeuwijk, 2002). The size fractions were defined as clay (<2 μm), silt (2-20 μm), fine sand (20-200 μm) and coarse sand (200-2,000 μm).The soil and plant samples were analyzed for total mercury content using Direct Mercury Analyzer DMA 80 Milestone.

Statistical analysis

Data were statistically processed by analysis of the main descriptive parameters and correlation coefficients ($p \leq 0.05$) using STATISTICA for Windows version 12 (Dell Inc. 2016).

Results and Discussion

Mercury in soil

Obtained values of total mercury content in this study vary in a wide interval, from 0.003 to 0.37 mg kg^{-1}. The results fall into the interval that is most often cited for mercury content in the soil without a known nearby source of pollution, and which amounts to 0.01–1 mg kg^{-1} (Shi et al., 2013). Median value of mercury in this study is 0.06 mg kg^{-1}±0.06 (Table 1), which also complies with the most cited mercury content in worldwide soils of 0.06 mg kg^{-1} (Adriano, 2001). Obtained values match with national average for China soils of 0.065 and Liaoning Province of 0.064 mg kg^{-1} (Shi et al., 2013).

Based on analyses of 1,370 samples of agricultural soil of Vojvodina, median for this province of the Republic of Serbia is of somewhat lower value and amounts to 0.05 mg kg-1 (Ninkov et al., 2017). High variation of coefficient of variation CV (72.5%) points out high heterogeneity of tested soil samples (Table 1); but this value is lower than the obtained for studies in Vojvodina (CV=119.9%).

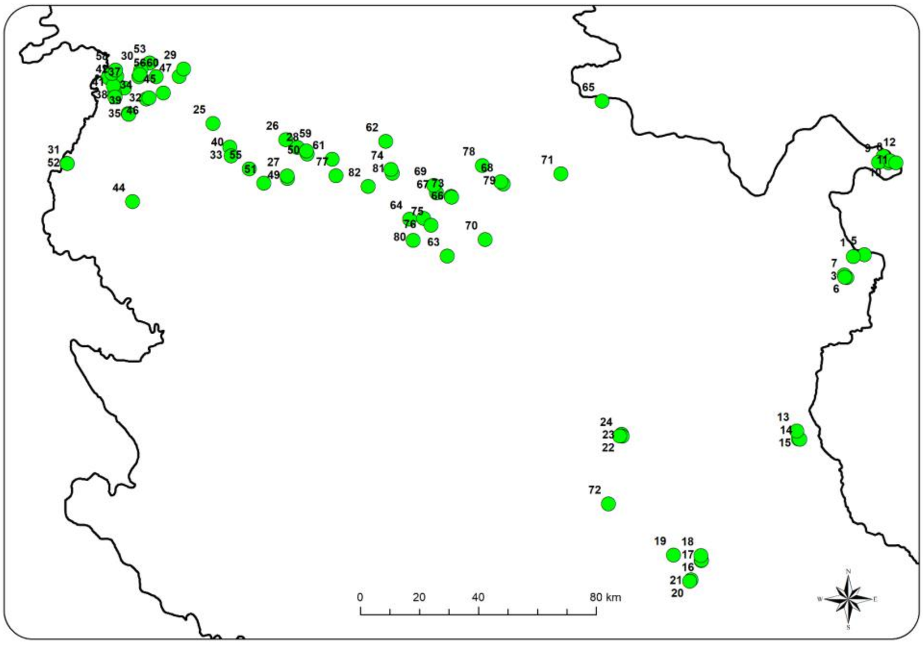

Figure 1. Layout of 84 taken soil and field crop samples in central Serbia

Based on GEMAS project (Ottesen et al., 2013), where 2,148 agricultural soil samples from a large part of Europe were analyzed (33 countries, 5.6 million km^2) at an average density of 1 sample site/2,500 km^2, median for mercury for agricultural arable land amounted to 0.03 mg kg^{-1} (Ap horizon, 0–20 cm). Median for Central Europe, where the Republic of Serbia belongs, amounted to 0.05 mg kg^{-1}. It was determined within GEMAS project that the values of Hg in Central Europe are the highest, compared to Scandinavia, Baltic Countries and Southern Europe. On the map of Hg distribution across Europe (Ottesen et al., 2013), part of Serbia, which is the subject of present study, ranged from 0.0476 to >1 mg kg^{-1}, while obtained values in our study are somewhat lower, i.e. Hg content in 25-75% percentiles were from 0.04 to 0.09 mg kg^{-1} (Table 1).

Mercury concentrations in soil of the United States of America ranged from 0.0091 to 0.37 mg kg^{-1} (Shacklette and Boerngen, 1984 *in* Wentz et al., 2014), similar to present research. Studies have shown that mercury concentrations in surface soil in the east part of USA are typically about twice those in the west part (Gustavsson and others, 2001 *in* Wentz et al., 2014). High mercury concentrations in the Eastern USA generally reflect greater rates of atmospheric mercury deposition in this region (Wentz et al., 2014).

Content of Hg in all tested samples was below the threshold of 2 mg kg^{-1}, which is the maximum allowable concentration (MAC) for agricultural soils as prescribed by the laws of the Republic of Serbia (OG 23/1994). There is only one sample which is above of threshold limit of 0.3 mg kg^{-1} according to Decree on Limit Values in soil (OG 30/2018). The obtained results also indicated that the measured levels of Hg in the soil are not limiting factors for safe food production in Serbia.

Table 1. Statistical summary of total mercury content (mg kg $^{-1}$) in agricultural soils

Statistical parameters	Value
Number of samples	84
Minimum value	0.003
Percentiles 25%	0.04
Median	0.06
Percentiles 75%	0.09
Maximum values	0.37
Average	0.08
Standard deviation	0.06
Coefficient of variation (%)	72.47

Silt fraction content was in significant positive correlation with Hg content, while there were no positive statistically significant correlations with other soil fractions content (Table 2). Generally, as for the other elements, heavier soils have higher Hg content and lighter soils have lower Hg content. Mean concentrations of Hg in podzols, sandy soils and desert sands is 0.06, while the mean concentration in loamy soils is 0.13 mg kg^{-1} (Hooda, 2010). In general, clays present negative charges, tend to undertake sorption of cations, and are associated with Hg retention in soil. It was found that mercury and mercury salts can adsorb strongly to soil particles, particularly clay minerals and iron oxides within neutral pH range (He et al., 2015). According to Roulet et al. (1998), the fine fraction (<63 μm) was two to seven times more concentrated in Hg than the medium (63-210 μm) and coarse (> 210 μm) fractions. However, some studies found no statistically significant differences that indicated a direct influence of clay contents on Hg concentrations in soil (Odumo et al., 2014).

Table 2. Correlation coefficients between content of mercury (mg kg^{-1}), various size soil fractions (%) and basic soil chemical properties

	Coarse sand %	Fine sand %	Silt %	Clay %	pH in H_2O	pH in KCl	$CaCO_3$ %	Organic matter %
Hg	-0.065	-0.083	0.220*	-0.040	0.262*	0.274*	0.634*	0.175

clay (<2 μm), silt (2-20 μm), fine sand (20-200 μm), coarse sand (200-2,000 μm)
* p≤0.05, significantly correlated

According to the established correlations shown in Table 2, Hg content was in statistically significant positive correlation to pH value (both, active in H_2O and substitution in 1MKCl, acidity), and $CaCO_3$ content, while statistically significant correlation was not found with organic matter content. In the previous studies (Rodriguez Martin et al., 2009; Ninkov et al., 2017) negative correlation between pH value and Hg content was established. The both listed studies were on alkali soils, while observed soils in present research were mainly acidic soils with median value of pH 5.41±1.21. In the study of Rodriguez Martin et al. (2009), negative correlation of Hg with $CaCO_3$ was found, while in some studies (Rodriguez Martin et al. 2013; Ninkov et al., 2017) significant correlation between $CaCO_3$ and Hg content was not found.

Statistically significant correlation was not found between organic matter and Hg content, which is the opposite of previous studies (Rodriguez Martin et al. 2009, 2013; Shi et al. 2013; Ninkov et al., 2017). The strong link between Hg concentration and organic material content was also confirmed in study of Ottesen et al. (2013), especially in the relatively high Hg levels in the Scandinavian soils.

Mercury in plant materials

As the result of high sensitivity of Direct Mercury Analyzer that was used in present study, mercury was detected in all samples of plant material, but in very small concentrations. The highest value in the study was 0.9 μg kg^{-1} in maize (Table 3). This value is still 10 times lower than allowed for animal feed, which, according to the Rulebook on the quality of animal feed (OG 39/2016), amounts 0.1 mg kg^{-1}. According to Rulebook on food (OG 90/2018), the limit for mercury content in fresh fruit and vegetables is 20, and for cereals and flour 30 μg kg^{-1}, while values obtained in present study are far lower (Table 3). According to the average Hg content in plant species, obtained results were classified from highest to lowest, respectively: bean, alfalfa, maize, soybean, rapeseed, sunflower, barley, pepper, wheat, rye and tomato (Table 3).

Statistical importance was not determined in the relation of Hg content in the soil and plant material. The majority of observed samples included the seed that plant protects from accumulation of harmful substances as its own generative part. Besides, observed vegetables and alfalfa were exposed to atmospheric deposition of Hg. Results of Niu et al. (2013) study indicated that Hg concentrations in the leaves of lettuce, radish, alfalfa and perennial ryegrass were the function of air Hg concentrations and time.

Table 3. The concentration of Hg in plants (μg kg^{-1} of originally fresh matter)

μg kg^{-1}	wheat	barley	maize	soybean	sunflower	rapeseed	alfalfa	tomato
N	17	5	26	6	10	3	12	2
Min.	0.0001	0.0150	0.0001	0.0001	0.0001	0.0001	0.2818	0.0001
Max.	0.0552	0.0504	0.9087	0.0679	0.2252	0.0580	0.8519	0.0001
Average	0.0122	0.0285	0.0507	0.0322	0.0286	0.0307	0.5296	0.0001

N number of samples

N=1: Rye 0.0001; pepper 0.0131; bean 0.8416 μg kg^{-1}

Conclusions

The obtained results of Hg content in the soil were within interval 0.003-0.37 mg kg^{-1}. The average concentration of Hg was 0.08, with median 0.06±0.06 mg kg^{-1}. This interval complied with the most cited range in soils up to 1 mg kg^{-1} without known nearby contamination sources. It was found that Hg content is positively correlated with pH value, slit soil fraction, $CaCO_3$. The obtained results also indicated that the measured levels of Hg in the soil are not the limiting factor for safe food production in Serbia. The obtained results of Hg content in plant – field crops, were within interval 0.0001-0.9087 μg kg^{-1}. Obtained maximum value is still far lower than maximum allowable concentration for feed and food. According to the average Hg content in plant species, obtained results were classified from highest to lowest, respectively: bean, alfalfa, maize, soybean, rapeseed, sunflower, barley, pepper, wheat, rye and tomato.

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VARIATION OF SOIL STRUCTURE IN THE FOOT AND TOE SLOPES OF MT. VUKAN, EAST-CENTRAL SERBIA

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Abstract
This paper presents the variation of soil structure along the foot and toe slopes of Mt. Vukan, East-Central Serbia. The analysis of aggregate size distribution and structure indices were conducted by means of soil units, characteristic soil horizons and elevation differences along the study area. Soils of Great Field located at different elevations were found to have significant variation in ASD and soil structure indices. Topsoil horizon of Eutric Cambisols have higher MWD after dry sieving, but at the same time it has the highest variation in MWD after wet sieving, indicating low water stability, which is opposite to the coefficient of aggregability. We share an opinion that change in MWD better depicts soils structure stability to water. The results of correlation analysis indicated that clay content is correlated more to structure indices compared with SOM content. SOM is significantly correlated with ASD and soil structure indices only in Calcomelansols, whereas the significant correlation of clay content and soil structure is more evident in Eutric Cambisols and Non-calcaric Chernozems, compared with other soil units. Soil structure variation along the lowest chain of Catena might be strong, and that it has to be analyzed from the point of view of soil unit and their corresponding soil horizons.

Keywords: *Soil structure, aggregate stability, aggregate size distribution, structure indices*

Introduction
Soil aggregation is one of the most promising indicators of soil quality, although quantitatively not always perfectly defined. It refers to natural organization of soil particles into various forms as a result of pedogenic processes. Soil structure is formed with the involvement and interaction of physical, chemical, mineralogical, and biological factors, the role of which depends on the soil type and the organization level of the soil particles (Alekseeva et al., 2009). Soil aggregates behave variously in respect to water impact and mechanical pressure. Aggregate stability depends on internal cohesiveness between constituent colloidal particles, among which the most important binding agents are humic substances, sesqui-oxides, Ca^{++} ions, clay minerals, calcium carbonate, earth worms and funghi. Soil structure degradation produce a decrease of soil porosity and infiltration, favors soil crusting, enhance reducing conditions, impede seedlings growth, favors runoff and water erosion, leads to water logging. There is no universally accepted way to measure soil structure (Diaz-Zorita et al., 20012). Common method used to analyze soil structure in Serbia is Savvinov's method (Savvinov, 1931).
Aggregate size classes and their distribution is one of the most important soil property, because of its enrolment in soil fertility and resistance to erosion, compaction and sealing. Many indices worldwide obtained from the sieving analysis are used to describe soil structure and soil aggregability, such as structure coefficient (Shein et al., 2001), geometric mean diameter (Campbell, 1993), mean weight diameter (Hillel, 1993), or coeficient of aggregability (Vershinin, 1958). The importance of soils structure and their linkage and

homogenity with soil classification units are presented in Serbia by different authors (Djordjevic, 1993; Ciric et al., 2012).
The aim of this study is to: 1) to perform quantitative assessment of soil structure after dry and wet sieving by means of soil type, 2), to compute different indices of soil aggregation in order to assess stability of soil structure, 3) to find relation of soil structure with soil organic matter and clay content, and 4) to assess the impact of soil spatial position on aggregation.

Material and Methods

Site description

This study was conducted in the East-central Serbia about 100 km southeast of Belgrade. The study area called Great Field is located at the elevation between 175-210 m a.s.l. The area has temperate climate and presents the foot and toe slopes of mountain Vukan. Foot and toe slopes have uniform gradient of within the 2.5 km distance. Field crops production is presented more in the western part of the area, whereas the eastern parts are used mainly for grazing.

Soil sampling

Total number of 42 soil profiles was opened in respect to elevation alterations within the study area. Disturbed soil samples were taken approximately at 20 cm depth within the horizons. A total number of 161 samples characterized the whole area.

Basic soil analysis and soil classification

In the laboratory, the following measurements were conducted: particle-size distribution of the soils was determined by combining sieving and the pipette methods (Rowell, 1997); soil organic carbon concentration (SOC) was determined using the dichromate method (Rowell, 1997); SOM percent was calculated by multiplying %SOC by a factor of 1.724; total carbonate content was measured volumetrically after treatment with 6 N HCl (Nelson, 1982). The soils are classified according to national classification system (Skoric et al., 1985). General soil characteristics of the five soil units and their corresponding soil horizons are presented in Tab. 1. For additional details regarding the studied soils and further analytical data refer to Zivotic (2016, 2017).

Aggregate-size distribution and aggregate stability

Soil aggregation was characterized by the dry aggregate size distribution (ASD) after dry and wet sieving. Savvinov's method (Savvinov, 1931) uses dry and wet sieving procedures. As a result of dry sieving, eight classes of soil aggregates by size were determined: mega-aggregates (>10 mm), macro-aggregates (10–5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 mm) and micro-aggregates (<0.25 mm). As a result of wet sieving six aggregate size classes were determined: 3–2, 2–1, 1–0.5, 0.5–0.25 and <0.25 mm (micro-aggregates).

Structure indices

The results of dry and wet sieving were used to determine structure indices: coefficient of structure (Ks), dry mean weight diameter (dMWD), wet mean weight diameter (wMWD), and dry geometric mean diameter (dGMD), wet geometric mean diameter (wGMD) and coefficient of aggregability (Ka). Ks was calculated as the ratio between the content of the agronomically valuable fraction (0.25–10 mm) and the sum of aggregates higher than 10 mm and lower than 0.25 mm, after dry sieving (Shein et al., 2001). Soils with Ks value above 1.5 have a good structure, while those with Ks lower than 0.66 have with weak (bad) structure.
The MWD is the sum of the weighted mean diameters of all size classes, with the weighting factor of each class which is a proportion of the total sample weight. Both dMWD and wMWD were calculated by the following equation (Hilel, 2004):

$$MWD = \sum_{i=1}^{n} x_i \cdot w_i \qquad \text{Equation 1}$$

Where: xi is the mean diameter of any particular size range of aggregates separated by sieving, and w_i is the weight of the aggregates in that size range as a fraction of the total dry weight of the sample analyzed.
Geometric mean diameter was calculated as index of Mazurak (1950) after dry and wet sieving using following equation:

$$GMD = \exp\left\{\frac{\sum W_i \cdot \log X_i}{\sum W_i}\right\} \qquad \text{Equation 2}$$

Where: W_i is the weight of the aggregates of each size class (g) and X_i is the mean diameter of the i_{th} size class (mm).

Table 1. The mean (± standard deviation) values of and particle-size distribution, SOM content and $CaCO_3$ content in five different soil units and their characteristic horizons

Soil type*	Horizon designation*	Sand (%)	Silt (%)	Clay (%)	SOM (%)	$CaCO_3$ (%)
Colluvial Soils	A	26.8±12.0	46.8±3.7	26.4±9.6	3.17±0.61	19.5±21.6
Calcomelanosol	A	21.6±8.6	46.5±6.3	25.9±6.2	4.02±0.94	12.3±11.8
Calcaric Chernozem	A	26.8±7.4	48.5±3.2	24.7±8.2	3.65±0.43	19.7±12.9
	AC	31.6±11.5	45.9±6.8	22.6±8.3	2.64±0.62	30.7±19.5
	C	29.1±7.0	48.2±4.1	22.7±4.7	2.02±1.03	34.4±14.5
Non-Calcaric Chernozem	A	15.8±2.8	53.8±3.4	30.4±3.7	2.60±0.91	-
	AC	13.6±2.3	51.7±5.2	34.7±4.6	1.43±0.43	-
Eutric cambisol	A	15.3±2.7	52.2±4.0	32.6±3.8	2.36±0.61	-
	(B)	12.1±2.8	49.6±3.8	38.3±5.0	1.15±0.42	-

*according to national classification

Differences in MWD and GMD, after dry and wet sieving, were used to describe aggregate stability to saturated water conditions. High wMWD values imply greater aggregate stability, whereas high values of aggregates <0.25 mm implies low aggregate stability. Differences in the content of micro-aggregates between wet sieving and dry sieving was also used to express aggregate stability. Aggregate stability is expressed through coefficient of aggregability (Vershinin, 1958), as ratio of wet aggregates higher than 0.25 mm, and lower than 0.25 mm.

Statistical analysis
Descriptive statistic was used to present the results of soil structure for separate soil units. Six different elevations zones (180, 185, 190, 200, 205-210 m a.s.l) across the study area presented statistical treatments, whereas six profiles within the zone presented replications. The differences in soil characteristics within elevation groups were conducted by means of one-way ANOVA. The LSD test was used to assess the differences within the means of soil groups topsoil characteristics. Correlation analysis was performed to investigate the relationships between clay and SOM content and structure indices. The correlations were expressed as Pearson's linear coefficients with the indication of statistical significance (* for $P<0.05$, ** for $P<0.01$). All statistical analyses were performed using SPSS 18.0 software (SPSS, 1995).

Results and Discussion

Aggregate-size distribution and aggregate stability
Humus-accumulative horizons of Eutric Cambisols are characterized with the highest amount of aggregates >10 mm (58.7±13.7%), followed by their subsoil horizons (42.7±10.2%) and topsoil horizons of Non-calcaric Chernozems (42.2±7.2%). The lowest content of mega-aggregates is found in Calcomelanosols, 22.5±10.9%. The content of micro-aggregates in all

soil units is low. It is the highest in C horizon of Calcaric Chernozems, topsoil horizon of Colluvial Soils, and intermediate horizon of Calcaric Chernozems, 5.2±3.1%, 4.0±4.1%, and 3.3±2.1%, respectively. Ciric et al. (2012) found significant differences in ASD and structural indices among different soil types in Vojvodina. The lowest value of Ks is found in topsoil horizon of Eutric Cambisols, topsoil horizon of Non-calcaric Chernozems, and subsoil horizons of Eutric Cambisols, 0.8, 1.4 and 1.4, respectively. All the other soil horizons have coefficient of structure higher indicating good aggregation. MWD is different within soil units. It is the highest in Eutric Cambisols A horizon, (9.01±1.09 mm), followed by subsoil horizon of Eutric Cambisols, and topsoil horizon of Non-calcaric Chernozems, 7.76±0.85 and 7.70±0.59 mm, respectively. Calcomelanosols topsoil has the lowest MWD between soil units, 5.66±1.01 mm.

The results of wet sieving indicate the highest increase in the content of micro-aggregates in Non-calcaric Chernozems, whereas the lowest change was detected in topsoil horizon of Calcaric Chernozems, indicating the most stable structure. On one side, wMWD is the highest in topsoil horizon of Calcomelansols, which had the lowest MWD after dry sieving. On the other side, the lowest wMWD was found in the subsoil of Eutric Cambisols and in the A and AC horizons of Non-calcaric Chernozems, 1.44±0.31, 1.45±0.53, and 1.51±0.58 mm. These horizons had dMWD between 7–8 mm. Changes in MWD after wet sieving are presented in Fig. 1 below. Soil units presented on the right part of the figure exhibited higher changes in MWD. Soil units closer to dashed line exhibited lower changes. Calcomelanosols are followed by Calcaric Chernozems and Colluvial Soils by means of water stability. Located more on the right in the Figure 1 are Non-Calcaric Chernozems, and subsoil horizons of Eutric Cambisols, whereas topsoil horizon of Eutric Cambisols is located far away from the dashed line.

Ka values are high in all soil units described, ranging from 2.94 in C horizon of Calcaric Chernozems to 4.95 in A horizon of Eutric Cambisols. Coefficient of aggregability (Ka) results that we obtained does not correspond to the results of change in MWD after wet sieving. According to Ka, the highest values are found in Eutric Cambisols, which in turn have the highest change in MWD after wet sieving. Therefore, there is discrepancy among these indices. We share an opinion that change in MWD better present stability of soil structure.

Clay and SOM content vs. structural indices

The results indicate that SOM content and different structural indices have better correlation in Calcomelanosols compared with other soil units. SOM in Calcomelanosols has high positive correlation with content of water stable macro-aggregates (0,586**) and high negative correlation with content of water stable micro-aggregates (-0,601*). SOM is also positively correlated with the difference in the content of micro aggregates after wet sieving, and wMWD (0.629**) and Ka (0.722**). These significant correlations between SOM and structural indices of Calcomelnosols after wet sieving might be related to higher content of SOM in Calcomelanosols compared with other soil units. Similar findings about the correlation between SOM and soil structure after wet sieving was found in Serbia on Fluvisols (Gajic et al., 2010). On the contrary to their findings, SOM was not found to be correlated with dry micro-aggregates, whereas there were no significant correlation between SOM and dry mega-aggregates. In other soil units, significant SOM correlation with investigated structure indices is rare.

On the contrary to SOM content, clay content have shown much higher and more often correlations within different soil units. Clay content is highly negatively correlated with the content of micro-aggregates after dry sieving in Colluvial Soils (-0.842*), A horizon of Calcaric Chernozems (-0,609*), and C horizons of Non-calcaric Chernozems (-0.740*). Ks is positively correlated with clay content only in A horizons of Eutric Cambisols (0.514*). Clay content is highly negatively correlated with micro-aggregate content after wet sieving in Calcomelanosols (-0.478*), A horizon of Non-Calcaric Chernozems (-0.516*), AC horizon of Non-Calcaric Chernozems (-0.641**), and both horizons of Eutric Cambisols (-0.554* in A, and -0.812** in (B)). Clay content is positively correlated with dMWD in Colluvial soils (0.816*), AC horizons of Calcaric Chernozems (0.769*), and AC horizon of Non-calcaric Chernozems (0,459), whereas it is negatively correlated in A horizons of Eutric Cambisols (-0.560*). Clay content is positively correlated with wMWD in A horizons of Calcaric Chernozems (0.800**), A horizons of Non-calcaric Chernozems (0.579*), AC horizon of Non-calcaric Chernozems (0.678**), A horizons of Eutric Cambisols (0.493*) and subsoil horizons of Eutric Cambisols (0.641**). A positive correlation with Ka in A horizons of Calcaric Chernozems (0.808**), AC horizons of Calcaric Chernozems (0.781*), AC horizon of Non-calcaric Chernozems (0.556*), and subsoil horizons of Eutric Cambisols (0.843**) is found.

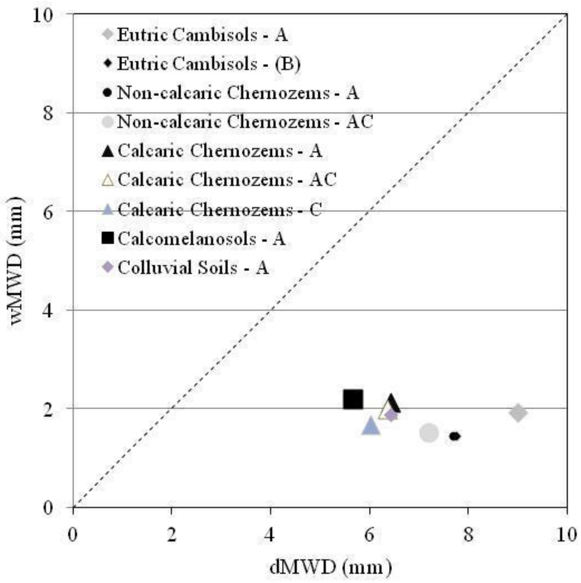

Figure 1. Scatter diagram of dMWD and wMWD of soil aggregates in five different soil units and their characteristic horizons

Changes in soil structure along the elevation changes

The spatial position of soils along the foot and toe slopes was found to be important for aggregation. There are significant differences between the content of micro-aggregates, mega-aggregates and several fractions of macro-aggregates between the soils along different elevations. These differences are mainly related to the presence of Eutric Cambisols as dominant soils on the lowest elevations and Calcomelanosols as the dominant soils at the higher elevation. Also, there is also significant difference in dMWD and dGMD between the same elevation positions. There are no significant differences between the soils at different elevations in respect to wet sieving.

Conclusion

Soils of Great Field located at different elevations were found to have significant variation in ASD and soil structure indices. These differences are mainly related to Eutric Cambisols found in the toe slopes and Calcomelanosols located at the foot slopes. Topsoil horizon of Eutric Cambisols have higher MWD after dry sieving, but at the same time it has the highest variation in MWD after wet sieving, indicating low water stability. The results of correlation analysis indicated that clay content is correlated more significantly to structure indices compared with SOM content. SOM is significantly correlated with ASD and soil structure indices in Calcomelansols, whereas, the correlation of clay content and soil structure is more evident in other soil units. The overall conclusion of this work is that soils structure variation along the lowest chain of Catena might be strong, and that it has to be analyzed from the point of view of soil unit.

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COST AND ENVIRONMENTAL EFFICIENCY IN MEASURING THE ECO-EFFICIENCY PERFORMANCE OF WINTER TRITICALE PRODUCTION

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Abstract

Important issue in a sustainable production is a concept of eco-efficiency. Excessive application of agricultural inputs in fodder cereals production may affect both their economic performance and put an excessive pressure on the environment. The aim of the work was to estimate the eco-efficiency of winter triticale production by assessing the cost efficiency of applied inputs and the environmental efficiency. The research was carried out in a group of different types of farms located in two regions of Poland (Lubelskie and Wielkopolska), in the years 2017-2018. For efficiency measurement, the non-parametric Data Envelopment Analysis was implemented. The introduced model was focused on the input oriented efficiency that reflects the ability of using minimal amounts of inputs to obtain a given amount of output. Farms specialized in the milk farming reached the highest value of cost efficiency, while field crops farms displayed the full environmental efficiency. Cost efficiency of winter triticale production was higher in farms of lower economic size. Farms with higher environmental efficiencies were concentrated in groups of middle economic size. The results obtained suggest that the strategies of increasing the cost and environmental efficiencies of winter triticale production should be individualized dependent on the farming type and the economic size of farm. By applying DEA approach to the eco-efficiency analysis of winter triticale production, farms obtain an important source of information on an extent of indispensable changes with respect to improvement in cost efficiency and mitigation of their negative environmental effects.

Keywords: *Eco-efficiency, Cost efficiency, Environmental efficiency, Data envelopment analysis, Winter triticale*

Introduction

For a long time, one of the main objectives of agricultural policy in many countries was the increase in agricultural production. This increase was mainly due to the development of energy-intensive technology, use of mineral fertilization and plant protection products. It turned out, however, that the use of modern production factors on a wider scale results in an excessive burden on the environment. It is believed that maintaining further production growth without providing conditions for sustainable development will generate many ecological problems. The practical direction of implementing the idea of sustainable agriculture is the rational, economical use of agricultural production factors and at the same time exerting the lowest possible environmental pressure. Current views on sustainable agriculture assume equally important treatment of economic and ecological objectives. An operational way to achieve these goals is to carry out production tasks with the lowest cost and beneficial ecological effects. A useful tool for assessing and monitoring progress in the implementation of sustainable development principles is considered the eco-efficiency method (Huppes and Ishikawa, 2005, Kuosmanen and Kuosmanen, 2009). A multidimensional assessment of the effectiveness of inputs and environmental effects is possible by the use of non-parametric, Data Envelopment Analysis, DEA (Seiford and Zhu, 1999, Chen et al., 2005).

The purpose of the work was to assess the cost efficiency of the inputs used and environmental efficiency in the production of winter triticale in two different regions of Poland in farms representing various agricultural types of production and diversified economic size. The rationale for undertaking such assessment is that the triticale crop is an important Polish fodder plant being a component of compound feeds both for cattle and pigs. The widespread cultivation of this crop results from a good nutritional value of the grain and a suitability for cultivation on lower quality soils, compared to winter wheat.

Material and Methods

Data to the eco-efficiency analyses of winter triticale were collected during the years 2017-2018 among the group of 69 commercial farms, located at two provinces of Poland (Lubelskie and Wielkopolska). The surveyed farms with triticale cultivation represented different farming activities: milk farming, pig farming and field crops. They were differed in respect to the economic size, measured in the standard output units. Besides the monitoring of production costs, the analysis program also included the assessment of nitrate leaching, pesticide emission into the environment and ammonia emissions from mineral and natural fertilizers. The model described by van Beek et al. (2003) was used to calculate the nitrate leaching. The tier 1 default emissions factors from fertilizers were used to estimate ammonia emissions (IPCC, 2006). The quantities of pesticide emission have been determined following the method described by Margni et al. (2002).

The cost efficiency of winter triticale production was determined based on the DEA model, with input oriented approach and under the assumption of constant return to scale (Cooper et al., 2004). It is described by equations: (1a) - (1d). In the analysis, the following variables were taken into account on the input side of the model: direct costs including seeds, fertiliser and pesticide costs, maintenance and repair costs of machines and vehicles, labour costs and other costs including fuel costs, lubricants and service costs, expressed in PLN per ha. On the output side there was the variable of winter triticale value, as determined by sale prices (PLN/ha AL). The classic version of the DEA model was used, assuming that all factors of the input side are controllable, i.e. susceptible to changes, therefore they can be minimized. Finding the solution for this model involves searching for the possibility of radial, proportional reduction of all inputs without producing less output. In the model used, the goal is to indicate whether a given production volume can be realized with fewer inputs. This efficiency is relative and is determined in relation to the estimated frontier (best practices). The efficiency coefficients range from 0 to 1, $0 < \theta_o \leq 1$. An efficiency ratio of less than 1 means that it is possible to reduce inputs while leaving the output (production effect) at its current level. In the constructed model variable inputs are expressed in the form of costs, therefore the analysed technical efficiency can be characterized as cost efficiency (Chavas and Aliber, 1993).

DEA model describing the DEA model of efficiency (Cooper et al., 2004):

$$Min\,\theta_o, \qquad (1a)$$

$$Subject\ to:$$

$$y_o \leq \sum_{j=1}^{J} \lambda_j\, y_j, \qquad (1b)$$

$$\theta_o\, z_{io} \geq \sum_{j=1}^{J} \lambda_i\, z_{ij}, \quad i = 1,...,I, \qquad (1c)$$

$$\lambda_j \geq 0, \quad j = 1,...,J \qquad (1d)$$

where: θo = optimal, relative efficiency of object „o", yo – output value of object „o", yj – output value of object „j", j = farms (j = 1,...,J), zio – value of conventional ith input of object „o", zij = value of conventional ith input used by object „j", λ = nonnegative scalars.

The DEA model with identical structure as for cost efficiency was applied to calculate the environmental efficiency of the winter triticale production. The following variables, causing threats of environmental degradation, were introduced to the model: ammonia emission from mineral fertilization and manure (kg NH_3/ha AL), leaching of nitrates (kg NO_3/ha AL) and emission of pesticides to the environment (kg active substance/ha AL). The model assumes minimizing the values of indicators in relation to the unit of AL. By applying the DEA model of environmental efficiency, the possibility of equally proportional reduction in all environmental pollutants in relation to the efficient objects described as benchmarks is determined (De Koeijer et al. 2002). Both types of efficiencies are calculated by solving the linear programming task for each farm.

Results and Discussion

The cost and environmental efficiencies of winter triticale production in a group of farms located in two regions of Poland are shown in Figure 1.

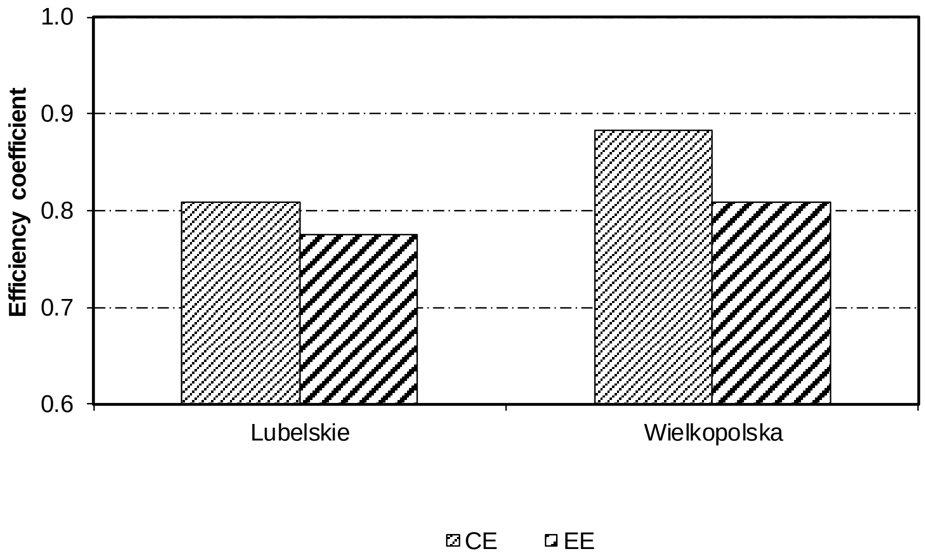

Figure 1. Cost (CE) and environmental efficiency (EE) for the analysed farm population in Lubelskie and Wielkopolska provinces.
Source: Authors' own calculations.

On average, farms from Wielkopolska province (Middle West region of Poland) achieved higher cost and environmental efficiency compared to farms from Lubelskie province (South East part of Poland). In the whole group, consisted of the analysed farms from both provinces, the average cost and environmental efficiencies of triticale production were equal to 0.845 and 0.792, respectively. Noticeable differences in cost and environmental efficiencies occurred between the identified agricultural types of farms (Figure 2). The highest level of cost efficiency of triticale cultivation was characteristic for farms oriented to milk production. The average value of the coefficient for this group was 0.884. The pig farms had an inferior cost efficiency of growing triticale by 6.7 percentage points. The lowest level of cost efficiency was achieved by farms of field crops type. The average efficiency for this this group was 0.742. This means that the cost of inputs for winter triticale production in this type of farms should be reduced by nearly 26 percentage points in order to be able to achieve the full efficiency while maintaining the current level of output value. On farms specializing in milk production, a simultaneous reduction of all costs by the average of the 11.6 percentage points would be sufficient to obtain the full efficiency of triticale production. It expected that an improvement in the cost efficiency of triticale will be one of the main factors determining

the ability to profitability gain of its production in the near future, increasing thus the competitive potential of triticale production in the Polish feed grains market.
The values of environmental efficiency coefficients for triticale cultivation in types of farms specializing in the production of milk and pigs were lower compared to the cost efficiency (Figure 3).

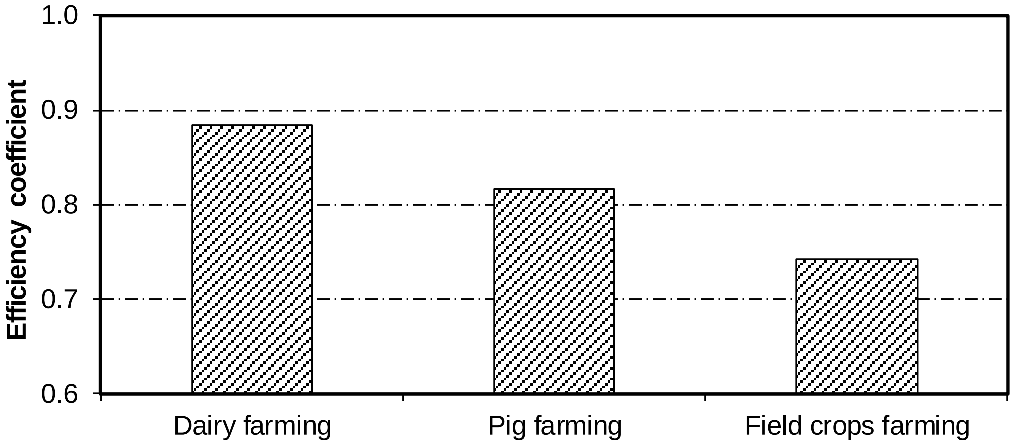

Figure 2. Cost efficiency of winter triticale production according to farming types.
Source: Authors' own calculations

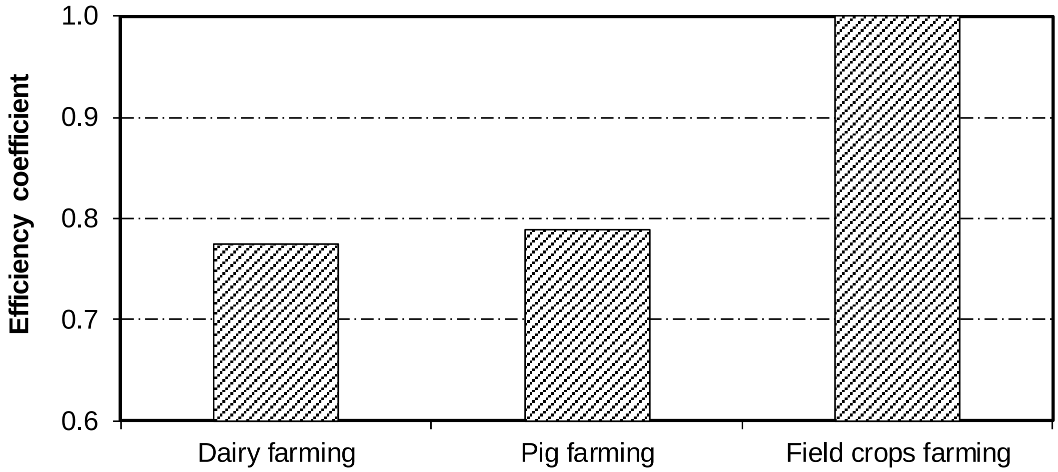

Figure 3. Environmental efficiency of winter triticale production according to farming types.
Source: Authors' own calculations

The production process of triticale in farms specializing in field crops was carried out in a way that ensured a full environmental efficiency compared to the milk and pig farming types. The group of milk farms was characterized by the lowest environmental efficiency (0.774). It would be possible to achieve a full environmental efficiency of triticale production, provided that the value of emission factors has been improved by an average of 22.6 percentage points. Generally, lower values of environmental efficiency coefficients for triticale production in farms with milking cows and also with pigs suggest that the conditions for achieving a full environmental efficiency are more difficult to meet by them than managing the costs of triticale production ensuring a better cost efficiency. The results also indicate that animal farms use nitrogen less economically by possibly applying excessive amounts of nitrogen to triticale fields and that they use larger amounts of pesticides, compared to the field crops farms. Animal farms have available manure which is utilised for fertilizing different crops, including also winter triticale, leading often to nitrogen balance surpluses that contribute to its excessive emissions to the environment (Bieńkowski and Jankowiak, 2006).

Table 1. Cost and environmental efficiency of winter triticale production according to the groups of economic size.

Specification	Economic size group (thous. Euro)			
	I (38,0)	II (38,1 – 96,0)	III (96,1 – 185,6)	IV (> 185,6 ha)
Cost efficiency	0.895	0.862	0.770	0.844
Environmental efficiency	0.782	0.817	0.798	0.763

*Source: Author's own calculation

The factor determining the diversification of cost and environmental efficiencies of triticale production was also the economic size of farms, calculated on the basis of the standard output value, expressed in Euro, (Commission ..., 2008). Table 1 shows the efficiency coefficients for separate farm groups of different economic size. The boundaries for division into four groups were determined by standard output, grouping 25% of farms ranked in order of increasing their output values. The farms in the first two groups achieved the highest cost efficiency coefficients for the triticale production. The difference in cost efficiency between the 1st and 3rd size group with the lowest cost efficiency was 12.5 percentage points. In turn, higher environmental efficiency was found to be concentrated in the middle size groups (2nd and 3rd). The results presented show that in smaller farms it is relatively easier to obtain better cost efficiency than the environmental efficiency. The largest farms showing inferior performance with regard to environmental efficiency of triticale production should take into account the introduction of technological changes optimizing a better utilisation of nitrogen fertilizers and plant protection products in relation to their production effects.

Conclusions

Farms specializing in milk production achieved the highest cost efficiency of winter triticale production, and at the same time they had the lowest environmental efficiency. Opposed values of efficiency coefficients had field crops farms in which the production of triticale was characterized by the highest environmental efficiency and the lowest cost efficiency. Milk farms should modify their activity to become more efficient by rationalizing their operations which limit the amount of nitrate available for runoff in fields with triticale. Second way of environmental efficiency changes in milk farms should be focused on decreasing the use of plant protection products contributing to lower emissions of active substances to environment. For the field crops farms, the preferential direction of changes in the triticale production should be the reduction of costs. The results of the research indicate that an important challenge for increasing the eco-efficiency of winter triticale cultivation may be the implementation of the environment-friendly farming practices and minimizing the production costs in different farming types .

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IMPACT OF GRAZING ON SOIL ORGANIC MATTER AND PHYSICAL PROPERTIES OF A FLUVISOL IN NORTWEST SERBIA

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Abstract

The effects of long-term (>20 yr) grazing on the selected physical properties of a non carbonated silty-clay Fluvisols were studied in the region of the Kolubara Valley, Northwest Serbia. Two adjacent land-use types (native deciduous forest and natural pasture soils converted from forests for more than 20 years) were chosen for the study. Disturbed and undisturbed soil samples were collected from three sites at each of the two different land-use types from the depths of 0–15, 15–30 and 30–45 cm. In relation to the soil under native forest, soil organic matter content, total porosity and air-filled porosity were significantly reduced after long-term of grazing. The bulk density (0.99–1.48 g cm^{-3}) and the saturated hydraulic conductivity ($6.9 \cdot 10^{-2}$–$3.2 \cdot 10^{-4}$ cm s^{-1}) were significantly lower in forest compared to the adjacent pasture (ex-forest) soil (1.49–1.55 g cm^{-3} and $3.4 \cdot 10^{-4}$–$5.5 \cdot 10^{-4}$ cm s^{-1}, respectively). In addition, forest had significantly lower dry mean weight diameter (7.0–9.2 mm) and greater wet mean weight diameter (2.0–2.6 mm) for 0–45 cm depth compared with the pasture (8.8–9.4 mm and 1.8–2.3 mm, respectively). The decrease of soil organic matter content and reduction in aggregate stability under long-term grazing rendered the soil more susceptible to compaction. In conclusion, the results of this study indicate that removal of permanent vegetation in the conversion process from forest areas to pasture land may lead to loss of soil productivity and serious soil degradation. Obviously, there is a need for greater attention to developing sustainable land use practices in management of these ecosystems to prevent further degradation of pasture soils in the region.

Keywords: *Pasture, Trampling, Soil structure, Hydraulic conductivity, Total and air-filled porosity.*

Introduction

It is known that grazing can affect soil physical properties. Changes to soil physical properties caused by grazing animals have received little attention compared with of arable soils (Greenwood and McKenzie, 2001). The longer-term effects of livestock grazing on soil physical properties are complex. According to Sharrow (2007), they reflect the balance of restorative and compactive processes at work on grazed and ungrazed areas. The actual impact of change depends on several factors such as the type of pasture ecosystem, soil type, mineralogy, texture, soil depth, initial soil properties, the nature, duration and intensity of the grazing, animal species and age, stocking density and management practices (Willatt and Pullar, 1983; Sparling *et al.*, 2000; Sharrow, 2007; Bormann and Klaassen, 2008; Reszkowska *et al.*, 2011), and long-term site-specific factors (e.g. parent material, topography and climate – Ogle *et al.*, 2005; Khormali *et al.*, 2009).

The conversion of natural forest into pasture is known to deteriorate soil properties, especially reduce SOC and change the soil structure (Yong-Zhong *et al*., 2005; Reszkowska *et al*., 2011). Grazing affects the grassland environment by a reduced vegetation cover followed by decrease in SOM content in the topsoil (Yong-Zhong *et al*. 2005). Due to shearing intensive grazing animals leads to rearrangement of soil particles as well as to soil homogenization (Drewry, 2006). Thus, animal trampling results in an increase in soil bulk density and hardness, decrease in pore continuity, reduction in soil porosity, hydraulic and air conductivity (Horn, 1986; Sharrow, 2007; Reszkowska *et al*., 2011). According to Greenwood and McKenzie (2001), the stresses exerted by livestock have similar influence on soil as agricultural machinery, however, due to smaller contact area, the soil deformation restricts only to the upper 15 cm soil layer. This zone is very important in determining soil hydrology and plant growth/vigor.

It is not clear from the literature whether heavy grazing leads to a deterioration of physical parameters of topsoils in steppe ecosystems (Steffens *et al*., 2008). In contrast, Lu *et al*. (2015) reported that short-term grazing exclusion has no impact on soil properties of degraded alpine grassland in Tibet, Chine.

The main purpose of our work was to evaluate the long-term (>20 yr) effects of grazing on soil quality by measuring the differences in the SOM and some physical properties of noncarbonated silty clay Fluvisol in the lowland ecosystems of Western Serbia.

Materials and Methods

Site description

The study was conducted in the Kolubara Valley in northwest Serbia (44°36′ N and 20°17′ E), in spring 2016. The area has continental climate, with a mean annual precipitation of 730 mm and a mean annual air temperature around 11°C. The area is geomorphologically plain and nearly flat. The soil was noncarbonate Fluvisols (FAO, 2006), developed on a poorly carbonated alluvium of the Kolubara River. The textural classes of soils at the 0–45 cm depth are: silty clay with 5.4–5.8% sand, 44.9–46.1% silt, and 48.2–49.7% clay for the forest; silty clay with 7.6–7.7% sand, 49.6–50.5% silt, and 41.8–42.5% clay for the pasture. More detailed information on the study area is presented by Gajić (2013). Three locations were selected in the Central Kolubara River Basin. At each location, sites of forest and pasture land were selected in close proximity (at a distance of ≈75 m) and of similar topography. The natural vegetation of the deciduous forest sites is characterized by a community of common oak and common ash (*As. Querceto-raxinetum serbicum, Rud*.). In the pasture, in addition to the grasses (*Festuca ovina* and *Poa pratensis), Ranunculus* and *Lathyrus* sp. were also dominating herbs. The pasture has been used over the last 20 years for grazing animals. The pasture was traditionally grazed by ewe and cattle around the 20 year. Stocking rates for the experiments ranged from 5 to 8 ewes ha^{-1} and 2–3 cattle ha^{-1}.

Soil sampling

Six sites were sampled, three within each of the two adjacent land-use types of forest and pasture. For each land-use type and its three sites, one monolithic (about 20 cm×15 cm×10 cm) soil sample of ≈ 4 kg was taken for each of the depth ranges of 0–15, 15–30, and 30–45 cm. These soils were collected to determine organic carbon (SOC), particle density, particle size distribution, wilting point, dry mean weight diameter (dMWD) and wet mean weight diameter (wMWD). To determine bulk density (BD), total porosity (TP), field capacity (FC) and saturated hydraulic conductivity five soil cores from each depth were collected using a 100 cm^3 cylindrical steel sampler (height 5.1 cm, and inner diameter 5.0 cm).

Soil chemical and physical analyses

In the laboratory, the monoliths were carefully broken up by hand along natural planes of weakness into aggregates of < 25 mm. After visible plant materials were removed, all soil samples were air-dried and ground to pass 2 mm sieve for the following measurements.
Soil organic carbon (SOC) concentration was determined using the dichromate method (Rowell, 1997). Particle-size distribution of the soils was determined by combining sieving and the pipette methods (Rowell, 1997). Bulk density was determined using the core procedure (Rowell, 1997). Particle density was measured with a pycnometer (Rowell, 1997). Total porosity (TP) was calculated using dry bulk density and a particle density. To obtain the water stored at field capacity (FC) and wilting point (WP), all undisturbed soil samples were saturated and equilibrated to a matric potential of −33 kPa using ceramic porous plates and to matric potential of −1,500 kPa using the pressure method, respectively (Schlichting *et al*., 1995; Hartge and Horn, 2009). The air capacity (AC) was defined as difference between TP and FC. The plant available water (PAW) was calculated from the difference between volumetric water content at FC and WP. The dry mean weight diameter (dMWD) and wet mean weight diameter (wMWD) as indices of soil aggregation were determined by the procedure outlined in Gajić et al. (2010). Saturated hydraulic conductivity was measured by the falling-head method according to Klute and Dirksen (1986). Data analyses were carried out using SPSS software (version 15.0). The LSD procedure was conducted to compare means of the soil properties at $P < 0.05$.

Results and Discussion

The effects of grazing on SOC and soil physical properties are shown in Table 1. The conversion of natural forest soil into soils under pasture resulted in significant reductions in the concentrations of SOC. Soil organic carbon of pasture land in the 0–15 cm, 15–30 cm and 30–45 cm soil layers was lower than in the forest plots, but the differences were not statistical significant in the 15–30 cm and 30–45 cm soil depth ($P > 0.05$). The SOC was about 3 times less under pasture compared with forest in the 0–15 cm soil layer. The lower values in pasture are probably due in differences in C inputs. In contrast to our results, in the Flooding Pampa of Argentina, cattle grazing did not affect SOC (0–10 cm depth) in an upland site, but increased it by 28% in a lowland site (Chaneton and Lavado, 1996), suggesting that topographic position modulated grazing effect on carbon cycling. Moreover, according to them, grazing effects may depend on the soil pool being sampled.
The long-term grazing had a great effect on the bulk density at all the three depths. The bulk density was 4.7–50.5% higher in pasture than in forest soil, and varied from 0.99–1.48 g cm^{-3} for forest to 1.49–1.55 g cm^{-3} for pasture, in the 0–45 cm soil layer.
Total porosity of the forest (61.0, 50.8 and 44.6% in the 0–15-, 15–30- and 30–45-cm layers, respectively) was significantly greater (4.9–36.5%) compared with pasture.
The air capacity was also significantly affected by grazing. After the conversion natural forest to pasture, air porosity decreased to 85.3%, 59.5% and 32.0% in the 0–15-, 15–30- and 30–45-cm depth, respectively. The air capacity at all three depths of pasture soil had average soil air-filled pore volume below the 10% value often cited (Rab, 2004) as the point where soil anoxia becomes a problem.
Similar to this study, Abbasi *et al*. (2007) reported that soil from grassland had 10% lower porosity than the soil from the forest at 0–15 cm depth in the hilly and mountain ecosystem in the northeast of Pakistan. In contrast, Celik (2005) reported no differences in the total porosity values between forest and pasture in the 0–10 cm layer but differed significantly in the 10–20 cm layer to ~ 7%.

Table 1. Comparison of the mean values of the studied soil properties in two studied land uses (forest – FO and pasture – PA) and depths.

Depth (cm)	Land use	SOC[†] (w%)	BD ($g\ cm^{-3}$)	TP (v%)	AP (v%)	dMWD (mm)	wMWD (mm)	FC (v%)	WP (v%)	PAW (v%)	K_f ($cm\ s^{-1}$)
0–15	FO	6.1a	0.99b	61.0a	17.5a	7.0b	2.5a	46.3a	18.7b	24.8a	$6.9 \cdot 10^{-2}$a
	PA	3.6b	1.49a	44.7b	2.6b	8.8a	2.3b	42.2b	23.6a	18.6b	$3.4 \cdot 10^{-4}$b
15–30	FO	1.8a	1.29b	50.8a	9.9a	9.2ab	2.6a	46.4a	22.1a	18.8a	$5.0 \cdot 10^{-3}$a
	PA	1.9a	1.49a	44.5b	4.0b	9.4a	2.1b	40.5b	23.0a	17.5a	$3.9 \cdot 10^{-4}$b
30–45	FO	1.0a	1.48b	44.6a	4.8a	8.1b	2.0a	41.3a	26.0a	13.8a	$3.2 \cdot 10^{-4}$b
	PA	0.9a	1.55a	42.5b	3.2b	9.4a	1.8ab	39.3a	24.2a	15.1a	$5.5 \cdot 10^{-4}$a

[†]SOC, soil organic carbon; BD, bulk density; TP, total porosity; AP, air porosity; dMWD, dry mean weight diameter; wMWD, wet mean weight diameter; FC, field water capacity; WP, wilting point; PAW, plant available water capacity; K_f, saturated hydraulic conductivity. Numbers with the similar letters are not significantly (P < 0.05) different in two land uses in each depths.

In pasture land average dMWD values are significantly higher (8.82–9.41 mm) compared to forest (6.99–9.21 mm) at a depth of 0–45 cm which is mainly attributed to animal trampling. In the 0–15 cm soil layer, long-term pasture had 26% greater mean dMWD than forest.

As in the case of dMWD, grazing effects on wMWD. However, wMWD values in forest soil were significantly lower in pasture (1.80–2.29 mm) then in forest (2.04–2.64 mm) which is mainly attributed to the high SOC content of this soil. In forest soil at a depth of 0–15 cm, mean values of wMWD are 7% higher than in the same depth zone of pasture land. This decrease in wMWD was mostly due to a decrease in the proportion of water-stable aggregates > 3 mm. It appeared that macroaggregates of larger sizes are more sensitive to animal trampling than those of smaller sizes when a virgin soil is brought under long-term pasture.

After 20 yr of grazing, in 0–30 cm layer, the water content at field capacity was significantly lower (8.8–12.7%) in the pasture than in forest. There was no significant difference in FWC values for the 30–45 cm layer.

In 0–15 cm layer, there was an increase of 26.6% in the water content at wilting point in pasture treatment, over that of the forest soil. There was no significant difference in wilting point values for the 15–45 cm layer.

Plant available water was significantly altered by the grazing treatment in the 0–15 cm layer of the pasture land (P < 0,05), but it was not significantly altered at the 15–45 cm. Namely, results show that PAW at 0–15 cm depth significantly decreased (25.0%) due to grazing.

In contrast to our results, Sharrow (2007) found that stored soil water at field capacity tended to be numerically higher on pastures than in forests.

Differences in soil bulk density and porosity were strongly reflected in saturated hydraulic conductivity. After the conversion forest to pasture and long-term grazing, the saturated hydraulic conductivity was significantly reduced. The average value of the saturated hydraulic conductivity in the surface layer (0–15 cm) in the pasture land have decreased by 20 times, in the subsoil (15–30 cm), they became 13 times less. At a depth of 30–45 cm, the average value of the saturated hydraulic conductivity is 1.7 times higher than in the same depth zone of the forest.

Also, Singleton *et al*. (2000) found greater reductions in soil hydraulic conductivity (50–90%) under intensive cattle grazing on wet soils in New Zealand, especially on soils that were

previously damaged. Grazing compaction often results in low saturated hydraulic conductivity (Gifford and Hawkins, 1978; Willat and Pullar, 1983).
Higher soil bulk densities and a lower water content, proportion of stable aggregates, and water permeability, as a result of increased animal trampling, have been observed for different grazing animals in different grassland ecosystems (Daniel *et al*., 2002; Binkley e*t al*., 2003; Ilan *et al*., 2008). Our results were therefore consistent with previous research.

Conclusions

The degradation of the lowland soils by the grazing seriously impaired soil properties and resulted in significant increases bulk density and decreases in the soil organic carbon, aggregate stability, mean weight diameter and the hydraulic conductivity. Decreases of organic matter up to 66% and its crucial effects on soil physical properties well explained the vulnerability of the structure and function of the lowland ecosystems. In conclusion, when natural forest systems are converted for pasture without the use of proper practices of securing organic matter and soil stability, they are easily threatened. Therefore, the measures should be implemented urgently to sustain lowland ecosystems and reinstate the degraded lands in the northwest region of Serbia.

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FOOD SECURITY IN QATAR: THE BLOCKADE OF 2017 AS AN OPPORTUNITY TOWARDS A PRODUCTIVE AND SUSTAINABLE LOCAL FOOD PRODUCTION

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Abstract

Food insecurity concerns are as old as humanity. Food security exists when all population, at all times, has access to sufficient, safe and nutritious food. It is built on four pillars, namely food availability, food access, food utilisation, and stability. While it is widely admitted that food security increases with economic development, also rich countries in the Near East and North Africa (NENA) region, such as Qatar, face specific challenges. Therefore, this review paper analyses the state, determinants and perspectives of food security in Qatar. Since 2007-2008 global food crisis, Qatar's food security has been an ongoing challenge as the growing population is making significant pressure on the government. Based on its fiscal strength, the Qatari government adopted three important strategies: increasing local production, foreign agro-investments and long-term arrangements for food imports. As a result, in 2018, Qatar was ranked first in the Arab world and 22nd globally in the Global Food Security Index. However, denied food supplies from Saudi Arabia following the blockade in June 2017 exposed the high dependence of Qatar on imports, limits of import-based food policies and the need to increase the local production. Since then the Qatari agriculture sector has been under growing pressure to increase the local food production and to realize the highest possible level of self-sufficiency. However, agriculture is limited by several natural conditions, such as scarce water resources and poor soils, and aquifers have been heavily exploited above the average natural recharge. In addition, local food production is limited by some structural factors such as low use of modern agricultural techniques and equipment, and the poor sectoral coordination and integration within the governmental institutions. The paper makes the case for promoting a productive and sustainable agriculture, with high resources use efficiency, to increase food security in Qatar.

Keywords: *food security, food self-sufficiency, blockade of 2017, sustainable agriculture, Qatar.*

Introduction

Achieving a world without hunger and malnutrition is one of the aims of the 2030 Agenda for Sustainable Development (United Nations, 2015). In fact, ensuring access to safe, nutritious and sufficient food for all (Target 2.1) and eliminating all forms of malnutrition (Target 2.2) are prominent targets of the second Sustainable Development Goal (SDG) of the 2030 Agenda (i.e. End hunger, achieve food security and improved nutrition and promote sustainable agriculture). Moreover, the achievement of SDG2 depends on and also contributes to the attainment of many other goals of the 2030 Agenda and sustainable development as a whole (FAO et al., 2018).

Food security concept has evolved and been expanded over recent decades (Du and King, 2018; Committee on World Food Security, 2012; Gross et al., 2000; Ingram, 2011; Lang and Barling, 2012; McMichael, 2014) with a change of focus from increasing food production to improving food access. Indeed, it is increasingly recognised that attaining food security is more complicated than just producing more food, as the fundamental issue concerns access to nutritious and safe food (Dumont and Rosier, 1969; George, 1976; OECD, 2013; Prosekov and Ivanova, 2018; Sen, 1981). The 1996 World Food Summit definition of food security is still widely used (FAO, 1996). It was officially reaffirmed in the 2009 Declaration of the World Summit on Food Security (FAO, 2009a, 2009b), with the addition of social access to food: "*Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life*" (FAO, 2009a). Food security is built on four pillars (Committee on World Food Security, 2012; Ericksen, 2008; FAO et al. 2013; United Nations System High Level Task Force on Global Food Security, 2011): *food availability* (i.e. sufficient quantities of food available on a consistent basis); *food access* (i.e. having sufficient resources to obtain appropriate and nutritious foods); *food use/utilisation* (i.e. appropriate food use based on knowledge of basic nutrition and care); and *stability* in food availability, access and utilization.

The most recent report on the *State of Food Security and Nutrition in the World* (FAO et al., 2018) shows that the number of undernourished people has been growing and was estimated to nearly 821 million in 2017 (17 million more than in 2016), so around one out of every nine people in the world. The situation is worsening in South America and most of Africa. Conflicts and climate change are among the key drivers behind the recent uptick in global hunger. Indeed, climate change threatens to erode and reverse gains made in ending hunger and malnutrition. Moreover, food insecurity contributes to overweight and obesity and the three burdens of malnutrition (undernutrition, overweight/obesity and micronutrient deficiencies) coexist in many countries (FAO et al., 2018).

Food insecurity and malnutrition are still relevant issues in the Near East and North Africa (NENA) region (FAO, 2015). In their discussion paper on "Food Security and Economic Development in the Middle East and North Africa", Breisinger et al. (2010) put that "…*the region's longstanding challenges persist; yet taking immediate action is more urgent in light of the recent, global food, fuel, and financial crisis and projected severe impacts of climate change*" (p. 7). While it is widely admitted that food insecurity decreases with economic development, also rich countries in the region (e.g. Gulf Cooperation Council – GCC – countries i.e. Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates) face specific challenges for achieving long-term, sustainable food security. Therefore, the present review paper analyses the state, determinants and perspectives of food security in Qatar not only in a changing climate but also unstable geopolitical context. Qatar is a small country located in the Persian Gulf covering an area of approximately 11,437 km^2, with a population of 2.7 million. The territory is surrounded by Gulf waters with a sole land border with Saudi Arabia. Qatar has the third largest gas reserves in the world, after Iran and Russia. Enormous hydrocarbons reserves compared with a modest national population, have made Qatar the richest country in the world with GDP per capita at USD 120,000 in purchasing power parity (PPP) terms in 2016 (Qatar National Bank (QNB), 2017).

Food security in Qatar: Limits and drawbacks of import-based food policies

Food security is an important challenge in Qatar (Box 1). Historically, food production in Qatar and in the Gulf Region was limited and based mostly on fishing, Bedouin animal husbandry, date farming and small-scale vegetable production. Since the 1970s, Qatar relies heavily on food imports to sustain its booming population. Due to its robust fiscal position, Qatar, like the other GCC countries, has been less vulnerable to price risk than other food importers (Efron et al., 2018) and able to bridge the shortfall in domestic production (Ismail, 2015a).

However, in the wake of the 2007–2008 global food crisis, food security became an ongoing challenge. Based on its fiscal strength, the Qatari government adopted three important strategies to offset fluctuations in global supply: increasing local production to realize the highest possible level of self-sufficiency through the National Food Security Programme (QNFSP), foreign agro-investments and long-term arrangements for food imports (Al-Ali Mustafa, 2017). In 2008, the government adopted the ambitious QNFSP, with the goal of increasing self-sufficiency from 10 to 70 percent by 2023. A key element of QNFSP is the deployment of advanced technologies by investing in four key areas: renewable energy, desalination and water management; agricultural production, and food processing and management (Ismail, 2015a). However, the financial and environmental costs which are expected to outweigh the cost of importing food, forced the government to abandon the plan and concentrate on foreign agro-investments and long-term arrangements for food imports (Ismail, 2015a; Al-Ali Mustafa, 2017). In 2008, the agricultural arm of Qatar's sovereign wealth fund, Hassad Food, was established in order to contribute to food security in Qatar through foreign agro-investments (McSparren et al., 2017). Since then, Hassad acquired lands in Africa and Australia, among other places (Fuchs, 2012), invest in existing agricultural business and export the produce to Qatar (Salacanin, 2013) so that it can maintain control over the food supply chain. Today, Qatar is one of the biggest regional investors (along with Saudi Arabia and the UAE) in agricultural land abroad (Al-Ali Mustafa, 2017).

Box 1. Research on food security in Qatar.

Most of the existent studies related to food security in Qatar focus merely on some aspects of food security. The first group of literature focused on the environmental aspects of food security in Qatar such as the Qatar's National Food Security Program (QNFSP) and its financial and environmental consequences (Ismail, 2015a); the water-energy-food nexus (Mohtar and Daher, 2015; Ismail, 2015b); or the sustainability of Qatar's self-sufficiency (Al-Ali Mustafa, 2017). The second group of researches focused on the geopolitical aspects of food security such as foreign agro-investment policies (Tétreault et al., 2014); or the effects of the June 2017 blockade on food supply in Qatar (Miniaoui et al., 2018).

In 2018, Qatar was ranked 1st in the Arab world and 22nd globally in the Global Food Security Index (The Economist Intelligence Unit, 2018). Notwithstanding, Qatar still imports more than 90 percent of its food and the future of food security in Qatar is challenged by two factors. Firstly, the impacts of climate change could significantly affect agriculture production through yield reductions (FAO and OECD, 2018). As a result, agricultural commodity prices are expected to remain volatile for the near future, which could result on export restrictions and speculation (World Bank, 2011). Secondly, since nearly all food imports come through the Saudi border and Hormuz Strait, the geopolitical instability of the region, threatens the security of Qatar's food supply (McSparren et al., 2017). Any disruptions to food shipments due to conflict in the Hormuz Strait would have a devastating impact on Qatar's food security (Ismail, 2015a). Furthermore, denied food supplies from Saudi Arabia following the blockade of June 2017 has exposed the high dependence of Qatar on imports.

In June 2017, Saudi Arabia, the UAE, Bahrain, and Egypt cut off diplomatic relations with Qatar and impose an air, sea, and land blockade. Prior to the blockade, Saudi Arabia and UAE accounted for 27.4 percent of Qatar's total value of food products. Meanwhile, about 80% of Qatar's food imports passed through a neighboring country, with 40% coming through the Saudi border and 60% of dairy products imported by Qatar coming from Saudi Arabia and the UAE. In response to the blockade, Qatar has adopted a range of strategies to ensure its food security. Firstly, Qatar arranged alternative trading routes and food supply chain with new partners, most notably Iran, Oman, Turkey and Pakistan (Efron et al., 2018; Miniaoui et al., 2018; Bouoiyour and Selmi, 2019). Qatar also upgraded its ports to prepare for additional shipping with the expanding of the new Hamad port. Inaugurated officially on the 5th of September 2017, the port has reached, within a short period of time, a large global connectivity coverage with 40 ports spanning over three continents (Kumar, 2018). Secondly, the blockade showed the limits of import-based food policies and the need to increase the local production in Qatar; "*One of the positive outcomes of the blockade is that it has galvanised Qatar to exploit its own agricultural production potential*" (Miniaoui et al., 2018:7).

Food production in Qatar: Limiting natural factors and structural challenges

Achieving food self-sufficiency in Qatar is not an easy task. Since Qatar is located in one of the driest regions in the world (Ismail, 2015a), the local food production is limited by several harsh natural conditions, such as scarce water resources, limited arable land and high temperatures (Abu Sukar et al., 2007). Furthermore, most of the water used in irrigation is groundwater, with a low use efficiency. Subsequently, aquifers have been heavily exploited above the average natural recharge, thus increasing water and soil salinity (FAO, 2009). Meanwhile, additional local food production requires an important increase in water and energy resources as well as intensive land use (Mohtar and Daher, 2015).

In addition, local food production is limited by some structural factors. Farming methods in Qatar are outdated and badly matched to local conditions (General Secretariat for Development Planning (GSDP), 2011). Low use of fertilizers and modern agricultural techniques and equipment, and inappropriate mixes of crops and livestock have reduced the efficiency and the productivity of the agricultural sector. Consequently, agricultural yields are generally lower than those of other Gulf States. Furthermore, laws and legislation on agricultural production do not keep pace with the country's food security strategies (GSDP, 2018). Finally, as highlighted by the Second National Development Strategy (2018-2022), the poor sectoral coordination and integration is another major institutional challenge in Qatar. This challenge reflects the lack of clarity in the overall sectoral orientation of some government sectors, such as food security and food production. Poor coordination is due to the lack of "planning culture" and "team work" that require joint action and coordination of efforts (GSDP, 2018).

Achieving food security in Qatar: Innovation in agriculture as a promising solution

Very clearly, since the blockade of 2017, the Qatari agriculture sector is under growing pressure to enhance the local food production to realize the highest possible level of self-sufficiency without depleting the natural resources. In arid and semi-arid countries, such as Qatar, where natural resources are scarce, there is a need for strategies to produce more food with less land and water. There is, hence, a need for a transition towards sustainable food systems that ensure food and nutrition security (Capone et al., 2014; El Bilali et al., 2019). In this context, new technologies and innovative practices such as hydroponics, greenhouses, modern irrigation systems (e.g. drip irrigation), and appropriate crops that would suit the local climatic conditions, can be used to increase the productivity and the sustainability of agriculture systems (Anderson et al., 2016; Sixt and Poppe, 2019); within a positive enabling environment supported by efficient policies (Hussein and El Harizi, 2013). Further, another issue related to agriculture innovation is the adoption and integration of new technologies (cf. innovations) into agricultural production. Low adoption rates of new and potentially beneficial agricultural technologies in many countries continues to contribute to food insecurity and low agricultural productivity (Ngigi, 2003). In order to identify where interventions can be targeted to optimize the functioning of the agriculture sector, it is particularly important to understand and analyze how farmers in Qatar innovate, interact to innovate, adopt new technologies and how they absorb external knowledge. In addition, it is important to analyze and understand the factors that may support or hinder technology adoption and innovation, especially water and land saving technologies. However, data about types of innovations adopted by farmers to improve food production in Qatar is scarce.

Conclusions

Since the blockade of 2017, the Qatari agriculture sector is under growing pressure to enhance the local food production to realize the highest possible level of self-sufficiency without depleting the natural resources. There is, hence, a need for a transition towards sustainable food systems that ensure food and nutrition security. In this context, new technologies and innovative practices that would suit the local climatic conditions can be used, to increase the productivity and the sustainability of agriculture systems. However, the paucity of current research on the dynamics and characteristics of innovation and technologies adoption within the agriculture sector in Qatar and its link to food security and sustainability leaves a major and worrying gap in the knowledge base needed to form effective policies. Further, studies detailing the factors that influence the interactions between the different stakeholders and what does this imply for knowledge production and exchange, and consequently innovation, are lacking. There is a need for research to identify the main obstacles and the factors that influence innovation, technologies adoption, knowledge sharing and cooperation within the agriculture sector in Qatar. Furthermore, the blockade of 2017 and the need to increase the local food production is an opportunity for a systemic and holistic approach of innovation that considers the several aspects of food production, including agricultural production, economic development, environmental sustainability, and institutions, taking into account at the same time all the elements, their interconnections and related effects. A more system-oriented understanding of how innovation occurs is critical to promoting dynamism in agriculture and, ultimately, to enhancing agri-food productivity and sustainability in Qatar.

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GEOGRAPHICAL ORIGIN OF INVASIVE PLANT SPECIES IN THE WORLD AND SERBIA

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Abstract

Invasive species are regarded as one of the biggest biodiversity threat on the planet. Globaly considered and having in mind the organisms of all the taxonomical categories, the highest number of invasive species is of Euro Asian and South American origin. However, analyzing the content of invasive flora in different regions, different species presentation, due to specific geographical origin, can be noticed because of the climate characteristics of each region. Although plant species of Old World are regarded competitively superior in relation to plant species of the New World, percentage predominance of European species is recorded only in the parts of the world with similar climate characteristics, such as the parts of North America. Among invasive plant species in Serbia, the most dominant are those of North American (approx. 67%) and Asian origin (approx. 24%). South American origin has been found in about 9% invasive plant species, European in about 6%, African in about 4%, while 3% of invasive species are hybrids. Those species with native distribution in more than one continent are assigned to each of the continents. Although North American species, on the global level, do not definitely belong to species groups with the biggest invasive potential, they are predominant according to their presentation among invasive species in Serbia. It can be explained by climate similarity of the parts of North America and Serbia, phylogenetic distance of North American and Serbian flora and economic-trade connections of Europe (therefore Serbia, too) with North America.

Keywords: *Invasive plant species*, *Geographical origin*, *Serbia*.

Introduction

Since invasive species present bigger and bigger problem worldwide, it is very important to know the basic characteristic patterns of their distribution. There is an accepted opinion that the Old World plants, especially European, have higher invasive potential than the others, making them dominant among alochton flora of other regions. According to Rapaport (2000), practically all European species introduced in other parts of the world have bigger chances to become adventive in comparison to the species that originate from other regions. Di Castri, in his book from 1989, marked European plants as "highly invasive". However, some of the mentioned examples point out that hypothesis of invasive superiority of European plants can't be applied universaly.

According to Lambdon *et al*. (2008), the highest number of naturalized European species have European origin, i.e. even 53,2% of the total registered naturalized species in Europe are native for some other part of Europe. According to Khuroo *et al*., (2012) more than one third (35%) alochton flora of India are species that originate from Latin America, most of them from Mexico and Brasil. However, when only invasive species are considered (as subset of alochton species), even more than a half (52%) originate from Latin America. Among naturalized and invasive species of China, the most are of American origin (Weber *et al*. 2008; Huang *et al*, 2009; Wu *et al*., 2010), i.e. as in India the most invasive plants originate from the tropic parts of Central and South America. Most plants that are invasive in Brasil have European origin (Zenni, 2014). However, if we regard Europe as donor region separated from Asia, it can be noticed that European species are presented in a small number of species

compared to the species from other continents. According to Castro *et al*. (2005), 323 alien species in Chile have EuroAsian origin, 63 American, 42 species originate from the other parts of the world. The biggest role in flora homogenization of North and South America have the species of European and Asian origin. In North America the most widely distributed species originate from some other continent: approx. 85% have European origin, 7% EuroAsian, 3% Asian (Stohlegren *et al*., 2011).
Geographical analysis of Australian invasive species was the subject of Philips *et al*. (2010), but with a little bit different approach: they examined percentage part of invasive species in the total number of alochtonic species presented in Australia, for each donor region separately. Beside this, they took into consideration residence time that passed from introduction of each species in Australia. This method can lead to more correct conclusions about species invasion (sensu stricto) in some regions in contrast to comparison of the presented invasive species number. It is interesting that Australia has more invasive species from South America than it is expected, and less from Europe and Australia.
The aim of this paper is to determine the regions in the world from which the largest number of invasive species come to Serbia, to show the content of invasive flora in Serbia according to geographical origin as well as to compare the presentation of invasive species of different geographical origin in Serbia to world class data.

Material and Methods

Preliminary list of invasive species in Serbia according to Lazarević *et al*. (2012) was used for the purpose of this paper. The authors of it noted 67 taxons as invasive plants in Serbia dividing them into three groups: highly invasive, sporadically invasive and potentially invasive. The data of these species origin are taken from the site Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC, 2018) and Invasive Alien Species in Vojvodina (IASV, 2018).

Results and Discussion

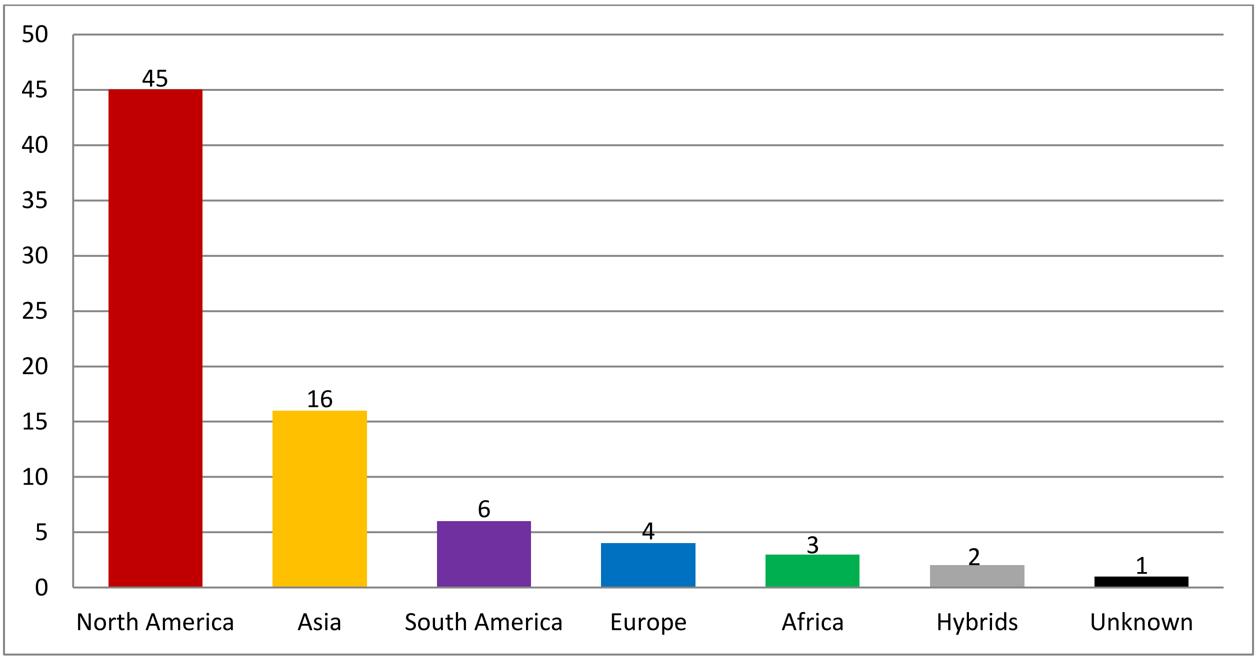
Analysis results of the geographical origin of invasive plant species in Serbia mostly depend on the choice of the list that is used. In other words, different sources note different species as invasive making the term "invasive" not used universally. Foreign sites dealing with this subject are not usable in this case since they don't have complete data when Serbia is in question. So, for example the site Global Invasive Species Database (GISD) for Serbia notes only three invasive species: *Impatiens glandulifera*, *Luzula campestris* and *Salsola tragus*[8], while Delivering Alien Invasive Species Inventories for Europe (DAISIE) list of invasive plant species does not exist in Serbia. According to Turbelin *et al*. (2017) there are 18 types of all taxon category organisms in Serbia making that number not real having in mind that only Pannonian part of Serbia has even 152 species of alien invasive neophytes (Anačkov *et al*., 2013).
The list of invasive taxons (according to Lazarević *et al*., 2012) with their geographical origin (according to CABI ISC, 2018 and IASV, 2018) is given in Table 1.

[8] Neither domestic author state *Luzula campestris* as invasive, but according to the site Euro + Med Plant Base, it is regarded as native in Serbia. According to the same site, *Salsola tragus* is not noted for Serbia, but is native for some surrounding countries.

Table 1. Invasive plant taxons in Serbia (according to Lazarević *et al.*, 2012) and their geographical origin (according to the data from the site CABI ISC 2018 and IASV 2018)

INVASIVE SPECIES (TAXON)	GEOGRAPHICAL ORIGIN							INVASIVE SPECIES (TAXON)	GEOGRAPHICAL ORIGIN						
	N. America	Asia	S. America	Europe	Africa	Hybrid	Unknown		N. America	Asia	S. America	Europe	Africa	Hybrid	Unknown
Acer negundo	x							*Impatiens noli-tangere*	x	x		x			
Ailanthus altissima		x						*Impatiens parviflora*		x					
Amaranthus retroflexus	x							*Iva xanthifolia*	x						
Ambrosia artemisiifolia	x		x					*Juncus tenuis*	x						
Amorpha fruticosa	x							*Lycium barbarum*		x					
Armoracia rusticana				x				*Matricaria discoidea*		x					
Artemisia verlotiorum		x						*Oenothera biennis*	x						
Asclepias syriaca	x							*Oenothera depressa*	x						
Aster × versicolor						x		*Oxalis stricta*	x						
Azolla filiculoides	x		x					*Parthenocissus quinquefolia*	x						
Bidens frondosa	x							*Paspalum distichum*	x		x				
Broussonetia papyrifera		x						*Phytolacca americana*	x						
Bryonia dioica					x			*Prunus padus*		x		x			
Celtis occidentalis	x							*Prunus serotina*	x						
Cenchrus spinifex	x							*Reynoutria japonica*		x					
Cuscuta campestris	x							*Reynoutria sachalinensis*		x					
Cyperus strigosus	x							*Reynoutria x bohemica*						x	
Datura stramonium							x	*Robinia pseudacacia*	x						
Echinochloa crus-galli		x						*Rudbeckia laciniata*	x						
Echinocystis lobata	x							*Solanum elaeagnifolium*	x						
Eleusine indica		x			x			*Solidago canadensis*	x						
Elodea canadensis	x							*Solidago gigantea*	x						
Elodea nuttallii	x							*Sorghum halepense*		x		x	x		
Erigeron annuus	x							*Symphyotrichum lanceolatum*	x						
Erigeron canadensis	x							*Symphyotrichum novi-belgii*	x						
Erigeron sumatrensis			x					*Symphyotrichum salignum*	x						
Euphorbia maculata	x							*Symphyotrichum tradescantii*	x						
Fraxinus americana	x							*Ulmus pumila*		x					
Fraxinus pennsylvanica	x							*Veronica persica*		x					
Galinsoga parviflora	x							*Vitis riparia*	x						
Galinsoga quadriradiata	x							*Xanthium orientale ssp. italicum*	x		x				
Helianthus decapetalus	x							*Xanthium spinosum*			x				
Helianthus tuberosus	x							*Xanthium strumarium*	x						
Impatiens glandulifera		x													

Among invasive plant species in Serbia the most numerous are those of North America origin, i.e. native range for even 45 to 67 taxons is North America (approx. 67%), while 4 among them are native both for North and South America. The second place belongs to taxons where Asia is their native range (16), but some of them are regarded as native both for Europe and Asia. South America is considered as native range for 6 taxons, while those that are "unique" for South America (alochton for North America) are only 2. Among alien species that are invasive in Serbia are 4 species that are native for some other part of Europe. Mostly these species, beside Europe, are regarded native for Asia, or both for Asia and Africa. For entirely three species native range is Africa, while only 1 has African origin exclusively, i.e. is regarded alochton in Europe and Asia. Two species are hybrids, while for one, primary areal has not been definitely determined yet (Graphic 1).



Graphic 1. Geographical origin of invasive plants in Serbia (those with native distribution in more than one continent were assigned to each of the continents)

North American species on the global level definitely do not belong to the group of species with the highest invasive potential, i.e. the largest number of invasive species originate from South America and EuroAsia. However, North American invasive plants are predominant in Serbia, which means that Serbia does not suit to global pattern. The reason for this can be found in climate similarity between Serbia and some regions in North America (Fig. 1).

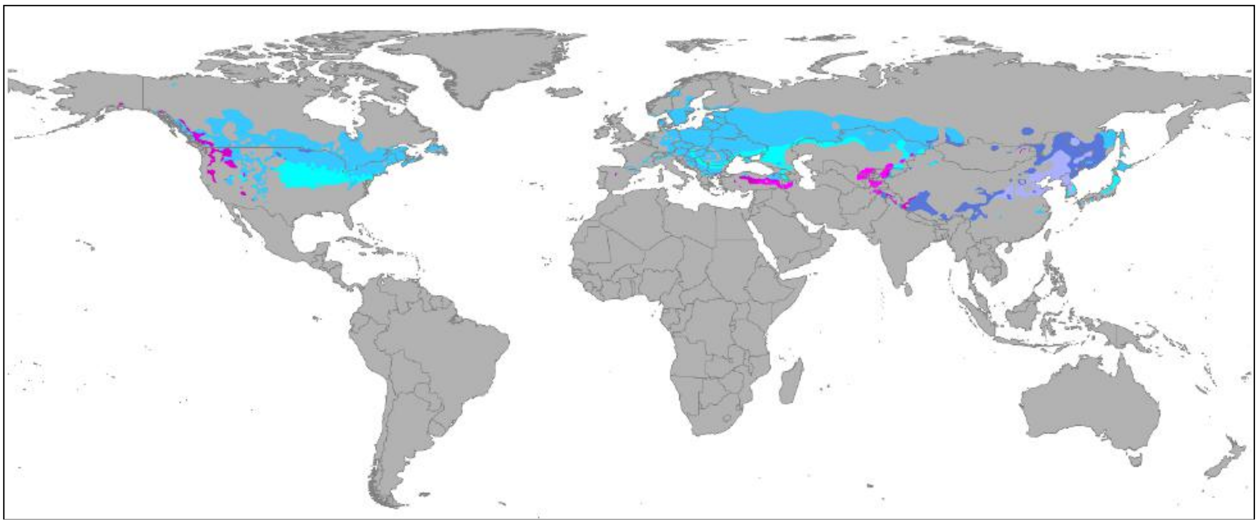


Fig. 1 The map that presents regions with different varieties of moderate continental climate in the world, according to Koppen-Geiger climate classification (according to Peel *et al*., 2007)

Although the regions with similar type of climate exist in Asia, they are not enough far from the territory of Serbia, so they have a large number of the same native species. Also, North American flora is for sure phylogenetically less related to the flora in Serbia, compared to flora of some parts in Asia with similar climate, and according to Strauss (2006) "exotic taxa that are less related to native ones are more invasive than the others". Beside all these, high level of alien species introduction from North America into Europe (being the consequence of these two continent economic-trade connections), directly or indirectly into Serbia too, is very important fact so when we take it into consideration, it is clearly obvious why North American species are so numerous and considered invasive in Serbia.

Climatic mis-match can be considered as the basic reason why South American species are less presented in Serbia in spite of phylogenetic distance of these flora, meaning that climate is the key factor on which geographical origin of invasive plants in Serbia depends. African species are obviously less presented than South American because besides climatic mis-matching there is a fact that African and European flora is phylogenetically closer to each other.

Conclusions

In Serbia invasive species that are mostly presented are those from North America continent and the reasons for that are as follow:

- Climate similarity of North America and Serbia parts;
- Phylogenetic distance of North America and Serbia flora;
- Economic-trade connections of Europe (therefore Serbia too) with North America.

Biogeographical patterns of invasive species distribution are very complex and under the influence of many factors. What is important on global level, need not be important on regional when climate characteristics of each regions are of great importance. It is not possible to mark some species as those with universaly high invasiveness, for what is invasive for one climate, need not be invasive in the other. So, when we talk about the invasion of some plant (or group of plants) it is necessary to have in mind geographical, i.e. climate characteristics related to that invasion. Also, the attention must be paid on the fact that each case of invasion is unique and depends on species characteristics, as well as the environmental ones. All this indicates the importance of risk assessment during plant species introduction in new regions and dangers of making wrong decisions if made on stereotype conclusions. Because of this, as well as the fact how invasive species are dangerous for biodiversity, examinations of all the aspects of invasive species (biogeographical too) will be of great importance in future.

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DIVERSITY AND SPECIFICITY OF BIOTA IN TUNISIAN ARTIFICIAL RESERVOIRS IN A MANAGEMENT CONTEXT

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Abstract

Due to the scarcity of water and for efficient use, many reservoirs, hillside dams and hillside lakes have been built mobilizing more than 90% of surface waters. These man made environment have biological potential that deserves to be better well known, monitored, exploited and managed. This review attempts to collect information on some dam reservoirs biodiversity in Tunisia from bibliographic studies for sustainable management. Investigations conducted in some Tunisian reservoirs revealed the presence of 185 phytoplanktonic species. Cyanobacteria branch was the most diversified with 52 species, some of which are potentially toxic and can be considered as a nuisance for water usages like drinking water, irrigation, fish farming or recreation. The Autochtonous tunisian freshwater fish biodiversity is low and limited to five families: Anguillidae, Cyprinidae Cyprinidontidae, Gobidae and Pœciliidae. Autochthonous marine fish species (Mullets) as well as freshwater fish species native from Eastern Europe and Western Asia have been introduced in few reservoirs for the diversification of livestock and the promotion of inland fishing. The avifauna of reservoirs in Tunisia is quite diversified. Some have a special status and are considered vulnerable to endangered species. Most of the reservoirs in Tunisia are poor in both submerged and emerged macrophytes which may affect diversity and abundance of water birds. The environmental and ecological conditions in these reservoirs seem to favor the growth of species belonging to different biological compartments but resource management is not sufficiently efficient.

Keywords: *Reservoirs, Biodiversity, Management, Tunisia.*

Introduction

Tunisia is one of the countries particularly vulnerable to climate change, which will intensify the variability and aridity that now affect the largest part of the country. The average rainfall over the entire territory is only 230 mm / year but the extreme annual averages vary from more than 1500 mm in the northwest of the country to less than 50 mm in the southwest (Henia and Benzarti, 2005). The characteristics of the water resource (scarcity, irregularity) in addition to population growth and especially the urban population and the evolution of the economic sectors that consume large amounts of water and the country's low adaptability are all factors that contribute to the fragility of this resource in the face of climate change and basic management that needs to be reconsidered. In this context, many dams have been built last decades in Tunisia mainly in the wettest area, particularly in the north and centre of the country mobilizing more than 90% of surface waters (ITES, 2014). Based on water quality, these reservoirs were used for many purposes: consumption, irrigation, fish farming, groundwater recharge, downstream infrastructure protection and energy production, allowing rural populations to settle and benefit from employment opportunities.
In Tunisian inland waters, inventories of fauna and flora species and trophic networks linking species to each other and their environment are still poorly documented, thus hindering the

management of ecosystems as a whole. Thereby, several studies on the eco-biology and exploitation of freshwater fish have been undertaken in particular for commercially valuable fish (Djemali et al., 2009; Hajlaoui et al., 2016; Mili et al., 2016; Laouar and Djemali, 2018) however, only a few studies have focused on the other communities such as macrophytes, invertebrates and avifauna thus limiting the further exploitation of data. This review attempts to gather information on some dam reservoirs biodiversity in Tunisia from bibliographic studies for sustainable management that would be compatible with water availability and quality.

Material and Methods

This study is based on bibliographic data on the biological potential of dam reservoirs in Tunisia located in different watersheds in an attempt to improve their management. These data, collected over the period 1983-2019, yielded useful information on various fauna and flora communities.

Results and discussion

Water surface management

Tunisia, a semi-arid country, remains very vulnerable to water resources where the exploitable volumes of surface water mobilized are about 2500Mm3/year**.** Water potential is highly dependent on rainfall which is rare and irregular. In addition to extended periods of drought, most wet precipitation occurs during short periods of heavy rainfall, which causes strong runoff with excessive erosion of soils, part of which will be carried to dam reservoirs (Saadaoui, 1995). Silting of reservoirs is causing a reduction in their storage capacity that remains a major problem despite the redevelopment and maintenance works (Abid, 2003; Ben Mammou and Louati, 2007). Thus, for improved management of water storage in reservoirs, it is necessary (i) to limit runoff through cultural practices and vegetation cover. However, a collective reflection must be carried out and implemented at the scale of the catchment area. (ii)to consider the surpluses of rainy years, which are not yet under control, to minimize losses and ensure better water mobilization and consequently better availability and exploitation.

Phytoplankton communities

In aquatic ecosystems, phytoplankton is the first link in the food chain. Its development conditions that of the primary consumers which regulate in turn the development of secondary consumers. Thus, because of its position in aquatic food webs and its high sensitivity to changes in environmental conditions, phytoplankton is a real tool designed for decision support and management of evolving water bodies. Several studies have shown that phytoplankton from Tunisian inland waters is highly diversified where more than 187 taxa belonging to 7 classes have been identified (Ben Rejeb Jenhani et al., 2019). Cyanobacteria branch was the most diversified with 52 species, some of which are potentially toxic and can be considered as a nuisance for water usages like drinking water, irrigation, fish farming or recreation with many health, ecological and economic consequences (figure 1). Cyanobacterial blooms have been observed in summer-autumn period following the proliferation of one or a few species under favorable hydroclimatic conditions (stability of the water column, high temperature, drought) and in particular nutrient enrichment from watersheds (Ben Rejeb Jenhani et al., 2006; El Herry et al., 2008; Fathalli et al. 2010; Fathalli et al.2015). Thus, to preserve water quality for multiple uses, it is necessary, through coherent management actions, to adopt a strategy to prevent eutrophication by upstream monitoring of nutrients, by managing water flows through regular desilting and cleaning actions and by screening toxic species through spatial and temporal monitoring. The strengthening of fundamental scientific knowledge on phytoplankton communities will make it possible to better elucidate the determinism of the appearance of toxic blooms.

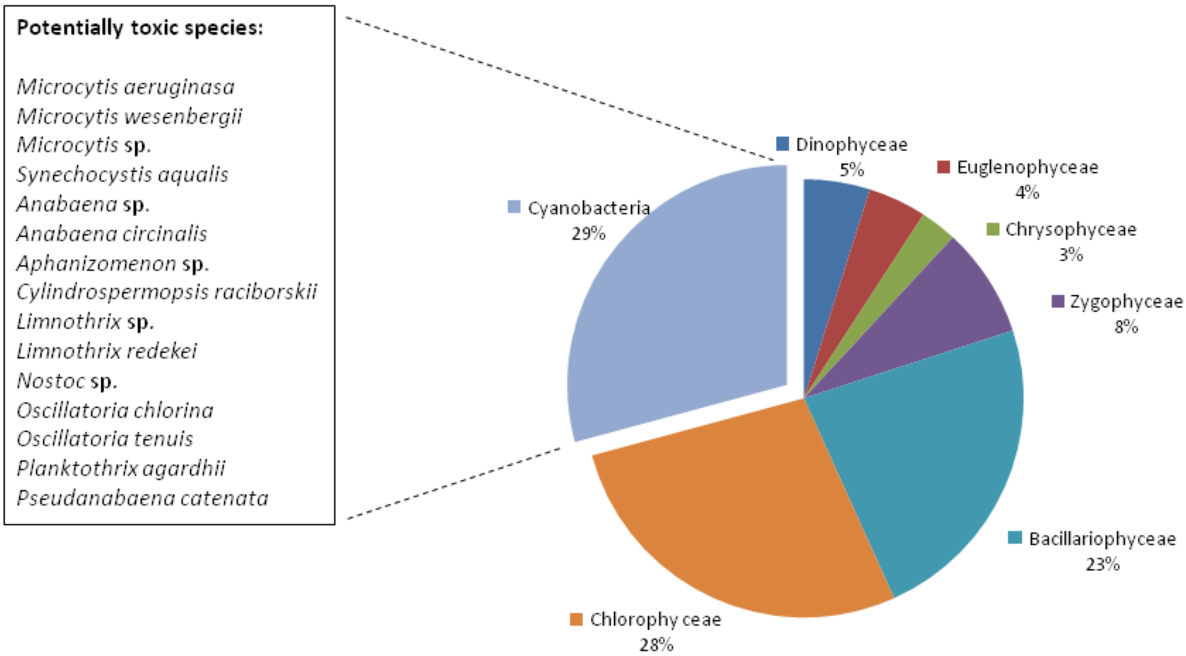

Figure 1: Rate of phytoplankton classes and potentially toxic cyanobacteria identified in Tunisian reservoirs

Fish communities

Tunisian fresh waters are poor in fish (Kraim, 1983). Several attempts have been made to introduce in reservoirs various species of Asian, European and African origin in order to diversify the livestock population, as forage or commercial fish. Indigenous and introduced fish stocks belong mainly to 8 families: Mugilidae, Percidae, Pœciliidae, Siluridae, Cyprinidae, Cyprinodontidae, Gobidae and Anguilladae**, (**table 1). Only about thirty of the more than forty dams are affected by fishing activity, which is based on an active fleet of 250 non-motorized boats and a population of about 500 fishermen (DGPA, 2016). The total production in these reservoirs over the last decade is between 870 and 1350 t/year. This production comes mainly from the Sidi Salem (51.5%), Sidi Saad (19.5%) and Sidi Barrak (7.5%) reservoirs. The distribution of production by species shows the predominance of mullet (34%), carp (28%) and pike-perch (17%,) (DGPA 2016, Laouar, 2019). This production is related to the stocking efforts of mullet fry and the transfer of spawners, which have sometimes been found to be deficient, causing a decrease or stagnation in production, in addition to illegal fishing activity that has not yet been controlled (Hajlaoui et al., 2017). Moreover, the fisheries statistics that are the foundation of any long-term planning strategy remain unreliable.

Table 1. Autochtonous and introduced fish in Tunisian reservoirs

Family	**Species**	**Status**	**Diet**
Anguilladae	*Anguilla anguilla*	Autochtonous	Omnivorous
Mugilidae	*Mugil cephalus*	Autochtonous	Omnivorous
	Liza ramada	Autochtonous	Omnivorous
Percidae	*Sander lucioperca*	Introduced	Carnivorous
Siluridae	*Silurus glanis*	Introduced	Carnivorous
	Barbus callensis	Autochtonous	Omnivorous
Cyprinidae	*Cyprinus carpio*	Introduced	Omnivorous

	Scardinius erythrophthalmus	Introduced	Omnivorous
	Rutilus rubilio	Introduced	Omnivorous
	Pseudophoxinus chaignon	Autochtonous	Omnivorous
Pœciliidae	*Gambusia offinis*	Introduced	Insect larvae and small invertebrates
Cyprinodontidae	*Aphanius fasciatus*	Autochtonous	Omnivorous
Gobidae	*Pomatoschistus marmoratus*	Autochtonous	Omnivorous

Water Birds communities

Furthermore, dams, by creating artificial water reservoirs, are rather beneficial for birds, especially migrants who find new or compensatory resting and breeding areas. The avifauna of reservoirs in Tunisia is quite diversified and is spread over several families. Nine of reservoirs in Tunisia are, due especially to their ornithological richness, included in the Tunisian list of the international Ramsar agreement (www.ramsar.org) and five as Important Bird Area (www.birdlife.org). Lebna reservoir is the most visited with more than thirty species represented by several tens of thousands of individuals (FDR 2007). Some have a special status and are considered vulnerable to endangered species: *Platalea leucorodia*, *Marmaronetta angustirostris*, *Aythya nyroca* et *Oxyura leucocephala* (table2). The last two species, recorded in Lebna, exceeded the 1% threshold of their biogeographic population in Tunisia during the 2009 census (Feltrup-Azafzaf and Azafzaf, 2009). Birds censuses are generally variable according to years and are largely dependent on climatic, anthropogenic and environmental hazards. Most of the reservoirs in Tunisia are poor in both submerged and emerged macrophytes which are represented by reed stands (Thyphaceae, Poaceae) on the banks and farther, in the deeper zone, we find *Potamogeton* (Potamogetonaceae) and *Myriophyllum* (Haloragaceae). Filamentous algae such as *Chaetomorpha* (Cladophoraceae) are also reported (Loss et al. 1991). Riparian and aquatic vegetation, which is essential for water-birds, is often subject to variations in water levels due to different uses (irrigation, hydroelectric production, drinking water requirements) and to drought thus disrupting the presence of birds, adds illegal hunting, poaching, stocking and eutrophication. Despite annual bird censuses, management plans remain deficient in Tunisia. National plans, in collaboration with international species conservation actions, should be implemented for the protection of threatened species and habitats.

Table 2: Avifauna inventory in Tunisian reservoirs

Order	Family	Specific diversity	Species status (UICN, 2012)
Gaviiformes	Gaviidae	1	
Podicipediformes	Podicipedidae	3	
Procellariiformes	Diomedeidae	1	
Suliformes	Phalacrocoracidae	1	
Ciconiiformes	Ardeidae, Ciconiidae	6	
Pelecaniformes	Threskiornithidae	2	*Platalea leucorodia* (NT)
Phoenicopteriforme	Phoenicopteridae	1	
Anseriformes	Anatidae	16	*Marmaronetta angustirostris* (VU) *Aythya nyroca (EN)* *Oxyura leucocephala (EN)*
Gruiformes	Rallidae, Gruidae	5	

Charadriiformes	Haematopodidae, Recurvirostridae, Burhinidae, Charadriidae, Scolopacidae, Laridae, Phalaropodinae, Sternidae	43	
Coraciiformes	Alcedinidae	1	

(NT): Near Threatened; (VU): Vulnerable; (EN): Endangered

Conclusion

Tunisia is a semi-arid country with a very high vulnerability to climatic changes leading to a high environmental vulnerability of aquatic ecosystems especially in periods of prolonged drought or heavy floods. These unpredictable phenomena favour the siltation of reservoirs and the installation of favorable conditions for harmful algal blooms reaching different levels of aquatic food webs. For the preservation of available water resources, an improvement in water storage conditions is required (desilting during floods, optimization of cultural practices, reforestation, watershed management, protection perimeters). In addition, the development of tools for forecasting and managing the response of aquatic ecosystems to climate variability (monitoring indicators, mathematical models, etc.) will preserve biodiversity, habitats and environment.

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RESOURCE ASSESSMENT OF ADONIS VERNALIS IN REPRESENTATIVE NATURAL LOCALITIES IN WESTERN BULGARIA

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Abstract

Genus *Adonis* comprises 32 species distributed in Europe and Asia. *Adonis vernalis* commonly called Pheasant's eye is a valuable medicinal plant characterized by high content of cardiac glycosides. Due to its toxicity, nowadays it is not among the most popular medicinal plants but is used frequently as homoeopathic remedy. *A. vernalis* grows on open limestone habitats with steppe character. Its distribution in Bulgaria is scattered in the northern and western parts of the country. It is an important part of the natural habitats of European importance included in the network Natura 2000: 40A0 Subcontinental Peri-Pannonic Scrub and 6210 Semi-natural dry grasslands (Festuco-Brometalia). Resource assessment was performed in natural localities situated in three floristic regions: Sredna gora, Znepole region and Sofia region. Floristic composition is rich and is typical for the calcareous sites. The predominant species are *Dichanthium ischaemum, Satureja montana, Amygdalus nana, Rosa spinosissima, Veronica austriaca, Syringa vulgaris, Pulsatilla montana, Inula oculus-christi*, but also some rare and endemic species occur: *Anacamptis pyramidalis, Ophrys cornuta, Gymnadenia conopsea, Anthylis aurea, Edraianthus serbicus*. The projective cover of *A. vernalis* is 15 – 20% and the population status of the species is stable. The resources were assessed and the estimated yield was 18 g/m^2. Continuous monitoring on the population status will provide valuable information for its sustainable use and conservation.

Key words: *sustainable use, medicinal plants, conservation*

Introduction

Conservation and sustainable use of the natural plant resources is a complex task, requiring coordination of numerous efforts and activities. This issue encompasses broad range of studies and inventories of the available plant resources, evaluation of their population status, possible threats and finally – development of strategies and plans for conservation and management for particular plant species in particular regions (Heywood & Iriondo, 2003). Monitoring and inventory of the natural resources are an integral part of each conservation and management plans. The issue is of particular importance also for the resources of medicinal and aromatic plants (MAPs), which are subjected to exploitation (Carvalho and Frazão-Moreira, 2011).

Bulgaria is among the richest countries in medicinal plant resources in Europe (Evstatieva et al., 2007). Almost ¼ of the natural plant species occurring in the country are being used, or can be used, as MAPs. There are 745 species listed in the annexes of the Medicinal Plant Act of Bulgaria (Anonymous, 2000) and this number reflects the great plant diversity in the country, which is due at least to the very diverse environmental conditions, resulting in a great diversity of habitats. And because different habitats are expected to be influenced differently by the environmental, including climate changes, the monitoring on plant resources should focus primarily to the most sensitive and threatened plant species and habitats. One of the species selected for monitoring and inventory of its natural resources in Bulgaria is *Adonis vernalis* L. (Figure 1).

Depending on the taxonomic concept applied, genus *Adonis* contains between 20 and 30 (32) species, distributed in Europe and Asia (Tutin 1964; Shang et al., 2019). There are about 10 species naturally occurring in Europe (Euro+Med Plantbase, 2019) and six species – in Bulgaria (Panov & Assenov, 1970). Some of the species are characterized by high content of cardiac glycosides and are used in the folk medicine (Shan et al., 2019). Of perennial species, *A. vernalis* is the most popular one in Europe, even though nowadays it is not among the most popular medicinal plants, due to its toxicity. It is used, however, as a homoeopathic remedy.

Figure 1: *Adonis vernalis* in its natural locality

A. vernalis grows on open limestone habitats with steppe character. Its distribution in Bulgaria is scattered in the northern and western parts of the country (Panov & Assenov, 1970). Even though it is not subjected to exploitation, its populations could be threatened by the human pressure – construction works, livestock breeding and other agricultural activities.

In the light of the above statement the objective of the present study was to perform an inventory of representative localities of the species in Bulgaria. The information could be of use for the need of monitoring of species' resources and the effect of environmental changes.

Material and methods

Three natural localities were included in the pilot study. They were selected to represent typical populations of the target species (*Adonis vernalis*) and also, to reflect the available diversity of site conditions. Two of the experimental plots are in the region of Sofia, and one – in the region of Pernik in Bulgaria. The field studies were performed in April and May, 2019. The geographic coordinates of experimental plots are:

1. Lozenska Mts – near the village Lozen, 42°35' 52" N; 23°29' 12" E, altitude 760 m a.s.l. (called hereafter L-site)
2. Golo Bardo – near the village Studena, 42°31'27" N; 23°05'49" E, altitude 1000 m a.s.l. (called hereafter G-site)
3. Beledie Khan – near the village of the same name, 42°54'44"N; 23°09'54"E, altitude 900 m a.s.l. (called hereafter B-site).

Experimental plots (50 x 20 m) were set in the estimated central part of the populations of *Adonis vernalis*. In each plot, number of individuals of the target species were scored, and plant species composition was inventoried. The projective cover was estimated based on smaller experimental plots (1 x 2 m). The species composition was based on one visit to the experimental plots – in April, during the flowering period of the target species. Some species were added based on other visits to the sites. Doubtless, the species composition could be (and will be) updated during the next inventories of the experimental plots. In the present paper we report only the results of the first-year observation.

The phytogeographic affiliation of the species followed these of Walter (1985) with slight modifications.

Resource evaluation was performed following Shrëter & al. (1986), with slight modifications.

Results and discussion

Total of 84 species were identified in the three experimental plots (Table 1). This species diversity can hardly be evaluated as high, given the peculiarities of the sites where the populations grow, but as stated above, the inventories were done mostly in the spring, when *A. vernalis* occurs and represent mostly the spring aspect of the flora. It is expected that the floristic inventory and analysis will be enlarged and deepened. Twenty-eight species were recorded in the locality in Lozenska Mts (L), 45 species in Golo Bardo (G) and 57 species in Beledie Khan (B) localities. There were 18 trees and shrubs, including small shrubs like *Satureja montana* and *Hyssopus officinalis*. However, the tree species were represented by single individuals and small groups scattered across the area and did not represent forest community, where *A. vernalis* could not have occurred.

Table 1. List of the species recorded in the three study sites.

Species	Study sites		
	Lozenska Mts (L)	Golo Bardo (G)	Beledie khan (B)
Trees and shrubs			
Acer campestre L.	+	+	+
Amygdalus nana L.			+
Chamaecytisus hirsutus (L.) Link.			+
Corothamnus procumbens (Waldst. & Kit.) C. Presl		+	
Crataegus monogyna Jacq.	+	+	+
Euonymus europaeus L.			+
Euonymus verrucosus Scop.			+
Fraxinus ornus L.	+	+	+
Hyssopus officinalis L.			+
Prunus fruticosa Pall.			+
Prunus spinosa L.			+
Quercus pubescens Willd.		+	+
Rosa canina L.	+		
Rosa pimpinelifolia L.			+
Satureja montana L.		+	+

Syringa vulgaris L.			+
Ulmus minor Mill.			+
Viburnum lantana L.		+	+
Herbs			
Achillea ageratifolia (Sm.) Boiss.		+	
Achillea serbica (Nyman) Petrovič		+	
Adonis vernalis L.	+	+	+
Agrimonia eupatoria L.	+		
Ajuga chamaepitys Schreb.		+	
Anacamptis pyramidalis (L.) Rich.	+	+	+
Anemone sylvestris L.			+
Anthoxanthum odoratum L.	+		+
Anthyllis aurea Welden ex Host.		+	
Anthyllis montana L.		+	+
Artemisia alba Turra		+	+
Asphodeline liburnica (Scop.) Rchb.		+	
Berteroa incana (L.) DC	+		
Bromus squarrosus L.	+	+	+
Carex caryophyllea Latourr.	+	+	+
Cephalaria transsylvanica (L.) Roem. & Schult.	+		+
Cichorium intybus L.	+		
Cirsium ligulare Boiss.	+		
Comandra elegans (Rochel ex Rchb.) Rchb. f.			+
Cynodon dactylon (L.) Pers.	+		
Dactylis glomerata L.	+		
Dichanthium ischaemum (L.) Roberty	+	+	+
Dipsacus fullonum L.	+		
Draba lasiocarpa Rochel		+	
Echium vulgare L.	+		
Edraianthus serbicus (Kern.) Petrovic		+	
Elymus repens (L.) Gould.	+		
Festuca valesiaca Schleich. ex Gaudin	+	+	+
Filipendula vulgaris Moench.	+	+	+
Fumana procumbens (Dunal) Gren. & Godr.		+	+
Globularia aphyllanthes Crantz		+	
Gymnadenia conopsea (L.) R.Br.	+	+	+
Gypsophylla glomerata Pall. ex M. Bieb.		+	
Helleborus odorus Waldst. et Kit.	+	+	+
Hesperis tristis L.			+
Hyacinthella leucophaea (Steven ex Kunth.) Schur		+	
Hypericum perforatum L.	+		+
Hippocrepis comosa L.			+
Inula oculus-christi L.		+	+
Iris reichenbachii Heuff.		+	+
Lamium bifidum Cyr.			+
Linum austriacum L.			+
Melica ciliata L.			+
Mercurialis ovata Sternb. & Hoppe			+

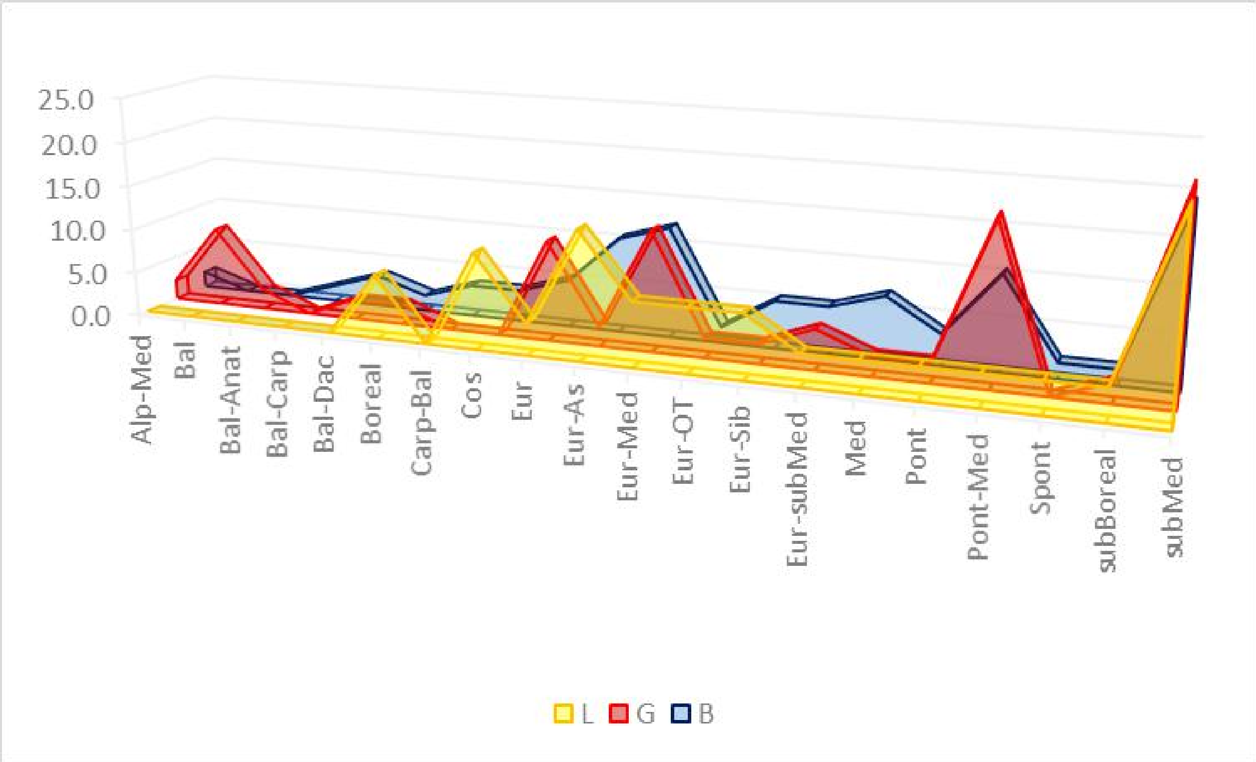
Muscari tenuiflorum Tausch			+
Odontites serotina (Lam.) Dumort.	+	+	+
Onosma visianii Clementi		+	
Ophrys cornuta Steven		+	
Ornithogalum pyrenaicum L.			+
Papaver dubium L.			+
Plantago subulata L.		+	+
Poa pratensis L.	+		+
Potentilla cinerea Chaix ex Vill.		+	
Pulsatilla montana (Hoppe) Reichenb.		+	
Sesleria rigida Heuffel ex Rchb.			+
Stippa capillata L.		+	+
Teucrium chamaedrys L.	+	+	+
Teucrium polium L.		+	+
Thymus callieri Borbás ex Velen.			+
Thymus pannonicus All.		+	+
Tragopogon balcanicum Velen.			+
Trigonella gladiata Steven ex M. Bieb.		+	
Trinia glauca (L.) Dumort.		+	+
Veronica austriaca L.			+
Vinca herbacea Waldst. et Kit.		+	+
Viola riviniana Rchb.		+	

Besides the fact that locality in Lozenska Mts is poorest in species composition, it seems most affected by the human pressure, which is easily explained by the close proximity of the village of Lozen and a bit further – of Sofia. The human pressure is reflected in the species composition, including some cosmopolitan and ruderal species, like *Cynodon dactylon*, *Poa pratensis Cirsium ligulare* (Table 1). The other two localities are richer in rare species, including few endemics and sub-endemics, among them *Achillea ageratifolia*, *A. serbica*, *Anthyllis aurea*, *Edraianthus serbicus*, *Tragopogon balcanicum*. Sørensen-Dice affinity coefficients were lower (0.38 and 042) between L, and G and B, respectively, while between G and B it was 0.55. This could be explained again by the anthropogenic influence on the L site.

The phytogeographic analysis (Figure 2) showed that the most common were the submediterranean phytogeographic elements - they predominated in all experimental plots. Second position in the sites G and B was occupied by the Pontic-Mediterranean elements, but not in the site L, where the Euro-Asiatic elements were also well represented.

Generally, the species with Mediterranean component (Med, subMed, Eur-Med etc.) were the most represented phytogeographic elements – 39 % in L, 62 % in G, and 56 % in B, respectively. Elements with Pontic component were the second most numerous and constituted 11 %, 22 %, and 15 % in the experimental plots L, G, and B, respectively. These patterns of phytogeographic origin fit completely to the peculiarities of the plant communities (Velchev, 1962).

Figure 2. Phytogeographic origin of the species recorded in the three study sites. L – Lozenska Mts, G – Golo Bardo, B – Beledie khan



The projective plant cover in the three localities varies from 70 to 90 %, and that of *A. vernalis* in all localities ranges roughly from 15 to 20 %.

The natural habitat where the experimental plots were set is 40A0 Subcontinental peri-Pannonic scrub (Gussev, 2009; Tzonev & Gussev, 2015). Moreover, *A. vernalis* is one of the species characterizing the habitat type. At least half of the characteristic species listed by Tzonev & Gussev (2015) was recorded during the field survey.

A. vernalis occurs in Bulgaria as a part of some other habitats, too; however, in one of them it has more visible and substantial presence: 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*). No experimental plots were set in this habitat, but it was registered during the process of preliminary inventory of the species locality in Bulgaria (Tzonev, 2009). *A. vernalis* was found also in some other habitats, exclusively on calcareous soils, but with sporadic occurrence, and therefore, of little interest for monitoring and resource investigations.

The resource assessment revealed that the highest stock – 25 g/m^2 – was recorded in the site B, while in the other two sites the results were practically the same – 14 and 15 g/m^2 in the sites L and G, respectively.

Conclusion

The results of the study provide information, which could be useful in relation to future monitoring and assessment. It will allow following the trend of development of populations and habitats and will provide important insight regarding their conservation, management and sustainable use.

Acknowledgment

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CLIMATE CHANGE ADAPTATION OPTIONS IN LATVIA'S AGRICULTURE

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Abstract

Environmental impacts of agriculture under a changing climate are considered more and more important. To avoid or at least reduce the negative effects and exploit possible positive effects of climate change, adaptation strategies and measures should be developed and implemented. Adapting agriculture to climate change has linkages with other policy areas, including water management, economic policies, climate change mitigation policies, as well as infrastructure, health, and other sectors and policies that foster sustainable development and increase resilience of communities as a whole. There are various widely accepted and evidence based effective climate change adaptation options proposed by scholars and experts for agriculture sector. Most important of them, for example, are the following: agricultural input efficiency; improving (i.e., less energy-intensive) farming; reducing N surplus and N use efficiency via fertilizer usage; crop and soil management strategies' change, such as tillage practices, permanent crops; crop rotations and use of cover crops or catch crops, organic farming, green manuring; as well as reducing and/or completely removing agrochemicals; carbon sequestration and improve soil physical, chemical and biological properties. Furthermore, implementation of agro-ecological and conservation agriculture principles that affect biodiversity conservation (agrobiodiversity; i.e., crop genetic diversity) and supports many ecosystem services. Adaptation measures include technological advancements, adaptive farming practices, etc. The paper presents results of current situation's evaluation, analysing above-mentioned measures implementation status, as well as provides proposals for implementation of most promising adaptation measures in Latvia's agriculture. Moreover, necessary policy decisions and public financial support (i.e., RDP 2014-2020, RDP post 2020) are indicated.

Keywords: adaptation, climate change, agriculture, Latvia.

Introduction

The impacts of climate change become increasingly evident for the agricultural sector, as well as rural areas in general (Olesen et al., 2011; Shelton et al., 2018). From other side recent (intensive) agriculture is an important contributor to climate change. To avoid or at least reduce the negative effects of climate change, adaptation strategies and measures should be developed and implemented. Both mitigation and adaptation are aimed to reduce undesirable consequences; and can jointly contribute to the climate change strategies. There is a growing argument that synergistic approaches to adaptation and mitigation could bring substantial benefits at multiple scales in the land use sector (Duguma et al., 2014), which rises a growing interest by scholars and practitioners of agriculture and landscape management for it (Locatelli et al., 2015).

Lal et al. (2011) stress that being a source and a cause of the problem, agricultural ecosystems can be a sink of atmospheric CO_2 and reduce GHG through the adoption of sustainable land management options. An ecosystem-based approaches are recognised as most promising, which address the crucial links between climate change, biodiversity and sustainable resource management; and, by preserving and enhancing ecosystems, enable society to better mitigate and adapt to climate change. Besides, some mitigation/ adaptation options, which are typically best management practices already, raise resilience of farms, and lower farm emissions,

simultaneously also contributing to cost savings and thus increasing farm profitability (Frelih-Larsen & Dooley, 2015).
The aim of presented study was twofold: 1) to evaluate current situation, analysing implementation of mitigation/ adaptation measures to climate change; 2) to propose of most promising adaptation measures for Latvia's agriculture and landscape.

Material and Methods

The principal materials used in the studies are as follows: various sources of literature, e.g., scholars' articles, the reports of institutions (esp. EU), etc. The data were obtained from Eurostat database (http://ec.europa.eu/eurostat/data/database) and database of Central Statistical Bureau – CSB (http://www.csb.gov.lv/en/dati/).
Despite the Baltic Sea region countries are also non-EU countries, only EU member states—Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, and Sweden—are included for state and trends' evaluation; and are indicated as the Baltic Sea countries.
The mixed methods, combining suitable qualitative (monographic; analysis and synthesis, etc.) and quantitative (correlation-regression analysis) research methods have been used.

Results and Discussion

Intensification of agricultural production has caused several undesirable consequences: an increase of monocultures, application of agrochemicals (i.e. pesticides, mineral fertilizers); reduction the biodiversity (i.e., agricultural, wild and soil) (Tsiafouli et al., 2015; Melece & Shena, 2018). These processes contribute to further climate change.
Nitrogen management – is aimed to reduce and optimise usage of N fertiliser. Indicator—gross nitrogen balance (GNB)—balance between N added to agricultural land and that removed from it. GNB provides: an indication of the potential surplus of N; trends of N inputs and outputs; and characteristics of soil N resources (EEA, 2018; Eurostat, 2018).
Between 2000-2003 and 2012-2015, the GNB in most of the EU countries, as well as the Baltic Sea countries showed a decrease. Conversely, two of the Baltic Sea countries – Latvia and Poland show an increase (Fig. 1), although the average of allowed N dose still remain below.

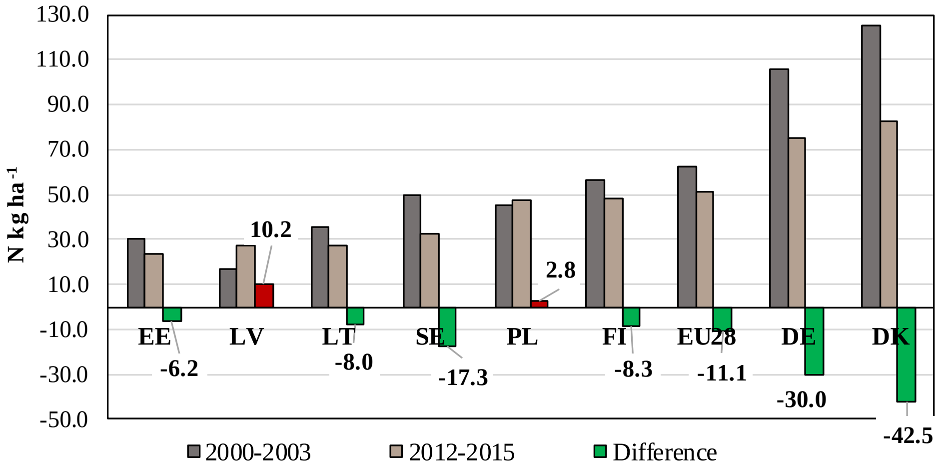


Figure 1. GNB and it changes in the Baltic Sea countries, 2000-2003 and 2012-2015
Source: authors' elaboration based on EEA, 2018

A surplus of N indicates potential climate change through: emissions of GHG (N_2O) and ammonia; and N leaching, which increase soil degradation process. High N inputs tend to decrease biodiversity (i.e., soil) within and outside the farmed area (Tsiafouli et al., 2015; D'Acunto et al., 2018; EEA, 2018).

Another important indicator—nitrogen use efficiency (NUE)—total N outputs (as yield) divided by total N inputs provides an indication of utilisation of N (EU Nitrogen Expert…, 2015; Eurostat, 2018). It may be useful to evaluate different strategies' contribution towards utilisation of N.

The highest value of NUE does not mean the best result. Rates that may be close to or above 1.0 would indicate a risk of soil depletion, as the N uptake by crops exceeds the amount of N applied to the soil, and cannot be considered sustainable (EU Nitrogen Expert…, 2015). NUE changes among the EU countries between periods 2000-2004 and 2010-2014 show that only in four EU countries, including Latvia, NUE decreases (Fig. 2).

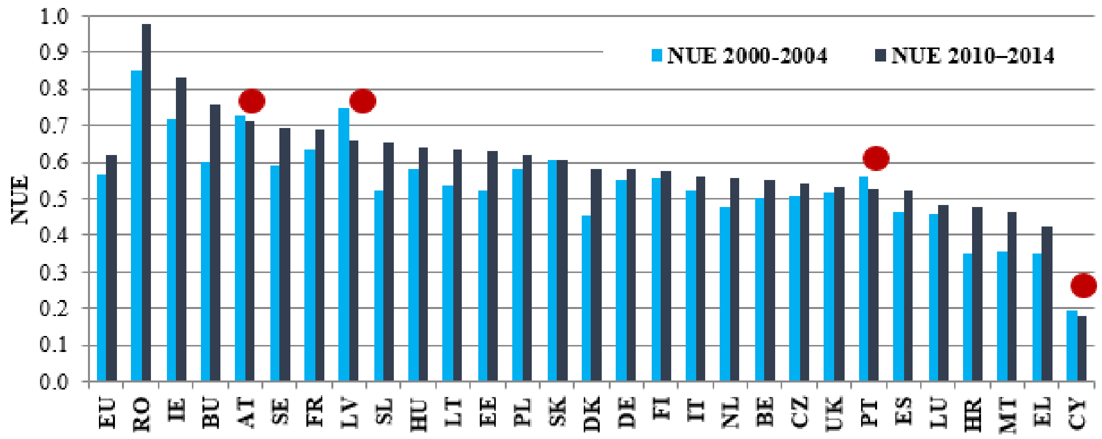
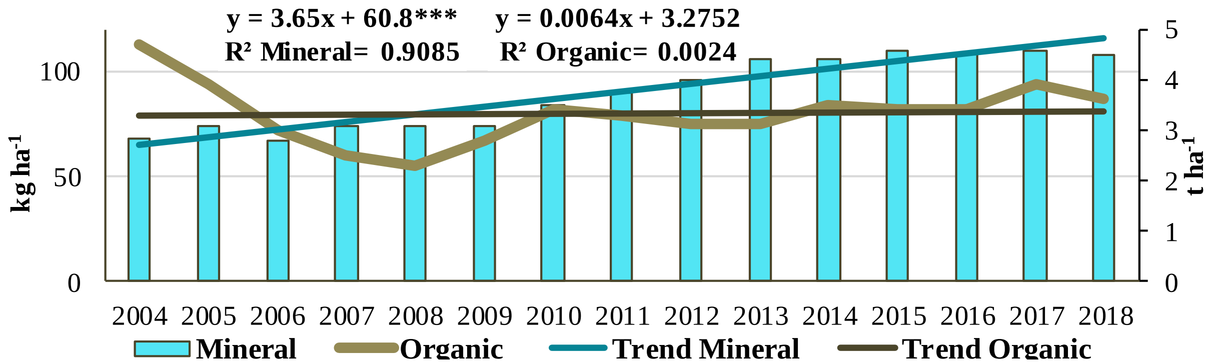


Figure 2. Nitrogen use efficiency and it change in the EU countries, 2000-2004 and 2010-2014

Source: authors' elaboration based on Eurostat, 2018

A range of preferable NUE is between 50% and 90% (0.5-0.9), where lower values increase potential N losses, but the higher values risk mining of soil N stocks or reserves (EU Nitrogen Expert…, 2015). NUE allows assessing agricultural production systems in terms of resource use efficiency, productivity, and environmental impact at the same time (Brentrup & Lammel, 2016).

In Latvia negative impact of agricultural intensification (i.e., monoculture, specialization of farming—crop farming, crop rotation disregard, decrease of crop diversity, etc.) causes significant increases of mineral N fertilizer usage (Fig. 3).

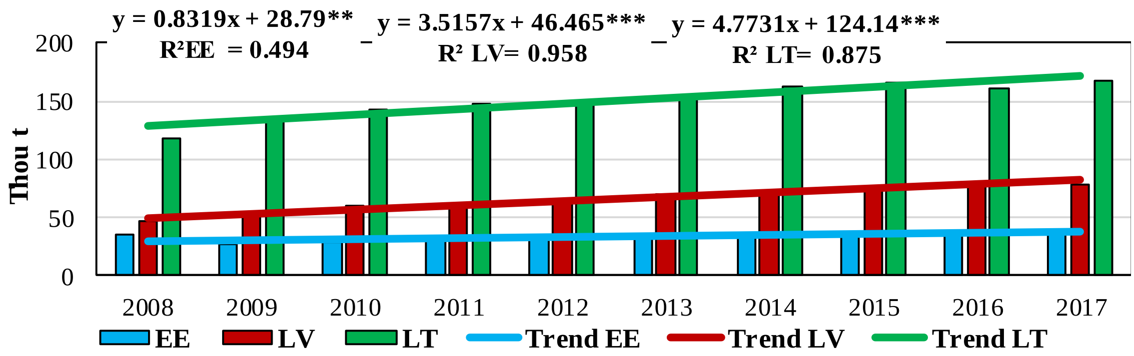


*** - α<0.01

Figure 3. Usage of mineral nitrogen fertilizer (kg) and organic fertilizer (t) per ha of sown area and it trends in Latvia, 2004-2018

Source: authors' calculation based on CSB data

Notwithstanding in all Baltic States the significant increase of mineral N fertilizer's consumption is detected, a more obvious trend is observed in Latvia (Fig. 4).



** - α<0.05; *** - α<0.01

Figure 4. Consumption of mineral N fertilizer and it trends in the Baltic States, 2008-2017
Source: authors' calculation based on Eurostat data

As result of agriculture intensification the diversity of crops decreased; and noticeably increased the production of most marketable crops, i.e., used as energy crops. Wheat and rape are exported, but green maize is used for biogas production. Moreover, growing tendency toward expansion of commercial monocultures is observed (Table 1).

Table 1. The changes of various crops' area from 2005 to 2017

Crop	Sown area, thou ha		Changes 2017/2005, %	
	2005	2017		
Winter wheat	129.1	331.2	**157%**	↑
Rye	45.1	34.0	-25%	↓
Spring wheat	55.4	140.4	**153%**	↑
Winter triticale	13.3	7.0	-47%	↓
Spring barley	145.9	78.2	-46%	↓
Oat	58.0	70.9	22%	↑
Buckwheat	10.4	30.9	197%	↑
Mixed cereals and pulses	8.1	5.3	-35%	↓
Field beans	0.4	42.5	**10525%**	↑
Winter rape	22.9	90.0	**293%**	↑
Spring rape	48.5	27.3	-44%	↓
Fodder roots	3.8	0.2	-95%	↓
Potatoes	45.1	22.7	-50%	↓
Open field vegetables	12.9	8.0	-38%	↓
Forage crops	372.2	297.6	-20%	↓
Perennial grass	360.6	270.3	-25%	↓
Crops for green feed & silage	8.7	1.6	-82%	↓
Maize for silage & green feed	2.9	25.7	**786%**	↑
Green manure crops	3.7	0.6	-84%	↓

Source: authors' elaboration based on CSB data

Dramatic increase of field beans' cultivation in Latvia, was encouraged by the introduction of green payments of the CAP. A similar tendency is observed in other EU countries. Implementation of such kind 'greening' measures has been criticised by scholars and experts, i.e., on EU level (ECA, 2017), because restriction or ban to use pesticides in greening areas lacking. Besides, it is recognised on EU level that Latvia's implementation choices for the CAP 2014-2020 involved political and historical factors, and was influenced by significant lobbying from the farmers' side (Ecorys, 2016). In particular, large farm owners.

Analysing recommended measures, we conclude that main part of them are noted as mandatory or voluntary requirements in RDP. Due to limited space, most suitable for Latvia adaptation measures to climate change on farm level are reported as a summary (Table 2).

Table 2. Farm level adaptation measures to climate change

Adaptation option/measure	Reference
Biodiversity (crops, soil, etc.)	Tittonell, 2014; Kazakova-Mateva & Radeva-Decheva, 2015; Tsiafouli et al., 2015; Hart et al., 2016; EEA, 2019; IPCC, 2019
Permanent grassland & permaculture	Ferguson & Lovell, 2014; Tittonell, 2014; Kazakova-Mateva & Radeva-Decheva, 2015; Hart et al., 2016; IPCC, 2019
Crop rotation, i.e., extend perennial phase	Niggli et al., 2009; Kazakova-Mateva & Radeva-Decheva, 2015; Hart et al., 2016; EEA, 2019; IPCC, 2019
Crop diversification, i.e., intercropping	Niggli et al., 2009; Kazakova-Mateva & Radeva-Decheva, 2015; Hart et al., 2016; Hart et al., 2017; EEA, 2019; IPCC, 2019
Use cover crops; mulching	Niggli et al., 2009; Kazakova-Mateva & Radeva-Decheva, 2015; Hart et al., 2016; EEA, 2019; IPCC, 2019
Catch crops & green manure	Frelih-Larsen & Dooley, 2015; Hart et al., 2016
Mixed farm strategies, i.e., agricultural diversification	Niggli et al., 2009; Tittonell, 2014; Kazakova-Mateva & Radeva-Decheva, 2015; Richards et al., 2015; Hart et al., 2016; EEA, 2019; IPCC, 2019
Nitrogen management (reduce & optimise use of N fertilisers, improve N use efficiency)	Niggli et al., 2009; Olesen et al., 2011; Frelih-Larsen & Dooley, 2015; Kazakova-Mateva & Radeva-Decheva, 2015; Hart et al., 2016; Hart et al., 2017; Blandford & Hassapoyannes, 2018; Delzeit et al., 2018; EEA, 2019; IPCC, 2019
Fertile soil maintenance, i.e., keep & increase quality	Niggli et al., 2009; Olesen et al., 2011; Richards et al., 2015; Hart et al., 2016; Blandford & Hassapoyannes, 2018; EEA, 2019; IPCC, 2019
Conservation tillage (reduced or no tillage)	Niggli et al., 2009; Olesen et al., 2011; Frelih-Larsen & Dooley, 2015; Blandford & Hassapoyannes, 2018; Hart et al., 2016; EEA, 2019; IPCC, 2019
Crop residues management	Niggli et al., 2009; Frelih-Larsen & Dooley, 2015; Blandford & Hassapoyannes, 2018; IPCC, 2019
Agroecology, i.e., organic agriculture	Niggli et al., 2009; Ferguson & Lovell, 2014; Tittonell, 2014; Kazakova-Mateva & Radeva-Decheva, 2015; Richards et al., 2015; Hart et al., 2016; EEA, 2019; IPCC, 2019

Conclusions

CAP policy and RDP measures regarding climate change and environment issues have various support to farmers to implement measures oriented to mitigate and adapt to climate change, as well as requirements such as Good Agriculture Practice, which should be fulfilled to receive payments. Nevertheless, proper nitrogen management, prevention of biodiversity decline, and soil and land degradation, etc., and related farming practices are not implemented or are implemented unsuccessfully. Since CAP (i.e., financial support) should contribute to soil, climate and biodiversity targets, it is necessary to develop more precise rules, requirements and procedures to receive green and agri-environment payments.

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CHEMICAL COMPOSITION OF FLOODPLAIN SOILS OF THE LOWER DNIESTER RIVER

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Abstract

The chemical composition of the floodplain soils are caused by erosion-accumulative activity of the river and the nature of the basin soil cover. As a result of biogenic accumulation in the uppermost horizon of the soil, in addition to organic matter, a number of chemical elements are concentrated. The Dniester floodplain accounts for more than 50 thousand hectares. With a relatively low content of organic matter, the Dniester floodplain soils differ in significant humus reserves, which is associated with a large capacity of humiferous horizons in both modern and buried soils. In the 0-10 cm soil layer, the humus content is about 2.0%; in the 20-40 cm - 3.0%, with a gradual decrease to 0.5% at a depth of 185 cm. The soils of the Dniester floodplain are carbonate. The carbonate content (up to 7.5%) is inversely related to the amount of clay particles (<0.001 mm). The largest amount of SiO_2 is 56-81%. Alluvial silty-loamy riverbed soil contains 62.6-81.4% of SiO_2 in the layer with the highest silt content. Despite the light soil composition, the total P_2O_5 content is relatively high - up to 0.23%. The distribution of Na_2O is more uniform in soil - 0.8%. The soils are a high content of CaO (4-6%) and MgO (2.0-2.4%). Compared to the soils of the terraces, the floodplain soils contain a significant large number of nutrients - K_2O and P_2O_5.

Keywords: *Chemical composition, Dniester River, Floodplain soils, Moldova.*

Introduction

Lower Dniester is a protected area of the Republic of Moldova, a wetland of international importance. The total area is 60.000 ha (Kuharuk & Leah, 2017). In the floodplain of the Dniester river the whole range of alluvial soils is found, and in the valleys - diluvial soils. There are more than 60 of soil varieties registered in the floodplain of Dniester River (Sulin, 1970). The alluvial (meadow) soil is a representative one in the soil cover of the area. Flood meadow soils are new formations of alluvial soil. The alluvial processes include on the one hand the erosion of the riverbed, which causes the periodic "rejuvenation" of the riverbed and the adjacent sectors of the meadow, and on the other hand accumulations on the meadow surface the solid drainage of the river. In the Dniester meadow occurs the periodic flooding of its meadow with water from the river; in such cases the soils and the entire layer of alluvial deposits are considerably moistened. An important feature of alluvial soils is their high biogenesis, conditioned by the acceleration of the circuit of substances and the process of soil genesis (Leah, 2014; 2018b). The chemical composition of floodplain soils is determined not only by the processes of the floodplain soil formation, but to a large extent by the erosion-accumulative activity of the river and the nature of soil cover of its basin (Zakharova, et al, 2018). As a result of biogenic accumulation, in the uppermost soil horizon, in addition to organic matter, a number of chemical elements are concentrated, most of which are necessary for plant nutrition (Zakharova, et al, 2018). But this topsoil is primarily eroded. In Moldova, up to 50 tons of soil are washed off from 1 ha of cultivated slope lands in some cases, with which a large amount of humus and other substances is lost annually. These substances in the composition of solid and liquid runoff of the river are carried to the Black Sea (Kuharuk & Leah, 2017). Some of them accumulated in the sediments of the bases of slopes, beams and ravines, small river floodplains. Settling in completely different conditions, they become the arena of a new process of soil formation. Floodplain soils in Moldova occupy about 250 thousand hectares, which is 8.5% of the entire territory of the republic. Along with the researches on the zonal (principals) soils of Moldova, research is also being carried out on the floodplain soils (Leah, 2018a; 2018c; Leah, et al., 2018). However, many questions are not fully disclosed, since the soil of the floodplains is a very heavy object for research. In particular, little attention was paid to studying the chemical composition of these soils. The conditions of soil formation throughout the floodplain vary within the widest limits. Soils of all stages of hydromorphism are widespread here: from primitive underwater soils to steppe - meadow and forest - meadow soils, which are represented by different varieties of mechanical composition. Features of the mechanical composition are determined by the conditions of sediment deposition in the main geomorphological areas of the floodplain (Leah, et al., 2018). The lighter ones are confined to the riverbed area, the heavier ones are associated with the central area and terraces. In this regard, the soils developing on these sediments are differentiated by mechanical composition from the riverbed to the root bank and from the head of river to the mouth of the river.

The research purpose - to study chemical composition of the floodplain soils for their use in sustainable agriculture.

Material and methods

Recently, studies have been conducted on the chemical composition of the Lower Dniester river floodplain soils. A fully developed floodplain consists of three geomorphological parts or areas; the near riverbed, the central or plain, and the near-terrace (flat-lowered). In the near-river area of the floodplain, the most active is the alluvial process and minimally - floodplain process. Alluvial light soddy layered, often roughly stratified, soils arise here. In this area the alluvial light soddy stratified soils are formed, often coarsely - stratified soils. The area - is 13.3 thousand ha. Profile 1 (P1): *Alluvial-soddy, stratified, silty-clayey soil.*

The central area of the floodplain is characterized by the calm passage of flood waters. They attract mainly fine fractions of fine material - dusty and silty particles. Here alluvial-soddy granular and soddy granular gleyed soils are formed, which are most favorable in their properties for cultivating meadow grasses, planting vegetables and other crops. Their area is 22 thousand hectares. Profile 2 (P2): *Alluvial granular, clayey soil.*

In the near-flat lowland (near-terrace) area of the floodplain, clayey and colloidal particles gradually settle from the slowly flowing waters, flooding with a powerful layer the entire lowered territory of this part of the terrace. Under such conditions, the most severe medium- and heavily gleyed soils are formed. Their area is about 13.5 hectares. Profile 3 (P3). *Alluvial meadow, clayey - loamy, gleyed soil.*

Soil samples were taken from the most characteristic profile layers to the level of groundwater. Soil research occupies about 80% of the entire floodplain area and is characterized these three genetic areas: near-riverbed, central, and near- terrace.

Chemical composition includes determining of the hygroscopic water content, loss of calcinations, and the content of elements that make up the mineral part of the soil: Si, Al, Fe, Ca, Mg, Na, K, P, S, Ti, Mn. The sum of the oxides of these elements is usually more than 99% of the mineral part of the soil. In the carbonatic soils, in addition, the content of CO_2 carbonates is determined. Total composition of analyzed elements is determined by the classical method.

Results and discussions

The soils of the Dniester floodplain are low-humus. However, with a relatively low content of organic matter, they differ in its significant reserves, which is associated with the large thickness of the humus horizons of both modern and buried soils. The distribution and content of humus along the profile is uneven and is primarily due to the influence of energy material of different mechanical composition deposited by the river during the flood.

The smallest reserves of organic matter are characterized by the soil of the near-riverbed (P1), where they are formed on a light, low-humus riverbed alluvium. In the 0-15 cm layer, the humus content is about 2.0%, in the 20-40 cm layer it reaches 3.0% with a gradual decrease to 0.5% at a depth of 185 cm. Even lower the amount of humus increases again and at about a depth of 300 cm is 1.8%. Such a distribution reflects only the lithological (granulometric) stratifying of the soil profile and is not related to the modern process of soil formation.

The mechanical composition of the soil (texture, granulometric composition of soil) is the content in the soil of elementary particles of different sizes. This is one of the leading environmental (regulatory and limiting) factors on which other soil properties also depend - moisture capacity, porosity, thermal stability, and consequently, the soil fertility.

So, in the layer of 0-10 cm the content of particles <0.001 mm is 16% in P1, 32% - P2 and 48% - P3. In a layer of 90-100 cm - 14%, 37%, 58%, below the mechanical composition is gradually lightened, and then heavier again. In the Profile 1 the stratification is clearly highlighted; in the P2 - the composition indices are evenly distributed on the whole depth; and in the P3 - a slightly uniform distribution up to the depth of 150 cm, from the 160 cm stratification is evidently (Figure 1, 2, 3).

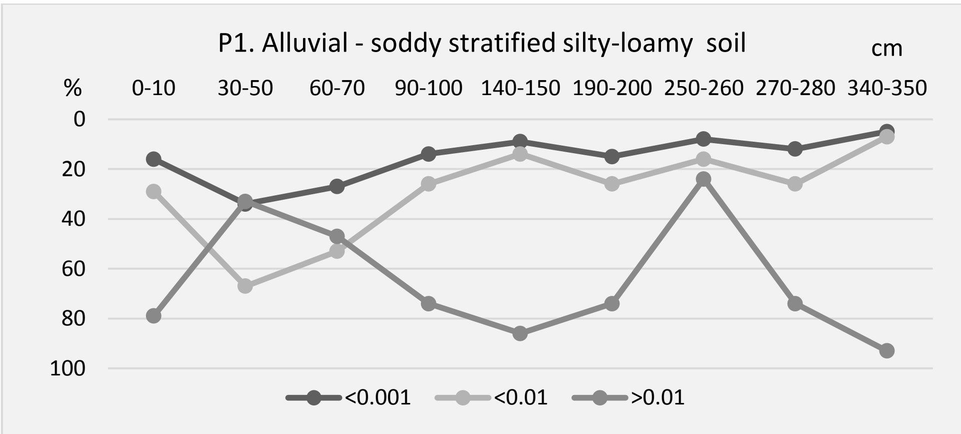

Fig.1. The fraction composition (<0.001, <0.01, >0.01) of Profile 1.

The soil from central floodplain in terms of mechanical composition is heavier and more homogeneous. The humus content is higher, and its distribution along the profile is more even. A layer of 0-10 cm contains 4.4% of humus; in a layer of 10-60 cm - 3.5%, below its amount, as the mechanical composition is facilitated, decreases in heavier layers.

The studied soils of the Dniester floodplain are carbonated. The carbonate content is inversely related to the amount of silt particles (<0.001), as well as the soils are mechanically layered so that their distribution along the profile is uneven. This is especially noticeable in stratified soils (P1), in the profile of which several maxima of carbonates are observed. In light layered soils of the near-terrace, the distribution of $CaCO_2$ is more uniform and content is highest.

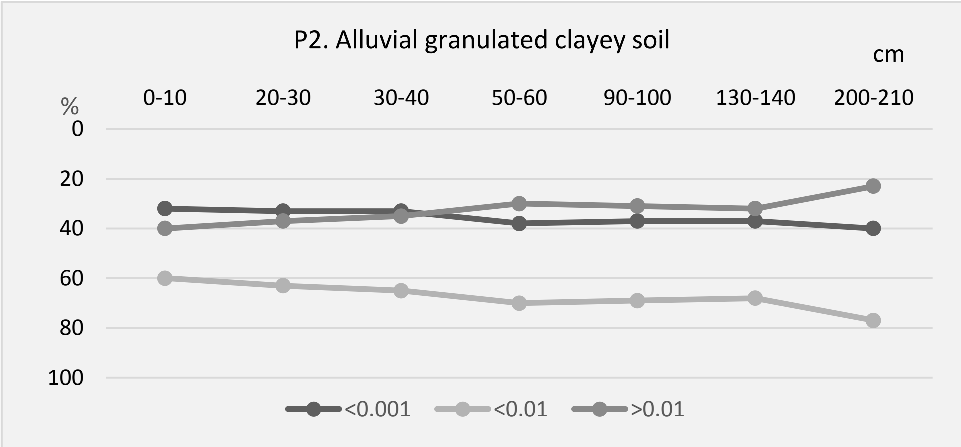

Fig.2. The fraction composition (<0.001, <0.01, >0.01) of Profile 2.

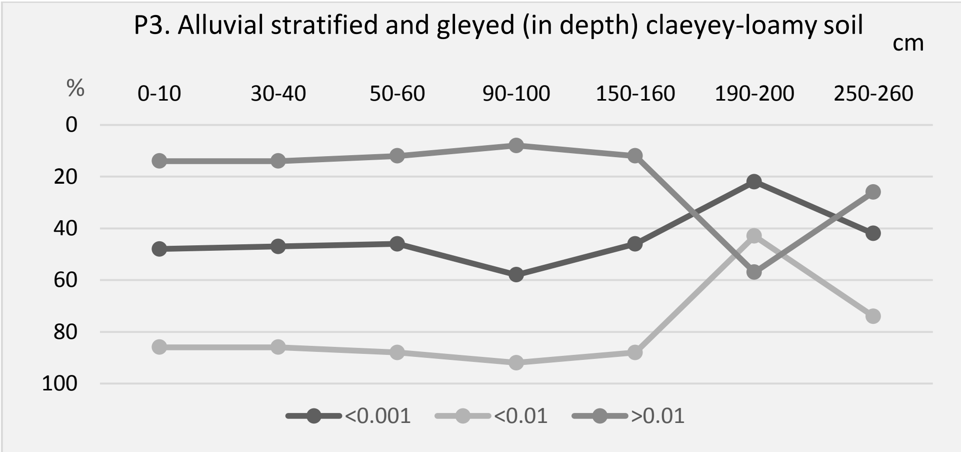

Fig.3. The fraction composition (<0.001, <0.01, >0.01) of Profile 3.

In soils with a homogeneous mechanical composition, according to the content of individual elements of the aluminum-silicate part, can be traced the presence or absence of mineral destruction processes and the profile movement of destruction products. In contrast its, the granulometric heterogeneity of the soil profile of floodplain soils makes it difficult to identify changes in their chemical composition that have occurred as a result of soil formation, and makes it possible only to ascertain the presence of an element in individual layers of the profile. The content of most elements in soil and alluvial deposits depends mainly on the mechanical composition. In this regard, their distribution in the soils of floodplain areas, as well as in the profile of individual soils, is uneven.

The greatest amount of SiO_2 is observed in the soils of the riverbed (P1). So, for example, in layers of 250-260 cm, where particles <0.001 mm contain 8%, the amount of silicon reaches 76%. In the layer of 140-150 cm, where silt particles account for 9%, its content drops to 78%. The near-terrace soil (P3) contains less SiO_2 with a minimum of 56.2% in the layer with the highest silt content - 46%. The soils of the riverbed also have a high content of calcium (4.1-5.6%) and magnesium (1.3-2.1%). The distribution of these elements along the all profile varies relatively little - 3 - 6% (CaO) and 1.2 - 2.1% of (MgO). Compared to the soils of the central and riverbed area the near-terrace soil (p3) is characterized by a lower content of elements - MgO, CaO, and highest content of Al_2O_3, Fe_2O_3, K_2O, P_2O_5, TiO_2 (Table 1).

Table 1. Chemical composition of the alluvial soils

Depth, cm	LAC*	SiO_2	Al_2O_3	Fe_2O_3	MnO	Na_2O	K_2O	CaO	MgO	P_2O_5	SO_3	TiO_2
Riverbed floodplain. Alluvial - soddy stratified silty-loamy soil, Profile 1.												
0-10	7.24	73.01	8.69	2.65	0.08	0.90	1.43	4.31	6.96	0.16	0.18	0.48
30-50	10.03	62.65	13.28	4.75	0.13	0.84	2.15	4.57	1.31	0.23	0.08	0.43
60-70	8.01	68.71	11.75	4.45	0.12	0.83	1.63	4.14	1.48	0.19	0.08	0.60
90-100	7.20	74.63	7.94	2.39	0.08	0.87	1.31	5.35	1.73	0.12	0.18	0.40
140-150	6.08	78.27	6.01	1.71	0.05	0.83	1.09	4.97	1.83	0.10	0.23	0.40
190-200	7.08	72.29	8.00	2.65	0.08	0.88	1.32	5.57	1.83	0.12	0.18	0.52
250-260	6.46	76.51	6.38	1.97	0.05	0.83	1.12	4.77	2.09	0.10	0.18	0.41
270-280	6.36	75.73	6.90	2.23	0.06	0.83	1.59	5.40	1.79	0.12	0.19	0.40
340-350	5.16	81.41	4.95	1.45	0.04	0.88	0.60	4.69	1.96	0.09	0.08	0.34
Central floodplain. Alluvial granulated clayey soil, Profile 2.												
0-10	9.04	64.21	12.70	4.12	0.14	0.72	1.76	5.38	2.34	0.21	0.30	0.55
20-30	3.38	64.52	12.21	3.99	0.15	0.58	1.46	5.24	1.52	0.19	0.07	0.64
30-40	9.13	62.60	14.13	3.91	0.13	0.63	1.59	5.38	1.23	0.19	0.09	0.64
50-60	9.61	60.51	13.97	4.79	0.13	0.54	1.56	5.21	1.35	0.30	0.13	0.76
90-100	10.10	62.49	14.13	3.99	0.13	0.72	1.57	4.94	1.39	0.24	0.21	0.49
130-140	9.60	63.10	15.10	5.24	0.21	0.49	1.63	3.20	1.73	0.30	0.21	0.64
200-210	8.08	62.36	14.28	4.99	0.12	0.51	1.62	3.92	1.36	0.32	0.08	0.65
Terrace floodplain. Alluvial stratified and gleyed (in depth) clayey-loamy soil, Profile 3.												
0-10	10.92	59.64	15.70	5.84	0.09	0.59	2.97	2.90	1.32	0.27	0.32	0.63
30-40	11.19	59.28	15.30	5.86	0.10	0.71	1.88	3.38	1.54	0.26	0.20	0.74
50-60	9.65	58.77	17.67	6.56	0.11	0.58	1.88	2.79	1.50	0.28	0.35	0.58
90-100	10.88	59.33	17.59	5.94	0.09	0.60	1.68	2.70	1.64	0.15	0.45	0.52
150-160	8.61	56.20	16.55	6.85	0.43	0.74	1.74	6.03	1.54	0.16	0.45	0.47
190-200	9.16	61.03	14.00	6.17	0.23	0.87	1.32	5.46	1.86	0.25	0.32	0.36
250-260	13.61	59.95	14.21	4.27	0.06	0.72	1.64	3.41	1.70	0.15	0.20	0.54
LAC*- losses after calcinations. The LAC are used to calculate the total content of minerals in the soil and recalculate the content of nutrients in the mineral part of the soil to a calcinated sample.												

The titanium content in soils is distributed relatively uniformly along the profile. In a layer of 60-70 cm, its amount reaches 0.6% (P1). In clayey (P2) and clayey-loamy soil (P3) the TiO_2 content is higher and is clearly dependent on the size of the silt fraction. Despite the heavy composition of these soils, the total P_2O_5 is relatively high and ranges from 0.15-0.30%.

Soils of the near-terrace floodplain contain less SiO_2, compared with near-riverbed. The amount of it, even in the layers of the most light mechanical composition does not rise above 61% (P3). One and a half oxides are much larger. The distribution of the listed elements along the profile is quite uniform, especially in soils with a uniform mechanical composition (P2). The content of calcium and magnesium is slightly lower, and phosphorus, potassium and sulfur are much higher. In some layers, the amount of P_2O_5 reaches 0.27%.

The uniform distribution of Na_2O in all alluvial soils, on average not exceeding 0.9% (P1, 0-10 cm) is noteworthy. However, in soil (P2 and P3, 0-10 cm) is 0.72% and 0.59%. Such a sharp increase in this element in the upper horizon is explained by sodium sulfate salinization.

The soil of the near-terrace area (P3) is characterized by the lowest content of SiO_2 - 59.6%, and the highest content of Al_2O_3 - 15,7% or 1,8 times more than in P1 (0-10 cm). Calcium and magnesium are less here, and K_2O, SiO_2 and P_2O_5 are slightly more. All other elements are evenly distributed along the profile (Table 1).

An analysis of the latest alluvial deposits shows that the content of many elements in them corresponds to the content of those in the soils that are formed on them. The distribution and content of chemical elements in the soils of the Dniester floodplain is not uniform in profile and tends to repeat the regularity of the physical clay content. Soils of the riverbed and central floodplain are most enriched with elements compared with near-terrace floodplain soil. The distribution and content of total elements along the profile of the studied soils is in accordance with stratification and depends on the mechanical composition.

Conclusions

The forming soils in the Dniester floodplain are confined to zones of accumulation of energetically material carried away from the watersheds and slopes of the river basin. Differentiation of alluvial deposits according to the mechanical composition in the floodplain, from the riverbed to the root bank entails the differentiation of floodplain soils in the same direction. The accumulation of humus, phosphorus, potassium and other nutrients is mainly due to input from the outside and only part of them is formed as a result of the soil formation process in the floodplain itself. An increase in the content of many chemical elements in the floodplain soils is observed from near-riverbed area to near-terrace and downstream of the river. Compared to the zonal soils of the river basin, in its floodplain, the soils contain a significantly larger number of nutrients.

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IMPACT OF O*LEA EUROPAEA*SUBSP*EUROPAEA*VAR*SYLVESTRIS*ON ABUNDANCE AND DISTRIBUTION OF SOIL INVERTEBRATES IN NORTH ALGERIA

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Abstract

The biological diversity of an ecosystem is a good indicator of its quality and its capacity to resist deterioration due to the external factors. The biodiversity conservation is a key element of environmental protection. The aim of this work was to evaluate the physical, chemical and biological characteristics of soils under oléastre in Kabylian region Algeria. We sampled soil of seven shrubs on four levels. The physical and chemical soil parameters as well as the enumeration of the soil fauna according to Berelèse-Tullgren method on four levels were measured. Soil was a cambisol (W.R.B. 1998). To do so, soils as well as soil invertebrates were analysed. The soil was silty loam and with sandy texture, with a slightly acidic to neutral pH, with low rates of organic carbon by means against the levels of available phosphorus are very low. This count allowed us to examine six orders of animals, springtails, beetles, annelids, nematodes, centipedes and beetles larvae. This work showed that the environmental conditions, in particular soil characteristics, hadan important impact on the presence and/or absence of certain soil invertebrates. The main result of this work lies on the hierarchization of factors driving invertebrate communities in Kabylian soils. However, the composition of soil fauna should be considered for rehabilitation. The results presented in this study are valid for this region only. To make a more general statement about the results, this type of studies needs to be applied to different soils, orchards and other climates in Northern Africa.

Keywords:*Olea europaea subsp europaea var sylvestris, invertebrates, carbon, soil quality.*

Introduction

Soil is a natural resource that represents all the products of physical degradation and then chemical alteration of the parent rock (Deprince, 2003). It consists of three phases ;a solid phase consisting of an inorganic fraction and an organic fraction; a liquid phase and a gaseous phase. Soil fauna presents an important taxonomic diversity, it is currently estimated at more than 23% of the animal biodiversity described today (Lavelle et al., 2006). These living beings are indicators of soil quality and should be considered as a resource for improving ecosystems (Boudiaf NaitKaci, 2014).African wild olive is a valuable tree in the dry Afro-montane forest, capable of regenerating naturally (Arests et al., 2006). This shrub generally grows on relatively poor soils with rugged terrain, in semi-arid zones with temperate climates, such as in southern Spain and northern Africa, it can also be found on the banks of streams temporary (Durand and Terral, 2006). The olive tree counts among the most important oil-producing crops throughout the Mediterranean region. The century-long presence of olive trees in most producing countries can lead to misunderstanding as to the sustainability, hardiness, longevity and plasticity of their cultivation. Soil management in olive has been oriented for centuries to insure productivity and survival of the plantation under conditions of limited, and highly variable, rainfall using a combination of low plant density, limitation of tree canopy size by pruning and elimination of adventitious vegetation to limit competition for soil water (Fernández-Escobar et al., 2013).

The aim of the present work was to hierachize factors recognized as important drivers for soil invertebrate community: the physical, chemical and biological soils characteristics under *Olea europeae* sub sp *sativa*in Kabylian region Algeria. We hypothesized that soil invertebrates are controlled by interactions between these three factors.

Material and methods

Study sites

Olea europeae sub sp *sativa* population has been selected in TiziRached Northern Algeria (36° 41'71" N ; 4°12'39,27" E), the altitude is 216 m.Our study site is a 70-year-old olive grove, where the oleaster has settled spontaneously following a fire in olive-growing orchards a decade ago. It rests on a sandy mother rock.Climate is sub-humid and the dry period begins in June and ends in September.

Soil sampling and characterization

The soils were Cambisols (WRB, 1998). Sampling was carried out on seven *Olea europeae* sub sp *sativa*, on four depths, the trees were chosen according to the vegetation cover and the homogeneity of the lithotopesquence. Soil sampling in January 2016, on four levels : h1 (0-10 cm), h2 (10-20 cm), h3 (20-30 cm) andh4 (30-40 cm).

Soil characteristics were determined according to standard methods proposed in Jackson (1967). Particle size distribution was measured according to the Robinson pipette method (*i.e.* organic matter oxidation by H_2O_2, shaking in a sodium hexametaphosphate solution). Soil pH was measured in a 1/5 soil distilled water suspension. The $CaCO_3$ content was determined using the HCl 1M volumetric method. Organic C was determined by sulfochromic oxidation. Bioavailable phosphorus was extracted by shaking soil samples in $NaHCO_4$ 0.5 M solution at pH = 8.5 (soil/solution ratio of 1/10) during 1 h (Olsen in Jackson, 1967). The suspension was then filtrated and phosphorus concentration in the solution was determined by colorometry.

Invertebrate sampling and identification

Invertebrate samplings were done during winter 2016 following the methodology of Coineau, (1974). In each selected shrub, four depths were sampled (0-10, 10-20, 20-30, 30-40 cm). In the laboratory, large invertebrates were hand-sorted. Afterwards, remaining invertebrates were extracted from soil by Berlese-Tullgren method (Pesson, 1971). After extraction, all invertebrates were preserved in 70° alcohol for ulterior identification. Giving the low taxonomic expertise on Algerian soil fauna, invertebrates were indentified at order level in most cases.

Statistical analyses

Kruskal-Wallis rank sum test were used for testing soil differences between depths and trees. Afterwards, multiple comparisons were done to identify groups that differed (Siegel and Castellan, 1998). We also tested the influence of the factors 'soil', 'level' and 'shrubs' and their interactions on the density and the diversity of invertebrates. For this, analyses of variance were performed on log-transformed data. Tukey's 'Honest Significant Difference' method was used to test for means multiple comparisons.

Results and discussion

Soil characteristics

Soils have a silty-sandy loam texture. This is due to the presence of a sandstone bedrock (Rudel and Treilhou, 1974). Soils pH are neutral (fig. 1a). These variations can be due to the buffering capacity of the soils and the numerous reactions occurring therein, which regulate the actual acidity (Hinsinger et *al*., 2003). In the case of the studied soils, the passage of a fire and the destruction of the organic matter and the mineral substances found there reduce the acidity of the soil (Viro, 1974).

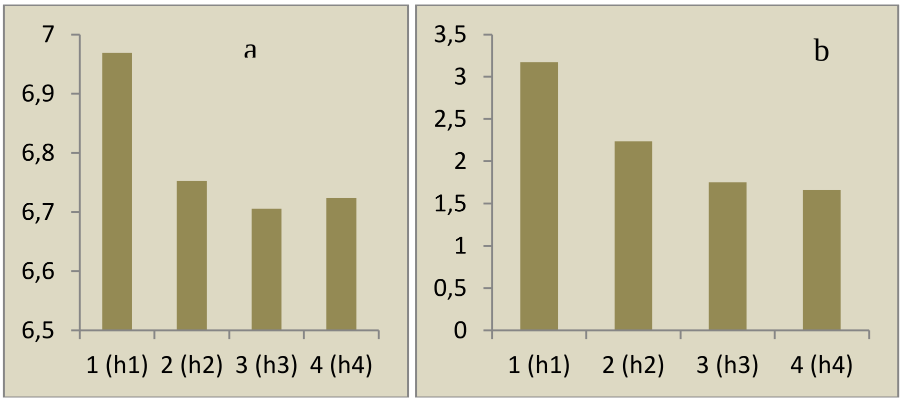


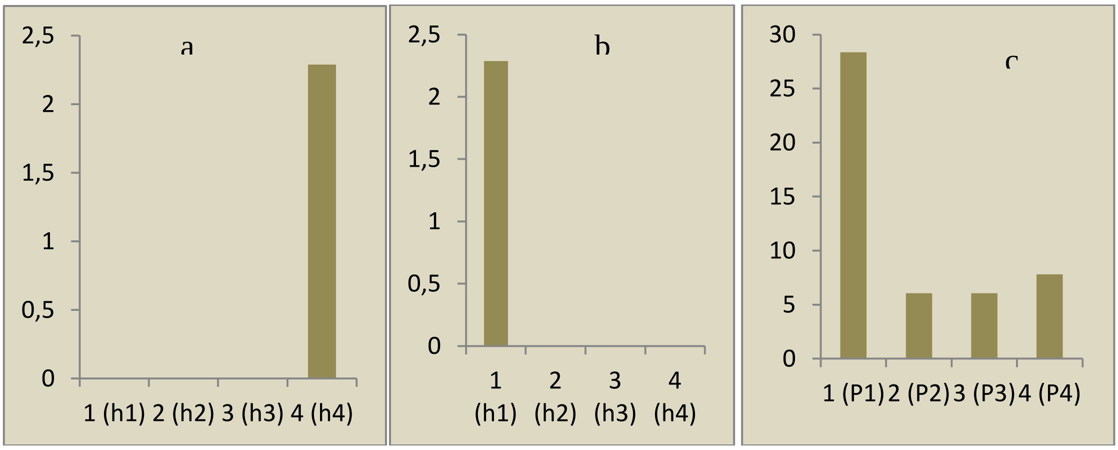
Figure 1. Mean soil characteristics of the 7 studied trees (a : pH ; b: organic matter)

Organic carbon levels are low to medium (fig.1b). The lowest rates can be related to the degradation of the olive grove that has undergone fires. Pansu et *al*., (2009) demonstrated that fires cause loss of organic matter.The Olsen phosphorus contents are very low and vary between 0.483 and 1.32 ppm. This variation can be explained by the fact that the roots have an important part of the root system compared to its low diffusion to the soil and its slow transfer to the roots (Stengel and Gelin 1998 and Jaillard et al., 2000). A clear difference between 1st and 2nd level is to be noted, this may be due to the passage of a fire. Viro, (1974) reports that assimilable phosphorus is richer in the surface layers of the soil after the passage of a fire.

Invertebrate density and diversity

The enumeration of the invertebrates allowed us to count a total of 2240 ind / m^2, grouped in six orders. The annelids are strongly represented with a dominance of 48%, the myriapods comprise 20% of the elements recorded, the beetles are represented with a dominance of 14% of which 13% are represented by beetle larvae. On the other hand, nematodes and mites are less abundant, respectively 11% and 7% of the total population. Collemboles are weakly present with a 1% strength.

The invertebrate harvesting in the soils of this station has shown that collembola are present only in level 4 (fig. 2a). These invertebrates have adaptation to the deep layers of the soil, they are hydrophilic species and the lack of moisture causes. Their desiccation explains their abundance in level 4 because they shun heat towards the deeper habitats (Davet, 1996; Ait Mouloud, 2006).Coleopterans are observed only in the first level (fig. 2b). Deprince, (2003) showed that they are located particularly in the humic horizon and in the soil annexes.



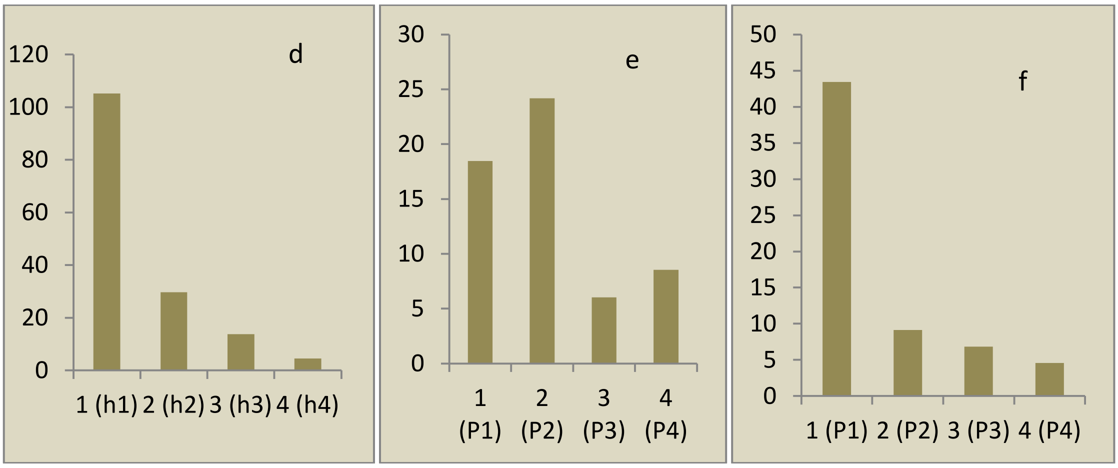


Figure 2. Mean density of invertebrates asa function of depth (a : Springtails ; b : Coleoptera ; c : Beetle larvae; d : Annelids ; e : Nematodes ; f : myriapods)

Coleoptera larvae are found mainly in the first ten centimeters of the soil nevertheless, they are also present in the 2nd, 3rd level, with a slight increase in the 4th level (fig. 2c). This is consistent with the fact that beetles show their influences in the soil by their larvae (Deprince, 2003; Gobat et *al*., 2003).

Annelids are very abundant by contribution to the other groups, they are distributed mainly in the first 10 centimeters of the ground, however they are present in depth (fig. 2d). Annelids descend to the depths in dry and wet periods, but remain active throughout the year (Abdul and Abdul, 1994). The works of Monroy (2006), show annelids prefer to live in decomposed logs. They contribute to litter decomposition and have a direct effect on nutrient mineralization (Morin, 2002). Nematodes are mostly present in the first 20 centimeters (fig. 2e), but also present in the other horizons, they are aquatic animals that live in the rhizosphere and decaying matter (Chaussod, 1996), which explains their displacement via leaching waters which can easily lead them deeply (Bachelier, 1978).

Myriapods are essentially concentrated in the top 10 cm of soil (fig. 2f)., which is consistent with the fact that they live in litter and play a very important role in the decomposition chain, or consume up to 25% of the litter (Gobat et *al*., 2003).

Hierarchy of factors

The main result of this work lies on the hierarchization of factors driving invertebrate communities in Kabylian soils. It clearly appeared that the factors soil and depth profoundly affected the total density of soil invertebrates. Based on the ACP it is found that there are significant values at the threshold of $\alpha = 0.05$ between annelids and pH, CO and myriapods and annelids (fig.3).

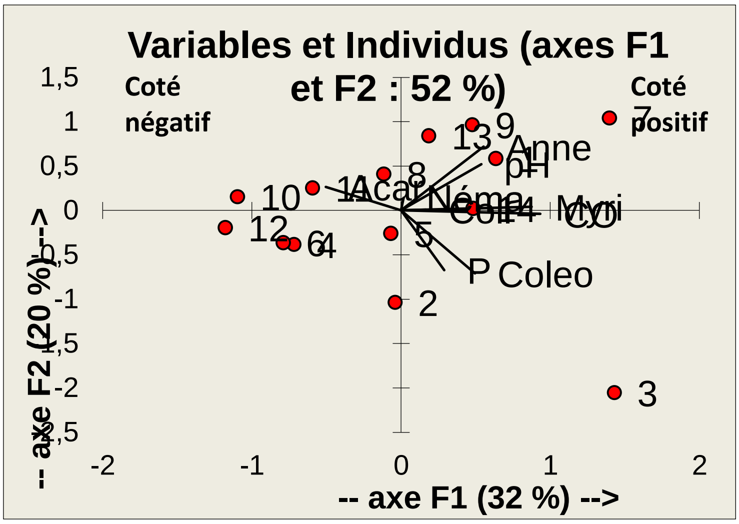


Figure 3. ACP

pH is a chemical constraint that largely influences the biological activity of soils (Davet, 1996), it intervenes in the presence and absence of fauna (Raphael, 2007), annelids prefer soils at pH between 5-7.4.Myriapods belong to the first trash compartment and live in litter (Gobat et *al*., 2003), while coarsely fragmenting organic matter (Deprince, 2003), hence the correlation between myriapods and organic carbone. The myriapods and annelids break down and fragment organic matter (Jungmann, 2014), which justifies their presence and their cohabitation in *Olea europaea* subsp *europaea* var *sylvestris* soils.

Conclusion

The objective of this work is a physical chemical and biological characterization of a soil under a population of *Olea europaea subsp europaea var sylvestris* appeared spontaneously after a fire. The morphology and description of the profile allow us to classify this soil as a Cambisol (WRB, 1998).This study gathers the original data concerning the abundance of invertebrate communities. It has shown in terms of variation and abundance that invertebrates are structurally different from one tree to another and from one level to another, Another in an oleo-lentisic ecosystem, which is a sign of degradation of Mediterranean soils. This census allowed us to examine six orders of animals: collembola, beetles, annelids, nematodes, myriapods and beetle larvae. We were interested in understanding the biofunctioning of this fragile environment as well as the vertical distribution of its fauna. Several factors have been considered to explain the distribution of invertebrates in these soils, without neglecting the impact of the fire on the physical, chemical and biological changes of this environment.Soil degradation, most frequently soil erosion but also a decrease of soil nutrients and organic matter, and soil compaction, is systematically quoted as one of the major threats to sustainability of oleaster.

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CALIBRATION OF SALTMED MODEL FOR INDUSTRIAL TOMATO CULTIVATION IN CENTRAL GREECE USING A DRIP IRRIGATION SYSTEM

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Abstract

Irrigation plays a key role in increasing agricultural productivity. Irrigated agriculture uses about 70% of global fresh water consumption. The continuing increase in world population and fresh water demands, along with the climate change effects are further threat for limited fresh water resources especially in arid and semi-arid regions. Models can be very useful tools in agricultural water management. They can help in irrigation scheduling and crop water requirements calculation and also to predict yields and soil salinization. Most of the existing models are designed for a specific irrigation system and are single-process oriented, such as models for water and solute movement, infiltration, leaching or water uptake by plant roots or a combination of them. SALTMED model has been developed for generic applications. In this research, the SALTMED model was calibrated using data from an industrial tomato cultivated field in Larissa region, central Greece, irrigated with fresh water at different water levels, using a drip irrigation system, corresponding to 100%, 80% and 60% of ET_c (deficit irrigation). The model successfully illustrated the effect of the irrigation application on soil moisture distribution and crop yield.

Keywords: *SALTMED model, Crop yield.*

Introduction

Irrigation plays a key role in increasing agricultural productivity. As a matter of fact, agriculture is the largest water user worldwide. Irrigated agriculture uses about 70% of global fresh water withdrawal (FAO, 2017). The continuing increase in world population and fresh water demands, along with the climate change effects are further threat for limited fresh water resources especially in arid and semi-arid regions (Lekakis and Antonopoulos, 2015). Improving agricultural productivity, while conserving natural resources as water, is essential for sustainable food production. Models can be very useful tools in agricultural water management. They can help in irrigation scheduling and crop water requirements calculation and also to predict yields and soil salinization. Most of the existing models are single-process, oriented, such as: a) models for infiltration (Bresler, 1975; Vogel and Hopmans, 1992), b) models for root water uptake (Cardon and Letey, 1992a; Coelho and Dani, 1996), c) models for leaching or water and solute transport (Antonopoulos, 1997; Vanclooster et al., 1996; Babajimopoulos *et al*., 1995; Cardon and Letey, 1992b), or d) models for specific applications, i.e. certain irrigation system, soils, region or a crop (Noborio *et al*., 1996; Minhas and Cupta, 1993; Cardon and Letey, 1992b; Ragab *et al*., 1990).

The SALTMED model is a comprehensive generic model (Ragab 2002, 2005, 2015) that can be used to predict final yield, dry matter, soil moisture and soil salinity profiles, plant water and nitrogen uptake, soil nitrogen transformation and content, drainage flow and evapotranspiration. The model is suitable for rainfed and irrigated agriculture, as well as for different irrigation systems and irrigation strategies, and it accounts for the presence of drainage systems and shallow groundwater. Moreover, the model can handle different crops, soil types and N-fertilizer applications.

One of the most demanding crops for irrigation is the industrial tomato, as it requires approximately 600 mm water per year. Consequently, the optimization of the irrigation conditions of the industrial tomato production is of paramount importance.
The objective of the present study was to calibrate the SALTMED model according to the soil moisture and crop yield results obtained for an industrial tomato crop cultivated on a sandy clay loam soil in central Greece, using drip irrigation with fresh water, with amounts of water equal or less than the full crop requirements (deficit irrigation).

Material and Methods

A field experiment was established at the farm of University of Thessaly in Larissa, central Greece. The soil at the experimental site was a sandy clay loam. Industrial tomato (*Heinz S3402*) was transplanted at the stage of 6-7 real leaves, on 1st and 2nd of May 2012. Each experimental plot contained 4 crop rows, spaced 1.05 m apart. The dimensions of each experimental plot were 3 m x 5 m (15 m^2). Industrial tomato was irrigated with fresh water. Three irrigation levels (treatments) were applied representing 100%, 80% and 60% of the design irrigation depth. Table 1 shows the amount of water applied, as irrigation water and total water (sum of irrigation plus rainfall). The experimental plan included randomized groups with four replicates.
The irrigation network consisted of a main delivery PE ∅32 pipe and three PE ∅20 secondary ones (one for each treatment). The 16-cm in diameter drip lines of the drip system were 5 m in length and spaced 1.5 m apart, with built-in pressure-compensating and self-cleaning emitters, spaced 0.70 m apart, with a flow rate of 2.2 L h^{-1} at an operating pressure range of 0.5 to 3.5 bars, with a double self-cleaning mechanism and two outlet channels to prevent clogging. The irrigation system control unit consisted of a collector equipped with 3 electric valves connected to an irrigation controller and 3 water meters. Each valve with its water meter was connected to the secondary pipes, each one of them fed four experimental plots. Each experimental plot contained 2 drip lines. The depth of the water applied was calculated from the readings on the water meters and the area of each experimental plot.
Crop harvest was carried out at the red maturation stage according to the USDA (1997) ranking, on 24th of August 2012, by hand. From each experimental plot, a total of 10 plants was harvested and weighed separately for each line. Crop yield was calculated.

Table 1. Total irrigation water and total applied water depths for each treatment (irrigation level).

Irrigation level	Irrigation water (mm)	Total water applied (mm)
100% ET_c	587.18	697.00
80% ET_c	469.74	580.14
60% ET_c	352.31	462.71

The version of SALTMED model that was used in this study is the one presented by Ragab (2015) version 2019. A detailed description of the SALTMED model is provided in Ragab (2002; 2015). Some key parameters of the model are presented below.
Crop evapotranspiration ETc was calculated as:

$$ET_c = ET_0 (K_{cb} + K_e) \quad (1)$$

where K_{cb} is the crop transpiration coefficient and K_e the bare soil evaporation coefficient. The values of K_{cb} and K_c, (the crop coefficient) for each growth stage, and the length of each

growth stage for large number of crops, are available in the model's database. K_e is calculated as the difference between K_c and K_{cb}.
Evapotranspiration (ET_0) has been calculated using the Penman–Monteith equation according to the modified version of the equation by the Food and Agriculture Organization of the United Nations (FAO) (Allen et al., 1998):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma\frac{900}{T+273}U_2(e_s - e_a)}{\Delta + \gamma(1 + 0{,}34U_2)}$$
(2)

where ET_0 is the reference evapotranspiration (mm day^{-1}); Rn, the net radiation (MJ m^{-2} day^{-1}); G, the soil heat flux density (MJ m^{-2} day^{-1}); T, the mean daily air temperature at 2 m height (°C); Δ, the slope of the saturated vapour pressure curve (kPa $°C^{-1}$); γ, the psychrometric constant, 66 Pa $°C^{-1}$; e_s, the saturated vapour pressure at air temperature (kPa); e_a, the prevailing vapour pressure (kPa); and U_2, the wind speed at 2 m height (m s^{-1}).
Actual water uptake rate has been calculated based on the formula suggested by Cardon and Letey (1992), which determines the water uptake S (mm day^{-1}) as follows:

$$S(z,t) = \left[\frac{S_{max}(t)}{1 + \left(\frac{a(t)h + \pi}{\pi_{50}(t)}\right)^3}\right]\lambda(z,t)$$
(3)

where
$\lambda(z) = 5/\ 3L$ for $z \leq 0{,}2L$
(4)
$\lambda(z) = 25/\ 12L \times (1 - z/L)$ for $0.2L < z \leq L$
(4a)
$\lambda(z) = 0{,}0$ for $z > L$
(4b)

$S_{max}(t)$ is the maximum potential root water uptake at the time t; z, the vertical depth; λ, (z,t) the depth and time-dependent fraction of total root mass; L, the maximum rooting depth; π, the osmotic pressure head; h, the matric pressure head; $\pi_{50}(t)$, the time-dependent value of the osmotic pressure at which $S_{max}(t)$ is reduced by 50%, and a(t) is a weighing coefficient that accounts for the differential response of a crop to matric and solute pressure. The coefficient a(t) equals $\pi_{50}(t)/\ h_{50}(t)$ where $h_{50}(t)$ is the matric pressure at which $S_{max}(t)$ is reduced by 50%.
The potential water uptake $S_{max}(t)$ is calculated as:

$S_{max}(t) = ET_0(t) * K_{cb}(t)$
(5)

The rooting depth was assumed to follow the same path of the crop coefficient K_c and was therefore described as follows:

$$Root\ depth(t) = \frac{[Root\ depth_{min} + (Root\ depth_{max} - Root\ depth_{min}) \times K_c(t)}{K_{c\,max}}$$
(6)

The relative crop yield (RY) is estimated as the sum of the actual water uptake over the season divided by the sum of the maximum water uptake, i.e. the water uptake under no water and salinity stress, as:

$$RY = \frac{\sum S(x,z,t)}{\sum S_{max}(x,z,t)}$$
(7)

where x and z are the horizontal and vertical coordinates, respectively, of each point in the root zone that contain roots. The actual yield (AY) based on RY is calculated as:

$AY = RY * Y_{max}$
(8)

where Y_{max} is the maximum yield recorded in a given region under stress-free optimum conditions.

Results and Discussion

The SALTMED model was calibrated using data from an experimental field of industrial tomato cultivated on sandy clay loam soil at the farm of University of Thessaly in Larissa, central Greece, irrigated with freshwater. The meteorological data were taken from the Larissa meteorological station.

The plant coefficients k_c and k_{cb} were 0.6 and 0.18, respectively, for stage (1) of 33 days. During stage (2) of 40 days, they both increased linearly, k_c from 0.6 to 1.15 and k_{cb} from 0.18 to 1.1. During stage (3) of 45 days, k_c and k_{cb} were 1.15 and 1.1, respectively. At the final stage (4) of 27 days, both coefficients decreased linearly, k_c from 1.15 to 0.7 and k_{cb} from 1.1 to 0.7.

Crop parameters, such as the minimum and maximum depths of the root zone, which were 0.1 and 0.6 m, respectively, were determined by field measurements. Soil parameters, such as saturated hydraulic conductivity were also obtained from field measurements, while the characteristic curve of the soil was determined in the laboratory. Soil water characteristic curve in conjunction with the RETC program (Van Genuchten et al., 1991), were used to calculate parameters such as the residual moisture content θ_r and the saturation moisture content θ_s. Their values were found to be 0.54 and 0.003, respectively. The parameters, α, n and λ (soil resource allocation coefficient) were found to be 0.0012, 1.3 and 0.3, respectively. The bubbling pressure value used by the program database for sandy clay loam soil was 37.037 cm. Volumetric moisture content measurements were carried out using the Trime device, which operates with the TDR method.

Figure 1 shows the evapotranspiration of the crop, the crop transpiration and the bare soil evaporation throughout the growing season in the year 2012. Figure 2 presents the simulated by the SALTMED model industrial tomato yield, indicatively for the treatment of 100% ET_c, as well as the actual and dynamic intake of water by the plants' roots. As shown in Figure 2, the maximum crop yield was set at 100 tn/ha and was reduced accordingly to the water stress.

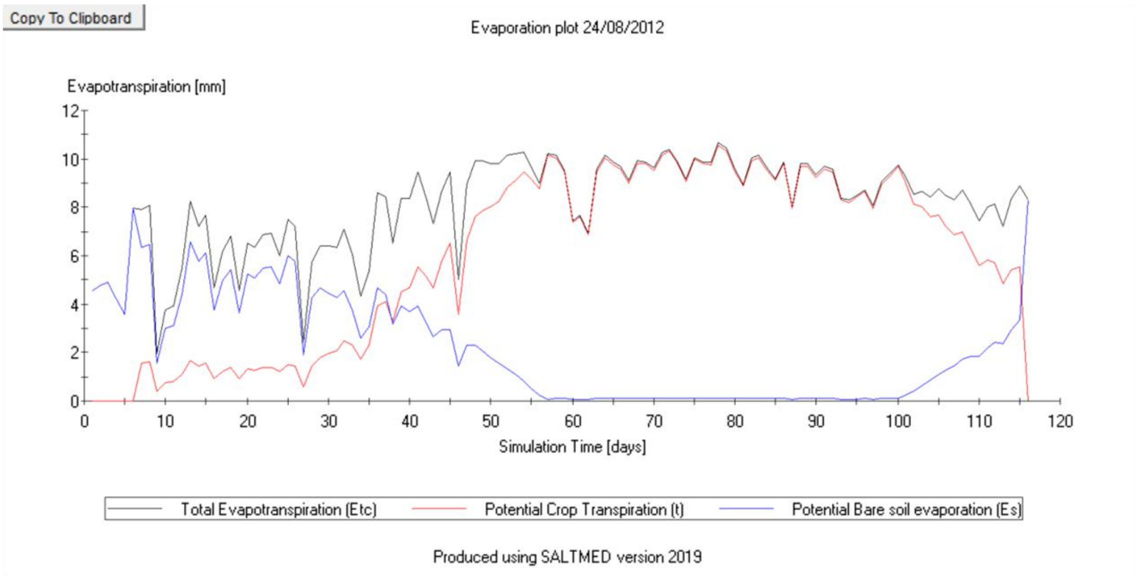


Figure 1. Evapotranspiration, crop transpiration and bare soil evaporation.

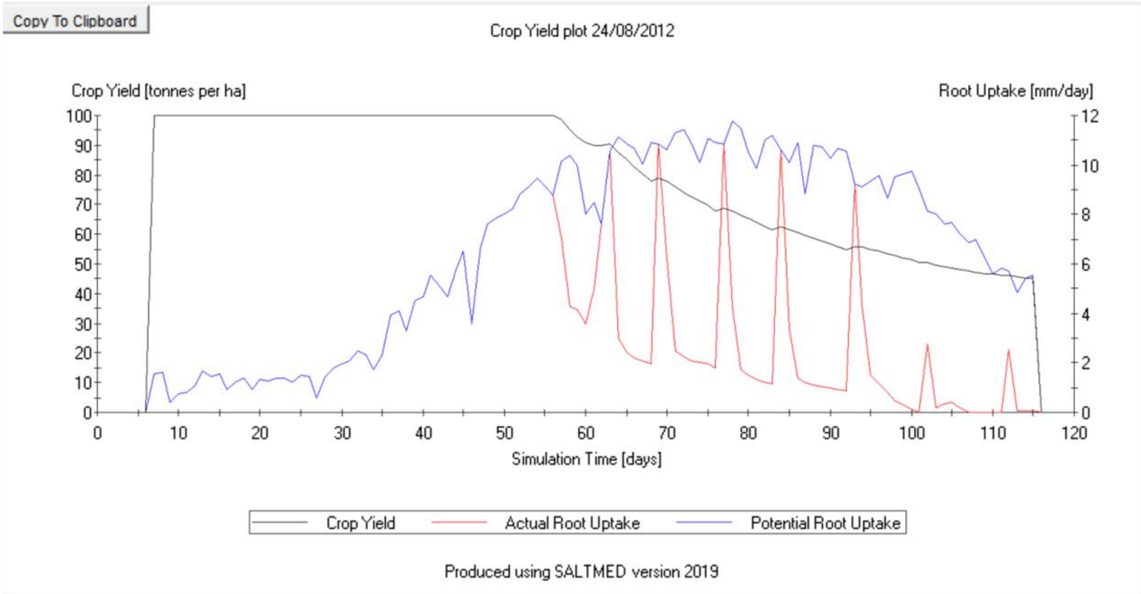


Figure 2. Simulated industrial tomato yield by the SALTMED model for the treatment of 100% ET_c.

Industrial tomato yield, simulated and measured, for each treatment is presented in Figure 3. The simulated tomato production values do not deviate from the measured ones, at all levels of irrigation tested, thus suggesting that the SALTMED model can simulate well industrial tomato yield. Also, the results illustrate different yields under different irrigation strategies. The more deficit irrigation strategy resulted in the lower yield, suggesting that industrial tomato is indeed a highly water-demanding crop and irrigation water reductions up to 20% of ET_c can reduce crop production by about 28%. Therefore, other irrigation strategies, such as the use of treated wastewater, or saline water, should be considered in order to achieve fresh water savings whilst ensuring maximum industrial tomato yields to meet the future food demand.

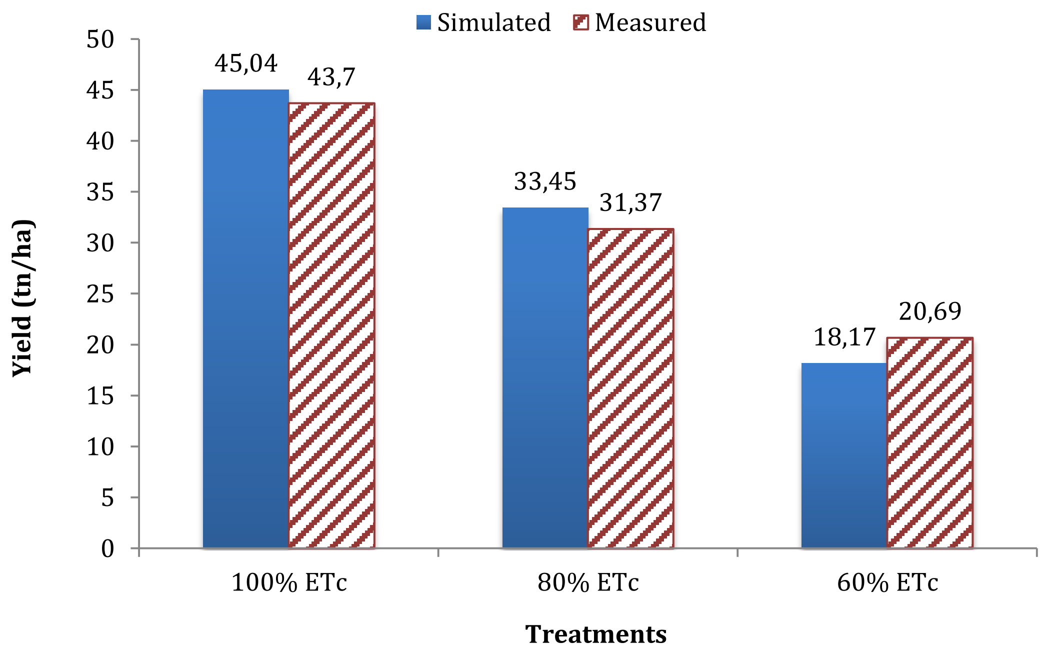


Figure 3. Comparison between simulated and measured industrial tomato yields under different irrigation treatments (100% ET_c, 80% ET_c and 60% ET_c).

The irrigation was carried out by using the automatic weather station, after the humidity was exhausted, as shown in Figure 4 indicatively for treatment of 100% ET_c. The SALTMED model during its implementation has the capability to graphically visualize the soil moisture profile at different distances from the emitter to a daily step.

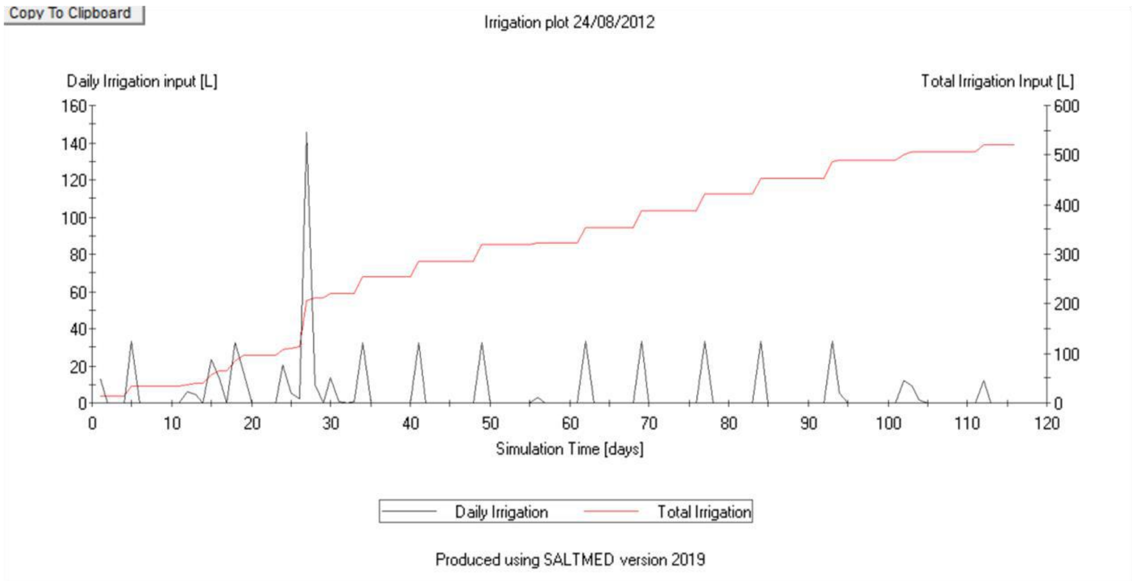
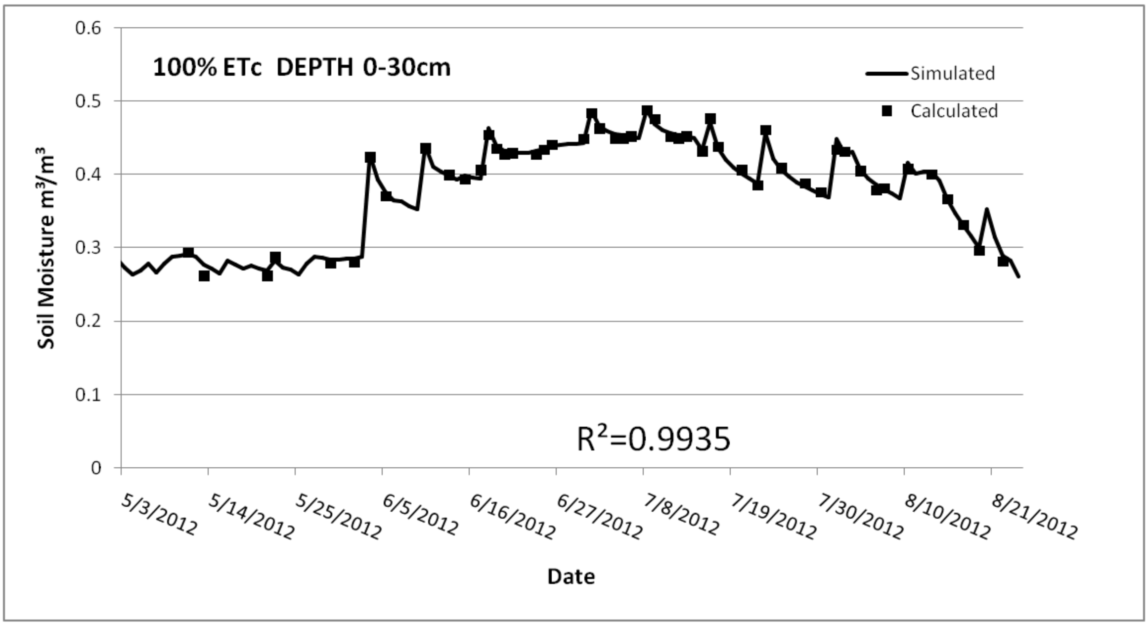
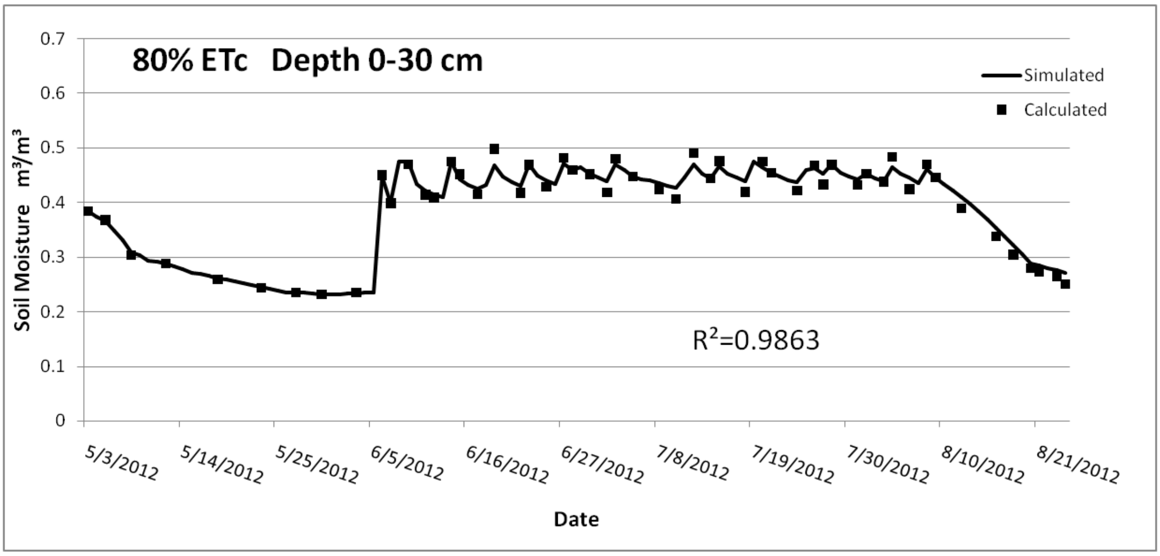


Figure 4. Volume of irrigation water added daily and in total for treatment 100% ET_c.

Figure 5 graphically represents the soil moisture at the location of the dripper at the depth between 0 cm and 30 cm with the measured and simulated values of soil moisture before and after irrigation with a very satisfactory approach. The model has shown a good fit of the simulated soil moisture when compared with the observed values.





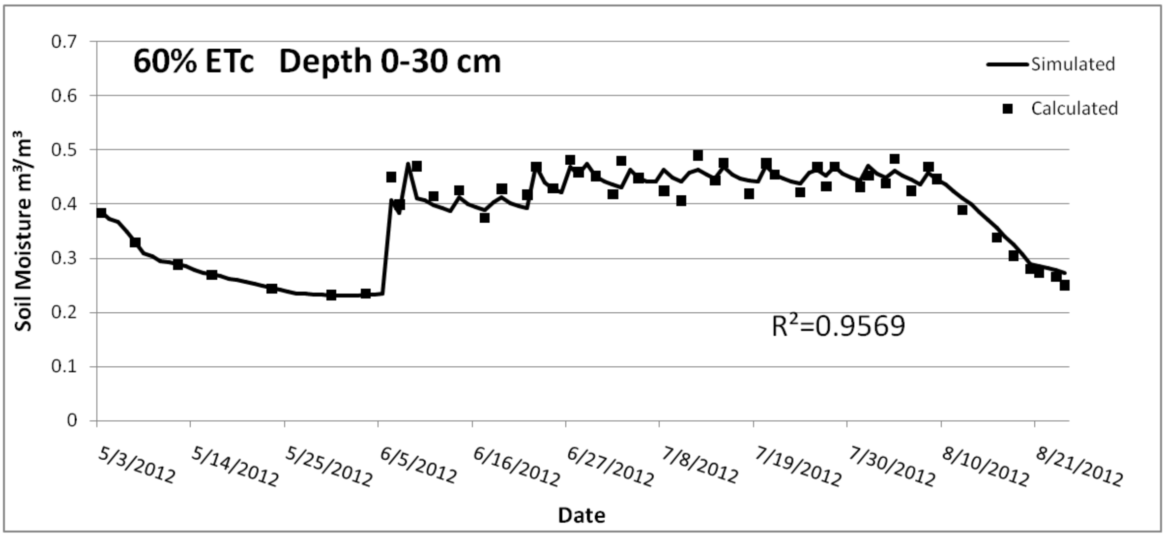


Figure 5. Graphic representation of measured and simulated soil moisture for industrial tomato within the top 30 cm depths, for the different treatments (100% ET_c, 80% ET_c and 60% ET_c).

Conclusions

In terms of model simulations, the model showed a strong relationship between the measured and simulated soil moisture and industrial tomato yield in a drip irrigation system. The mathematical model SALTMED adequately described the effect of the irrigation on the soil water balance, which makes it an effective tool in the irrigation water management practice.
A next paper will present the model's results using data from crops irrigated with saline water and different irrigation systems.

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RECYCLING OF PACKAGING MATERIALS TO OBTAIN AN OENOLOGICAL ADJUVANT

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Abstract

Circular economy is aimed to create a *zero-waste* system by the reduction of wastes and their exploitation by turning them into by-products to be used as inputs for new production processes. In this perspective, packaging materials (glass and aluminium) were used to synthetize zeolitic materials to be evaluated for oenological application. A potassium (Zeo-K) and a sodium (Zeo-Na) zeolitic material (Zeo-K and Zeo-Na) were obtained, containing 16% and 30% of crystalline materials respectively. Both materials were then tested as oenological adjuvants, evaluating their ability to remove riboflavin from wines. Riboflavin is responsible of a photoxidation process leading to the onset of the light-struck taste in wines. Preliminary experiments were performed using a model wine solution, enriched with 300 μg L^{-1} of riboflavin. Each zeolite was added to the riboflavin-enriched solution at the concentrations of 1 g L^{-1} (Zeo-K1, Zeo-Na1) and 10 g L^{-1} (Zeo-K10, Zeo-Na10). For comparison, two bentonites were also tested at the concentration of 1 g L^{-1}. A control without either zeolites or bentonites was also run. The obtained results showed that riboflavin concentration remained unvaried in the control, whereas it decreased from 4% (Zeo-K1) to 37% (Zeo-Na10) in the treatments. Both zeolitic materials appeared less efficient than bentonites at the same concentration. Nevertheless, synthesizing zeolites with higher crystallinity is likely to lead to materials with higher efficiency, almost comparable to bentonites. Efforts are being undertaken to produce more effective zeolites from waste materials for oenological applications and to evaluate possible secondary effects on wine properties.

Keywords: *Circular economy, packaging materials, zeolites, wine, riboflavin, light-struck taste*

Introduction

Circular economy is aimed to turn goods that are at the end of their service life into resources for others, closing loops in industrial ecosystems and minimizing waste (Stahel, 2016). It is ultimately linked to resource cycling and to the 'waste-as-food concept', wherein unwanted outputs of one industrial process are used as raw materials in another industrial process (Murray, Skene, & Haynes, 2017). Business motivations for shifting from a linear to a circular economy are manifold. Economic advantages, namely cost reduction, new revenue sources and employment creation, have been identified and quantified for whole sectors or regions (Stewart, Niero, Murdock, & Olsen, 2018). Throughput materials, in particular, become end-of-life waste within a year, and the largest part of this fraction is potentially available for recycling after use (Haas, Krausmann, Wiedenhofer, & Heinz, 2015). Among these, food packaging can be a relevant source of raw materials for other products. Metal cans and glass bottles/jars, composing 5.8 and 0.6 % of municipal solid wastes (Consonni & Viganò, 2008) can provide, therefore, aluminium and silica as raw materials.
Aluminium and silica are also at the basis of zeolites' composition. Zeolites are tectosilicates characterized by high cation exchange capacity and a complex system of micropores and channels. Several synthesis processes have been developed in the last decades, in order to

produce zeolites with useful chemical and structural properties. Among the materials used for the synthesis of these aluminosilicates, glass and aluminium derived from food and drink packaging recycling have been recently proposed (Terzano, D'Alessandro, Spagnuolo, Romagnoli, & Medici, 2015). Thanks to their high sorption capacity, they are used in a number of industrial applications and also in the agri-food sector, especially after their classification as 'non-toxic' by International Agency for Research on Cancer (IARC) and 'safe for human consumption' by the Food and Drug Administration (FDA). Their applications range from storage pest management to food additives (Eroglu, Emekci, & Athanassiou, 2017). Some researchers proposed natural zeolites as oenological adjuvants, for protein/tartaric stability (Mercurio et al., 2010), phenolic off-odor removal (Lisanti, Gambuti, Genovese, Piombino, & Moio, 2017), prevention of light-struck taste due to riboflavin-induced photoxidation (Fracassetti et al., 2017).
The objective of the present work is to evaluate the potential of two synthetic zeolitic materials, obtained by an alkaline hydrothermal treatment of glass and aluminum recovered from drink and food packaging, for the removal of the photosensitizer riboflavin in a model wine system.

Material and Methods

White glass from drink bottles and food jars, and aluminum from cans of commercial soft drinks were used to synthesize a potassium zeolitic material (Zeo-K) and a sodium zeolitic material (Zeo-Na). Both starting materials were characterized for the chemical composition by wavelength dispersive X-ray fluorescence spectrometer (WD-XRF; Supermini 200, Rigaku Corporation, Tokyo, Japan). The zeolite synthesis was carried out according to the procedures reported by Terzano et al. (2015). Briefly, Zeo-K was obtained adding 1.6 L of a 5M KOH solution to 10 g of aluminum pieces in HDPE vessels, which were stored for 24 h at 25°C. Then, the aluminum solution was filtered with Whatman 1 and 160 g of glass fragments (Ø<2mm) were added to the filtrate. The suspension was stirred for 2 h and finally stored at 90°C for 7 days. The synthesis of Zeo-Na required the use of a 2.5M NaOH solution instead of KOH solution, and a crystallization temperature of 60°C instead of 90°C. In both cases, after the incubation time, the samples were cooled to room temperature and centrifuged at 5500 rpm for 15 min. The pellet obtained was washed three times with deionized water, then dried at 105°C for 24 h, and sieved at 500 μm. The materials obtained were analysed by X-ray powder diffraction (Miniflex II X-ray diffractometer, Rigaku Corporation, Tokyo, Japan) to assess their mineralogical composition.
The ability of the two zeolitic materials in reducing the concentration of riboflavin was preliminary assessed in a model wine solution, prepared with 5 g L^{-1} tartaric acid, 12% ethanol (v/v) and corrected for pH at 3.2 with NaOH. Riboflavin was added at the concentration of 300 μg L^{-1} in the model solution. Each zeolite was added to the riboflavin-enriched solution at the concentrations of 1 g L^{-1} (Zeo-K1, Zeo-Na1) and 10 g L^{-1} (Zeo-K10, Zeo-Na10). For comparison, two commercial bentonites (Bent1, Bent2) were also tested at the concentrations of 1 g L^{-1} for the capacity in removing riboflavin. A control without either zeolites or bentonites was also run. All the samples were stirred in the dark for 24 h at 25°C, then they were filtered at 0.45 μm and analysed by HPLC. All the treatments were replicated three times.
Riboflavin was detected using an Agilent 1200 Series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1311B quaternary pump, G1329B auto-sampler injector (30 μL sample loop), G1316A Thermostatted Column Compartment, G1315D Photo diode array detector, in a series configuration. The stationary phase was a RP C18 Acclaim^{TM} 120 column (3 μm, 150 × 3 mm) (Thermo Fisher, Waltham, MA, USA) at a column temperature of 25 °C. The mobile phases were (A) 90% 50 mmol citrate buffer at pH

2.5 and 10% methanol (v/v) and (B) 10% 50 mmol citrate buffer at pH 2.5 and 90% methanol (v/v). The elution program was: from 100% A to 30% (0-8 min), from 30% A to 100% (1 min), 100% A (9-15 min); flow rate of 0.6 mL min^{-1}. PDA detector was monitored at 440 nm. Quantitative analysis was performed according to the external standard method on the basis of calibration curve obtained by injection of solutions at known concentrations in the range of 0.01-3 mg/L (R^2=0.9998). Results were expressed as mg L^{-1}.

Results and Discussion

Both glass and aluminium used for the zeolite synthesis did not contain any potentially toxic element, as revealed by the WD-XRF analysis. The XRPD patterns of the two zeolitic materials are shown in Fig. 1. The Zeo-K synthesis produced the formation of 16% edingtonite, whereas the Zeo-Na synthesis caused the formation of 30% zeolite-A. The broad hump between 20 and 30° 2θ in XRPD patterns suggested the presence of a considerable amount of amorphous phases, consisting of cryptocrystalline and poorly ordered fractions. Both zeolitic materials were characterized by a high cation exchange capacity (260 and 390 $cmol_+$ kg^{-1} for Zeo-K and Zeo-Na, respectively). The other chemical and structural properties of zeolites synthetized are reported in Terzano et al. (Terzano et al., 2015).

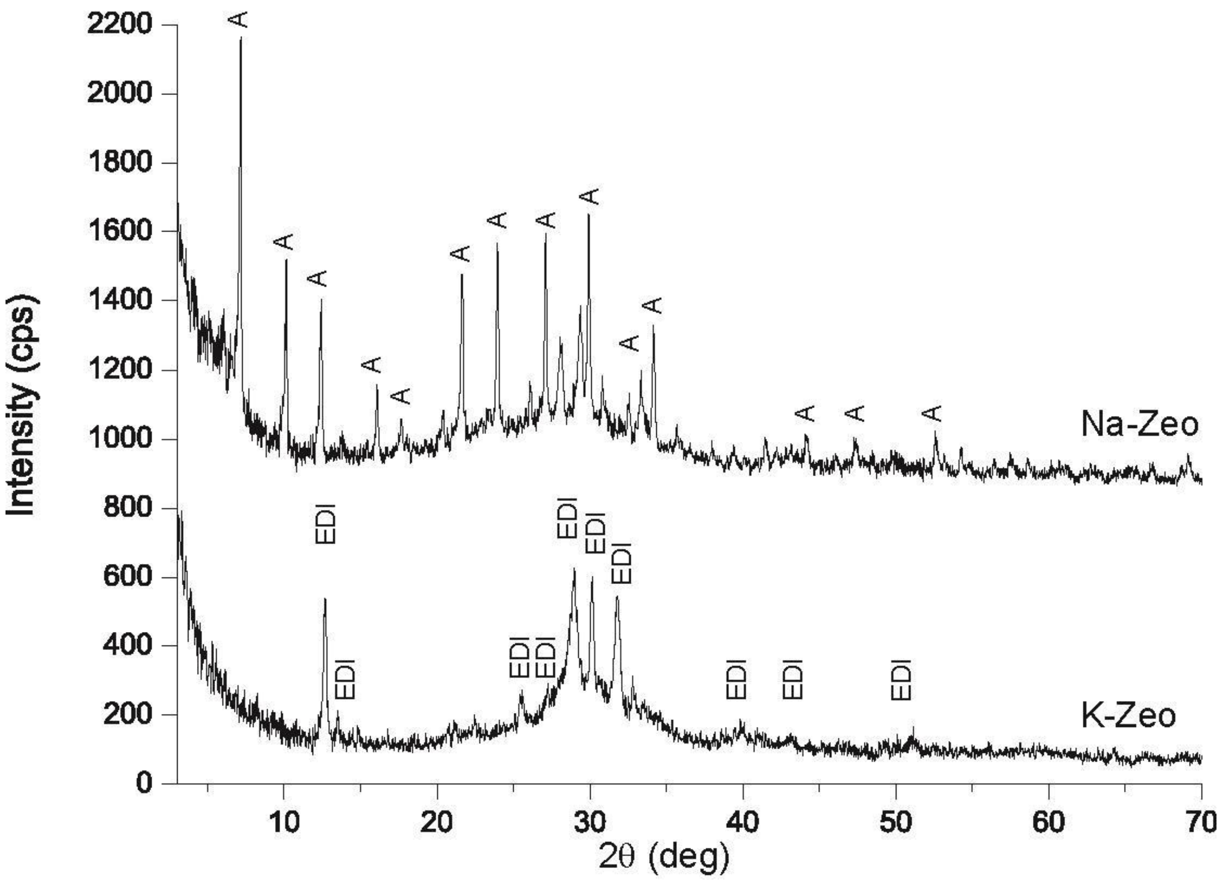

Fig.1 Diffraction patterns of Zeo-Na and Zeo-K, and main reflections of zeolites detected in the two samples (A: zeolite-A; EDI: edingtonite).

The model wine system was spiked with an amount of 300 μg L^{-1}, that is in the upper end of the concentration range of riboflavin found in wine, as reported in literature (Fracassetti et al., 2017). Two different commercial bentonites, commonly used as wine fining agents, were used to compare the ability of the zeolitic materials to remove riboflavin.

The HPLC analyses revealed that the riboflavin concentration remained unchanged in the control and in the treatment Zeo-K1, whereas it significantly decreased in all the other treatments (Figure 2). Bentonites reduced riboflavin amounts by 14.7% and 20.9% Fracassetti et al. (2017) previously obtained better performances from commercial bentonites (1 g L^{-1}), removing about 40% of riboflavin in a model wine system containing higher amounts of photosensitizer (350 μg L^{-1}). The reduction due to Zeo-Na1 was only 8.3%. Nevertheless, it should be considered that commercial bentonites for oenological use have a legal content of active crystalline aluminosilicates (montmorillonite) of at least 80% (90-95 % in the tested bentonites, according to the technical sheets). The lower removal ability of both zeolitic materials might be ascribed, therefore, to their low content in zeolites. In these preliminary tests on the synthetized zeolitic materials, their addition in higher amounts was also evaluated. Zeo-Na10 and Zeo-K10 resulted the most effective treatments, causing 37% and 26% of removal, respectively. In all treatments, however, the residual amount of riboflavin was higher the target that would avoid the appearance of light-struck taste in wines (100 μg L^{-1}).

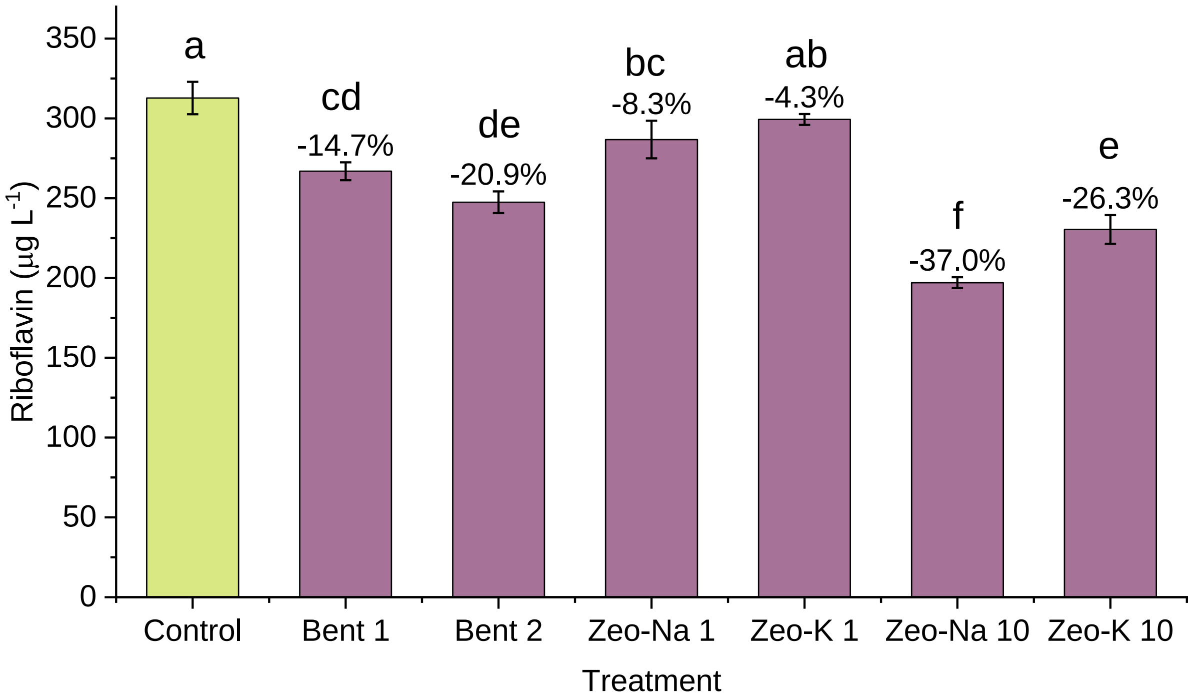

Fig.2 Amounts detected by HPLC-DAD analysis in the control model wine system and in the samples treated with bentonites and zeolitic materials. For treatment codes see Materials and methods. Means ± standard deviation of fhree independent replicates. Different letters mean a significant difference at $p < 0.05$.

However, the ability of zeolitic materials obtained from packaging materials was proven. Further research is needed to obtain more effective zeolitic materials, by either increasing their purity or tailoring their structural properties. Moreover, secondary effects on wine composition and sensory properties should be evaluated. In fact, in case of bentonites, the tested amounts (1 g L^{-1}) could result detrimental for wine sensory properties (Fracassetti et al., 2017). As regards zeolites, tests on natural zeolites applied to wine have been carried out at quite higher concentrations (up to 8 g L^{-1}) (Mercurio et al., 2010) and no data is available at this moment on the highest tolerable amounts that could be used without affecting wine properties.

Conclusions

Two zeolitic materials obtained from food packaging were tested for the first time as possible oenological adjuvants, with the aim to reduce the levels of the photosensitizer riboflavin. Both materials resulted able to partially remove riboflavin (up to 37% less than control), though with lower efficiency compared to commercial bentonites. Further research will be focused on improving their purity and efficiency, and to evaluate possible secondary effects on wine properties.

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THE ECOLOGICAL FORMULATION OF A PESTICIDE MANUFACTURED BY JSC SCHELKOVO AGROHIM (RUSSIA) ON THE EXAMPLE OF THE HERBICIDE ZONTRAN CSC

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Abstract

The results of studying the effectiveness of the herbicide Zontran CSC, containing the active substance metribuzin 250 g/l in a preparative form (formulation) named Colloidal Solution Concentrate (CSC) are presented. Experiments were carried out for two years in 2011–2012 on soybean crops with mixed type of weeds: annual dicotyledonous and annual grasses. The biological effectiveness of Zontran CSC was compared with a product containing metribuzin 700 g/kg in a formulation Wettable Powder (WP). It was established that the pre-emergence application of Zontran CSC on soybean crops at a dose rate of 1.2 l/ha was comparable to the biological effect of the product, consisting of metribuzin at 2.3 times more, but in the WP formulation. It was observed that Zontran CSC in comparison with the product containing a higher amount of metribuzin, but in the WP formulation, was at the level, and in some cases exceeded the biological effectiveness of this product. Thus, the formulation of the colloidal solution concentrate allowed, without loss of biological effectiveness and with obtaining reliable yield increases, to reduce the amount of metribuzin per unit of cultivated area, which makes the use of pesticide environmentally friendly, since minimizes its release into the environment.

Keywords: *Herbicides, metribuzin, colloidal solution concentrate, formulation, soybean.*

Introduction

Intensive application of the mineral fertilizers and pesticides are the cause to the yearly entry into the biosphere of various chemicals. In this regard, the problem of environmental protection, especially when using pesticides, is important (Ganiev and Nedorezkov, 2006). The use of pesticides ensures to save crop yield by controlling the number of harmful objects. However, applying pesticides during or before the vegetation periods leads to the fact that chemicals that can adversely affect the soil biota and terrestrial organisms are released into the environment and accumulated in the form of residues in agricultural products. Therefore, reducing the pesticide accumulation on nature is actual and significant.

Biological efficacy of the products depends on the active substances in their composition, as well as the amount of active ingredient that interacts with the harmful objects, which is determined by the dose rate of the pesticide. Preparative form of pesticide (Formulation) is the composition of the active substance of the pesticide and auxiliary substances, ensuring the delivery of the active substance to the target object.

The different biological efficacy is achieved with the same active ingredient and its equal amount but depending on the type of formulation. The dose rate decreasing of active ingredients may lead to a decrease in biological effectiveness.

JSC Schelkovo Agrohim set a goal to create products that would have a biological efficiency comparable to the level of traditionally used pesticides, but contained a smaller amount of the same active ingredient per unit of cultivated area in order to minimize the pesticide environmental accumulation. This effect was supposed to be obtained by means of a new formulation, which is a special case of the emulsion concentrate (EC) - the colloidal solution concentrate (CSC). CSC is an EC which, when preparing the working solution, forms a colloidal system. A colloid system is a system in which discrete particles, droplets or bubbles of a dispersed phase, having a size in at least one dimension from 1 to 100 nm, are distributed in another phase, usually continuous, different from the first in composition or aggregative state and called dispersion phase. The popularity of colloidal systems lies in their unique properties that are transmitted to colloid-based products: the system is homogeneous, there is no sediment (the product has a transparent colour), a high coverage ratio, wetting is ensured, which cause to a significant increase in the degree of absorption of active substances, and as a result, optimization of biological activity.

A whole line of the products of CSC formulation was created, one of them is the herbicide Zontran CSC containing 250 g/l of metribuzin.

Metribuzin is included as an active ingredient in many pesticides. It is used pre- and post-emergence to control weeds on following crops: potatoes, wheat, tomatoes, carrots, soybeans, and sugarcane. The mechanism of action is based on the inhibition of the photosystem II (Metabolic Pathways of Agrochemicals: Herbicides and plant growth regulators, 1998).

The objective of this research was to study the biological effectiveness of the herbicide Zontran CSC and compare it with its analogue (hereinafter referred to as Analog) containing 700 g/kg of metribuzin in the formulation Wettable Powder (WP).

Material and Methods

Biological trials were carried out for two years in 2011–2012 in three regions of the Russian Federation on the soybean varieties Annushka, Bara, Svetlaya. The Zontran CSC was applied pre-emergence, on vegetative weeds with dose rates of 0.6, 0.8 and 1.2 l/ha, which corresponds respectively to the application of metribuzin 150, 200 and 300 g/ha consequently. At the application time, weeds were in the phase of cotyledons - two leaves.

The biological efficiency of Zontran CSC was compared with the Analog containing 700 g/kg metribuzin, formulation Wettable Powder (WP), in dose rates of 0.5 and 1.0 kg/ha, which corresponds to 350 and 700 g of metribuzin on ha respectively. The trials were provided in accordance with the "Guidelines for registration testing of herbicides in agriculture" (Dolzhenko V.I., 2013) under conditions of small plots of 0.25 m^2 in the mixed type of weediness. The names of weed plant species were given in accordance with the work of P.F. Mayevsky "Flora of the middle zone of the European part of Russia"(Mayevsky P.F., 2014).

The study was conducted by an independent source namely All-Russian Institute for Plant Protection (VIZR), the results of this research Zontran CSC received an extension of official registration of pesticides in the Russian Federation for soybeans.

The determination of the particle size of the Zontran CSC was carried out on a Photocor Compact instrument using photon correlation spectroscopy. Particle size analysis of the Analog (WP) has been done on a Fritsch Analysette 22 MicroTec Plus instrument by laser diffraction. The surface tension of each of the products were determined by the method of ring detachment on a Kruss K6 tensiometer.

Results and Discussion

In case of pre-emergence application of the Zontran CSC in soybean crops following weeds were effectively suppressed: *Stellaria media L.* (85%), *Amaranthus retroflexus L.* (94,6%), *Chenopodium album L.* (94%), *Echinochloa crus-galli L.* (81%) *Thlaspi arvense* (82%) (Fig. 1).

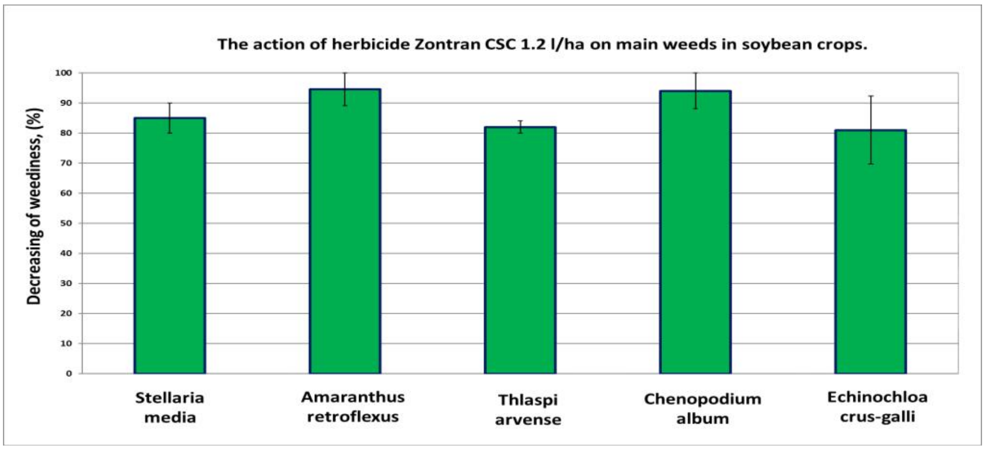


Fig. 1. Efficacy of pre-emergence application of the Zontran CSC herbicide on the main types of weeds in soybean crops.

The Zontran CSC effectiveness of action on annual dicotyledonous weeds in dose rates of 0.6 and 0.8 g/l (metribuzin 150 and 200 g/ha respectively) was comparable with the efficiency of the Analog in the dose rate of 0.5 kg/ha (metribuzin 350 g/ha), and at a dose rate of 1.2 l/ha (metribuzin 300 g/ha), the effect of Zontran CSC approaches to the Analog effect in a dose rate of 1 kg/ha (metribuzin 700 g/ha) (Fig. 2). By the effectiveness of the action on annual grass weeds, the use of the herbicide Zontran CSC in dose rates of 0.8 and 1.2 l/ha was comparable to the efficiency of the Analog in the dose rate of 0.5 kg/ha (metribuzin 350 g/ha) and 1 kg/ha (metribuzin 700 g/ha), in the dose rate of 0.6 l/ha the effectiveness was lower. (Fig. 3). In variants with the Zontran CSC application of 0.8 and 1.2 l/ha the weight loss of annual dicotyledonous weeds was 98-100%, the weight of annual grasses species - 94-100%, which corresponded to efficiency of Analog in the dose rate of 1 kg/ha. Weight loss of weeds with the use Zontran CSC of 0.6 l/ha was at the level of Analog efficiency of 0.5 kg/ha, which was 89-98% for annual dicotyledonous weeds and 88-93% for grasses.
Elimination of competition with weeds in the initial period of crop vegetation allowed to save from 26.1 to 58.7 % of the soybeans harvest an medium weediness of crops (annual dicotyledonous weeds 48-49 ind./m^2 (194 g/m^2), annual grasses 60-63 ind./m^2 (360 g/m^2)), and from 28.2 to 32.9 % with high weediness (annual dicotyledonous weeds 242-256 ind./m^2 (580 g/m^2), annual grasses 151-154 ind./m^2 (315 g/m^2)). (Table. 1)

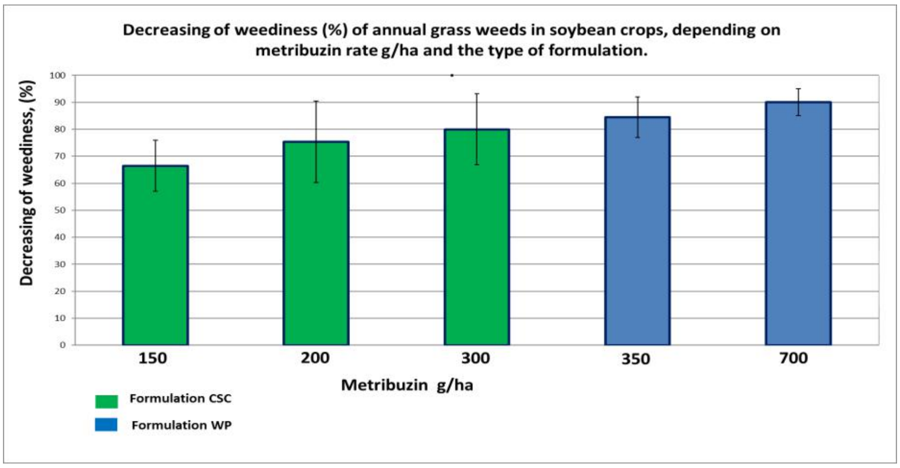


Fig. 2 The biological effectiveness comparison of Zontran CSC and another metribuzin-containing product Analog WP on annual dicotyledonous weeds in soybean crops, depending on the application rate of metribuzin g/ha and the type of formulation

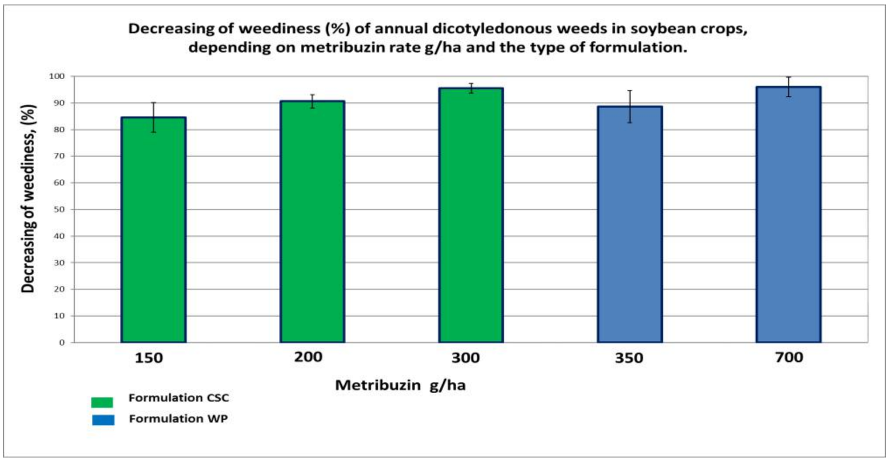


Fig. 3 The biological effectiveness comparison of Zontran CSC and another metribuzin-containing product AnalogWP on annual grass weeds in soybean crops, depending on the application rate of metribuzin g/ha and the type of formulation.

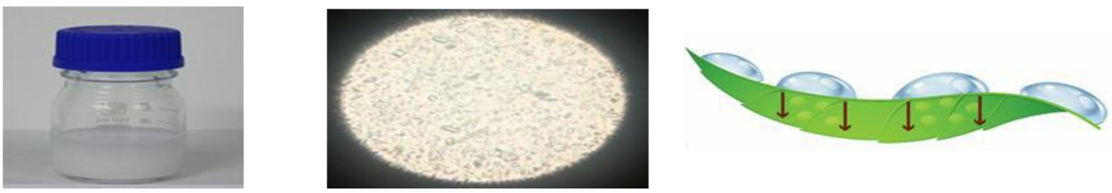
Table 1. The influence of herbicides of different formulations on the saved yield of the soybean crops (variety Annushka) with a high and medium degrees of weediness

Weediness	Yield in control hundredweight /ha	Saved yield (%) when applied Zontran CSC 1,2 l/ha	Saved yield (%) when applied Analog WP 1 l/ha
High	4,6	26,1 - 58,7	32,6 - 58,7
Medium	14,9	28,2 -32,2	30,2 - 35,6

By the photon correlation spectroscopy method, the particle size of Zontran CSC was determined as 1.327 nm. A small size of particles carrying the active substance leads to suggest that the "targeting" of metribuzin delivery of Zontran CSC will be higher than the metribuzin from Analog formulation WP with a particle size of 5 mkm. The stability of an emulsion depends on many factors, for example, surface tension, viscosity of the dispersion medium, temperature, particle dispersion, etc. But of all the above, the main factor of emulsion stability is the size of the emulsified particles, that is, its dispersion. When the size of the droplets decreases, the effect of gravitational forces decreases and the forces that keep them in a stably suspended state begin to prevail (Kireev,1978).

Colloidal Solution Concentrate (CSC)

A **B** **C**



A **B** **C**

Wettable Powder (WP).

Fig. 4 Comparison of two formulations: CSC and WP.

A- is the appearance of the working solution,

B- is a view of the formulation homogeneity by the light microscope (1600x),

C- is the intended effect on the surface of the plant leaf.

It was proved that the surface tension of a droplet containing the active substance in the CSC form will be less (37 mNm) than the WP formulation (46 mNm), which means that the droplet spreading over the surface and holding it on the plant leaf for CSC will be higher than the WP (Fig. 4) Due to the above factors, the efficiency of using the active substance in the CSC formulation will be higher than in the WP formulation, which allow to reduce the dose rate of the active ingredient per unit of cultivated area and, using colloidal formulation, achieve a similar or increased biological efficiency compared to standard forms.

Conclusions

The effect of application of Zontran CSC on soybean crops at a dose rate of 1.2 l/ha was comparable with product contenting at 2.3 times more metribuzin but in WP formulation, while the application rate of metribuzin per 1 ha is 300 g/ha and 700 g/ha, respectively. Thus, the CSC formulation allows reducing the amount of the active substance per unit of cultivated area without loss of biological efficiency and with obtaining reliable yield increases. The product Zontran CSC was included in the State Catalog of Pesticides and Agrochemicals permitted for use on the territory of the Russian Federation (State catalog of pesticides and agrochemicals, 2018) in soybean crops with a dose rate of 0.6-1.2 l/ha.

Acknowledgement

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THE PLACE OF GREEN PRODUCTION IN ENVIRONMENTAL PROTECTION AND NATURAL RESOURCE MANAGEMENT

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Abstract
The rapid increase of the population every day, it also increases production with consumption. Recycling and green production concepts gained importance as a result of the negative consequences of the wastes released to the environment during the production in the soil, water and air. With the implementation of these concepts, waste from the environment has been reduced with the recycling of waste, on the other hand, natural resources are preserved. at this point, there is a social responsibility for both producers and consumers. Reducing the damage to the environment as much as possible in production, the use of renewable resources in production rather than natural resources is a major task for producers; consumers also prefer to use environmentally friendly green products while consuming. This choice forces manufacturers to produce green. In this study, studies on green production related to environmental protection and non-consumption of natural resources were evaluated. In addition, the contribution of the sample companies that match the green production to the environment, natural resources and the country's economy has been examined.

Keywords: *Green Production, Environmental Protection, Eco-Friendly Product, Natural Resources.*

Introduction
In order to survive, people interacted with the environment continuously and made it easier for them to use natural resources. However, the rapid increase of the population, the construction of giant factories with the industrial revolution, global warming, rapid industrialization with mechanization, harmful wastes, unconscious resource use have caused air, water and soil pollution. All of these have caused both depletion of resources and deterioration of ecological balance. As a result, consumers have realized that natural resources are rapidly depleting and that natural life is now moving towards uninhabitable points and that act within the framework of this responsibility. These consumers, who are considered as green consumers, are more sensitive about waste of resources, environmental pollution and recycling of products (Haden et al., 2009; Kükrer, 2012).
Rapidly increasing environmental problems are no longer an individual problem and have become a concern for the whole society. Within the framework of these problems, it is clearly seen that the sensitivity of consumers towards the environment has increased and it is necessary to reflect this sensitivity to the behaviors of people. However, although the majority of consumers are worried that natural resources are rapidly depleting and nature is being destroyed, it is seen that this is not only a matter of concern, but does not reflect how they should be treated in the process of buying, consuming and following the use (Hussein & Cankül, 2010). In the face of such a problem, it is important to raise awareness of consumers against environmental problems and to ensure that less harmful products are consumed.
The use of the world's resources as if they were never exhausted, and environmental problems that threaten people's lives and ecosystems have pushed states to work to protect and sustain the natural environment. In fact, it is necessary to develop international cooperation and environmental policies since the countries understand that the problem cannot be solved alone and improvement works will not be enough. In line with these developments, the pressures of

production and service companies to pay more attention to the environmental-based results of the products and services they produce and the processes they apply are increasing exponentially (Kleindorfer et al., 2005).

Green Product Concept

Product; color, brand, packaging, design, after-sales services are the aggregate of concrete and abstract qualities including the image of the seller (Altunışık et al., 2016).

Green product; Although it is expressed as more environmentally friendly, the recovery or disposal of wastes can be defined as the product that uses the energy sources at the lowest level, which helps to protect the natural environment (Ottman et al., 2006).

In order to qualify a product as a green product, there are some features that it must carry. These features are briefly (Moisander, 2007; Elkington et al., 1990);

- ✓ Manufacturing, consumption and after-consumption processes should be in a way that will not harm the environment,
- ✓ Not disproportionately consume energy and other resources in production, consumption and storage,
- ✓ Do not contain harmful materials that do not harm the environment or species, do not cause environmental pollution, and contain reusable packages,
- ✓ It must be of a quality that will not harm human and animal health,
- ✓ Should not cause unnecessary waste / waste in nature due to excess packaging or short-term use,
- ✓ Should not be used unnecessarily and should not torture animals,
- ✓ It should not harm the environment during storage.

When the previous researches are examined, it is understood that 70% of the damage caused by a product occurs in the production and design stages of the product (Polonsky & Rosenberg, 2001).

Therefore, the green product, will not pollute the environment, all or a large majority can be re-nature, packaging, brand, design, after-sales services, water and energy saving, taking into account a number of features such as the use of raw materials to production and consumption so that the process should be designed to cause minimal damage to nature (Mahlangu, 2014).

Green Production Approach

Green production includes production processes that use inputs with little impact on the environment, do not generate waste or generate very little waste and do not generate environmental pollution. Atlas and Florida examined green production alternatives under five headings (Atlas & Florida, 1999): change in production inputs, internal use of waste, change in product, better management and change in production processes.

Green production of enterprises, that is, the management level to act with a green management approach, will provide serious advantages in terms of sustainability by focusing on continuous improvement of the enterprise (Kautto, 2006).

Economic and Environmental Effects of Green Production Practices

In 2016, 18 projects realized within the scope of energy efficiency activities at 4 refineries within Tüpraş saved 30,186 TJ of energy and 92,675 tons of CO_2 emissions. The amount of greenhouse gas emissions reduction in the last 10 years has reached 2.1 million tons.

In 2016, Tüpraş continued its water recovery activities. Thanks to these efforts, 20 million m^3 of water was recovered.

In 2016, Tüpraş recovered 67% of 41,506 tons of waste, of which 30,872 tons were hazardous and 10,534 tons were non-hazardous. Non-recyclable wastes were disposed of by licensed companies with the norms and methods specified in the legal regulations.
Within the scope of afforestation activities, Tüpraș planted 600 saplings in 2016 and prevented emissions to 200 tons of CO_2 (Tüpraş).

PINAR

Thanks to the optimization efforts in its production processes, Pınar Süt has created less waste water and reduced water consumption by 9%. It also reduced the amount of paper used in cheese and yogurt cartons.
Pınar Et reduced its total carbon emissions by 11% compared to 2010. Within the scope of box improvement works, it has reduced the amount of paper used per box by 30% in some of its products. The carbon emissions from shipping parcels were also reduced. With the change of design in the boxes of the open-end sliced products; It has used 104 tons less paper, resulting in a 6.4% reduction in the use of paper packaging. Annual carbon emissions from these products decreased by 87%.
Pınar AŞ planted 1 tree for the employee who brought 10 batteries with his "voluntary employee project". A total of 1000 trees were planted. Thanks to the non-disposable batteries, 6,000 m2 of soil and 600 million liters of water are prevented from being polluted.
Pınar Su has reduced its carbon emissions by 13% over the previous year (Pınar, 2019).

opet

Opet prevented 104 tons of CO_2 greenhouse gas emissions in 2014 and 106 tons of CO_2 in 2015 as a result of its energy efficiency efforts.
With the establishment of the solar power plant at the Mersin Terminal, it supplies 8% of its annual energy need from a renewable energy source.
In 2015, 80% of hazardous wastes generated at its terminals and 85% of non-hazardous wastes were recycled.
The volume of water recycled and reused in 2015 was 84,571 m3 with a recovery rate of 57% (Opet, 2019).

Ford

Ford Otosan attaches importance to providing the energy they use in production from renewable energy sources. With the installation of wind turbines and solar panels, it produced 89 Gj of energy in 2017. This ratio increased to 2,368 Gj in 2018. Thanks to the energy efficiency projects it has implemented, it has saved 79,869 Gj of energy and prevented 4,860,6 tons of CO_2 emissions. With the projects developed within the scope of water management, 1.17 million m3 of water was consumed in 2018 and 25% of this was recovered. In 2019, they redesigned their water management projects in order to recover 75-80% of the water used. With the optimization of the production process, the consumption of the chemicals used was reduced and productivity was achieved. Accordingly, they reduced solvent consumption per vehicle by 18%. In 2018, a total of 95,364 tons of waste was recycled (Ford, 2019).

Unilever explains that by 2025, 100% of the plastic packaging used in its products will be recyclable. With its new factories, design and production systems, the company is implementing a 50% reduction in carbon emissions and water consumption compared to similar production centers. At the end of 2017, 56% of agricultural raw materials were obtained from sustainable sources (Unilever, 2019).

In 2018, Tofaş achieved 79,935 GJ of energy savings and 5,387 tons of CO_2 greenhouse gas emissions reduction through energy efficiency projects. The company has recovered 64,555,900 m3 of water used in vehicle production with its innovations. 100% of the packaging wastes are recycled and reused (Tofaş, 2019).

Thanks to effective water management practices, it saved approximately 199,862 m^3 of water in six countries. This figure replenishment rate reached 150% in Turkey. In other words, for each liter of water used in production, it replaces 1.5 liters of water. This equals approximately 4,271 million liters of water. As a result of the projects carried out by Coca Cola, the company achieved an improvement of 468 million kWh in energy consumption in its factories in 6 countries. This corresponds to a reduction in CO_2 emissions of approximately 78,711 tons. This figure corresponds to the amount of CO_2 absorbed by approximately 6.6 million trees a year. Total waste recycling plant in all of the Company 96.0% in Turkey, Jordan, 91.90%, 90.0% in Kazakhstan, 97.8% in Azerbaijan, Kyrgyzstan is 94.44% and 97.7% in Pakistan (Cci, 2019).

With its solar, water, wind and biogas fuel cell projects, Apple meets the power requirement of all its facilities with 100% renewable energy. Since 2011, the use of renewable energy has reduced CO_2 equivalent emissions of its worldwide operations by 54% and prevented

approximately 2.1 million metric tons of CO_2 equivalent emissions into the atmosphere. Even in 2017, the company reduced its CO_2 equivalent emissions by about 590,000 metric tons (Apple, 2019).

Starbuck saves energy and water by using environmentally friendly materials and systems that reduce carbon emissions through environmentally friendly stores. One of the notable campaigns of Starbucks is the project of transforming cardboard glasses into plastic cups. Significant water savings are achieved in the stores with the renewed water tank system. Energy saving bulbs are used to reduce energy requirements and carbon emissions. Used coffee grounds can be used as fertilizer to feed plants by offering them free of charge (Starbucks, 2019).

Turkey's first hybrid bus carrying the Hybrid Avenue feature uses the Siemens ELFA Hybrid Systems. These systems exhibit an environmental approach with lower carbon dioxide emissions and zero emissions. TEMSA Hybrid Avenue saves up to 25 percent in fuel consumption and carbon dioxide emissions compared to diesel buses (Capital, 2019).

Türk Telekom provides carbon savings through its efforts to use alternative energy sources such as solar energy and wind energy. In 2010, Türk Telekom became the first Turkish telecommunications company to report to the CDP (Carbon Disclosure Project). In 2016, carbon emissions decreased by 16% with efficiency and abatement projects compared to the previous year (Turktelekom, 2019).

The recycling rate of ARÇELİK A.Ş. products varies between 83.9% and 98%. Approximately 1650 trees were cut down by 85% and 60% reduction in the rate of wood used in the packaging of the products. Logistics "Environmentally Responsible Networks Network" approach is adopted. In this context, is made with approximately 70% of the sea's exports to Turkey, arrival ports after also preferred that is sensitive to the environment in all possible destinations railway and-borne transport. At the same time, low-emission vehicles are preferred in the vehicles used within the scope of Authorized Services and Consumer Services, and vehicles over the age of 5 are renewed and continuous emission improvement is provided. (Arcelikas, 2019).

The company has saved more than 1.8 billion liters of water and recycled more than 129 million liters of water so far by reusing the water used during production and switching to new finishing methods that require less water than traditional production processes (Levis, 2019).

Conclusions

The rapid depletion of natural resources, the state's sensitivity to the environment and consumer preferences for environmental products increase competition conditions day by day. Consumers have begun to demand products that are renewable, create less pollution, are safer for the ecosystem and are recyclable. At this point, the presence of consumers who behave more consciously than in the past provides an environment for producers to be environmentally friendly. Another point that needs to be considered here is that the conscious consumer group, who conducts research and questioning, wants to know how nature is protected not only from environmental certificates on products but from the production stage to the consumption stage. In other words, whether the factories where the products are produced does not harm the environment, whether the products are raw materials, packaging,

whether the energy used is environmentally friendly or even the transportation operations used during the delivery of the product to the final consumer affects the product preferences of the conscious consumers. Therefore, consumers' preference situations will make significant contributions to the planning of the enterprises for their environmentalist approaches, the structuring of production strategies, production structuring and marketing management.
Starting from this point of view, developing state strategies to raise awareness of consumers about green consumption and carrying out awareness-raising activities in particular to increase the environmental sensitivity of the young generation will encourage consumers to receive environmentally sensitive products and services from production to consumption. In this case, it will require producers to produce environmentally friendly products in order to survive.

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POTENTIALS OF PRODUCED WATER *BACILLUS SP.* FOR PHA PRODUCTION AND PLANT GROWTH PROMOTION

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Abstract

Produced Water (PW) is the major waste water stream of petroleum industry. This produced water, due to its unique chemical composition, supports many hydrocarbon-degrading microorganisms. The presence of high carbon content and low nitrogen content indicated that produced water was an ideal environment for many bacterial species that produce polyhydroxyalkanoates (PHA), an intracellular storage polymer. The main purpose of this study was to isolate bacterial strains that can produce large amounts of PHA on different carbon sources. While *Bacillus* sp. producing PHA used for Plant microbe interaction studies carried out with wheat crop.Produced water sample was collected from Potwar oil fields in sterilized bottles and stored at 4^{o}C. Sample was appropriately analyzed for many parameters including temperature, pH, odor, texture and color. Isolation of bacterial strains was performed according to serial dilution method. Isolated strains were screened for PHA production by using PHA detection media supplemented with Nile blue. Plant growth promotion experiment was done on PHA producing *Bacillus* sp. with wheat crop (*Triticum aestivum*).The use of glucose as sole carbon source resulted in highest production of PHA (43%), after 24 hours cultivation by *Bacillus sp.*(MH142143). It showed significant results in bioremediation of hydrocarbons. Maximum germination of 93% was observed in wheat seedlings when supplemented with*Bacillus sp.* with marked increase of 72.87cm in wheat seedling length in lab scale experiment. Results of this study helps in the elimination of hydrocarbon from produced water by *Bacillus sp.* and make it suitable for agriculture.

Key words: *Produce water, Bacillus, Triticum aestivum, polyhydroxyalkanoates, Plant microbe interaction.*

Introduction

The use of petroleum and petroleum base polymers has increased to an alarming degree. Polyhydroxyalkanoates (PHA) are biodegradable plastics that have the potential to replace these petroleum-based polymers.Polyhydroxyalkanoates (PHAs) are inclusion bodies, used as storage reserves by the cell. Most common PHAsare polyhydroxybutyrate (PHB) (Santhanam & Sasidharan, 2010), produced by *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Rhodococcus*, *Agrobcterium*, *Comamonas*, *Hydrogenophaga*, *Ralstonia* etc. (Lee, Kang, & Choi, 1995). These bio-polyesters are biodegradable as well as biocompatible (Povolo et al., 2013).

The properties of PHAs are strongly dependent on their monomeric composition and structures. Poly (3-hydroxybutyrate-co-hydroxyvalerate) has lower crystallinity and melting point than poly (3-hydroxybutyrate). Whereas polyethylene has low tensile strength and malleability as compared to poly (4-hydroxybutyrate) (Povolo et al., 2013). Increase in poly (3-hydroxybutyrate) concentration among PHA monomers increases melting point. Incorporation of specific monomers tends to enhance thermal stability (Khosravi-Darani, Mokhtari, Amai, & Tanaka, 2013).

The chemical composition of PHA is dependent onbiosynthetic pathway and PHA productionenzymes. Classification of PHAdivides them into short chain length PHA (scl

PHA), medium chain length PHA (mcl PHA) and long chain length PHA (lcl PHA) units. Scl PHA, ranging from C3 to C5, include 3-hydroxypropionate, 3-hydroxyvalerate etc. are produced mainly by *Ralstonia* and *Alcaligenes* species (Pantazaki, Papaneophytou, & Lambropoulou, 2011). Mcl PHA, ranging from C6 to C14, include 3-hydroxyhexanoate, 3-hydroxytetradecanoate etc. are produced mainly by *Pseudomonas* species. Whereas lcl PHA have >C14 PHA units (Povolo et al., 2013). Some bacteria also produce copolymers of PHA (Khosravi-Darani et al., 2013). There are four main classes of PHA synthases. Scl PHA are mostly produced by bacteria having synthases belonging to Group I PHA synthases while mcl PHA are typical produced by bacteria having Group II PHA synthases (Povolo et al., 2013). There are three main pathways of PHA production. All three pathways along with other pathways, without exception, need PhaC for successful production of PHA (Tsuge, 2002).

Materials and Methods

Produced water sample was collected and properly labeled with collector's name, site of collection, time of collection and date of collection. Sample was stored at 4oC and transported to laboratory for further characterization. Sample was appropriately analyzed and characterized.Dilutions of sample were prepared by serial dilution method and aseptically spread on Luria Agar plates. Plates were incubated at 37^{o}C for 24 hours. After incubation, colony count was estimated using colony counter. CFU/ml was calculated by using following formula: CFU/ml = $Number of colonies Dilution Factor x Volume plated$

Colonies with distinguishing features were selected from the mixed culture plates to obtain pure colonies. Plates were incubated at 37oC for 24 hours. After incubation, colonial characteristics such as shape, size, elevation, margin, pigmentation, etc. of cultures were recorded.

Isolated strains were screened for PHA production by using PHA detection media supplemented with Nile blue or Nile red(Greenspan et al., 1985), followed by staining of screened colonies with Sudan black dye to visualize PHA granules(Kitamura & Doi, 1994). After incubation, plates were observed under UV illuminator for fluorescence around culture growth(Chaudhry et al., 2011). Bacterial smears were prepared from 24-hour culture of isolated strains cultivated on PHA detection media. Smear was flooded for 15 minutes with Sudan black dye and decolorized with xylene. Slides were washed and counterstained with safranin. Slides were visualized under microscope using magnification 4X, 10X, 40X and 100X (Kitamura & Doi, 1994).

Polyhydroxyalkanoate (PHA) was produced on PHA detection media and extracted from cell mass by using different cheap, renewablecarbon sources e.g. glucose, waste glycerol and animal fat oiland incubated at 37oC for 96 hours. Growth kinetics of isolates were observed and culture densities were measured at 600nm by using spectrophotometer at regular intervals. (Teeka et al., 2010)**.**PHA was extracted using Sodium hypochlorite-SDS method.Dried weight of PHA was weighted and recorded. Percentage of PHA was calculated as follows: % $PHA{=}Weigh\ tof PHA/Weigh\ tof biomass X100$.

PHA production by strains was time profiled at regular intervals over a period of 96 hours.

Selected bacterial strains were used to promote growth of wheat plant, in these experiments produced water was used. The experiments were conducted both at lab and field scale (Khan et al). Polyhydroxyalkanoate (PHA) samples were analyzed by FT-IR around scan range 400 to 4000 cm-1, at Research Centre, Lahore Women's University.

Results and Discussion

Strains were screened for PHA production and 6 out of 13 were found positive. These six strains gave fluorescence on Nile blue and Nile red supplemented PHA detection media. On Nile red, green fluorescence was observed while on Nile blue, blue fluorescence was observed. Binding of dye molecules to PHA granules gives fluorescence (Figure 1). On Sudan staining of these strains, black granules of PHA were observed against pink background (Figure 2).

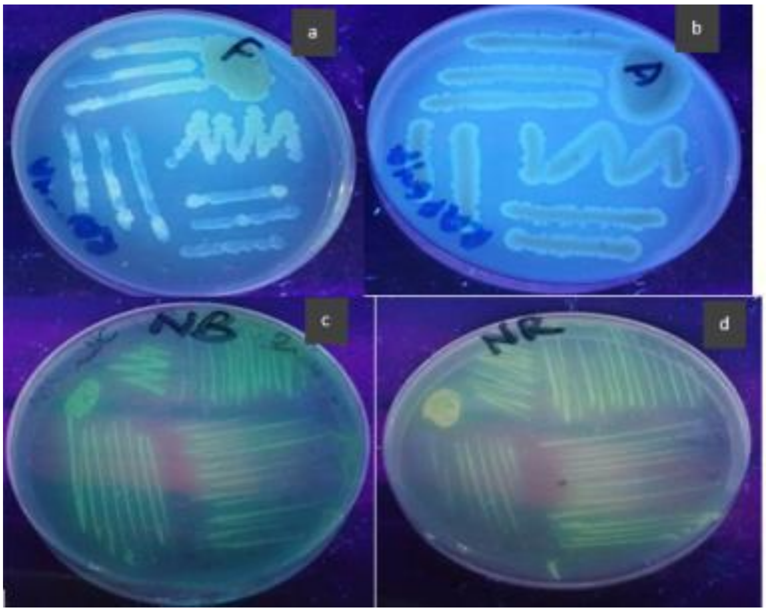

Figure 7 PHA fluorescence on Nile Blue (a, b), Nile red (c, d).

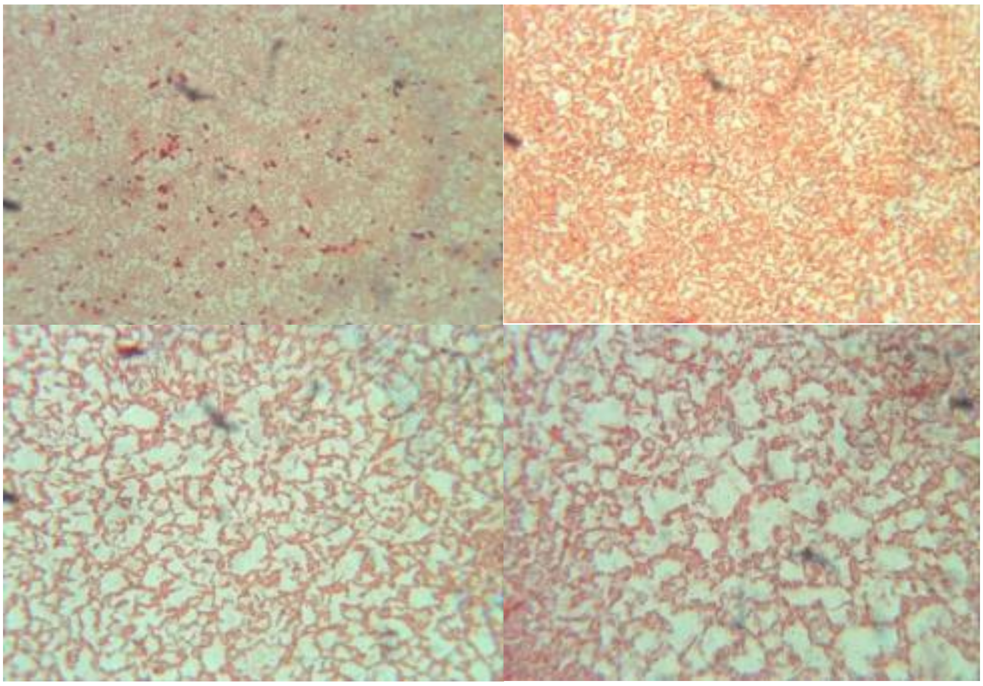

Figure 8 Sudan Staining of PHA granules

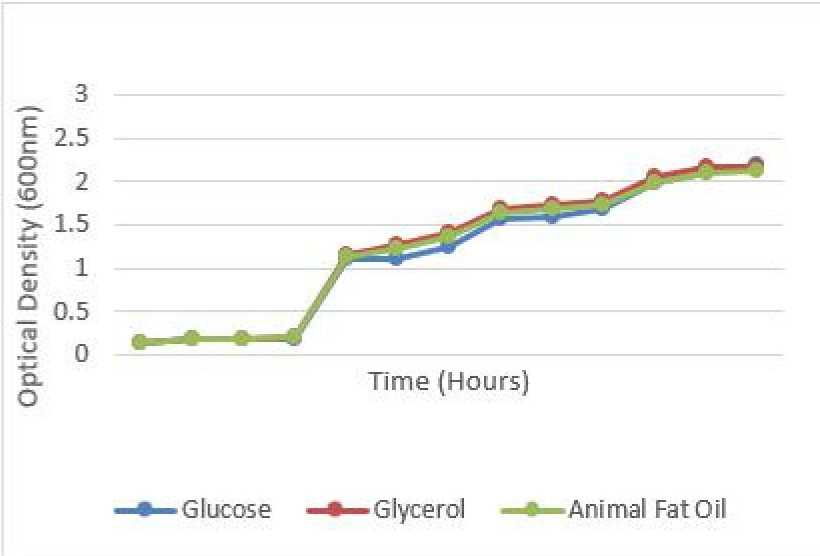

Figure 9 Growth Kinetics of PWA on PHA Detection Media

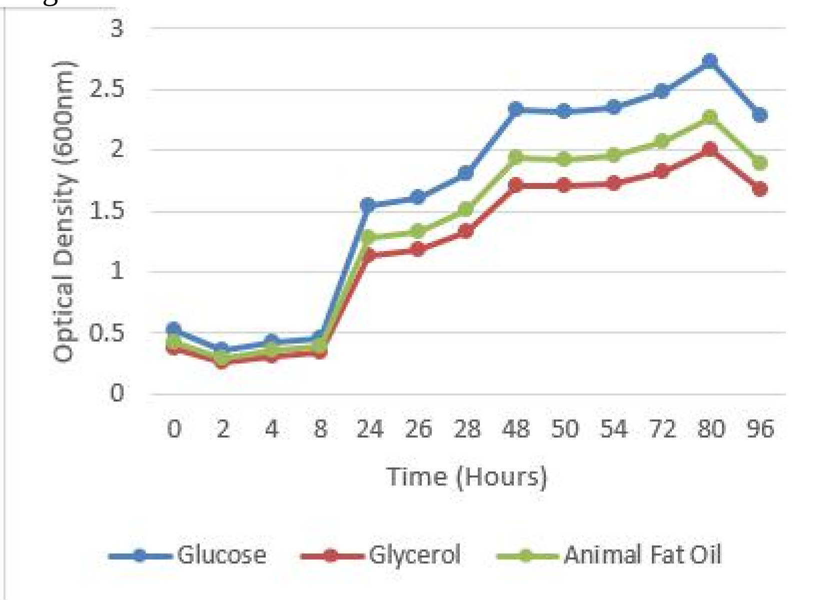

Figure 10 Growth Kinetics of PWC on PHA Detection Media

Overall, highest growth was observed on glucose, followed by animal fat oil and least growth was observed on glycerol supplemented on PHA detection media. All isolated strains were able to grow efficiently on PHA detection media supplemented with these three carbon sources. PWA showed almost equal growth rates on PHA detection media (**Error! Reference source not found.**). PWC showed highest growth rate on glucose, followed by animal fat oil and lowest growth rate on waste glycerol supplemented PHA detection media (**Error! Reference source not found.**). Growth rates of PWC on PHA detection media were slightly higher than growth rates on minimal media.

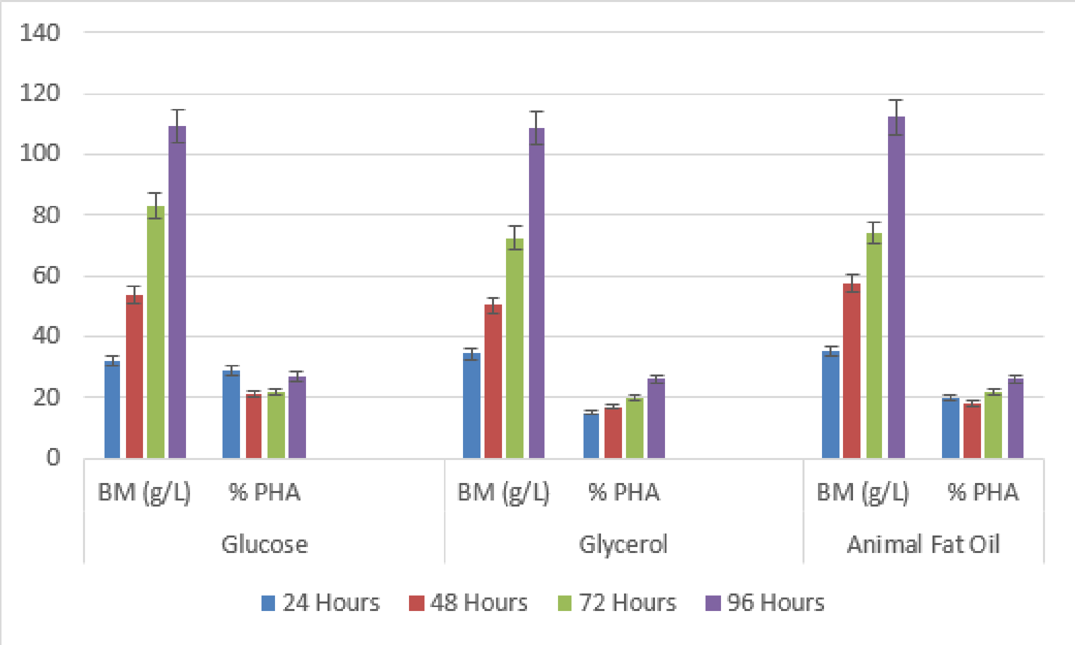

Figure 11 Time profiling for PHA production by PWA on PHA detection media

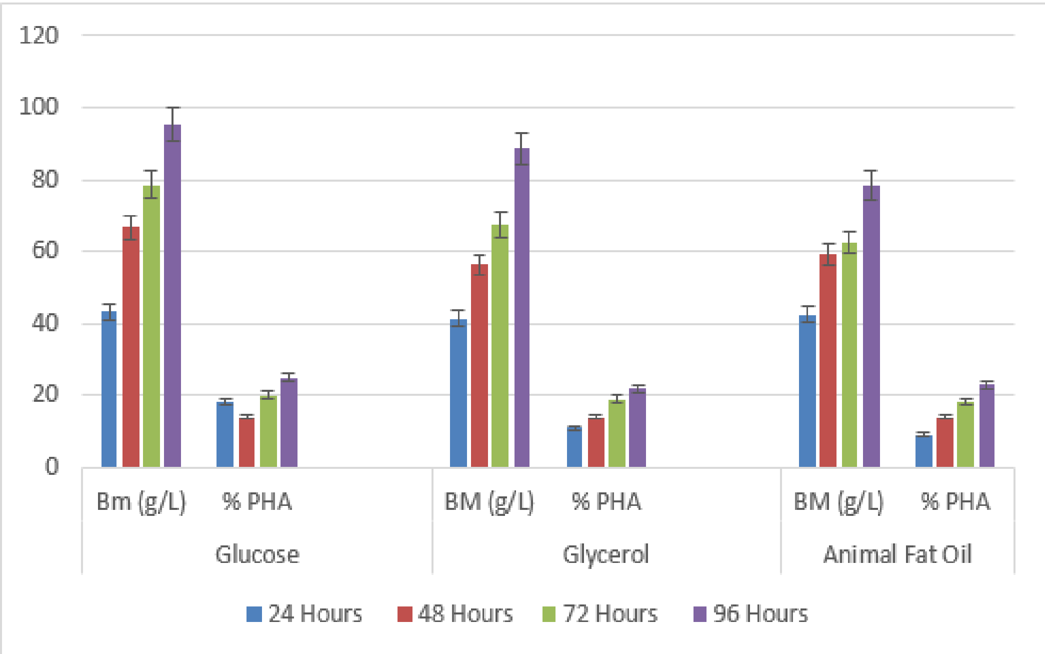

Figure 12 Time profiling for PHA production by PWC on PHA detection media

Time profiling for PWC and PWA indicated overall highest PHA production(Figure 5,6) on using glucose (27%). Films of PHA were obtained for PWA and PWC (Figure7).

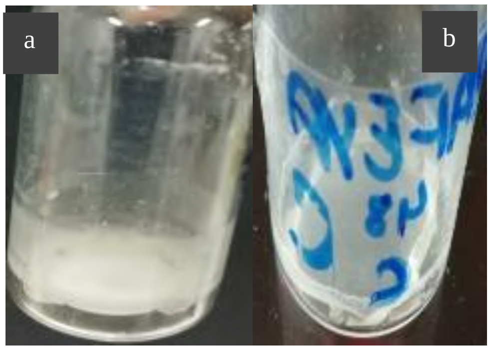

Figure 13 PHA Produced by PWA (a), PWC (b).

Experiments were conducted with wheat for plant microbe interaction (Figure 8). Selected microbial strains PWA and PWC showed an increase in plant growth. Plant microbe interaction experiments were conducted at both lab and field level. Different produced water sample (PW site 1, 2A, 2, 3) were used for these experiments against a control – tap water. Bacterial strains used were PWA and PWC, previously found to be PHA positive strains. These experiments showed more than 90%increase in seed germination rate as compared to control.

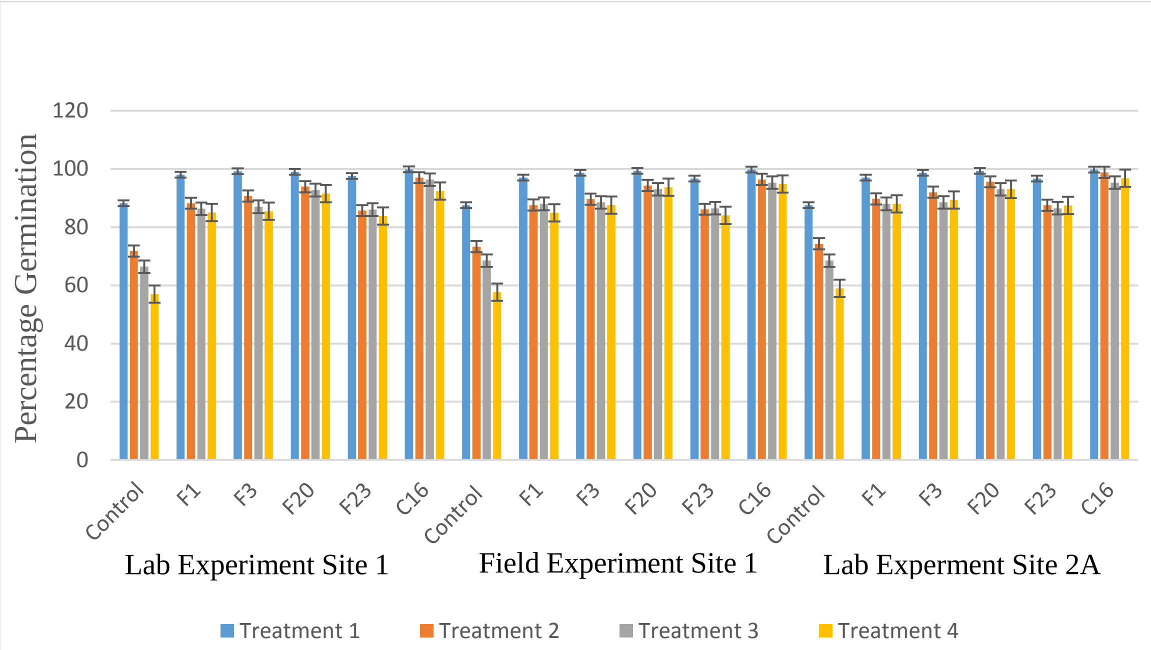

Figure 14 Percentage Germination of Wheat Seedlings in Lab Experiment (with PW Site 1), Field Experiment (with PW Site 1) and Lab Experiment (with PW Site 2 A) of each selected strains

In lab scale experiments maximum increase of 15.78 cm was recorded in shoot length of wheat seedlings. In field experiment the increase was 62.55 cm in shoot length, soil was treated with produced water from site 3. Maximum seedling length of 72.88cm was recorded when PWA and PWC bacterial strains were used along with produced water from site 1.

Conclusions

This study showed the use of complex hydrocarbons by Bacillus sp. from produced water – treated water can be used further in agriculture. Wheat plant – staple food for many countries showed 93% germination rate and 72.87 cm increase in seedling length. Selected strains were also able to produce PHA – a biodegrade-able plastic.

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LITHUANIAN COMPOST QUALITY: PHYSICAL CHARACTERISTICS, NUTRIENT CONTENT, HEAVY METALS AND ORGANIC POLLUTANTS

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Abstract

The aim of this study was to investigate the quality of composts produced in Lithuania. Physical characteristics, nutrient, heavy metals and organic pollutants contents were determined in green waste, sewage sludge, mixed municipal waste and mixed municipal waste composts after mechanical biological treatment. After analysing physical and chemical parameters of compost quality, it was found that sewage sludge compost contained the highest amounts of nitrogen (2.98%), phosphorus (4.44%) and organic matter (47.6%), and the highest potassium content (1.20%) was found in mixed municipal waste composts after mechanical biological treatment. Having analysed green waste compost samples, it was found that the green waste compost samples of the first year were the most contaminated with heavy metals. Those samples manifested the highest concentrations of all metals investigated during all the research years, except for chromium (Cr). The content of polychlorbiphenyls in different composts varies from a minimum of 0.92 μg kg^{-1} found in green waste compost up to a maximum of 505 μg kg^{-1} in mixed municipal waste compost. PAHs of high molecular weight are predominant in green waste composts, and the ratio between high and low molecular weight PAHs in sewage sludge composts is almost equal. The distribution of low and high molecular weight PAHs in mixed municipal waste composts and composts from mixed municipal waste after mechanical biological treatment is uneven, depending on the different research years this ratio ranged from 93:7 % to 11:89 %.

Keywords: *compost, persistent organic pollutants, heavy metals, nutrients content.*

Introduction

The increasing amount of waste causes the problem of waste disposal, therefore, there is an on-going search for ways to reduce the negative impacts of accumulated waste. The regional waste management centres established in Lithuania implement Directive 2008/98/EC of the European Union, which provides for the reduction of biodegradable waste going to landfills. Each such centre is equipped with composting sites, which compost both tree leaves fallen in autumn and other types of biodegradable waste collected from streets and individual urban residents. Sewage sludge and mixed biodegradable municipal wastes are composted in many regions of Lithuania. Composting is an alternative way of managing biodegradable waste (Saveyn and Eder, 2014, Staugaitis et al., 2015). During composting, biodegradable waste is affected by microorganisms and decomposed, thus a new valuable product – compost – is obtained. High-quality compost is a valuable fertiliser and soil improver. The incorporation of compost into the soil improves its structure, sorption properties, enriches it with the nutrients and helps to fight against soil organic matter degradation (Srivastava et al., 2016). Therefore, as this type of organic fertiliser is gaining popularity, it is particularly important to ascertain both positive and negative effects of it on the environment, soil and plants. Compost can be a source of pollution with heavy metals, persistent organic pollutants and microorganisms (Alvarenga et al., 2017; Gusiatin and Kulikowska, 2014).

The aim of this study was to investigate the quality of composts produced in Lithuania. Physical characteristics, nutrient, heavy metals and organic pollutants contents were determined in green waste (GWC), sewage sludge (SSC), mixed municipal waste (MMWC) and mixed municipal waste composts after mechanical biological treatment (MMWCABMT).

Material and Methods

All chemical parameters of composts were carried out in 2015-2017 years at Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry.
PH_{H2O} was measured by a combined (mixed) electrode according to EN 13037:2012 (Soil improvers and growing media –determination of pH). Organic matter in the composts was determined by the gravimetric method according to the standard EN 13039:2012 (Soil improvers and growing media – Determination of organic content and ash), total nitrogen (N) was determined by the Kjeldahl nitrogen distiller in accordance with the standard EN 13654-1:2012 (Soil improvers and growing media – Determination of nitrogen. Modified Kjeldahl method), total phosphorus (P_2O_5) and sulphur (S) were determined in *aqua regia* by the spectrometric method according to EN 13650:2001 (Soil improvers and growing media – Extraction of *aqua regia* soluble elements) and EN ISO 11885:2009 (Water quality – Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)) using an atomic-emission spectrometer Optima 2100 DV (Perkin Elmer, USA). Organic carbon (C_{org}) was determined by the dry combustion method using a total carbon analyser Liqui TOC II (Elementar, Germany). Total potassium (K) was determined according to EN 13650:2006 (Soil improvers and growing media – Extraction of *aqua regia* soluble elements) and ISO 9964-3:1998 (Water quality – Determination of sodium and potassium – Part 3: Determination of sodium and potassium by flame emission spectrometry) using a flame emission photometer PFP7 (Jenway, UK). Heavy metals in composts were determined by the standards ISO 11466:2016 (Soil quality – Extraction of trace elements soluble in *aqua regia*) and ISO 11047:2015 (Soil quality – Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc – Flame and electrothermal atomic absorption spectrometric methods) using the atomic-emission spectrometer Optima 2100 DV (Perkin Elmer). Non-instrumental methods were used for the analyses of dry matter, electrical conductivity and laboratory compact bulk density. The quantity of polycyclic aromatic hydrocarbons (PAHs) and polychlorbiphenyls (PCBs) was determined according to laboratory-developed methods LVP G-11(Determination of polycyclic aromatic hydrocarbons in soil improvers by liquid chromatography) and LVP G-12 (Determination of polychlorbiphenyls in soil improvers by gas chromatography with an electron capture detector) respectively.
Composition and preparation of composts are described in the publication (Barcauskaitė, 2019).

Results and discussion

After analysing physical and chemical parameters of compost quality, it was found that sewage sludge compost contained the highest amounts of nitrogen (2.98%), phosphorus (4.44%) and organic matter (47.6%), and the highest potassium content (1.20%) was found in mixed municipal waste composts after mechanical biological treatment. Of all the composts tested, green waste composts had the lowest content of the following nutrients: nitrogen, phosphorus, potassium, calcium, magnesium and sulfur. In all tested composts, pH was moderately alkaline, except for sewage sludge compost during the second year of research. Concentrations of 7 heavy metals (Cr, Zn, Cd, Ni, Pb, Cu and Hg) were determined in composts of different origin. The results obtained are presented in Tables 1–4.

Table 1. Total amount of heavy metals in green waste compost, mg kg^{-1} d.w.

Heavy metals	Year		
	2015	2016	2017
Chromium (Cr)	12.5±0.028	13.8±6.24	12.1±0.42
Zinc (Zn)	475±24.8	166±61.3	168±9.9
Mercury (Hg)	0.62±0.028	0.01±0.024	0.02±0.001
Cadmium (Cd)	0.34±0.021	0.24±0.080	0.22±0.007
Nickel (Ni)	7.93±0.318	6.61±3.324	6.53±0.424
Lead (Pb)	40.9±0.21	15.1±5.01	16.8±2.90
Copper (Cu)	72.4±1.77	23.6±11.54	30.8±1.27

Table 2. Total amount of heavy metals in sewage sludge compost, mg kg^{-1} d.w.

Heavy metals	Year		
	2015	2016	2017
Chromium (Cr)	79.3±0.85	23.8±14.86	56.3±4.67
Zinc (Zn)	1031±40.3	678±429.5	1363±42.4
Mercury (Hg)	0.53±0.106	0.03±0.023	0.02±0.14
Cadmium (Cd)	2.02±0.028	1.26±0.688	2.66±0.269
Nickel (Ni)	41.7±0.71	20.1±11.05	45.0±2.40
Lead (Pb)	62.9±2.69	26.2±15.96	68.3±3.89
Copper (Cu)	183±21.2	129±72.2	237±13.4

Table 3. Total amount of heavy metals in mixed municipal waste compost after mechanical-biological treatment, mg kg^{-1} d.w.

Heavy metals	Year		
	2015	2016	2017
Chromium (Cr)	6.30±0.113	55.1±22.23	8.32±1.909
Zinc (Zn)	1087±14.1	1365±138.4	1305±7.1
Mercury (Hg)	0.34±0.021	n.d.	n.d.
Cadmium (Cd)	1.83±0.212	1.01±0.418	0.25±0.042
Nickel (Ni)	57.7±1.98	45.3±22.45	5.08±2.192
Lead (Pb)	92.0±2.33	70.9±17.8	7.49±1.294
Copper (Cu)	502±2.1	231±102.4	23.6±1.63

Table 4. Total amount of heavy metals in mixed municipal waste compost, mg kg^{-1} d.w.

Heavy metals	Year	
	2015	2016
Chromium (Cr)	54.3±2.83	74.5±2.75
Zinc (Zn)	1068±49.5	1188±314.9
Mercury (Hg)	0.27±0.071	0.15±0.173
Cadmium (Cd)	1.40±0.071	1.85±1.353
Nickel (Ni)	37.0±0.85	41.6±11.98
Lead (Pb)	116±7.1	117±39.8
Copper (Cu)	298±9.9	338±153.3

Having analysed green waste compost samples, it was found that the green waste compost samples of the first year were the most contaminated with heavy metals. Those samples manifested the highest concentrations of all metals investigated during all the research years, except for chromium (Cr).

The amounts of seven PCBs most commonly described in scientific articles have been investigated in green waste, sewage sludge, mixed municipal waste composts and composts of mixed municipal waste after mechanical biological treatment. The amounts of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180 have been determined. The qualitative analysis for various PCB homologues is equally important to perform as is to study the total amount of them. The durability level and resistance to the effects of microorganisms in PCB molecules depend on the number and position of chlorine atoms (Correa et al., 2010; Pěnčíková et al., 2018). The average distribution of seven different PCBs in composts of different origin produced in Lithuania is depicted in Figure 1.

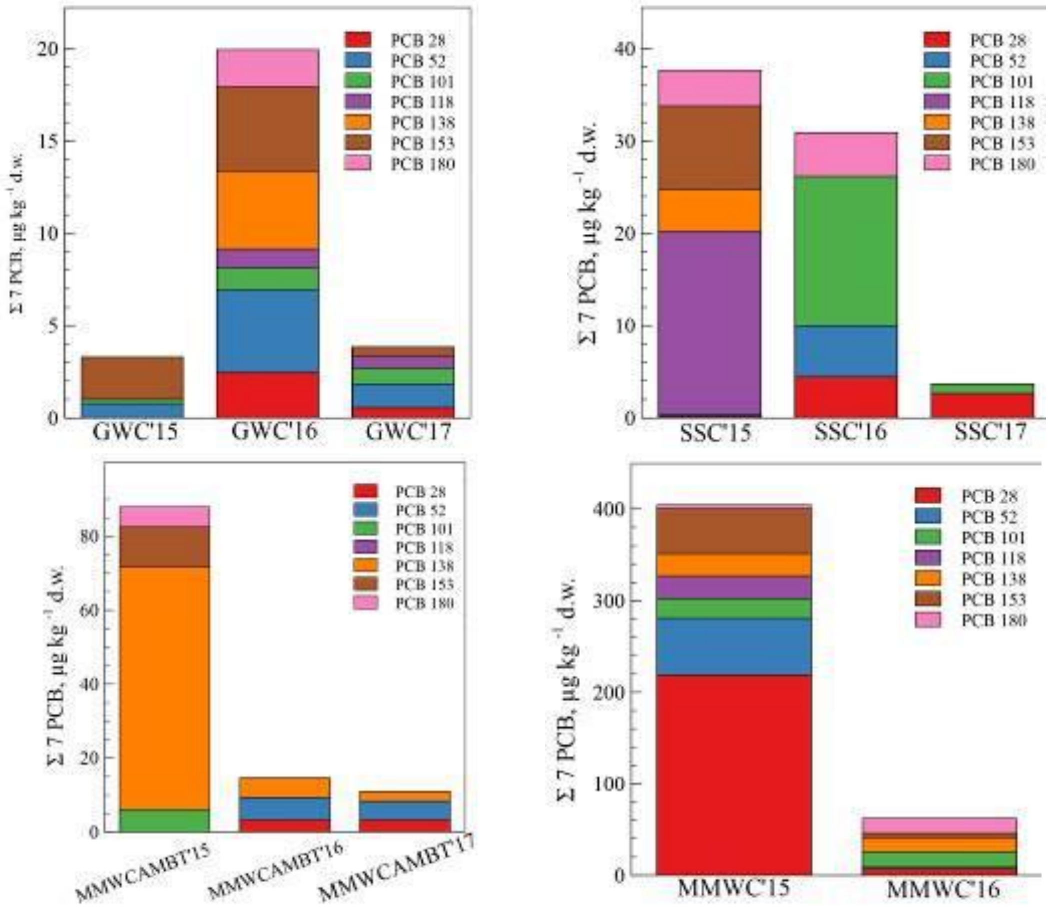

Figure 2. Average distribution of seven different PCBs in composts of different origin

During the research period (2015–2017), a quantitative analysis was performed on 15 PAHs included in the list of priority pollutants by US Environmental Protection Agency (Kafilzadeh, et al., 2015). The dominant PAH homologues in green waste composts were fluoranthene in the first research year, and pyrene and benzo(g,h,i)perylene during the second and third year, respectively. The highest amount of dibenzo(a,h)anthracene was found in sewage sludge composts during the first study year, in the second year the dominant compounds were fluoranthene and phenanthrene compounds, and during the third year, they were dibenzo(a,h)anthracene and fluoranthene. For mixed municipal waste composts after mechanical biological treatment, fluorine dominated during the first and second year of research, which accounted for 82% and 24% of the total PAH content found, respectively. In the third research year the highest levels of pyrene were found. Fluoranthene, pyrene and fluorine were dominant in mixed municipal waste composts.

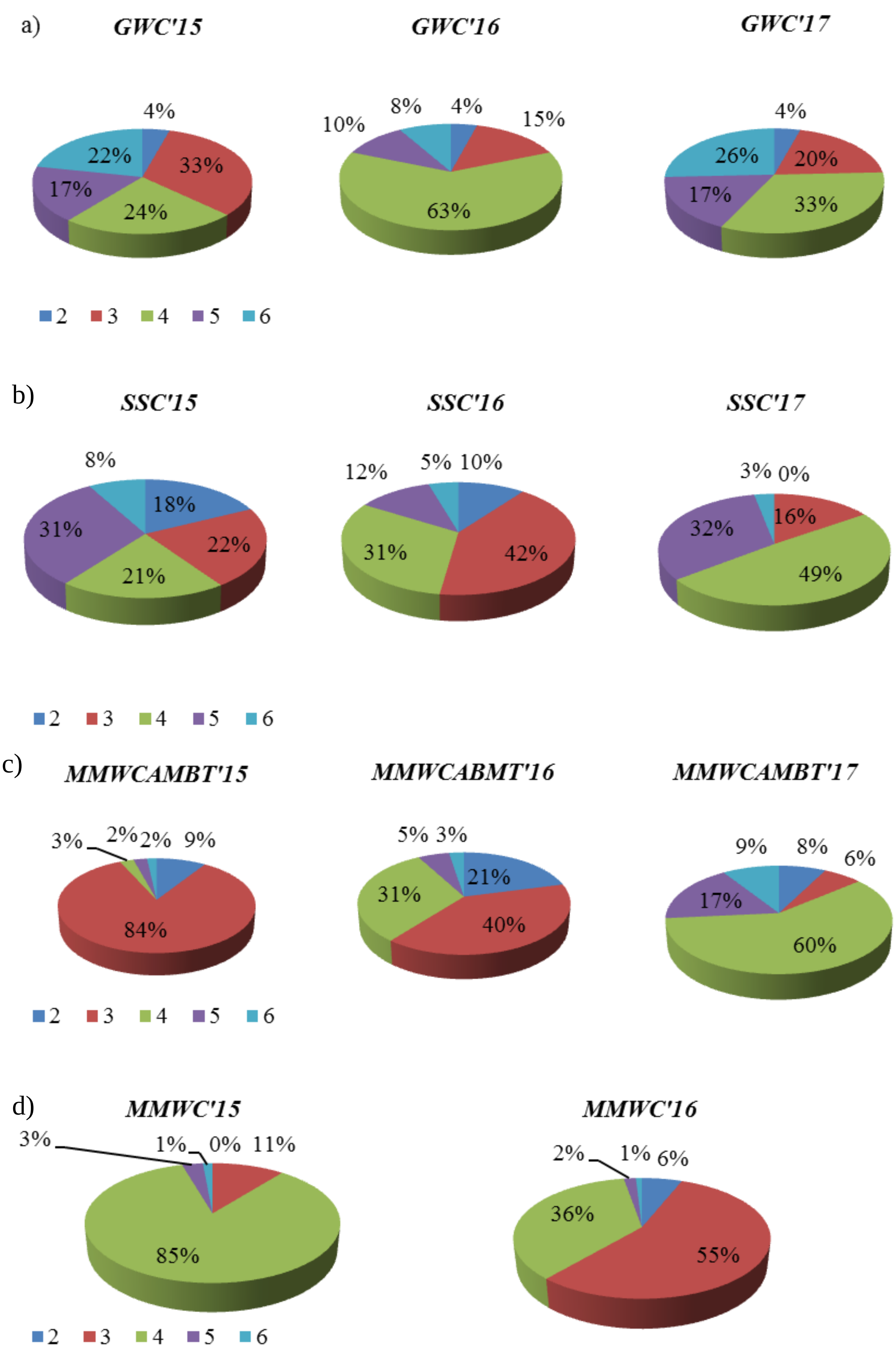

Figure 2. Distribution of polycyclic aromatic hydrocarbons in different kinds of compost according to benzene ring number

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According to the number of aromatic rings, polycyclic aromatic hydrocarbons are classified as low molecular weight (2–3 rings) and high molecular weight (4–6 rings) compounds (Wang et al., 2007). The number of aromatic rings in a polycyclic aromatic hydrocarbon molecule determines their biological and physical biodegradation properties (Melnyk et al., 2015). As shown in Figure 2, high molecular weight PAHs dominated in green waste composts (a). They accounted for 63%, 81% and 76% in a respective research year. Low and high molecular weight PAHs were distributed almost equally (40% and 60% in 2015, 52% and 48% in 2016) in the analysed sewage sludge composts (b), except for 2017, when low molecular weight PAHs were determined to account for 16 %, while high molecular weight PAHs – 84%. Low and high molecular weight PAHs were not distributed evenly in mixed municipal waste composts and composts from mixed municipal wastes after mechanical biological treatment. For mixed municipal waste composts, low and high molecular weight PAHs were found to be 93% and 7% in the first year, 61% and 39% in the second year, and 14% and 86% during the third year, respectively.

Conclusions

Having performed the chemical analysis of composts made from using green waste, sewage sludge, mixed municipal waste after mechanical biological treatment and mixed municipal waste, the highest amounts of nitrogen, phosphorus and organic matter were found in sewage sludge composts, and highest potassium levels – in mixed municipal waste compost after mechanical biological treatment. The content of polychlorbiphenyls in different composts varies from a minimum of 0.92 μg kg^{-1} found in green waste compost up to a maximum of 505 μg kg^{-1} in mixed municipal waste compost. PAHs of high molecular weight are predominant in green waste composts, and the ratio between high and low molecular weight PAHs in sewage sludge composts is almost equal. The distribution of low and high molecular weight PAHs in mixed municipal waste composts and composts from mixed municipal waste after mechanical biological treatment is uneven, depending on the different research years this ratio ranged from 93:7 % to 11:89 %.

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5. ANIMAL HUSBANDRY

EFFECTS OF GENOTYPE AND SEX ON PRODUCTIVE PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKENS

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Abstract
In this experiment, 180 day-old chickens of Ross 308 and Cobb 500 broiler hybrids were used in a 42-day experiment to determine the effects of genotype and sex on productive performance and carcass characteristics. Chickens were housed in a 3-etage cage technology with proportion 75x50 cm (0.375 m^2) for each cage in half-operating conditions. Feed and water were provided ad libitum. We recorded body weight, body weight gain, feed intake, feed conversion ratio and mortality rate. Genotype had a significant effect ($p<0.05$) on feed conversion ratio, Cobb 500 being a better efficient in converting feed than Ross 308. Males were heavier at 42 days of age, gained more body weight, consumed more feed and utilized the feed more efficiently compared with females but had a higher mortality rate. There were significant genotype × sex interaction effects in 42-day body weight, body weight gain, feed intake and mortality rate. In case of carcass characteristics, genotype significantly affected ($p<0.05$) liver and gizzard weights with a heavier values in Cobb 500 than Ross 308. Sex significantly affected ($p<0.05$) slaughter, carcass, breast, legs, liver, gizzard weights with higher values for males than females. In contrast, abdominal fat weight was significantly higher ($p<0.05$) for females compared with males. Genotype × sex interaction effects significantly influenced carcass, breast, legs and liver weights.

Keywords: *chicken, genotype, sex, performance, carcass*

Introduction
The modern broiler chicken production is an extensive and rapidly developing sector, supplying the market with relatively cheap and high-quality dietetic food. Due to contemporary selection programmes, a considerable improvement of weight gain, feed conversion, slaughter yield and breast meat yields were achieved during the past decades (Hristakieva *et al.*, 2014). The aforementioned traits depend on numerous factors, including the genotype and the sex. Many researchers have reported a substantial effect of the genotype on body weight (Razuki *et al.*, 2011), feed conversion, carcass composition (Marcato *et al.*, 2006), carcass weight (Rondelli *et al.*, 2003) and abdominal fat (Hristakieva *et al.*, 2014).
It has been reported that sex affects performance traits of chickens such as body weight, body weight gain, feed intake and feed conversion ratio (Ajayi and Ejiofor, 2009). Sex also significantly affects carcass traits such as carcass composition, proportion of wing, thigh, neck and carcass fat and carcass meat with higher values in males than females (Siaga *et al.*, 2017).
The aim of this study was to assess the effect of genotype and sex on productive performance and carcass characteristics broiler chickens reared under cage technology.

Material and Methods

The experiment was realised in experimental base of Department of Poultry Science and Small Farm Animals on two broiler chicken hybrids (Ross 308 and Cobb 500). Broiler chickens were housed in a 3-etage cage technology (MBD, Czech Republic) consisted of 18 cages with proportion 75x50 cm (0.375 m^2) for each cage. Chickens were fed *ad libitum* with complete feed mixtures – starter (CP min. 210.00 g/kg, ME min. 12.00 g/kg) in powdery form from Day1 to Day 21 and grower (CP min. 190.00 g/kg, ME min. 12.00 g/.kg) in granular form from Day 22 to Day 42 (Boskop, a.s., Trencin, Slovak Republic).

Temperatures were maintained at 33 °C in the 1^{st} week and this was reduced by 2 °C every week then decrease gradually until reach 23 °C in the 6^{th} week. Moisture was retained during fattening period between 50 to 60%. Lighting in the poultry house first day was 24 hours and by starting the 5 day became permanent and 23 hours, used the 40 watt bulbs.

Totally 45 male and 45 female one-day-old chickens each of Ross 308 and Cobb 500 hybrids were kept in this experiment. During the experiment broiler chickens were weighted for individual body weight at 1, 7, 21, 35 and 42 day of age and body weight gain were calculated as the difference between the final and initial chicken weight. Feed consumption, feed conversion ratio and mortality rate were recorded. In 42 day of fattening, representative 10 chickens with body weight similar to the mean were chosen from each group for slaughter weighed and subjected to a 12-hours feed withdrawal. After slaughter, carcasses were weighed and subjected to simplified dissection. The breast, leg (drumstick + thigh), back and wings were collected and weighed. In addition to carcass weight, liver (without gall bladder), gizzard (empty gizzard), heart and abdominal fat weights were recorded individually.

Data were subjected to analysis of variance using one way ANOVA procedure of the statistical system at JASP 0.8.6 software (JASP, 2018). Differences between means were ranked by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

As shown in Tables from 1 to 3, broiler genotype significantly affected Day 7 and Day 21 body weights and feed consumption. In contrast, body weight gain, feed conversion ratio and mortality rate were no influenced by genotype. Chicken genotype affected feed conversion ratio but did not affect Day 35 body weight - Cobb utilized feed more efficiently than Ross, but the genotypes did not differ in Day 42 body weight, body weight gain, feed consumption, and mortality rate. There were significant genotype × sex interaction effects on body weight gain, feed intake, feed conversion ratio, and mortality rate. Our results are similar to those published Benyi *et al.* (2015) but no consistent with findings of Castellini *et al.* (2014), who reported significant differences in feed conversion ratio among hybrids of broiler chickens.

Males were heavier at Day 7, consumed more feed, utilized the feed more efficiently, gained more body weight, and were heavier in Day 21 in comparison with females. We recorded significant genotype × sex interaction effects on Day 21 body weight and body weight gain from Day 7 to Day 21 ($P<0.05$). Sex influenced Day 35 body weight, body weight gain, feed consumption, and feed conversion ratio with better means for males than females.

The two sexes however did not differ significantly in mortality rate. Males consumed more feed and utilized it less efficiently. There were significant genotype × sex interaction effects on 35-day body weight, body weight gain, feed consumption, and feed conversion ratio. Sex significantly affected Day 42 body weight, body weight gain, feed consumption, and feed conversion ratio with a better performance in males than females. There were significant genotype × sex interaction effects in Day 42 on body weight, body weight gain and feed consumption (Table 1 to 3). Similar findings recording Thutwa *et al.* (2012).

The data of carcass characteristics in Table 4 shows that genotype had no significant effects on all the traits. Sex affected wing weight, carcass weight, back weight, and leg weight but did not affect breast weight. Males were heavier than females in carcass, back, wing, and leg weights. Genotype × sex interactions significantly affected carcass, breast, back, wing, and leg weights. Similarly results found Fernandes *et al.* (2013) who found no significant differences in carcass yields of Ross, Cobb, Hubbard and Arbor Acres chicken hybrids.

The results in Table 5 show that broiler chicken genotype affected liver weight. Sex influenced liver, gizzard and abdominal fat weights but we noted no significant effect on heart weight. Males were heavier than females in liver and gizzard weights but deposited less abdominal fat than females. Genotype × sex interactions affected liver weight but had insignificant effects on gizzard, heart and abdominal fat weights. With regard to giblets, our results agree with that of Olawumi *et al.* (2012) that breed × sex interactions have insignificant effects on liver weight and gizzard weight, both sexes within and between breeds recorded similar mean values, and this result is contrary to the results of Ojedapo *et al.* (2008) who reported significant strain × sex interaction effects on these traits.

Table 1 Effects of genotype and sex on body weight and body weight gain of broiler chickens

Genotype	Sex	Body weight (g)				Body weight gain (g)		
		Day 7	**Day 21**	**Day 35**	**Day 42**	**Day 7-Day 21**	**Day 22-Day 35**	**Day 36-Day42**
Ross 308	Males	166.09±41.38[a]	842.76±98.34[a]	1886.24±164.98[ab]	2425.57±199.28[ab]	676.67±62.79[a]	1043.48±89.29[a]	539.33±59.72[a]
	Females	158.28±39.67[b]	798.25±92.76[b]	1691.79±161.52[bc]	2188,07±189.76[bc]	639.97±61.28[b]	893.54±86.28[b]	496.28±55.38[b]
Cobb 500	Males	152.74±41.24[bc]	836.37±96.23[a]	1959.88±162.78[a]	2511.53±193.51[a]	683.63±63.91[a]	1123.51±88.82[a]	551.65±58.47[a]
	Females	146.38±38.42[c]	788.87±93.11[c]	1676.17±163.71[c]	2177.41±181.67[c]	642.49±62.53[b]	887.30±87.52[b]	501.24±56.62[b]
Genotype		**	**	ns	ns	ns	ns	ns
Sex		**	**	**	**	**	**	**
Genotype × Sex		ns	*	**	**	*	**	**

Results are presented as Mean ± SD/ SEM
[a, b, c] means with the same superscripts are significantly different at P<0.05
ns not significant P>0.05; *P<0.05; **P<0.01

Table 2 Effects of genotype and sex on feed consumption and feed conversion ratio of broiler chickens

Genotype	Sex	Feed consumption (g/bird)				Feed conversion ratio (g/bird)			
		Day 7	**Day 21**	**Day 35**	**Day 42**	**Day 7**	**Day 21**	**Day 35**	**Day 42**
Ross 308	Males	146±0.41[a]	1124±1.78[a]	2912±2.38[a]	4036±3.98[a]	1.19±0.02[b]	1.38±0.02[b]	1.63±0.03[b]	1.74±0.03[b]
	Females	142±0.39[b]	1108±1.89[b]	2856±2.27[b]	3876±3.73[b]	1.23±0.02[a]	1.42±0.03[a]	1.66±0.03[a]	1.82±0.04[a]
Cobb 500	Males	144±0.40[a]	1125±1.81[a]	2905±2.41[a]	4002±3.88[a]	1.16±0.01[b]	1.36±0.02[b]	1.60±0.02[b]	1.71±0.03[b]
	Females	139±0.33[b]	1112±1.77[b]	2888±2.39[b]	3869±3.75[b]	1.19±0.01[a]	1.43±0.02[a]	1.64±0.03[a]	1.78±0.03[a]
Genotype		*	*	*	*	ns	*	*	*
Sex		**	**	*	**	*	**	**	**
Genotype × Sex		ns	ns	ns	ns	ns	ns	ns	ns

Results are presented as Mean ± SD/ SEM
[a, b] means with the same superscripts are significantly different at P<0.05
ns not significant P>0.05; *P<0.05; **P<0.01

Table 3 Effects of genotype and sex on mortality rate of broiler chickens

Genotype	**Sex**	**Mortality rate (%)**			
		Day 7	**Day 21**	**Day 35**	**Day 42**
Ross 308	Males	0.02	0.02	0.09[a]	0.09[a]
	Females	0.02	0.00	0,00	0.02
Cobb 500	Males	0.00	0.02	0,12[a]	0.12[a]
	Females	0.02	0.00	0.02	0.04
Genotype		ns	ns	ns	ns
Sex		ns	ns	ns	ns
Genotype × Sex		ns	ns	ns	ns

ns not significant P>0.05

[a] means with the same superscripts are significantly different at P<0.05

Table 4 Effects of genotype and sex on carcass characteristics of broiler chickens

Genotype	**Sex**	**Carcass characteristics (g)**				
		Carcass weight	**Breast weight**	**Legs weight**	**Back weight**	**Wings weight**
Ross 308	Males	1895.91±89.28[b]	580.81±24.87[a]	590.19±28.28[a]	511.89±19.87[a]	237.57±11.46[a]
	Females	1707.47±88.28[ab]	562.99±22.32[a]	531.53±27.11[b]	460.89±18.36[b]	213.44±11.22[b]
Cob 500	Males	1880.98±86.29[a]	576.15±23.29[a]	585.54±27.98[a]	488.42±18.21[a]	235.12±12.35[a]
	Females	1668.88±87,11[b]	579.37±22.38[a]	528.86±28.02[ab]	458.69±19.76[b]	212.36±11.67[b]
Genotype		ns	ns	ns	ns	ns
Sex		*	ns	*	**	*
Genotype × Sex		**	**	**	**	**

Results are presented as Mean ± SD/ SEM

[a, b] means with the same superscripts are significantly different at P<0.05

ns not significant P>0.05; *P<0.05; **P<0.01

Table 5 Effects of genotype and sex on edible giblets and abdominal fat of broiler chickens

Genotype	**Sex**	**Edible giblets and abdominal fat (g)**			
		Liver	**Gizzard**	**Heart**	**Abdominal fat**
Ross 308	Males	38.72±3.62[b]	21.17±1.23[a]	6.17±0.18[a]	46.76±3.78[b]
	Females	39.11±3.29[b]	20.88±1.31[a]	6.15±0.22[a]	66.21±4.11[a]
Cob 500	Males	44.76±3.82[a]	20.49±1.25[a]	6.18±0.19[a]	51.68±4.12[b]
	Females	38.89±3.51[b]	20.62±1.29[a]	5.82±0.16[a]	63.29±3.98[ab]
Genotype		*	*	ns	ns
Sex		*	*	ns	**
Genotype × Sex		*	*	ns	ns

Results are presented as Mean ± SD/ SEM
[a, b] means with the same superscripts are significantly different at P<0.05
ns not significant P>0.05; *P<0.05; **P<0.01

Conclusion

In conclusion, we can state that broiler chickens no significantly affected final body weight, body weight gain, feed intake, and mortality rate, although, Cobb 500 chickens utilized feed more efficiently compared Ross 308 chickens. In cause of sex, males were heavier in the end of fattening period, gained more body weight, utilized feed more efficiently but consumed more feed and had a higher mortality rate in comparison with females. From characteristics, hybrid influenced liver and gizzard weights. Males deposited less abdominal fat compared with females. We recorded significant genotype × sex interaction effects on all the carcass traits measured except gizzard, heart, and abdominal fat weights.

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THE EFFECT OF APPLIED CROSSBREEDING ON INCREASING OF MILK PRODUCTION IN GOATS

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Abstract

The aim of research was represented by conducting of some research activities aiming to evaluate the lactogenic capacity for the experimental batches, as an effect of cross-breeding between goats belonging to local breed Carpatina and bucks from Anglo Nubian and French Alpine breeds. Estimation of milk production was done in according with International Committee for Animal Recording (AT4 method) and statistical analysis was performed using the Restricted Maximum Likelihood Method. In case of effectuated analysis for evaluation of ameliorative effect of milk production obtained from F1 half-breed females, resulted by cross-breeding of Anglo Nubian breed bucks with local females, a real absolute difference for total milk production of 17.74 milk kg could be observed, difference which have an increased statistical significance degree for $p<0.01$. In the case of batch formed by F1 half-breed females, resulted by cross-breeding of French Alpine breed bucks with Carpatina local goats, a real absolute differences of 23.30 milk kg was found, being distinctively significant for $p<0.01$. The obtained results show that effect due to heterosis have a higher manifestation on productive performances for F1 half-breed females resulted by cross-breeding of Carpatina breed females with French Alpine bucks. According to this observation we could say that Frennch Alpine breed have a higher ameliorative degree on milk production.

Keywords: *Romanian goats, Carpatina goat, Cross breeding, Milk production, Small ruminants*

Introduction

Sheep and goat farming in the EU faces some economic, structural and other difficulties, which have led to a steady decrease in total staff in many member countries. Thus, at the level of the European Union, the decrease in the number of herds was 16% during period 2000-2016, being accelerated in the periods when some contagious diseases broke out, as well as after some major changes of the financing schemes of the farms from the Union European or national resources. In order to encourage the revival of activities in this field, there are several EU support instruments to support production capacity and deliver not only food, but also public goods such as the use of natural resources and the preservation of biodiversity. However, given the low profitability and the fact that small ruminants are concentrated mainly in less favoured areas, some support measures that are more attractive for farmers need to be developed and implemented in order to revival the growth activity (Pascal, 2015). In the current context, the discussion on the Common Agricultural Policy concerns the post-2020 period and aims to adopt communication and promotion measures to strengthen the sector's position in the EU and respect for consumer demand in this geographical area (European Parliamentary Research Service, 2017). At the level of the European continent, the Mediterranean and Black Sea countries are less important for dairy goats than for sheep milk. These countries produce 10.5% of world production of goat milk and produce 19.1% of goat's milk in the world (FAOSTAT, 2018). Among them, France (20.7%, 715 l/doe), Spain (14.1%, 328 l/doe), Greece (13.2%; 134 l/doe) and Turkey (11.8%, 73 l/doe) are the current leaders in the production of goat milk in the area followed by the Russian Federation (8.6%, 330 l/doe), Ukraine (7.8%, 500 l/doe) and Algeria (7.9%, 85 l/doe) (Pulina et al., 2018). In this context, Romania wants to join these countries and has adopted a number of support measures that have led to an increase in total goat population in 2015 compared to 2005 by more than 27% (Pascal et al. 2017). The level reached has also led to the application of research and technical activities to attract and improve milk production. For this purpose, this research was also carried out, the main objective being to assess the effect of heterosis due to crosses between native females of Carpatina breed with Anglo Nubian and French Alpine goats.

Material and Methods

The biological material was represented by different populations of goats belonging to different breeds, namely the Carpatina native breed (M) and some half-breeds from the first-generation resulting from the crossbreeding of the local female breed with Anglo-Nubian (L1) and French Alpine (L2) males.

In order to eliminate the influence of external factors on productive performances homogeneous lots belonging to the three researched populations were constituted, each batch consisting of 25 females at the first lactation. The maintenance, feeding conditions, as well as the evaluation period of the researched characters, were performed within the same time frame.

In order to compare the results and to determine the cross-breeding effect, in particular on the milk production capacity, the data obtained as a result of the application of the control of the productive performances placed at equal intervals in the lactation was used.

The performance evaluation for milk production resulted in the analyzed lactation was based on the application of successive periodic inspections, and using for the lactation period the Nica method and for the milking period was exclusively applied the method AT_4 in compliance with the technical specifications suggested by International Committee for Animal Recording. Estimation of the average total production of milk was carried out using the Fleischmann method.

$$\text{Milk yield [kg]} = \mathrm{L}_1.\mathrm{int}_1 + \sum_{i=2an}\left(\frac{\mathrm{L}_i + \mathrm{L}_{i-1}}{2}.\mathrm{int}_i\right) + \mathrm{L}_n.14$$

where:
L_1 = milk yield of the 1st monthly test;
L_i = milk yield of the ith monthly test (i = 1,..., n);
L_n = milk yield of the last test;
int_1 = number of days from kidding to 1st monthly test;
int_i= number of days between monthly tests (i-1) and i (i = 1,...,n);
n = total number of monthly test for a specific animal.

Data were statistically evaluated with the algorithm REML (REstricted Maximum Likelihood), which provides the achievements of the statistical parametric estimators within the normal range.

Results and Discussion

Genetic improvement programs for goats are a common practice, the goal being to identify solutions, methods, ways and technologies that can increase the lactogenic potential. Typically, these programs include characters and features that are economically important to achieve greater productivity, higher income, and lower cost per product unit (Taftă et al., 1997).

For practice, the ameliorative effect due to the application of cross-breeding programs is positive, but the final effect depends mainly on the participating breeds, but also on their genetic baggage for the characters that are subject to change. The option of introducing into the crossbreeding scheme of the breeds was motivated by the fact that the literature shows different results showing that these breeds improve not only the milk production (Serradilla, 2001) but also the precocity and growth intensity (Moman M.S. et al., 2012), but also some

reproductive indices specific to the reproduction activity (Aboul Nagaa et al., 2016) in different goat populations in less favourable areas.
Also, in an extensive study, Ricordeau (1981) analyzed the results of increased milk production as a result of crossbreeding between indigenous breeds of specialized breeds and concludes that when it is desired to increase milk production in a shorter time; crossing is a favourable technical solution.
As regards to the Anglo Nubian goats, their role has increased over the last period of time because it has been found to react positively to crossings with local breeds that are less productive. This is the reason why Nubian goats have been continuously imported into China for the past decades, to improve the performance of local breed production (Yuan et al., 2017). Understanding the genetic basis underlying the distinct phenotypes of these two breeds will be a perquisite for new breed selection and customizing strategies for cross breeding.
After the statistical processing of the data obtained from the application of the productive control, there was a tendency to show a positive type of heterosis for the quantity of milk in both half-breed female populations (L1 and L2) compared to the performance obtained from the females of Carpatina breed (M), being at the first lactation.
This finding is supported by the fact that both half-bred female groups gave milk yields higher with 10.36% in the case of those resulted from the crossing with Anglo Nubian goats and with 13.18% at the group obtained with the French Alpine (table 1 and figure 2), both differences recording a high degree of statistical significance for $p \geq 0.001$.

Table 1. Total average milk production obtained in the controlled lactation (kg)

Specification		M	L1	L2
n		25	25	25
$\overline{x}$		153.48	171.22	176.78
$\pm s_{\overline{x}}$		1.18	1.51	2.31
V%		14.43	13.34	13.09
s		11.031	14.221	14.305
Limits	Minimum	95.41	108.45	147.10
	Maximum	198.33	189.02	236.50
Difference and its signification for the average total milk production				
Batches	Difference ±	Statistical significance of the difference		
M – L1	- 17.74	*** - F (21.6622) > Fα (12.0831) for 0.001		
L1 – L2	+ 30.33	*** - F (28.2822) > Fα (11.3487) for 0.001		
M – L2	- 49.41	*** - F (31.2247) > Fα (16.6120) for 0.001		

In the case of the analysis performed to determine the ameliorative effect of milk production obtained from the first generation in the half-breed females resulted from the crossing of the Anglo Nubian goats with the local Carpatina females, there is a real absolute difference between the total milk production obtained from the controlled lactation, of 17.74 kg of milk, a difference with a high degree of statistical significance for $p<0.01\%$.

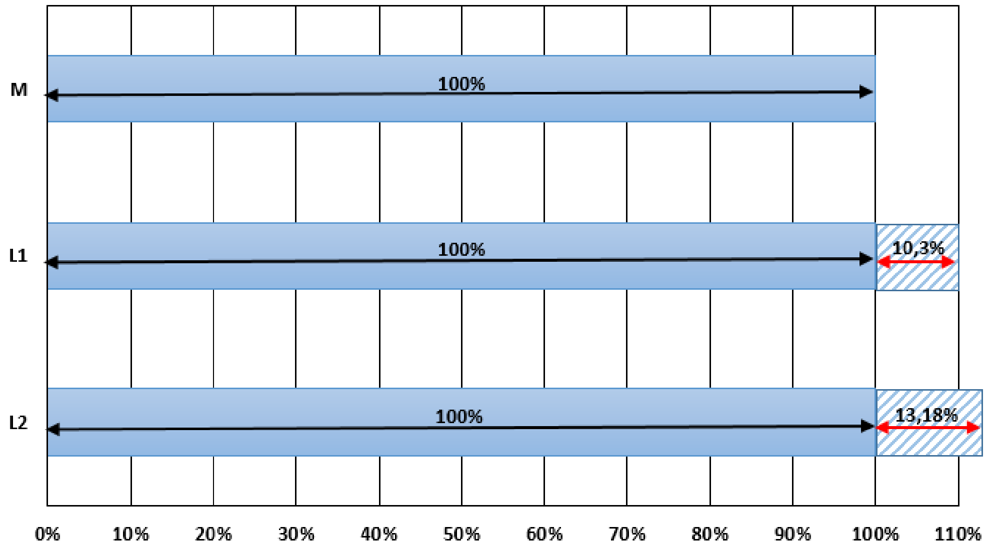

Figure 1. Graphical representation of the differences between groups for productive performance recorded in the controlled lactation

In the case of crossbreeding of French Alpine goats with local females, the real difference between the milk production level is of 23.30 kg of milk and was distinctly significant for $p<0.01$. However, results obtained in other researches, but carried out on other groups of half-breed females obtained using French Alpine goats, show the ability to improve milk production right from the first lactation. Thus, in a study conducted in 2017 by Anghel et al., 2017, it is found that for the half-breed goats of French Alpine x Carpatina it is noted an increase of the milk quantity starting with the second month of lactation, when the maximum was reached. The experimental data proved that both half-breed French Alpine x Carpatina (table 1), and also those of Saanen x Carpatina reach the prediction value at the establishment of the ratio, of 2 kg milk/day, starting with the second day of lactation.

Conclusions

Because in both half-bred lots there is a manifestation of the positive type of heterosis for the quantity of milk obtained at the first lactation from the half-bred females resulted in F_1 it can be said that there is a high degree of genetic combinability among the participating breeds;
Both half-breed female lots gave milk yields higher with 10.44% for those resulted by the use of Anglo Nubian goats and with 11.56% for the lot obtained with the French Alpine;
The fact that the half-bred population obtained by using French Alpine goats produces a production of more than 200 litres in a normal lactation, recommends its use in the improvement of this character in goats.

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MICROELEMET PROFILE ANALYSIS OF GRAPE BY-PRODUCTS FROM SLOVAKIA AND AUSTRIA

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Abstract

The aim of this study was to determine the microelements content of grape pomace, grape stem and grape bunch of three different cultivars of *Vitis vinifera sp*. (Green Veltliner, Pinot Blanc and Zweigelt) from two countries as a possible sources for animal nutrition. Mineral profile analysis was performed by using the High Resolution Continuum Source Atomic Absorption Spectrometer contrAA 700 for zinc, copper, iron, manganese. Significant differences ($P<0.05$) in mineral composition of analyzed samples were found between the countries, as well as between the cultivars within countries. The grape pomace samples from Slovakia and Austria had significantly different ($P<0.05$) content of all the studied microelements, except for zinc. In the case of grape stems significant differences ($P<0.05$) for copper and manganese content were found. The grape bunches from two counties significantly differed ($P<0.05$) in copper, iron and zinc content. The Austrian grape pomaces, as well as grape stems had significantly different ($P<0.05$) copper and iron content, while the bunch samples differed significantly ($P<0.05$) only in iron content. Between Slovakian grape pomaces significant differences ($P<0.05$) in copper and zinc content were found. All the stem samples from Slovakia differed significantly ($P<0.05$) only in zinc content and the bunches had significantly different ($P<0.05$) copper content. These results indicate a significant impact of the grape variety and location on the mineral profile of grape by-products.

Keywords: *Microelements, Grape pomace, Grape stem, Grape bunch.*

Introduction

Grape is the world's largest fruit crop, with an annual production of more than 75 million tonnes (OIV, 2017). The vast majority of the total grape production (75%) is used to produce wine (García-Lomillo, Gonzáles-San José, 2017). The two main by-products of grape processing are pomace and stalks (Makris et al., 2007). Grape pomace accounts for about 20-25% of the weight of the grape crushed for wine production (Yu, Ahmedna, 2013), the quantity of stems varies between 1.4% and 7% of the raw matter processed (Souquet et al., 2000). These by-products are currently not considered to be profitable and are most commonly used as fertilizer, left on open spaces or burned, which can lead to environmental problems ranging from surface and groundwater pollution to foul odours (Rondeau et al., 2013, Bekhit et al., 2016). The use of these products for fertilizers or composts on soils can cause an increase in nitrogen leaching and oxygen depletion due to the presence of tannins and other compounds (Bekhit et al., 2016). With respect to animal feed, the nutritional value and the digestibility of these by-products is, due to high fiber content, generally low. However, because of the growing interest in environmentally friendly waste-free technologies and minimizing the cost of processing by-products from wine industry, innovative procedures are being introduced (Brenes et al., 2016, Gálik et al., 2018). Many experiments showed, that these products can be used a substantial source of certain nutrients and biologically active compounds in animal nutrition (Viveros et al., 2011, Nistor et al., 2014, Teixeira et al., 2014, Chamorro et al., 2015, Domíngues et al., 2016, Kerasioti el al., 2017, Chedea et al., 2018). This alternative may contribute to reduce winery residuals, improve environmental aspects, so as to reduce production costs and offer a new way to diversify the production (García-Lomillo, Gonzáles-San José, 2017). The composition of these by-products may vary depending on extrinsic factors such as edaphoclimatic conditions and viticultural practices, as well as intrinsic factors such as variety and maturity of the grapes (García-Lomillo, Gonzáles-San José, 2017). Therefore, their composition should be determined on a case-by-case basis (Ziarati et al., 2017). In this study, we hypothesize that the mineral profile of grape pomace (GP), grape stem (GS) and grape bunch (GB) is closely related to the grape variety and differences in their composition can be found due to the country of origin. The aim of this study was to determine the microelements content of GP, GS and GB of three different cultivars of *Vitis vinifera sp*. - Green Veltliner (GV), Pinot Blanc (PB) and Zweigelt (ZW) from two countries as a possible sources for animal nutrition.

Material and Methods

The pomace, as a by-product of juice pressing in wine industry, mainly contained of residual grape skin, seeds and pulps. GS were only rachis, peduncle and pedicels after removing grape berries. In total, 54 samples from 3 varieties from 6 different locations were analysed. Laboratory samples were processed in the Laboratory of Quality and Nutritive Value of Feeds at the Department of Animal Nutrition at the Slovak Agricultural University in Nitra by standard laboratory methods and procedures (EC No 152/2009). The contents of mineral nutrients were determined by High Resolution Continuum Source Atomic Absorption Spectrometer contrAA 700 (ANALYTIK JENA). The determination of individual elements content was based on the absorptions measured at the following wavelengths: zinc (Zn) at 213.9 nm, copper (Cu) at 324.7 nm, iron (Fe) at 248.3 nm and manganese (Mn) at 279.5 nm. To calculate basic statistic characteristics, to determine significance of differences and to compare results one-way ANOVA and t-test were performed at $P<0.05$ level. The SAS statistical package was used (SAS Inc., New York City, USA).

Results and Discussion

Microelemet profile of grape by-products from Slovakia in shown in Table 1. Between GP significant differences ($P<0.05$) in Cu and Zn content were found. GP from GV had the highest Cu and Fe content and the lowest content of Mn and Zn. On the other hand, the concentration of these microelement was exactly the opposite in ZW, except for Zn. The highest amount of Zn was detected for PB. All stem samples from Slovakia differed significantly ($P<0.05$) only in Zn content. Similarly to GP, GS from GV had the highest Cu and the lowest Zn content and stem from ZW was the richest in Mn content with the lowest Fe concentration. Stem from PB contained the highest amount of Fe and Zn and the lowest amount of Cu and Mn. Slovakian bunches had significantly different ($P<0.05$) Cu content. The lowest concentration of Mn was found for GV, bunch from PB had the lowest amount Cu, Fe and Zn. The highest content of the studied microelements was measured for ZW. Interestingly, the lowest number of measured trace elements was detected in bunches compared to their contents in pomaces and stalks.

Table 1. Microelemet profile of grape by-products from Slovakia (mg.kg^{-1})

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean±Standard Deviation		
Cu	pomace	25.26±0.6^{a}	21.29±0.48^{b}	16.68±0.71^{c}
	stems	30.78±0.32^{a}	12.90±2.22^{b}	13.07±0.71^{b}
	bunch	9.67±0.37^{a}	4.93±0.32^{b}	16.24±0.01^{c}
Fe	pomace	152.86±2.82^{a}	103.49±0.57^{b}	100.38±0.02^{b}
	stems	115.03±1.08^{a}	153.77±19.12^{b}	98.72±1.28^{a}
	bunch	35.36±2.39^{ab}	31.79±1.85^{b}	39.30±0.13^{a}
Mn	pomace	14.35±0.54^{a}	16.95±1.06^{b}	17.81±0.63^{b}
	stems	42.32±1.81^{a}	40.11±0.21^{a}	47.72±0.80^{b}
	bunch	3.68±0.15^{a}	5.01±0.04^{b}	5.06±0.34^{b}
Zn	pomace	18.48±0.92^{a}	37.59±2.33^{b}	23.14±0.53^{c}
	stems	17.50±0.33^{a}	43.55±1.11^{b}	37.54±3.66^{c}
	bunch	5.83±0.07^{a}	5.50±0.38^{a}	16.44±0.18^{b}

Values followed by different letters within a row are significant at the level 0.05.

The situation with microelemet profile of grape by-products from Austria was completely different (Table 2.). The Austrian GP, as well as GS had significantly different ($P<0.05$) Cu and Fe content, while the bunch samples differed significantly ($P<0.05$) only in Fe content. From pomace samples, GV was characterised with the lowest concentration of all the microelements, PB had the highest Fe and Zn content and for ZW the highest amount of Cu and Mn was detected. Stem from GV had the lowest Mn and highest Cu and Fe content, while the concentration of these microelement was exactly the opposite for ZW. In addition, ZW had the lowest Zn content. The highest Zn content was measured for PB. Bunches from GV and ZW had practically identical Cu concentration, the highest amount of this microelement was detected for PB. This variety had also the highest Fe content. Bunch from GV was characterised by the highest Mn and Zn concentration. The lowest amount of Zn was detected for ZW. The most Austrian bunches were also characterized by the lowest content of studied microelements.

Table 2. Microelemet profile of grape by-products from Austria (mg.kg^{-1})

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean±Standard Deviation		
Cu	pomace	17.79±0.78^{a}	35.28±0.15^{b}	60.53±0.72^{c}
	stems	29.41±1.19^{a}	38.82±0.22^{b}	44.12±0.79^{c}
	bunch	13.31±0.11^{a}	18.63±0.11^{b}	13.36±0.08^{a}
Fe	pomace	42.37±2.88^{a}	95.49±3.06^{b}	70.45±1.37^{c}
	stems	42.86±1.27^{a}	103.04±0.19^{b}	229.21±2.16^{c}
	bunch	60.66±0.73^{a}	74.76±0.51^{b}	34.03±0.08^{c}
Mn	pomace	7.11±0.22^{a}	7.89±0.05^{a}	13.89±0.67^{c}
	stems	24.43±0.24^{a}	23.92±0.75^{a}	21.02±0.22^{b}
	bunch	6.33±0.13^{a}	4.10±0.25^{bc}	3.98±0.00^{c}
Zn	pomace	18.92±0.42^{a}	41.32±0.65^{b}	21.24±0.01^{ac}
	stems	24.67±0.53^{a}	36.26±0.73^{b}	23.13±1.84^{a}
	bunch	61.94±3.39^{a}	16.72±0.64^{b}	12.56±0.75^{b}

Values followed by different letters within a row are significant at the level 0.05.

The total comparison of microelement profile of grape by-products from Slovakia and Austria is shown in Table 3. The GP samples from Slovakia and Austria had significantly different ($P<0.05$) content of all the studied microelements, except for Zn. In the case of GS significant differences (P<0.05) for Cu and Mn concentrations were found. The GB from two counties significantly differed (P<0.05) in Cu, Fe and Zn content.

Table 3. Comparison of microelement profile of grape by-products from Slovakia and Austria

		Slovakia	Austria	Significance
		Mean±Standard Deviation (mg.kg^{-1})		
Cu	pomace	21.08±3.76	37.87±18.62	0.017
	stems	18.92±8.97	37.45±6.49	0.000
	bunch	10.28±4.92	15.10±2.65	0.020
Fe	pomace	118.91±25.54	69.44±23.12	0.001
	stems	122.51±26.30	125.04±82.37	0.931
	bunch	35.49±3.59	56.48±17.92	0.003
Mn	pomace	16.37±1.70	9.63±3.23	0.000
	stems	43.38±3.53	23.12±1.64	0.000
	bunch	4.58±0.70	4.80±1.15	0.634
Zn	pomace	26.40±8.72	27.16±10.68	0.871
	stems	32.86±11.97	28.02±6.30	0.299
	bunch	9.26±5.39	30.41±23.78	0.019

The level of significance was set at P<0.05.

The mineral profile of GP is well documented in the literature. Ziarati et al. (2017) revealed high trace elements content in 5 different GP from South Iran with significant differences between the cultivars. Bennemann et al. (2016) studied the mineral content of 9 GP from Brasil, also with significant differences between the varieties. These authors also declare an interesting amount of Cu, Mn and Fe in GP. Chikwanha et al. (2018) state, that GP can be a good source of Cu and Fe for animals. In comparison with Ordonez et al. (2015) higher concentration of Cu in GP was detected, however Ziarati et al. (2017) and Bennemann et al. (2017) reported much higher amount of this trace element in GP (49.87 mg.kg^{-1} and 89.24 mg.kg^{-1} respectively). Results obtained for Fe in GP are lower than reported by Ziarati et al. (2017), Bennemann et al. (2017), Ordonez et al. (2015), Wadhwa el al. (2015), Ribeiro et al. (2015) and Corbin et al. (2015). According to Ziarati et al. (2017) and Ordonez et al. (2015) higher Mn content in pomaces was detected (3.10 mg.kg^{-1} and 11.5 mg.kg^{-1} respectively), but lower than published by Ribeiro et al. (2015) and Bennemann et al. (2017) (31.21 mg.kg^{-1} and 25.43 mg.kg^{-1} respectively). Wadhwa el al. (2015) reported similar Zn concentration in GP (24 mg.kg^{-1}), while Ziarati et al. (2017), Ordonez et al. (2015) and Corbin et al. (2015) found higher (42.73 mg.kg^{-1}, 30.60 mg.kg^{-1}, 31.56 mg.kg^{-1} respectively) and Bennemann et al. (2017) lower (10.04 mg.kg^{-1}) amount of this microelement in GP. Recently, only a limited number of papers has been published on the content of microelements in GS and GB. Spigno et al. (2013) studied the mineral composition of 6 GS from Italy and found significant differences in Zn, Fe and Cu content between the cultivars. According to Spigno et al. (2013) lower amount of Cu and higher concentration of Fe and Zn was detected in GS. A justification for this difference between the mineral content of grape-by products would be the difference in the composition of the soil and climate of the growing regions. Elements such as the soil and weather factors, especially temperature, humidity and solar radiation, exert great influence on the development, production and grape quality Bennemann et al. (2016).

Conclusion

The aim of this study was to determine the microelements content of GP, GS and GB of three different cultivars of *Vitis vinifera sp*. from two countries as a possible sources for animal nutrition. Significant differences (P<0.05) in mineral composition of analyzed samples were found between the countries, as well as between the cultivars within countries. Despite the different composition of the by-products of grape processing from both countries, some similarities can be seen in the mineral profile of the same cultivars. For example, both Slovakian and Austrian GP and GS from PB had the highest Zn content and the bunches of this variety also had the highest Cu and Fe concentration. Pomaces form GV were the richest in Mn and Zn content, and ZW pomaces were characterized by the highest amount of Mn for both countries. Overall, it can be concluded that the pomaces from Slovakia were richer in the content of Fe and Mn, the stems from this country had a higher content of Zn and Mn but the bunches from Austria were characterized by a higher content of all monitored microelements. These results indicate a significant impact of the grape variety and location on the mineral profile of grape by-products.

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HAPLOTYPE BLOCK STRUCTURE IN THE GENOME OF SLOVAK PINZGAU CATTLE

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Abstract

The aim of this study was to analyse the haplotype structure of Slovak Pinzgau cattle genome to identify and describe most frequent haplotype blocks reflecting the effect of selection in particular genomic regions. The high-density genomic data of 150 animals (BovineSNP50 BeadChip v2), covering nucleus of Slovak Pinzgau breed, were used to call haplotypes. After quality control of the data, the database included information of 41,068 SNP markers covering 2.50 Mb of the cattle genome. The average SNP spacing was 48.86±42.97 kb. The coordinates of haplotype blocks were generated from the phased SNP data based on the non-overlapping sliding windows of ten markers. The analysis indicated totally 4097 haplotype blocks with coverage rates of autosomal genome 44.82%. The majority part of blocks was concentrated on BTA1, BTA2 and BTA6. The longest haplotype block located on BTA12 (70.18 Mb – 76.95 Mb) included overall of 95 protein-coding genes. The shortest block was found on BTA14 (66.72 Mb – 68.80 Mb). To describe the most frequent haplotypes in Slovak Pinzgau genome, the frequency of each haplotype in blocks was plotted against the start position. From the identified haplotypes overall of 131 segments reached the frequency higher than 50%. Of these, two haplotypes on BTA3 (79.76 Mb – 80.47 Mb) and BTA6 (86.31 Mb – 87.53 Mb) were identified in more than 95% of animals. Inside these two regions several genes responsible for genetic control of economically important traits were detected; e.g. LEPR, CSN3, CSN2, CSN1S1. The beneficial effects of these haplotypes could be used to enhance genetic gain trough selection of animals with specific haplotypes depending on farmer's requirements.

Keywords: *autozygosity, cattle, genotyping data, selection signatures.*

Introduction

In livestock, the use of high-throughput genotyping technology allows to estimate the pattern of genetic variation at genome-wide level. The analysis of linkage disequilibrium (LD) and haplotype block structure can help to fully understand the history of the livestock populations and their genetic background (Jasielczuk *et al.*, 2016; Xu *et al.*, 2019). Various studies demonstrated that even if the linkage disequilibrium tends to decrease over larger genomic distances the complete LD can be observed among distant SNP loci as well. Moreover, Wall and Pritchard (2003) showed that extend of LD can differ significantly from one to another region in the genome. Such SNP loci showing strong LD are usually organized into the discrete blocks of haplotypes that are separated by possible hot spots of recombination (Veroneze *et al.*, 2013). Therefore, the understanding of haplotype block structure in genome can considerably facilitate the analysis of LD (Niu *et al.*, 2016).

Generally, the haplotype blocks are defined as long stretches of SNPs along a chromosome that have low recombination rates, which characterized by relatively few haplotypes (Luikart *et al.*, 2003). Clarifying the structure of haplotype block across genome can bring important considerations for genome-wide association (GWA) and genomic selection (GS) studies, such as the possibility of selecting a set of SNPs with the prospect of reducing the information of several SNPs into the information of a haplotype block, reducing the number of SNPs in a

coherent way, and optimizing the design of GWAS (Mokry *et al.*, 2014). Moreover, the analysis of haplotype block structure can be used to identify the genomic regions under strong selection pressure that occurred during the grading-up process of target population or breed.
The aim of this study was to evaluate the haplotype block structure in the genome of Slovak Pinzgau cattle and to identify and describe most frequent blocks arising probably due to the strong selection pressure on a given regions in the genome.

Material and methods

The genome of Slovak Pinzgau cattle represented genomic information of overall 152 animals (37 sires, 115 dams of sires) that were genotyped for 54609 SNPs using Illumina BovineSNP50 BeadChip v2 in commercial lab. Animals for genotyping were selected based on the pedigree analysis in order to select the sample representing the gene pool of Slovak Pinzgau cattle.
Before the scan of haplotype block structure, the genomic data were subjected to quality control (QC) using PLINK 1.9 software (Chang *et al.*, 2015). Only autosomal loci with known chromosomal position, call rate >90 % and minor allele frequency >1 % across samples were retained in dataset. All of animals with more than 10 % of missing data were filtered out. After QC the dataset consisted of 150 animals and 41,068 SNP loci covering 2.50 Mb of the autosomal genome, with average SNP spacing 48.86±42.97 kb.
To call haplotypes and identify haplotypes block structure across the autosomal genome phased genotypes were used. The initial haplotypes were determined by using SHAPEIT software version v2 (O’Connell *et al.*, 2014) based on default options. The R package GHap (Utsunomiya *et al.*, 2016) was then used to infer the haplotype blocks. The coordinates of each block across autosomes were generated based on the non-overlapping sliding windows of 10 SNP markers, following Mészáros *et al.* (2019). To identify the genomic region under strong selection pressure the frequency of haplotypes was plotted against the starting position. The selection signatures were defined based on the haplotypes with frequency higher than 50 % in the entire sample (Fig. 2). Each of region were examined in detail using the web based tool Biomart from Ensembl database (https://www.ensembl.org/).

Results and discussion

The applied QC of data resulted in final database that included 41,068 SNPs covering 2.50 Mb of the genome. The obtained average spacing among adjacent SNPs (48.86±42.97) was in accordance with previous studies in cattle (Mancini *et al.*, 2014; Randhawa *et al.*, 2016; Kukučková *et al.*, 2017). Subsequent analysis of haplotype blocks coordinates derived from phased data was based on the non-overlapping sliding windows of ten markers. The results revealed overall 4097 haplotype blocs with average size 0.55±0.24 Mb that covered 44.82 % of autosomal genome expressed by SNPs on the genotyping array. The majority part of blocks was concentrated within the autosomes BTA1, BTA2 and BTA6 (Fig. 1). The longest haplotype block located on BTA12 (70.18 Mb – 76.95 Mb) included overall of 95 protein-coding genes. The shortest block was found on BTA14 (66.72 Mb – 68.80 Mb).

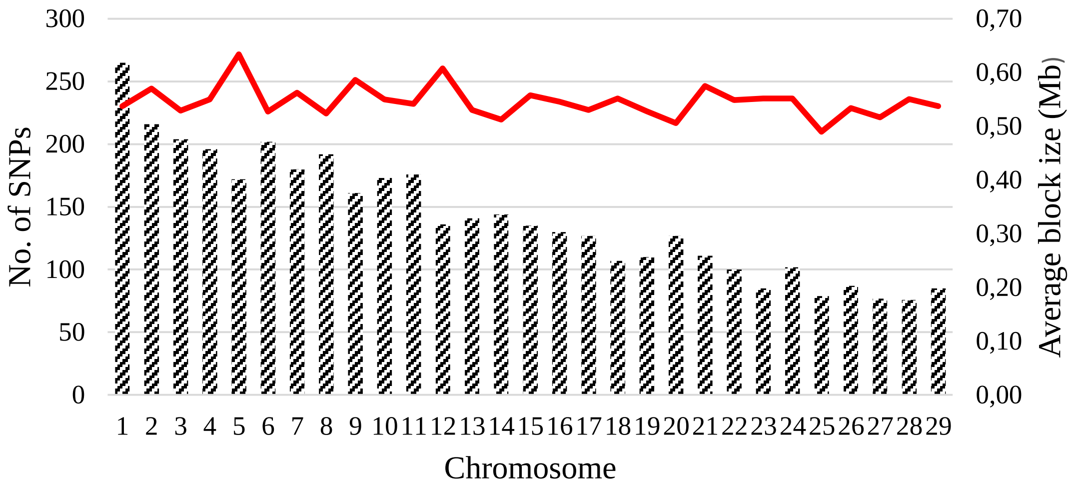

Figure 1. Distribution of haplotype blocks across autosomal genome of Slovak Pinzgau cattle

Assuming that the most frequent haplotypes in the genome of Slovak Pinzgau cattle resulted from the selective breeding for traits of interest, the haplotypes with proportion higher than 50 % in the entire sample were recognized as signals of selection. To visualize most significant genomic regions the frequency of haplotypes was plotted against the starting position (Fig. 2). From the identified haplotypes in total of 131 segments showed the proportion higher than 50 %. Top ten signals of selection are described in table 1. Of these, two haplotypes on BTA3 (79.76 Mb – 80.47 Mb) and BTA6 (86.31 Mb – 87.53 Mb) were identified in more than 95% of animals. Inside explored regions various protein-coding genes was identified (Tab. 1).

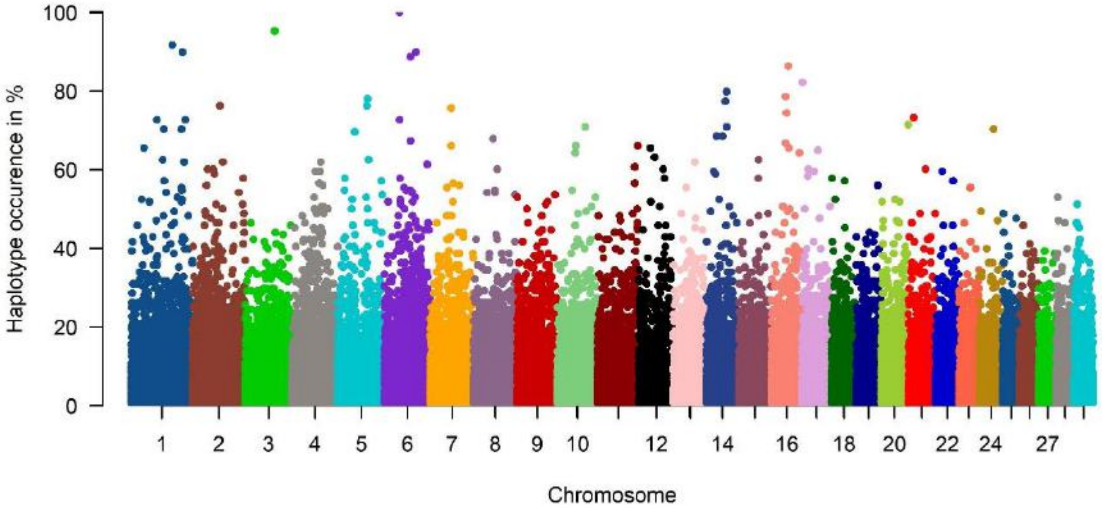

Figure 2. Genome-wide distribution of haplotypes in Slovak Pinzgau cattle

Table 1. Ten most frequent haplotypes in the analysed population

BTA	Start position (Mb)	End position (Mb)	Allele	Freq. (%)	Protein-coding genes
6	38689886	39172862	TTACGCCCCC	100.00	-
3	76481717	76797698	TAGCCGACTG	95.24	DEPDC1, LEPR
1	105134888	105477614	AATTGGACCA	91.67	LOC100141216
1	131533041	131865531	CCGGGCTTGG	89.88	TRNAG-CCC

6	81368675	82078166	AGGCTTCATG	89.88	EPHA5, CSN1, CSN2, CSN3
6	66991502	67239769	CCAGAATGAG	88.69	SLAIN2, SLC10A4, ZAR1, FRYL
16	43847822	44459269	CACTTCCCGA	86.31	PIK3CD, TMEM201, SLC25A33, SPSB1, MIR34A, GPR157, CA6
17	761685	1261843	GGGCTGGCCT	82.14	LOC617123, LOC101903170, LOC112442105
14	53246073	53532230	AACTGTTACC	79.76	TRNAE-UUC, LOC112449580, TRNAC-ACA
16	37151009	37391539	TCCCCTGCGT	78.57	F5, SELP, SELL, SELE

In the detected region on BTA1 the TRNAG-CCC gene is located. Santana *et al.* (2015) suggested that this gene is associated with body weight gain in Bos indicus cattle.
On the BTA3, the LEPR gene responsible for production traits in dairy and beef cattle was found. Trakovická *et al.* (2013) reported that both leptin receptor (LEPR) and leptin (LEP) genes significantly affected milk production and reproduction traits in Slovak Spotted and Slovak Pinzgau cattle. Moreover, these genes are involved in the control of feed intake and energy homeostasis via neurotransmitters such as neuropeptide Y and proopiomelanocortin (Lisa *et al.*, 2013).
On the BTA6, the casein family genes (CSN1, CSN2, CSN3) were identified. The casein and whey proteins are considered as two major protein groups in milk (Massella *et al.*, 2017). Various study showed that casein genes are very good genetic markers for marker assisted selection in cattle in order to select animals with higher genetic potential for target quality and quantity of milk (Miluchová *et al.*, 2018; Hristov *et al.*, 2018; Zhou *et al.*, 2019). Among other genes located within identified regions on BTA6, it was showed that the ZAR1 gene play a role in functions of oocytes in ovarian folliculogenesis, fertilization and embryogenesis (Wu *et al.*, 2003).
Functional analysis of genes located in genomic region on BTA14 revealed that one of the most biologically important genes is the TRNAC-ACA gene. The experimental study of Medeiros de Oliveira Silva *et al.* (2017) showed that this gene is associated with rump fat thickness in Nelore cattle.
On the BTA16, the MIR34A gene was found. Slabáková *et al.* (2018) reported that MicroRNA miR-34a is recognized as a master regulator of tumor suppression in humans.

Conclusion

This study showed that the most frequent haplotypes in the genome of Slovak Pinzgau cattle are located directly or very close to the genes associated with milk and beef production traits. Thus, the identified haplotype blocks reflects mainly the selection strategy of this breed that is in Slovakia bred in dual-purpose direction. In addition, this study could be used to enhance genetic gain trough selection of animals with specific haplotypes. Currently, the analysis of haplotype block structure in the genome is considered as one of the most important steps for successful application of genomic selection in livestock populations.

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EFFECT OF SPRAYING SULPHUR, MAGNESIUM, ZINC AND, ON FRUITING OF SUPERIOR SEEDLESS GRAPEVINES

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Abstract

During 2017 and 2018 seasons, Superior seedless grapevines received four concentrations of boric acid (17% B) namely a1) 0.0%, a2) 0.025%, a3) 0.05% and a4) 0.1% and eight treatments from single and combined applications of Sulphur, Magnesium and Zinc. The main target of this investigation was studying the effect of spraying Sulphur, Magnesium, Zinc and Boron on some vegetative growth characters, as well as physical and chemical characters of Superior seedless grapevines. Selecting the best combination of these essential nutrients responsible for overcoming the problems of small berries and shot berries accompanied with improving yield quantitatively and qualitatively was also considered. From economical point of view, it is recommended to spray Superior grapevines with a mixture containing Sulphurat 0.1%, Magnesium Sulphurat 0.5%, chelated –Zn at 0.01% and Boric Acid at 0.05% four times before bloom, just after berry setting and at two weeks intervals for improving growth, vine nutritional status, yield and quality of the berries in addition to produce filled and low shot berry clusters. This recommendation was true under experimental and resembling conditions.

Key words: Shot Berries, Superior Grapevines, Magnesium, Zinc, Sulphur, Boron.

Introduction

The importance of grapes as agricultural crop in general and as fruit crop, in particular, is doubtless when it cultivated area and its production of fruits are concerned. It is considered the first major fruit crop all over the world (Bacha 1984 and F.A.O., 2006).

Superior grapevine cultivar is considered a prime and popular grapevine cv, successfully grown under Egypt conditions. Such cultivar ripens early sometimes in the last week of May under sandy soil conditions. In addition, it has a greater potentiality of export to foreign markets due to its early in ripening character which reduced competition. In spite of introducing numerous grapevine cvs recently to Egypt such grapevine cv is still one of the prime, main, popular and the most profitable grapevine cv.

The inferior on quality due to the highest production, the small of berries and the presence of higher shot berries in Superior grape cv represent the most important serious problems which face the production and produce unfavourable clusters from the consumer point of view. Shot berries in the clusters of such grapevine cv consider a serious defect, since they detract from eye appeal and reduce at the lower extent the production and the potential for exportation. These shot berries were produced from unfertilized flowers, unfavourable nutrient and environmental conditions (Bacha, 1984 and Chapman, 1990). These problems were produced as a result of deficiency and unbalanced use of specific essential macro and micro nutrients. Previous studies showed and emphasized the possibility of using S, Mg, Zn and B for partially solving these drawbacks.

Macronutrients especially S and Mg as well as micronutrients namely Zn and B play a key role in the nutrition of the fruit crops especially grapevines (Christensen, 1977).

Sulphur (S) is beneficial on decreasing soil pH and soil salinity and increasing the availability of most nutrients and protein biosynthesis (Nijjar , 1985 and Mengel, 1985).

Magnesium is not usually applied to grapevine as a routine matter. The quick leaching of this element applied via soil aggravates the problems of magnesium deficiency. Magnesium is vital to the production of chlorophylls because every molecule contains magnesium ion in structure. It plays an important role as activation of several enzymes involved in carbohydrate metabolism and also was associated with enhancing transport of P in plant tissues(Mengel, 1985)

Zinc plays many important regulatory roles in plant development. It activates various enzymes involved in plant metabolism, enhances the biosynthesis of some organic foods and IAA and stimulates cell division, cell enlargement, water and nutrient transport (Price et al. 1972)

The different functions of boron for fruit trees according to Stile, 1961; Edmond et al., 1964; Sister et al., 1965; Christensen, 1977; Reed 1977; Adriano, 1985; Mengel, 1985; Vanekova and Madhal, 1985; Christensen, 1986 and Cook, 1986).

The present study aimed to throw some lights on the effect of foliar application of Sulphur, Magnesium, Boron and Zinc on growth and vine nutritional status, percentage of berry setting, yield, shot berries % as well as physical and chemical properties of Superior grapes. Also, the effect of these nutrients on solving the problems of inferior quality and shot berries which reflected in facilitating the possibility of marketing such grapevine cv. to local markets are also concerned.

Materials and Methods

This study was carried out during two successive seasons on Superior seedless grapevines grown in a private vineyard located at Kaluobia Governorate where the soil is silty clay, well drained and water table not less than two meters deep. Vines are spaced at two meters (between vines) x 3 meters (between rows). The selected vines (96 vines) were chosen as uniform in vigour as possible and devoted to achieve this study. The chosen vines were pruned during the first week of January in both seasons. Cane pruning system using Y shape supporting method was followed. Vine load for all the selected vines was 66 eyes (in the basis of six fruiting canes x nine eyes plus six renewal spurs x two eyes). Surfaces irrigation system was followed. The trees were irrigated through surface irrigation system. Soil analysis was done according to the procedure that outlined according to Chapman and Pratt (1965) and Black et al., (1965)

All the chosen trees received all horticultural practices that were commonly applied in the orchard except those dealing with Boric Acid, Sulphur, Magnesium and Zinc.

This study involved 32 treatments from two factors (A & B). The first factor (A) consisted from four concentrations of boric acid. (17% B) namely a1) 0.0%, a2) 0.025%, a3) 0.05% and a4) 0.1%. While the second factor (B) contained the following eight treatments from single and combined applications of Sulphur, Magnesium and Zinc:

1- Control (vines sprayed with tap water)
2- Spraying elemental sulphur at 0.1 %.
3- Spraying magnesium sulphate at 0.5 %.
4- Spraying chelated zinc at 0.5 %.
5- Spraying elemental sulphur + magnesium sulphate at the same previous concentration.
6- Spraying elemental sulphur + chelated zinc at the same previous concentration.
7- Spraying magnesium sulphate + chelatd zinc at the same previous concentration.
8- Spraying the three fertilizers together at the same previous concentrations.

Each treatment was replicated three times, one vine per each. Therefore, 96 uniform in vigour Superior grapevines (four concentrations of boric acid x eight treatments of S, Mg and Zn x three replicates x one vine) were devoted for achieving of this investigation. Boric acid (17% B), elemental sulphur (100% S); magnesium sulphate (9.6% Mg) and chelated zinc (14% Zn)

were sprayed four times during each growing season. The first spray was conducted before bloom. The second was carried out immediately after berry set. The third and fourth sprays were established at two weeks intervals just after fruit setting. Sulphur, magnesium and zinc were applied in the sources of elemental sulphur, magnesium suphate and chelated zinc at fixed concentrations namely 0.1, 0.5 and 0.05%, respectively (according to Ahmed et al., 1991a and 1991b; Zaki, 2006 and others). Triton B as a wetting agent was applied at 0.05% to all nutrient solutions. The selected vines were sprayed with these nutrients till runoff.
The following parameters were recorded.

1. Percentage of shot berries was recorded by dividing number of small berries by the total number of berries per cluster and multiplying the product by 100.
2. Yield expressed in weight (kg.) per vine was recorded at harvesting date (first week of June) when T.S.S./ acid ratio in the berries of the check treatment reached at least 22- 24 (at the 3rd week of June in both seasons) (according to Weaver, 1976) .
3. Average cluster weight (g.).Five clusters were taken at random from the yield of each vine for determination of berry weight (g.), total soluble solids, total sugars (Lane and Eynon method, 1965) and total acidity (as g tartaric acid/ 100 ml juice, (A.O.A.C., 1995).
4. Statistical analysis: all the obtained data were tabulated and statistically analyzed using New L.S.D. at 5 % for made all comparisons among the investigated treatment means (according to Steel and Torrie, 1980 and Mead et al., 1993).

Results and Discussion

1- Growth characters:
It is obvious from the obtained data that application of boron in the form of boric acid had an announced and significant effect on growth characters of Superior grapevine. There was a gradual promotion on the three growth characters namely main shoot length, number of leaves per main shoot length with increasing concentrations of boric acid from 0.0 to 0.1%. Significant differences on these growth characters were observed among all boric acid concentration except among the two higher concentrations namely 0.05 and 0.1%. The maximum values were recorded on the vines that received four sprays of boric acid at 0.1%. Untreating the vines with boric acid gave the lowest values. Similar results were announced during the two experimental seasons.
The beneficial effects of boron on the biosynthesis and translocation of carbohydrates as well as its promotive effects on cell division and the formation of organic foods could explain its impact on stimulating growth characters (Mengel, 1985).
The stimulating effect of boron on growth characters of Superior grapevines was supported by the results of El- Sawy (2009) on Superior grapevines
2- Percentages of Mg and Zn:
It is evident from the obtained data that foliar application of boric acid at 0.025 to 0.1% four times significantly stimulated leaf content of Mg and Zn as compared to the check treatment. The promotion was associated with increasing boric acid concentrations. Increasing concentrations of boric acid from 0.05 to 0.1% had no significant promotion on the essential nutrients namely Mg and Zn. The maximum values were recorded on the vines treated four times with boric acid at 0.1%. The control vines produced the minimum values.
The effect of boron on encouraging root development and absorption of water could result in enhancing the uptake of most elements (Nijjar, 1985).
These results are in agreement with those obtained by El- Sawy (2009) on Superior grapevines. The results of Kamel (2002) who worked on Flame seedless grapevines, Ahmed and Abd El- Hameed (2003) who worked on Red Roomy grapevines and Shoeib and El- Sayed (2005) who worked on Thompson seedless grapevines, supported the present results.
3- Berry setting:

It is quite clear from the obtained data, that foliar application of boric acid at 0.025 to 0.01% significantly was accompanied with improving percentage of berry setting of Superior grapevines as compared with the check treatment. The promotion was associated with increasing concentrations. Negligible stimulation was observed among the two higher concentrations namely 0.05 and 0.1%. Using 0.1% boric acid maximized the percentage of berry setting. The untreated vines showed the minimum values.

The promoting effect of boron on berry setting was confirmed by the results of Zaki (2006) and El- Sawy (2009) on Superior grapevines, Ali (2000) and Kamel (2002) on Flame seedless grapevines and Abd El- Hameed and Abo El- Ez (2004) on Ruby seedless. The same trend was reported by Ahmed et al., 1991a and 1991b; Ahmed and El- Dawwey (1992) and El-Ashram (1993) on Red Roomy grapevines.

4- Quality of the berries:

The interaction between the four essential nutrients namely S, Mg, Zn and B had significant effect on both physical and chemical characters of the berries. Increasing concentrations of boric acid from 0.05 to 0.1% under the same S, Mg and Z treatments had no significant promotion on quality of the berries. Therefore, the best results with regard to quality of the berries were obtained when the vines received four sprays of a mixture containing sulphur at 0.1%, magnesium sulphur at 0.5, chelated –zn 0.05% and boric acid at 0.05%.

5- Yield/ vine (kg.)

It is clear from the obtained data that using sulphur, magnesium and zinc either singly or in all possible combinations was significantly favourable in improving the number of clusters in the second season of study and the yield expressed in weight (kg.) in both seasons rather than non- application.. The great increment on the yield was observed on the vines that treated with Magnesium, Zinc or Sulphur in descending order. Combined application of these nutrients was preferable than using each nutrient alone in improving the yield.

These results regarding the beneficial effect of sulphur on the yield are in agreement with those obtained by Masoud (2008) on Superior grapevines as well as El- Sayed (2001); Abd El- Hady et al., (2003) and Abd El- Motty – Elham et al., (2006) on Flame seedless grapevines.

The results of Zaki (2006) and El- Sawy (2009) on Superior grapevines, Usha and Singh (2002) on Perlette grapevines and Majer (2004) on Thompson seedless grapevines confirmed the great merits of magnesium on the yield.

Table (1) Effect of spraying single and combined application of Sulphur, Magnesium , Zinc and Boron on Berry setting % and Yield / vine (Kg.) in the leaves of Superior seedless grapevines during 2017 and 2018 seasons.

Sulphur (S), Magnesium (Mg) and Zinc (Zn) treatments (B)	Berry setting %									
	2017					2018				
	Concentrations of boric acid % (A)									
	a1 0.0	A2 0.025	A3 0.05	a4 0.1	Mean (B)	a1 0.0	a2 0.025	a3 0.05	a4 0.1	Mean (B)
b1 Control	8.2	8.8	9.5	9.6	9.1	8.0	8.6	9.2	9.3	8.8
b2 Sulphur at 0.1%	8.7	9.6	10.3	10.3	9.7	8.6	9.3	9.9	10.0	9.5
b3 Mg SO_4 at 0.5%	9.7	10.5	11.2	11.2	10.7	9.9	10.5	11.2	11.3	10.7
b4 Chelated Zn at 0.05%	9.2	9.8	10.9	11.0	10.2	9.1	9.6	10.2	10.3	9.8
b5 S + Mg	10.6	11.3	12.0	12.1	11.5	11.2	11.9	12.5	12.6	12.1
b6 S + Zn	10.1	10.5	11.2	11.2	10.8	10.5	11.1	11.7	11.9	11.3
b7 Mg + Zn	11.2	12.0	12.6	12.7	12.1	11.8	12.4	13.0	13.1	12.6
b8 S + Mg + Zn	11.8	12.8	13.8	13.9	13.1	12.9	13.5	15.0	15.1	14.1
Mean (A)	9.9	10.7	11.4	11.5		10.3	10.9	11.6	11.7	
New L.S.D. at 5%	A 0.4		B 0.5		AB 1.0	A 0.5		B 0.5		AB 1.0
	Yield / vine (Kg.)									
b1 Control	7.8	8.7	9.5	9.5	8.9	8.1	9.1	10.2	10.2	9.4
b2 Sulphur at 0.1%	8.1	8.7	9.3	9.4	8.9	8.4	9.7	10.4	10.5	9.8
b3 Mg SO_4 at 0.5%	9.5	10.1	10.6	10.6	10.2	10.2	11.6	12.5	12.5	11.7
b4 Chelated Zn at 0.05%	8.7	9.3	10.1	10.2	9.6	8.8	9.8	10.9	10.9	10.1
b5 S + Mg	10.8	11.4	12.2	12.2	11.7	12.7	13.9	15.9	15.4	14.3
b6 S + Zn	10.0	10.8	11.4	11.4	10.9	11.3	12.7	13.5	13.5	12.8
b7 Mg + Zn	11.4	12.5	13.2	13.2	12.6	13.5	14.4	14.4	14.4	14.2
b8 S + Mg + Zn	12.2	13.3	13.4	13.4	13.1	14.1	14.2	15.7	15.7	14.9
Mean (A)	9.8	10.6	11.2	11.2		10.9	11.9	12.9	12.9	
New L.S.D. at 5%	A 0.6		B 0.6		AB 1.2	A 0.5		B 0.6		AB 1.2

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THE DIFFERENCES IN MACROMINERAL PROFILES IN WINE BY-PRODUCTS FROM SLOVAKIA AND AUSTRIA

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Abstract

In general, grape pomace is known as good source of nutrients with high antioxidant activity and high concentration of lignin that can affect the digestion of nutrients. However, scientific sources focused on macrominerals profile of grape by-products are scarce. Thus, the aim of the research was to compare the concentration of macrominerals in wine industry grape by-products (bunch, stem, pomace) from 3 varieties of Vitis vinifera sp. (Green Veltliner - GV, Pinot Blanc - PB, Zweigelt - ZG) from Austria (AT) and Slovakia (SK). In the samples by High Resolution Continuum Source Atomic Absorption Spectrophotometry the content of macroelements - calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K) were examined. Significant ($p<0.05$) differences between the countries only in the Mg and K content in bunch were observed. However, in the stem of varieties, significant differences ($p<0.01$) in the content of all macroelements between the countries were found. Similar results in the pomace macroelements profile were observed, thus the content of all macroelements was statistically significant. Between all varieties in AT samples main significant differences in the content of Ca and Mg in the bunch, in the content of Ca, P and Na in the stem and in the concentration of every macroelement in the pomace were observed. In SK, main significant differences between every variety only in Na concentrations in the bunch and in P, Mg and Na concentrations in the stem were found.

Keywords: *grape pomace, grape bunch, grape stem, minerals.*

Introduction

Increased efforts are now being directed towards a broader revaluation of polyphenol-rich residues from wine processing for obtaining high value by-products (grape pomace, seeds, skin and polyphenols) (Brenes *et al.*, 2016). Ivanišová *et al.* (2018) found high antioxidant activity in grape samples with high content of total polyphenols. The nutritional composition of grape samples shown that it varies widely, depending on the grape variety, grape origin and the conditions of fertilization (Brenes *et al.*, 2016). Also, the nutritive value of grape by-products is determined by ratio of seeds and pulps (Guerrera-Rivas *et al.*, 2016). The mineral content of grape seeds depends on winemaking process that produce grape residues which can be important source of nutrients and essential elements (Lutterodt *et al.*, 2011). Minerals in grapes are usually classified in groups depending on their mobility in phloem. Potassium, phosphorus, sulfur, and magnesium show high mobility and are accumulated and mainly localized in the skin of the grape berry during ripening. In consequence, grape skins present higher levels than grape seeds, mainly due to their high content of potassium salts localized in grape skins, specifically in the hypodermal cells (Rogiers *et al.*, 2006). In contrast, seeds are the strongest reservoir of calcium, phosphorus, sulfur, and magnesium (Coombe 1987; Gül *et al.*, 2013; García-Lomillo *et al.*, 2014). The present study has shown that GP has moderate protein, crude fat and some minerals (potassium, calcium, phosphorus, copper, iron and sulfur) (Chikwanha *et al.* 2018). As previous studies shown, there are several factors affecting the mineral composition, thus the aim of the study was to compare the concentration of macrominerals in wine industry grape by-products (bunch, stem, pomace) from 3 varieties of Vitis vinifera sp. (Green Veltliner - GV, Pinot Blanc - PB, Zweigelt - ZG) from Austria (AT) and Slovakia (SK).

Material and Methods

Wine industry grape by-products (bunch, stem, pomace) from 3 varieties of Vitis vinifera sp. (Green Veltliner - GV, Pinot Blanc - PB, Zweigelt - ZG) from Austria (AT) and Slovakia (SK) were collected. After that, grape bunch and grape pomace and stem characterised as a result of winemaking industry after juice pressing in Laboratory of Quality and Nutritive Value of Feeds at Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra were analysed. After transportation to the Laboratory of quality and nutritional value of feeds of the Department of Animal Nutrition at the Slovak University of Agriculture in Nitra were samples prepared for nutrition analysis in accordance to standard analytical laboratory methods. The contents of mineral nutrients were determined by High Resolution Continuum Source Atomic Absorption Spectrometer ANALYTIK JENA contrAA 700 (Ca, Mg, Na, K) and 6400 Spectrophotometer (P). The determination of individual element content was based on the absorptions measured at the following wavelengths: Ca content was detected at 422.7 nm, P at 666 nm, Mg at 285.2 nm, Na at 589.0 nm and K at 766.5 nm. Results were statistically evaluated with IBM SPSS v. 20.0. Descriptive statistics and statistical significance of differences between grape varieties using One-way ANOVA (Tukey test) were generated. Then, statistical significance of results between the countries using Independent samples T-test was realised.

Results and Discussion

In both countries in grape bunch the content of K was the highest (Table 1). The highest content of K in AT in PB variety and in SK in ZG variety was determined. However, significant differences ($p<0.05$) in the K content only between the GV and PB, ZG and PB variety in AT were found. In SK, significant differences between the ZG and PB, ZG and GV were observed. The second most abundant mineral in grape pomace in both countries was Ca. Moreover, significant differences between the Ca concentrations in all varieties were found ($p<0.05$). In contrary, in SK significant results only between GV and ZG were determined ($p<0.05$). Then, the variety of GV bunch had in both countries the highest content of Ca. Third highest concentration in grape bunch had P. Though, there were no significant differences in AT between the grape bunch varieties. On the other hand, in SK there were significant differences between the ZG and GV, ZG and PB variety ($p<0.05$). The highest content of Na in AT in ZG variety and in SK in GV variety was observed. But, significant differences only between PB and GV, PB and ZG in AT were found ($p<0.05$). However, in SK significant differences between the all grape bunch varieties were observed ($p<0.05$). The lowest concentrations from minerals in the case of Mg in both countries were determined. In AT grape bunch samples were Mg concentrations between the varieties different ($p<0.05$). On the other hand in SK samples, significant differences only between the GV and PB, GV and ZG were observed ($p<0.05$). Then, the highest Mg content in AT in PB variety and in SK in GV variety was found. Significant differences between the AT and SK samples only between Mg, Na and K content were found ($p<0.05$). There are few studies about the mineral content of the whole grape bunch. Thus, the mineral content of grape bunch depends on the proportion of rachis, pulps, skins and seeds in grape. Ziarati *et al*. (2017) determined mineral content consisted of pulps, seeds and peels of 5 different grapes as follows: Ca 845.6 $mg.kg^{-1}$, Mg 321.5 mg.kg^{-1}, K 1 348.6 mg.kg^{-1}, P 112.0 mg.kg-1 and Na 1.36 mg.kg^{-1}. In comparison with recent study, the content of minerals in AT and SK samples was higher. According to Arrobas *et al.* (2014) in the pulp of grape is the lowest content of minerals in grape seed, then pulp and highest in the stem. Average concentration of minerals calculated from 3 different (pulp, seed, rachis) parts in their research was K 13 100 mg.kg^{-1}, Ca 4 400 mg.kg^{-1}, P 1 500 mg.kg^{-1}, Mg 1 133 mg.kg^{-1}, that is comparable to all samples from AT and SK except of Mg content which was lower in recent study.

Table 1. Mineral content of grape bunch varieties from Austria and Slovakia

C	M	GV		PB		ZG		T	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
AT	Ca	1865.73^{a}	11.62	1451.19^{b}	33.74	1245.56^{c}	35.22	1520.83	274.72
	P	1392.93	69.34	1407.12	70.41	1397.71	1.73	1399.25	49.81
	Mg	419.33^{a}	12.8	545.94^{b}	5.5	472.81^{c}	8.19	179.36^{A}	55.63
	Na	694.28^{a}	23.77	458.50^{b}	0.22	779.66^{a}	68.46	644.15^{A}	148.55
	K	12765.52^{a}	113.98	13777.09^{b}	287.93	12831.19^{a}	273.97	13124.6^{A}	532
SK	Ca	1468.00^{a}	22.54	1431.57^{ab}	7.2	1410.86^{b}	10.41	1436.81	28.19
	P	1322.68^{a}	68.17	1168.31^{a}	66.88	1670.81^{b}	67.20	1387.27	230.43
	Mg	656.39^{a}	10.33	586.84^{b}	14.39	558.51^{b}	10.79	44.83^{B}	44.83
	Na	646.51^{a}	6.11	261.09^{b}	0.22	366.75^{c}	3.42	172.51^{B}	172.51
	K	11527.99^{a}	507.56	11403.00^{a}	472.17	12942.04^{b}	228.18	11957.68^{B}	825.31

Abbreviatons: C - country, M - mineral, GV - Green Veltliner, PB - Pinot Blanc, ZG - Zweigelt, T - Total summary, $\bar{x}$ - mean, SD - standard deviation, AT - Austria, SK - Slovakia, Ca - Calcium, P - Phosphorus, Mg - Magnesium, Na - Natrium, K - Potassium. Different small letters in the columns indicate significant differences between the varieties at the 0.05 level (Tukey Test). Different large letters in the columns indicate significant differences between the countries at the 0.05 level (Independent Samples T-Test).

Similarly like in grape bunch, the most abundant mineral in grape stem in both countries was K (Table 2). However, in AT significant differences only between the PB and GV variety, PB and ZG variety were found ($p<0.05$). On the other side, in SK grape stem samples significant differences between the GV and PB variety and GV and ZG variety were observed ($p<0.05$). The highest K content in AT samples in ZG variety and in SK samples in PB variety was found. Then, again like in grape bunch, the second highest concentrations from minerals in the case of Ca were determined. In AT samples, significant differences between the samples were found, however, in SK samples significant differences only between PB and GV and PB and ZG were observed ($p<0.05$). Then, in AT the highest Ca concentrations in GV variety and in SK in ZG variety were monitored. The highest P concentrations in both countries in samples of ZG variety were found. Moreover, in both countries there were significant differences between the all varieties ($p<0.05$). The Mg content of grape stem samples in both countries in GV variety was the highest. However, significant differences in AT only between GV and PB and GV and ZG were observed ($p<0.05$). On the other hand in SK samples, all varieties had significant different content of Mg. Similarly like in grape bunch, the lowest content from minerals was formed from Na. Moreover, in both countries were differences between the varieties in Na concentration significant ($p<0.05$). However, in AT the highest concentrations of Mg in the ZG and in SK in the GV grape stem were found. Overall, significant differences between the AT and SK samples in the content of Ca, Mg, Na and K were observed. Nowadays, research focused on the mineral composition of grape stem is rare but several studies described concentration of minerals in the grape stem and their pattern. For example, Arrobas *et al*. (2014) found lower K content in grape stem in comparison with SK and AT samples (28 600 mg.kg^{-1}). Lower concentrations of K found also Spigno *et al.* (2013) from 25 800 to 31 700 mg.kg^{-1}, thus only GV sample in SK was in their found interval. Arrobas *et al.* (2014) observed the higher content of Ca 5900 mg.kg^{-1} in comparison with all AT samples but lower compared to all SK samples. However, Spigno *et al.* (2013) determined the Ca content in grape stem from 4 800 to 9 400 mg.kg^{-1} that was corresponding with SK samples, however concentrations of Ca in PB and ZG variety from AT were lower. The content of P in grape stem samples from AT and SK compared to Arrobas *et al.* (2014) (1 800 mg.kg^{-1}) was higher in all analysed samples. Wide range of Mg content determined Spigno *et al.* (2013) from 800 to 3 400 mg.kg^{-1} which was comparable to all samples of grape stem from AT and SK. On the other side Arrobas *et al.* (2014) observed comparatively higher content of Mg (1 400 mg.kg^{-1}) in comparison with AT samples, however the content of Mg in SK stem samples was higher. In overall is possible to detect in ash of grape stem more than 20 minerals with the major content of K, followed by Ca, Mg and Na (Prozil *et al.*, 2012).

Table 2. Mineral content of grape stem varieties from Austria and Slovakia

C	M	GV		PB		ZG		T	
		x	SD	x	SD	x	SD	x	SD
AT	Ca	5119.36^{a}	15.01	4839.97^{b}	21.78	4397.57^{c}	24.49	4785.63^{A}	315.70
	P	2112.23^{a}	67.15	2581.16^{b}	73.6	3105.42^{c}	1.48	2599.60	433.16
	Mg	1406.17^{a}	4.71	979.70^{bc}	10.73	926.34^{c}	0.44	1104.07^{A}	227.82
	Na	227.91^{a}	10.43	655.78^{b}	38.99	762.62^{c}	18.74	548.77^{A}	246.06
	K	39031.07^{a}	1310.21	34809.14^{b}	956.36	39539.82^{a}	1147.76	37793.34^{A}	2458.66
SK	Ca	7984.53^{a}	118.01	6261.87^{b}	64.85	8173.72^{a}	77.33	7473.37^{B}	915.61
	P	2931.44^{a}	69.49	2652.43^{b}	122.16	3226.61^{c}	0.53	2936.83	258.4
	Mg	2093.59^{a}	22.64	1774.26^{b}	10.75	1470.85^{c}	6.22	1779.56^{B}	269.99
	Na	736.32^{a}	12.35	2631.28^{b}	26.24	1501.13^{c}	69.61	1622.91^{B}	826.47
	K	29431.54^{a}	1047.97	33829.17^{b}	57.62	33494.83^{b}	698.11	32251.85^{B}	2211.88

Abbreviatons: C - country, M - mineral, GV - Green Veltliner, PB - Pinot Blanc, ZG - Zweigelt, T - Total summary, $\bar{x}$ - mean, SD - standard deviation, AT - Austria, SK - Slovakia, Ca - Calcium, P - Phosphorus, Mg - Magnesium, Na - Natrium, K - Potassium. Different small letters in the columns indicate significant differences between the varieties at the 0.05 level (Tukey Test). Different large letters in the columns indicate significant differences between the countries at the 0.05 level (Independent Samples T-Test).

In both countries the highest content in grape pomace from minerals had K in ZG variety (Table 3). While in AT samples were differences between the all varieties significant, in SK samples significant differences only between the GV and ZG were found ($p<0.05$). Then, Ca content in the both countries was also the highest in ZG variety. However, significant differences between all varieties only in AT samples were found ($p<0.05$). In SK samples were different results in the content of Ca only between ZG and GV, ZG and PB ($p<0.05$). Similar findings were also in the case of P content. The highest P concentrations in AT ale in ZG variety but in SK samples in PB variety were found. Then, significant results between the all varieties in AT samples were found ($p<0.05$). In contrary, in SK samples were significant differences only between the PB and GV, PB and ZG ($p<0.05$). Then, the highest Mg concentrations in AT and SK grape pomace samples in GV variety were found. Significant differences between the all varieties in AT samples were found, but in SK samples only between ZG and GV, ZG and PB ($p<0.05$). Finally, the lowest content from minerals formed Na. In both countries, the highest Na content in PB variety was determined. However, similarly in comparison with other minerals in AT samples, significant differences between the grape pomace varieties were observed. Differences between the grape pomace Na content from SK were significant only between the ZB and PB and ZG and GV ($p<0.05$). High variability in the concentration of K is observed between the studies. In comparison with Corbin *et al.* (2015) higher content of K in AT samples (20267 – 27333 mg.kg^{-1}) but similar in SK samples was found. Lower content of K observed Chikwanha *et al*. (2018) (15000 – 25300 mg.kg^{-1}) compared to AT samples, however in SK samples was K concentration in their found interval. Corbin *et al.* (2015) found the P concentrations from 2367 to 2733 mg.kg^{-1}. In AT samples of grape pomace, similar P content except of ZG variety was found. In contrary, compared to SK samples lower K content in their research was observed. Chikwanha *et al.* (2018) determined the maximal P concentration in grape pomace 3420 mg.kg^{-1}, which was higher compared to AT and SK analysed samples except of PB variety in SK. In comparison with Bennemann *et al.* (2016), the P content in the grape pomace was higher in all analysed samples from both countries (330.5 – 497.6 mg.kg^{-1}). Similar concentration of Ca found Corbin et al. (2015) (2170 mg.kg^{-1} – 3867 mg.kg^{-1}) compared to AT samples. However, in SK grape pomace samples higher content of P except of GV variety was determined. Chikwanha *et al.* (2018) found also similar concentration of Ca in grape pomace compared to AT samples but also lower in comparison with SK samples (2290 – 3730 mg.kg^{-1}). In contrary, Botelho *et al.* (2018) observed almost the same level of Ca compared to both countries except of ZG variety from SK (1580 – 4295 mg.kg^{-1}). Chiwkanha *et al.* (2018) observed higher Mg content (950 – 1370 mg.kg^{-1}) compared to AT samples, however SK samples were in their found interval. On the other side, Bennemann *et al.* (2016) determined in grape pomace similar Mg content from 587.3 to 1547 mg.kg^{-1}. Then, Corbin et al. (2015) found the content of Mg similar to AT samples (710 – 987 mg.kg^{-1}). Lower content of Na compared to SK and AT samples found Corbin et al. (2015) from 58 to 61 mg.kg^{-1}. On the other side, Chikwanha *et al.* (2018) observed similar Na content in grape pomace except of GV and PB varieties from SK (328 – 1210 mg.kg^{-1}).

Table 3. Mineral content of grape pomace varieties from Austria and Slovakia

C	M	GV		PB		ZG		T	
		x	SD	x	SD	x	SD	x	SD
AT	Ca	2225.58^{a}	73.73	3054.35^{b}	10.01	3286.60^{c}	31.72	2855.51	484.72
	P	2259.71^{a}	65.74	2712.80^{b}	67.75	3160.03^{c}	108.82	2710.85	396.45
	Mg	855.78^{a}	6.18	687.40^{b}	6.35	748.63^{c}	8.37	763.94	74.06
	Na	361.45^{a}	26.68	649.83^{b}	56.64	475.37^{c}	31.15	495.55	130.56
	K	26826.49^{a}	108.03	30122.56^{b}	752.56	58549.82^{c}	729.13	38499.63	15114.41
SK	Ca	3782.00^{a}	74.98	3939.39^{a}	143.02	5366.94^{b}	109.82	4362.78	762.48
	P	3006.57^{a}	1.35	3797.84^{b}	120.76	3200.71^{a}	66.82	3335.04	363.74
	Mg	1173.35^{a}	19.01	1153.86^{a}	16.39	1102.36^{b}	18.43	1143.19	35.38
	Na	1517.80^{a}	53.17	1555.59^{a}	0.25	341.30^{b}	19.36	1138.23	598.59
	K	23825.40^{a}	1287.83	24732.24^{ab}	4.01	25780.62^{b}	316.1	24779.42	1075.94

Abbreviatons: C - country, M - mineral, GV - Green Veltliner, PB - Pinot Blanc, ZG - Zweigelt, T - Total summary, $\bar{x}$ - mean, SD - standard deviation, AT - Austria, SK - Slovakia, Ca - Calcium, P - Phosphorus, Mg - Magnesium, Na - Natrium, K - Potassium. Different small letters in the columns indicate significant differences between the varieties at the 0.05 level (Tukey Test). Different large letters in the columns indicate significant differences between the countries at the 0.05 level (Independent Samples T-Test).

Conclusions

The overall concentration of minerals in both countries is the highest in the stem, then pomace and the lowest in grape bunch. However, larger differences between the countries are in the mineral content of grape bunch and pomace. Also, proportion of minerals in grape products is affected by variety and their origin. But the same concentrations patterns are observed in all by-products, thus there is the major content of K, then Ca, P, Na and the lowest content of Mg. However, it is necessary to realise further analysis focused on the mineral content of grape by-products because of lack information about this problematic in recent science.

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THE CHEMICAL COMPOSITION AND QUALITY OF MEAT FROM POLISH NATIVE PIG BREEDS

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Abstract

The native breeds of pigs are those that grow in a certain region and / or country as local, autochthonous breeds. In Poland there are 3 breeds: Pulawska, Zlotnicka White and Zlotnicka Spotted pigs. The aim of work was to analyse the meat quality of Polish native breed of pigs. We performed chemical analysis of obtained samples as well as colour, shear force and thermal loss analysis. Highest fat content (%) were found in Zlotnicka Spotted while the lowest in Zlotnicka White. Highest L* value and thermal loss were found in Pulawska breed. Highest shear force value were found in Zlotnicka White and differ significantly compared to other breeds. The meat of Pulawska breed, produced for project BIOSTRATEG was characterized by better quality parameters in comparison to meat of fatteners of the same breed bought in a popular shop-network. The meat of Pulawska, Zlotnicka White and Spotted pigs is a good quality raw material which is used for production of traditional and regional meat products gaining high sensory scores and good recognition among consumers. Promotion of traditional products obtained from above breeds will favour the development of their breeding.

Key words: *fatteners, native pig breeds, meat, quality*

Introduction

The domestic animals' breeds accompany human more than 10 thousand years. They have given not only food but also helped with field work, were the components of agri-landscape playing the immanent part of nature, of culture and even ethnography (Litwińczuk, 2017). Nowadays, in Poland according to data obtained for 2016, the altogether number of registered and preserved with special *in situ* programs, is 83 breeds, varieties and lines, where 43 are mammals, 35 domestic birds and 5 bees lines. They are breed in 3259 farms (Krupiński *et al.*, 2017). The native breeds of pigs are these, which are grown up in the certain region and/or country for example, Mangalica, Moravka, **Resavka,** Iberian, Casertana, Alentejana, Bazna, Mora Romagnola, Krskopolje, Turopolje. In Poland as native breeds there are 3 breeds: Pulawska, Zlotnicka White and Zlotnicka Spotted pigs (Szyndler-Nędza *et al.*, 2011). These breeds are characterized by worse fattening, slaughter and traits but their advantages are good quality raw meat, lower feeding demands, natural resistance for worse environmental conditions and higher resistance against illnesses and stress in comparison to commercial breeds. They are outstanding for high reproduction traits and well adaptation in difficult environmental conditions (Krupiński *et al.*, 2017). Also they are known for good maternal traits (Szyndler-Nędza *et al.*, 2011). The pigs of Polish native breeds can be used for breeding of heavy fatteners, because even afterwards obtaining of high body weight the meat shows the very good quality (Babicz *et al.*, 2009; Martyniuk, 2010; Szyndler-Nędza, 2012; Cebulska, 2015). The meat of these breeds can be characterized by advantageous muscle fibres structure and the amount of intramuscular fat favourably influencing the meat marbling and sensory properties. Thanks to above the meat of these pigs breeds is used for production of regional meat products (Florowski *et al.* 2006; Blicharski, 2007; Szyndler – Nędza *et al.* 2011). In 1996, the Pulawska, Zlotnicka White and Zlotnicka Spotted pigs were included in Genetic Resources Conservation Programme in order to preserve biodiversity and in 2010 Polish Pig Breeders and Producers Association POLSUS cooperation with the Auchan hipermarket network was launched, which in their offer included the meat of Puławska breed pigs

The aim of work was to analyse the meat quality of Polish homeland traditionally breed pigs.

Material and methods

In scope of the project *"The uses and the conservation of farm animal genetic resources under sustainable development"* co-financed by the National Centre for Research and Development within the framework of the strategic R&D programme "Environment, agriculture and forestry" – BIOSTRATEG, contract number: BIOSTRATEG2/297267/14/NCBR/2016 were analysed meats of native Polish pigs' breeds. The fatteners of Polish native breeds were obtained from farms specially dedicated for this type of breeding-these were namely: Pulawska, Zlotnicka White and Zlotnicka Spotted. The fatteners with weight from about 120 to 130 kg were slaughtered in professional slaughter-houses. The experimental material was pork loin (*m. longissimus dorsi*) obtained from 10 purebreed fatteners (5 gilts and 5 barrows).

Also there were analysed loins (*m.longissimus dorsi*) and hams (*m. semimembranosus)* of 6 Pulawska breed pigs' meat bought in shop-network delivering meat of fatteners specially breed for the BIOSTRATEG project. The obtained samples of meat were minced and subjected to physico-chemical analyses in laboratory of Department of Animal Product Technology University of Agriculture in Kraków. In the raw meat samples:

• water content according to the standard *PN-ISO 1442:2000*,
• fat content according to the standard *PN-ISO 1444:2000*,
• protein content by Kjeldahl method *(PN-75/A-04018)*,
• total ash content according to the standard *PN-ISO 936:2000*,
• total carbohydrates content was calculated assuming that the all total solids and water stand for 100%

To analyse meat weight cooking loss samples were heated to reach the internal temperature of 75ºC in the geometric centre of the sample. The results were computed from the difference between the weight before and after cooking,

• the measurements of colour of meat samples were obtained using CIELab system. Lightness [L*], redness [a*] and yellowness [b*] of meat were determined using a Konica Minolta CM – 600d spectrophotometer, for the D65 illuminant and a 10° standard observer,
• the pH of the muscle was measured with the use of pH-meter Benchtop Electrochemistry Meter (Belgium).
• The measurement of shear force was performed with Warner-Bratzler triangle edge knife. From each lot were cut out 5 cylindrical samples (diameter 14mm , height 15mm) and the force needed for rectangular samples cutting was measured. The test blade speed was 2mm/s.

Statistical analysis

All samples were obtained at least in duplicates. All results were analysed with ANOVA and present as means with standard deviation. The calculations were performed with Statistica 6.0 *(StatSoft, 2003)*.

Results and discussion

In Table 1 is presented the chemical composition of meat loin of fatteners. The content of protein in the loin of native pigs ranged from 22.10% (Zlotnicka Spotted breed) to 23,51% (Zlotnicka White). Szulc and Skrzypczak (2016) analysed chemical composition of muscle *longissimus dorsi* of different pigs breeds show that protein content in Zlotnicka Spotted breed meat ranges from 22.3% to 24.54%, and in Pulawska breed meat from 22.0 to 23.37%. The intramuscular fat (IMF) level in Zlotnicka White loin was 2.51% whereas in Zlotnicka Spotted 3.40%. Szulc and Skrzypczak (2016) reported that IMF content in *longissimus dorsi* of Zlotnicka Spotted breed ranges from 1.87 to 3.44%, whereas in Pulawska *longissimus dorsi* meat from 2.20 to 3.70%. Although the high intermuscular fat content has advantages influence on flavor and texture of meat, but consumers may not accept IMF content Wood *et al.*, (1999), Daszkiewicz *et al.*, (2005) and Tyra and Mitka (2015) reported that the optimum taste, tenderness and juiciness show the meat with fat content ranging from 2.5 to 3.0%. The lower amounts (1,19 ÷ 2,20%) of intramuscular fat and paralely lower energy value of loins of Pulawska pigs was obtained by Piórkowska *et al.* (2010) and Kasprzyk *et al.* (2013). Above described differences arised from different ways of feeding and breeding conditions of Pulawska breed pigs, in compared assessments. The ash content in meat of analysed fatteners was similar and did not differ statistically.

In Table 2 are presented selected quality traits of analysed breeds. pH24 value was similar to results obtained by other authors Szulc and Skrzypczak (2015, 2016). The meat of Pulawska breed pigs was characterized by higher values of parameter L* (56.73) the higher values of parameter a* (9.79) (was more red). The lighter colour and the higher value of parameter L* were, the most probably, the effect of higher fat content; the higher contents of fat make the meat brighter. Szulc and Skrzypczak (2016) revealed that the meat of native breeds is characterized by the lightness value L* at level 45-50. They also mentioned that the differences in intermuscular fat content influence on muscles , the results of colour measurements so then on lightness and share of red colour. Szulc *et al.* (2012) reported the low share for red colour (4.06) whereas Jankowiak *et al.* (2010) and Bocian *et al.* (2012) found the bigger shares of red colour (16.82–17.34) in meat of Zlotnicka Spotted pigs' breed. After the thermal treatment (roasting), there were obtained, in meats of both fatteners groups, similar thermal lossess, although in loins of Pulawska breed these losses were observed as lower but that difference was not statistically significant.

Table 1. Chemical composition of loins of native breeds of fatteners

Component	Fattener's breed		
	Pulawska	Zlotnicka White	Zlotnicka Spotted
Water	72.37±2.72	72.60±0.89	72.70±0.75
Total solids	27.63±0.78	27.40±0.86	27.30±0.72
Protein	23.02±5.55	23.51^{a}±3.97	22.10^{b}±3.52
Fat	3.10±0.49	2.51^{a}±0.84	3.40^{b}±0.64
Ash	1.10±0.07	1.08±0.09	1.20±0.13
Carbohydrates	0.41±0.01	0.30±0.02	0.60±0.04

Mean values in rows marked with a different letters differ statistically significantly at $p \leq 0.05$

Szulc and Skrzypczak (2016) revealed that the thermal loss for local pigs' breeds is slow. But Wojtysiak and Pawłowicz (2014) obtained, the comparable to ours, thermal loss for Pulawska breed pigs' meat 35.92 %. Roasted meat of Zlotnicka Spotted breed was characterized by a smaller shear force than meat of Zlotnicka White and Pulawska pig's breeds.

Table 2. Selected quality characteristics of loins of analysed breeds.

Parameters	Fatteners breeds		
	Pulawska	Zlotnicka White	Zlotnicka Spotted
pH_{24}	5.60±0.03	5.58±0.03	5.52±0.02
Colour parameters			
L*	56.73^{a}±4.71	50.74^{ab}±3.89	46.43^{b}±4.03
a*	9.79^{a}±1.77	5.03^{b}±1.08	8.20^{a}±0.92
b*	8.65^{a}±1.57	3.87^{b}±0.56	2.95^{b} ±0.44
Thermal losses %	32.88^{ab}±2.98	34.16^{a}±3.86	29.32^{b}±3.17
Shear force of roasted meat [N]	18.16^{a}± 3.01	22.63^{b}± 3.41	17.06^{a}± 2.59

Mean values in rows marked with a different letters differ statistically significantly at p≤0.05

In Table 3 are presented chemical composition and selected quality characteristic of loin and ham of Pulawska breed bought in shop-network and produced for BIOSTRATEG project. The meat of Pulawska pigs, bought in the shop, was characterized by bigger thermal loss and higher shear force in comparison to meat of pigs breed in used for BIOSTRATEG project. The meat bought in the shop was originated from fatteners with high meatness (lower fat content), and the high thermal loss points for crossbreed with Pietrain breed. Janiszewski *et al.* (2015) assessed the quality of meat and of whole carcasses of Zlotnicka Spotted breed and of its crossbreeds with PLW (Polish Large White) and Duroc. The crossbreeding of Zlotnicka breed pigs with PLW and Duroc caused growth of meat content in the carcass and lowering of lard amounts. Florowski *et al.* (2016) found that the pig's breed is the significantly differing factor for many meat characteristics.

Table 3. Chemical composition and selected quality traits of loins and hams of Pulawska breed pig.

Parameters	Muscles			
	Loin – (*m. longissimus dorsi*)		Ham (*m. semimembranosus*)	
	Produced for BIOSTRATEG project	Bought in shop - network	Produced for BIOSTRATEG project	Bought in shop - network
Chemical composition %:				
Total solids	27.63±2.78	26.76±2.98	27.47±3.03	26.43±3.21
Protein	23.02±5.55	23.41±2.75	22.42±3.58	22.69±3.23
Fat	3.10^{a}±0.49	1.95^{b}±0.43	3.45^{r}±0.67	2.24^{s}±0.38
Ash	1.10±0.07	1.11±0.06	1.22±0.11	1.18±0.12
Carbohydrates	0.41±0.01	0.29±0.03	0.38±0.04	0.32±0.03
Thermal losses %	32.88^{a}±2.98	37.10^{b}±2.96	34.21±3.02	35.99±1.95
Colour parameters				
L*	56.73±4.71	54.10±2.54	50.80±3.43	54.17±3.17
a*	9.79^{a}±1.77	2.16^{b}±0.33	16.77^{r}±1.06	3.78^{s}±0.43
b*	8.65±1.57	10.93±1.12	11.54^{r}±0.96	11.34^{s}±1.12
Shear force for roasted sample [N]	18.16^{a}± 3.01	22.61^{b}± 2.44	12.80^{r}± 2.07	24.72^{s}± 3.11

^{a,b} Mean values for loin in rows marked with a different letters differ statistically significantly at p≤0.05
^{r,s} Mean values for ham in rows marked with a different letters differ statistically significantly at p≤0.05

Among all pig's breeds present in Poland the most valuable meat (better sensory scores) is in Duroc and native breeds (here belongs Pulawska), whereas less valuable is meat obtained from Pietrain breed. With lowering of fat content in fatteners the lossess of preservation and preparation for consumption of meat are growing (Trombetta *et al.*,1997).

Conclusions

The meat of Pulawska, Zlotnicka White and Spotted breeds of pigs is a good quality raw material which is used for production of traditional and regional meat products gaining high sensory scores and good recognition among consumers. The meat of Zlotnicka Spotted breed deserves the special attention because of advantages intramuscular fat content, lower thermal losses and smaller shear force. The meat of Pulawska breed, produced for project BIOSTRATEG was characterized by better quality parameters in comparison to meat of fatteners of the same breed bought in a popular shop-network. In order for meat from the Pulawska porkers sold in the shop network to compete for meat of very fine meat fatteners, it is necessary to change the requirements for porkers of the Pulawska breed and eliminate the crossing of the Pulawska breed with pietrain boars. Promotion of traditional products obtained from above assessed breeds will favour the development of their breeding and stocks rising.

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EFFECT OF LACTATION STAGE ON THE CONCENTRATION OF SELECTED ESSENTIAL AND TOXIC ELEMENTS IN MILK OF SHEEPS FROM AREA OF SLOVAKIA WITH SLIGHTLY DISTURBED ENVIRONMENT

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Abstract

The aim of this study was to determine the lactation stage effect on the concentration of selected essential and toxic elements in the sheep milk from area of Slovakia with slightly disturbed environment and to find the actual contamination of selected areas, in view of its environmental character, and to refer to the suitability of the use of milk from this area to other food processing. The research was conducted with 300 sheep (Tsigai breed), average age of 6 years, where the milk samples were taken during the spring season (early lactation stage), summer season (middle lactation stage), and autumn season (late lactation stage). Sheep were reared on the extensive pastures, reared indoors afterwards, fed with pasture *ad libidum*. Milk samples were collected after morning and afternoon milking. Despite the fact that there was large number of animals on the farms, average milk samples were obtained from milk tanks at the end of milking. The samples of milk were analyzed by atomic absorption spectroscopy and atomic emission spectrometry. Significant increase ($P<0.05$) of Ca, Mg, Se and Fe concentrations were found in the milk during the lactation. In case of Mg, Se and Fe increase of concentration was found only in the last stage of lactation. Statistically significant difference ($P<0.05$) in concentration of Zn were found during the lactation stages, but the concentration of Zn decreased between the spring and summer lactation and increased between the summer and autumn lactation. Concentration of essential element (Cu) and toxic elements (As, Cd, Hg, Ni, Pb) in milk was low, below the LOQ. It can be concluded, that the use of milk of sheep from this area for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

Keywords: *sheep, milk, environmental burden, essential elements, toxic elements, lactation stage.*

Introduction

Milk and dairy products are important components of the human diet. Milk has been described as a complete food because it contains vital nutrients including proteins, essential fatty acids, lactose, vitamins and mineral in balanced proportions. However, milk and products of milk can also contain chemical hazards and contaminants, which constitute a technological risk factor for dairy products, for the related commercial image and, above all, for the health of the consumer (Licata et al., 2004).
The importance of milk in human diet is widely established and its regular consumption is recommended, especially for young children. In recent decades sheep milk has assumed an increasingly important role in the human diet, not just for infants but also for adults and especially nursing mothers (Sanz Ceballos et al., 2009; Kapila et al., 2013)
Sheep milk may contain various elements of nutritional or toxicological importance, and their levels can vary according to intrinsic factors (lactation stage, animal species and health status), and extrinsic factors (season, feeding and environment). Because the nutritional habit of these small ruminants to graze plants and grass, they may be considered such

environmental bio-indicators and their milk a good matrix to monitor the pollution status. Heavy metals such as arsenic, cadmium, lead, nickel and mercury whose toxicity is well known (Llobet et al., 2003), are widely dispersed in the environment and their contamination sources are various: grazing animals are exposed to their accumulation by ingestion of water, grass and feed (Hilali et al., 2011; Rahimi, 2013; Najarnezhadand and Akbarabadi, 2013).
Toxic heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg) and nickel (Ni) have negative effects on livestock health (Lane et al., 2015), as well as harmful effects on human health (Perween, 2015). This problem is more important for children, who consume large amounts of milk and are the most vulnerable population. Pb and Cd are not essential for animals and plants. These metals are potentially toxic, causing hematologic, neurotoxic, and nephrotoxic effects even at low concentrations. Human exposure to these heavy metals has a negative effect on specific organs that may lead to metabolic disorders, fatigue, heart failure, and cancer. Furthermore, both chronic and acute exposure to Pb can result in encephalopathy (vomiting, depressed consciousness and lethargy), and it also decreases the learning ability in childhood (JECFA, 2011; EFSA, 2012).
Micronutrient elements such as iron, copper and zinc are essential for many biological functions. Deficiencies of such elements contribute significantly to the global burden of disease; however, if present at higher levels, they can have a negative effect on human health. Both toxicity and necessity vary from element to element. Milk and milk products are considered very poor sources of iron and copper and can supply smaller quantities of zinc (Kazi et al., 2009).
Thus, the aim of the study was to determine by monitoring of environment the content of essential elements (Ca, Mg, Fe, Se, Cu, Zn) and toxic elements (Cd, As, Pb, Hg, Ni) in milk from selected farm of Slovakia according to different stages of lactation. To find the actual contamination of selected area to refer to the suitability or unsuitability of the use of milk from these areas, to other food processing.

Material and Methods

The monitoring of area was realized during the lactation stages on selected farm of Slovakia. According to the Ministry of Environment of Slovak Republic (SR) and Slovak Environmental Agency, regions of SR are divided into the three types of environmental quality: 1st environmental quality – regions with undisturbed environment and convenient environment; 2nd environmental quality – regions with a slightly disturbed environment, areas with disturbed environment and areas with widely disturbed environment; 3rd environmental quality – regions with heavily disturbed environment (Bohuš and Klinda, 2008). For monitoring in this study, the village Klátova Nová Ves (Western Slovakia) which is characterized as area with widely disturbed of environment was selected. This region, also called Horná Nitra, is typical with contamination of soil by heavy metals (As, Hg). Contamination soil and environment of this region is caused by power station in Nováky, where the coal is burned. The ash from the burning of low quality coal contains high amounts of As.
The research was conducted with 300 ewes (Tsigai breed), average age of 6 years. Total number samples of milk were 15 and about 500 mL sample of milk were taken during the spring season (early lactation stage), summer season (middle lactation stage), and autumn season (late lactation stage). Sheep were reared on the extensive pastures, reared indoors afterwards, fed with pasture *ad libidum*. Milk samples were collected after morning and afternoon milking. Despite the fact that there was large number of animals on the farms, average milk samples were obtained from milk tanks at the end of milking.
Milk samples for determination of Ca, Zn, Fe, Mg, Cu, Ni, Pb, Cd were prepared by mineralization with microwave decomposition with HNO3 and H2O2 (microwave oven

MARS 6 240/50). Milk sample for determination of Se was prepared by mineralization with microwave oven with HNO3 and H2O2 (microwave oven MARS 6 240/50), removal nitrous gases, cooling, followed addition solution of HCl, reduction from Se6+ to Se4+ by heated at 90°C. Milk samples for determination of As were prepared by dry mineralization with oxidation mixture (oxygen, oxides of nitrogen, ozone), heated at 300 – 400°C. The ash was re-diluted in solution of HCl. As and Se in milk were analyzed using the hydride generation atomic absorption spectroscopy (HG-AAS) method with Spectr AA-220 FS (The Netherlands). Ca, Fe and Mg in milk and feed were detected using the inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 720-ES, USA). Cd, Pb and Ni in milk and feed were analyzed using the electro thermal atomization atomic-absorption spectrometry (ETA-AAS, Agilent DUO AA 240Z/240FS, USA). Zn and Cu in milk and feed were analyzed using the (F-AAS, DUO AA 240Z/240FS, USA). Hg in milk and feed was analyzed using the Advanced Mercury Analyzer and atomic-absorption spectrometry (AMA-AAS, Altec CR) without the need for chemical preparation of the sample. All analyses were conducted in certified testing laboratory Eurofins/Bel Novamann (Nové Zámky, Slovak Republic).

Data were analyzed with statistical software IBM SPSS Statistics 20. The results are presented as arithmetic mean with standard deviation and maximum and minimum value of concentrations of selected metals. Means of concentrations of metals during the lactation stages were compared using Tukey HSD test and differences between lactation stage effect declared significant at $p<0.05$ level.

Results and Discussion

The average concentrations of elements in feed and sheep´s milk as well as other statistical measurements were determined (Table 1 and Table 2). During the spring season content of essential elements in feed (Ca, Zn, Fe, Mg, Cu) and toxic metals (As, Cd, Hg, Ni, Pb) was higher than during the autumns season. In case of autumn season, content Se and all analyzed toxic metals was below the LOQ (limit quantification). Bushra et al. (2014**)** studied concentrations of toxic elements in feed from rural and urban areas. They found, that the content of Cd, Ni and Pb was 0.27, 1.68, 4.11 mg.kg-1 in urban areas and in rural areas 0.037, 0.024, 4.52 mg.kg-1, respectively. Compared to results of their study, on farm of Klátova Nova Ves (widely disturbed environment), content of Pb and Cd was lower and content of Ni was higher. Lower content of Ni, As, Cd, Pb in feed state Zhou et al. (2017) compared to results of this study.

Animal studies demonstrate that nickel has negative effects on the structure and function of the testis, seminal vesicles and prostate gland; there is similar report on adverse effect on spermatozoa (Pandey et al., 2000; Forgacs et al., 2001). Lukáč et al. (2014) reported the negative effect of nickel on spermatogenesis. The decrease in the relative volume of germinal epithelium indicates on alterations of the spermatozoa production. Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects (Kukner et al., 2007; Massányi et al., 2007).

Tab. 1 Concentrations of mineral and selected toxic elements in feed of sheep (mg/kg)

Green forage											
Elements	Ca	Zn	Se	Mg	Fe	Cu	As	Cd	Hg	Ni	Pb
Spring season	7400.0	42.2	0.065	3050.0	333.0	11.2	1.2	0.15	0.2	6.8	3.8
Autumn season	1140.0	5.3	<0.03*	314.0	72.0	1.6	<0.03*	<0.01*	<0.3*	<0.10*	<0.30*

*concentrations with this index are below the LOQ (limit quantification)

A positive result of this study was, that content of toxic metals, which detected in feed on farm, did not affect on their occurrence in sheep milk. Content of all toxic elements (As, Hg, Ni, Pb, Cd) in milk was below the LOQ (Table 2). The content of Pb in milk in this work was significantly lower than in milk samples from Iran (Najarnezhadand and Akbarabadi, 2013; Rahimi, 2013) and very low in comparison to the results of Anastasio et al. (2006) and Licata et al (2012).

Tab.2 Descriptive statistics for concentrations of essential and toxic elements in sheep´s milk

Element, mg/kg	Mean±SD	Min	Max
Ca	1730.00±769.61	1040.00	2560.00
Mg	151.67±54.42	89.00	187.00
Zn	4.33±3.22	2.00	8.00
Se	<0.030	-	-
Fe	0.67±0.58	0.1	1.0
Cu	<0.50	-	-
As	<0.030	-	-
Cd	<0.0040	-	-
Pb	<0.010	-	-
Ni	<0.10	-	-
Hg	<0.002	-	-

SD- standard deviation; Min- minimum value; Max- maximum value

It is evident (Table 3) that milk of sheep from farm of Klátova Nová Ves was found significant increase concentrations of Ca during the lactation and significant increase of Mg between early and middle lactation stage and significant decrease between middle and late lactation stage. Concentrations of Zn were identical during the early and middle lactation stage and in the last stage of lactation significantly decrease of Zn concentrations was recorded. Concentrations of Se were below the LOQ during the lactation stages and the highest concentration of Fe was found in middle stage of lactation. Cu and toxic elements (As, Cd, Hg, Pb, Ni) during the lactation stages were below the LOQ.

Compared to present study, Antunovič et al. (2016) in sheep´s milk in Croatia found increased concentrations Ca, Mg during the lactation and increased concentrations Fe and Se during the early and middle stage of lactation. Al-Wabel (2008) recorded in sheep´s milk in Saudi Arabia lower concentration of Zn (3.09 mg/kg), Ca (822.50 mg/kg) and higher concentration of Cu (0.62/mg) and Fe (5.01 mg/kg). In milk of Bulgarian breeds of sheep (Strednostaroplaninska and Tetevenska sheep) Gerchev and Mihaylova (2012) found similar concentrations of Ca (223 and 208 mg/100g) as well as higher concentration of Fe (0.181 and 0.12 mg/100g).

The concentrations of elements in raw milk are also affected by animal forage, feed and water (Dobrzański et al., 2005; Al-Wabel, 2008). Animal feed with elevated levels of these elements causes also an increase of their level in milk (Bushra et al., 2014). Concentrations of health-beneficial elements, e.g. Fe, Zn in milk are dependent on the animal species, feed, milk sample collection time, environmental conditions and manufacturing processes (Herwing et al., 2011). Changes in composition of milk can also be affected by many genetic (breed, herd) and physiological factors (lactation, age, animal health), but also the environment (food, climate, season, method of milking) (Komperej et al., 1999).

Tab. 3 Concentrations of essential elements in sheep´s milk during different stages of lactation

Element mg/kg	Stage of lactation (Mean±SD)		
	Early	Middle	Late
Ca	2560.00±1.58[a]	1040.00±1.58[c]	1590.00±1.58[b]
Mg	187.00±1.58[a]	1040.00±1.58[c]	179.00±1.58[b]
Zn	8.00±0.16[a]	8.00±0.16[a]	2.20±0.16[b]
Se	<0.030	<0.030	<0.030
Fe	<0.50	1.30±0.16	0.81±0.002

SD- standard deviation; [a,b,c] means within a row with different superscripts differ (p<0,05); [a] means within a row with the same superscripts- non significant

Conclusions

The results indicate that content of selected essential elements and toxic metals in feed and milk changes depending on the season of year and lactation stage. The work showed significantly increase content of Ca, Mg, Se and Fe during the lactation stage. Concentration of essential element (Cu) and toxic elements (As, Cd, Hg, Ni, Pb) in milk was low, below the LOQ. It can be concluded, that the use of milk of sheep from this area for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

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THE CHEMICAL CONTENT OF BEEF MEAT AT THE DIFFERENT FEEDING

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Abstract

Both, genetic and feeding factors, affect beef quality. Although breed or type contributes significantly to the genetic variation in beef quality, nutrition is one of the most important factors. The aim of our research was to study the influence of feeding of pea on beef meat quality. We had two groups with different feeding: ordinary feed ration and feed ration with pea. Three calves from each group were slaughtered (live weight 250 kg) at a commercial slaughterhouse via electrical stunning, followed by exsanguinations, and carcasses were dehaired via scalding, eviscerated, and split vertically down the midline. Hot carcass weights were obtained and measured. The samples of meat were taken from the *musculus longissimus lumborumetthoracis* 24 hours *post mortem* and subsequently subjected to the chemical analysis. The carcass traits in our studies were not influenced by peas included in beef cattle calves diets: carcass outcome weight was from 58 to 62%, muscle-eye area 62-76 cm^2. Higher results were in pea cattle group. The chemical content of meat also did not differ significantly, except crude protein, 24.1±0.16 and 24.9±0.31, respectively. The higher was in pea cattle group ($p<0.05$). The other indices were similar in both groups: crude fat 1.7±0.19 and 1.6±0.20, cholesterol, mg 100g - 56.0±3.99 and 58.2±4.79, ph 5.4±0.11 and 5.3±0.15. The pH of muscle considered "normal" was 5.6. Muscle color, texture, water-holding capacity and tenderness were influenced by pH. The pH in beef meat ranged from 5.3-5.4 in our research, a little less than in"normal" meat. This indicates that the animals may have been killed in stress. The concentrated feed with peas in cattle diets make beef carcasses more muscular.

Key words: *beef meat, pea, feeding, carcass traits.*

Introduction

Field pea contributes significant amounts of protein, carbohydrates, and amino acids to all species, but is increasingly considered an excellent ingredient in beef, dairy, pig and poultry rations due to their nutrient density. The crude protein content of field pea may vary due to the influence of variety and environment. Because of this variation, field pea should be tested for protein for inclusion in balanced livestock rations. Field pea compares favorably with other grains and co-products for several nutrients. Pea are considered a crude protein source in most diets. Energy levels are similar to corn for most livestock species with starch (54%) and digestible fiber (hemicellulose fraction 7%) accounting for most of this fraction. Fat is a modest contributor at 1.55% (Walhain et al. 1992). Amino acids are important to pig and poultry, but not a major concern to ruminants as microbes in the rumen provide the required amino acids for beef and dairy cattle and sheep. However, rate and extent of ruminal degradation for both starch and protein are important to ruminants. In cattle nutrition, utilization of pea seeds is limited, particularly in high-producing cows, where the need for undegradable protein is likely to be high. Therefore, pea protein is characterized by high solubility and ruminal effective degradability (Walhain et al. 1992). Protein value attributed to pea in the Feed Tables, which is largely influenced by effective degradability, is very low. The effective degradability value is obtained from the nylon bag method, which is strickly and carefully standardized. In this method, tested feeds are ground through a 0.8-mm screen. A more coarse grinding, as in practice, could reduce ruminal effective degradability and improve protein value. Several experiments have been conducted to investigate the effects of particle size on rate and extent of breakdown in the rumen of different feeds for example barley, corn, soy, wheat, lupin seeds (Kibelolaud et al. 1991). There is lack of data in the literature on the effect of grinding on rumen escape of pea crude protein and starch (Bayourthe et.al., 2000). The aim of the our research was to determine the efficiency of feeding of pea into concentrated feed mixture of the beef cattle calves rations and to study its effect on meat quality.

Materials and methods

Animals and housing. The twenty pure Charolais breed beef cattle calves were allotted to trial. The research was done in one farm in Latvia. Two groups of growing beef cattle calves with their mothers were conducted for trial in summer time. The calves received concentrated feed in special feeding equipment.The animals were 90 days old at the start of the experiment. The calves were raised from 110 kg to weaning weight. The one calves group received pea 15% (trial group) blended into a mixture of concentrated feed, but control group only mixed concentrates. We used field pea, which harvested as grain, with chemical content: crude protein 25.87%, acid detergent fiber 9.03%, neutral detergent fiber 17.97%, starch 48.16%, calcium 0.09%, phoshorus 0.57% in dry matter. The field pea (15%) we mixed together with barley (27.5%), wheat (27.5%) and oats (30%). This concentrate feed received trial beef cattle group (10 calves in growing period), but control group only mixed grains barley (27.5%), oats (45%) and wheat (27.5%). Control and trial groups animals received mixed feed with chemical content, respectively, crude protein 11.0% and 14.3%, starch 55.9% and 54.4%, acid detergent fiber 8.81% and 8.18%, neutral detergent fiber 22.59% and 18.15%, the energy content 8.8 and 7.9 NEL, MJ kg $^{-1}$ in dry matter. The basic feed was grazing grass with crude protein 10.3% for both beef cattle groups.

The chemical analyses of the pea, the prepared concentrated feed and grass were determined in the laboratory. During the study the live weight of calves was monitored, and the feed consumed was counted.

Three calves from each grop were slaughtered at a commercial slaughterhouse via electrical stunning, followed by exsanguinations, and carcases were dehaired via scalding, eviscerated, and split vertically down the midline.

Hot carcass weights were obtained and was measured muscle-eye area with the planimeter. The carcass was devided into fractions for determination muscle and fat. The samples of meat were taken from the *musculus longissimus lumborum et thoracis* 24 hours *post mortem* and subsequently subjected to the chemical analysis.

Analytical Methods. Diets and ingredients were milled through a 1-mm screen before analysis. The dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), starch, fat, Ca and P contents were analyzed based on standard methodology, respectively DM with ISO 6496: 1999, CP with LVS EN ISO 5983-2: 2009, Ca with LVS EN ISO 6969: 2002, P with ISO 6491: 1998, ADF with LVS EN ISO 13906:2008 and NDF with LVS EN ISO 16472:2006. The neto energy (NEL) were calculated according to equation (Mc Donald et.al., 2002; Morgan,1975). The quality indicators for nutrients were determined by the accredited laboratory of Agronomic analysis of the Latvia University of Life Sciences and Technologies. The chemical analysis of meat were determined by LVS ISO 1442:1997 for water, LVS ISO 1443:1973 for fat and LVS ISO 937:1978 for crude protein content, LVS ISO 2917:2004 for ph and BIOR-T-012-132-2011 for cholesterol content in laboratory of Food and Environmental Investigations (BIOR) in Latvia.

Statistical analysis. All data were statistically processed to determine the differences between diets. Statistical analysis was performed according to the General linear Model procedure of

SAS/STAT 9.22 software package (2010). Most data was reported as aritmetic means with the pooled SEM. The treatment means were compared using Student's *t-test.* Statements of statistical significance were based upon $P<0.05$.

Results and discussion

The results showed that 15% inclusion of pea in beef cattle calves diets signicantly increase daily liveweight gain (Table1.). The daily liveweight gain were 1.1±0.04 kg and 1.3±0.55 kg, respectively in control and trial calves groups.

Table 1. Beef cattle calves growth rates (n=12)

Indices	Control group	Trial group
Liveweight at the beginning of the growing period, kg	110±24.2	118±25.6
Liveweight at the end of growing period, kg	245±23.5*	281±22.0*
Liveweight gain, kg	135	163
Growing period, days	123	123
Liveweight gain per day, kg	1.1±0.04*	1.3±0.55*
Liveweight at the age of 200 days, kg	202.7±12.9*	249.6±21.9*

*p<0.05

The increase of liveweight gain per day was 0.2 kg higher for a group fed concentrate with pea. Beef cattle live weight was significantly higher also *(p <0.05)* at 200 days of age by 46.9 kg. Field pea is a very palatable feedstuff for all classes of beef cattle. This feed may best be used in diets where nutrient density and palatability are important, such as creep feeds (Bayourthe et al., 2000). Feeds with 33% to 67% field peas produced optimum animal performance and return. This formulation may provide excess crude protein as creep feed recommendations call for no more than 16%. Weaned calves can be fed pea at essentially any proportion of the concentrate when grains and supplements make up 60% or less of the total diet. Dietary crude protein requirements for growing steers and heifers are based on gain goals, with higher protein required for faster growth. Maximum recommendations are 13.5 to 14% crude protein in the diet. Peas fed at more than 25% of the total diet will probably result in excess crude protein, but like the creep feed trials, slightly improved performance was observed over the control diet when peas were included at 50% or more of the concentrate. The economics of using field peas at levels higher than 25% of the total diet should be carefully considered (Bayourthe et al., 2000). Finishing cattle have demonstrated some improved performance traits with up to 20% field peas in the diet. Field pea works well in beef cow supplements at most any level. The nutrient density will provide additional benefits as fewer finances of feed will be required for the same nutrition, resulting in lower transportation and storage costs. Field pea may be fed in place of range cake as a protein and energy source for wintering cows or incorporated into range cake at any level required. Field pea makes an excellent binder for pelletting or cubing. No anti-nutritional traits were observed in field pea fed to feedlot and breeding beef cattle at up to 76% of total dry matter intake. Both starch and protein from field peas degrade slowly but relatively thoroughly in the rumen, with only modest levels of escape protein (Bayourthe et al., 2000).

Nevertheless, to peas inclusion in feed mixture, the consumed concentrated feed for one calves during the our research period in control and trial group were, respectively 49.5 and 51 kg and feed consumption per 1 kg liveweight gain did not differ significantly between the research groups. The concentrated feed costs per 1 kg live weight gain was similarly (0.05 EUR).

We know that both genetic and environmental factors affect beef quality. Although breed or type contributes significantly to the genetic variation in beef quality, but nutrition is one of the most important environmental factors (Melton, 1990). Nutrition significantly affects the rate of conditioning and consequently carcass composition, conformation, meat yield and meat and fat quality. In addition to tenderness, the importance of fat quality has increased because it contributes towards the appearance, palatability, nutritive value, processibility, shelf life and ultimately the acceptability of beef. Emphasis is increasingly placed on the production of edible lean with a minimum of excess fat, but reducing fat to too low levels may adversely affect eating satisfaction. Beef quality can be manipulated by a variety of nutritional interventions, many of which have been implemented successfully in feedlots world-wide. The carcass traits in our studies did not influenced by peas inclusin in beef cattle calves diets (Table 2.).

Table 2. Carcass traits (n=6)

Carcass indices	Control group	Trial group
Carcass evaluation (SEUROP)	U	U
Carcass outcome weight,%	58	62
Muscle-eye area, cm^2	62	76
Muscle in carcass, %	69.9	71.1
Fat in carcass, %	2.5	3.0

The chemical content of meat also did not differ significantly, except crude protein, 24.1±0.16 and 24.9±0.31, respectively. The higher was in pea cattle group ($p<0.05$). The other indices were similar in both groups (Table 3). In the other scientist research, fifteen experiments which compared forage and grain finished beef at the same carcass weight or degree of fatness, have been selected from the literature (Muir et al., 1998). When compared at similar carcass weights or the same degree of fatness, the type of feeding system had no effect on tenderness, juiciness, lean meat colour, marbling, or pH. In eight out of twelve experiments where flavour was assessed, panellists could not distinguish an effect of diet on flavour. Effects on fat colour were variable and, in six of the nine experiments where fat colour was measured, grain feeding failed to "improve" fat colour. It is concluded that there is little scientific justification for the claim that grain feeding is necessary to produce high quality beef. Beef of comparable quality can be obtained from cattle finished on forage based diets (i.e., pasture) provided that acceptable carcass weights and degrees of finish can be achieved at a young age (Muir et al., 1998).

Table 3.The chemical content of meat in dry matter (n=6)

Chemical content	Control group	Trial group
Dry matter,%	29.8±0.11	29.6±0.61
Crude protein, %	24.1±0.16*	24.9±0.31*
Crude fat,%	1.7±0.19	1.6±0.20
Ph	5.4±0.11	5.3±0.15
Cholesterol, mg 100g	56.0±3.99	58.2±4.79

*$p<0.05$

The pH of muscle considered "normal" is 5.6 (pH is the negative log of the hydrogen ion concentration; the higher the pH, the less acidic is the muscle). Muscle color, texture, water-holding capacity and tenderness are influenced by pH. The Ph (Table3.) in beef meat were from 5.3-5.4, a little less then in meat" normal".This indicates that the animals may be were killed in stress.

Conclusions

The daily liveweight gain of beef cattle were 0.2 kg higher for the group fed peas. The live weight of the young cattle at the age of 200 days differed significantly *($p<0.05$)*. The pea groups cattle were 46.9 kg heavier. The concentrates feed with peas in cattle diets make beef carcasses more muscular.

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IMPROVING REPRODUCTIVE PERFORMANCES IN PRIMIPAROUS AND MULTIPAROUS COWS

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Abstract

Main goal in cattle reproduction is to achieve high fertility with economical justification for duration of open days or intercalving period. This period in optimal conditions should last 12-13 months, which in return gives best milk production in lactation year. Utilisation of biotechnological measures in control of reproductive functions, it is possible to increase reproductive performances in dairy cows. Induction and synchronisation of estrus usualy require utilisation of hormones for control of ovarian activity and to reduce unsuccessful artificial inseminations. Final goal is to reduce open days/service period to prevent or reduce financial losses. Our study has included 187 cows. Anamnestic data have been taken from reproductive/health card. Before hormonal treatment, all cows had clinical examination of reproductive system. First group had primiparous and multiparous cows which have received prostaglandin F2α in period 40-80 days postpartum, while second group had primiparous and multiparous cows which have received GnRH in period 10-14 days postpartum. Status of ovaries, uterus and pregnancy, have been done with manual and sonographic transrectal examination, while progesterone levels have been assessed using RIA method. Prostaglandin F2α application to multiparous cows, had reduced open days period for 20 days, but same application to primiparous cows had increased open days duration for 30 days. Treatment of multiparous cows with GnRH had signifficant shortening of open days period for 90 days, but same treatment in primiparous cows had reduced percent of conception. Our study has shown that optimum of reproductive performances could be achieved with application of GnRH hormone soon after parturition and timely application of prostaglandin F2α after 40 days postpartum, when histological regeneration of uterine endometrium is complete.

Keywords: *primiparous, multiparous, cow, hormone, reproduction, service period*

Introduction

Main mission of reproduction in cattle husbandry is to achieve high fertility and pregnancy rate. Intercalving period is one of the most representative sign of reproduction success and it should be ideally 12-13 months long, when cows have best reproductive and productive performances. Using breed selection to maximize their genetic potential, signifficant increase in milk production have been achieved in recent decades (Butler, 1981)

Management of reproduction is one of the most important steps toward economical success of any dairy farm. Numerous studies have shown that reproductive ability in dairy cows is compromised, mainly because of late postpartum ovarian cyclicity. Prolonged postpartum anestrus have negative influence to productive ability and profitability of dairy herd (Diskin,

2008). Main negative effect of prolonged postpartum anestrus is seen in longer intercalving period. Increasing intercalving period in dairy cows means, increase of open days period (service period), which in return decrease profitability of those animals. When cows are out of optimum time period to achieve pregnancy, milk production starts to decrease which make them less profitable and increase rick of culling.
Many studies have proven decrease of reproductive efficiency in high yield dairy herds, seen as longer anestrus period, increase of cows with silent and short estrus, low conception rates, uterine abnormalities, which altogether results in more frequent unsuccessful arteficial inseminations (AI) (Iglesia, 1996). Utilisation of appropriate biotechnology methods in control of reproductive functions, it is possible to have influence in reproductive performances. Most often methods of induction and synchronisation of estrus are used to shorten anestrus periods and increase AI success (Chebel, 2004).
Control of ovarian cyclicity in cows and heifers is achieved with utilisation of hormones like GnRH, prostaglandins, estrogens, progesterons or mainly hormones which stimulates release of pituitary hormones.
Goal of this study was to investigate utilisation of hormones to induce ovulation and synchronise estrus in primiparous and multiparous cows in farm conditions and to reduce time period from partus to fertile estrus.

Material and methods

Our study has included 187 cows from free stall dairy farm „Spreča", which have 800 dairy cows, located in Gornje Vukovije – Tuzla Canton. All anamnestic notes were taken from reproductive/health card and all experimental and control cows had manual/ultrasonography transrectal examination of reproductive system.
First group was consisted of primiparous and multiparous cows to receive injection of PGF2α (Estrumate 10 ml, MSD Animal Health) and one control group:

- Multiparous cows (n=38) to receive PGF2α in period 40-80 days pospartum
- Primiparous cows (n=40) to receive PGF2α in period 40-80 days pospartum
- Control group of multiparous cows (n=8) and primiparous cows (n=12)
  Second group were consisted of primiparous and multiparous cows to receive GnRH (Receptal - MSD Animal Healt) at 10. – 14. days postpartum and one control group:
- Multiparous cows (n=34) to receive GnRH at 10.-14. days postpartum
- Primiparous cows (n=38) to receive GnRH at 10-14. days postpartum
- Control group of multiparous cows (n=10) and primiparous cows (n=7)

AI have been performed one time in period 11 to 13h, but if cows had signs of observed estrus next day, they were inseminated again. Pregnancy examination have been done 34 to 38 days after insemination. Norms for comaparation of results have been length of open days period and conception rate. Statistical data processing was done using software Microsoft Excel 2010, method of descriptive statistics and t-test to determine significant differences. All differences from $p<0,05$ are considered as significant.

Results and Discussion

Table 1. Length of postpartum period and conception rate after first AI in treated primiparous and multiparous cows

	Period postpartum multiparous cows	**Period postpartum primiparous cows**	**Conception rate multiparous cows**	**Conception rate primiparous cows**
Average	51,94	55,15	0,39	0,52
Standard Deviation	1,49	2,01	0,08	0,07
Min	39	27	-	-
Max	73	78	-	-
%	/	/	39,48%	52,5%
Total	326	190	326	190

Postpartum period in multiparous cows is a little bit shorter (3.21 day) compared to primiparous cows. Conception rate in primiparous cows is 52.5% and represents significant difference compared to multiparous cows, which have conception rate of 39.48%.

Table 2. Primiparous and multiparous cows with PGF2α applied at 40. – 80. days postpartum, which had conception after first, second or third artificial inseminations (AI).

	First AI	Second AI	Third AI
Multiparous cows (n=38)	*9/38* *23,68%*	*16/38* *42,1%*	*7/38* *18,42%*
Primiparous cows (n=40)	*20/40* *50%*	*11/40* *27,5%*	*5/40* *12,5%*
Control multiparous cows (n=8)	*3/8* *37,5%*	*3/8* *37,5%*	*--/8*
Control primiparous cows (n=12)	*4/12* *33,3%*	*3/12* *25%*	*1/12* *8,33%*

In table 2. it is seen that most multiparous cows treated with PGF2α in period 40. – 80. days postpartum had successful conception in second AI (42,1%), while from the first much less (23,68%) and least from the third AI (18,42%). Primiparous cows had best conception in first AI (50%), then second (27,5%) and then in third AI (12,5%). Control primiparous and multiparous cows have had lower results, compared to experimental group, what could be sign of lower estrus detection.

Table 3. Primiparous and multiparous cows with GnRH applied 10. – 14. days postpartum which had conception after first, second or third artificial inseminations (AI).

	First AI	Second AI	Third AI
Multiparous cows (n=34)	*6/34 17,64%*	*18/34 52,94%*	*5/34 14,70%*
Primiparous cows (n=38)	*11/38 28,94%*	*17/38 44,73%*	*3/38 7,89%*
Control multiparous cows (n=10)	*1/10 10%*	*5/10 50%*	*2/10 20%*
Control primiparous cows (n=7)	*1/7 14,28%*	*3/7 42,85%*	*3/7 42,85%*

In table 3. it is seen that most multiparous cows treated with GnRH 10. – 14. days postpartum had successful conception in second AI (52,94%), while from the first (17,64%) and third (14,70%) much less. Primiparous cows had most conception after second AI (44,73%) and less after first (28,94%) and second AI (7,89%).

According to McDougall (2006) reproductive performances in dairy cows have continuous decrease in farm conditions, as well as in pasture. To accomplish intercalving period of 12-13 months, high level of reproductive performances have to be achieved. Same author quote that out of 80% cows observed in estrus and first time inseminated after 60-80 open days period, conception achieve 55 do 65% cows.

From available literature it is clear that for optimal open days/intercalving period, reproductive efficiency have to be provided (Mutevelić, 2002). Timely estrus detection and AI after parturition is one of the most important measure to enhance reproductive performances, because every non-observed estrus prolong open days period for another 18-25 days. Many factors which depend of each other must be fullfilled to achieve these goals, because none of them working alone is not enough (Crowe, 2008).

Islam et al. (2013) had treated 40 cows with PGF2α at 60 days postpartum and AI had been done in observed estrus. In their study 75% cows had positive response after 2-3 days, which coincides with our results where we had most positive responses at second and third day after application (67,94%). In same study conception was signifficantly higher in treated cows (57,5%) compared to control group ($P<0,05$), which did not happen in our study, where we had better conception in multiparous cows after second AI (42,1%) and better conception in primiparous cows after first AI (50%). Out of 38 treated multiparous cows in our study, 5 cows or 13,15% did not had response to PGF2α application and reason is dystocia ie. uterine dislocation after parturition. One cow without positive response had caesarean section, while other 5 cows have had inflammation of uterus and prolonged postpartal interval.

Important role of GnRH utilisation is prophylactic and therapeutical, which should help in reactivation of ovarian cyclicity in dairy cows in postpartum period, which should have positive effect to earlier AI and better conception (Mlaćo. 2000).

According to Bosu et al. (1987) first postpartum ovulation is after 15-30 days, while 10-15% cows have abnormal ovarian activity during first 2 months postpartum and pituitary response to GnRH application increase with longer time period after parturition. Their conclusion was that GnRH application 2 weeks after parturition, should reactivate ovarian cyclicity. In our study, primiparous and multiparous cows after GnRH application 10 – 14 days postpartum, had clinicaly observed estrus in period 40-80 days postpartum and conception of 19,44%, which coincide with results from Bosu et al.

Conclusions

- Prostanglandin F2α application to multiparous cows have satisfactory results with shortening open days period, but same procedure in primiparous cows leads to longer service period.
- Multiparous cows treated with GnRH have better conception after second AI and shorter service period, while in primiparous cows same procedure have less success.
- During puerperium period, regardles on parturition difficulty, all cows should have routine control of reproductive system as well as frequent utilisation of different hormones to achieve shorter intercalving period.

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EFFECT OF DIFFERENT NITROGEN FERTILIZER APPLICATION LEVELS ON YIELD AND QUALITY OF THE GREEN FODDER *TRICHANTHERA GIGANTEA*

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Abstract
The purpose of this study was to determine the effect of different nitrogen (N) fertilizer application levels on leaf yield and quality of the green fodder *Trichanthera gigantea* as material for leaf meal production for poultry feed in order to improve meat and egg quality. The experiment included five formulas (NT) representing five different nitrogen applications, namely NT1: 0kgN, NT2: 20kgN, NT3: 40kgN, NT4: 60kgN and NT5: 80 kgN/ha/cutting. All five formulas were arranged in a complete block randomized design with five replicates for each formula. The other factors such as plantation density, cutting height and cutting intervals were similar among the treatments. The results showed that from NT1 (0kgN) to NT5 (80kgN/ha/cutting), the leaf dry matter yield was 10.33, 11.22, 11.85, 12.13 and 12.15 tons/ha/year, respectively; the crude protein yield was 2.41, 2.70, 2.95, 3.15 and 3.24 tons/ha/year, respectively. Thus, the increase of nitrogen application from 0kgN to 80kgN/ha/cutting had decreased 10.8% dry matter in the fresh leaf, increased 14.1% crude protein on leaf dry matter and decreased 11.6% crude fiber on leaf dry matter. Based on the statistical analysis of leaf dry matter, crude protein yields and the chemical composition of leaves, it is recommended that the application of nitrogen fertilizer for *Trichanthera gigantea* should be at 40 – 60kg N/ha/cutting.

Keywords: *Nitrogen fertilizer levels, Foliage yield, quality, Trichanthera gigantea*

Introduction
In order to introduce a green fodder variety as the ingredient into animal feed, it normally takes two steps: *i)* studying on plantation and nutritive value; *ii)* studying on its utilization. For the first phase, it is usually to study a number of factors which have major effect on plant yield and quality such as plant varieties, plantation and harvesting technique, fertilizer application etc. in which, nitrogen fertilizer was the priority to evaluate. The study on optimal level of N fertilizer application for the green fodder *T.gigantea* is the first phase on plantation research. It was believed that Nitrogen fertilizer is the major factor which makes a great contribution to the yield and quality of the green fodder. Kien et al. (2011), Hoan et al. (2011), Tuan et al. (2011), Quang et al. (2012), Hong et al. (2014), Kien and Hoan (2014) Nhung et al. (2018) have reported that the optimal increase of N fertilizer not only significantly improved green fodder yield but also improved its quality, eg. Improved crude protein content, decreased crude fibre content in dry matter basic . A low level of application may give lower efficiency, but a high level of application may give negative effects on the development of the plants such as weakness, falling or death (Hien et al., 2002). Therefore, it is necessary to investigate the most suitable level of Nitrogen fertilizer application for *T. gigantea.*

Materials and methods
This study focused on green fodder *Trichanthera gigantea* and it was conducted at Thai Nguyen University of Agriculture and Forestry, Thai Nguyen Province, Vietnam from March 2017 to March 2019.The experiment included 5 treasments representing 5 different Nitrogen fertilizer levels, which abbreviated as NT1: 0kgN, NT2: 20kgN; NT3: 40kgN; NT4: 60kgN and NT5: 80kgN/ha/cutting. The short name of treatments was as 0N; 20N; 40N; 60N and

80N. The plantation area of each treasments was 24 m^2 with 5 replicates, and arrangeed in a completely random block design. All 5 treatments had the similar plantation density, cutting intervals, cutting height and fertilizer application level (except N - fertilizer).

The monitoring parameters include productivity and yield of biomass, fresh leaves, dry matter, crude protein and chemical composition of the *T. gigantea* leaves. These parameters were determined by the method from Hien et al. (2002). The chemical composition of the green fodder was analysed following A.O.A.C. (1990). Each criterion was analyzed with 5 replicates (n=5).Collected data was processed following the method from Oanh and Phu (2012) with IRRISTART 5.0.2009 software.

Results and discussion

Effect of nitrogen application levels on productivity of *T.gigantea*

The green fodder productivity was determined by following parameters: *i)* biomass productivity, *ii)* fresh leaf productivity and *iii)* dry matter productivity. These parameters were expressed as weight of biomass or fresh leaves or dry matter received per each harvest per ha, the unit expressed as quintal/ha/cutting (or harvesting). The average productivity of biomass, fresh leaves, dry mater/ha/cutting of 2 experimental years are presented in Table 1.

Table 1. The productivity of *T.gigantea* at different N- levels (quintal/ ha/ harvest)

N - levels	Biomass	Fresh leaves	Dry matter
0 N	182.04^{c}	113.88^{c}	18.78^{b}
20 N	203.35^{b}	127.21^{b}	20.41^{ba}
40 N	220.46^{ab}	137.92^{ab}	21.54^{a}
60 N	232.23^{a}	145.29^{a}	22.05^{a}
80 N	240.11^{a}	150.21^{a}	22.10^{a}
SEM	11.122	6.958	1.087
P	0.000	0.000	0.000

Note: 1quintal = 100 kg

Effect of nitrogen application levels on biomass productivity

The biomass productivity includes the entire stems, branches and leaves of green forage crops obtained on 1 ha in a harvest. It is an important parameter to calculate fresh leaves and dry matter productivities. When nitrogen application level increased from 0N to 80N/ha/cutting, the average biomass productivity per cutting of the two years increased from 182.04 to 240.11 quintal/ ha/ cutting.The relationship between N application level and biomass productivity was expressed by the following equation: $Y = 0.725\ x + b$; R= 95.5%. In which, *Y is biomass productivity (quintals/ha/cutting); X is nitrogen application level (kg N/ha/cutting).* This equation showed that the level of N application and biomass productivity has a significant correlation (R=0.95).

The results of statistical analysis and comparision showed that the average biomass productivity per cutting of the two years for the N application level of 20N was not significantly different from that of the 40N level; the data also showed no significant difference among 60N, 80N levels and 40N level. It can be explained as follows: the application level of 60N and 80N satuated the nitrogen requirement, thus the biomass productivity was not remarkedly increased. The following collected data proved this phenomenon: The average biomass productivity of the 2 years in 20N level was higher than that of 0N by 11.7%; that of 40N was higher than that of 20N by 8.4%; that of 60N was higher than that of 40N by 5.3%; that of 80N was higher than that of 60N by 3.4%. It can be seen that the increase of N application levels was not strongly correlated with the increase of the biomass productivity.

Other studies such as those on different N application levels for *P. atratum* grass from Kien et al. (2011), for cassava leaves production from Hoan et al. (2011), for some grass varieties from Quang et al (2012), for green fodder *M. oleifera* from Nhung et al. (2018) also showed the similar results. Accordingly, the increase of N application level resulted in increasing biomass productivity; however, when the N application was too high, this productivity even decreased.

Effect of nitrogen application levels on fresh leaf productivity

Leaf meal supplemented into chicken and rabbit complex diets was produced from green fodder leaves when stem and branches were removed and leaves were dried then ground to fine powder. Thus, it is very important for the producers to have knowledge on fresh leaf productivity of the green fodder. Fresh leaf productivity can be calculated by multiplying biomass productivity with the proportion of fresh leaves per biomass. The proportion in this experiment was determined as 62.56%. The average fresh leaves production of 2 years increased from 113.88 quintal (NT1) to 150.21 quintal/ha/cutting (NT5). Statistical analysis showed that the average productivity of fresh leaves of 2 years production of those received N fertilizer was significantly higher than those which did not receive any N fertilizer (0N) ($p < 0.001$); among those receiving N fertilizer, NT2 (20N) and NT3 (40N) were not significantly different; NT3 (40N), NT4 (60N) and NT5 (80N) also differed insignificantly ($p > 0.05$) due to the above mentioned reason.

Effect of nitrogen application levels on dry matter productivity

Dry matter productivity was calculated by multiplying fresh leaves productivity with dry matter content in fresh leaves. The dry matter content in fesh leaves was different in negative correlation with different N application levels; the lower application level of N, the higher dry matter content and vice versa; particularly, from NT1 to NT5 this contents were 16.49%; 16.04%; 15.62%; 15.18% and 14.71 % respectively. The average dry matter productivity of 2 years increased from 18.78 quintal (0N) to 22.10 quintal/ ha/ cutting (80N). The difference in productivity of dry matter in NT5 (80N) compared to NT1 (0N) was notably lower than that of fresh leaves productivity in these 2 treatments. It can be illustrated as: the difference in dry matter productivity between NT5 and NT1 was 11.68%, whereas that of fresh leaves was 31.90%. It might due to that when the application of N level increased, the dry matter content in fresh leaves decreased. For example, DM content of fresh leaves in 0N and 80N was 16.49% and 14.71%, respectively.

Trichanthera gigantea production yield at different N application levels

Production yield is the total biomass or fresh leaves productions, dry mater, crude protein harvested per ha per year. Production yield can be estimated by two means, namely *i)* accumulating productivity of all cuttings/ha/year, or *ii)* taking the average productivity of the cuttings to multiply by the number of cuttings per year, then converting from quintals/ha/year to tons/ha/year. Crude protein yield was calculated by multiplying DM yield with CP content in DM. Average yield of above parameters of the 2 years were presented in Table 2.

Table 2: *T.gigantea* yields at different N application levels (ton/ha/year)

N - levels	Biomass	Fresh leaves	Dry matter	Crude protein
0 N	100.122^{c}	62.636^{c}	10.329^{c}	2.412^{d}
20 N	111.841^{b}	69.968^{b}	11.223^{bc}	2.700^{c}
40 N	121.253^{ab}	75.856^{ab}	11.849^{ab}	2.950^{bc}
60 N	127.729^{a}	79.907^{a}	12.130^{a}	3.125^{ab}
80 N	132.062^{a}	82.618^{a}	12.154^{a}	3.239^{a}

SEM	6.117	3.827	0.598	0.149
P	0.000	0.000	0.000	0.000

Table 2 showed that the increase of N levels from 0N to 80N per ha per cutting resulted in the increase of biomass yield and fresh leaf yield by 31.9%, DM yield increased by 17.7% and CP yield increased by 34.3%. The difference in DM was smaller than that of the biomass and fresh leaves because when N level increased, DM content in the leaves decrease. Whereas, the difference seen in CP was larger than that of biomass and fresh leaves because when N level increased, CP content in fresh leaves (or DM) increased. In this study, CP content in DM from NT1 to NT5 was 23.35%; 24.06%; 24.90%; 25.75% and 26.65%, respectivlely.
The results of statistical analysis revealed that biomass and fresh leaves, DM yields of treatment 60N, 80N were significantly higher than that of 0N, 20N with $p<0.001$, however, this difference was not seen when compares to that of 40N treatment ($p>0.05$); that of 40N was significantly different from that of 0N ($p<0.001$). CP yield was insignificantly different between 80N and 60N, between 60N and 40N; between 40N and 20N; however, all the treatments with application of N resulted in the increase of CP yield compares to those did not receive any N (0N) with $p<0.001$. Thus, increased N application level significantly increased CP yield. The DM yield of the 40N treatment was not significantly different from that of 60N and 80N treatments; however, CP yield of this level was only remarkably lower than that of 80N; whereas, the CP yield of 60N and 80N was not significantly different. Thus, the N application levels of 40N and 60N were selected as the most suitable levels.
Research results on some other green food plants indicated that the average yields of dry matter and crude protein (ton/ha/year) of casava for leaf production were 9.23 and 2.21 (Hien and Trung, 2016), of S. guianensis grass were 7.25 and 1.35 (Hien et al, 2017), of L. leucocephala were 8.49 and 2.36 (Hoan et al, 2017), of M. oleifera were 9.40 and 3.16 (Nhung et al, 2018). Thus, dry matter and crude protein yield of *T. gigantea* in this experiment was greater than or similar to that of some other food crops which are often used to process leaf meal in Vietnam as reported by previous authors.

The DM and CP production efficiencies of the different treatments

The DM and CP production efficiencies of the different N application levels were estimated by taking DM and CP yields from NT2, NT3, NT4 and NT5 substract to DM and CP yields of NT1, converting the results from ton/ha/year to kg/ha/year then dividing by the total N used for 1 ha per year. The results were shown in Table 3.

Table 3. The efficiency of different N application levels at 2 years production

Parameters	Unit	NT2 20N	NT3 40N	NT4 60N	NT5 80N	SEM	P
DM added	Kg	894^{c}	1520^{b}	1801^{a}	1824^{a}	42.256	0.009
CP added	Kg	288^{d}	539^{c}	713^{b}	827^{a}	1.919	0.000
N/ha/year	Kg	110	220	330	440		
DM/N Efficiency	KgDM/kg N	8.13^{a}	6.91^{b}	5.46^{c}	4.15^{d}	0.154	0.000
CP/N Efficiency	Kg CP/kg N	2.62^{a}	2.45^{b}	2.16^{c}	1.88^{d}	0.006	0.000

Data in Table 3 showed that at the higher levels of N application, the addition of DM and CP per ha per year were increased significantly ($p<0.001$). In contradiction to that, the dry matter and crude protein per 1kgN was decreased significantly ($p<0.001$). It should be noted when applying N fertilizer for *T.gigantea*, if more DM, CP yields per ha per year is desired regardless of production cost then higher level of N application should be used (60 – 80 kgN/ha/cutting); if cost of production is taken into consideration then N application of 40 N – 60 kgN/ha/cutting should be the suitable levels.

Effect of different N application levels on the quality of *T.gigante*

Effect of different N application levels on *T.gigantea* quality was determined via chemical analysis of the leaves, such as: dry matter (DM), crude protein (CP), crude fat (ether extract - EE), crude fiber (CF), total mineral (Ash), nitrogen free extract (NFE). Obtained results were presented in following table.

Table 4. Chemical compostion of *T.gigantea* leaves

N levels	DM in fresh leaves, %	% DM				
		CP	EE	CF	Ash	NFE
0 N	16.49^{a}	23.35^{a}	2.18^{a}	11.16^{a}	21.59^{a}	41.72^{a}
20 N	16.04^{ab}	24.06^{ab}	2.37^{ab}	10.72^{a}	22.26^{ab}	40.59^{ab}
40 N	15.62^{abc}	24.90^{bc}	2.43^{b}	10.50^{ab}	22.98^{bc}	39.19^{ab}
60 N	15.18^{bc}	25.76^{cd}	2.57^{bc}	10.01^{b}	23.72^{cd}	37.94^{bc}
80 N	14.71^{c}	26.65^{d}	2.72^{c}	9.86^{b}	24.61^{d}	36.16^{c}
SEM	0.677	0.519	0.130	0.369	0.581	1.595
P	0.004	0.000	0.000	0.000	0.000	0.000

Data in Table 4 showed that when the application level of N fertilizer increased from 0 to 80 kgN, DM content in the leaves decreased by 1.78% (from 16.49% to 14.71%); in dry matter content, the CP was increased by 3.3% (from 23,35% to 26.65%), fiber content was decreased by 1.3% (from 11.16% to 9.86%), the total ash was increased by 3.02% (from 21.59% to 24.61%) and the nitrogen free extract (NFE) was decreased by 5.56% (from 41.72% to 36.16%). Thus, increase in N application level did not only improve biomass productivity but also improve biomass quality, such as increasing CP content and decreasing CF content in the feed.

The increase or decrease of these nutrients were only significantly different ($p<0.001$) when there was a difference of 40kgN/ha/cutting or more. For example: the N application level of 40N compared to 0N, 60N compared to 20N and 80N compared to 40N.

Several authors who studied on N fertilizer application for different green fodders also stated that N fertilizer had significant effect on the chemical composition, especially, on CP and CF contents in the green fodders. Kien et al. (2011) reported that the increase of N fertilizer from 0 kgN to 60 kgN/ha/cutting for B decumbens increased CP content in DM from 8.77% to 13.18%, decreased CF in DM from 39.18% to 35.16%. Hong et al. (2012), Tuan et al. (2011), Hoan et al. (2011), Kien and Hoan (2014), Nhung et al. (2018) studying on different N application levels on *Setaria* grass, cassava for leaf production, *B. brizantha*, *M. oleifera* also had similar results.

Conclusion

Increasing N fertilizer application from 0kgN to 80 kgN/ha/cutting increased dry matter yield from 10.329 tons to 12.154 tons and crude protein yield from 2.412 tons to 3.239 tons/ha/year, increased crude protein content by 3.3% and decreased crude fiber by 1.3% in leaves dry matter. Based on the results of dry matter, crude protein yields and chemical composition of the leaves, it is recommended that nitrogen fertilizer application level for *Trichanthera gigantea* should be at least 40 kgN/ha/cutting or more, the most suitable level is 60 kgN/ha/cutting.

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IMPACT OF TEMPERATURE-HUMIDITY INDEX (THI) ON THE EGG WEIGHT OF HYBRID „HISEX BROWN" LAYING HENS

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Abstract

The paper establishes how average egg production changes in the course of one year (per month) and how egg production is independently influenced by maximum daily temperature and maximum relative ambient air humidity, as well as what their joint impact is. For the purpose of investigating into the impact of average annual temperature, air humidity and daily temperature on production properties, egg laying capacity and weight of laid eggs, the authors conducted a relevant experimental research study on the farm Šurik Invest. The research subject comprised of 25200 heads of the HISEX BROWN light line hen hybrid that were 30 weeks old at the beginning of the experiment, i.e. 47 weeks old at the end of the research period. The research lasted for 18 weeks in the period between May, 1st 2016 and August 31st 2016. By means of statistical data analysis performed by calculating the ambient air humidity index and by means of various analytical models and nomograms it was ascertained that the THI in different months and seasons of the year was in correlation with the number of laid eggs and their weight and that its intensity had impact on the productivity of laying hens. Namely, in May, when the THI was >74 and productivity was 79.13%, egg weight was 68.06 g, i.e. in August, when the THI was >84; and productivity was 68.63%, average egg weight was 67.51 g. Likewise, the number of cracked eggs increased due to low food consumption and low protein intake, i.e. from 1. 65% in May to 4.40% in August.

Key words: *laying hens, eggs, productivity, egg weight, temperature humidity index.*

Introduction

Production of eggs for human consumption is a demanding technological process which has acquired the properties of industrial production in the scope of intensive poultry farming.However, one cannot but observe that no attention whatsoever has been paid to the fundamental biological needs of poultry but merely to the matters of production efficiency and profitability. Hence, conditions created by keeping laying hens in conventional (traditional) cages, as well as ambient conditions, nutrition and external factors (air humidity, temperature, etc.) comprise the main factors which impact a flock´s productivity, as well as production results of laying hens (the number of laid eggs, egg weight, eggshell stiffness, etc.).

The greatest impact on a flock´s production results is exerted by the manner of exploitation with all the accompanying equipment which indirectly influences laying hens´ health and productivity. Whether the breeding conditions for laying hens comply with legal regulations is indeed disputable. However, one can rest assure that new regulations promise to introduce great changes in the production of table eggs in Serbia, since it is neither effortless nor cost-effective to change breeding methods and the manner of production.

There are two phases in the system of table eggs production. The first phase implies breeding of pullets for approximately 18 weeks, while the second phase comprises exploitation of hens from the beginning of the reproductive period to their exclusion, which most frequently takes place between 72 and 76 weeks of age. Hybrids which are nowadays used for producing table eggs have a very high genetic capacity. However, the extent to which this capacity is to be

exploited depends first and foremost on breeding conditions for laying hens, i.e. on the breeder.
In the production phase the optimum temperature is between 18°C and 22°C. In our region problems occur during summer months when outdoor temperature is extremely high. The consequences of high temperatures of over 33°C are reflected in a decreased food consumption and slower egg production. During intensive heat the eggshell tends to be soft, while the percentage of eggshell cracking suddenly increases followed by an intensive decrease in egg weight.
The optimum air humidity in a facility is obtained by means of proper ventilation. Low humidity has a negative impact, causing nervousness and dehydration in laying hens, as well as decrease in production. High humidity also causes nervousness in laying hens, wherebytheir feathers become conglutinated and egg production decreases. In both events hen resistance decreases and they become susceptible to infections. Optimum air humidity should be around 65% to 75%, while the required amount of air is 4 to 6 m^3 of air per hour per kilogram of live body weight.

Literature review

One of the most commonly observed occurrences in intensive poultry farming is the weakening of form and physique (Karabasil *et al*., 2013). The processes which lead to the weakening of general and specific body resistance, as well as of adaptation and acclimatisation power are unfortunately dictated by breeders and producers who removed animals from their natural ambient by being dedicated for centuries to a rather unilateral goal. They raised animals in a modified environment void of natural selection vectors such as nutrition depending on seasonal and climate cycles, the effects of pathogenic micro- and macro-organisms, livestock transportation and other factors which have been confirmed (Vecerek *et al*., 2006; Warriss *et al*., 1990).
In the process of studying laying hens, stress factors are devoted special attention. Among them one needs to emphasise environmental conditions and food quality, i.e. nutrition. Theintensity of impact of these factors on animals and the manner in which they will react in stressful situations depends first and foremost on endogenous condition of animals. Therefore it is important to establish the intensity of stress impact on hens for each farm (Ritz *et al*., 2005).
In the case of intensive breeding of laying hens, i.e. in the case of the system of keeping hens in a closed space throughout the year, one of the main issues, aside from spatial properties, are ambient conditions. Regardless of whether one encountersthe floor housing system or cage breeding, the movement of hens is largely limited which frequently causes stressful reactions in hens (Rakonjac *et al*., 2014)?
Under unfavourable ambient conditions, primarily with respect to the ventilation system, one encounters unfavourable microclimate conditions which also negatively influence animals´ mood, appetite, productivity and general health. Hens are animals with a slightly increased body temperature in comparison to other animals (optimal body temperature is 41°C), which is also related to a higher optimal ambient temperature. Numerous results indicate that the optimal air temperature for laying hens ranges between 18°C and 21°C.
In all animals as well as in hens the heat stress impacts the functions of adrenal glands and secretion of corticosteroids. The problem which one encounters when studying the functions of adrenal glands in hens is the fact that, unlike mammals, this gland is diffused in hens, which makes the complete separation impossible (Milošević *et al*., 2012).
The heat stress is doubtless one of the main stress factors which causes great losses in poultry farming.

The first symptoms of heat stress in laying hens can be observed at a temperature of 22°C, while serious problems with appetite and behaviour occur at a temperature of over 27°C. When air temperature exceeds 30°C hens stop taking food which inevitably leads not merely to productivity disruption, but also to an increased number of dirty eggs due to a more fragile eggshell, to decreased egg weight and eventually to an increase in hen demise (Perić *et al.*, 1998).
Low and especially high temperatures are a significant stress factor which influences not merely egg production but also hens´ general health.
Temperature humidity index (hereinafter: THI) has been introduced as a generally acceptable parameter. This index establishes a connection between maximum daily temperature and momentary relative humidity from the standpoint of the risk of heat stress. With an increase in temperature and ambient humidity the THI inclines towards 100. In order to establish the risk of stress various analytic models and nomograms have been defined.

The most frequently used model in research studies is as follows:

$$THI = (0{,}8\,Tmax) + \left(\frac{\varphi}{100}\right)(Tmax - 14{,}4) + 46{,}4$$

φ – Relative air humidity

THI values lower than 74 indicate that there are optimum conditions for egg production. Under these conditions there are virtually no recorded cases of stress.
When THI values are between 75 and 78 there are cases of productivity decrease. When THI values are between 79 and 84 there are intense stress reactions which significantly influence hen metabolism. Under these conditions homeostasis is infringed, which causes a decrease in productivity. When THI values are higher than 84, one is a witness to serious health disorders while productivity is almost entirely reduced
The intensity of reduced productivity as a consequence of stress caused by high THI values depends on animal age. The most sensitive are pullets at the beginning of their reproduction period (Bogosavljević-Bošković *et al.*, 2012). Aside from real THI values it is also important to establish when high values occur and how long these conditions last.
Likewise, it has been established that sudden changes in THI values as well as the length of stress do have impact on productivity. Thus, for instance, a sudden increase in these values can cause a decrease in productivity by 15%.

Material and Methods

For the purpose of examining the impact of average annual temperature, air humidity and daily temperatures on production properties, egg quality, productivity and egg weight various experimental research studies have been performed. The experiment was performed at the foot of the mountain Čemernik, at the Šurik Invest farm (municipality of Crna Trava), Republic of Serbia, in the period between May 1st, 2016 and August 31st, 2016. Geographical co-ordinates of the location are 42º44'06"N and 22º16'38"E, at an altitude of 1638 m.
At the experimental farm there are 25200 heads of theHISEX BROWN light line hen hybrid which produced 2.264.924 eggs in 123 days (May-August) whereby the average productivity was 74.28%.
Statistical data analysis was performed by calculating ambient humidity index and by applying various analytical models, was used SPSS statistical package together lith Student's *t*-test for data procesing and drawings showing the results without computation.

Results and Discussion

In May, during dry days, the THI was less than 74. Under such conditions average S class egg weight was 4.8 g, M class 8.3 g, L class 62.76 g, while average XL class egg was 22.35 g. In the course of this month average weight of cracked eggs was 1.75 g.

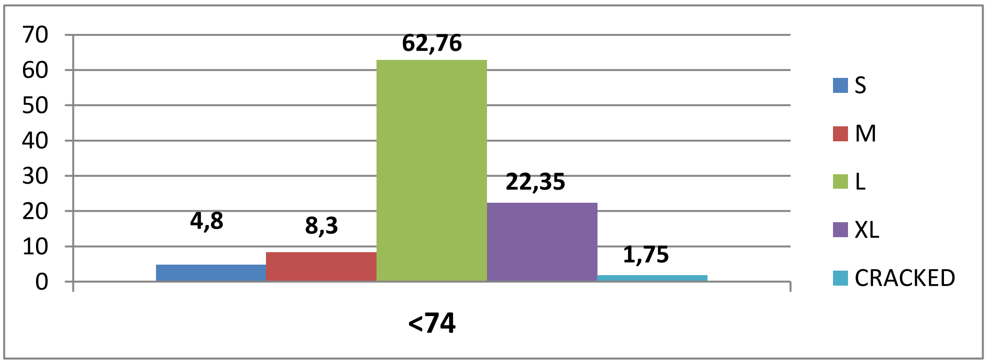

Chart 1: Egg weight per class depending on THI values in May

In June, THI values were expressed through three temperature intervals. Under the most favourable conditions, when THI value was under 74, average S class egg weight was 3.52 g, M class 4.93 g, L class 67.87 g, while XL class egg weight was 11.04 g. In this interval average weight of cracked eggs was 1.45 g.

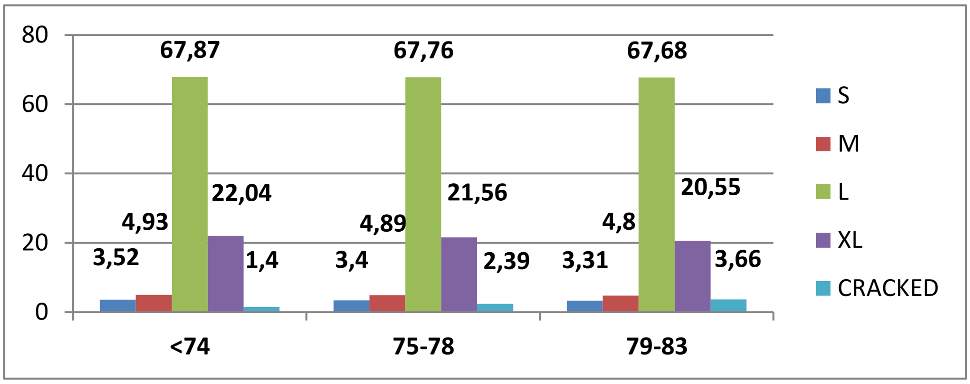

Chart 2: Egg weight per class depending on THI values in June

With the increase in temperature interval the egg weight per class decreased, whereby average egg weight in the temperature interval between 75 and 78 for S class was 3.4 g, for M class 4.89 g, for L class 67.76 g, while for XL class it was 21.56 g. With the increase in interval average weight of cracked eggs increased as well and it amounted to 2.39 g in this interval.
In the temperature interval between 79 and 83 laid eggs tended to lose on weight. Thus, average S class weight was 3.31 g, M class 4.80 g, L class 67.68 g, and XL class was 20.55 g. In this interval the weight of cracked eggs was 3.66 g.
In July, THI values were expressed through three temperature intervals. In the interval between 75 and 78 average S class egg weight was 3.74 g, M class 4.78 g, L class 68.48 g and XL class was 20.66 g. In this interval the weight of cracked eggs was 2.34 g.

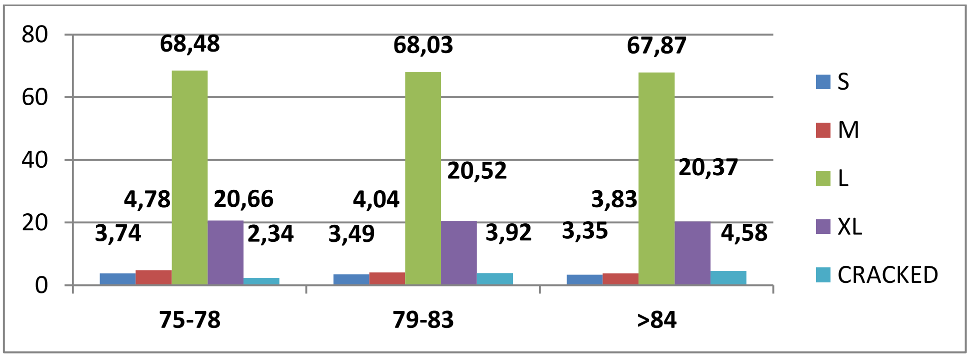

Chart 3: Egg weight per class depending on THI values in July

With the increase in temperature interval the egg weight per class decreased, whereby average egg weight in the temperature interval between 79 and 83 for S class was 3.49 g, for M class 4.04 g, for L class 68.03 g, while for XL class it was 20.52 g. With the increase in interval average weight of cracked eggs increased as well and it amounted to 3.92 g in this interval.
In the highest interval with average index values of over 84 laid eggs tended to lose on weight. Thus, average S class weight was 3.35 g, M class 3.83 g, L class 67.87 g, and XL class was 20.37 g. In this interval the weight of cracked eggs was 4.58 g.
In August, THI values were expressed through three temperature intervals. In the interval between 75 and 78 average S class eggsweight was 3.65 g, M class 6.92 g, L class 67.61 g, and XL class was 18.79 g. In this interval the weight of cracked eggs was 3.03 g.

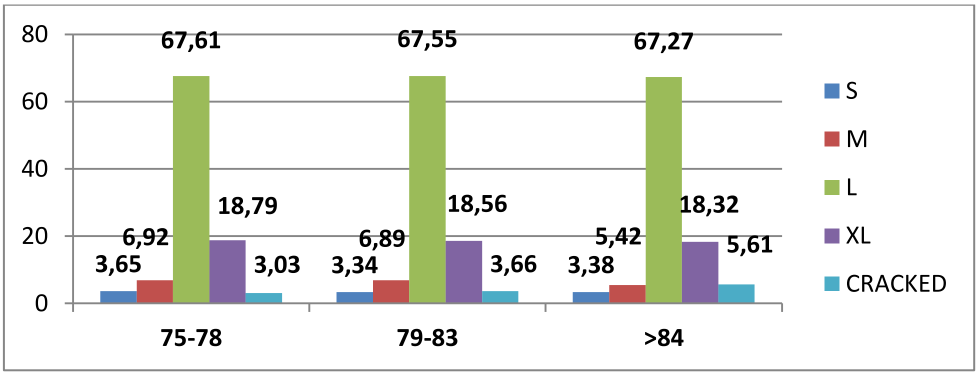

Chart 4: Egg weight per class depending on THI values in August

With the increase in temperature interval the egg weight per class decreased, whereby average egg weight in the temperature interval between 79 and 83 for S class was 3.34 g, for M class 6.89 g, for L class 67.55 g, while for XL class it was 18.56 g. With the increase in interval average weight of cracked eggs increased as well and it amounted to 3.66 g in this interval.
In the highest interval with average index values of over 84 laid eggs tended to lose on weight. Thus, average S class egg weight was 3.38g, M class 5.42 g, L class 67.27 g, and XL class was 18.32 g. In this interval the weight of cracked eggs was 5.61 g.

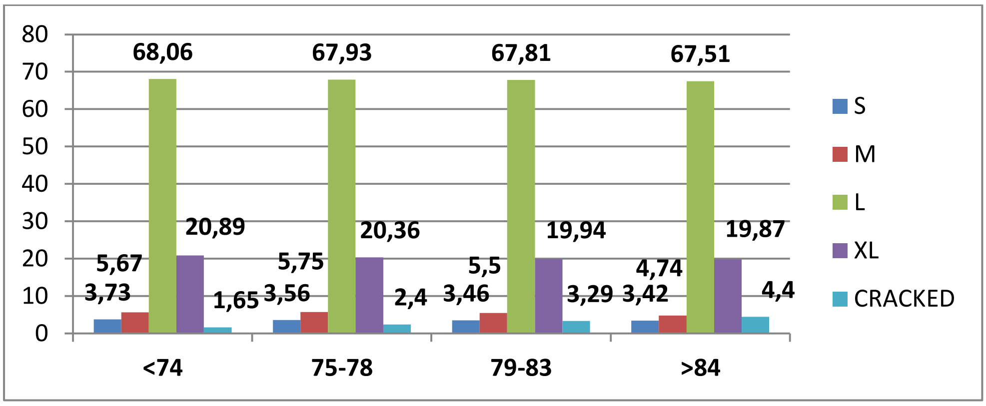

Chart 5: Egg weight per class depending on THI values in the period between May and August

Likewise, (Pešić *et al*., 2015) established that temperatures in poultry breeding facilities have the greatest impact on production results and animal health. Optimum temperature for mature poultry is between 19°C and 27°C. Temperatures higher than 28°C require that birds spend additional energy in order to cool down, which leads to heat stress. In the interval with maximum index over 84 average S class egg weight was 3.42 g, M class 4.74 g, L class 67.51 g, while XL class was 19.87 g. In this interval the weight of cracked eggs was 4.4 g.
Microclimatic conditions are a significant factor in egg production and in exploitation of laying hens, which is presented in Chart 2, where one can see the productivity interval for the observed period. With an increase in temperature over 21°C, egg production and average egg weight decrease. Furthermore, eggshell quality decreases due to lower calcium levels, which leads to eggshell cracking, described in the papers by Đukić *et al*.,(2009).
Hristov *et al.*,(2006) asserted in his paper that fighting heat stress was essential for farmers. He further asserted that the heat released by chickens was the greatest source of heat in the facility, whereby the negative effect of high temperature was enhanced in combination with high humidity. The damaging effect of high temperatures can be diminished primarily by constructing solid and insulated facilities, by installing quality equipment, and then by introducing quality nutrition and securing sufficient amount of clean and fresh water, which were the points of agreement in everyone´s research.

Conclusion

On the basis of the obtained results one can draw the following conclusions: With an increase in THI values the egg weight in all indexed intervals decreases. The largest egg weight is in the interval when there is no heat stress, i.e. when there is no THI change for a longer period of time. Such is the case with the month of May, when the interval has always been <74. Opposite to egg weight, cracked eggs weight increased with the increase in the interval which implies that high temperatures make the eggshell thinner. Thus, the largest weight of cracked eggs was in the interval with the greatest stress caused by an increase in THI values. This was especially conspicuous in the period when the stress was present for a longer period of time. The largest decrease in egg weight was observed in the heaviest class where the egg weight was decreased by an average of 1g, while in lighter classes the decrease in weight was less than 1g.

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EVALUATION OF LACTOSE, MILK PRODUCTION, SOMATIC CELLS COUNT AND CALVING INTERVAL IN SELECTED FARMING CONDITIONS OF THE SLOVAK SPOTTED DAIRY COWS

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Abstract

Lactose is a major component of milk (typically around 5% of composition) that is not usually directly considered in national genetic improvement programs of dairy cattle. Lactose percentage and other traits of milk production in percentage may be used in indirect selection for health and fertility traits, which are economically important, but of low heritability. The aim to this study was to evaluate relation among percentage of lactose and others traits of milk production, somatic count cells and calving interval in selected breeding herds of Slovak Spotted dairy cows (S_0). A total of 16,334 control milk samples from 796 Slovak Spotted dairy cows born from 2001 to 2013 were used for estimation of parameters for percentage of lactose content (LC) and correlations with other milk yield (MY), somatic cell count (SCC) and calving interval (CI). These data were analysed using the Statistical Analysis System (SAS) version 9.3 and linear model with fixed effects: herds (H), years-season of control (YS), order of lactation (OL) and sire (S). By evaluation of milk yields we found out that the average of control samples of milk production during the examined period in all 16,334 control samples in 796 dairy cows were for lactose content 4.76±0.25%, 26.09±9.51 kgs of milk, 351.89±1,014.07 cells/ ml.10^3 of somatic cell count and 405.07±81.41 days of calving interval. The correlation among the traits of milk production, the somatic cells count and calving interval, for example lactose in % with milk in kgs, somatic cells count in cells/ml.10^3 and calving interval, was found as follows r = 0.45574, r = -0.29403 and r = -0.05025. These coefficients were statistically highly significant p <0.0001. The analyses by the effects on percentage of lactose (LC) reviled higher effect of sire R^2 = 0.158213 than effect of order of lactation R^2 = 0.082009. These effects were significant (P<.0001).

Keywords: *Slovak Spotted dairy cows, traits of milk, somatic cells count, calving interval, coefficient of determination.*

Introduction

Lactose is a major component of milk (typically around 5% of composition) that is not usually directly considered in national genetic improvement programs of dairy cattle (Haile-Mariam, Pryce, 2017; Costa et al., 2019). Lactose percentage and other traits of milk production in percentage may be used in indirect selection for health and fertility traits, which are economically important, but of low heritability (Satoła et al, 2017; Miglior et al., 2017). Costa et al. (2019) shows that lactose percentages has potentiality to be used as indicator trait to improve udder health in Fleckvieh dairy cows. Miglior et al. (2007) reported that lactose percentage was negatively genetically correlated with somatic cell score (-0.20) and may be used in selection for resistance for mastitis together with somatic cell score. Similar conclusions have been made by other authors such as Kasarda et al. (2015), Alessio et al., (2016), Bujko (2018b), Martin et al. (2019) and others. Some authors investigated the relationships between lactose percentage and fertility traits (Melendez et al., 2000; Martin et al., 2019). The contents of lactose in milk is influenced by multiple factors as are genetic and no-genetic (Alessio et al., 2016; Bujko et al., 2018a and others). The relationship of lactose percentage with production traits has been the subject of many studies (Ptak et al., 2012; Sneddon et al., 2015; Costa et al., 2018; Bujko et al., 2019). Factors affecting on lactose percentages shows in publications Alessio et al. (2016), Costa et al. (2019) and others. The aim to this study was to evaluate relation among percentage of lactose and others traits of milk production, somatic cell count and calving interval in selected breeding herds of Slovak Spotted dairy cows (S_0).

Material and Methods

The material for evaluation of milk components, somatic cells count and calving interval in selected breeding herds of Slovak spotted breed was provided from the database of Breeding Services of the Slovak republic.

A total of 16,334 control milk samples from 796 Slovak Spotted dairy cows born from 2001 to 2013 were used for estimation of parameters for percentage of lactose content (LC) and correlations with other milk yield (MY), somatic cell count (SCC) and calving interval (CI).

We divided dairy cows only breed-type S_0 - cows with genetic proportion of pure Slovak Simmental blood into 87.5 % and divided dairy cows by order of lactation the "I" - the first, "II"- the second, "III"- the third, "IV"- the fourth, "V"- the fifth and higher lactation.

The basic statistical and variability characteristics (least square means, standard deviations) were evaluated using the Statistical System (SAS) version 9.3 (TS1M2) Enterprise Guide 5.1 (SAS, 2011). The mixed procedure was used for the final analysis. The value of statistical significance (P), the value of the F-test and the coefficient of determination (R^2) of the above mentioned of milk components, somatic cell count and calving interval were evaluated with its relation to: herd, year and season of controls, order of lactation and sire.

For actual computation a linear model with fixed effects was used:

$$y_{ijklm} = \mu + H_i + YS_j + OL_k + S_l + e_{ijkl}$$

, where: μ = mean value of depended variable, H_i = fixed effect of herd (1 - 5), YS_j = fixed effect of years-season of control (1 - 34), P_k = fixed effect of order of lactation (1 - 5), S_l = fixed effect of sire (1 - 81), e_{ijkl} = residual error.

Statistical evaluations of the differences between traits were tested at the levels of statistical significance: $^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001 or $^{-}$P>0.05.

Results and Discussion

The basic traits of lactose content (LC), milk (MY), somatic cell count (SCC) and calving interval (CI) are presented in Table 1. The mean of LC, MY, FC, PC, SCC and CI in selected herd of Slovak Spotted dairy cows were similar to the national average for evaluated traits dairy cows in Slovak Republic (The Breeding Service of the Slovak Republic. S.E., 2016).

Table 1. Statistical characteristic of LC, MY, FC, PC, SCC and CI in dairy cows of Slovak Spotted cattle

Traits	**Statistical parameter**					
	n[1]	$\bar{x}$ [2]	**SD**[3]	**CV**[4]	**MODE**[5]	**MEDIAN**[6]
Lactose in (%)	16,334	4.76	0.25	5.34	4.68	4.80
Milk in (kg)		26.09	9.51	36.47	29.3	26.2
Fat in (%)		4.06	0.73	18.03	3.81	4.03
Proteins in (%)		3.59	0.36	10.13	3.54	3.58
SCC in (ml^{-1} x 10^3)		351.89	1,014.07	288.18	21.0	99.0
Calving interval in (day)		405.07	81.41	20.1	342.0	385.0

[1]number of observation, [2] mean, [3]standard deviation, [4]coefficient of variation, [5]mode (value that appears most often in a set of data), [6]median (value separating the higher half from the lower half of a data sample)

By evaluation of milk yields we found out that the average of control samples of milk production during the examined period in all 16,334 control samples in 796 dairy cows were for lactose content 4.76±0.25%, 26.09±9.51 kgs of milk, 351.89±1,014.07 cells/ ml.10^3 of somatic cell count and 405.07±81.41 days of calving interval. The mean of lactose content (4.76 %) was comparable in this study than that reported in literature (4.49%–5.09%, Welper and Freeman, 1992; Sneddon et al., 2015 and Satoła et al., 2017). These results are similar with conclusions as Miglior et al. (2007), Bujko (2011), Løvendahl and Weisbjerg (2017), Costa et al. (2018) and others.

Table 2. Relation between LC and other traits (MY, SCC and CI)

Traits	**Milk in kg (MY)**	**Fat in % (FC)**	**Proteins in % (PC)**	**Somatic cell count (SCC)**	**Calving interval (CI)**
Lactose in % (LC)	0.45574^{+++}	-0.15658^{+++}	-0.34094^{+++}	-0.29403^{+++}	-0.05025^{+++}

$^{+++}$ P<0.001

The correlation among the traits of milk production, the somatic cells count and calving interval, for example lactose in % with milk in kgs, fat in %, protein in %, somatic cells count in cells/ml.10^3 and calving interval, was found as follows r = 0.45574, r = -0.15658, r = -0.34094, r = -0.29403 and r = -0.05025. These coefficients were statistically highly significant p <0.0001. These results are correspondence with Melendez et al. (2000), Haile-Mariam and Pryce (2017), Bujko et al. (2018a), Martin et al. (2019) and others. Miglior et al. (2007) shows correlation coefficients among LC with SCC (r_p= -0.23, r_g= -0.2), than Sneddon et al. (2015) among LC with SCC (r_p= -0.19, r_g= -0.07).

Table 3. Factors affecting of lactose contents (LC) in Slovak Spotted dairy cows (S_0)

Sources of variability	**DF**[1]	**Mean Square**	**F Value**	**Pr> F**	**R-Square** [2]
					Lactose contents
Herd (H)	4	4.17442983	65.47	<.0001	0.015784
Years-season (YS)	34	1.84745117	30.21	<.0001	0.057631
Order of lactation (OL)	5	21.68854719	364.69	<.0001	0.082009
Sire (S)	81	2.0920902	38.18	<.0001	0.158213

[1] degrees of freedom, [2] coefficient of determination (R^2)

The linear model to represent coefficients of determination on lactose contents with all fixed effects R^2 = 0.215936 %. These effects were significant (P<.0001). In Table 3 showed the analyses by the effects on percentage of lactose (LC) reviled higher effect of sire R^2 = 0.158213 than effect of order of lactation R^2 = 0.082009. These effects were significant, P<.0001 (Table 3). These results are similar with results Alessio et al. (2016), Tančin et al. (2018), Costa et al. (2019) and others. Alessio et al. (2016) shows that the lactose content in milk is influenced by somatic cell count, parity, and season.

Conclusions

In conclusion, correlation between lactose in % with milk in kgs was medium high positive. Correlations between lactose in % and somatic cells count in cells/ml.10^3 as well as lactose in % and calving interval were found low negative. The analyses by the effects on percentage of lactose (LC) reviled higher effect of father than effect of order of lactation.

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EFFECT OF SEX ON GROWTH OF ABERDEEN ANGUS CALVES

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Abstract

The aim of the experiment was to evaluate growth ability of Aberdeen angus calves breed in organic farming system in the area of White Carpats (southeastern part of Czech Republic). Total number of animals used in experiment was 181 – 82 bulls and 99 heifers. Evaluation of growth ability was made according to methodology of Czech Beef Breeders Association. Average birth weight of calves was 37 ± 3.18 kg. Bulls had significantly ($p<0.01$) higher birth weight (39 ±3.72 kg), than heifers (36 ± 2.53 kg). In the age of 120 days there was significant difference ($p<0.01$) between gender at the range of 14 kg in favor of bulls (187 ± 30.42 kg). In the age of 210 days differences between sexes deepened even more. Average weight of bulls was 280 kg and of heifers was 257 kg. Same growth trend was also maintained at the age of 365 days, when difference ($p<0.01$) between bulls and heifers was 79 kg. Mean value of daily gain of bulls, from birth to 365 days, was 1424 $g.day^{-1}$, of heifers was 943 $g.day^{-1}$. In the age of 210 days hip height was also measured. Between gender there were no significant ($p>0.01$) differences.

Keywords: *Aberdeen Angus, growth of calves, bulls, heifers*

Introduction

Breeding meat livestock breeds is a dynamically developing sector in the Czech Republic. At present, 222,000 pieces of cows of beef cattle breeds are raised in the Czech Republic according to the Czech Statistical Office. The Aberdeen Angus (AA) breed is the second most numerous beef cattle breed (the efficiency inspection includes 4022 pieces of cows). Bures et al. (2004) evaluated slaughter parameters of bulls of the AA, Hereford, Charolais and Meat Simental breeds. Results confirmed a higher growth intensity in the group of breeds with a large body frame (Charolais, Meat Simental), while animals of the medium frame (AA and Hereford) were rather characterised by a higher beef quality. Mazzucco et al. (2016) states that the breed is highly adaptable and suitable for raising in most continents of the world. According to Gregory and Cudiff (1980) this breed is ideal both for pure-bred breeding, and for utility cross breeding with numerous domestic cattle breeds. This statement is supplemented by French et al. (2000); Garcia et al. (2008) and Dinh et al. (2010) who point out, in addition to the high growth ability of AA, an excellent quality of slaughter treated body and meat. The authors particularly advise of the representation of fatty acids and their positive effect on the human health. Hornick et al. (2000) point out excellent growth abilities of the breed when individuals achieve a high compensatory growth after the period of insufficient quantity or worsened quality of the grazing area. Bown et al. (2016) compared a growth ability of the bulls of Holstein cattle, AA and Hereford breeds, where the authors did not prove a statistically significant differences ($p<0.05$) in growth intensity among the breeds. However, Schenkel et al. (2004) advise of the difference in the composition of the slaughter body in individual cattle breeds. In the Czech Republic, the evaluation of the growth of beef cattle is carried out according to the Methodology of animal recording beef Efficiency Inspection of Cattle without Market Milk Production, issued by the Czech Beef Breeders Association. The methodology is in compliance with the rules International committee for

animal recording. Appointed person determines a birth weight of calves and a weight at the age of 120, 210 and 365 days.
The goal of this experiment was to compare the growth intensity of bulls and heifers of the AA breed raised in the organic farming system.

Material and methods

The experiment was carried out at a farm operating in the area of the White Carpathians in the southeaster part of Czech Republic. Since 2006 the farm has been certified for the ecological management system focused on breeding beef cattle and production of oats and hay. The total area of the farm consists of 272 ha of meadows and 12 ha of arable soil. The farm is situated at the altitude from 380 to 560 m above he sea level. The farm raises the AA breed, where the basis herd consists of 200 pieces of cows of the black and red colour (an approximate ratio of 1 : 1). The reproduction of breeding cows is ensured by a combination of natural reproduction and insemination. The farm uses the system of seasonal calving from the middle of January to the end of March. Most deliveries occur in the wintering grounds. The pastoral period start at the end of March and runs until the end of November. Calves are not additionally fed by grain fodder. Animals have a mineral lick available at the pasture and they have an unlimited access to water. Outside the pastoral period animals are stalled in the wintering grounds in separates stables for bulls, heifers and cows. All stables use a deep bedding. Hay, grass ensilage, mineral lick and water are used for feeding ad lib. The growth intensity was evaluated according to the "Methodology of Efficiency Inspection of Beef Cattle Breeds" issued by the Czech Beef Breeders Association that is authorised by the breeding activity of beef cattle bred in the Czech Republic. Total number of animals used in experiment was 181 – 82 bulls and 99 heifers. During the experiment, the birth weight and the weight at 120, 210 and 365 days of calves' age was determined by weighing on a stationary cattle scale with a built-in weight indicator. An average daily increment was calculated from the detected weights from the birth up to 120 days, from 120 to 210 days, from 210 to 365 days and from birth to 365 days of the age of animals.
In addition to the weight, the sacrum height was determined at the age of calves of 210 days. Measurements were performed using a Lydtin's measuring rod.
The data statistical analysis was carried out using the STATISTICA 12.0 statistical software using the ANOVA method:

$$Y_{ij} = \mu+G_i + e_{ij}$$

where:

μ = dependent variable
G = gender (bull, heifer)
e = residue

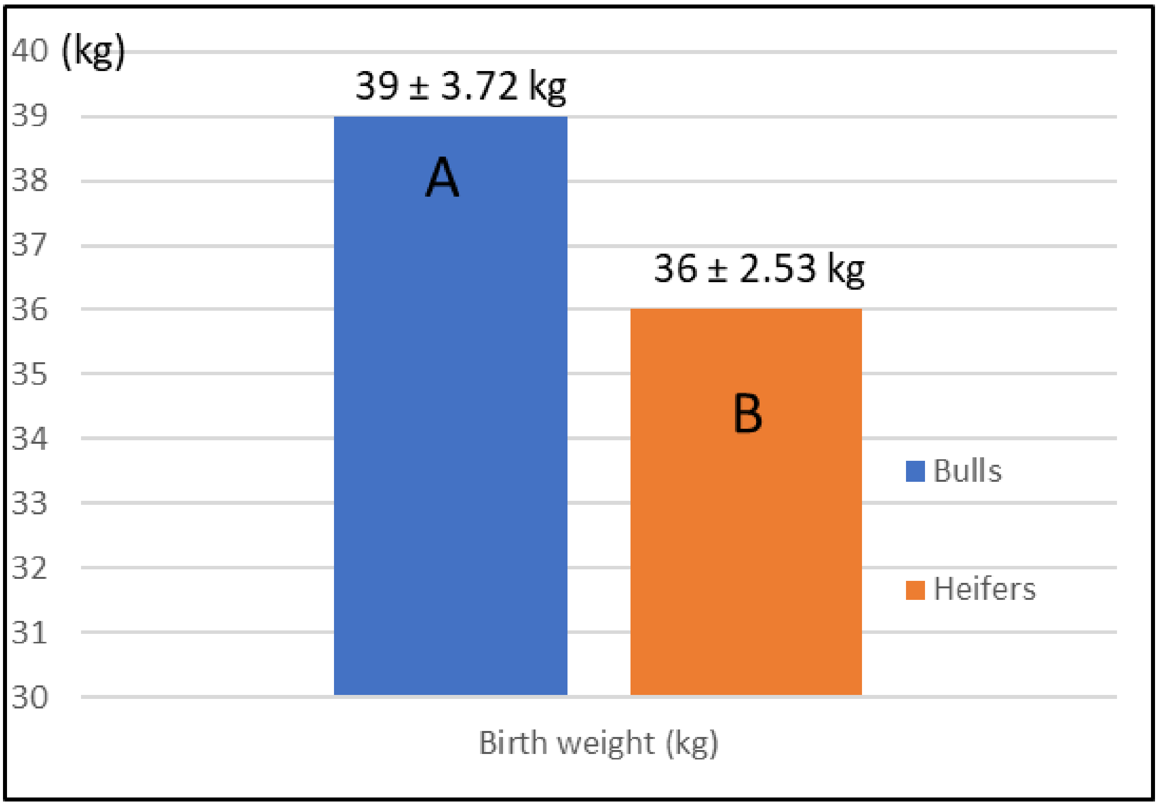
Differences between gender were compared using Tukeyś post-hoc test.

Results and Discussion

An average birth weight of the newborn calves was approximately 37 kg with a difference of ± 3.18 kg (Fig. 1). A statistically provably higher ($p<0.01$) birth weight was determined in bulls (39 ± 3.72 kg) compared to heifers (36 ± 2.53 kg). Casas et al. (2012) confirmed our results in their research stating that bulls have a higher birth weight compared to heifers. Bormann et al., (2006) specify birth weights of heifers of the AA breed raised in the U.S.A. of 34.9 kg, which corresponds to our results. In contrast, Berger et al., (1992) published birth weights of heifers only of 30 kg. An average calf weight at the age of 120 days was 178 kg with a difference of ± 28.05 kg, where bulls reached an average weight of

187 kg (Fig. 2). The weight of heifers at the same age was determined to be 14 kg ($p<0.01$) lower (173 ± 25.56 kg).

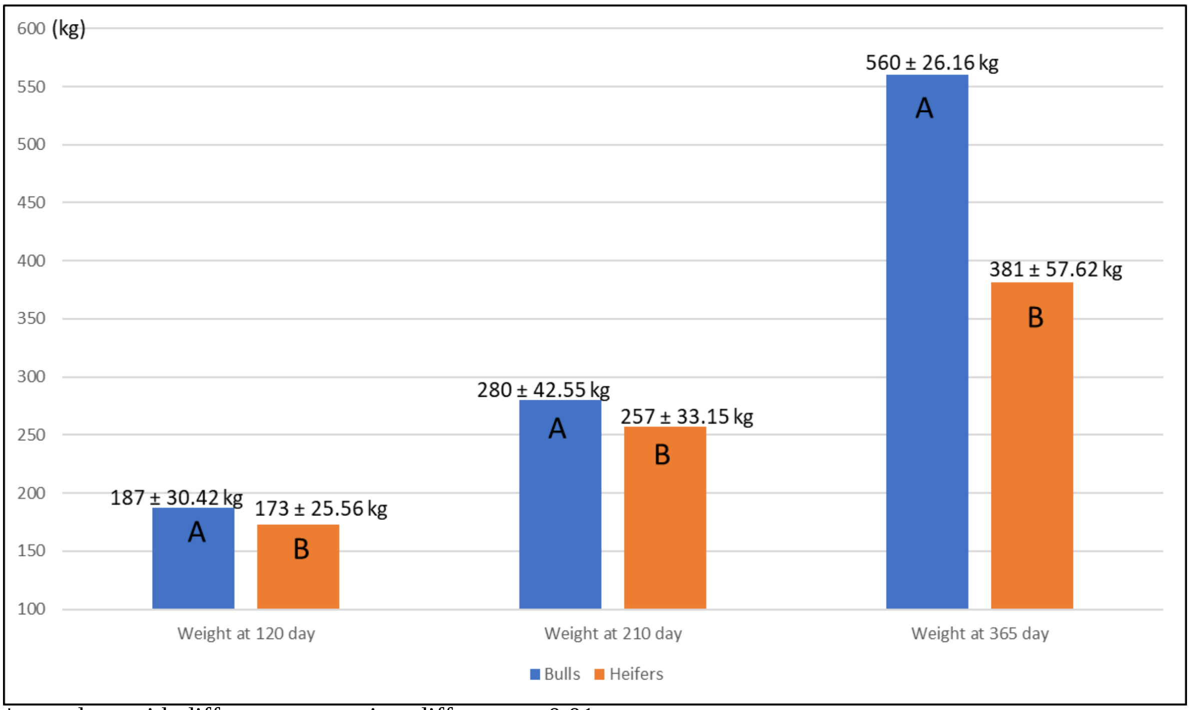
Fig. 1 Birth weight of calves



*A, B values with different superscripts differ at $p < 0.01$
** LSM ± S.D.

At the next measurement within the efficiency inspection at 210 days of animals' age, average weights of animals detected were 265 kg, when even more significant ($p<0.01$) difference was proven between genders compared to the previous weighing. Heifers reached the weight of 257 ± 33.15 kg. Bulls reached the weight of 280 ± 42.55 kg. Variability between individuals also occurred within both genders. This relates to the effect of individuality of every animal to their own growth ability which is intensively manifested at this age of animals because the effect of mother has been ceasing (ending of lactation). Our results correspond to the data specified in the paper of Casas et al. (2012) who evaluated offspring of beef cattle breeds. Significantly lower weights of bulls (204 kg) and heifers (192 kg) at the age of 200 days were published by Gregory et al. (1991). At the age of 210 days the hip height was determined in both genders, which ranges in the variation range from 116 to 138 cm (Fig 4). The hip height was inconclusively ($p>0.05$) determined for bulls of (128 ± 9.27 cm. A significantly lower values of the hip height were published by Gregory et al. (1991), when the authors specified the hip height for bulls of 117 cm and for heifers of 113 cm. Only 97 animals were evaluated at the next weighing at 365 days of age (50 bulls and 47 heifers). The reason for evaluating a lower number of animals was the fact that at the end of the pastoral period a part of animals were sold due to capacity reasons. An average weight of one year-old animals was determined of 444 kg, and bulls reached the weight of 560 kg ± 29.16 kg, while heifers weighted only 381 ± 57.62 kg. A statistically provable ($p<0.01$) difference between the genders was also proven in this weighing.

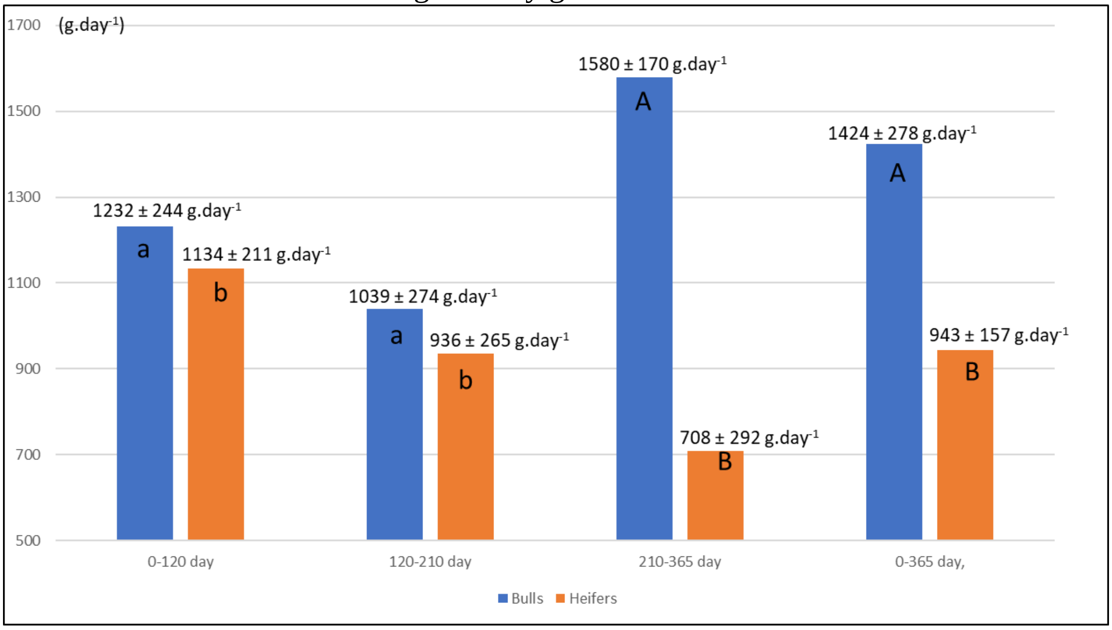
Fig. 2 Effect of gender on growth of calves



*A, B values with different superscripts differ at $p < 0.01$
** LSM ± S.D

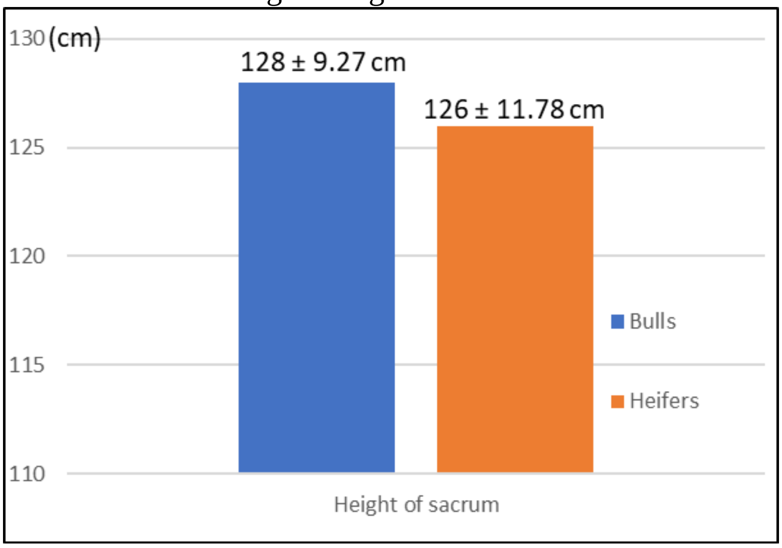
Average daily increments in calves in the period from birth to 120 days were in the amount of 1168 kg with a difference of ± 227 g.day^{-1} (Fig. 3). A significantly higher growth intensity in this period was proven in bulls (1232 ± 244 g.den^{-1}). In the period from 120 to 210 days a decrease of the growth intensity was registered in both genders, caused especially by the end of mother's milk and a transfer to the full plant fodder. An average daily increment of bulls was calculated to be 1039 ± 274 g.day^{-1} (a decrease in the growth intensity by approximately 100 g.day^{-1}). The decrease in heifers was almost by 200 g.day^{-1} (936 ± 265 g.day^{-1}). The growth intensity of females was continuously decreasing with the increasing age. In the period from 210 to 365 days of age an average daily increment was 708 ± 292 g.day^{-1}. In contrast, the trend was opposite in bulls. In the period from 210 to 365 days of age the bulls reached the highest growth intensity (1580 g.day^{-1}). Overall, it can be stated that in the period from birth to 365 days of the age of bulls put on weight significantly ($p<0.01$) faster (1424 ± 278 g.day^{-1}) than heifers (943 ± 157 g.day^{-1}). Similar values of average daily increments in bulls were published by Albertí et al., 2008; Bures and Barton (2018).

Fig. 3 Daily gain of calves



*a, b values with different superscripts differ at $p < 0.05$; A, B values with different superscripts differ at $p < 0.01$
** LSM ± S.D

Fig 4. Height of sacrum



* LSM ± S.D

Conclusions

The goal of this experiment was to compare the growth intensity of bulls and heifers of the AA breed raised in a farm managed at the ecological agricultural regime. A higher birth weight and a higher growth intensity of bulls in the period from birth to 365 days of age was confirmed. The difference of 179 kg between the weight of bulls and heifers was proven at 365 day ages. The growth intensity of heifers was highest in the period from birth to 120 days of age and then there was a linear decrease of average daily increments. On the other hand, the growth intensity of bulls was highest in the period from 210 to 365 days of age. No significant differences were proven in the evaluation of growth to the height of sacrum between cattle genders at 210 days age. It can be stated according to the reached results of the growth intensity of bulls and heifers that this breed is suitable for breeding under conditions of the Czech Republic; animals are also able to reach adequate growth ability in ecologically managed farms.

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EFFECT OF DIFFERENT DIETARY PROTEIN CONTENT ON PRODUCTION PARAMETERS OF CROSS-BRED FATTENING LAMBS

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Abstract

The aim of the study was to examine the effect of different dietary protein content on the production parameters of cross-bred fattening lambs. The study was conducted on 20 cross-bred lambs from different Pramenka types, divided in two groups. The lambs were at the age of 55 - 60 days, and the fattening period lasted five weeks. The first group of lambs were fed with a mixture of cereals and sunflower meal that contained 12.26% of crude protein, while the second group was fed with a concentrate mixture with crude protein level of 14.69%. During the fattening period the lambs from both groups were fed with hay ad libitum. Fresh water was offered with no restriction during the study. Lambs were weighed at the starting and at the end of every week of the study, and the results were statistically analyzed. The body weight gain was determined within the both groups according to age of lambs during fattening. At the end of fattening period higher average weight and weight gain with no significance ($p>0.05$) were observed in lambs fed with a mixture of cereals and sunflower meal. However, similar values were determined for feed to gain ratio in both groups of lambs. The obtained results indicated that the cross-bred fattening lambs fed with a mixture of cereals and sunflower meal had achieved better, but not significantly better production parameters that may have economical benefit for lamb meat production.

Key words: *lamb, feed mixture, production parameters, fattening*

Introduction

Sheep breeding in Bosnia and Herzegovina generally plays an important role from an economic, cultural, and environmental point of view. Breed structure is quite varied although it is dominated by indigenous breed Pramenka that has a large number of strains of different phenotypic and breeding characteristics. Pramenka, like the rest of the sheep, belongs to a group of sheep of combined production qualities. The economic significance of the breeding of pramenka is based on their biological characteristics, which enable the relatively scarce vegetation of pastures to turn into highly valuable products: meat, milk, wool. Production of lamb meat in Bosnia and Herzegovina is important for the nutritional needs of the local consumers.

The quantity and quality of the lamb meat is strongly influenced by the genotype, sex, age, body weight at slaughter and numerous other non-genetic factors (Ružić-Muslić *et al*., 2005; Mioč *et al*., 2007; Petrović *et al*., 2009). In order to increase production and quality of meat are carried out different types of crossing local sheep with imported noble breeds. There are numerous references on fattening and slaughter traits of different sheep crosses and mostly they have better production parameters (Konyves *et al*., 2014; Petrović *et al*., 2015). However, Kozarovski *et al.* (2000) reported that crossing the merino type Ovčepoljska sheep and Merinolandschaf did not give expected results regarding birth body weight, daily weight gain and slaughter traits.

Nutrition is one of the most important factors that influence the growth of lambs during the fattening and it depends on the type of breed, rearing system and management strategy. Sheep

are the most efficient converters of the hilly-mountain vegetation into high quality animal protein. Fattening of early weaned lambs lasts short and provide good meat quality using hay and cereals or concentrate mixture.
The aim of the study was to examine the effect of different dietary protein content on production parameters of cross-bred fattening lambs.

Materials and Methods

The study was conducted on 20 cross-bred lambs of Dubska and Sjenica Pramenka. The lambs were divided into groups and they were 55 – 60 days old at the start of the experiment. The fattening period lasted five weeks. During the experiment the first group (Group I) of lambs were fed with a mixture of cereals (corn, barley and wheat bran) and sunflower meal with no mineral-vitamin premix that contained 12.26% of crude protein, while the second group (Group II) was fed with a concentrate mixture for older lambs with 14.69% crude protein. The lambs from both groups during fattening were fed hay *ad libitum*. The chemical analysis of cereals mixture, concentrate mixture and grass hay are presented in Table 1.
The body weight and cereals and concentrate mixture consumption of lambs were recorded weekly during the trial period. The body weight gain was calculated weekly. Feed consumption and feed to gain ratio were calculated at the end of the fattening period (5th week).
The obtain results were statistically analysed using Microsoft Excel 2010, module Data Analysis by the t-test.

Table 1. Chemical composition of cereals mixture, concentrate mixture and hay (as-fed)

Nutrients, %	Cereal mixture	Concentrate mixture	Grass hay
Dry matter	87.29	87.71	86.95
Crude protein	12.26	14.69	8.02
Crude fiber	6.87	5.88	27.64
Crude fat	3.52	3.35	1.42
Ash	3.94	4.81	7.41
Calcium	0.64	1.00	0.86
Phosphorus	0.52	0.62	0.18

Results and Discussion

Body weight, weight gain, feed consumption and feed to gain ratio are presented in Table 2.
The results of this study show that the lambs fed mixture of cereals (corn, barley and wheat bran) and sunflower meal with no mineral-vitamin premix (Group I) achieved higher body weight and average daily weight gain compared to group II (lambs fed with concentrate mixture for older lambs) with no significance ($p>0.05$). The average body weight of lambs obtained in our research is in accordance with the results that have been conducted on cigaja breed lambs in organic breeding and during the fatteing lambs were fed mixture of grain and alfalfa hay *ad libitum* (Antunović *et al.* 2010). The results of Softić *et al.* (2017) indicated that lambs fed with cereals mixture and sunflower meal achieved better with no significance ($p>0.05$) all linear body measures comparing to lambs fed with feed mixture.

Table 2. Growth performance of lambs during fattening period

Parameter	Group I	Group II
Body weight (kg)		
Initial	18.90	18.40
1st week	20.50	19.80
2nd	22.20	21.50
3rd	24.10	23.40
4th	25.80	25.00
5th	27.20	26.40
Daily weight gain (g)		
1st week	232.86	208.57
2nd	237.14	241.43
3rd	278.57	268.57
4th	231.43	232.86
5th	207.14	194.29
Average	237.43	229.14
Feed consumption, mixture (g/day/head)	785.43	779.86
Feed to gain ratio, mixture (g/g)	3.31	3.36

Group 1 – group fed with mixture of cereals
Group 2 – lambs fed with feed mixture

Marić *et al.* (2013) conducted similar research on lambs breed Dubrovačka ruda using feed mixture with 16% protein and corn with wheat bran. The lambs fed with feed mixture obtained higher body weight and average daily gain compared to group fed with corn and wheat bran without significance ($p>0.05$) but these results are lower than in our study (both tested groups achieved higher body weight and average daily gain). In the study that was conducted by Dabiri and Tonney (2004) there was little difference in average daily gain or feed efficiency between lambs fed the diets with 15 or 17% CP, suggesting that a crude protein level near 15% based on supplemental soybean meal would be optimal for 25- to 40-kg growing Finnsheep × Dorset lambs.
Body weight (28.87 kg) of lambs breed Lička Pramenka before slauthering at average age of 158 days (Kaić *et al*. 2011) and is similar to results that reporeted by Bedeković *et al.* (2007) for lambs Travnička pramenka at age of 3,5 months (28.05 kg). The results of both researches are in agreement with our results obtained on crooss-bred lambs of Pramenka (27.20 and 26.40 kg). These results show influence of breed and age of lambs on body weight.
The average daily gain of lambs from Group I were 237.43 g and 229.14 g from Group II and these results are very similar with other authors experiment data (Zervas *et al.,* 1999; Softić *et al*., 2002; Ramljak *et al.,* 2005). Cmiljanić *et al*. (2003) reported higher average body weight gain (299 g and 251 g) during the all period of fattening in cross-bred lambs of Pirot Pramenka and Wurttenberg in semi-intensive and extensive feeding system, respectivelly. However, several authors (Kozarovski *et al*., 2000; Kaić *et al.,* 2011; Marić *et al*., 2013) have

reported lower daily weight gain in fattening lambs comparing with the results obtain in our study.

Based on the results of the cereals and feed mixture consumption, it is evident that lambs of Group I and Group II consumed almost the same amount of feed/day/lamb (785.43 and 779.86 g, respectivelly) during the fattening (differences < 1%) and calculated feed to gain ratio were similar in both groups (3.31 and 3.36) that is in agreement with the results of Softić *et al.*, (2002).The results suggest that the gain weight and feed to gain ratio are satisfactory for used diet in fattening and could be useful in futher breeding operation for producing a domestic lamb meat.

Cmiljanić *et al*., (2003) reported that the lambs in semi-intensive feeding system consumed 15.09 % hay less and 10.94 % less concentrate for unit of gain in comparison with lambs in extensive feeding system. Onk et al. (2016) were conducted the study for the purpose of comparing male Tuj lambs fed under extensive, semi-intensive and intesive fattening condition. Lambs in the extensive group were grazed in pasture. In addition to pasture, concentrated feed was given to lambs in the semi-intensive group. At the end of 90 days of fattening period the result show that the lambs of semi-intensive group obtained higher body weight 41.22 kg, and average daily gain 229.66 g compared to group of extenisive and intensive groups (body weight were 31.19 and 40.56 kg, respectivelly and average daily gain were 117.52 and 221.11 g, respectivelly. The results are in agreement with our results that lambs could obtained higher body weight even if they fed with lower amount of concentrated feed (200 g vs. 400 g in intensive groupFood conversion ratio was in favor of lambs kept indoors in individual pens and fed 200 g of hay/lamb/day and concentrates ad libitum (10.75 vs. 6.52 kg DM/kg BW gain) comparing to lambs kept on pasture, but feeding and labor costs were in favor of lambs of kept on pasture (Zervas *et al.*,1999), and author concluded that the system of fattening lambs on pasture can allow for a higher margin to the farmer because of the lower inputs and the products of higher value, compared to the indoors fattening system.

Conclusion

Results obtained in this study indicate that cross-bred lambs of Dubska and Sjenica Pramenka fed with a mixture of cereals (corn, barley and wheat bran) and sunflower meal with no mineral-vitamin premix that contained 12.26% crude protein can achieve better, but not significantly better production parameters that could have beneficial effects for economical lamb meat production with traditional character.

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NATURAL RADIONUCLIDES IN POULTRY FEED AND ASSESSMENT OF RADIATION RISK

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Abstract

The researches of radioactivity in poultry feed are particularly important because a part of the quantity of radionuclides in the food that animals ingest could be transmitted to people by means of the path of radionuclides in the food chain.

The study was conducted in order to detect the natural radioactivity in poultry feed and its values were compared with the measured values with poultry feed which is being produced in other parts of the world. The samples were analyzed by means of an instrument – gamma spectrometer (Canbera Packard) with a high purity germanium detector. The measurement was performed in a hermetically sealed container, whereby the spectra obtained from the measurement were analyzed by using the program GENIE 2000.

The results showed that the activity concentrations in feed supplements are within the range from 59.6 to 127.4 Bq/kg for 40K, 12.7 to 18.5 Bq/kg for 226Ra and 4.6 to 11.7 Bq/kg for 232Th, while those of the compounded feed are within the range from 64.7 to 172.0 Bq/kg for 40K, 9.7 to 29.4 Bq/kg for 226Ra, and 19.6 to 42.1 Bq/kg for 232Th. As it was expected during the analyses, the obtained values for the compounded feed were higher than the values for the feed supplements, which is expected, since some of these supplements are used for compounding feeds. In addition, anthropogenic radionuclides were not detected which shows that there was no contamination due to artificial radionuclides. The values of the specific activity obtained in this study do not exceed the safety limits, emphasizing the insigificant danger of radiation that arises from the Earth's radionuclides that are naturally present.

Key words: gamma spectrometry, feeds, natural radioactivity.

Introduction

Animal feed is developed from an organic basis (plants or animals) and is intended to provide the fullest diet possible (Filho et al., 2016). Very often, in order to improve the nutritional value of animal feed, it is supplemented with substances that could increase the level of activity of radionuclides.

In Macedonia, the most commonly used poultry feed is soybean, maize and "Premix Vitamínico Mineral", a bone meal and a fish meal. Therefore, control of radionuclides of these specimens is required, especially in phosphate stone, which is another raw material used in the production of animal feed additives (Casacuberta et al., 2010), which provides calcium for domestic animals such as poultry. One of the most common ways in which radionuclides reach poultry is ingestion, that is, consumption of foods that may contain a specific level of radiation, which would impose concerns in regard to such used poultry feed.

Considering the fact that through the food chain radioactive substances reach the human, more attention has been paid to these problems in the last decades in terms of the radiation safety of the population.

People consume eggs and poultry meat, therefore it is expected to monitor the levels of radiation in poultry feed, because a part of the amount of radionuclides in the food that

animals ingest could be passed on to people through the path of radionuclides in the food chain (Mc Donald et al., 1999; Breuninger et al., 2002; Hernandez et al., 2004). For this reason, due to the transfer of radioactive substances from the environment to agricultural, livestock and fish products, radioactive contamination of food and its health effects have become a major concern for people (Ramasany et al., 2006; Kaplan et al., 2011).
The study was conducted in order to detect natural radioactivity in poultry feed and the results were compared with measured values of poultry feed produced in other parts of the world.

Material and methods

2.1 Sampling

The poultry feed samples were taken from several producers, and several samples were directly purchased from the market in Macedonia. All samples were homogenized in a blender and then placed in Marinelli (hermetically sealed containers with a weight of approximately 450 grams).
The Marinelli were stored for 10 days in order to achieve a balance of radium and thorium with their daughters.

2.2 Instrument

The spectral analysis of the radionuclides of these samples was conducted by applying a γ-ray spectrometer with high purity germanium (HPGe) detector with 30% relative efficiency and energy resolution (FWHM) of 1.8 keV for 1.33 MeV reference passage of ^{60}Co (Verdoya et al., 2009).
The detector was protected with 9cm-thick lead with an internal line with a 0.5 cm-thin copper panel covered by 1 mm aluminum in order to absorb the x-rays from the lead and the copper. The internal size of the cavity of the shell was 30 x 30 x 30 cm. The detector was given a high voltage through a preamplifier which was then connected to an amplifier with a computer based channel analyzer through an ADC (analogue to digital converter). The software used for obtaining the data is Canberra software package Genie-2000, including search of maximal value and modules for identification of nuclides. The system was regularly calibrated for energy and efficiency. The gamma rays of interest were within a range of 50-3000 keV. The prepared Marinelli glasses (samples) were placed on a final detector at a distance of approximately 10 mm. Every sample was measured within a period of around 62000s in order to get good statistics and the constant time was lower than 10%. The measurements with an empty Marinelli glass, in identical conditions were also conducted in order to determine the basic recounts. Then they were deducted from the measured spectrums of every sample in order to obtain the net activities of the radionuclides.

Results and discussion

The activity concentrations of ^{40}K, ^{226}Ra and ^{232}Th were assessed and they were shown in Table 1 and Table 2 as well as in Figure 1 and Figure 2. The results showed that the activity concentrations in feed supplements range from 59.6 to 127.4 Bq/kg for ^{40}K, 12.7 to 18.5 Bq/kg for ^{226}Ra and 4.6 to 11.7 Bq/kg for ^{232}Th, while that of the compound feed range from 64.7 to 172.0 Bq/kg for ^{40}K, 9.7 to 29.4 Bq/kg for ^{226}Ra and 19.6 to 42.1 Bq/kg for ^{232}Th.
Compound feed had higher values than poultry feed supplements, which is expected, because some of these supplements are used for combining the food. The results on the natural radioactivity were compared with the results from different countries of the world and were relatively higher compared to other such studies (Harb et al., 2010; Filho et al., 2016).
Presence of anthropogenic radionuclides was not found, which shows that there is no contamination due to artificial radionuclides.

Table 1. Activity concentrations of poultry feedstuff (Bq/kg)

Sampling sites	^{40}K	^{226}Ra	^{232}Th
Feed supplement	67.24±2.12	14.79±3.00	4.60±1.30
Feed supplement	111.60±2.54	12.70±2.00	10.21±1.50
Feed supplement	89.24±5.00	15.22±5.20	7.02±2.30
Feed supplement	127.40±3.00	12.98±3.00	7.12±2.50
Feed supplement	102.10±2.40	18.50±1.20	11.70±1.50
Feed supplement	113.17±4.20	17.54±1.95	5.10±1.65
Feed supplement	66.51±4.21	17.02±3.63	9.68±2.30
Feed supplement	111.35±2.50	13.22±1.95	5.55±2.50
Feed supplement	59.60±5.20	16.47±2.50	4.90±1.70
Feed supplement	63.06±2.60	14.02±3.80	4.60±1.30
Feed supplement	121.15±4.50	13.29±2.50	9.26±3.50
Feed supplement	73.30±3.55	15.45±3.50	9.01±1.50
Feed supplement	124.56±2.30	17.98±3.11	5.47±2.50
Feed supplement	68.07±2.50	13.27±5.00	7.62±2.50

procedure included the subtraction of the background spectrum.

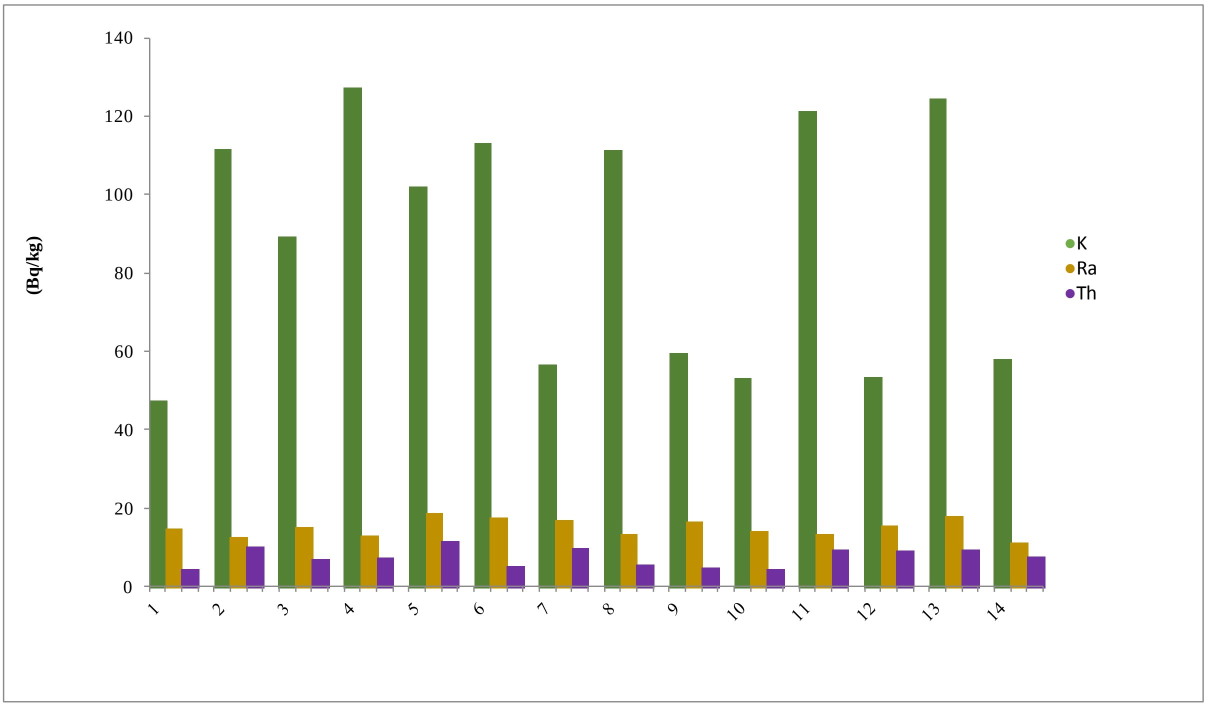

Figure 1. Activity concentration of K-40, Ra-226 and Th-232.

Table 2. Activity concentrations of compounded feed (Bq/kg)

Sampling sites	^{40}K	^{226}Ra	^{232}Th
Compounded feed	77.11±2.00	16.00±2.50	24.30±1.50
Compounded feed	172.00±2.50	25.13±2.22	42.10±1.70
Compounded feed	99.14±2.50	15.22±5.20	21.52±2.50
Compounded feed	127.40±3.00	29.40±1.50	38.22±2.55
Compounded feed	114.22±2.20	17.32±1.50	35.11±1.50
Compounded feed	113.17±4.20	15.14±1.90	21.10±1.80
Compounded feed	82.37±3.22	9.70±2.00	20.17±2.30
Compounded feed	154.35±5.50	23.12±1.90	39.55±1.50
Compounded feed	137.11±5.00	21.07±2.50	34.36±2.60
Compounded feed	64.70±5.20	10.35±3.50	19.60±1.50

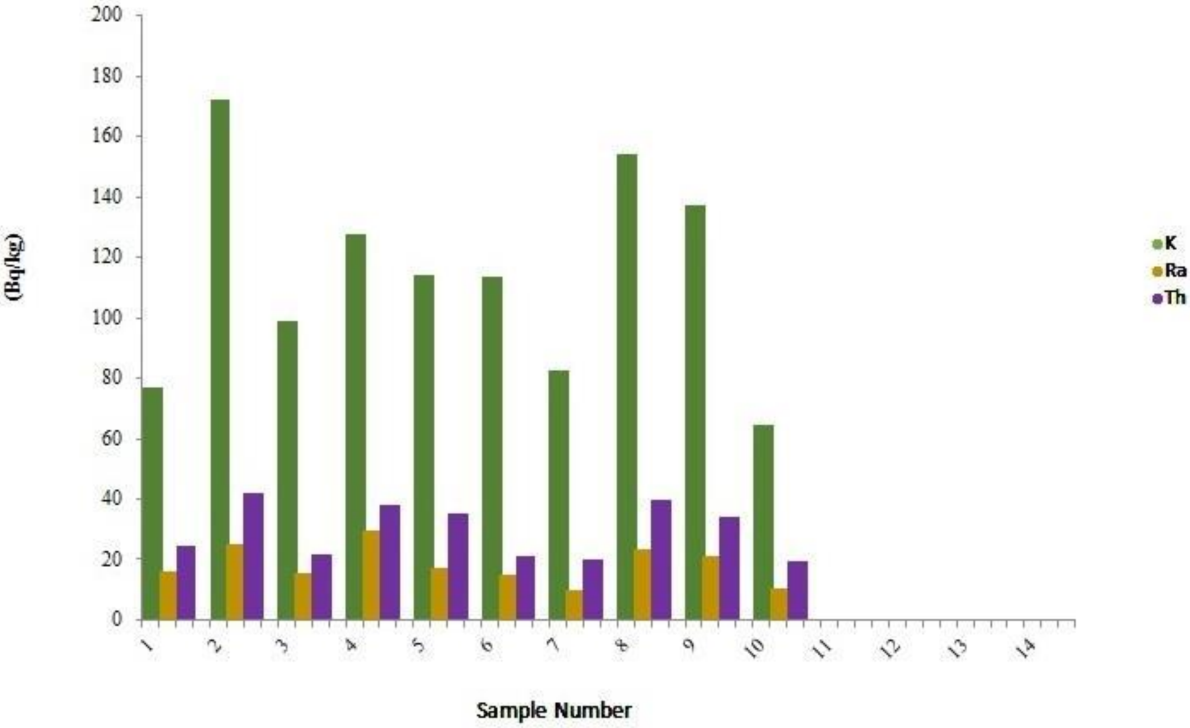

Figure 2. Activity concentration of K-40, Ra-226 and Th-232.

Conclusion

The values of the specific activity in this study did not exceed the safety limits, emphasizing the insignificant radiation hazard arising from naturally occurring earth radionuclides.

The results were compared with other poultry feed in other parts of the world, which shows that at the present level of radioactive contamination, there would be no need to take measures related to the reduction of radioactive contamination. The conclusion in this study is that the transfer of such levels to poultry meat and, ultimately, to the human, in the path of radionuclides, will not pose a danger when the public will eventually consume the meat and eggs from the poultry that consume such feed.

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COMPARISON OF THE VEAL QUALITY AND CARCASS VALUE UNDER TWO FEEDING CONCEPTS

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Abstract

The aim of this study was to evaluate the carcass and qualitative parameters of veal from Holstein bull calves - two groups of 10 calves each. Calves of the 1st group received a basal diet (milk replacer and concentrate) for the first stage of experiment (60 days). Then, they received feed mixture (maize silage, alfalfa silage, concentrate). The 2nd group was during whole experiment fed with the milk replacers and the starter concentrate, while after 35 days, liquid milk was replaced with pellets form. Calves were slaughtered after about 4 months.The 1st group of calves had higher slaughter weight ($P<0.05$), but lower dressing percentage ($P<0.05$). Significantly higher proportion of kidney fat ($P<0.05$) exhibited calves of the 2nd group. No significant, but higher proportion of meat from right-half carcass was revealed in the 2nd group. Contrariwise, right-half carcasses of 1st group had higher proportion of bones and separable fat ($P>0.05$). According to the CIE colour scale of MLT measured 24 hours *post mortem*, calves of the 1st group had darker (lower L^*; $P>0.05$) and redder (higher a^*; $P<0.05$) meat. The MLT from calves of 1st group had higher pH_{24} value ($P<0.05$), electrical conductivity ($P>0.05$), but lower drip loss value ($P>0.05$). Chemical composition of MLT showed higher moisture content ($P<0.05$) in the 2nd group of calves. No significant differences in protein and intramuscular fat content were revealed. We found minimal differences in WB shear force between monitored groups ($P>0.05$).

Keywords: *Holstein veal, milk pellets, carcass value, physical - chemical parameters, redness*

Introduction

For a modern consumer, the taste and nutritional value of the meat are two important quality traits. The tendency is to focus on producing consumable lean meat with minimal excess of visible fat (Webb and O'Neill, 2008). Traditionally, many European consumers include veal to the highest quality. Such meat is associated with a healthy product, low fat content and smooth, delicate taste (Vieira *et al*., 2005). Dairy calves for the meat production are generally separated from the cows at birth, receiving a nutritionally balanced feed. In most cases, these calves are a by-product of dairy farms (Fluharty *et al.*, 2000). The consumer may also define a required feeding system, however, that must comply with applicable country-specific regulations. In general for veal production may be used suckling (*mother-fed*), milk replacers (*formula-fed*) or combinative feeding, which may include whole milk, milk replacers, silage, cereals, grains or other plant products. Under the legislation of specific EU member States, feed should not contain fishmeal, animal origin supplements, growth promoters or products derived from genetically modified organisms (UNECE, 2011). In terms of animal health and welfare, it is important for veal producers to optimize the growth of rosé calves after weaning. In particular, a fluent weaning from liquid dairy feed mixtures to solid feed is required, as

well as its effective utilization, which is mainly influenced by management before weaning and after weaning (Drake, 2017). Feeding with milk and concentrated solid feed could affect the utilization of nutrients in the feed, especially at an early age of animal when microbial fermentation and rumen development is restricted (Brscic *et al*., 2014). Several articles describe systems of early weaning from liquid milk replacers and its replacement with granulated milk (Terré *et al*., 2016; Fletcher, 2016). In general, the positive impact of this method (mainly on heifers) on growth, increased intake of solid feed and health status is described, which allows to increase of milk production in the next generation of dairy cows.
The aim of this work was to verify a modified feeding method of calves with granulated dairy feed mixture in the Holstein bulls and its influence on meat production and quality compared to traditionally fed calves.

Material and methods

For this experiment bull calves from Holstein breed (n = 20) were studied. Calves were born within a week. Calves were housed in individual outdoor crates and divided into two groups with different diet. Calves of the 1st group received a basal diet (liquid dairy feed mixture and starter feed mixture) for the first 60 days. From 60 days to 75 days they were gradually adapted to solid feed mixture. Calves were fed with dairy feed mixture once per day, starter feed mixture *ad libitum* and total mixed ration (TMR) in small amount. After this stage they were fed with the feed mixture (maize silage, alfalfa silage and concentrate) ad libitum; drinking water was available ad libitum. Calves of the 2nd group were during whole experiment fed with the milk replacers and the starter feed mixture. For the first 35 days they received liquid milk. Subsequently, they were habited on pelleted milk (1x liquid milk, 1x pelleted milk and starter feed mixture) up to weight 85 kg. The experimental period of both group started at initial body weight 85 kg in group barns, 10 calves each. All animal experiments and procedures were approved by the Ethics Committee of the Slovak University of Agriculture Animals were slaughtered after about 4 months of treatment in the experimental abattoir of Slovak University of Agriculture (SUA) in Nitra. At slaughter were carcass composition recorded. After 24 hours chilling, detailed dissection from the right - half carcass into meat cuts was carried out. Immediately, slices of loin and top round muscles were removed from right-half carcass for the proximate composition and physical technological parameters of veal. Weight and percentage of lean meat, separable fat and bones (technical, marrow, pelvis, scapula) from the hindquarter and forequarter of right-half carcass were determined. Meat cuts were separated according to 1st quality class meat including top round, sirloin, tenderloin, shortloin, shoulder; 2nd quality class meat including neck, fore - shank, brisket, rib, short plate, flank, hind – shank. Parameters associated with the mat quality (pH, electrical conductivity, drip loss and colour measurements) were evaluated 24 hours *post mortem*. Analyses of qualitative and physical technological parameters were performed using standard instruments in the laboratories of SUA in Nitra. For statistical evaluation of results were calculated basic variability and statistical characteristics using the Statistical Analysis System (SAS) version 9.3 (TS1M2) Enterprise Guide 5.1. (SAS INSTITUTE Inc., 2011).

Results and discussion

Experimental group (2nd) of calves fed with granulated milk replacer was lighter at the end of fattening ($P<0.05$) as well as at slaughter ($P<0.05$), as shown in Table 1. The carcass weight was higher in the first group of calves ($P>0.05$). Dietary effects on growth rate were revealed, while they may consist in different rumen development or rate of muscle deposition (Noon *et al.,* 1998). Diet slightly significant influenced dressing percentage ($P<0.05$) as well as proportion of kidney fat ($P<0.05$); higher results were determined in the calves of second group. Variations in dressing percentage may depend on the dry matter content of milk or solid feed when feeding calves with diet, including mix diets (Vieira *et al*., 2005). Xiccato *et al*. (2002) found +10 kg higher final weight in calves fed with the maize grain supplement compared to exclusively milk fed calves. Moran *et al*. (1988) found in calves fed with whole milk with carcass weight of 66.7 kg weight of kidney fat 1.26 kg.

Table 1. Average slaughter characteristics of Holstein male calves with different feeding system.

	Weight (kg)		*Sign.*	Percentage (%)		*Sign.*
	Group I	Group II		Group I	Group II	
Final weight	138.2 ± 2.25	121.36 ± 9.32	*			
Slaughter weight	128.7 ± 1.10	114.38 ± 9.77	*			
Carcass weight	66.30 ± 1.91	63.31 ± 5.46	*ns*			
Carcass yield				51.51 ± 1.32	55.41 ± 2.97	*
Kidney fat	0.42 ± 0.13	0.88 ± 0.30	*	0.33 ± 0.09	0.76 ± 0.21	*

Values are mean ± standard deviation, * P<0.05, ns – not significant

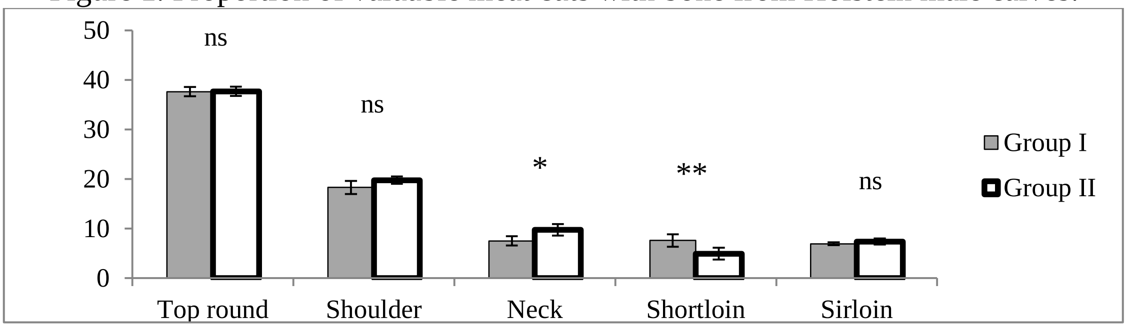
Table 2 describes weight of meat quality classes from right – half carcasses of calves with different feeding system. Higher proportion of the 1st choice meat as well as 2nd choice meat were determined in the calves fed with granulated milk, results were not significant (P>0.05). Feeding system had little effect on longissimus area (P<0.05). Our results in MLD area of the 1st group were similar to those of Titi *et al*. (2008).

Table 2. Meat quality grades from Holstein male calves in different feeding system.

	Weight (kg)		*Sign.*	Percentage (%)		*Sign.*
	Group I	Group II		Group I	Group II	
Right-half carcass	32.14±0.53	30.04±4.11	*ns*			
1st choice meat	22.44±0.63	21.16±2.85	*ns*	69.81±1.07	70.48±2.01	*ns*
1st choice meat[1]	11.47±0.76	11.42±1.62	*ns*	35.68±1.94	38.04±2.01	*ns*
2nd choice meat	13.98±0.36	13.54±1.68	*ns*	43.48±1.09	45.18±2.50	*ns*
2nd choice meat[1]	9.55±0.36	9.50±1.20	*ns*	29.71±1.35	31.68±1.87	*ns*
MLD area (cm^2)	26.16±2.82	35.38±4.99	*			

Values are mean ± standard deviation, * P<0.05, ns – not significant; [1] boneless

Figure 1. Proportion of valuable meat cuts with bone from Holstein male calves.



** P<0.01, * P<0.05, ns – not significant

The proportions of most valuable meat cuts from right – half carcass of calves fed with different diets are presented in Figure 1. Feeding with granulated milk influenced proportions of 1st choice meat including top round, shoulder and sirloin as well as neck from 2nd meat quality grade (P>0.05). Contrariwise, significant higher proportion of shortloin (P<0.05) was found in the 1st group. Our results are similar to those of Holló *et al*. (2013) in buffalo male calves (when calculated proportion of boneless meat cuts).

Minimal differences (P>0.05) in the proportion of the hindquarter and forequarter from the right – half carcass were recorded between groups (Table 3). Feeding system influenced proportion of meat from hindquarter, while a significantly higher proportion had calves of the 2nd group (P<0.05). Traditionally fed calves had higher proportion of bones (technical, marrow, pelvis and scapula) of both the quarters from right – half carcass (P<0.05). Calves of the 2nd group had higher proportion of meat (P<0.05), lower proportion of bones (P>0.05) and separable fat (P>0.05) from hindquarter of right – half carcass. According to Moran and Curie (1992) Holstein calves are often considered unsuitable for the meat production due to their poor conformation of the hindquarters and thus highly priced meat of carcasses with lower muscularity. In contrast with our findings, Moran and Currie (1992) reported weight of forequarter 14.27 kg and forequarter meat 7.62 kg.

Table 3. Individual tissues from quarters of right – half carcass of Holstein male calves.

	Weight (kg)		*Sign.*	Percentage (%)		*Sign.*
	Group I	Group II		Group I	Group II	
Forequarter	13.95±0.71	13.21±2.01	*ns*	43.40±1.63	43.88±1.00	*ns*
Hindquarter	18.19±0.36	16.71±2.32	*ns*	56.60±1.63	55.61±0.73	*ns*
Meat from FQ[1]	8.24±0.51	8.10±0.97	*ns*	25.62±1.34	27.03±0.79	*ns*
Meat from HQ[1]	11.12±0.68	11.12±1.45	*ns*	34.57±1.83	37.05±0.93	*
Bones from FQ	4.89±0.45	4.29±0.66	*ns*	15.22±1.32	14.25±0.48	*ns*
Bones from HQ	4.78±0.16	4.27±0.52	*ns*	14.86±0.45	14.24±0.58	*ns*
SPF from FQ	0.82±0.21	0.90±0.27	*ns*	2.56±0.63	2.96±0.51	*ns*
SPF from HQ	1.41±0.42	0.89±0.25	*ns*	4.39±1.37	2.91±0.51	*ns*

Values are mean ± standard deviation, * P<0.05, ns – not significant, [1]boneless meat, FQ – forequarter, HQ – hindquarter, SPF – separable fat

The pH taken on the loin muscle at 24 hours post mortem (Table 4) showed slightly higher values in the 1st group (P<0.05). Meat from calves fed with milk replacers usually had higher content of fat and lower pH value (Ngapo and Gariépy, 2006). No significant differences were found in the pH values on *M. semimembranosus* (P>0.05). According to Florek *et al*. (2015) the ultimate pH value of veal (usually measured within range between 2 days and 12 days *post mortem*) ranged from 5.54 to 5.64, which indicate the proper glycolysis process post mortem. Titi *et al*. (2008) reported lower values of pH_{24} (5.61) in Holstein calves. Feeding system had no effect on electrical conductivity of both the muscles (P>0.05). The loin muscle from calves fed with granulated milk had higher drip loss than those from traditionally fed (P>0.05). No significant differences in shear force were determined in MLT (P>0.05). Lower results in shear force were described in Gottardo *et al*. (2002). Schaefer (2007) did not found effect of diet composition and nutritional management to sensory characteristics of Holstein beef.

Table 4. Parameters associated with the veal quality of Holstein male calves.

	MLT		*Sign.*	SM		*Sign.*
	Group I	Group II		Group I	Group II	
pH_{24}	6.15 ± 0.13	5.95 ± 0.08	*	6.01 ± 0.17	5.82 ± 0.11	*ns*
EV-2 (μS)	2.36 ± 0.51	2.64 ± 0.65	*ns*	3.04 ± 0.79	3.54 ± 0.40	*ns*
Drip loss (%)	1.23 ± 0.36	1.59 ± 0.55	*ns*			
Shear force ($kg.cm^{-2}$)	5.57 ± 1.41	5.13 ± 1.32	*ns*			
Lightness	43.52±2.17	44.2±4.13	*ns*	45.32±2.46	45.25±2.61	*ns*

Redness	4.37±1.36	1.20±2.41	*	5.28±0.54	3.9±3.83	*ns*
Yellowness	10.1±1.10	8.83±0.45	*ns*	10.69±0.89	10.36±1.88	*ns*

Values are mean ± standard deviation, * P<0.05, ns – not significant; SM - *M. semimembranosus*

Colour measurements of MLT (Table 4) designated light and pink meat. A higher L* value was measured in 2nd group (P>0.05). Significant differences in redness of loin were confirmed between two feeding groups (4.37 vs. 1.20). The rate of post mortem protein degradation is directly dependent on the ultimate value of pH. In addition, degradation contributes to the spread of scattering on the meat surface resulting to increase in colour measurements (Florek *et al*., 2015). In contrast with our findings Scheeder *et al*. (1999) reported L* value 44.1 and b* value 7.5 in MLT of calves fed with milk replacer. Opposite to our findings Titi *et al*. (2008) found in Holstein calves with slaughter weight 370.5 kg markedly darker (L* - 32.7) and slightly redder meat (a* - 5.0). In agreement with our findings, the study of Ngapo and Gariépy (2006) show that grain fed calves produce darker meat compare to exclusively milk fed calves, due to a higher content of haemoglobin. Calves in study of Scheeder *et al*. (1999) were fed with milk replacers or maize silage and concentrate feed mixture. They found markedly darker meat (L*:a*:b* 44,1: 12,3: 7,5) in the second group.

Table 5. Proximate composition of the loin muscle from Holstein male calves.

	Group I	Group II	*Sign.*
Moisture (%)	75.13±0.02	76.85±1.07	*
Protein (%)	22.38±0.49	22.06±0.56	*ns*
Intramuscular fat (%)	1.75±0.71	1.79±0.15	*ns*
EV (kJ.100g^{-1})	440.96±0.58	437.07±5.69	*ns*

Values are mean ± standard deviation, * P<0.05, ns – not significant

Chemical composition of MLT samples from Holstein male calves are described in Table 5. The results showed significant effect of the treatment on moisture content (P<0.05). It was determined that meat from 1st group of calves had slightly higher protein content (P>0.05). According to Xiccato *et al*. (2002) increasing in consumption of milk in diet resulted to higher fat percentage in carcasses. We found minimal differences in energy value as well as in intramuscular fat of monitored groups (P>0.05) (P>0.05). Holló *et al*. (2013) reported in buffalo suckling male calves content of protein 20.99% and fat content 0.63%.

Conclusion

Recently, several foreign papers describe the effect (especially in heifers) of so-called early weaning from liquid milk replacers and its replacement with granulated dairy feed mixture. Papers mentioned faster growth, increased intake of solid fee, greater rumen development, greater health and consequent higher utility of dairy cows. In our experiment we tested the liquid dairy mixture replacement with a granulated milk mixture for the meat performance of the Holstein calves. Although we found in the fattening indicators lower average daily gains of the experimental group (group II) in comparison to traditionally fed calves, variability of monitored indicators were lower. On the other hand, calves of the experimental group in the given weight category, had greater most of carcass values, which was also reflected in the larger body shapes of calves. In terms of meat quality indicators, the most important influence of the feeding system shown to be in lighter and pinker meat colour. However, mentioned results need to be verified in a greater extent and with a higher number of animals.

Acknowledgment

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NITROGEN USE EFFICIENCY IN DAIRY CATTLE

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Abstract

In this paper we considered the possibilities for controlling or reducing the N losses and increasing N use efficiency in dairy cattle by using optimized feeding strategy and diet formulation. Ruminants have a low efficiency of N utilization compared with non-ruminants, whereas the N use efficiency in dairy cows is usually between 22 and 33%. The ruminal ammonia-N concentration between 6 and 18 mM is required to maximize microbial protein synthesis. Dietary strategies to reduce N losses should focus on an optimal supply of rumen degradable protein (RDP) and optimal efficiency of absorbed amino acid utilization for milk protein synthesis. Synchronization of the supply of rumen available protein and energy is an important factor for improving the utilization of dietary N. The optimal ratio of N to rumen fermentable OM is around 25 g/kg. A crude protein (CP) concentration in diets for lactating cows should be reduced to 15% DM to improve N efficiency and reduce environmental impact. Increased ratio of energy to protein improves N utilization and milk protein content as well as decreases milk urea N (MUN). Feeding adequate readily fermentable carbohydrates is critical for the efficient microbial capture of rumen available N. Improving the utilization of dietary protein is important for significant improvements in whole farm N balances, increasing the conversion of dietary N to animal products and providing opportunity for reducing environmental N losses.

Key words: *Dairy cows, Nutrition, Protein, Energy, Urea.*

Introduction

The low efficiency of dietary nitrogen utilization in ruminants is attributable primarily to the effects of the rumen microbes on nitrogen utilization. Dairy cows have specific requirements for amino acids that must be supplied either directly by the diet, or by rumen microbes flowing out of the rumen in the digesta. Feeding excess CP can result in unnecessary feeding expenses with no return in milk or milk protein yield. Approximately 75-85% of the excessive protein provided in rations are excreted, mostly in the urine. There is a direct relationship between the level of CP in dairy cattle rations and the amount of nitrogen excreted (Stojanović *et al*., 2004). The level of nitrogen excreted in the feces of animals is generally constant and cannot be significantly altered. In contrast, the level of nitrogen in the urine can be effectively controlled by balancing protein and energy needs of cows. Urea is the most variable component in urine, contributing from 50-90% of all N in urine. Dairy cows remove approximately 2.5-3.0% of the total amount of urea by produced milk.

Strategy to reduce N losses should focus on an optimal supply of rumen degradable protein (RDP) and optimal efficiency of absorbed AA utilization for milk protein synthesis (Stojanović *et al*., 2010). Rumen microbes need energy to be able to capture ammonia, and use it as an N source for growth. Carbohydrates fermented in rumen are the main source of energy required by microbes to utilize dietary and endogenous N, and increase the supply of AA to the small intestine (Stojanović *et al*., 2006). Synchronization of the supply of rumen available protein and energy is an important factor for improving the nitrogen use efficiency (NUE) from rumen degradable protein (RDP). Providing the high quantities of energy from readily fermentable carbohydrates in rumen, in synchrony with produced ammonia reducing the lag in energy supply as fibrous carbohydrates are fermented (Stojanović *et al*., 2014). Ruminants have significant capacity to recycle N absorbed from the digestive tract, and dietary manipulation should be aimed to provide the appropriate energy supply which corresponds with amount of ruminally available N.

Monitoring of blood or milk urea nitrogen (BUN, MUN) can be used for measuring protein and energy status in dairy cattle as these values are positively associated with rumen NH_3 concentrations. Blood urea nitrogen is highly correlated with ruminal ammonia, and MUN is highly correlated with BUN (r^2 = 0.72-0.98), and their values are indicators of the protein to energy ratio in the diet (Stojanović *et al*., 2007).

The aim of this paper was to show the main pathways of N metabolism and losses in dairy cattle, and to consider the possibilities for controlling or reducing the N losses and increasing N use efficiency by using optimized feeding strategy and diet formulation.

Efficiency of nitrogen utilization in dairy cattle

Ruminants have a low efficiency of nitrogen utilization compared with non-ruminants, with approximately 72% of N intake excreted in manure. Rumen metabolism is the most important factor contributing to the inefficient use of N in ruminants. Nitrogen losses in dairy cattle include: urinary excretion of urea synthesized from ammonia produced in the rumen; undigested microbial protein excreted in feces; microbial nucleic acids synthesized in the rumen and excreted mainly in urine; fecal and urinary excretion resulting from endogenous secretions; and urinary excretion related to maintenance and milk protein synthesis. The theoretical upper limit of nitrogen use efficiency (NUE) in a lactating cows is 0.40-0.45, but usually is well below this maximal value, between 0.22 and 0.33 (Dijkstra *et al*., 2013). Cows with higher DM intake and milk yield on diets contain a lower CP and higher non-fibre carbohydrates (NFC) have higher NUE.

The ruminal ammonia-N concentration between 6 and 18 mM is required to maximize microbial protein synthesis (Reynal and Broderick, 2005). However, benefits of supplying amino acids and peptides on rumen microbial protein synthesis and efficiency are significant. The efficiency of synthesis of microbial CP in the presence of AA and peptides, compared with NH_3 is about 20% higher when expressed per unit of fermented organic matter (OM), (Dijkstra *et al*., 2013).

The lowest loss of N (35 g/day) is at an efficiency of microbial CP synthesis of 25.2 g microbial N/kg rumen fermentable OM (Bach *et al*., 2005). Dietary rumen available N content should be 15 g/kg DM or 94 g CP/kg DM. Surplus of rumen degradable protein is excreted as urea-N in urine. Bacterial CP synthesis in rumen was estimated to be 130 g/kg TDN (NRC, 2001).

Ruminants have the ability for nitrogen recycling and by that to save the urea N from excretion and transfer it back to the gastrointestinal tract. This is provided by the urea transfer from the blood across the epithelial tissue or through the saliva, and is a significant source of N for rumen microorganisms. Depending on dietary CP concentration, urea recycling back to the gut can be from 19-96% of endogenous production, and of this, 35-55% may be used for microbial protein synthesis (Lapierre and Lobley, 2001). The recycling of N to the rumen and incorporation into microbial protein is especially significant for the efficient use of diets with low CP content. Holstein heifers capture approximately 43% of the N recycled back to the digestive tract when fed low CP diets compared to 6% when fed high CP diets (Marini and Van Amburgh, 2003).

Rumen microbial synthesis of nucleic acids (20-25% of rumen microbial N) leads to considerable losses of N particularly in urine, and there is no possibility to reduce losses of N related to nucleic acid synthesis in the rumen. The major end products of catabolism of

pyrimidines are β-amino acids and ammonia, as well as of catabolism of purine are allantoin, uric acid, xanthine and hypoxanthine.
The digestibility of microbial true protein and rumen undegradable protein (RUP) in the small intestine of cows is 80-85% (Stojanović and Grubić, 2008).
About the 65% of AA digested in the small intestine is recovered in the portal vein, whereas the losses are the endogenous protein synthesis (replacement of sloughed epithelial cells and synthesis of digestive enzymes) and oxidation of AA across the gut wall (Lapierre *et al*., 2006). The utilization efficiency of absorbed AA for milk protein synthesis is 64-68%. The utilization of available AA by the mammary gland is related to the supply of energy. Increased supply of metabolizable energy may reduce N losses in post-absorptive tissues.
Losses of N related to the maintenance are mostly unavoidable and include tissue maintenance requirements, skin secretions and scurf, and hair losses.

Strategies to increase nitrogen use efficiency in dairy cows

The most commonly used approaches to improve N utilization in dairy cows including reducing dietary CP content, increasing ruminal energy supply, synchronizing ruminal energy and protein supply, and balancing the supply of AA to the duodenum.
The catabolism of absorbed essential amino acids by the liver and other body tissues is largely determined by the extent to which they are absorbed in excess of requirement, which is largely determined by the rate of protein synthesis and secretion by the mammary gland (Reynolds and Kristensen, 2008). The key factor determining post-absorptive nitrogen use efficiency is the rate of milk protein secretion by the mammary gland relative to the rate of absorption of the individual AA required for milk protein synthesis.
The extent, to which ammonia is used to synthesize microbial protein in rumen, is largely dependent upon the availability of energy generated by the fermentation of carbohydrates. On average, 20 g of bacterial protein is synthesized per 100 g of OM fermented in the rumen. Bacterial protein synthesis may range from less than 400 g/day to about 1500 g/day, depending primarily on the digestibility of the diet.
With increase the supply of energy to rumen microbes while the RDP content does not change, less ammonia-N is formed and lost as urea-N in urine. The N use efficiency of a low energy and high protein diet can be improved from 0.20 to 0.24 when either supply of protein is reduced or supply of energy is increased, and can be improved to 0.28 with both (Rius *et al*. 2010).
The increase of dietary CP from 15.1 to 16.7% at the low and medium energy levels, significantly increase milk and milk protein yield, and there is no effect of increasing CP beyond 16.7% (Broderick *et al*. 2003). Milk and milk protein yield is also increased with ration energy increasing. Grings *et al*. (1991) found increase in milk yield with increasing dietary CP content from 13.8 to 17.5% but determined little benefit from increasing CP above 17.5%. Castillo *et al*. (2000) concluded that CP concentrations should be reduced to 15% DM to improve N efficiency and reduce environmental impact, and compared to diets containing 20% CP, this would reduce N excretion in the feces by 21% and N excretion in the urine by 66%. An increase in dietary CP from 13 to 18% DM led to a higher concentration of urea in milk from 7.0 to more than 15.0 mg/dL (Kebreab *et al*. 2002).
When low protein diets for lactating cows is used, to sustain high levels of production and milk protein yield, the rumen protected methionine and lysine should be used, because it has been shown for these essential AA to be the most limiting AA for milk production when dietary protein supply is low (Stojanović *et al*., 2010).
Increased ratio of energy to protein improves N utilization and milk protein content as well as decreases MUN. Feeding a concentrate based on ground shelled corn at the same time as high quality fresh pasture decreased the concentration of NH_3 in the rumen by 33% (Kolver *et al*.,

1998). Addition of a barley-sugar beet pulp based concentrate to cows (up to 6 kg) fed fresh pasture increases a microbial protein available for absorption and decreases MUN (from 50.4 to 38.9 mg/dL), (Sairanen *et al*., 2005). Cows consuming only pasture had a NUE of around 21.0% while supplemented grazing cows with carbohydrate feeds improved their NUE by storing in the milk 24.6% of the ingested N (Keim and Anrique, 2011).

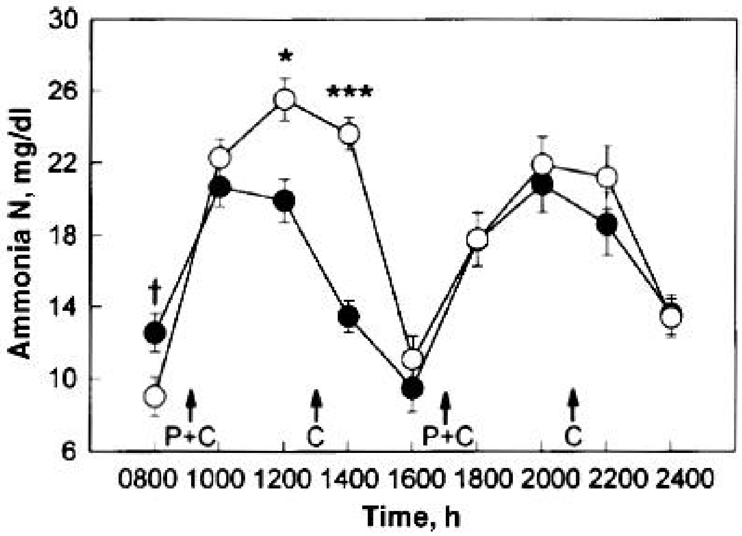

Figure 1. Diurnal pattern of ruminal ammonia N (mg/dL) of cows on pasture fed energy : protein synchronous (●) or asynchronous (○) diet (Kolver *et al*., 1998).

The relationship between milk protein content and dietary energy intake is much stronger than that with dietary protein intake. This is attributed to increased flows of microbial protein to the duodenum, but could also be explained by endocrine changes that affect the use of AA by the mammary gland (Griinari *et al*., 1997). Energy status affects insulin and the IGF (insulin like growth factor) system which have important roles in nutrient partitioning and milk protein synthesis.

Tannins as bioactive plant factors have high affinity towards proteins, and by forming the complexes reduce soluble protein fractions amount. The tannin-protein bonds are cleaved under the acidic conditions in the abomasum, and protein can further be digested in abomasum and small intestine. Lactating cows fed with silage containing different levels of condensed tannins from birdsfoot trefoil (*Lotus corniculatus*) or with added tannin extract at dosages of 15 and 30 g/kg DM had reduced milk urea nitrogen, ruminal ammonia and urinary N excretion (Davidović *et al*., 2018).

For MUN content, it is generally accepted that the range of 15.0-25.0 mg/dL is optimal in terms of protein and energy balance in rations for dairy cattle. In high producing dairy cows, BUN or MUN concentrations of less than about 15 mg/dL indicate a relative deficiency of dietary protein.

Urea nitrogen concentrations of greater than 19 to 20 mg/dL have been associated with reduced conception and pregnancy rates in dairy cows (Stojanović *et al.*, 2007). Urea has a significant impact on the percentage of pregnant cows after the first insemination procedure. The fertility is by 21.4% higher in the group of cows with a milk urea level <19.0 mg/dL, when compared to cows which milk contained >19.0 mg/dL of MUN (Butler *et al.*, 1996).

Conclusion

The nitrogen utilization efficiency in ruminants depends primarily of the effects of the rumen microbes on dietary CP utilization. In order to improve the efficiency of dietary CP utilization and minimize nitrogen excretion and environmental impacts, the most effective approach is to decrease concentration of protein in ration for dairy cattle. A CP concentration between 14 and 16% DM is probably adequate to meet the requirements of lactating dairy cows. Increasing ruminal energy supply, synchronizing ruminal available energy and protein supply, and balancing the supply of AA to the duodenum are also effective for increasing N use efficiency. With low protein diets for lactating cows, the rumen protected methionine and lysine should be used, as these essential AA are the most limiting for milk production. Reducing the soluble protein fractions in ration, lowers milk urea nitrogen, ruminal ammonia and urinary N excretion. The range of MUN between 15.0-25.0 mg/dL is optimal in terms of protein and energy balance in rations for dairy cattle.

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EFFECT OF BLUE LUPINE ON THE GROWTH RATE, CARCASS AND MEAT QUALITY OF HEAVY-TYPE TURKEYS

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Abstract

Soybean meal is the commonly used source of dietary protein in poultry feed. However, around 98% of soybean meal is produced from genetically modified plants. An alternative protein source might be lupines (*Lupinus* spp.). However, the available information is insufficient to develop the diets with blue lupines. The purpose of our study was to determine the effects of soybean meal replacement with different amounts of blue lupine in the diets of turkeys on the growth rate, carcass and meat quality. Three hundred and sixty cross BIG-6 turkeys were allotted to control and five experimental groups of turkeys. The control group of turkeys was fed with the diet containing soybean meal, whereas the trial groups were offered different amounts (from 20 to 30%) of lupines. Group 4 and 5 were additionally given probiotic mixture Bio Plus 2B and allzyme SSF. Soybean oil replacement from 20 to 30% lupine in the diet had no influence on the growth rate, dressing percentage, edible parts and abdominal fat content of turkeys. The study indicated that lupines in the diet of turkeys had a different effect on the meat quality of different genders. Lupines did not have any negative effect on the meat quality of female turkeys and 30% lupines even improved the protein value index of breast muscles. However, 20–30 and 25–30% lupines in male turkey diets lowered dry matter and protein contents in breast muscles. The results of the study showed that the negative effect on the male breast muscle quality might be avoided using Bio Plus 2B or allzyme SSF additives in the male diets containing 30% lupines.

Keywords: *Anatomic carcass dissection data, chemical indicator, growth rate, lupine, turkey.*

Introduction

Soybean meal is the commonly used source of dietary protein in animal and poultry feed formulations around the world. However, it is indicated that 98% of soybean meal or cake are produced from genetically modified plants (Sieradzki *et al.*, 2006). The diet containing exceptionally high protein content (up to 26–28%) is needed for growing heavy-type turkey poults. So, the necessity arises to search for the alternative protein sources in poultry feeds that could completely or partially replace soya components in poultry feed formulation (Nalle, 2009). An alternative protein source for soybeans might be lupines (*Lupinus* spp.). Lupines possess good agronomic characteristics and they have no antinutritive factors, such as trypsin-inhibitor (Olver, 1987). The protein from lupines has all indispensable amino acids and well-digestible (Sujak *et al.* (2006). However, the worldwide production of grain lupine seeds is low. Predictable, the areas with lupines will be increasing in Lithuania, whereas currently the production of sweet lupines in our country is low. One of the most popular species of lupines in our country is the narrow-leafed lupine. 'Bora' is a narrow-leafed alkaloid-free variety of blue lupines. Several studies can be found on the efficiency of lupines in the diets of different species of animals and birds but there is no unanimous agreement regarding the highest possible amount of blue lupines in the diets of heavy-type turkeys. The studies of Mierlita (2014), Zdunczyk et al. (2014) and Krawczyk et al. (2015) indicated that from 6 to 30 % lupine in the turkey diets had no influence on their body weight if compared with lupine-free diets. Mierlita (2014) concluded that substituting soybean protein meals with white lupine beans in turkey broilers at a rate from 20 to 30% had no adverse effects on the slaughter indices, however, the diet containing over 30% of lupines had a negative impact on carcass features. Zdunczyk *et al.* (2014) indicated that the slaughter value of turkeys was not affected by feeding them 6 to18% sweet yellow lupine meal in comparison with the diet containing soybean meal. The studies of effect of lupines on the chemical indicators of goslings and chicken's meat showed that 5-20% of lupines in the feed had no influence on the meat indicators and on the contents of tryptophan and oxyprolin (Morkunas *et al*., 1995; Morkunas, 2002).

Currently, the demand for organic fowl is increasing both in Europe and Lithuania. However, the available information is insufficient to develop the diets with blue lupines that have no negative influence on the qualitative and quantitative indicators of meat.

The purpose of our study was to determine the efficiency of different amounts of blue narrow lupine seed meal in the diets for hybrid cross BIG-6 turkeys, to analyse the effects of the lupine content on the growth rate of turkeys, anatomic carcass dissection data and chemical indicators of breast and thigh muscles, and to determine the contents of tryptophan and oxyprolin in above muscles.

Material and methods

The study was carried out in turkey farm in the district of Anyksciai, Lithuania. A total three hundred sixty cross BIG-6 turkeys were assigned to control group and five experimental groups of 30 one-day-old turkeys each. The diet of the control group turkeys contained soybean meal, which for the turkeys was replaced by respectively 20, 25 and 30% 'Bora' variety blue narrow-lupine in Trial group 1, Trial group 2 and Trial groups 3, 4 and 5. Besides, the diet of the turkeys in Trial Group 4 was supplemented with 200 g of probiotic mixture Bio Plus 2B per 1000 kg of feed at all age periods. The diet of the turkeys in Trial group 5 was supplemented with 200 g allzyme SSF per 1000 kg of feed. Starting from 13 week, there was no genetically modified soybean meal in the diets of turkeys in Groups 3, 4 and 5. For all the groups, the amounts of lysine, methionine and threonine were, respectively, 16, 10.5 and 10.4 g per kg feed at the age of 0–4 weeks, from 5 to 8 weeks it was 14, 9.0 and 9.0 g, , from 9 to 12 weeks 12.1, 6.5, 7.9 g and from 13 to 16 weeks – 9.0, 5.2 and 6.3 g. During experimental period, water and feed were provided *ad libitum.* All the birds were reared under the same conditions, in accordance with the law for animal welfare and handling, and a substaatutory act by the State Food and Veterinary Service of Lithuanian Republic regarding the confirmation of the order on the animals for experiments, research, storage, maintenance and operating requirements (State News, 2012). Birds of all groups were weighed at 4, 8, 12 and 16 weeks of age. At the age of 16 weeks, three males and three females of average weight were chosen from each group for control slaughtering. Prior to slaughter, the birds had not been fed for 12 hours. Carcasses were anatomically dissected according to the methodological recommendation of anatomic carcass dissection and organoleptic evaluation of poultry. The chemical composition of meat was analyzed by standard AOAC methods (1990). The contents of tryptophan and oxyprolin in meat were determined by the methods of Marina & Shut (1970) and Kolar (1990). All analytical studies were carried out at the Analytical Laboratory of the Animal Science Institute of Lithuanian University of Health Sciences.

Processing of the data was performed using software Statistica (Data Analysis Software System, Version 7.0; StatSoft, Inc., Tulsa, OK, USA). The statistical evaluation of the results was performed using descriptive statistics and Student's t test for independent samples. The probability level of $p < 0.05$ was considered to be statistically significant.

Results and discussion

The variety of lupines used in our study contained 1% bitter lupine seeds, 94.12% dry matter, 5.12% crude fat, crude protein 37.22%, crude fibre 13.32%, crude ash 3.09%. In our study, there was no significant difference between turkeys' weights at the age of 4, 8, 12 and 16 weeks in both control and treated groups. The results of our study are in agreement with those of Mierlita (2014), Zdunczyk *et al.* (2014), Krawczyk *et al.* (2015) who indicated that lupine in the diet of heavy-type turkey**s** had no adverse effect on their body weight. However, our results do not agree with the findings of Mikulski *et al.* (2014) who indicated that the use of blue lupine in the diet had resulted in significantly higher body weight of turkeys. The different amounts of blue lupines in the diets of turkeys had no effects on the dressing percentage (Table 1), except for the significant difference between the control and Group 4 male turkeys fed diets containing 30% lupines and probiotics (+3.95%; $p<0.05$).

Table 1. Effect of different amounts of blue lupine in the diets of turkeys on anatomic carcass dissection data, %

Item		Dressing percentage, mean±SEM	Internal edible parts, mean±SEM	Abdominal fat, mean±SEM
Control group	♂	82.32±0.74	3.39±0.05	0.56±0.24
	♀	85.03±1.04	2.80±0.20	1.53±0.42
Group 1	♂	80.26±2.48	3.73±0.58	0.34±0.08
	♀	82.19±4.21	3.05±0.48	2.80±0.25
Group 2	♂	80.62±0.49	3.77±0.32	0.27±0.07
	♀	84.86±1.42	3.05±0.13	1.62±0.35
Group 3	♂	81.91±1.45	4.46±0.22	0.77±0.22
	♀	84.39±0.64	3.96±0.37	1.37±0.19
Group 4	♂	78.37±0.48*	4.36±0.13	0.62±0.39
	♀	84.93±1.11	3.02±0.22	1.97±0.38
Group 5	♂	81.27±1.07	3.61±0.28	0.29±0.11
	♀	84.63±0.67	2.92±0.34	2.27±0.50

*– $p<0.05$; ±SEM – standard error

Feeding chickens lupines or no-lupines resulted in no significant difference for the dressing percentage in the studies by Nalle (2009), Morkunas *et al.* (1995), Olver (1987), Bekric *et al.* (1990) and Orda *et al.* (2006). Similar findings were observed in the studies by Witak *et al.* (2006) on feeding duckling's 2.5–15% yellow lupine, Mierlita (2014) on feeding male turkeys 20–30% white lupine, Mikulski *et al.* (2014) on feeding male turkeys 6–18% blue lupine and Krawczyk *et al.* (2015) on feeding female turkeys 8–24% yellow lupine. Contrary findings, i. e. higher dressing percentage values when feeding lupines, were reported by Morkunas (2002) in his study with goslings and Mierlita (2014) in the trials with male turkeys.

In our study, there were no differences found for the internal edible parts in treatment turkeys of both genders if compared with the control group ($p>0.05$). This is in disagreement with the data of Orda *et al.* (2006) and Mierlita (2014) who reported a significantly higher amount of internal edible parts in chickens fed 5–20% yellow lupines and turkey poults fed 40% white lupines. There was no correlation determined between the content of abdominal fat and the composition of the feed offered to both trial and control groups of turkeys of both genders. This is in agreement with the findings of Witak *et al.* (2006), Suchy *et al.* (2010) and Orda *et al.* (2006). On the contrary, Mikulski *et al.* (2014) indicated that 18% blue lupine in the diet of male turkeys had resulted in higher content of abdominal fat. Krawczyk *et al.* (2015) have also found that the content of abdominal fat tended to increase when female turkeys were given 8–24% yellow lupine. However, Bekric *et al.* (1990) reported that 23% lupines in the diet of chickens had influenced lower abdominal fat percent. The dry matter content in the male breast muscles (Table 2) trial groups 1, 2, 3 was from 1.95 to 2.31% lower (p<0.05–p<0.025), in Group 4 tended to decrease (–1.58%; p=0.08) in Group 5 was statistically insignificantly lower (–1.30 %; p>0.05) than that of male turkeys in the control group. The protein content in male breast

Table 2. Effect of different amounts of blue lupine in the diets of turkeys on chemical composition of male's breast muscles

Control group	Group 1	Group 2	Group 3	Group 4	Group 5
Dry matter % mean ±SE					
27.50±0.62	25.21±0.53*	25.19±0.17**	25.55±0.16*	25.92±0.26	26.20±0.54
Protein % mean ±SE					
25.14±0.52	22.71±0.91	23.01±0.29**	23.28±0.14*	23.45±0.37	23.50±0.34
Fat % mean ±SE					
1.29±0.20	1.43±0.39	0.82±0.03	1.16±0.15	0.79±0.04	0.90±0.18
Ash, % mean ±SE					
0.99±0.03	1.05±0.01	0.93±0.09	1.04±0.01	1.04±0.01	0.97±0.01
Tryptophan mg 100 g mean ±SE					
369.01±31.76	350.50±20.74	383.57±9.94	352.43±41.73	370.71±15.57	424.25±43.38
Oxyprolin mg 100 g mean ±SE					
54.21±1.59	55.81±4.52	54.00±0.44	53.37±2.14	53.87±1.84	60.11±2.93
Tryptophan:oxyprolin ratio (protein value index)					
6.81	6.28	7.10	6.60	6.88	7.06

*p<0.05; **p<0.025; ±SE – standard error

muscles in trial (Groups 2 and 3 was from 1.86 to 2.13% lower (p<0.05–p<0.025), in Groups 1, 4 and 5 tended to decrease from –1.64 to –2.43% (p=0.06–p=0.08) in comparison with the control group. Our findings for the dry matter content are contrary to those by Morkunas (2002) who indicated that male chickens fed the diet containing 10–20% lupines had from 0.48 to 1.77% higher dry matter content in breast muscles. No other data have been found regarding the dry matter and protein content in the breast muscles of heavy-type turkeys fed lupines. In our study was found that diet supplementation with lupines had not affected the dry matter and protein content data in the female breast muscle, male and female thigh muscles (Table 3, 4, 5).

Table 3. Effect of different amounts of blue lupine in the diets of turkeys on chemical composition of female's breast muscles

Control group	Group 1	Group 2	Group 3	Group 4	Group 5
Dry matter % mean ±SE					
27.34±0.55	26.62±0.49	27.05±0.53	26.89±0.70	25.98±0.42	27.06±0.21
Protein % mean ±SE					
23.51±0.16	22.69±0.60	23.13±0.30	23.16±0.19	22.97±0.46	23.33±0.23
Fat % mean ±SE					
2.60±0.67	2.74±0.77	2.72±0.20	2.59±0.83	1.91±0.17	2.59± 0.15

Ash % mean ±SE					
1.10±0.04	1.12±0.03	1.08±0.02	0.97±0.06	1.03±0.04	1.10± 0.03
Tryptophan mg 100 g mean ±SE					
317.52± 10.09	329.85±13.95	328.72±15.29	309.31±13.07	295.50±11. 94	298.08±3.34
Oxyprolin mg 100 g mean ±SE					
65.55±1.38	52.89±0.13 *****	58.15±4.61	57.10±1.19 ***	53.98±0.49 *****	62.03±3.29
Tryptophan:oxyprolin ratio (protein value index)					
4.84	6.24	5.65	5.42	5.48	4.81

***p<0.01; *****p<0.001; ±SE – standard error

This is in agreement with the results of Olver (1987) in the study with chickens fed 40% white lupine regarding dry matter difference, Suchy *et al.* (2010) regarding protein content difference, Froidmont *et al.* (2004), who found no difference for the above indicators in thigh muscles and Sitko & Čermak (1998) who indicated no difference for the protein content in breast and thigh muscles. Moreover, no difference for the dry matter and protein content in the breast muscles were reported by Krawzcyk *et al.* (2015) in the study with female turkeys fed 8–24% yellow lupine and Mikulski *et al.* (2014) in the study with male turkeys fed 6–18% blue lupine.

Table 4 – Effect of different amounts of blue lupine in the diets of turkeys on chemical composition of male's thigh muscles

Control group	Group 1	Group 2	Group 3	Group 4	Group 5
Dry matter % mean ±SE					
25.36±0.80	25.99±0.18	24.51±0.62	26.16±0.37	26.43±1.28	25.78±1.02
Protein % mean ±SE					
21.08±0.74	20.65±0.08	20.10±0.07	20.44±0.14	20.19±0.58	20.44±0.45
Fat % mean ±SE					
3.17±0.34	4.30±0.27	3.33±0.57	4.66±0.48	4.83±1.15	4.29±0.58
Ash % mean ±SE					
1.07±0.01	1.01±0.04	1.05±0.02	1.02±0.00*	1.03±0.01	1.00±0.01**
Tryptophan mg 100 g mean ±SE					
320.56±12.3 8	298.14±11.5 6	302.73±11.0 1	303.22±6.20	275.55±12.3 0	288.23±21.5 5
Oxyprolin mg 100 g mean ±SE					
93.85±2.16	96.98±4.37	94.00±4.01	107.11±1.05*** *	103.53±3.79	88.28±2.37
Tryptophan:oxyprolin ratio (protein value index)					
3.42	3.07	3.22	2.83	2.66	3.26

*p<0.05; **p<0.025; ****p<0.005; ±SE – standard error

We found that the ash content in the thigh muscles of male turkeys was from 0.05 to 0.07% (p<0.05–p<0.025) and from 0.02 to 0.06% (p>0.05) lower in, respectively, Groups 3 and 5 and Groups 1, 2 and 4 in comparison with the control group. No significant differences for the ash content were found in the breast muscles of male and female turkeys and in the thigh muscles of female turkeys. This is in agreement with the findings of Suchy *et al.* (2010). The ash content in the breast muscles was in agreement with the results of Krawczyk *et al.* (2015) in the trial with turkeys fed from 8 to 24% yellow lupine. The oxyprolin content in the breast muscles of female turkeys was from 3.52 to 12.66 mg% lower if compared with the control group and statistically significant difference was found when compared with Groups 1, 3 and 4 ($p<0.01$–$p<0.001$). The protein value index in female muscles (Groups 1–4) was from 0.58 to 1.40 units higher in comparison with the control group. The oxyprolin content in the thigh muscles of male turkeys (Groups 1–4) was from 0.15 to 13.26 mg% higher in comparison with the control group and the significance was determined when comparing with Group 3 ($p<0.001$) and if compared with Group 4, this indicator tended to increase (+9.68 mg%, $p=0.09$).The protein value index in male thigh muscles was from 0.16 to 0.73 units lower in comparison with the control group. The differences of the results for the oxyprolin content in different male and female muscles are in agreement with the conclusion by Nalle (2009) that the gender of the bird might influence different meat quality indicators.

Table 5 – Effect of different amounts of blue lupine in the diets of turkeys on chemical composition of female's thigh muscles

Control group	Group 1	Group 2	Group 3	Group 4	Group 5
Dry matter % mean ±SE					
30.26±2.73	28.77±0.33	28.64±0.22	28.58±0.86	28.69±0.43	29.13±0.96
Protein % mean ±SE					
22.10±1.42	20.44±0.19	20.31±0.47	20.64±0.15	20.53±0.19	20.40±0.15
Fat % mean ±SE					
6.59±0.96	6.60±0.80	6.12±0.31	6.36±1.00	6.61±0.40	5.26±0.37
Ash % mean ±SE					
0.92±0.14	0.98±0.02	0.91±0.02	0.89±0.04	0.95±0.02	0.91±0.01
Tryptophan mg 100 g mean ±SE					
304.76±39.42	292.37±33.09	274.89±5.48	289.33±8.38	291.31±10.99	301.17±27.28
Oxyprolin mg 100 g mean ±SE					
99.07±1.71	104.82±5.36	102.99±5.71	100.37±6.61	94.65±10.64	111.51±6.50
Tryptophan:oxyprolin ratio (protein value index)					
3.08	2.79	2.67	2.88	3.08	2.70

±SE – standard error

On the contrary, our data on the oxyprolin content partly in female breast and male thigh muscles contradicts the findings of Barroeta (2007) who noticed that the composition of the feed has no influence on the amino acid content in poultry meat and also do not agree the results of Leikus *et al.* (2004), Leikus (2006) and Juodka *et al.* (2016) who indicated that the use of lupine or peas in the feed had no effect on the oxyprolin content in meat.

Conclusion

It can be concluded that soybean oil meal replacement with lupine content from 20 to 30% in the diets of turkeys had no influence on the growth rate, dressing percentage, edible parts and abdominal fat content, except for the significantly lower dressing percentage in male turkeys fed 30% lupine and probiotic supplement if compared with a lupine free diet. The study indicated that lupines in the diet of turkeys had a different effect on the meat quality of different genders. Lupines did not have any negative effect on the meat quality of female turkeys and 30% lupines even improved the protein value index of breast muscles. However, 20–30 and 25–30% lupines in male turkey diets lowered dry matter and protein contents in breast muscles but had no negative influence on the main quality indicators in thigh muscles. The results of the study showed that the negative effect on the male breast muscle quality might be avoided using BioPlus2B or allzyme SSF additives in the male diets containing 30 % lupines.

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THE EFFECT OF AGE OF PARENT YOUNG BROILER ON THE EFFICIENCY OF INCUBATION OF CHICKENS

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Abstract

By establishing reproductive-production characteristics of broiler parent hybrids Cobb 500 the research has been conducted on one parent flock during the period of 38 weeks of production. During the research, special attention was given to breeding eggs incubation and diurnal chicken production. The main goal of this paperwork was establishing the impact of broiler parents' age on chicken effective incubation. During the production cycle in 3 periods (AP_{23}, AP_{31}, AP_{42}) the following was observed: the breeding egg mass on deposition, the breeding egg mass on shuffling, diurnal chicken mass, length of diurnal chickens, and chicken to egg ration. According to the tested periods, broilers' parent in the largest number of cases had the best reproduction-production values during the middle of production cycle (AP_{31}), in relation to the first and third testing period (AP_{23}, AP_{42}). Broiler parents' age influenced the incubation values of the eggs and one-day chicks. The average mass of fresh layed eggs was the biggest in the tested period (AP_{31}) - 69.32 g, while the biggest mass of the eggs on transshipment was in the third tested period (AP_{42}) -60.44 g. The incubational values of one-day chicks during the incubation were increased along with the broilers parents' age which had the consequence that mass and weight of chick were the biggest in 3rd tested period (AP_{42}) where average mass was 42.41 grams, and length 16.80 cm. Chick share had its specific variations according to other characteristics. The biggest average share of the chick in the egg mass was in the 23rd week, or in the first testing period and it was 69.91%.

The differences in the examined traits were considered significant at levels *P<0.05; **P<0.01.

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Keywords: *age, parent flock, Cobb 500, incubation properties.*

Introduction

Production of one-day chickens as a final product includes two production cycles which are interconnected and conditioned by each other (and show some interaction), these are the breeding of the parent flock (the production of eggs for the plantation) and the artificial laying of chickens, that is, the incubation of breeding eggs. Production of one-day broiler chickens consists of several technological phases, "from eggs to chickens": breeding of the breeding flock of a heavy type of chicken, quality features eggs for plantations, the influence of genetic and paragenetic factors on their quality, the collection of eggs on the farm, selection and transport of breeding eggs from the farm to the incubator station, reception, storage (guarding) and preparation of eggs for incubation, investment of eggs in the incubator and general rules for artificial chick laying, egg process in the dressing room (up to 18 days of incubation), transferring eggs from the hens to the breeder, the process with eggs in the derivative (from day 18 to day 21) and laying (performing) chickens, extracting, grading, packing and dispatching one-day broiler chicks Stanišić (2012). The main goal of production is to find

optimal solutions in order to produce as high quality breeding eggs as each parent's flock, and therefore the maximum number of quality vital day-old chicks.
That the age of broiler parents affects the mass of eggs, that is, one-day chickens, the percentage of chickens in the weight of eggs, as well as the further development of broiler chickens, have been determined Shanawany (1987), Wilson (1991), Vieira and Moran (1998), Dalanezi *et al.* (2004), Vieira *et al.* (2005). The authors, among other things, conclude that with the age of broiler parents during the production cycle, as a rule, every week the mass of eggs increases.
Pinchasov (1991) states that the age of the laying hens has a lower effect on the weight of the chickens, that is, that the egg mass significantly has a greater effect on the weight of one-day chickens. The weight of the eggs affects the weight of the chickens and the percentage of chickens in the egg mass, according to Miclea and Zahan (2006), at the parent flock of the Ross SL 2000 hybrid grown from 35 to 49 weeks of age. Of the light eggs (average weight 54.59 g), chickens weighed 38.11 g, medium sized (58,89 g), chickens 40.74 g of heavy eggs (63.10 g) were chickens of average weight 43.18 g. Loss of embryonic mortality was higher in light (7.83%) and in heavy eggs (7.90%), compared to eggs of medium weight (6,67%).
Similar findings were obtained Traldi *et al.* (2011) and Wilson (1991), with the relative share of the chick mass in the egg mass ranged between 67% and 70%, or between 62% and 76%. Although Traldi *et al.* (2011) obtained in two experiments a similarly similar percentage of chickens in the mass of eggs in broiler hybrids Ross aged 29 and 55 weeks.
Abudabos (2010) found that Ross 308 and Cobb 500 hybrid parents found that with both the age of the flock both hybrids increase the mass of eggs and, therefore, the weight of one-day chickens. In the Cobb hybrid parent number of 26 weeks old, the weight of the eggs was 58,2 g, chickens 41.3g (percentage of chickens in the weight of eggs -71.00%), and in the age of 44 weeks eggs 75.8 g of chickens 51.6 g (71.50%), in the parents of hybrid Ross aged 32 weeks the average weight of eggs was 67.4 g, chickens 49.3 g (73.00%), and at the age of 36 weeks, the weight of eggs was 66,4 g and the weight of chickens was 48.3 g (73.10%).
Enting *et al.* (2007) examined the influence of the age of broiler parent hybrids Cobb 500 and the composition concentrates the concentrate on the incubation characteristics of the eggs for the plantations or broiler chickens. In the control group (fed normal-standard mixture) of broiler parents, the average weight of eggs at the age of 29 weeks was 55.4 g (day-old chickens – 35.9 g), at the age of 41 weeks, 67.0 g (41.8 g) and at the end of the production cycle (60th week of age), the weight of eggs on average was 71.4 g, and day-old chickens were 45.1 g. The authors conclude that with the age of broiler parents the weight of eggs and the weight of one-day chickens is increasing, while the percentage of chickens in eggs was the highest at the age of flocks of 29 weeks, 64.80%, then 60 weeks (63.16%), and the smallest in the 41st week (62.39%).
The main objective of this work and day-old chickens and to determine the impact of the age of the parent flock on the mentioned group of incubation properties through the three phases of the production cycle of breeding parent brooders of the Cobb 500 hybrid, or different ages of broiler parents (AP_{23}, AP_{31}, AP_{42}).

Material and Methods

The experimental part of the research was carried out on the poultry farm, i.e. the incubator station of the meat industry "Akova Group", Visoko, Bosnia and Herzegovina.
The initial experimental material was served by a flock of broiler parents of the Cobb 500 hybrid, grown on the parent farm from the 20th to the 60th week of age.
The monitoring of reproductive and production characteristics of broiler parents was carried out at the parent farm, and the determination of the efficiency of chick incubation in an incubator station within the said poultry farm.

An old 20-week-old parent's cattery from a nursery-growing facility was relocated to a breeding broiler facility. 28 128 pieces of laying hens and 3 380 pieces of roosters were brought into the breeding facility of broiler parents. 27 492 and laying hens 3 276 chicks were transferred to the production. Parental breeding began with the production of eggs at the end of the 22nd week of age, but eggs were used for incubation at the beginning of the 23rd week of age until the 60th week of age. During the cultivation (production of eggs) of the examined parent flock, the technology proposed by the hybrid selector Cobb 500 (www.cobb-vantress.com) was used. Nutrition, power, ventilation and lighting are automatically regulated.
In order to determine the influence of age of numerical parents on the efficiency of chick incubation during the production cycle, eggs were incubated three times, selected by the random sample method. In each week (AP_{23}, AP_{31}, AP_{42}), by random sample method, 200 eggs were taken, i.e. a total of 600 eggs. All eggs are stored in a storage room that is air conditioned with the possibility of humidifying the air with an air humidifier in the form of a water mist.
In each examined egg period, the eggs were individually measured before being incubated (incubator) and on the 18th day of incubation (before transferring to the derivative), while the chickens were measured directly at the bed (21st day of incubation). During the incubation, egg-shaping processes were performed to have an approximate insight into the state of fertilization of eggs.

Reproductive - production characteristics for which individual data are made are:

1. Weight of breeding eggs on investment, (g)
2. Weight of breeding eggs on postponement (Day 18), (g)
3. Mass of day-old chickens, (g)
4. Length of day-old chickens, (cm)
5. Relative share of chickens in egg mass (chick mass / egg mass) x100

Basic data processing was done in SPSS v. 22 package software. For the indicators that are processed individually, the following are calculated: mean ($\bar{x}$), standard error of the mean (S $\bar{x}$), standard deviation (S), and variation coefficient (C.V.)
Testing the significance of the difference between the incubation values tested was performed with a formula with the same number of repetitions Hadživuković (1973).

Results and Discussion

According to the plan and program of this study in the incubator station, the reproductive properties, that is, the incubation values of breeding eggs during the breeding of broiler rodents of hybrid Cobb 500 were investigated.
Table 1 gives the average values and variables for the investment egg mass, the weight of eggs on the shift of day 18, the weight and length of day-old chickens, and the share of chickens in the egg mass for the three examined periods.

Table 1. Average values and variability for the properties of eggs and day-old chicks for all three load periods, depending on the age of numerical parents.

Age of parents (AP)	Properties	$(\bar{x})$	S	$S(\bar{x})$	C.V. (%)
AP_{23}	The weight of eggs on investment	55.30	2.06	0.14	3.72
	The weight of eggs on postponement	49.47	2.37	0.17	4.79
	Mass of day-old chicks	38.46	2.46	0.17	6.39
	Length of day-old chicks	16.14	0.64	0.04	3.96
	Chicken share in egg mass	69.91	5.60	0.39	8.01
AP_{31}	The weight of eggs on investment	69.32	4.37	0.31	6.30
	The weight of eggs on postponement	58.52	4.11	0.29	7.02
	Mass of day-old chicks	42.05	2.56	0.18	6.08
	Length of day-old chicks	16.32	0.67	0.05	4.10
	Chicken share in egg mass	60.89	5.42	0.38	8.90
AP_{42}	The weight of eggs on investment	68.33	3.51	0.25	5.13
	The weight of eggs on postponement	60.44	3.10	0.22	5.12
	Mass of day-old chicks	42.41	2.87	0.20	6.76
	Length of day-old chicks	16.80	0.54	0.04	3.22
	Chicken share in egg mass	61.10	6.03	0.43	9.86

The average weight of all eggs originating from the young flock (AP_{23}) was 55.30 g, then increased with age (AP_{31}) to 69.32 g, and then fell again to 68.33 g at 42 weeks of age. Unlike the average weight of eggs on the investment, the egg-laying eggs (day 18) with the age of the parents increased throughout all three examined periods. Analogously to the mass of eggs, the weight of one-day chickens increased with the age of broiler parents. For AP_{23}, the average weight of day-old chickens was 38.46 g, for AP_{31} 42.05 g and for AP_{42} 42.41 g, which is in line with the studies carried out by Yildrim (2005), Christensen *et al.* (2002). The highest relative share of chickens in eggs was in the first study period (AP_{23} – 69.91 %), slightly lower in the third study period (AP_{42} – 61.10 %), while the smallest was in the middle of the production cycle (AP_{31} 60.89 %). To similar results, and the same contations, relative to the relative share of chickens in the egg mass, came Enting *et al.* (2007). In addition, the data in Table 1 shows that deviations from the average values of individual egg and chicken characteristics were small, i.e. the coefficient of variation ranged from 3.22% (length of day-old chickens – AP_{42}) to 9.86% (share of chickens in egg mass – AP_{42}).

Table 2 shows the significance of the calculated differences between the average values for the investment egg weight, the weight of the eggs on the shift of day 18, the weight and length of day-old chickens, and the share of chickens in the egg mass for the three examined periods.

Table 2. Significance of differences in the reproductive characteristics of eggs and day-old chicks, depending on the age of the parent (AP).

Properties	Age of parents. weeks (AP)	$(\bar{x})$	d	Significance
The weight of eggs on the investment (g)	$AP_{23} – AP_{31}$ $AP_{23} – AP_{42}$ $AP_{31} – AP_{42}$	55.30 – 69.32 55.30 – 68.33 68.33 – 69.32	-14.02 -13.03 0.99	** ** ns
The weight of eggs on postponement (g)	$AP_{23} – AP_{31}$ $AP_{23} – AP_{42}$ $AP_{31} – AP_{42}$	49.47 – 58.52 49.47 – 60.44 58.52 – 60.44	-9.05 -10.97 -1.92	** ** *
Mass of day-old chicks (g)	$AP_{23} – AP_{31}$ $AP_{23} – AP_{42}$ $AP_{31} – AP_{42}$	38.46 – 42.05 38.46 – 42.41 42.05 – 42.41	-3.59 -3.95 -0.36	** ** ns
Length of day-old chicks (cm)	$AP_{23} – AP_{31}$ $AP_{23} – AP_{42}$ $AP_{31} – AP_{42}$	16.14 – 16.32 16.14 – 16.80 16.32 – 16.80	-0.18 -0.59 -0.41	ns ns ns
Chicken share in egg mass (%)	$AP_{23} – AP_{31}$ $AP_{23} – AP_{42}$ $AP_{31} – AP_{42}$	69.91 – 60.89 69.91 – 61.10 60.89 – 61.10	9.02 8.81 -0.21	** ** ns

**P<0,01; *P<0,05; ^{ns}P>0,05

From Table 2 we can see that for the mass of eggs, significant meanings of average values were reported between AP_{23} and AP_{42}, and there were no statistical significances between AP_{31} and AP_{42}. The weight of eggs on the transfer of statistical significance was in the ratio between all three examined periods. When it comes to the mass of day-old chicks and the share of chickens in the egg mass, the same results were achieved as for the egg-weight of the investment, while for the length of one-day chickens statistical significance did not occur between any examined period. Similar results have been obtained by Enting *et al.* (2007), Abudabos (2010).

Conclusions

Based on the results of the study of the influence of the age of numerical parents (AP) on the incubation values of day-old chicks conducted on the Cobb 500 heavy line hybrid flock, the following conclusions can be drawn:

- Indicators of descriptive statistics for the characteristics of eggs and day-old chicks for all three load periods showed that the average weight of breeding eggs on investment was the highest in the second study period (AP_{31}) of 69.32 g while the smallest was in the first study period (AP_{23}) 55.30 g.
- The average weight of breeding eggs on postponement was the highest in the third study period (AP_{42}) of 60.44 g, and the smallest in the 23 weeks age (49.47 g).
- The highest average weight of day-old chicks was 42.41 g (AP_{42}), and the smallest 38.46 g (AP_{23}).

- The length of day-old chicks ranged from 16.14 cm in the 23rd to 16.80 cm at the 42nd week of the parents.
- The average share of chickens in eggs ranged opposite to other characteristics, so it was the highest in the 23rd week (69.91%) while the smallest was in the 31st week old age.
- The average value of egg masses on investment of significance was reported between AP_{23} and AP_{31}, as well as between AP_{23} and AP_{42} at $P<0.01$, while there were no statistical significances between AP_{31} and AP_{42} ($P> 0.05$).
- In the case of weight of eggs on the spread of statistical significance, they all occurred between all three examined periods at the level of $P<0.01$ and $P<0.05$.
- With the average values of the weight of one-day chicks and the share of chickens in the weight of eggs, the significance ranged at the same levels as the average values of egg weight on the investment.
- For the value of the length of day-old chickens of statistical significance, there was not a single study period ($P>0.05$).

From all of the above, it can be said that the age of the brooding parents of the Cobb 500 hybrid on farm conditions influenced in a certain way all the traits, except for the length of one-day chickens.

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CHARACTERISTICS OF LOW FAT YOGURT

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Abstract

Yogurt is very popular fermented dairy product, already considered to be healthy food. Due to low calorie value, low fat yogurts have gained popularity in recent years. Quality, rheological and sensory characteristics of yogurt depend on many factors including milk type and its fat content, the microflora used and the applied technology. In Serbia, low fat yogurt is consumed as stirred and Greek style yogurt. Many consumers experienced these products as sour, insufficiently viscous or grainy. Therefore this work aimed to determine composition, sensory quality and some rheological characteristics of low fat yogurts with milk fat (MF) in the range 0-2 % that could be found on Serbian market. As expected, due to specific production process, the highest levels of total solids (13.54% and 15.69%) and proteins (8.01% and 7.90%) were detected in Greek-style yogurts with 0% and 2% MF. Significantly higher ($p<0.05$) values of viscosity were recorded in Greek style yoghurts. Among stirred yogurt samples, the highest viscosity was determined in yogurts with 0.9% MF. The highest syneresis (44.15%) and the lowest viscosity were determined in samples with 1% MF. Greek-style yogurts with 0% and 2% MF had the lowest sensory ratings due to grainy consistency (as a result of high protein content) and higher acidity levels (1.38% and 1.32% l.a., respectively). According to all parameters of sensory quality, the best rated was yogurt with 0.9% MF.

Keywords: *Low fat yogurt, Viscosity, Synresis, Sensory characteristics.*

Introduction

Recent health trends favor the consumption of nonfat and low fat foods, especially dairy products. In yogurt, the reduction of fat has negative effects on yogurt characteristics such as lack flavor, week structure and syneresis (Kahkonen and Tuorila, 1999; Lucey and Singh, 1997), which led to lowering the consumer's acceptability of these products. During the past two decades, many attempts have been made to produce non fat and low fat yogurts which are similar in quality to full fat yogurt. Commonly used approaches for improving quality of low fat yoghurt are changing the formulation of the yoghurts (addition of fat replacers, thickening agents, milk fortifiers, etc.), using exopolysaccharide producing starter cultures, introducing modifications in the manufacturing process, addition of flavours or a combination of these methods (Prasanna et al., 2013; Torres et al., 2011; Trejo et al., 2014). Since all of the above increases to the cost of the production, many dairies produce stirred non fat and low fat yogurt from standardized milk by same technology used for full fat yogurt. On the other hand, Greek yogurt is characterized with high content of total solids. On the Serbian market it could be found as low fat Greek yogurt and as yogurt with high fat content in type of Greek yogurt. Methods for achieving required total solids content are different according to the type of Greek yogurt, and equipment and technology of the producers. All this indicates different quality of low fat yogurts consumed in Serbia. Thus the aim of this work was to investigate physico-chemical, some rheological and sensory characteristics of low fat yogurt available on Serbian market.

Material and method

Twelve samples of non fat and low fat yogurts of different companies that could be found on Serbian market were collected. Samples were transported in refrigerated vans and stored in refrigerators at 4^0 C, once at our laboratory. Basic analysis of physico-chemical composition of yogurt samples were done with the following methods: total solids (Carić et al., 2000), proteins (IDF 20B:1993), fat in yogurt (Carić et al., 2000), titrable acidity (IDF 105:1991), pH value with pH meter (Consort C931). Each analysis was done in triplicate. Syneresis was determined according to the Ramirez-Santiago et al. (2010): 40 g of yogurt is weighed in a cuvette and centrifuged at 220 g for 10 min at 4^0 C. After centrifugation, the separated whey was measured and shown as percent of 100 g of the sample. For these tests, Laboratory Centrifuges (Ependorf, USA) was used. These analyses were done in duplicate.

Measurements of viscosity were done with Visco Basic+R Viscometer (Fungilab, Spain) at constant speed of spindle rotation. At the beginning, during the first 30 seconds, the speed is 100 rpm and then adjusted to 20 rpm. Readings were performed every 30 seconds (a total of 6 readings). For each yogurt test was performed twice and the average viscosity was calculated.

The overall sensory quality was determined by point rating scale method (Radovanović and Popov-Raljić, 2001) by trained judges.

The effect of milk fat content on yogurt characteristics was analyzed using Statistica 6.0 software (Stat Soft. Inc., Tulsa, USA), using analysis of variance (ANOVA). Mean comparisons of the parameters were performed by t-test, with the level of significance at 0.05.

Results and discussion

Yogurt samples used in this study were produced from cow milk with different milk fat (MF) content in different geographical regions. Besides MF content, stirred and Greek-style yogurts are produced by different technological processes. Furthermore, each dairy plant has specific production process parameters regarding heat treatment, starter culture, fermentation, cooling and storage of yogurt. Therefore, differences in composition, sensory quality and some rheological characteristics of low fat yogurts are expected.

Physico-chemical characteristics of commercial yogurt samples with different milk fat content are shown in Table 1.

Table 1. Physico-chemical characteristics of Greek and stirred yogurts

Parameter Declared fat content (%)	TS (%)	MF (%)	TSNF (%)	Proteins (%)	Acidity (% l.a.)	pH
			Greek			
0	$13{,}54\pm0{,}05^{b}$	$0{,}00\pm0{,}00^{b}$	$13{,}54\pm0{,}05^{b}$	$8{,}01\pm0{,}11^{a}$	$1{,}38\pm0{,}01^{a}$	$4{,}41\pm0{,}00^{b}$
2	$15{,}69\pm0{,}12^{a}$	$1{,}87\pm0{,}16^{a}$	$13{,}82\pm0{,}04^{a}$	$7{,}90\pm0{,}27^{a}$	$1{,}32\pm0{,}01^{b}$	$4{,}51\pm0{,}00^{a}$
			Stirred			
0,5	$8{,}74\pm0{,}34^{d}$	$0{,}50\pm0{,}06^{f}$	$8{,}25\pm0{,}28^{cd}$	$3{,}45\pm0{,}19^{a}$	$0{,}93\pm0{,}01^{a}$	$4{,}34\pm0{,}05^{bc}$
0,9	$8{,}69\pm0{,}03^{d}$	$0{,}77\pm0{,}16^{e}$	$7{,}92\pm0{,}18^{d}$	$2{,}75\pm0{,}08^{b}$	$0{,}87\pm0{,}01^{c}$	$4{,}31\pm0{,}00^{c}$
1	$9{,}51\pm0{,}08^{c}$	$0{,}99\pm0{,}09^{d}$	$8{,}52\pm0{,}12^{bc}$	$3{,}24\pm0{,}20^{a}$	$0{,}88\pm0{,}01^{c}$	$4{,}39\pm0{,}03^{b}$
1,5	$10{,}27\pm0{,}07^{b}$	$1{,}54\pm0{,}00^{bc}$	$8{,}73\pm0{,}07^{ab}$	$3{,}38\pm0{,}09^{a}$	$0{,}88\pm0{,}01^{c}$	$4{,}37\pm0{,}03^{bc}$
1,6	$9{,}89\pm0{,}50^{bc}$	$1{,}65\pm0{,}09^{b}$	$8{,}24\pm0{,}53^{cd}$	$3{,}44\pm0{,}07^{a}$	$0{,}89\pm0{,}03^{bc}$	$4{,}38\pm0{,}05^{bc}$
2	$10{,}99\pm0{,}06^{a}$	$1{,}87\pm0{,}16^{a}$	$9{,}12\pm0{,}10^{a}$	$2{,}93\pm0{,}00^{b}$	$0{,}91\pm0{,}00^{ab}$	$4{,}48\pm0{,}00^{a}$

* TS – total solids, MF – milk fat, TSNF – total solids non fat

Different superscripts within the same column indicate that the means differ significantly ($p<0{,}05$).

Greek yogurts are characterized by high total solids and protein content which can be achieved by draining, centrifugal separation, ultrafiltration or addition of milk-protein based products. Therefore, as expected, relatively high TS contents were observed. Furthermore, lack of fat in fat-free Greek yogurt influenced significantly lower total solids content comparing to Greek yogurt with 2% MF ($p<0,05$). Although both yogurts had similar protein content, TSNF were higher in 2% Greek yogurt, probably due to higher lactose content. Acidity is a consequence of lactic acidification obtained at the end of incubation and post-acidification during storage. Stabilizers added in Greek yogurt production also have great influence on yogurt acidity. Differences in acidity of examined Greek yogurts could be a consequence of the type of used stabilizer, since yogurts with pectin or guar gum used as stabilizers show higher acidities than those containing xanthan or κ-carrageenan (Soukoulis et al., 2007). Comparing to stirred, Greek yogurts had much higher acidity (1,32 and 1,38% l.a.), which is also higher than the acidity of low fat Greek yogurts investigated by Serafeimidou et al. (2012).

Among stirred yogurts significantly lower protein contents were observed in yogurts with 0,9 and 2% MF ($p<0,05$). Higher protein levels indicate higher buffer capacity, so lower pH in yogurts with 0,9% MF (2,75% proteins) was expected. However, the highest pH was in samples with 2% MF and only 2,93% proteins. Since all yogurts were obtained from the market, possible explanation would be that these samples had shorter storage period.

Syneresis is separation of the liquid phase from the gel, and depends many factors such as: heat treatment of milk, total solids content, stabilizers used, rate and temperature of acidification, type of starter culture, acidity that occures as a result of the action of lactic acid bacteria, etc. Expulsion of whey (syneresis) of low fat yogurts is presented in Figure 1.

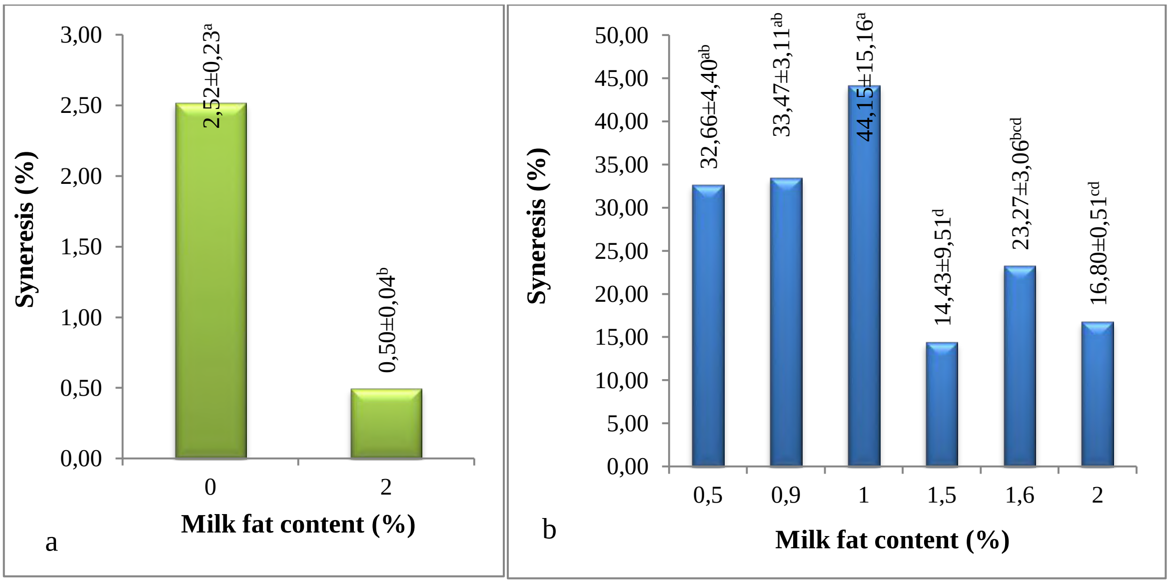

Figure 1. Syneresis of low fat yogurts (a – Greek yogurts, b – stirred yogurts)

*Different superscripts indicate that the means differ significantly ($p<0,05$).

Reduction of syneresis in yogurt could be achieved by the addition of casein-based ingredients (Guzmán-González et al., 2000) or whey protein-based products (Vučić et al., 2011). Greek yogurts had significantly higher protein content comparing to stirred yogurts, which could partially be due to addition of proteins, but they did not differ in protein content among each other. Therefore, approximately five times higher syneresis in non fat yogurt could be attributed to lower pH values. According to Harwalkar and Kalab (1986) lower pH values increase positive charge inside casein micelles and consequently intermolecular repulsion. As a result, pores in protein matrix are larger and therefore more intense whey separation occurs. Lowest expulsion of whey among stirred yogurts was observed in samples with 1,5% MF, while highest syneresis showed yogurts with 1% MF. However, those yogurts did not differ significantly regarding protein content and pH. It could be assumed that starter cultures that produce exopolysaccharides (EPS+ strains) were used in the production of yogurt with 1,5% MF, since EPS are characterized by a great water holding capacity which results in a reduced syneresis of yogurt.

Viscosity of yogurt is highly affected by milk composition, addition of proteins, heat treatment and type of starter culture. Hence, differences regarding viscosity between yogurts used in this investigation were expected. As previously mentioned Greek yogurts had similar protein content. But as a result of a higher fat content significantly higher viscosity ($p<0,05$) is observed in yogurt with 2% MF (Figure 2.a) Higher milk fat content leads to an increase in the viscosity of yoghurt, which has been confirmed by De Lorenzi et al. (1995).

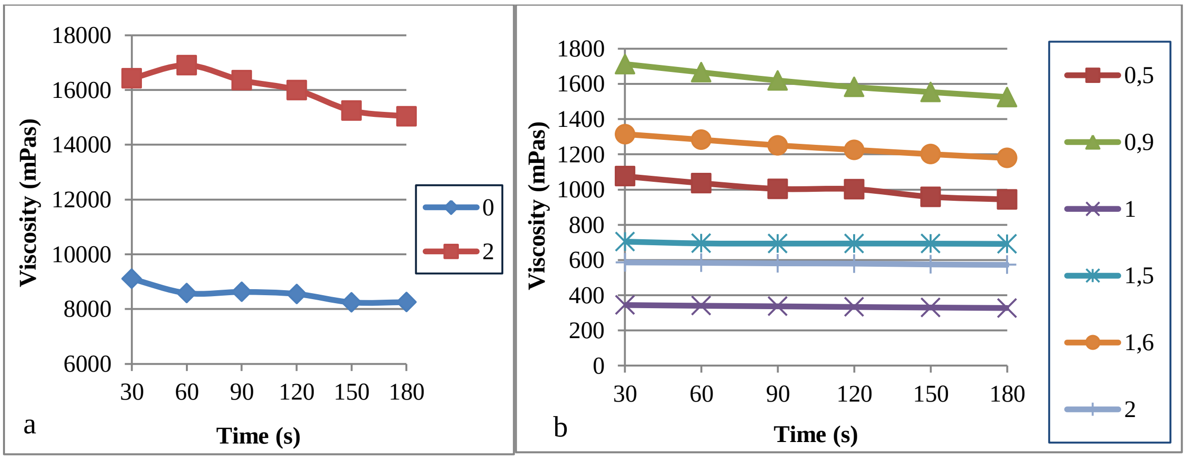

Figure 2. Viscosity of low fat yogurts (a – Greek; b – stirred)
* Numbers in Legend indicate declared fat content

Under the influence of the shear force, the viscosity decreases over time, and significantly lower viscosity is observed at the end of the measurement in yogurt with 2% MF. On the other hand, no significant change in viscosity was detected in yogurt with 0% MF which indicates the use of stabilizers in its production.

In stirred yogurt, breaking of gel structure under the influence of the shear force is less pronounced due to post-incubation operations (stirring, cooling and packaging) in which the structure of the gel has already been broken (Afonso and Maia, 1999). That is probably why viscosity hasn't significantly changed during measurement in all stirred yogurts, except in yogurt with 2% MF. Unexpectedly, the highest viscosity was observed in yogurt with 0,9% MF (Figure 2.b), although it had the lowest protein and TSNF contents. However, this yogurt also had the lowest pH, so it could be assumed that it had longer storage period. During storage, the protein matrix structure is rearranged, and the number of protein bonds within matrix increases leading to higher viscosity. Viscosity of yogurt with 0,9% MF was significantly higher comparing to yogurts with 1%, 1,5% and 2% MF ($p<0,05$). The lowest viscosity had yogurt with 1% MF which along with highest syneresis indicates week structure of protein matrix.

Acceptance of the product by consumers is mainly based on its sensory characteristics. The average score for each sensory attribute and the overall preference of the samples are shown in Figure 3. (a, b). Greek yogurts had lower sensory quality than stirred yogurts. Due to grainy consistency, acid and poor taste Greek yogurts had moderately low pondered mean values

(4,07 and 3,73, respectively). No significant differences were found in sensory quality of Greek yogurts regarding all parameters.

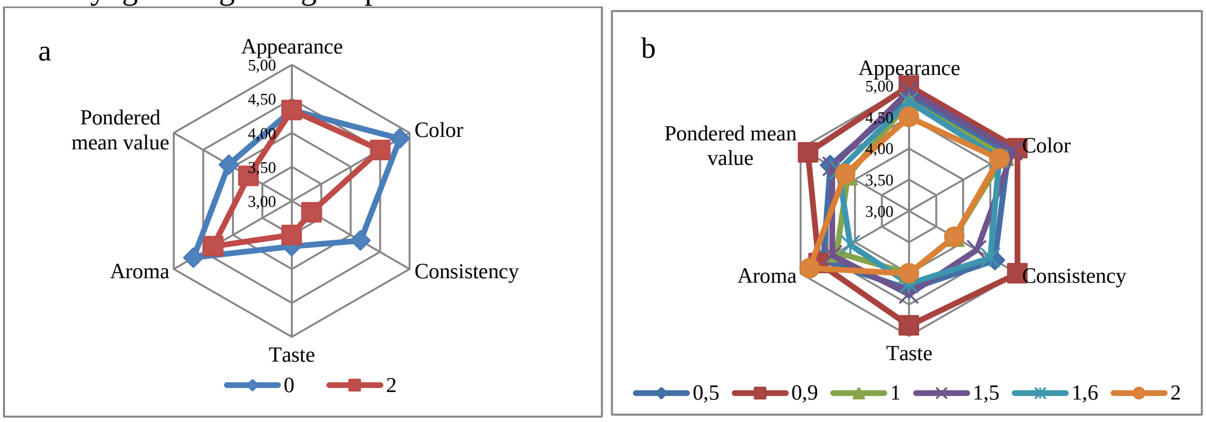

Figure 3. Sensory evaluation of low fat yogurt (a – Greek; b – stirred)
* Numbers in Legend indicate declared fat content

The sensory profile of all investigated stirred yogurts did not show any significant differences in the appearance, color, consistency, taste and aroma attributes. Scores obtained for the consistency were consistent with the results of viscosity measurement. Therefore yogurt with 0,9% MF had the highest consistency score. Also, this yogurt was evaluated as the best among investigated yogurts regarding all other sensory attributes except aroma. Consequently, the highest pondered mean value (4,86) was established in yogurt with 0,9% MF.

Conclusion

One way of utilization of skim milk in the production of fermented beverages is the production of low fat and non fat yogurt. Reduced energy value, slightly worse sensory characteristics, especially taste and consistency of these products require changes in the technological production process.

Investigations showed that two varieties of low fat yogurts (Greek and stirred) are present on Serbian market. Better rheological characteristics (significantly higher viscosity and very low syneresis – 0,5%) had Greek yogurt with 2% MF. Stirred yogurt with 0,9% MF had the highest sensory scores and therefore it was evaluated as the most acceptable.

Acknowledgement

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RELATIONSHIPS BETWEEN SOMATIC CELL COUNT AND SELECTED QUALITATIVE PARAMETERS OF SHEEP MILK

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Abstract

The aim of the study was to evaluate relationships between somatic cell count (SCC) and selected qualitative parameters of sheep milk (contents of fat (F), total protein (TP), casein (Cas) and lactose (L), pH, titratable acidity (TA) and clotting time (CT)). Determination of daily milk yield (DMY) and effect of the stage of lactation (SL) on selected parameters were also an integral part of the study. The study was carried out under operating conditions at a specialized sheep organic farm. Eighteen ewes of Lacaune breed were involved in the experiment. The SCCs insignificantly grew as lactation advanced, nevertheless, both mean counts (from 88 to 130 x 10^3/mL) and their counts in individual samples (from 36 to 420 x 10^3/mL) were relatively low during lactation. The SL also had no significant effect on pH and TA. On the other hand, the SL had a significant effect on all other monitored parameters. The SCC had a positive significant correlation with contents of F, TP and Cas. In contrast, between SCC and content of lactose a negative significant correlation was found. Many studies show that the content of lactose can be reduced with mastitis and SCC increase. However, in our opinion a negative significant correlation between SCC and lactose content was not affected by intramammary infection, because in all cases the SCCs were lower than the thresholds for subclinical mastitis in sheep. Our opinion also largely supports the fact, that all correlations between SCC and pH, TA, CT and DMY were insignificant.

Keywords: *Sheep milk, Somatic cell count, Milk composition, Milk acidity, Clotting time,*

Introduction

The somatic cell count in sheep's milk is generally higher than in cow's milk and lower than in goat's milk and their count significantly affects the quality of milk and products made from it. Contemporaneously it must be emphasized that their count also affects the price of milk.

Most studies focused on somatic cell count show that the most significant non-infectious factors that influence increasing of somatic cell counts (SCCs) in sheep milk are parity, stage of lactation, time of year, herd and handling of ewes. According to Bergonier et al. (2003) non-pathological factors are responsible for variation of SCC in this milk between 40 x 10^3 and 100 x 10^3 cells/mL. Nevertheless, SCC is also a good indicator of the existence of subclinical mastitis, whereby subclinical infection may result in significant losses of milk yield and changes in milk composition (Gonzalo et al., 2002; Leitner et al., 2004). In the United States the limit for sheep milk is below 1 000 000 SCC/mL (Paape et al., 2007), whereas there is no legal limit for this milk in European Union. Nevertheless, Bianchi et al. (2004) reported the limit for subclinical mastitis in sheep at level of 500 000 SCC/mL and Pirisi et al. (2007) stated that in Sardinia is used an incentive payment for sheep milk with lower SCC than 1 000 000/mL.

Many studies also show that the composition of sheep milk can be affected by a lot of factors such as nutrition, season, stage of lactation, and year while its composition also fundamentally affects milk coagulation properties (MCP) and the curd quality (CQ). Other important factors

that influence MCP and CQ are pH and titratable acidity of milk (Pellegrini et al., 1997; Jaramillo et al., 2008).
The main aim of our study was to evaluate relationships between somatic cell count (SCC) and selected qualitative parameters of sheep milk. Determination of daily milk yield (DMY) and effect of the stage of lactation (SL) on selected parameters were also an integral part of the study.

Materials and Methods

The study was carried out under operating conditions at a specialized organic sheep farm located in the Zlín region of the Czech Republic. Eighteen ewes of Lacaune (LC) breed on the second lactation were involved in the study.
The daily feed ration of ewes during the study consisted of pasture (*ad libitum*), organic oat (0.2 kg/ewe) and organic mineral lick (*ad libitum*). Throughout the whole experiment ewes were continuously kept on the pasture. During the study, all ewes were kept in one flock under identical conditions without any discernible differences in their nutrition or management.
Individual milk records and samplings were carried out after weaning of lambs, from May to September, on the mean 57, 92, 127, 162, and 197 day of lactation. Milk records were carried out from morning milking (6 a.m.) and evening milking (6 p.m.). Milk samplings were collected only from morning milking. Individual milk samples were cooled to 5–8°C and transported to the specialized milk laboratory at the Mendel University in Brno and to the accredited private Laboratory for Milk Analysis in Brno – Tuřany.
SCC was determined using fluoroopto-electronic apparatus BENTLEY 2500 (Czech State Standard EN ISO No. 13366-2). Fat (F) content was determined by Gerber's acidobutyrometric method (Czech Technical Standard ISO No. 2446). Total protein (TP) and casein (Cas) contents were determined using a PRO-MILK apparatus (Foss Electric, Denmark). Lactose (L) content was determined polarimetrically (Czech Technical Standard No. 570530). Active acidity (pH) was measured with the pH-meter WTW 95 with the probe WTW SenTix 97. Titratable acidity (TA) was determined by titration using the Soxhlet–Henkel method (Czech State Standard No. 570530). Clotting time was measured according to Berridge (1952).
Statistical analyses were performed using the STATISTICA software, version 12. ANOVA analysis was used to study the differences in all monitored parameters during lactation. To identify individual significant contrasts within the lactation Sheffe's test was used. To asses a level of the correlation coefficient among all particular variables Pearson's correlation was carried out. The differences were considered significant if $P<0.05$. Statistical analysis showed that SCC were normally distributed, therefore the actual SCC were not transformed to logarithm forms for further statistical analyses.

Results and Discussion

The stage of lactation (SL) had a significant effect on contents of all basic milk components and DMY (Table 1). These findings are consistent with data published by Matutinovic et al. (2011). In sheep it is well-known that as lactation progresses the contents of F, TP and Cas in milk increase and the DMY gradually decreases, when all these trends were also found in our study. The correlations between DMY and contents of F and TP were significantly negative in all cases (Table 2), which is in accordance with Ochoa-Cordero et al. (2002) and Mioc et al. (2009). In contrast to F and TP contents, the content of lactose (L) significantly decreased during lactation which is in line with Pugliese et al. (2000) and Matutinovic et al. (2011). During lactation the average L content ranged from 4.99 to 4.58% and these values are comparable with Kuchtik et al. (2008) and Kondyli et al. (2012). With regard to correlations

between L and F and L and TP, both of these correlations were significantly negative what is in line with Mioc et al. (2009).
The SL had not significant effect on pH, what is in line with Barron et al. (2001). All correlations between pH and the other parameters were also insignificant. Even though the values of titratable acidity (TA) increased during lactation from 9.0 to 10.0 ^{0}SH, the SL also had no significant effect on this parameter. However, the TA of sheep milk can be significantly influenced by protein content (Novotna et al., 2009), while the higher the TP content, the higher the TA and vice versa. The same trend was also found in our study. It seems interesting that the correlation between pH and TA in our study was insignificant, because Kuchtík et al. (2008) found out that this correlation is significantly negative.

Table 1.: Means, ranges of mean values and individual samples of SCC, BMC, pH, TA, CT and DMY throughout lactation and effect of the SL.

Trait	Mean	Range of mean values	Range of individual samples	SEM	Effect of SL
SCC (10^3/mL)	112.10	87.72 – 130.22	36.00 – 420.00	7.223	NS
Fat (%)	7.33	6.25 – 7.69	4.56 – 9.82	0.118	**
TP (%)	5.7	5.48 – 6.26	4.71 – 7.43	0.051	**
Casein (%)	4.26	4.02 – 4.76	3.21 – 5.68	0.049	**
Lactose (%)	4.76	4.58 – 4.99	4.16 – 5.28	0.027	**
pH	6.4	6.4 – 6.5	6.1 – 6.8	0.01	NS
TA (ºSH)	9.55	9.0 – 10.0	6.56 – 11.8	0.13	NS
CT (s)	164	143 – 188	102 – 411	4.6	**
DMY (L)	0.94	0.77 – 1.07	0.30 – 1.60	0.027	**

SCC: somatic cell count; BMC: basic milk compostion; TA: titratable acidity; CT: clotting time; DMY: daily milk yield; TP: total protein; SL: stage of lactation.
Significance levels: ** $P < 0.01$; NS: not significant.

Table 2: Correlations of all monitored parameters.

	SCC	Fat	TP	Casein	Lactose	pH	TA	CT	DMY
SCC	1.00	0.36**	0.36**	0.30**	-0.32**	-0.01	0.18	0.21	-0.15
Fat		1.00	0.33**	0.26*	-0.61**	-0.21	0.10	0.05	-0.42**
TP			1.00	0.82**	-0.23*	0.07	0.35**	0.42**	-0.29**
Casein				1.00	-0.15	0.04	0.26*	0.35**	-0.15
Lactose					1.00	0.09	-0.17	-0.13	0.31**
pH						1.00	0.13	-0.01	0.08
TA							1.00	-0.01	-0.26*
CT								1.00	0.13
DMY									1.00

SCC: somatic cell count; TP: total protein; TA: titratable acidity; CT: clotting time; DMY: daily milk yield; ** $P < 0.01$; * $P < 0.05$.

The SL had a significant effect on clotting time (CT), whereby its average time, except for the first sampling, extended with advanced lactation, what corresponds to Jaramillo et al. (2008). In our opinion, elongation of CT in our study was mainly affected by the gradual increase of the contents of TP and Cas during lactation, what is also confirmed by their significant positive correlations.
All ewes in our study were under permanent veterinary supervision and no clinical signs of mastitis were observed. This fact was reflected in relatively low both mean counts which ranged from 88 to 130 x 10^3/mL and their counts in individual samples which ranged from 36

to 420 x 10^3/mL during lactation, whilst the limit of SCC for subclinical mastitis, which was published by Bianchi et al. (2004), was not exceeded in any of the individual samples. Nevertheless, the SCCs in our study insignificantly grew as lactation advanced what is in line with Jaramillo et al. (2008). On the other hand Paape et al. (2007) and Matutinovic et al. (2011) reported a significant increase of SCC during lactation. In our opinion, the insignificant increase of SCC during the lactation could be linked with a reduced milk yield or a functional alteration of the mammary gland tissue caused by the shorter survival of epithelial cells that are supplied with fewer nutrients at the end of the lactation as it is published by Sevi et al. (1999). The SCC had a positive significant correlation with contents of F, TP and Cas, which is in line with the results published by Matutinovic et al. (2011). In contrast, between SCC and content of lactose a negative significant correlation was found which is in line with Nudda et al. (2003). In connection with the statement above, it should be noted, a lot of studies show that the content of lactose can be reduced with mastitis and SCC increase. According to Raynal-Ljutovac et al. (2007) the decrease in synthesis function of the mammary gland in case of mastitis results in lower lactose concentrations and also in a flow of minerals, sodium and chlorides, from blood to milk to maintain osmotic equilibrium. However, in our opinion a negative significant correlation between SCC and lactose content was not affected by intramammary infection, because in all cases the SCCs were lower than the thresholds for subclinical mastitis in sheep. Our opinion also strongly supports the fact, that all correlations between SCC and pH, TA, CT and DMY were insignificant.

Conclusions

As expected, the stage of lactation had a significant effect on contents of all basic milk components and daily milk yield. On the other hand, this factor had no significant effect on somatic cell count, pH and titratable acidity of sheep milk. The somatic cell counts in our study were relatively low both in mean counts and their counts in individual samples. The SCC had a positive significant correlation with contents of fat, total protein and casein. In contrast, between SCC and content of lactose a negative significant correlation was found. However, in our opinion a negative significant correlation between SCC and lactose content was not affected by intramammary infection, because the SCCs were lower in all cases than the limit for subclinical mastitis in sheep. Our opinion is also strongly supported by the fact, that all correlations between SCC and pH, TA, CT and DMY were insignificant. Finally, despite the relatively low number of ewes in our study, the results of our study suggest that the limit for subclinical mastitis in sheep at level of 500 000 SCC/mL is realistic.

Acknowledgements

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LIVESTOCK, SUSTAINABLE FOOD SYSTEMS AND THE SUSTAINABLE DEVELOPMENT GOALS

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Abstract
Animal production is an agriculture sub-sector together with crop production, fisheries and forestry. Agriculture is at the centre of the debate on sustainability and sustainable development. However, such a debate has, generally, two weaknesses; it ignores the contribution of livestock in agriculture and focuses on agriculture production rather than the whole food system. Therefore, the objective of this review is to analyse the relationship between livestock and sustainable food systems and to explore its implications in the context of the 2030 Agenda for Sustainable Development, including the Sustainable Development Goals (SDGs). After introducing the concept of sustainable food systems and providing an overview on the 17 SDGs, the paper explores linkages between agro-food systems and the SDGs. The analysis of the role of livestock in the context of the SDGs – both positive contributions and negative impacts as well as trade-offs – focuses on SDG 1 (No poverty), SDG 2 (Zero hunger), SDG 6 (Clean water and sanitation), SDG 13 (Climate action) and SDG 15 (Life on land). The review shows that livestock, sustainable food systems and the SDGs are strongly related. It also points out that intensive livestock systems, which are dominant nowadays especially in some developed countries, may jeopardise the achievement of the SDGs. Therefore, the paper argues that de-intensification – e.g. through conversion to environmentally-friendly livestock systems such as organic farming – can allow keeping the multifaceted and multidimensional benefits of the livestock sector while decreasing its negative externalities. This will contribute to the effective implementation of the 2030 Agenda as well as other regional agendas (e.g. CIHEAM Strategic Agenda 2025 in the Mediterranean region).

Keywords: *Climate change, Food security, Livestock, Sustainable Development Goals, Sustainable food systems.*

Introduction
Agriculture (crop production, animal production, fisheries and forestry) is central in the discussion on sustainable development. Meanwhile, there is an ongoing debate about the relation between livestock (including livestock products) and the sustainability of food systems (FAO, 2018a; HLPE, 2016a) from the environmental, economic, socio-cultural and nutrition-health points of view. This debate has become particularly relevant in the context of the 2030 Agenda for Sustainable Development and the related Sustainable Development Goals (SDGs) (United Nations, 2015) as well as other regional agendas such as the CIHEAM Strategic Agenda 2025 for the Mediterranean Region (CIHEAM, 2016). Indeed, it is widely recognized that there can be no achievement of SDGs without making transition towards sustainable food systems. This implies that the entire food chain - from production to consumption through processing and distribution - should become more sustainable in order to yield outcomes that contribute to a better world. However, it is not clear the role that

livestock will play in this sustainability transition journey. Therefore, the main objective of this review is to analyse the relationship between livestock and sustainable food systems and to explore its implications in the context of the SDGs.

Livestock in food systems

Livestock is commonly referred to all those domesticated animals raised in agricultural settings to produce benefits for human beings. Humans obtain labour from these animals and commodities such as meat, milk, eggs, leather, fur and wool (Encyclopaedia Britannica, 2019). The following table (*Table 1*) shows the amount of actual livestock's main species in the world. It is clear that Asia is the most important continent in terms of livestock production.

Table 1. Live animals, 2017.

	Cattle	Sheep	Goats	Pigs	Horses
World (Million head)	1,491	1,202	1,034	967	60,6
Africa (%)	23.23	31.70	40.87	3.93	11.81
Americas (%)	34.63	6.76	3.58	18.66	53.12
Asia (%)	31.52	42.26	53.29	57.64	25.56
Europe (%)	8.15	10.99	1.87	19.19	8.86
Oceania (%)	2.49	8.29	0.39	0.59	0.65

Source: FAO (2019).

Livestock is an important component of food systems. Indeed, according to HLPE (2014), "*A food system gathers all the elements (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food, and the outputs of these activities, including socio-economic and environmental outcomes*" (p. 29). Thus, a *food system* is intended as the sum of all the diverse elements and activities which, together, lead to the production and consumption of food as well as their interrelations (HLPE, 2014). The next step is to achieve a *sustainable food system* (SFS) defined as "*a food system that ensures food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition of future generations are not compromised*" (HLPE, 2014:31). The main objective of this definition is that a sustainable food system must ensure today's and tomorrow's food and nutrition security; if a food system does not ensure food security and adequate nutrition, it cannot be called sustainable. Livestock affects all dimensions of sustainability (environmental, economic, socio-cultural and nutrition-health) in food systems and diets (Dernini *et al*., 2013; Dernini *et al*., 2016; HLPE, 2016a; Layman, 2018).

Food systems in the context of the Sustainable Development Goals (SDGs)

The 2030 Agenda for Sustainable Development is a plan of action for people, planet, prosperity, peace and partnership (United Nations, 2015); it consists of 17 SDGs, 169 targets and 232 indicators (*Table 2*). The goals and targets aim to stimulate actions in areas of critical importance for humanity and the planet. Food and agriculture, as the prime connection between people and the planet, can help achieving the multiple SDGs. Indeed, according to FAO (2018b), "*Sustainable food and agriculture have great potential to revitalize the rural landscape, deliver inclusive growth to countries and drive positive change right across the 2030 Agenda*" (p. 7). Chaudhary *et al*. (2018) argue that food systems are at the heart of at least 12 of the 17 SDGs. Likewise, Caron *et al*. (2018) put that "*evidence shows the importance of food systems for sustainable development: they are at the nexus that links food security, nutrition, and human health, the viability of ecosystems, climate change, and social* *justice*" (p. 38). Even though, what is needed to emerge is a strategic narrative linking climate, agriculture and food, and calling for a deep transformation of food systems at large scale. For that, agriculture and food policies should be aligned to the 2030 Agenda (Caron *et al.*, 2018).

Table 2. Sustainable Development Goals (SDGs), targets and indicators.

No.	SDGs Short Name	SDGs Full Name	Number of Targets	Number of Indicators
1	No poverty	End poverty in all its forms everywhere	7	12
2	Zero hunger	End hunger, achieve food security and improved nutrition and promote sustainable agriculture	8	14
3	Good health and well-being	Ensure healthy lives and promote well-being for all at all ages	13	26
4	Quality education	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all	10	11
5	Gender equality	Achieve gender equality and empower all women and girls	9	14
6	Clean water and sanitation	Ensure availability and sustainable management of water and sanitation for all	8	11
7	Affordable and clean energy	Ensure access to affordable, reliable, sustainable and modern energy for all	5	6
8	Decent work and economic growth	Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all	12	17
9	Industry, innovation and infrastructure	Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation	8	12
10	Reduced inequalities	Reduce inequality within and among countries	10	11
11	Sustainable cities and communities	Make cities and human settlements inclusive, safe, resilient and sustainable	10	15
12	Responsible consumption and production	Ensure sustainable consumption and production patterns	11	13
13	Climate action	Take urgent action to combat climate change and its impacts	5	8
14	Life below water	Conserve and sustainably use the oceans, seas and marine resources for sustainable development	10	10
15	Life on land	Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss	12	14
16	Peace, justice and strong institutions	Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels	12	23
17	Partnerships for the goals	Strengthen the means of implementation and revitalize the global partnership for sustainable development	19	25

Source: United Nations (2015); United Nations (2017).

Livestock in the context of the Sustainable Development Goals

The following analysis focuses on 5 SDGs that are considered relevant in determining the impacts (positive, negative) of livestock on the implementation of the 2030 Agenda, namely SDG 1 (No poverty), SDG 2 (Zero hunger), SDG 6 (Clean water and sanitation), SDG 13 (Climate action) and SDG 15 (Life on land).

Livestock and SDG 1 (No poverty)

Since 1990, extreme poverty rates have decreased considerably, but the number of people living in extreme poverty globally remains unacceptably high. Studies reflect that 10.7 percent of the world's population in 2013 were living under the international poverty line (World Bank, 2017). According to the World Bank (2011), one in five people has to live with less than $1 a day. Poverty is still a rural phenomenon to a large extent; nevertheless, rural areas and their people have not received appreciation for their contributions to the achievement of sustainable development. Moreover, the health and well-being of rural agroecosystems and communities are considerably interlinked with the well-being, resilience and food security of cities (Anderson, 2015).

Livestock has been one of the fastest-growing agricultural sectors in developing nations and it is widely believed that agricultural growth has helped in decreasing the levels of poverty (Loayza and Raddatz, 2010). Indeed, Robinson *et al*. (2011) estimated that in 2010 the livelihoods of more than half a billion depended entirely or partially on livestock production. Livestock is essential in helping rural households to achieve their well-being expectancies (FAO, 2018; Herrero *et al*., 2013) as it affects all the assets and capitals of rural households. First, it enhances human capital by providing access to food, continued good health, life quality and labour. Second, it builds social capital, consolidating the cultural diversity and heritage of some ethnic groups and populations. Third, it contributes to the reserves of the natural capital that provides the resources and services needed to maintain and improve livelihoods. Fourth, it increases physical capital, providing transport, draught power and alternative energy for households to support and improve their productivity. Fifth, it improves the financial capital of families, providing an economic confidence for securing livelihood goals (FAO, 2018). Livestock is important not only for developing countries. For instance, in the European Union (EU) the livestock sector (meat, milk, eggs) makes up 40% of the agricultural production value. This part of the livestock market is nearly half (48%) of total EU's agricultural activity, with an estimated 130 billion EUR output value annually, and creates almost 30 million jobs (Animal Task Force, 2016).

Livestock and SDG 2 (Zero hunger)

The most recent report on the *State of Food Security and Nutrition in the World* (FAO *et al*., 2018) shows that the number of undernourished people has been growing and was estimated to nearly 821 million in 2017 (17 million more than in 2016), so one out of every nine people in the world. Moreover, food insecurity contributes to overweight and obesity and the three burdens of malnutrition (undernutrition, overweight/obesity and micronutrient deficiencies) coexist in many countries (FAO *et al*., 2018). Indeed, 2 billion people lack the essential nutrients they need to carry out healthy lives, while the number of overweight people has reached more than 1.9 billion adults, with more than 600 million classified as obese (HLPE, 2016) and the livestock sector (and animal-source foods) is blamed for these daunting figures (e.g. Wang and Beydoun, 2009). However, the livestock sector can also play a key role in ending hunger and malnutrition by helping the communities in different aspects and levels. At the household level, the main contribution of livestock is to well-nourish all household members, helping them to be healthy and to be able to improve their personal economies. It has been proved that livestock products contribute to the global human diet with 33 percent of protein intake and 17 percent of calorie intake (Rosegrant *et al*., 2009). Animal-source foods are very rich in nutrients, sources of energy, high-quality protein and essential micronutrients – such as vitamin B12 or calcium – which are difficult to obtain from only plant-based foods to keep a healthy diet (Murphy and Allen, 2003).

Livestock and SDG 6 (Clean water and sanitation)
Agriculture withdraws around 70 percent of all available freshwater, and livestock production uses approximately 30 percent of that quantity (Ran *et al*., 2016). Agricultural pressures on water quantity and quality come from all agriculture sub-sectors (crops, livestock, aquaculture) (Mateo-Sagasta *et al*., 2017). As for livestock, the total number has more than tripled (from 7.3 billion in 1970 to 24.2 billion units in 2011) (FAO, 2019) and that significantly increased the use of natural resources, including water. Nutrient runoff and leaching from livestock waste have serious detrimental effects on coastal areas. Therefore, the improvement in waste management on livestock farms can be a cost-effective way of reducing river-based nutrient loads, which often end up in marine systems and contribute to coastal eutrophication (Arheimer *et al*., 2004). Nevertheless, environmental services provided by livestock can be promoted through specific payments while water pollution can be avoided through better manure management (FAO, 2018). By carrying out these two main aspects, it should be possible to achieve a sustainable food system.

Livestock and SDG 13 (Climate action)
The UN recognised that climate change is the single biggest threat to development (FAO, 2018; United Nations, 2019). Evidence shows that 2016 was the third hottest year recorded in a row. That same year, the average CO_2 concentration in the atmosphere surpassed the unrealistic amount of 400 parts per million, a level never before reached in recorded history (more than 650,000 years). The current increase rate of greenhouse gases (GHG) is more than 100 times faster than when the last ice age ended (World Meteorological Organisation, 2017). These data should be shockingly enough to start trying to reverse the current situation.
Actually, the climate change impacts on agriculture and the implications for food security are alarming, because its symptoms will disproportionately fall on the poorest and most vulnerable people and countries. Climate change impacts livestock directly (the increasing temperatures also increases stress on animals and their mortality) and indirectly (by reducing the quantity and quality of feed and fodder, increasing the incidence of animal diseases) (FAO, 2018). Livestock sector contributes significantly to climate change in that direct livestock's GHG emissions, from manure and enteric fermentation, represented 2.4 gigatons of CO_2 equivalent in 2010, about 21 percent of total emissions from agriculture, forestry and other land uses, or about 5 percent of total anthropogenic GHG emissions (IPCC, 2014). Gerber *et al*. (2013) argue that global GHG emissions from livestock are very significant, representing 14.5 percent of all anthropogenic emissions.

Livestock and SDG 15 (Life on land)
Across the globe, natural resources are deteriorating, ecosystems are under stress and biological diversity is being lost (Millennium Ecosystem Assessment, 2005). The food sector is, worldwide, a dominant user of natural resources (UNEP, 2016). That is why unsustainable agricultural production consumes a large fraction of the world's available fresh water, and has contributed to increase deforestation, biodiversity loss, land degradation, and conversion of natural habitats. Changes in land use, including deforestation, result in the loss of valuable habitats, water pollution, land degradation, soil erosion and the release of carbon into the atmosphere. Such damage is mainly due to the conversion of forest or rangelands to other land uses such as agriculture (including pastures and land for feed production) and

infrastructure development (FAO, 2018). While the livestock sector plays a part in biodiversity reduction, land degradation and deforestation (FAO, 2006), it also provides invaluable services that promote sustainable use of terrestrial ecosystems, combat desertification, reverse land degradation and avoid biodiversity erosion (FAO, 2018).
In order to ensure sufficient food production without affecting ecosystem health, there is a need to make transition towards agro-ecological approaches such as organic farming. Indeed, organic agriculture – including organic animal production – delivers significantly higher public goods (e.g. soil fertility, biodiversity, quality of surface and drinking water) than conventional agriculture and has, consequently, great potential to contribute to the achievement of the SDGs (IFOAM, 2016; Setboonsarng and Gregorio, 2017).

Conclusions

Livestock, sustainable food systems and the SDGs are strongly related, and the contribution of livestock to both the sustainability of food systems and the SDGs is widely recognised. On the other hand, the intensive livestock system, which is dominant nowadays especially in industrialised countries, may jeopardise the achievement of the SDGs. De-intensification and "extensification" of livestock will contribute to keep the environmental, social, nutritional and economic benefits of the livestock sector and to decrease its externalities, thus contributing to the effective implementation of the 2030 Agenda for Sustainable Development. Finally, the conversion to organic livestock farming and other forms of environmentally-friendly livestock systems, can be considered as a strategy for the de-intensification and greening of the livestock sector, which is strongly necessary for the coming times.

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EFFECT OF SPERM STORAGE TIME AND TEMPERATURE ON SEMEN QUALITY IN THE RABBIT LINE ITELV 2006

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Abstract

The present study aims to study the effect of storage time and temperature on the semen quality of adult rabbits of the same age of the ITELV 2006 strain. In order to achieve our goal, 04 experiments are conducted to evaluate the semen quality of 10 adult rabbits. The semen samples are mixed the individual's ejaculation analysis and divided into fractions, and then diluted in a Tris diluent. They are then kept at four different temperatures for 96 hours. Samples are taken after 24, 48, 72 and 96 h. The experiment is repeated 4 times during 1 month at a rate of once a week. The microscopic parameters of ejaculation are evaluated before and after the preservation process. The results of the analysis showed that the percentage of motility and vitality of spermatozoa in fresh semen is 82.86% ± 7.82, 73.69% ± 1.29 respectively. For the conserved semen and for the same parameters, a decrease in the motility and vitality values is observed with the conservation time ($p<0.05$). For the storage temperature, the semen kept at 15 ° C for 24h has the best values compared to other temperatures (61.75% ± 2.05 and 66.5% ± 1.12 respectively for motility and vitality, $p<0.05$). We record a zero motility and vitality for the semen kept at 4 ° C from 72h.

Key words: *spermatozoa, refrigeration, Tris-based extender, vitality, motility.*

Introduction

Despite the widespread use of Artificial Insemination (AI) in large rabbit farms in several countries around the world, it has not become a common practice in Algeria. A limiting factor for a more extensive practical application is related to the conservation of the semen. The current practice of using freshly diluted sperm in a few hours following collection is mainly limited to AI at the farm where the male is located, resulting to such high conception rates as those obtained with natural mating. Short life imposes considerable stress on rabbit AI, as fast delivery is essential. Since frozen semen is currently not suitable for routine AI in commercial rabbit production (Morrell 1995 and Castellini 1996). The interest in preserving rabbit semen for a period of 24-72h has been the subject of several trials (Alvarino et al., 1996, Gottardi, 1993, perrier, theau-clément et al 1998, Santa Maria et al, 1989). Badù et al 2004, have shown that the semen kept 24h at 5 ° C in a saline solution (Tris- EDTA- citric acid) does not affect the mobility nor the straightness of the displacement of the cells. The storage temperature and its duration play an important role in sperm survival (Carluccio, and al., 2004) Temperatures of 5 ° C to 25 ° C are suitable for the preservation of rabbit sperm for 72 h (Johinke, D. and al., 2014; López, FJ and Alvariño, JMR 1998; Roca, J., and al., 2000). Mathur and al. (1992), Gottardi (1993) and Lopez and al. (1996) found that a storage temperature of 15 to 19 ° C is the most favorable for sperm survival. Sperm refrigerated and stored for 24 to 72 hours may facilitate their transport and subsequent widespread use of artificial insemination in rabbits while preserving the fertilizing capacity of sperm. In order to improve the management of reproduction, the objective of this study is to evaluate the effect of different storage times at different temperatures on the quality of the ITELV 2006 synthetic strain semen. This strain is created in 2003 as part of a breeding program cooperation between INRA and ITELV; it is obtained by an initial cross between the Algerian local population and the INRA 2666 strain (Gacem and Bolet 2005).

Materials and Methods

At the level of the animal department of the Department of Agricultural Sciences and Biotechnologies, Hassiba Ben Bouali University of Chlef, Algeria, 10 adult male rabbits of the synthetic hybrid strain ITELV 2006 brought from the Baba Ali Breeding Technical Institute of Algiers, aged 7.5 to 8 months, so at the time of sexual maturity. Indeed, sexual maturity, defined by the time when the number of spermatozoa harvested per day no longer increases, would be reached around the age of 32 weeks (Amann and Lambiase 1967). However, spermatozoa have been present in the ejaculation at puberty as early as 16 weeks of age (Martinet 1974). The average weight at the beginning of the test is 3.70 ± 0.13 kg and the same housing conditions are used. During the experimental period all animals receive, voluntarily, a commercial feed in the form of granules, containing maize, soybean meal, mill products, calcium, phosphates, amino acids, trace elements, poly vitamins, antioxidant, folic acid, oil soybean and alfalfa (Table 1) and watered ad libitum and placed in individual wire cages (75 cm long, 46 cm wide and 28 cm high) inside an air-conditioned building. The livestock building is a concrete room, with an area of 220.80 m^2 and a height of 4m. It consists of two separate rooms, one for the breeding of animals and the other in the form of a laboratory for carrying out various analyzes. The two dirty ones are opened in the light of the day by several lateral windows, having a system of cooling.

Table 1: Chemical composition of the standard feed

Component	Content. (% as is)	Reference of the method of analysis
Dry matter	91.42	NA 1291-1994
Mineral matter	7.51	NA 650 - 1994
Raw protein	14.5	NA 652-1992
Crude cellulose	9.49	NA 6138 -1991
Fat	3.38	NA 654 -1992
Calcium	0.89	NA 653 - 1992
Phosphorus	0.60	NA 657-1992

The sperm collection is carried out in a period of one month (March 2018) according to the following protocol: two successive ejaculates a single day a week. An interval of 10 min separated the 2 successive samples. The harvest of 2 consecutive ejaculates per day of collection and the choice of collection frequencies are initially motivated by the bibliographic knowledge. Males are trained to collect sperm using the artificial vagina as early as 5 months of age (Garcia and al 2004, Garcia-Thomas and al 2006b, Lavara and al 2008, Theau-Clément and al 2009). No sexual preparation is applied to the male before collection. A refusal of harvest is recorded if the male could not be harvested within ten minutes of contact with a first rabbit as well as with a second (Theau-Clément and al 1991 and 2009). Sperm collection is performed using an artificial vagina and in the presence of a receptive female teaser (Boussit 1989).

The methods of sperm analysis are as follows: Once the sperm has been collected, the total volume of ejaculate collected is measured by direct reading on the graduated tube used for collection. If the sperm collected contains gel, the gel is removed using a Pasteur pipette to determine the frost-free volume. Sperm pH is measured using a pH meter. Before dilution, macroscopic analyzes of each ejaculation were performed to assess the color and volume of the sample; Only white ejaculates had a volume greater than 0.25 ml, and had at least 70%

motility are used for the study. Then the mass and individual motility of spermatozoa are determined under a microscope. Mass motility is appreciated by placing a drop of pure sperm between slide and lamella observed at magnification (x10). A note is attributed to the movement of the mass of spermatozoa observed according to the Petitjean grid (1965) (Boussit 1989) ranging from 0 to 9. Individual motility is assessed after dilution of sperm in 1cc of saline. A drop of semen (diluted sperm) is observed between slide and coverslip at a magnification (x 40). The type of individual movements of spermatozoa is noted using the Andrieu (1974) scale (Boussit 1989) ranging from 0 to 4. The percentage of dead spermatozoa is determined by the preparation of a smear using eosin-nigrosine vital staining: a drop of sperm diluted in saline is mixed with a drop of dye, then the mixture is gently spread on a blade with a coverslip and dried in the open air (Baril and al 1993). Then it is observed under a microscope at magnification (x 40); 100 spermatozoa are counted, from which are estimated the percentage of colored spermatozoa corresponding to the dead spermatozoa whose damaged membrane is permeable to the pink coloring, whereas the living spermatozoa with their functional membranes do not diffuse the dye and therefore remain colorless (Boussit 1989). The percentage of abnormal spermatozoa is studied on the same slide used to count living spermatozoa. 100 spermatozoa are randomized from which abnormal spermatozoa are distinguished (Boussit 1989). The spermatozoa concentration (in million / ml) is determined using a Thoma cell type hemocytometer from a diluted drop of semen. Counting is performed under the microscope at × 40 magnification (Boussit 1989). All ejaculates are pooled to eliminate the differences, then the heterosperm samples are diluted (1/10) in a Tris diluent and the diluted sperm samples are divided into four equal fractions. was cooled gradually from 37 ° C to 5, 10, 15 and 20 ° C for 120 minutes (Mocé, E. and Vicente, JS 2009) and stored for up to 96 h. The evaluation of viability and motility of spermatozoa are determined at 24, 48, 72 and 96 h after the start of cooling This experimental procedure is repeated four times (once a week). In the experiment, all measurements are made within 4 weeks directly after harvest, 24, 48, 72h and 96h. Our statistical study of the obtained data is processed by a software XLstat 2016 for the calculations concerning the descriptive statistics. The characteristics of ejaculate are then studied using a fixed-effect model of variance analysis including the effects of storage temperature, shelf life and their interactions.

Results and Discussion

The color for all the results found in rabbits. According to the Roca and al (1993) sperm color chart, ranging from 0 to 3 (pearly white or ivory white sperm), a score of 3.0 is assigned for all ejaculates (Table 2). The average volume of all collections in our experiment is 0.48 ± 0.11 ml, lower than that collected in different breeds (Californian, New Zealand White and Chinchilla of Mexico) between 32 and 48 weeks of age (1.15 ml) (Salcedo-Baca and al 2004). According to Boulbina and al (2011) in the local Algerian rabbit, the total volume collected between the 24th and 33rd week of age is estimated at 1.2 ml. Furthermore, in hybrid rabbits, aged between 3 and 18 months, the mean sperm volume was 0.92 ml (Roca and al 2005). The pH of sperm collected during the experiment averaged 6.86. This value is considered normal and comparable to that reported by Brun and al (2006). A variability in sperm pH is revealed by the literature data between the different strains and races studied (from 6.94 to 7.63) (Garcia-Tomas and al 2006a, Brun and al 2002a, Brun and al 2009) . The measurement of the pH at the pH meter must be immediate because the semen is rapidly acidified following the formation of lactic acid, resulting from the use of sugars by the spermatozoa (Alvarino 1993, Arencibia and Rosario 2009). The concentration shows an average of 502 ± 29 million spermatozoa which is close to the values described by Boiti (2005) and Nabi (2013) for the same age. Our results are weaker than those recorded by Bencheikh (1993), Brun and al (2002), Castellini and al (2003), Theau-Clément and al (2009) and Brun and al (2006) in the L line (634x106 spermatozoa/ ml) and line H (738 x 10^6 spermatozoa / ml). Indeed, Boulbina (2011) found a higher mean value (642 x 10^6 spermatozoa and 735x10^6 spermatozoa / ml) in rabbits harvested from the 24th week of age to the 33rd age. Furthermore, Safaa and al (2008) indicate concentrations of 703 × 10^6 spermatozoa / ml for the Black Baladi breed and 590 × 10^6 spermatozoa / ml for the White New Zealand breed (in Egypt). However, significantly lower concentrations, of the order of 243 x 10^6 spermatozoa / ejaculate, are revealed in various strains selected by INRA (1077, 2066, 2666 and 1001) (Theau-Clément and al 2003; 2009) and of the order of 245x10^6 spermatozoa / ml for both lines C and R as reported by Garcia-Tomas and al (2006a). The mean scores of mass and individual motility were respectively 5.21 ± 0.77 out of 9 and 3.36 ± 0.56 out of 4. The whole of the ejaculates has an average percentage of spermatozoa abnormal of 14.4 ± 4.05%. Nevertheless, the average scores of mass and individual motility are low, below the threshold of good mobility (mass motility: appearance of waves (≥ 6/9) and individual motility: rapid progression (≥3 / 4) and desirable for inseminations (Boussit 1989). This weakness of motility can be a specific character of the strain, as it could be explained by the small number of observations recorded in a potentially short duration. The spermatozoa in sperm of synthetic rabbits are characterized by a lower mass motility than that measured in rabbits of different origins (Bencheikh 1995, Theau-Clément and al 2003, Brun and al 2006 and 2009). On the other hand, their individual motility is sometimes superior, sometimes weaker than those reported by the bibliographic data and this according to the genetic origin of the rabbit (Bencheikh 1995, Garcia-Tomas and al 2006a). Boulbina (2011) found a low rate of spermatozoa mobility in the local rabbit (64.2%) and 74.8% at older ages in the same population (between 24 and 33 weeks). Brun and al. (2006) reported a rate of 76% using males of selected lines on growth. The mobility of spermatozoa seems to improve with age (Salcedo-Baca and al 2004). In the rabbit, studies indicate that good mass motility of spermatozoa accompanies a good pregnancy rate (Brun and al 2002b). Whatever the characters considered (volume, motility, concentration), lower values are obtained than those recorded by Bencheikh (1993), Brun and al (2002), Castellini and al (2003), Brun and al (2006) and Theau-Clément and al (2009) (France). In fact, Boulbina (2011) found higher average values, namely volume (0.86 ml), mass motility (7.68), individual motility (3.57), and concentration (735x10^6 spermatozoa).). These differences in volume and sperm concentration appear to be related to the genetic origin of the rabbits and the selection program to which they would be subjected. It is important to note that the methods used by these different studies are based on observation and counting, which is likely to be an additional source of variability in the results.

Table 2. Macroscopic and microscopic features of fresh semen.

	N	*avg*	*ETM*	*Distr*
Color	80	3.00	0.00	3—3
pH	80	6.86	0.12	6.66—7.20
Volume (ml)	80	0.48	0.11	0.23—0.80
Massive motility	80	5.21	0.77	4—6
% MM	80	82.86	7.82	60--95
Individual motility	80	3.36	0.56	2—4
Percentage of live spz	80	73.69	1.29	70—76
con x10^6 spz/ml	80	502	28.9	437-564

n: number of ejaculates; avg: average; ETM: standard deviation of the mean; Distr: extent of distribution of spermatozoa per male; con: concentration; spz: spermatozoa; n: number of ejaculates; avg: average; ETM: standard deviation of the mean; Distr: extent of distribution of spermatozoa per male.

Mean values and global standard deviations of the different criteria for sperm analysis after storage are shown in Table 3.

The results obtained allow us to deduce that the overall motility decreases over the conservation time, from 5.21 ± 0.77 to 24h vs 0 to 96h, while variable values are recorded for the storage temperatures with deterioration of the characteristics studied from 72h, for the deferent storage temperature.

The effect of storage time and temperature on the motility and vitality of the semen is shown in Figure 1, the results showed that all spermatozoa parameters are decreased with storage time (P <0.05), regardless of the refrigeration temperature used. This is comparable to the El-Gaafary (1994) study, using a Tris-yellow diluent, which found that sperm are cooled and stored at 5°C for 24 h. had an average motility of 45% and dropped to 25% after 48 hours. This sharp decrease in sperm viability during the entire storage time may be due to the cooling temperature. Temperature as a cause of disturbance of rabbit spermatozoa has not been established, however studies comparing various conservation temperatures of rabbit sperm conclude that 15 ° C is more appropriate than 5 ° C (Roca and al., 2000). .

Table 3: The vitality and motility characteristics of the preserved semen.

	°C	fresh	24h	48h	72h	96h	ESM	*P*
TM (%)	4	82.86 ±7.82^{a}	25.50±1.12^{e}	07±2.24^{gh}	0.50±0.87^{i}	00^{i}	1.014	< 0.0001
	10		48.75±1.30^{c}	30.75±2.16^{d}	11±10^{fg}	2.75±0.83^{hi}		
	15		61.75±2.05^{b}	43.75±2.38^{c}	30±2.12^{de}	13.5±1.50^{f}		
	20		32.50±1.80^{d}	11±2.24^{fg}	03±0,71^{hi}	0.25±0.43^{i}		
MM	4	5.21 ± 0.77^{a}	03^{cd}	1.25±0.43^{fg}	00^{h}	00^{h}		
	10		3.75±0.43^{bc}	2.75±0.43^{de}	1.5±0.5^{fg}	0.75±0.43^{gh}		
	15		4^{b}	3.25±0.43^{bcd}	3^{cd}	2^{ef}		
	20		3^{cd}	1.75±0.43^{f}	0.75±0.43^{gh}	00^{h}		
MI	4	3.36 ± 0.56^{a}	2^{bc}	1de	00^{f}	00^{f}		
	10		2.75±0.43^{ab}	1.75±0.43^{cd}	1de	0.25±0.43^{ef}		
	15		3.25±0.43^{a}	2.75±0.43^{ab}	2^{bc}	1de		
	20		2^{bc}	1de	0.5±0.5^{ef}	00^{f}		
VIT (%)	4	73.69 ±1.29^{a}	36.75±1.78^{d}	9.75±1.09^{ef}	0.25±0.43^{g}	00^{g}		
	10		53±1.87^{c}	31.75±1.48^{d}	14.5±1.8^{e}	5±1.22^{fg}		
	15		66.5±1.12^{b}	53.75 ±2.38^{c}	36.75 ±5.17^{d}	15.25 ±2.77^{e}		
	20		33.5±2.18^{d}	15.75 ±3.11^{e}	5.75±0.83^{fg}	00^{g}		

(a, b, c, d , e , f , g , h , i) in the same row and for the same parameter, the assigned values of the same letter do not differ significantly (P> 0.05).

There are interactions between temperature and storage time (P <0.05) on all evaluated parameters. After 72 hours of storage, the quality of the semen stored at 15 ° C provided better results with significant deference (p <0.05) for all parameters studied compared to other temperatures used. However, the in vitro study by J. Roca (2000) demonstrated that 15 ° C may be a suitable temperature for storing rabbit semen when Tris buffer extensions are used. Castellini, 1996 concluded that inorganic buffers have limited buffering capacity while organic buffers, such as Tris, are more suitable for storage of rabbit sperm at low temperatures.

The effective use of chilled semen for AI depends on the vitality and motility of the sperm after storage. In our study the comparison of the quality of the semen kept at 4 ° C and 20 ° C in the same storage time, showed no significant difference (P> 0.05) between the spermatozoa examined for the viability parameters and motility.

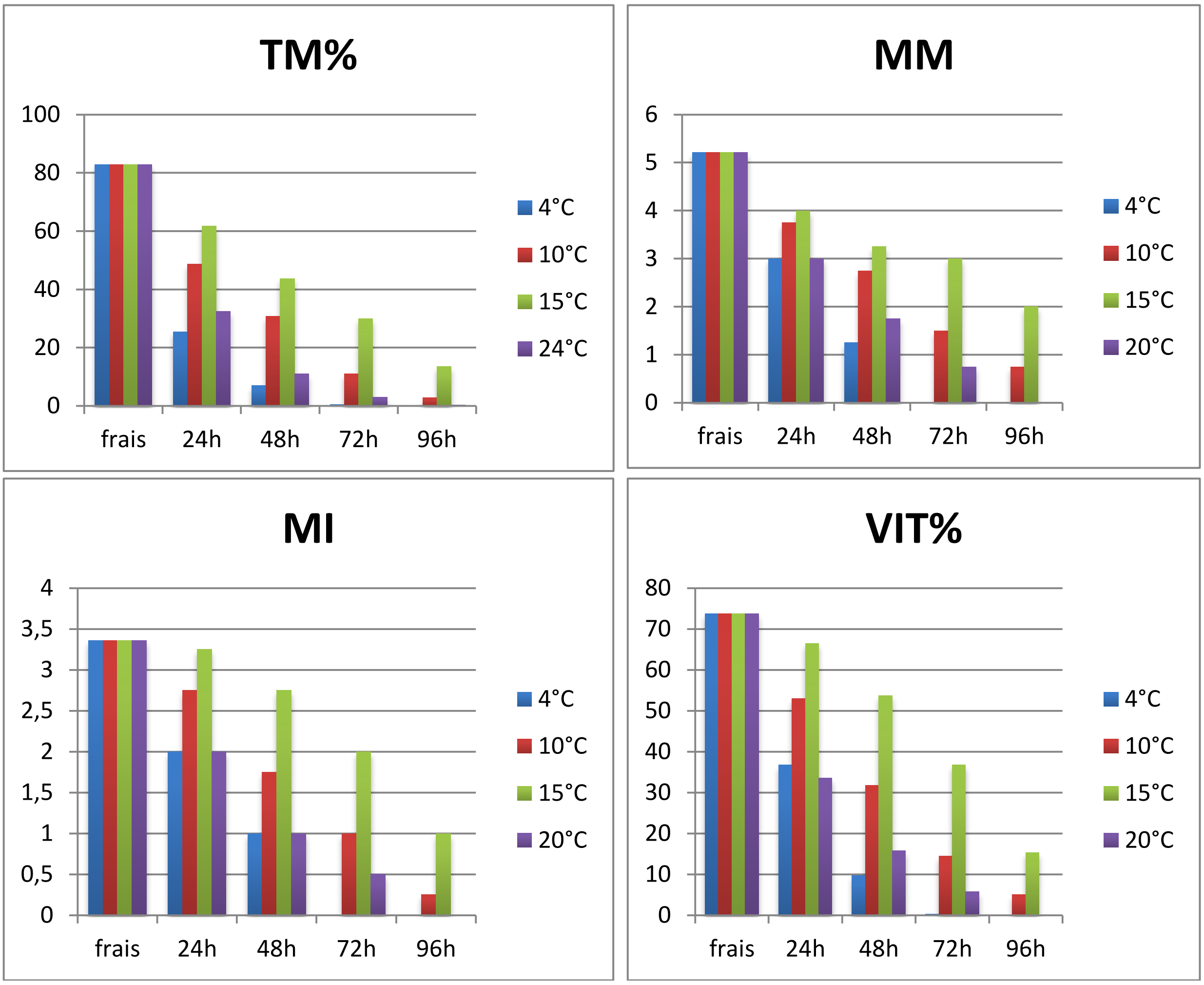

Figure 1: Evolution of semen variation parameters during the storage period.

The quality of spermatozoa is higher (P <0.05) for semen's kept for 24 hours compared to other durations. Spermatozoa examined at 96 h shows the lowest parameters (P <0.05).
Previous studies to evaluate the fertility of sperm stored in rabbits have been conducted under experimental conditions (El-Gaafary 1994, Maertens and Luzi 1995). Whereas the conception rate did not decrease when the semen was stored at 20 ° C for 6 h after dilution in a commercial diluent based on Tris Maertens and Luzi (1995), while El-Gaafary (1994) showed a fall birth rates when sperm were stored at 5 ° C for 24 and 48 h after dilution in a Tris-yellow diluent.
Sperm storage temperature is an important factor in maintaining the fertility of rabbit spermatozoa (López and al., 1996) and the temperature of 15 ° C seems to be better than the temperatures of 4 ° C, 10 ° C and 20 ° C. ° C with a shelf life of no more than 72h and the use of Tris buffer extensions.

Conclusion

It can be concluded that Tris buffer diluents are effective for dilution and storage of rabbit sperm at 15 ° C for 72h. The effect of storage time and temperature on semen motility and vitality shows that all spermatozoa parameters are decreased with storage time (P <0.05), regardless of temperature refrigeration used.
Nevertheless, after 72 hours, the quality of the semen kept at 15 ° C provided better results with a significant deference (p <0.05) for all the parameters studied compared to the other

temperatures used, this quality being higher (P <0.05) for semen's kept for 24 hours compared to other durations. So we can never go back to the original quality of the fresh semen.
The comparison of the quality of the semen kept at 4 ° C and 20 ° C in the same storage time, shows no significant difference (P> 0.05) between the spermatozoa examined for viability and motility parameters.
The temperature of 15 ° C seems to be better than the temperatures 4 ° C, 10 ° C and 20 ° C with a shelf life not exceeding 72h and the use of Tris buffer extensions, which allows to conclude that are effective for dilution and storage of rabbit semen at 15 ° C for 72h.

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CHARACTERISTICS OF BASIC BEHAVIOURAL FEATURES OF 'HORNLESS HEREFORD' LACTATING COWS RAISED ON A NATURAL GRASSLAND

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Abstract

A study was conducted on the main elements of the behaviour of lactating beef cows of 'Hornless Hereford' breed with their calves at the age 5-6 months on a natural pasture in the region of Troyan, Bulgaria. The pasture is situated at 386 m above sea level on a flat terrain with a local grassland. The meteorological indicators characteristic of the late spring were recorded. The behavioural reactions were examined by chronometry, such as: grazing, having a rest, lying and ruminating, moving, water intake, defecation, nursing of calves. It was found that cows were grazing from 5.36 to 5.51 hours on average during the experiment and the calves were suckling 4-6 times a day and 2 times at night, respectively. The time used for grazing is comparable to that recorded under other climatic conditions, grazing composition and meat cattle breeds. The physiological state, the climate elements, the alimentary conditions and the raising system had an impact on the behavioural reactions of lactating beef cattle. Animals tested were characterized by imitating behaviour.

Keywords: *Pasture, cows, behaviour, 'Hornless Hereford', grassland*

Introduction

Grazing on natural pastures of beef cattle is controlled by the farmer. It is the result of applied agro technical events that determine the amount (Iliev et al., 2017) and quality (Todorova and Churkova, 1998; Nikolova, 2012; Bozhanska et al., 2017) of the grassland that is used.

The cow grazes no more than 8 hours, as the immediate grass picking is about 5 hours on average. The rhythm of jaw movement in beef cows is 50 movements per minute on average (Wuazen, 1959; Roussev and colleagues, 1978; Nowicki 1981; Lubritz et al., 1989; Baskin and Chikurova, 2014).

Phillips (2002) found that beef cows are taking about 50000 bites of grass for twelve hours, as the bite frequency depends on the quality composition of the grass bite. When the grassland length is greater than 10 cm, the cow takes a pinch of 25-30 g of dry matter per a minute.

According to Albright and Arave (1997), cattle show increased activity at sunrise and sunset.

Hancock (1950) discovers and investigates genetically determined differences in the amount of grass, which was grazed, during twenty-four hours, resting, motor activity, frequency of defecation and urination, etc. in studies on meat cattle.

Grandin (2001) and Stoycheva (2014) emphasize that modern stockbreeding, respectively cattle breeding, obligatory involves a competent use of knowledge of animal behaviour and a ban on the use of physical force.

Kudrin et al. (2016) points out that a deficiency of many ethological studies is the lack of uniform criteria for assessing animal behaviour. It is almost impossible to compare data, as in most cases they are descriptive.

Sheveleva and Baharev (2003) assume that behavioural responses serve as important criteria for assessing the human-made technological conditions for animals as processes of optimal feeding, raising and realization.

The objective of the present study is to investigate the main elements of the behaviour of two groups of lactating beef cows of 'Hornless Hereford' breed, raised on a foothill, natural pasture, together with their calves.

Material and Methods

The study was carried out in the autumn of 2018 at Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) in Troyan, Bulgaria on a naturally grassed pasture, situated at 386 m above sea level on a flat terrain.

The available grassland found on the pastureland was: tall fescue (*Festuca arundinacea Schreb*) 33%, Kenthucky bluegrass (*Poa pratensis* L.) 12%, quack grass (*Agropyrum repens* L.) 15%, white clover (*Trifolium repens* L.) 10%, red clover (*Trifolium pretense* L.) 5%, sainfoins (*Onobrychis viciifolia Scop.)* 5%, bird's-foot-trefoil (*Lotus cirniculatus* L.) 5%, broadleaf plantain (*Plantago mayor* L.) 3% and weeds, such as thistle (*Cardunns spp.* L.), Bermuda grass (*Cinodon dactylon* L.), greater burdock (Arctium lappaL.), wild carrot (*Dancus carota* L.) 5% etc.

Meteorological data were taken from meteorological station. Data were taken from recordings twice a day (7.00 a.m. and 9.00 p.m.) of t˚ (dry and wet thermometer), relative air humidity, wind speed, cloudiness, and precipitation.

The pasture area was about 1.5 ha and was used by 10 pure-bred 'Hornless Hereford' cows from 29.05.2018 to 30.05.2018 inclusive. The cows were conditionally divided into two groups (1^{st}) and (2^{nd} control), of 5 animals by means of electric fencing. Water intake took place in a trough mounted on the grassland itself.

The animals were on the pasture for twenty-four hours for 2 days. The behaviour of cows and calves was observed from 7.00 a.m. to 7.00 p.m. during the day and from 7.00 p.m. to 6.30 a.m. at night. Cows were identified with numbers from 1 to 10 using a colour spray. The same identification was applied to calves. There were five main behavioural reactions – grazing, having a rest (lying, standing, ruminating), moving, water intake and nursering in cows, and one major behavioural reaction in calves as suckling. The acts of aggression to humans were further investigated. Observation of the animals over the course of two days gave us the opportunity to get information on the rhythm of the behavioural reactions. Their registration was recorded in protocols for every twenty-four-hour.

The ethologological responses were determined by chronometry of the various acts by Methods of Velikzhanin et al. (1975) and Velikzhanin (2000).

We have also determined the index of motor activity (IMA), the index of feeding activity (INA) and the index of total activity (ITA) according to the following formulas:

IMA = time spend for standing + time spend in ruminating and lying/1440

IFA = time spend in grazing + time spend in ruminating/1440

ITA = time the animal spend in activity/1440

The obtained data was biometrically processed and presented in diagrams and tables.

Results and Discussion

'Hornless Hereford' was introduced to Bulgaria in the mid-1980s in the town of Troyan. The breed has been acclimatized and raised and breeds in a number of countries around the world, including Bulgaria. It is relatively unpretentious to the conditions of raising and feeding.

In the study of behavioural reactions, the impact of the climate conditions of the studied area is of interest. Air temperature and humidity are key elements of the climate, and together with the other studied components, their values are shown in Figure 1 and Figure 2.

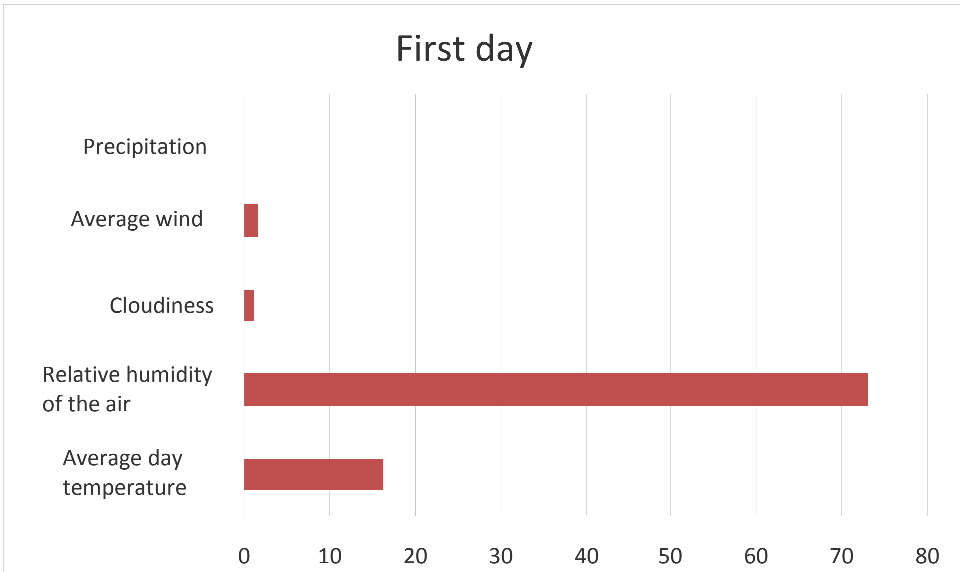

Figure 1. Components of climate and their values (1st day).

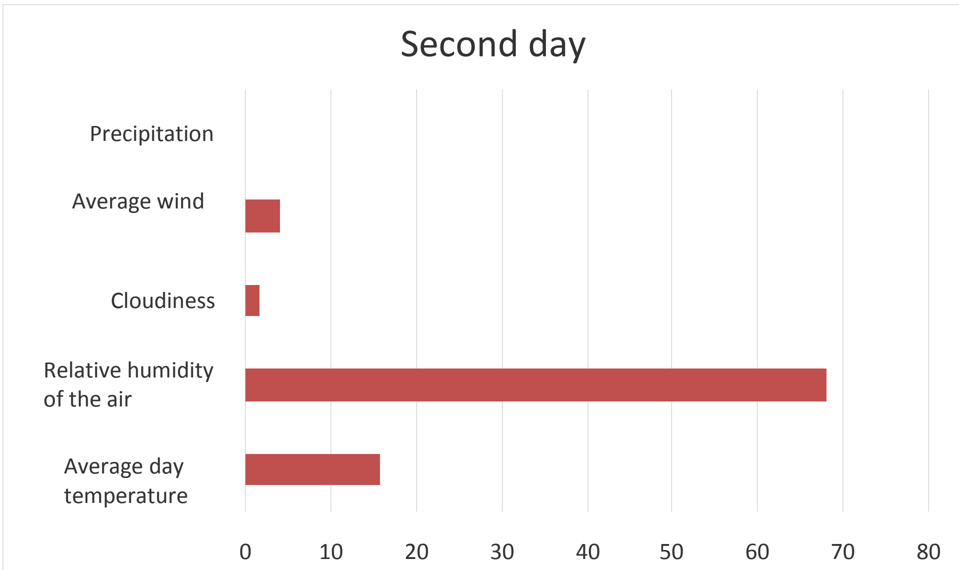

Figure 2. Components of climate and their values (2nd day).

The presented climatogram shows the average air temperature on the first day measured on a dry and wet thermometer 16.2 ° C (max. 25 °C and min. 17.5 °C). Average temperature amplitude was observed. The relative humidity is 73%, the cloudiness is 1.3 at maximum 100 and the average wind is 1.7 m/s (slight breeze), no precipitation. The weather is calm and the soil slightly damp. On the second day, the average t˚ is 15.8 °C (25 °C and minus 9 °C). Again there is average temperature amplitude. The relative humidity is 68%, the cloudiness is 1.7 at maximum 100, the average wind is 4 m/s and precipitation is 1.3 l/m². The weather is calm, the soil slightly damp. Reported temperatures are lower than the first day, the average t˚ is 0.2 ˚C less, the maximum t˚ is 25 ˚C, and the minimum t˚ shows higher values by 1.5 ˚C. No precipitation is observed. Air humidity is lower by 5%. The soil is slightly damp.
The values of the observed behavioural responses of lactating cows of 'Hornless Hereford' are shown in Table 1.

Table 1. Distribution of time for different behavioural responses in lactating cows of 'Hornless Hereford'.

Elements of behaviour	Lactating beef cattle 'Hornless Hereford' breed			
	1st group n=5		2nd group (control) n=5	
	min	%	min	%
Grazing	321.6±1.37*	22.4	330.6±1.45*	23
Having a rest: including	862.2±1.21	59.9	844.3±1.77	58.6
standing	261.4±2.86	18.2	277.4±2.44	19.3
lying	600.8±1.24	41.7	566.9±1.06	39.3
ruminating	322.6±1.17	22.4	328.1±1.25	22.8
moving	91.3±0.94*	6.3	95.4±1.04	6.6
water intake	23.5±0.04*	1.6	24.1±0.06*	1.7
nursering of calves	141.4± 1.14	9.8	145.7±1.19	10.1
Total	1440	100	1440	100

*P≤0.05

The cows of both groups observed were grazing 5.36 hours (321.6 minutes) and 5.51 hours (330.6 minutes) average per day (P≤0.05). The distance during grazing between the observed animals varied between 2.5-4 m, as the animals were in groups of 2 or 3. It is assumed that the grazing period is related to specific weather conditions. The difference between both groups (main and control) is observed during the period of rest, generally, which is 17.09 minutes or 1.3%. It is mainly attributed to the acts of standing, the difference is 1.1% as the difference for lying is 2.4%. In our opinion, this is due to the fact that different sections of the pasture were used by both studied groups at a relatively different time of day. The period for the rest was carried out on two sections of the pasture, mostly at its periphery during the day and at its center during the night. The cows were lying in a moon-like shape, with the calves in the center next to them. The value of period for rest was 862.2 minutes for the 1st group and 844.3 minutes for the 2nd group, or about 60% for twenty-four hours, as 45% of it was done during the day. The other behavioural reactions, such as ruminating, free movement and water intake are relatively close in values. During the nursing of calves the cows were standing and ruminating. Four of the mothers allowed calves of other cows to suckle from them for several times a day. There was also an aggression reaction to the people of two cows of 'Hornless Hereford', mostly in the dark of the day.

During the two periods of twenty-four hours, the calves suckled 4 to 6 times a day, and 1-2 times a night, as they were grazing or lying periodically at a distance of 2.5-4 m from their mothers.

Our data correspond and are similar in the results with the etchological studies of Novitski (1981) and Sheveleva and Baharev (2003).

Table 2. Indices for quantitative estimation of behaviour determined according to the formulas of Velikzhanin (2000).

Breed	Indices		
	IMA	IFA	ITA
Hornless Hereford (1st group)	0.823	0.447	0.625
Hornless Hereford (2nd group control)	0.814	0.457	0.641

The coefficients involved in creating the indexes used are expressed with specific values. The analysis of data from the calculated indices gives us a quantitative assessment of behaviour and shows that the index of motor activity (IMA) has high values for both groups tested, for

the first group is 0.823, and for the second one is 0.814, with a difference of 9 points in favour of the 1st group. The index of feeding activity (IFA) for the 1st group is 0.447, and for the 2nd group (control) is 0.457, or a difference of 10 points in favour of the 2nd group. For the index of total activity (ITA) the values are 0.625 for cows of the 1st group and 0.641 for the 2nd group (control), a difference of 16 points in favour of the 2nd group.
The two groups studied show lower values for the two main indices (IMA) and (IFA) that put the investigated animals in "Hyper passive" class. The decrease in the index of total activity is associated with a reduction in metabolic processes in the body and is explained by the climate, season, the productivity of the pasture used and the meteorological situation during the survey.
Our studies are similar to the results obtained in the studies of Kudrin et al. (2016) on lactating Ayrshire cows.

Conclusions

The physiological state, the elements of the climate, alimentary conditions and the system of raising have an impact on the behavioural reactions of lactating beef cows. The data obtained suggest that animals from 'Hornless Hereford' have good adaptive abilities for pasture farming in late spring in the conditions of the town of Troyan, the Republic of Bulgaria, a foot-hill terrain at 386 meters above the sea level of a natural pasture with predominant grassland of grasses and legumes and motley grasses with weeds. The time spent in grazing is comparable to that recorded under other climatic conditions, pasture composition and breeding of beef cattle. The animals of both groups tested were characterized by imitating behaviour, especially in grazing, resting and suckling of calves. Joint grazing with the mother is an important element in teaching of calf to graze. Lactating cows showed a good maternal instinct, as four of them allowed suckling of calves of other cows. Two of the lactating cows showed an aggressive behaviour in the form of attack when people approached.

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EFFECT OF SALINE WATER ON WATER INTAKE, GROWTH PERFORMANCE AND BLOOD METABOLITES OF BARBARINE LAMB

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Abstract

This experiment was designed to study the effect of drinking saline water on growth performance, water intake and blood parameters in local sheep reared under Tunisia conditions. Twenty of Barbarine lambs (initial body weight 23.5±4.5 kg). The lambs were divided into two homogenous groups regarding age and live weight. Animals are grazed in the open rangeland area of vetch. Control group had free access to a normal water, while the second group received water enriched with 10 g NaCl/l. The obtained results indicated that water intake, body weight, live body gain daily, plasma glucose and gamma glutamyl transferase were not affected by increasing salt concentration in drinking water. However, plasma cholesterol, total protein, albumin, creatinine, urea triglyceride, were significantly affected ($p<0.05$&0.01) by salinity. Barbarine local sheep could tolerate the salinity of water

Keywords: *sheep, salinity, intake, blood and rumen metabolism.*

Introduction

Sheep are considered one of the most important grazing animals that can affect the social and economic status of people inhabiting arid and semi-arid regions. Water is an essential nutrient; therefore, it is important for animals to have an adequate supply of good quality water to survive and maintain satisfactory production (Schoeman and Visser, 1995). Around 50% of all water resources have salinity levels below 1.5 g/l, thus could be used for human consumption and above 1.5 g/l. Shetty (2004) estimated that 30% of the shallow aquifers have a salinity level above 4 g/l.

In practice, it is difficult to quantify the amount of salt intake. Animals may consume halophytes and/or drink saline water of various salinity levels.

However, the available findings on the effect of water salinity on growth rate and blood parameters of sheep are limited.

In Tunisia, particularly in the arid and semiarid regions, groundwater salinity is increasing and it is currently in the range of 3000 to12000 ppm TDS. To the best of our knowledge, the response of grazing sheep to the high salinity level is not documented. Therefore, the present experiment aimed to determine the effect of drinking saline water on growth rate and blood metabolites of Barbarine lamb grazing vetch without any supplement.

Materials and methods

Animals and management

This experiment was carried out from June to September 2013 at the experimental station of the National Institute of Agricultural Research of Tunisia (INRAT) at Bourbia (25 km south of Tunis. The average temperature during this spring period is 36°C. Twenty male barbarine sheep were used to determine the effect of saline drinking water on growth performances, water intake and blood parameters. Animals were weighed and divided into four groups on basis of body weight. Animals in each group grazed the common vetch (a local variety). They

were 2 weeks of adaptation. Animals were treated for internal parasites prior to commencement of the experiment.
The experimental groups were:
Group 1 (NW) (grazing + drinking fresh water)
Group 2 (NW) (grazing + drinking water + 10g salt).

The chemical compositions of water are shown in Table 1.
Table 1. Chemical composition of the water utilized during the trial.

Item	*Water*	
	Control	*NaCl enriched*
Dry residue (g/l)	1.08	11.24
pH	7.01	6.90
Sodium (mg/l)	200	3700
Potasium (mg/l)	5	12
Calcium (mg/l)	85	230
Magnesium (mg/l)	34	26
Chlorures (mg/l)	350	6568
Sulfates (mg/l)	230	144
Carbonates (mg/l)	0	0
Bicarbonates (mg/l)	6	6
S.A.R	4.62	61.54
D°H	35	68

Water intake, body weight, weight gain and blood parameters

The animals were grazed in the morning (from 06:00 to 09:00h) and afternoon (from 15h to 16h) and then moved back to the individual barn to receive the normal water (NW) or salt water (SW).
A sample of vetch, at the start and end of the feeding period, were analysed for dry matter (DM,), ash and N (Kjeldahl-N) according to AOAC (1990). The procedure described by Van Soest et al. (1991) using the ANKOM220 system was used to analyse neutral detergent fibre (NDF). Acid detergent fibre (ADF) of feeds was determined and expressed exclusive of residual ash (AOAC, 1990). Lignin of feeds was analysed by solubilisation of cellulose with 720 g/kg sulphuric acid (Robertson and Van Soest, 1981). The contents of NDF, ADF and lignin were expressed as ash-free.
During the experimental period, the average of the morning and evening drinking was taken as a daily water intake. Animals were weighed every two weeks fasting to monitor body weight changes.
Blood samples, before the morning feeding, were obtained via Jugular vein puncture using Heparin as the anticoagulant and were then centrifuged at 3000×g at 4◦C. The plasma was separated, and samples stored at -20°C for the subsequent spectrophotometric analysis of the cholesterol, total protein, albumin, creatinine, urea triglyceride, gamma glutamyl transferase contents using the Biomaghreb laboratory kits

Statistical analysis

Data on body weight, blood parameters and water intakes were subjected to the analysis of variance the mixed model with repeated measurements (SAS, 1991). The model included effects of watering regimens, period, and their interaction. Means were compared by the least significant difference (LSD) and significant difference between treatment effects was considered when P-value is below 0.05.

Results and discussions

Composition of diets and water

The chemical compositions of vetch are shown in Table 2.

Table 2. The chemical composition of vetch (%, DM basis)

	Start of the feeding period	End of the feeding period
Dry matter	90.34	91.98
Organic matter	93.25	84.68
Crude protein	16.2	5.4
Neutral detergent fiber	62.44	65.23
Acid detergent fiber	39.03	52.64

DM, dry matter;

The results of this analysis show that the vetch is low in CP at the end of feeding period (5.4%) and rich in NDF and ADF (65.23% and 52.64 %).

Effect of salinity water on water intake

Water intake is influenced by several factors; of which is the water quality (salinity). Species adapted to arid and semi-arid areas may consume more water than others (Wilson, 1989). The data represented in table 3 showed the effect of water on water intake. Animals responded to increasing salinity level in drinking water by increasing water intakes. However, water intake was not affected by the treatment (P>0.05). These results agree with those reported by Wilson and Dudzinki, (1973), Gihad and *al.* (1993) and Attia-Ismail and *al.* (2008).

Table 3. Effect of water salinity on water intake.

Item	*Diet*		*SEM*	*P- value*
	NW	*SW*		
Liter/day	5.01	5.06	0.120	0.789
Liter/kg $BW^{0.75}$	0.365	0.377	0.017	0.612
Liter/kg OMI	14.63	18.20	5.015	0.633

OMI, organic matter intake; NW, water normal; SW, water salinity; BW, body weight; SEM = Standard error of mean

Effect of salinity water on growth rate

The data represented in Table 4 showed the effect of drinking saline water on body weight. These data indicated that, initial body weights, final body weights and average daily gain of lambs remained unaffected by salinity (P>0.05). The results agree with those reported by Said Shannan et *al.* (2010).

Table 4. Effect of salinity water on body weight.

Item	*Treatments*		*ESM*	*P*- value
	NW	*SW*		
Initial body weight (kg)	23.60	23.45	1.480	0.942
Final body weight (kg)	33.49	32.24	1.588	0.585
Average daily gain (g)	152.07	147.53	6.798	0.642

NW, water normal; SW, water salinity; SEM = Standard Error of Means

Effect of salinity water on blood parameters

Plasma metabolites are presented in Table 5. The concentrations of glucose and gamma glutamyl transferase were not influenced by the water salinity (P>0.05). Assad and El-Sherif (2002) reported similar findings. However, plasma cholesterol, total protein, albumin, creatinine, urea and triglyceride were significantly affected (P<0.05& 0.01) by salinity. This result may relate to dilute out the retained salt by the fact that water is held to the body fluids (Tietz, 1982; Ayyat and *al.*, 1991; Suckow and Douglas, 1997).

Table 5 Effect of salinity water on blood parameters

Item	*Period, treatments*					*P- value*		
	1		*2*					
	NW	*SW*	*NW*	*SW*	*ES*	*Period*	*Diet*	*Interaction*
Albumine (mg/l)	55.03	90.90	58.91	87.53	4.004	0.950	<0.001	0.372
Creatinine (mg/dl)	0.90	1.26	0.88	1.33	0.031	0.478	<0.001	0.109
Cholesterol (mg/dl)	47.00	69.94	61.08	71.87	4.042	0.463	0.005	0.792
Triglyceride (mg/dl)	17.60	15.29	21.05	17.46	0.551	<0.001	<0.001	0.255
Urea (mg/dl)	36.02	50.75	39.66	54.01	1.323	0.013	<0.001	0.886
Total protein (g/l)	88.05	72.20	98.78	80.90	2.817	0.001	<0.001	0.721
Glucose (mg/dl)	49.12	47.70	54.90	51.74	1.810	0.010	0.215	0.637
ɤ-gt (U/l)	10.53	8.23	8.69	6.36	0.995	0.070	0.025	0.990

NW, water normal; SW, water salinity; SEM = Standard error of means

Conclusion

The current study revealed that the effect of saline water on water intake, growth performance and blood metabolites of Barbarine lamb can affect some parameters but cannot modify significantly other parameters. The results indicated better tolerance of Tunisian local sheep to saline load. Further study is required to determine if other breeds of local sheep can tolerate these salinity levels.

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EFFICIENCY OF BEANS AND PEAS USED IN THE DIET OF DAIRY COWS

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Abstract

Lactating Holstein-Friesian Black-and-White dairy cows (n=4×5) were included in the trial in the initial lactation phase with the average milk yield of 30.00 kg per day, fat content 4.10% and 3.20% protein content in milk. The analyses of the chemical content of beans and peas showed, that crude protein and undegraded intake protein were higher in beans than in peas, respectively 29.97% and 25.04% of dry matter, undegraded intake protein content, respectively 40.51% and 39.69% of crude protein. The highest content of starch was 48.54% in peas, and 43.29% in beans, but it was the lowest in soybean meal - 7.62% of dry matter. There were 17 amino acids in total detected in beans, peas and soybean meal. The highest concentration of arginine, leucine, glutamic acid, aspartic acid and isoleucine was in beans - 0.76%, 0.58%, 0.67%, 0.42% and 0.29%, respectively which was more than in peas. Even though the daily milk yields decreased for all the cow groups during the experiment, which was normal during the lactation period, yet the milk yield decreased for the trial groups. The milk yield decreased for the trial groups (1st and 2nd) were smaller – 0.8 kg, 1.3 kg, respectively, compared with the initial stage of the experiment ($P< 0.05$). In contrast, group 4 showed a significant decrease in the average daily energy corrected milk yield (4.9 kg), compared with the initial stage of the trial. Compared with the control group, none of the dietary interventions showed significant ($P>0.05$) deviations. The highest total amount of amino acids in milk was detected in 3rd and 2nd trial cow groups, fed with fodder beans and peas respectively, 4.00 g/kg and 3.90 g/kg, respectively.

Keywords: *beans and peas, amino acids, dairy cows, milk.*

Introduction

Sustainable feed production and feeding are key factors in achieving resource-efficient and resilient livestock production systems with minimal environmental load and high product quality *Faba* bean is a protein-rich legume seed well adapted to most climatic areas of Europe and widely used for feed. A sustainable animal diet is characterized as a diet that is balanced in all nutrients and free from harmful components, meets production objectives, generates animal products safe for human consumption, and integrates the planet, people, profit, and ethical dimensions of sustainability. Among the possible alternatives, the *faba* beans and peas appears interesting for dairy cow diet (Makkar, 2016; Makkar and Ankers, 2014; Crépon, Pascal, Peyronnet, *et al*., 2010). The common reason for livestock producers to plant *faba* beans is to obtain a protein source that is home grown and that can easily be processed on-farm, as they contain little oil. They also do not contain anti-nutritional enzymes and therefore do not need roasting. Faba beans are related to lima beans and contain approximately 30% protein (Volpelli *et al*., 2010; Heeg, 2016). Field peas are highly digestible and highly fermentable in the rumen, but have a slower starch and protein fermentation rate than several other common feeds. Therefore, fodder beans and peas be used as an important forage legume to enhance feed values for dairy ruminants, especially of importance to today's high yielding dairy cows (Jensen, 2002; Ipharraguerre and Clark, 2005). The European Union policy on the protection and enhancement of biodiversity on agricultural holdings has contributed to an increase in the area sown with legumes in Latvia.

The objective of the present paper is to investigate the beans and peas in the diet of dairy cows' feed.

Material and Methods

Trials were carried out in the farm "Upites", Allazu Parish, Allazmuiza Municipality during the winter period from December to February (Latvia). For the trial, four analogue (according to yield, lactation phase, live weight, fat content and protein content) treatments groups of 20 animals of Holstein-Friesian Black-and-White cows were used in the study. Feeding trial was carried out 90 days. The experimental design is reported in Table 1.

Table 1. Scheme of the trial.

Group of cows	Number of cows	Feeding programme
1st trial	5	Basic feed + 10-12% *Pisum sativum*'Bruno' + 10 - 12 % *Vicia faba minora*'Lielplatone'
2nd trial	5	Basic feed + 20-24% *Pisum sativum*'Bruno'
3rdtrial	5	Basic feed + 20-24% *Vicia faba minora* 'Lielplatone'
4th control	5	Basic feed with soybean cake

The average live weight of cows in all groups were 650 kg the mean age was 3.0 lactations. The cows were in the initial phase of lactation were included in the experiment with the average yield of 30.00 kg per day, fat content 4.10% and protein content 3.20%. The cows are kept on the same farm, under equal feeding, housing and exploitation condition. During the trial, the dairy cows received the basic feeding ration (Table 2).

Table 2. Dairy cows feeding during the trials.

Feedstuffs	Amount, kg	1st trial	2nd trial	3rd trial	4th control
Silage	40	40	40	40	40
Hay	3	3	3	3	3
Concentrated feed	4	4	4	4	4
Complementary feed	4	4	4	4	4
Peas plus beans	-	1.82	-	-	-
Peas	-	-	1.9	-	-
Beans	-	-	-	1.7	-
Soybean meals	-	-	-	-	1
Mineral additive	0.15	0.15	0.15	0.15	0.15
Feed ration contains:					
Dry matter, kg	-	21.60	21.70	21.50	20.80
Crude protein, g	-	3266	3261	3276	3258
NEL, MJ	-	142.7	143.8	139	137.20
Calcium, g	-	153	157	155	162
Phosphorus, g	-	82.0	83.0	85.0	82.0

The feeding ration varied according to each cows' milk yield and physiological state, and was corrected monthly depending on the results of control milk yield, dry period and state of health. When elaborating the feeding rations, we took into consideration the following: amount of feedstuffs, dry matter (DM), net energy for lactation (NEL, MJ), amount of crude protein, calcium and phosphorus (Nutrient Requirements…, 2001).

A full value chemical analyses were conducted by the accredited Scientific Laboratory of Biotechnology, Department of Agronomic analyses of the Agronomical Analyses under the Latvia University of Life Sciences and Technologies. The biometric data were analysed by a Mann-Whitney test at the significance level $\alpha = 0.05$ to identify differences in comparison with the control group (Montgomery, 2012). All statistical analyses were performed using SPSS for Windows version 20.0.

Results and Discussion

The chemical composition of fodder beans, peas and soybean meal are presented in Table 3. As can be seen from data, the content of dry matter in peas (*Pisum sativum*) "Bruno" and fodder beans (*Vicia faba minor*) "Lielplatone" was high, 90.78% and 90.44% respectively but in soybean meal - 87.41%. Crude protein content was the highest in soybean meal - 50.61% and beans - 29.97%, while in peas - 25.04% of dry matter. Soybean meal had significantly high content of UIP - 73.99% of crude protein.

After summarizing reference data, undegraded intake protein (UIP) content in soybean meal in the rumen fluctuates within the range of 75 to 78 per cent (Singh *et al*., 2012). Relatively high percentage of UIP was also in fodder beans - 40.51%, whereas in peas it was 39.69% of the crude protein. According to Batterham and Egan (1986), the amount of UIP can make up 46% of crude protein content.

Table 3. The chemical composition, on dry matter basis, %.

Indices	Beans "Lielplatone"	Peas "Bruno"	Soybean meal
Dry matter, %	90.44	90.78	87.41
Crude protein, %	29.97	25.04	50.61
UIP of crude protein, %	40.51	39.69	73.99
Crude fat, %	1.09	1.22	1.60
Starch, %	43.29	48.54	7.62
Crude fiber, %	9.5	7.21	3.57
NDF, %	15.79	20.58	13.98
ADF, %	11.36	9.59	6.91
NEL, MJ kg^{-1}	7.7	7.84	7.89
Crude ash, %	3.61	3.28	1.76
Ca, %	0.12	0.07	0.42
P, %	0.66	0.43	0.71
Digestibility, %	80.00	81.40	81.80

The highest concentration of arginine, leucine, glutamic acid, aspartic acid and isoleucine was in fodder beans, respectively 0.76%, 0.58%, 0.67%, 0.42% and 0.29% of dry matter more than in peas (Table 4). Methionine, cystine, lysine, threonine, histidine, phenylalanine, tyrozine, glycine, serine and alanine amount in beans and peas was similar. The highest concentration of amino acids was shown soybean meal.

Table 4. The amino acids content, on dry matter basis, %.

Amino acids	Beans "Lielplatone"	Peas "Bruno"	Soybean meal
Methionine	0.21	0.22	0.61
Cystine	0.33	0.30	0.68
Lysine	1.71	1.50	2.80

Threonine	0.97	0.77	1.82
Arginine	2.54	1.78	3.35
Isoleucine	1.14	0.85	2.10
Leucine	2.05	1.47	3.45
Valine	1.21	0.95	2.21
Histidine	0.69	0.50	1.20
Phenylalanine	1.14	1.04	2.32
Tyrosine	0.84	0.64	1.63
Glycine	1.17	0.90	2.00
Serine	1.22	1.01	2.37
Proline	-	0.77	-
Alanine	1.10	0.92	2.06
Aspartic acid	2.82	2.40	5.27
Glutamic acid	4.42	3.75	8.29

However, compared to cereals, the content of lysine in beans is relatively high and the contents of the sulphur-containing amino acids cysteine and methionine are low. According to reference materials, beans mainly contain lysine, with minor concentrations of methionine and tryptophan (Crepon et al., 2010).
The greatest decrease in cow productivity (Table 5) was observed for the control group – by 3.98 kg of milk but a smaller decrease was observed for the 3 rd group – by 0.26 kg, of milk compared with the before the trial (P < 0.05).

Table 5. Cow productivity during the trials, on average, kg.

Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	Comparison between before and end values
1st trial	22.68	23.52	22.24	20.46	-2.22
2nd trial	23.48	21.58	21.76	21.38	-2.10
3rd trial	20.74	19.70	20.28	20.48	-0.26
4th control	24.62	24.92	21.96	20.64	-3.98
p–value (relative to control)					
Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	*P*-value (ralative to values at the before the trial)
1st trial	0.465	0.600	0.917	0.917	0.225
2nd trial	0.917	0.347	0.917	0.754	0.138
3rd trial	0.251	0.251	0.917	0.916	0.893
4th control	-	-	-	-	0.043 S

S significant differences (P < 0.05)

The content of total amount of amino acids in milk is shown in Table 6. The highest increase was found in the bulk milk samples of the 3rd and the 2nd trial group, 4.00 g/ kg and 3.90 g/ kg, respectively. The lowest increase was in the bulk milk samples of the 1st trial group 1.80 g/ kg, respectively, compared with the initial stage of the experiment. However, an increase in the total amount of amino acids was observed in the control group, respectively 2.30 g/kg. The trial group 3rd, which were fed beans, showed the amount of total amino acids in milk that was 1.70 g/kg higher than in control group that was fed soybean meal ($P<0.05$).

Table 6. The content of total amount of amino acids in milk, g/kg.

Group of cows	Before the trial	End of the trial	Comparison between initial and final values
Group of cows	30.2±0.44	32.0±0.68	+1.80
1st trial	29.2±0.49	33.1±0.71	+3.90
2nd trial	30.5±0.37	34.5±0.52	+4.00
3rdtrial	31.4±0.32	33.7±0.39	+2.30
4th control	30.2±0.44	32.0±0.68	+1.80

It is known that the cow farming technology, including cow feeding, can considerably affect milk yield. After examining the effects of different amounts of dietary legumes on productivity of dairy cows, Vander *et al*., (2008) demonstrated no significant changes when soybean flour is partially replaced by peas (150 g/kg). A similar finding was made by Tufarelli *et al*., (2012) who reported that replacing soybeans with faba beans (345 g/kg) in the diet for highly productive dairy cows (the average milk yield of 35 kg a day) did not influence the cows' productivity.

Conclusions

Obtained results of chemical composition of beans "Lielplatone", peas "Bruno" and soybean meal the content of crude protein content was the highest in soybean meal - 50.61% and beans - 29.97%, while in peas - 25.04% of dry matter. The highest content of starch was in peas - 48.54%, beans - 43.29% but the lowest in soybean meal - 7.62% of dry matter.

Upon the whole, it can be concluded that the highest rate of essential amino acids was detected in soybean meal and beans "Lielplatone", while the lowest - in peas "Bruno".

During the trial, the cow productivity indicators decreased for all the groups, which was normal during the lactation period, yet the daily milk yield decreases for the trial groups were smaller – 2.22 kg, 2.10 kg and 0.26 kg, respectively, compared with before the trial and the control group.

The highest total amount of amino acids in milk was detected in 3rd and 2nd trial cow groups, respectively, 4.00 g/kg and 3.90 g/kg, which was fed fodder beans and peas but the lowest amino acid amount was detected in trial group 1st - 1.80 g/kg, which was fed peas plus fodder beans compared with the initial stage of the trial.

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FIRST RECORDS OF *ICHTIOPHTHIRIUS MULTIFILIIS* ON CYPRINID FISHES FROM AQUACULTURE FACILITIES IN MACEDONIA

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Abstract

A total of 192 specimens of common carp (*Cyprinus carpio*), 38 specimens of grass carp (*Ctenopharyngodon idella*), as well as 26 specimens of bighead carp (*Hypophthalmichthys nobilis*), from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. This study was carried out by seasons. Protozoa *Ichthyophthirius multifiliis* is the largest known parasitic protozoan found on fishes. In our study, this protozoa was found in all seasons, except in winter. Total, prevalence with *I. multifiliis* in cyprinid fishes from aquaculture facilities in Macedonia, by seasons, was as following: spring - 0.79 %; summer - 2.99 % and autumn - 0.44 %, while mean intensity was: spring - 12.56; summer - 26.70 and autumn - 50.00. The records of *I. multifiliis* in grass carp and bighead carp in the present study is considered as the first records in Macedonia. Also, these two fish species is regarded as new hosts for *I. multifiliis* in Macedonian waters.

Keywords: *protozoa, Ichthyophthirius multifiliis, common carp, grass carp, bighead carp*

Introduction

One of the largest family of freshwater fishes in the world is family Cyprinidae, with several genera, among which *Cyprinus*, *Hypophthalmichthys* and *Ctenopharyngodon* are the most significant, with representatives of common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*) and grass carp (*Ctenopharyngodon idella*), respectively. These are warm water fish species which are successfully grown in aquaculture facilities in Macedonia.

Parasites are among the pathogenic factors that are very important for fish health and responsible for economic losses in the fish industry. According Blazhekovikj - Dimovska and Stojanovski (2015), parasitic diseases represent negative factor, which in intensive fish farming can cause considerable damages in aquaculture. Parasites in the waters where they are present sometimes cause fish reduction, thereby directly affecting on fish stocks in the country.

The ciliated protozoan, *Ichthyophthirius multifiliis*, the causative agent of ichthyophthiriasis or Ich, is one of the most important pathogenic parasites of cultured fish (Schäperclaus, 1991). This protozoan is widely distributed to all parts of the world and almost all of the freshwater fish are susceptible to infections. According Buchmann *et al.* (2001), the life cycle of this parasite is divided into three stages: a trophont, a tomont (which is divided into tomites) and a theront. The trophont is a parasitic stage that lives and feeds on the epidermis of the fish-host, where it can reach a diameter greater than 1 mm. A number of studies have shown that all these life cycle stages are extremely temperature dependent. The mature parasite is very large, up to 1000 μm in diameter, very dark in color due to the thick cilia covering the entire cell and moves with an amoeboid motion.

According Svobodova and Kolarova (2004), ichthyophthiriasis is one of the most serious parasitic diseases in freshwater fish which can cause large losses in fish population. The same authors noticed that high water temperatures, high stocking density, fish weakening as a result of malnutrition, are important conditional factors affecting on disease appearance. The disease appears in all facilities for intensive fish breeding.

In order to take certain preventive measures, it is necessary to know the composition of parasite fauna in economically most important fish species, their seasonal dynamics as well as prevalence and mean intensity of certain parasites in different age categories of fish in aquaculture facilities.

The aim of this study was to determine the presence of protozoan parasite *I. multifiliis*, prevalence, mean intensity, as well as, seasonal changes of parasite species in fish family Cyprinidae from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs. This study has chosen a protozoon which can impose a noticeable economic damages and losses to the fish breeding.

Material and methods

This parasitological study was carried out by seasons. A total of 192 specimens of common carp (*Cyprinus carpio*), 38 specimens of grass carp (*Ctenopharyngodon idella*), as well as 26 specimens of bighead carp (*Hypophthalmichthys nobilis*), from eight the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. These aquaculture facilities included fish farms Zhabeni, Bukri, Dolneni and Zhelezara, as well as cage culture systems on reservoirs Tikvesh, Mladost, Globochica and Gradche.

The fish were caught using net or hook by local fishermen. The specimens were placed in plastic tanks with ice and transferred to the research laboratory. The work on specimens was achieved immediately. Fish were killed by vertebral dislocation. The gill filaments, the eyes, the fins, the skin and the intestine of fishes were inspected by stereomicroscope and in second phase direct smears were prepared from probable lesions.

All parasites found in each individual fish were identified and enumerated. Preparation, fixing, staining and mounting of parasites were made by general methods used in parasitology. During the study period, data on parasite species were categorized according to season. Classical epidemiological variables (prevalence and mean intensity) were calculated according to Bush *et al.* (1997). The parasite specimens were identified according reference keys of Bykhovskaya-Pavlovskaya *et al.* (1962) and Gussev (1983).

During the examinations at Laboratory for fish diseases in Hydro - biological Institute in Ohrid (Macedonia), stereomicroscopes „Zeiss"- Stemi DV4 and „MBS 10", as well as light microscope „Reichart" were used.

The number of fish examined, fish infected, prevalence and mean intensity (total and by seasons) are given in tables.

Result and discussion

A total of 256 specimens of fish family Cyprinidae (192 specimens of common carp, 38 specimens of grass carp, as well as 26 specimens of bighead carp) from eight the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations and 53 fish (20.70 %) were infected with protozoan parasite *Ichthyophthirius multifiliis*. This parasitological study was carried out by seasons.

Protozoa *I. multifiliis* is the largest known parasitic protozoan found on fishes. In our study, it was found in 53 specimens of fish family Cyprinidae, from two cyprinid aquaculture facilities:

1. On fins and gills in 34 specimens of common carp (*Cyprinus carpio*) from cage culture system on reservoir Globochica, in summer season. The prevalence with *I. multifiliis* in

common carp was 3.55 %, while the mean intensity was 26.70.
2. On fins and gills in 14 specimens of grass carp (*Ctenopharyngodon idella*) from fish farm Zhabeni, in spring season. The prevalence with *I. multifiliis* in grass carp was 18.42 %, while the mean intensity was 25.93.
3. On gills in 5 specimens of bighead carp (*Hypophthalmichthys nobilis*) from fish farm Zhabeni, in autumn season. The prevalence with *I. multifiliis* in bighead carp was 9.43 %, while the mean intensity was 50.00.
In our study, this protozoan was found in all seasons, except in winter. Total, the prevalence with *I. multifiliis* in cyprinid fishes from aquaculture facilities in Macedonia, by seasons, was as following: spring - 0.79 %; summer - 2.99 % and autumn - 0.44 %, while the mean intensity was: spring - 12.56; summer - 26.70 and autumn - 50.00.
The records of *I. multifiliis* in grass carp (*Ctenopharyngodon idella*) and bighead carp (*Hypophthalmichthys nobilis*) in the present study is considered as the first records in Macedonia. These two fish species is regarded as new hosts for *I. multifiliis* in Macedonian waters. Also, this is first finding of *I. multifiliis* in common carp from cage culture system on reservoir Globochica.
Data on fish examined, fish infected, as well as the prevalence and mean intensity with *I. multifiliis* (total and by seasons) are given in Table 1 and Table 2.

Table 1. Number of examined and infected fish, percent of infestation, total prevalence (E) and mean intensity (I) with protozoan parasite *Ichthyophthirius multifiliis*

Fish species	Number of fish examined	Number of fish infected	Percent of infestation	Total	
				I	E (%)
Common carp ***(Cyprinus carpio)***	192	34	17.70 %	26.48	4.23
Grass carp ***(Ctenopharyngodon idella)***	38	14	36.84 %		
Bighead carp ***(Hypophthalmichthys nobilis)***	26	5	19.23 %		

Table 2. Prevalence (E) and mean intensity (I) with protozoan parasite *Ichthyophthirius multifiliis* in fish family Cyprinidae from aquaculture facilities in Macedonia, by seasons

Fish species	Spring		Summer		Autumn		Winter	
	I	E (%)	I	E (%)	I	E (%)	I	E (%)
Common carp ***(Cyprinus carpio)***	/	/	26.70	3.55	/	/	/	/
Grass carp ***(Ctenopharyngodon idella)***	25.93	18.42	/	/	/	/	/	/
Bighead carp ***(Hypophthalmichthys nobilis)***	/	/	/	/	50.00	9.43	/	/

During this study, infection with *I. multifiliis* was recorded on fish in all seasons with exception of winter (Table 2). Mean intensity of *I. multifiliis* was not varied among the spring and summer seasons, but it was significantly higher in autumn season. The greatest value of mean intensity was recorded in autumn (50.00) in bighead carp.
Prevalence (%) of *I. multifiliis* was the highest in spring (18.42) and lowest in summer (3.55) (Table 2).
I. multifiliis is a single cell infusoria size of 0.2 - 1 mm. It has a round or an egg shape, with thick cilia on the surface of the body, which uses for moving and attaching to the fish body. In

the middle of the body it is a fat macronucleus that has a crescent shape. This parasite is visible with eye.

Fig. 1. *Ichthyophthirius multifiliis* (original)

Moreover, in our study, we were unable to detect any trophont of *I. multifiliis* in the winter season, in all three fish species. Frequent ichthyophthiriasis epizootics at higher water temperature (24– 26ºC) were also reported from the farms of North America (Wood, 1979) and Europe (Valtonen and Keräen, 1981).
According to the present results, the prevalence and mean intensity of *I. multifiliis* in grass carp was 18.42 % and 25.93, respectively, and it is relatively high. However, different prevalence and mean intensity in various fish species has been reported in previous literatures (Kuperman *et al.,* 2002). Therefore, the diversity of hosts of this parasite and various prevalence and mean intensities are probably related to the different possible influences of environmental and nutritional factors.
Moreover, temperature changes have main effect on each stage of development e.g. encystment, division and maturity of *I. multifiliis* (Aihua and Buchmann, 2001). Fish species with high prevalence probably are living in a favorable temperature for parasite, because considerable changes in temperature will kill these ciliates (Nigrelli *et al.,* 1976). Buchmann and Bresciani (1997) demonstrated that *I. multifiliis* in traditional fish farms was more prevalent at high temperatures. Moreover, our study clearly shows that the development of *I. mulifiliis* was inhibited at lower temperatures and increased at higher temperatures.
The external parasites of fish were previously investigated in Macedonia. To date, *I. multifiliis* have been recorded in fish from Macedonian waters. According to the data from the previous parasitological research in Macedonia, Hristovski *et al.* (1999, 2001) have established the *I. multifiliis* of skin and gills in common carp from the fish farm Zhabeni. This parasite has been determined by Hristovski *et al.* (2006, 2012) in other fish species from Prespa Lake, including *Rutilus rubilio prespensis, Barbus cyclolepis prespensis* and *Leuciscus cephalus albus*. According to the literature data from the surrounding countries, findings on the presence of *I. multifiliis* in common carp and grass carp in Bosnia and Herzegovina were first published by Žitnan *et al.* (1969) in the fish farm "Vuchijak" – Prnjavor, in waters in Serbia by Cakić

(1992), while in Croatia this parasite was established at the fish population in the Krka River, by Valić *et al.* (2005).
Data on the presence of *I. multifiliis* on the gills, in the body and nasal cavity of common carp from fish farms in Romania, were published by Radu *et al.* (2008); in two-year-old carps from fish farms in the Czech Republic, by Ondračková *et al.* (2012); while Szekely & Molnar (1996 - 1997) established this parasite in common carp from Lake Balaton, Hungary. According to literature data from the world, the presence of *I. multifiliis* in common carp was determined by Cengizler *et al.* (2001) in the river Seyhan, Turkey; Raissy *et al.* (2010) and Bozorgnia *et al.* (2012) in fish farms in Iran; Ali *et al.* (1988), Al-Marjan and Shamall (2008) and Mansoor and Al-Shaikh (2010) in fish farms in Iraq and Subasinghe (1992) in fish farms in Sri Lanka.
According Abowei *et al.* (2011), the severe infestations with this parasite in fish result in anxiety, atrophy, body irritation and asphyxia. Morbidity can be 100%, while mortality occurs only in significant damage to the gills and skin. According Sao Clemente *et al.* (2000), in the more advanced stages of the disease, the fish become lethargic, stop consuming food and die from asphyxia.
Sao Clemente *et al.* (2000) and Dickerson (2006) considered that the *I. multifiliis* has a wide distribution and parasites in many freshwater fish in all stages of development, ranging from offspring to adult fishes, which is correlated with the results of our research.

Conclusions

Ichthyophthirius multifilis is an epizootiologically important parasite in all economically significant fish species that are grown in cyprinid aquaculture facilities, attacking the skin, gills and fins of the fish. Ichthyophthiriasis (Ich) or "white spot disease" is one of the most serious diseases of fishes and as a devastating pathogen in aquaculture, is responsible for substantial economic losses worldwide.
In our study, total, the prevalence with *I. multifiliis* in cyprinid fishes from aquaculture facilities in Macedonia, by seasons, was as following: spring - 0.79 %; summer - 2.99 % and autumn - 0.44 %, while mean intensity was: spring - 12.56; summer - 26.70 and autumn - 50.00. This protozoan was not found in winter.
The prevalence with *I. multifiliis* in common carp, grass carp and bighead carp was 3.55 %, 18.42 % and 9.43 %, respectively, while the mean intensity was 26.70, 25.93 and 50.00, respectively.
The records of *I. multifiliis* in grass carp (*Ctenopharyngodon idella*) and bighead carp (*Hypophthalmichthys nobilis*) in the present study is considered as the first records in Macedonia. These two fish species is regarded as new hosts for *I. multifiliis* in Macedonian waters.
Also, this is first finding of *I. multifiliis* in common carp from cage culture system on reservoir Globochica.
Since these parasites are dangerous and cause a strong negative impact on fish population, it is necessary to identify such parasites and take control measures, especially for important fish species such as common carp, bighead carp and grass carp.

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THE INFLUENCE OF FORAGER BEES NUTRITION WITH DIFFERENT POLLENS ON THE DEVELOPMENT OF HYPOPHARINGEAL GLANDS

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Abstract

The influence of the feeding of forager bees with the pollen of different quality on the size of the hypopharyngeal glands was studied in this paper. Forager bees, 1-2 days old, were sampled from the frames with an emerging brood and were kept in mini cages where they were fed with different types of pollen. Pollen was collected during the pollination of the red clover, and it was classified by color in 4 categories: beige, yellow, orange and gray. The beige pollen was from red clover while other colors were not identified. Pollen quality was determined by standard chemical methods, and the following parameters were studied: ash, crude protein, fats, cellulose, macro-elements (P, K) and certain micro-elements (Ca, Mg, Fe, Zn and Mn). The gray pollen had the highest ash and crude protein, while the beige one had the most crude fats and cellulose. Pollen was added into the sugar dough (cake). The control treatment was without pollen, and only the sugar dough was used. The largest glands have developed in the group of bees fed with the beige pollen (8.17 µm), and the smallest glands were found in bees fed only by sugar dough (the control, 6.53 µm) and with the gray pollen (6.84 µm), although it had the most protein. The differences between treatments were statistically significant. Three homogeneous groups were distinguished, and bees fed with red clover pollen were very different from other studied groups.

Key words: hypopharyngeal glands, nutrition, forager bees, pollen

Introduction

The royal jelly or hypopharyngeal glands of the honey bees are located in the front of the head, with the outflow vessels opening behind the tongue. Their basic function is to secrete the royal jelly which is used to feed worker and drone brood in the initial stage of development, while the queen bee keeps feeding in both the developmental and the adult stages of its life. The glands are set as a cluster and their highest activity is from 6 to 10 days of worker bee's age (Stanimirović *et al*., 2006). After this period, their function decreases and changes in the bees that begin to fly out in the field - forager bees (Kubo *et al*., 1996). Functional and morphological transition from the nursing bees to the forager bees is controlled by juvenile hormone (Jaycox, 1976; Robinson, 1987). In the worker bees hatched at the end of the summer and in autumn, the hypopharyngeal glands function throughout the winter, until spring (Plavša and Pavlović, 2018). For the normal growth and function of these glands, pollen feed is very important in both the larval stage (developmental period) and after hatching (Maurizio, 1961; 1962a). The lack of pollen in the feeding of young bees impedes the function and growth of these glands. If worker bees are later fed normally with pollen, the glands can grow and regain their function (Maurizio, 1962b). Standif (1967) proved that the feeding of bees with red clover pollen more favorably affects the development of the hypopharyngeal glands compared to feeding bees with the same concentration of alfalfa (*Medicago sativa*) and black poplar (*Populus nigra*) pollen. Ohashi *et al*. (2000) found that in

the colonies without queen bee, most of the older bees (80-90%) can retain or restore the function of these glands to the level of functioning as in the nursing bees (age 6-12 days). The amount of synthesized royal jelly was maintained at the level of a normal colony (colony with a queen bee) for at least two months.
The aim of the study was to determine how the additional honeybee bee feeding with pollen in their first days of life (1-10) influences the development of the hypopharyngeal glands. Particular attention was paid to the pollen of the red clover (*Trifolium pratense*) because the pollen-gathering colonies were in the selection stage for the increased foraging of pollen of this leguminous species for more successful pollination.

Material and methods

For this experiment, the honey bees from the apiary of the Institute for forage crops were used. The experiment was conducted in 2014. Bees of age 1-2 days were used for this experiment by separating frames with a capped brood (emerging brood). After 48 hours the bees were hatched from it. These bees were kept in small, glass, experimental hives and fed with a sugar dough in which different pollen was added (15%). Pollen was collected at the time on the red clover in bloom (late June and early July). Pollen was taken by pollen collectors which were set in the production honey bee colonies. After collection, the pollen was dried (in the pollen dryer at 42°C) and sorted by the color as B - beige, Z - yellow, N-orange and S - gray pollen. Pollen of beige color was collected on a red clover (determined by a microscope) while for the other groups of differently colored pollen the origin of the plant species could not be accurately determined. Control treatment was a group of bees fed only with a sugar dough. From each mini-hive, 50 worker bees were sampled for the measurement of the glands. The measurement was done 15 days after feeding. The size of the glands was measured under a microscope (Olympus), using the measurement tile. Total of 250 samples. For all groups of pollen, quality analysis was carried out according to standard methods. All analyzes were done according to the Rulebook (Official Gazette of RS 101/2015). Phosphorus was determined by spectrometric method SRP, ISO (6491/2003), and potassium, macro and micro elements were determined by the AAS method (AAS-Perkin Elmer 1100 B USA). Each sample was done in three repetitions. ANOVA was performed using the computer program Statistica 8.0 (StatSoft) in a completely randomized design in three replications. The treatments were compared to each other by a LSD test at a significance level of 0.01.

Results and discussion

It was found that the size of the hypopharyngeal glands averaged at 7.25 μm. The largest glands were found in bees that were fed with a sugar dough with added red clover pollen (beige pollen). In second place were bees fed with orange pollen, then yellow and at the end were the bee fed with the dough with added gray pollen (Table 1). The smallest glands were determined in bees fed only with the sugar dough (6.53 μm). No protein component (pollen powder) was added to the sugar dough used to feed these. The fact that the bees fed only the sugar component have the smallest glands was also found by DeGrandi-Hoffman *et al*. (2010). They have shown that supplementary diet of pollen can also reduce the additional losses of bee colonies, which is very important for wide beekeeping practice.

Table 1. Average values of the size of hypopharyngeal glands of worker bees (μm), number of bees and standard deviation.

Treatment	Average	Number	Standard deviation
B-beige	8.1674[a]	50	1.1009
N-orange	7.2796[b]	50	1.0962
Z-yellow	7.4208[b]	50	0.8402
S-grey	6.8418[c]	50	0.5814
Control	6.5308[c]	50	0.8163
All groups	7.2481	250	1.0601
F test	0245**		
Lsd 005 001	0.26 0.42		

* p < 0,05
* * p <0,01
ns - no significance
a, b, c –homogenous groups within which there are no significant differences, and there are significant or very significant differences between groups

After the monofactorial analyzes of variance and comparison by the LSD, it was found that three homogeneous groups were formed according to the size of the hypopharyngeal glands. The largest glands were in bees fed with a sugar dough with beige pollen (pollen of red clover). The size of the glands in this group of bees was an average of 8.17 μm and very significantly differed from other groups of bees. The size of the glands of bees fed with yellow and orange pollen did not differ from one another, and compared to the other two homogeneous groups, they differed significantly. The gland size in the bees fed with gray pollen and in the bees from control did not differ from each other, but they differed significantly from other treatments.

By chemical pollen analysis, it was found that the highest crude protein was in gray pollen (Table 2). This pollen had the highest amount of ash (3.15%), as well as certain macro (P, Ca and Mg) and micro elements (Fe, Zn). Pollen of red clover (beige color) had the most crude fat. The yellow pollen was the richest in K and Mn, and the pollen of orange color had the most crude cellulose.

When considering the influence of pollen quality on the development of the hypopharyngeal glands, it can be noticed that gray pollen, although it had the highest protein content, and most of the macro and micro elements examined did not yield the best results. Although this pollen had the most residue in the analysis, it had the most of the ash. Bees fed with this type of pollen had the least developed hypopharyngeal glands of all bees fed with food with added pollen. They had larger glands compared to control treatment where bees were fed with pure sugar dough. The largest hypopharyngeal glands were found in bees fed with the red clover (beige) pollen. This pollen contained the most crude cellulose and almost the same amount of crude fat as orange pollen (Table 3), but the ratio of other components was well balanced and gave very positive results in the development of the hypopharyngeal glands. This pollen, although it had 6.11% less crude protein and twice less phosphorus (P) and iron (Fe) compared to gray pollen, led to a significantly higher development of the hypopharyngeal glands (19.4%). Feeding bees with red clover has led to an increase in the size of hypopharyngeal glands by 26.1% compared to the control. It is interesting to note that the diet with gray gray, which according to its quality was at the level of bee pollen-bread (Anđelković *et al.*, 2011) and it led to an increase in the hypopharyngeal glands by 4.7% compared to control. This can also be attributed to the high amount of residues that this pollen (crude ash) has, as well as double the higher proportion of individual elements (P and Fe), which obviously have a negative impact on the development of the hypopharyngeal glands.

Table 2. Pollen quality, crude ash, protein, cellulose, fat (%), macro and micro elements.

Pollen color	Crude ash	Crude protein	Crude cellulose	Crude fat	K %	P %	Ca %	Mg %	Fe mg/kg	Zn mg/kg	Mn mg/kg
B	2.07	21.14	3.55	11.75	0.30	0.42	0.37	0.15	56.31	42.97	14.42
Ž	1.51	13.17	1.41	9.27	0.63	0.29	0.28	0.15	55.95	25.04	17.77
N	1.71	14.16	2.00	11.88	0.47	0.29	0.40	0.10	65.42	28.45	12.91
S	3.15	27.25	1.68	9.88	0.32	0.80	0.79	0.20	135.22	45.37	12.59
$\overline{X}$	**2.11**	**18.93**	**2.16**	**10.70**	**0.43**	**0.45**	**0.46**	**0.15**	**78.22**	**35.46**	**14.42**

Conclusion

After monitoring the influence of feeding bees with various types of pollen on the development of royal jelly (hypopharyngeal) glands, the following conclusions can be made:
The measured hypopharyngeal gland size was, on average, 7.25 µm and ranged from 6.53 µm in control to 8.17 µm in bees fed with the red clover pollen.
Three homogeneous groups were isolated from the treatment, and the size of the hypopharyngeal gland of the bees fed with red clover pollen was significantly different in comparison to other treatments.
Although the gray pollen showed the highest values for most quality parameters (crude proteins, macro and micro elements), bees fed with this pollen had larger hypopharyngeal glands compared only to the control treatment.
The size of the glands in bees fed with red clover pollen was 26.1% higher than in the control (bees fed only with the sugar dough).

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RESULTS ON CHROMIUM (Cr) CONCENTRATION IN WILD FISH SQUALIS CEPHALUS &BARBUS BARBUS TISSUES IN VARDAR RIVER, NORTH MACEDONIA

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Abstract

The concentrations of Chromium (Cr) in fish from the river Vardar have been investigated in order to assess safety for consumers and the level of contamination. The selected tissues (muscle**,** liver and skin) of two fish species: Europian Chub (*Squalius cephalus*) and Common barbel (*Barbus barbus*) from river Vardar stream, Republic of North Macedonia. Samples are collected in nine different points (Hot Spots) in total distance of 301km and approximate 33km between sampling sites. The effect of environmental conditions and urban discharges on Chromium (Cr) accumulation in muscles, liver and skin were investigated. The metal analyses were performed using Inductively coupled plasma mass spectrometry (ICPMS). The average of metal concentrations (micrograms per gram wet weight) in nine hot spots (HS) occurred in the following ranges:HS-1: muscles 0.25 - liver 1.00 - skin 0.10, HS-2: muscles 0.25 - liver 0.70 – skin 0.20, HS-3: muscles 0.10 - liver 0.30 - skin 0.10, HS-4: muscles 0.15 - liver 2.40 – skin 0.10, HS-5: muscles 0.10 - liver 0.54 - skin 0.04, HS-6: muscles 0.10 – liver 0.20 - skin 0.00, HS-7: muscles 0.10 - liver 1.00 - skin 0.10, HS-8: muscles 0.14 - liver 1.40 - skin 0.00 and HS-9: muscles 0.15 - liver 1.35 - skin 0.00. The lowest levels of the Chromium (Cr) were detected in the skin. The muscles and liver were found to accumulate the highest amounts of Cr. In the case of organs, the highest levels were found, as follows: liver > muscles > skin. Further investigation of heavy metals is recommended, including a survey of fish consumption frequency among the local inhabitants.

Keywords: *heavy metals, Chromium, fish tissue, Republic of North Macedonia*

Introduction

Fish is an important part of the human diet, but also a good indicator of trace metal pollution in the aquatic ecosystem. Fish samples are considered as one of the most indicative factors, in fresh water systems, for the estimation of trace metals pollution potential (Rashed,2001).Organisms retain Cr, through gastrointestinal system in to the liver, soft tissue muscle to deposite in the end at the kidney, gills, ovaries and testes. Pollution of water bodies is becoming a major cause of concern with respect to human health (Jarup, 2003).Heavy metal from anthropogenic pollution source are released in to the aqua systems ,and then heavy metal become serious threat because of their toxicity, long persistence bioacumulation and biomagnifications in the food chain (Eisler, 1988). Metals in waters may be of natural origin from the rocks and soil or from human activities, including industry, domestic wastewater, agricultural discharge, mine runoff, solid waste disposal and atmosphere aic deposition. Metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes.

Increase in the human population has greatly contributed towards the conversion of these water bodies to impending contamination sinks (Tarvainen et al., 1997; Stephen et al., 2000).

Heavy metals are well known to be non-biodegradable and when present at high concentrations, they tend to bioaccumulate (De Forest et al, 2007).Being non-biodegradable, metals can be concentrated along the food chain, producing their toxic effects at points often far away from the source of the pollution (Fernandez et al., 2000). Heavy metals can cause a variety of ailments in humans depending on the degree of exposure. These vary from minor skin irritation to severe damages of the liver, kidney, skeleton, nerve tissues and circulatory system. Liver and muscle are usually used as a targeted tissues for the analysis of the heavy metal concentration, according to the studies carried out with the different fish species have shown that trace metals are accumulate mainly in metabolic organs such as the liver, where metal are stored for detoxication through the metalothioniens (Carpene and Vasak,1989;Kargin and Erden 1991;Hogstrand and Howx,1991)

Chromium (Cr) is a heavy metal but is essential as an enzyme cofactor, which may become a very toxic when accumulating in the important organs like liver and spleen (Wagner and Boman,2003).Chromium is an essential nutrient metal, necessary for metabolism of carbohydrates. Chromium entering the aquatic ecosystem thru discharges from the lether industries, metal finishing, electroplanting, mining, printing industries, pharmaceutical industries, etc. Poor treatment of these effluents can lead to the presence of the Chromium in the water bodies as a Cr (VI) where it can be commonly found in the high level concentration and potentially harmful for the fish. Hexavalent Cr (VI) is considered to be toxic because of its power to potentially oxidate and ability to cross cell membranes (WHO, 1990).

River Vardar is the longest river in the Republic of North Macedonia and Republic of Greece with distance of over 388 km, with his source in the location Vrutok and stream at the Aegean sea. The river Vardar, with its tributaries, makes up a great part of the total water resource of North Macedonia. These tributaries and river Vardar are directly or indirectly connected with the mining areas and foundry for Chromium (Cr) in the locations. The first reported information about ichthyo fauna of river Vardar vas from Steindachner (1892) and then he describe the Leucos macedonicus from the river Vardar. Some fish species in the river Vardar (Squalius cephalus,Barbus Barbus, Vimba vimba) are good examination samples for pollution because they are in the all stream of river.The ichthiofauna of the Vardar river is important part of the aquatic food chain for their omnivores way of feeding. The river is widely used for fisheries (wild fish and fish farming), sports and recreation. Very little recent information is available regarding the contamination with metals in the Vardar river fish species. The river Vardar passes near and across the biggest cities including Gostivar, Tetovo, Skopje, Veles, Negotino, Demir Kapija and Gevgelija with possibility of environmental contamination from domestic and industrial sewage Hot point spots in Fig.1.

Fig.1 In the map of the Republic of North Macedonia, showing hot point spots

The aim of this study is to provide information and evaluate the level of Chromium (Cr) as a heavy metal in fish organs (muscle, liver and skin) from Europian Chub (*Squalius cephalus*) and Common barbel (*Barbus barbus*) fish species.

Material and methods

Samples were collected in nine (9) places with distance between them in around 33km along the river Vardar. From each place fishes were collected from both species with support from fishermen’s licensed for sport fishing in the North Macedonian fishing Federation.

Fish samples were transported with the plastic bags set in transport refrigerator in laboratory submitted for dissection of target organs were collected for study including skin, liver and muscle. Each sample of fish tissue has been measurement with analytical scale then set in plastic bag, marked with number which show the catching location and tissue then finally refrigerated below the -18ºC.Total numbers of samples were sixty (60) from Europian Chub and Common barbell.

The next step was the sample to set in the porcelain pots then the sample was heated in microwave in 105°C for 24h to drain. The next day the samples were taken out from microwave and they passed to the stove for 24h in 550°C where they were burned. After 24h the burned samples are prepare for digestion with HNO3 65%.Prepared sample after digestion are ready for reading and analyze by Inductivly coupled plasma mass spectrometry with the ICP-MS type Agilent 7500 series. All data are presented in the unit mg/kg wet weight of a sample tissue.

Results and discussion

Results of this study showed that the metal concentrations accumulated in the tissue samples were in descending order of liver > muscles >skin. In the study, we found that the concentration of Chromium (Cr) were different in the analyzed organs and differently in the sampling locations. The average of concentration of the Chromium (Cr) in the organs (muscle, liver and skin) and the locations (nine locations) is showed in Table 1.

Table 1. Mean Heavy Metal Content (mg/kg wet weight) in Fish muscle, liver and skin

Sampling point	Muscle	Liver	Skin
HS-1	0.25	1.00	0.10
HS-2	0.25	0.70	0.20
HS-3	0.10	0.30	0.10
HS-4	0.15	2.40	0.10
HS-5	0.10	0.54	0.04
HS-6	0.10	0.20	0.00
HS-7	0.10	1.00	0.10
HS-8	0.14	1.40	0.00
HS-9	0.15	1.35	0.00

Higher Chromium (Cr) concentrations were found in liver tissue, while the lowest were detected in skin tissues. This finding is in agreement with those of other studies regarding the differences between heavy-metal accumulation in fish tissue (N. Biba, J. Mavromati, 2014).
There guidelines on acceptable levels of in Chromium (Cr) the edible parts of fish suggested in North Macedonia is according to international standards 0.73 mg/kg (IAEA) and the 2< mg/kg (Chine). According to results, there is metal contamination, but it is higher than the guidelines, in the edible part of the examined fish. The examined fish were not associated with enhanced Chromium (Cr) content in their muscle and were safe within the limits for human consumption. From results it can be seen that authors obtained a different Chromium (Cr) concentration, and as we can see that highest level is in the HS-4 sampling site, which is the point where the river Lepenci is connected to the river Vardar, waste water and atmospheric water from capitol city Skopje is load to the river Vardar, the uper part between city of Veles and Skopje, so it is expected to have registered Chromium (Cr) pollution. In the most studies of similar analyzed samples, the liver accumulate the highest concentration of Chromium (Cr) in our study is shown also that is the samples of liver in sampling sites HS -4 and till the HS-9, showed that fishes in the part of river Vardar from Skopje till the border with Republic of Greece accumulated higher concentration of Chromium (Cr). In previous study, increased concentrations of these hazardous substances also, especially Pb and Cd, in water and sediment from the lower part of the Vardar River, influenced higher accumulations of metals in liver, gills and gonads of Gobio gobio L. (Nastova, et al. 2017), concentration of Chromium (Cr) in skin is not reported.
In our country the river pollution with heavy metal contaminations are in the concern because off potential thrived waste from domestic and industrial sewage, non-secured industrial landfill and mining fields. The Chromium (Cr) are regarded as potential hazards that can endanger both animal and human health. Knowledge of their concentrations in fish is therefore important both with respect to nature management and human consumption of fish as suggested in (Amundsen et al. 1997).
In Vardar river, among all river aquatic organisms the fish are the most interested for humans for sport activities in particular, source of food mainly for fisherman's and their families as well. Fishes are considered as indicators in river ecosystem for heavy metal contaminations with light level risk as a potential for human food consumption, because fish are in the top of aquatic food chain, heavy metal accumulation and possibly for the transfer heavy metals on the humans.

Conclusion

Preliminary results provide information for the levels of Chromium (Cr) in common fish species of the river Vardar. Results will contribute to the effective monitoring of both environmental quality and the health of the organisms inhabiting the river ecosystem. According to the fish sample analyses the range of concentration with Chromium (Cr) in the higher border with the range of international standards 0.73 - 2 mg/kg wet weight, and it shows that the fishes from investigated Vardar river are safe for human consumption.

Aquatic organisms have been widely used in biological monitoring and assessment of safe environmental levels of heavy metals. In this study concentration of Chromium (Cr) in the muscle of both fish species were used to investigate possible transfer of Chromium (Cr) to human populations via fish consumption. Since accumulation of metals in the biological system is dangerous to human beings in our country, there is a need for regular or continuous monitoring of heavy metals concentrations in the aquatic environments.

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GENOMIC DIVERSITY AND LEVEL OF ADMIXTURE IN THE SLOVAK SPOTTED CATTLE

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Abstract

For the assessment of the population structure, evaluation of the genetic relationships and possible admixture between breeds the Bayesian clustering algorithm was used. Genetic analyses of relationships and admixture were performed in the local maintained population of Slovak Spotted cattle (N=85). The loss of diversity due to the rapid decrease of the population's size and the unequal use of founders was indicated by pedigree analysis before. To analyse the level of admixture, genotyping information of other, closely related breeds involved in the formation of Slovak Spotted cattle were used; Holstein (N=99), Swiss Simmental (N=78), Pinzgau (N=151) and Ayrshire (N=10). After LD pruning ($r^2 \leq 0.05$) final dataset consisted of 423 animals and 5,770 SNP markers. Generally, the F_{ST} (in average 0.15) index showed low value of genetic differentiation among evaluated populations mainly due to their common historical origin and high values of gene flow. Unsupervised analysis of population structure resulted in the most likely expected cluster number K=3–5. The graphical visualization of K=5, the likelihood that the individuals belong to the respective five populations, allowed to describe genetic variability and admixture of respective breeds. Observed changes among membership probability pointed out that the Slovak Spotted is closest to the Slovak Pinzgau cattle. Even both breeds having different history, selected set of SNPs could reflect the relationship to the authentic cattle living in Carpathians. Gene flow of Holstein, Ayrshire and Swiss Simmental in formation of Slovak Spotted could be proposed.

Key words: *admixture, Slovak Spotted cattle, population structure, Wright fixation index.*

Introduction

The genetic diversity is an important tool which allows for the genetic improvement of animals with respect to their economically important traits (Melka *et al.*, 2013). For the estimation of genetic diversity are used multiple methods including evaluation of genetic structure. According to Andam *et al.* (2017) the genetic structure represents the organization of genetic variation which is directed by combined effects of mutation, genetic drift, recombination, demographic history and selection.

To analysing population structure was developed of several statistical methods, among the most common belong admixture-based clustering (Pritchard *et al.*, 2000; Alexander *et al.*, 2009). Various methods are available for the evaluation of genetic admixture proportions in populations; often used the Bayesian algorithm STRUCTURE (Pritchard *et al.*, 2000) and principal components analysis (Patterson *et al.*, 2006). Analysis of admixture represents an important tool which can be applied on genomic data to study genetic background of local population. It can be defined as a phenomenon that can occur as a result of introgression and hybridization of individuals, populations or species (Kadlečík *et al.*, 2017b). Using this indicator, it is possible to explain the historical or geographical origin of an animal. According to Kasarda *et al.* (2015) different histories among the populations can affect the frequency of allele among populations of the genome and this deviation may be analysed for example by F_{ST} index. Wright's F_{ST} index (Wright, 1931) is an indicator that represents the degree of population differentiation resulting from the genetic structure. At the beginning, the

F_{ST} index was defined as the relationship between two random sampled gametes from the same subpopulation in the case the correlation between two sampled gametes from the total population was set to zero. Currently, more definitions of F_{ST} are available that are based on a variety of different conceptual formulations but with a focus on rating some aspect of population differentiation (Holsinger and Weir, 2009). Development of new technologies was increased potential for exploring genetic diversity, population structure as well as the common history of populations. To ensure the breeding goal of Slovak spotted cattle, it is advisable to know the extent of admixture of other breeds. In this study, SNP high-density data were used for evaluation of genetic diversity, population structure and admixture in four cattle breeds, which have contributed to the formation of Slovak Spotted cattle.

Materials and Methods

The analysis consisted of a total of 423 cattle genotypes from 5 breeds and was comprised of an SNP array dataset, which was created from a combination of new data and previously published data. The genomic information about Holstein (N=99), Swiss Simmental (N=78), Slovak Pinzgau (N=151), Ayrshire (N=10) and Slovak Spotted (N=85) was obtained by two platforms: Illumina BovineSNP50v2 BeadChip and ICBF International Dairy and Beef v3. (McTavish *et al.*,2013a). The quality control of SNP data was done by using program PLINK v1.9 (Chang *et al.*, 2015). From the database were excluded SNPs with unknown chromosomal position according to the latest bovine genome assembly (Btau 5.0.1) and markers located on sex chromosomes (Moravčíková *et al.*, 2017). The remaining individuals were then subjected to the next quality control. SNPs with a call rate of less than 0.90 and minor allele frequency (MAF) of 0.05 were excluded from the dataset and remained 39 934 SNPs. Some SNPs that were in high linkage disequilibrium (LD) were pruned out using command indep-pairwise 50 5 0.05 by software PLINK v1.9. This command left 5 770 SNPs for further analysis. Kijas *et al.* (2009) reported that pruning of SNPs in high LD prevents ascertainment bias and generate a meaningful comparison between breeds. To infer the genetic structure and the degree of admixture were used the Bayesian clustering procedure adopted in STRUCTURE v2.3.4 software (Pritchard *et al.*, 2000). The analyses were run in ten replications separately from K=1 to K=10. The individual simulations consisted of the burn-in period of 1000 followed by Monte Carlo Markov chain (MCMC) simulations steps of 10 000 iterations. The results were exported to STRUCTURE HARVESTER (Earl and von Holdt, 2012), which implements the Evanno's method to infer optimal number of clusters (K) in analysed dataset (Evanno *et al.*, 2005). The value of the K and values of log probability of the data (L [K]) for each cluster were estimated.
Genetic differentiation among populations was estimated using Wright fixation index according to Weir and Cockerham (1984). Akey *et al.* (2002) reported that the F_{ST} index is one of the most appropriate parameter to describe the genetic differentiation among subpopulations (values move range from 0 to 1). For calculation of F_{ST} index, program R and package StAMPP (Pembleton *et al.*, 2013) was used.

Results and Discussion

The algorithm was primarily focused on identifying related individuals without a reference to prior information of the genetic division. The aim was to determine the degree of genetic admixture among the 5 evaluated cattle populations. For determination of the genetic population structure of each evaluated breed and estimation of admixture level among them the correlated allele frequencies model was used. The results of Delta K showed that the optimal number of clusters, which represents most similarly ancestor of breeds, ranged from K=3 to K=5. The membership probabilities at the K=3 indicated formations of three genetically separated clusters; first composed from Swiss Simmental, second from Holstein and Ayrshire, and the last one composed from Slovak Spotted and Slovak Pinzgau cattle (Figure 1A). At the K=5 (Figure 1B), Slovak Spotted and Slovak Pinzgau breed were divided into two separate clusters. In Figure 1, each animal represents one vertical line which is split into K coloured segments. The mixed colours reported information about the level of admixture for individually predefined populations of K among 3 and 5.

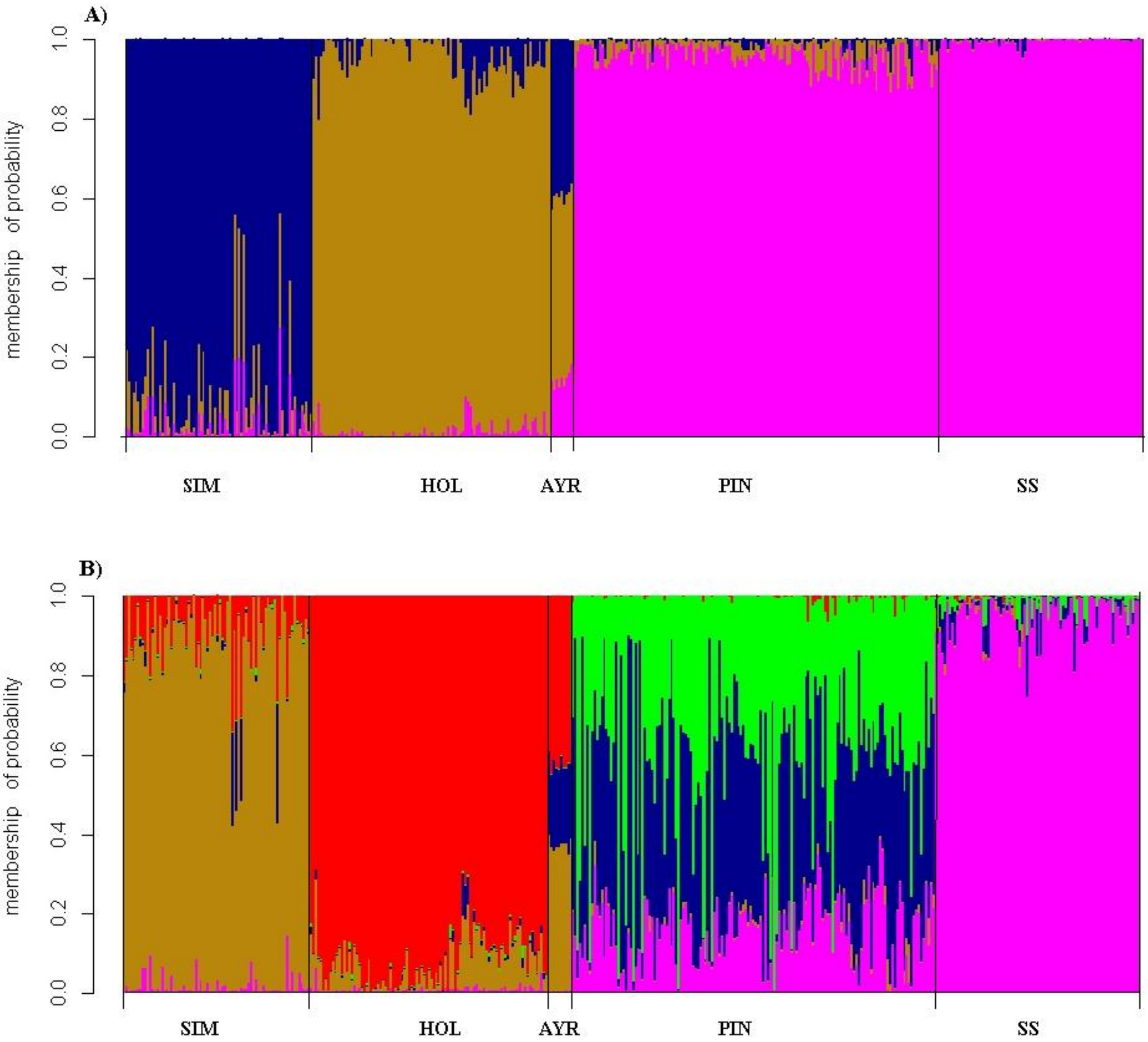

Figure 1 Results of the Bayesian clustering analysis implemented in STRUCTURE v 2.3.4 (Pritchard *et al.* 2000) analyses inferred using the ΔK statistic of Evanno *et al.* (2005) for the five cattle breeds. A. K=3 yields the highest value of ΔK. Three groups corresponding to K=3 are shown here. B. K=5 yields the highest value of ΔK. Three groups corresponding to K=5 are shown here. Each individual is represented by a vertical line, which is divided into K coloured segments that represent the individual's estimated membership proportion in each of the clusters. SIM – Swiss Simmental, HOL – Holstein, AYR – Ayrshire, PIN – Slovak Pinzgau, SS – Slovak Spotted.

Figure 1A showed among the evaluated breeds low level of the admixture, especially for the Slovak Spotted and Pinzgau breeds, with neglectable representation of other breeds. Slovak

Spotted and Slovak Pinzgau had the highest membership probability in the fourth and fifth cluster.
In the first cluster, the highest membership probability of Swiss Simental was found, while within the other clusters this breed showed only a low proportion, except for the Ayrshire breed. In the second cluster, the highest proportion reached the Holstein breed. This cluster showed the representation of Simental and Ayrshire breeds, but the values of membership probability not exceeded 20%. The Ayrshire breed in the third cluster pointed out a higher proportion of admixture, but the small number of individuals (N=10) it is not enough to fully confirm the genetic composition of this breed. The highest value of membership probability for Ayrshire breed was assigned in the fourth cluster, while this breed was involved in the grading-up process of Slovak Pinzgau cattle (Figure 1B). On the contrary, in the K=5 (Figure 1B) it can be seen, that the Slovak Pinzgau cattle in the fourth cluster had a high level of admixture. The Ayrshire breed reached highest representation, which exceeded more than 50%. In addition, the low representation of Simental and Holstein can be seen, but neither breed exceeds 20%. The last cluster represents Slovak Spotted cattle. Figure 1A indicated the low representation of Swiss Simental and Holstein, but Figure 1B showed representation of all evaluated populations.
The results reflected mainly the history of cattle breeding in Slovakia. The current genepool of the Slovak Spotted and Slovak Pinzgau cattle was affected by various breeds including extincted autochthonous Carpathian Red and Carpathian Grey cattle (Kasarda *et al.* 2015) with Swiss Simmental, Austrian Pinzgau, Holstein and Ayrshire. Based on our results the Slovak Spotted is more related to Slovak Pinzgau (Figure 1A) than Ayrshire in comparison to Swiss Simental. The reason may be a significant "grading-up" in recent years, as stated by Šidlová *et al.* (2015). Especially after 1972 due to efforts to increase milk production, bulls of the Ayrshire and Holstein breeds were used in the breeding. This can also be seen in Figure 1B. Obtained results pointed out certain signals of admixture between the Holstein and the Swiss Simmental breed. Signer - Hasler *et al.* (2017) reported admixture, where Swiss Simmental was clearly divided from Holstein breed, although those breeds share common ancestors. Kim and Rothschild (2014) examined admixture between Holstein and Ayrshire, which are used in improvement of multi-purpose breeds globally and have common ancestors. Results reflects the history of these two breeds. Similar conclusions were reported by Šidlová *et al.* (2015).

Table 1 Wright F_{ST} index among evaluated breeds

	SS	**AYR**	**HOL**	**SIM**	**PIN**
SS					
AYR	0.198				
HOL	0.157	0.089			
SIM	0.192	0.102	0.085		
PIN	0.032	0.21	0.167	0.192	

Wright (1978) and Hartl and Clark (1997) introduced that the F_{ST} values up to 0.05 indicated low genetic differentiation among evaluated populations. The F_{ST} values in range 0.05–0.15 reported about moderate differentiation and value from 0.15 to 0.25 suggested relatively high genetic differentiation in metapopulation. The values >0.25 indicated very high genetic differentiation.
The results showed that the F_{ST} values were lowest between two Slovak breeds (Slovak Spotted - Pinzgau cattle) compared to other (Table 1). The highest differentiation was found between Ayrshire and Pinzgau breed. The highest differentiation was found between Ayrshire and Pinzgau breed. The lower value was expected due to the fact, that the Pinzgau breed

undergone grading up with Ayrshire breed among 1971–1989. In the same period, Pinzgau breeding bulls and semen from Austria were imported which could cause such a high differentiation. Kasarda *et al.* (2018) confirmed a common genepool of Slovak and Austrian Pinzgau (F_{ST} = 0.13). Kadlečík *et al.* (2017a) which evaluated pedigree data of Slovak Spotted cattle through Wright fixation index confirmed genetic differentiation (F_{ST} =0.098) among subpopulations.

Obtained results of F_{ST} values for other evaluated breeds expressed high differentiation, which was based on how these breeds were involved in the formation of Slovak Spotted breed. Signer – Hasler *et al.* (2017) estimated slightly higher F_{ST} index between Swiss Simmental and Holstein (0.142). This could be related to the fact that Holstein was involved in formation Slovak Spotted breed, but not as much as up to that extent like for the Swiss breeds. Based on our results Ayrshire breed reached values of F_{ST} close to 0.2 which could indicate higher incidence of heterozygotes in the subpopulations. Our results were consistent with the results of Kim and Rothschild (2014) which confirmed the higher incidence of heterozygotes in the F1 generation of evaluated breeds. The F_{ST} results confirm the occurrence of genetic differentiation between the subpopulations. However, none of the subpopulations has reached a higher representation than 0.25. Our results reached slightly lower values, which indicates a higher presence of homozygotes in the evaluated subpopulations. Between two local Slovak Spotted and Slovak Pinzgau populations low genomic differentiation was found. This information can be used in the selection strategy or conservation programs for the Slovak Spotted Breed within the strategy of small homeland heritage.

Conclusion

Results showed low level of genetic differentiation as well as particular gene flow among analysed breeds. This study provides new information about the level of admixture and genetic structure of evaluated breeds occurring in Danubian region. In five evaluated breeds were found discrete genetic features, which should be taken into account in conservation programs in regard of preservation of genetic diversity. The negligible value of genetic differentiation was found within the Slovak Spotted and Slovak Pinzgau cattle genome, which can be due to the common selection history. In the future, priority on increasing genetic diversity and population size should be given, including the active exchange and use of proven sires of sires and sires of dams.

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RELATION BETWEEN THE FREE SALIVA TESTOSTERONE LEVELS AND BEHAVIOR IN NEW ROOM IN THE PIGLETS UNDER STRESS

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Abstract

The aim of this study was to evaluate changes in the free testosterone saliva (TSL) in pigs under stress and its relation to behavior. Fifty-five pigs were tested (28 gilts and 27 barrows). Males were castrated in 2nd week after birth. Behavior in new room was tested in open-field arena. Test lasts 20 minutes and was recorded by one camera. Saliva samples for TSL analyses were sampled in calm state before testing and immediately after test. Behavior was analyzed from records using Noldus Observer XT software. TSL were measured by ELISA method. TSL in calm state varied from 78.91 pg/mL to 621.37 pg/mL and from 93.12 pg/mL to 1589.36 pg/mL after stress situation in new room. There was not difference in TSL between gilts and barrows. TSL after stress was higher than TSL in calm state in 34 animals (group I), and in 21 animals (group D) TSL after stress was lower than TSL in calm state. For group I TSL in calm state did not correlate with TSL after stress ($r = 0.191$, $P = 0.278$). For group D TSL in calm state correlate with TSL after stress ($r = 0.603$, $P = 0.004$). Analysis did not show difference in behavior between I and D group. Despite no difference in behavior, correlation analysis detected small difference in relation between evaluated behavior traits and TSL in calm state as well as after stress. ANOVA by TSL quartiles in calm state did not show difference between pigs with low and high TSL values. ANOVA by TSL quartiles after stress discovered significant difference in total duration standing between pigs with the lowest (Q_1) and the highest (Q_4) TSL.

Keywords: *Pig, Piglet, Behavior, Open-field, Testosterone.*

Introduction

The testosterone plays role in many physiologic and metabolic processes, from the prenatal period to the end of life. The testosterone has impact to the development of male reproductive tissues, promotion to secondary sexual traits, body growth and mass, health status and well-being (Prader 1962, Colenbrander et al., 1978, Mooradian et al., 1987, Traish, 2014, Caliber and Hackett, 2019). The testosterone level is affecting the brain development, social and sexual behavior, as well as orientation behavior (Arnold and Breedlove, 1985, Cronin et al., 2003, Fredriksen, et al., 2004, Albrecht et al., 2012). The testosterone become play important role in the pork production in the recent years. Public as well as scientists concerns to the surgical castration as source of the pain with issues to the growth and welfare (Dunshea et al., 1993, Prunier et al. 2005, Fredriksen et al., 2011, De Briyne et al., 2016). The entire males number for pork production is increasing in the Europe, but it leads to risk boar-tainted carcasses (Schanbacher et al., 1985, Bee et al., 2015). Heritability of the boar taint components allows use effective selection in the breeding programs (Willeke et al. 1980, Ducro-Steverink, 2006, Squires 2006, Tajet et al., 2006, Winding et al., 2012, Robic et al., 2012, Lervik et al., 2013, Haberland et al. 2014). Promptly prediction of the steroid levels in young animals could be useful for the selection beneficial for elimination the boar taint problem, particularly in the breeding. Testosterone changes under stress are often investigates mainly in human, but results are inconsistent. A source of this inconsistency may be a nature of stress triggers (Bedgood et al., 2014). Choi et al. (2012) reported the significantly decrease salivary testosterone in men under stress from pain. On the contrary Bedgood et al. (2014) presented increasing testosterone level under social stress in men, but in connection to basal levels of cortisol. Higher levels of the testosterone found Lane et al. (2015) in men after physical exercise. Psychological stress significantly increased the testosterone levels in men but no significant decrease in women (Afrisham et al., 2016). Some animal model studies are reported in study Edinger and Frye (2005). Authors suggested effect of the testosterone anti-anxiety and analgesia and in behavior change under stress. The aim of this study was to evaluate changes in the salivary testosterone levels under stress and its relationship with behavior in new, unknown room in pig.

Materials and methods

Animals and housing

Experiment was carried out at the Experimental Centre for Farm Animals (ECFA) of Slovak University of Agriculture in Nitra. Fifty-five pigs Large White with live weight from 18.5 kg to 27.5 kg were tested (28 gilts and 27 barrows). Males were castrated in 2nd week after birth. All procedures related to animals were performed in accordance with guidelines of the Slovak University of Agriculture Ethics Committee. Pigs were housed in pairs in the pens measuring 3100 mm × 1070 mm. The pen has solid floor without bedding, metal walls, metal barns in front and metal gate in back. Floor was cleaned twice a day. Feed was provided ad libitum due automatic feeding system. Feed mixture was prepared individually for each pen by average daily gain. Gain was calculated from weekly weighting. Water was provided due nipple drinker, one in each pen.

Behavior test

Behavior in new room was tested in the open-field arena in same stall at ECFA. The open-field arena measured 4000 mm × 4000 mm is acoustic insulated, has solid walls, door and one window with mirror glass above animal area, window border is 120 cm above floor. Adjacent room is observation room, observer can see to the open-field arena though window. Arena was cleaned between tests to reduce odor from the pig in the preceding test. For the behavior test each pig was picked up from the pen, gently moved to the opened entrance door to open-field arena and let to enter it and door was closed. Test lasts 20 minutes. After 20 minutes assistant enter open-field area and collects saliva. After saliva collection pig was moved back to the pen. Behavior was recorded from CCTV system, camera was mounted above arena and connected to PC in observation room. The Noldus Observer XT 11.5 software was used for behavior analysis. Latency of standing (LS) and locomotion activity was scored: moving (M), standing (S) and lying (L). Initiate behavior was M. Behavior was recorded by continuous recording, M, S and L behavior was scored as state event, latency of standing as point event.

Saliva collection and testosterone analysis

Saliva was collected in calm state (C), approximately 30 minutes before open-field test and after stress (S), immediately at the end of open-field test. Saliva was collected by allowing the pig to chew sterilized cotton tampon until it was moistened (about 60 seconds). Tampons were pre-prepared and stored in sterilized laboratory glass tubes. Moistened tampon was placed back to tube and centrifuged 15 minutes at 3000 rpm. Collected saliva was filtered and stored at -20ºC until analysis. The testosterone level was measured by ELISA method, commercial kit Dia Metra Testosterone saliva was used. Optical absorbance was measured by Microplate Reader Model DV 990BV4, UniEquip Deutschland.

Statistical analysis

Difference in testosterone levels in calm state (TSLC) and in stress state (TSLS) between gilts and barrows (tested by t-test for independent samples) was not significant and therefore disregarded in all other analysis. The TSLS was higher than the TSLC in 34 pigs (group I), and in 21 pigs (group D) the TSLS was lower than the TSLC. Difference in the sex ratio in I and D group was tested by chi-square test and was not significant. Differences in the TSLC and the TSLS in whole group (n = 55) and groups I (n = 34) and D (n = 21) were compared by paired t-test. Difference between I and D groups in TSLC and TSLS was tested by t-test for independent samples. Relation between TSLC and TSLS was evaluated by Pearson Correlation coefficient. Behavior in open-field arena was evaluated by LS, total duration (TD), mean duration of bout (M), total number of bouts (TN) of M, S and L. Difference in behavior between I and D groups was tested by t-test for independent samples. Relation between behavior and TSLC as well as TSLS was evaluated by Pearson Correlation coefficient. Difference in behavior among pigs with various TSLC and TSTS was tested by One Way Analysis of Variance (ANOVA), with quartile classification as fixed factor. Statistical analysis was performed with IBM SPSS version 20.

Results and discussion

The TSLC in whole group (n = 55) ranged from 78.91 pg/mL to 621.37 pg/mL, mean value was 297.714 pg/mL and s.d. was 130.671 pg/mL. Moya et al. (2008) reported very similar testosterone levels in pig from 3 weeks to 8 weeks of age (mean = 290 pg/mL, s.d. = 40 pg/mL). The TSLS in whole group (n = 55) ranged from 93.12 pg/mL to 1589.36 pg/mL. The mean value TSLS was significantly higher than TSLC (TSLS mean = 394.98 pg/mL and s.d. = 273.102 pg/mL, $P < 0.05$). Six piglets had TSLS higher than average TSL in non-castrated males in paper Moya et al. (2008). Similar changes in levels of testosterone after stress in men reported Choi et al. (2012), Bedgood et al. (2014), Lane et al. (2015) and Afrisham et al. (2016). Choi et al. (2012) presented decrease but other authors increase. Testosterone level in women after stress was lower, but no significant in paper Afrisham et al. (2016). Interesting fact in our investigation is the TSLS was higher than the TSLC only in 34 pigs and in 21 pigs the TSLS was lower than the TSLC. Difference between groups I and D in mean values of TSLC and TSLS was significant as well as difference between TSLC and TSLS in groups I and D (Tab. 1). Difference between gilts and barrows ratio in group I and D was not found (Tab. 2).

Table 1. Saliva testosterone levels in group I (n = 34) and group D (n = 21).

	Mean TSLC [pg/mL]	s.d. TSLC [pg/mL]	Mean TSLS [pg/mL]	s.d TSLS [pg/mL]
Group I	248.611*	113.084	493.513*	306.414
Group D	377.213*	119.446	235.451*	56.879

* P < 0.001

Table 2. Gilts and barrows ratio crosstabulation by TSL change after stress (n = 55).

		Group I	Group D	Total
Gilt	Count	17.0	11.0	28.0
	Expected Count	17.3	10.7	28.0
Barrow	Count	17.0	10	27
	Expected Count	16.7	10.3	27.0
Total	Count	34.0	21.0	55.0
	Expected Count	34.0	21.0	55.0

$\chi^2 = 0.029$, $P = 0.864$

The TSLC and the TSLS correlate only in group D ($r = 0.603$, $P < 0.01$), in whole group (n = 55) and in group I did not correlate ($r = -0.068$, $P > 0.05$, $r = 0.191$, $P > 0.05$ resp.).

Comparison of means of evaluated behavior traits by t-test did not show difference between I and D group. Despite no difference between means, correlation analysis discovered some minor differences in relation between TSL and some behaviors. The TSLC significantly correlate with TDS (r = -0.464, P < 0.01), ML (r = 0.369, P < 0.05), TNM-0.469, P <0.01) and TNS -0.501, P < 0.01) in group I. The TSLS significantly correlate with TDL (r = 0.402, P < 0.05), ML (r = 0.463, P <0.01) and LS -0.367, P < 0.05) in group I. The TLSC significantly correlate only with ML (r = 0.481, P < 0.05) and the TSLS significantly correlate with MM (r = 0.469, P < 0.05) and TNS (r = -0.507, P < 0.05) in group D. Because of small frequency of lying (28 pigs do not lie down and 19 piglets showed one lying bout) important difference is in TDS, TNM, MM and LS. The correlation between the ML and the TSLS over all group (r= 0.434, P = 0.001, n = 55) suggests connection between lying and testosterone level in stress. Comparison of behavior by ANOVA with fixed factor TSLS quartile classification discovered significant difference in TDS and LS between pigs with the highest TSL and pigs with low TLS in stress. Animals with the highest TSLS shower significantly shorter TDS and LS (Fig. 1, 2).

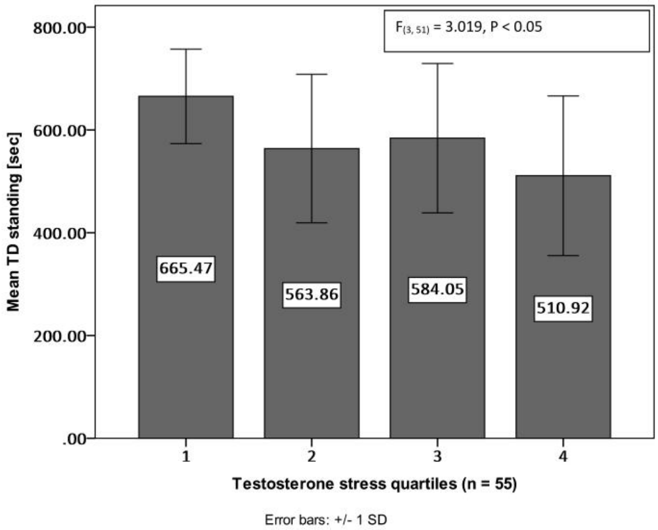

Figure 1. Mean values of the total duration standing in piglets divided to groups by the saliva testosterone level in stress quartiles.

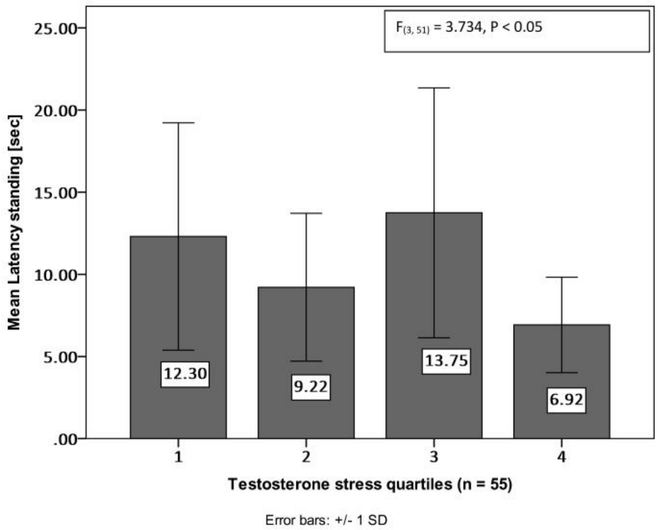

Figure 2. Mean values of the latency standing in piglets divided to groups by the saliva testosterone level in stress quartiles.

Behavior of pigs with the highest TSLS (shorted standing and longer lying) can be considered as expression of less anxiety. The role of higher levels of TS and other androgens in modulation anxiety in human as well as animals suggests many authors (Choi et al. 2012, Bedgood et al., 2014, Lane et al., 2015, Afrisham et al., 2016, Edinger and Frye, 2005). Rats with higher TSL in the open-field tests showed more exploratory behavior, less freezing (Edinger and Frye, 2005. Unfortunately, difference in the behavior is not enough to reliable detection of the pigs with high TSLS, because of variability (Fig. 1, 2). Comparison of behavior by ANOVA with fixed factor TSLC quartile classification did not show difference between pigs with low and high TSL values.

Conclusion

In summary, results suggest the TSL in pigs is changed in stress. Generally, TSL after stress is higher than in calm state, but in some animals is direction of change opposite and TSL after stress is lower. Source of this change is unknown and should be investigated. There is not difference between gilts and barrows in direction of change. The TSL in stress in some animal is higher than in non-castrated males. Change is probably very short in time, but in case of long term stresses the relation of quality of pork and TSL should be investigated in next research. The TSL correlates with some behaviors, but relationship is not very strong. Difference in evaluated behavior traits between animals with low and high TSL is not very expressive. I next research not only the total duration of behaviors but the course in time series during tests should be considered.

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6. RURAL DEVELOPMENT AND AGRO-ECONOMY

CROP PRODUCTION AND FOOD CONSUMPTION FOR FARMERS' WELFARE IN RWANDA

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Abstract

Agriculture is a source of livelihood for increasing population in the world. It provides mainly food and is expected to avail enough income to farmers and thus improve their livelihood through the increased yield. This study attempted to assess the effects of crop production and food consumption on farmers' welfare. We used secondary data collected at national level during the Fifth Integrated Household Living Conditions from October 2016 to October 2017. For data analysis, Chi-Square test, Pearson's correlation coefficient, t test, and ordinary least-squares (OLS) methods were used. The results revealed that the crop output increased with the increase in inputs (labour, fertilizers, pesticides, and seeds). The return to scale of crop production was 1.06, which implies that the crop production system scored increasing returns to scale. The OLS estimates indicated that food consumption was positively influenced by the age and the marital status of the household head, the household size, farm income, land size, crop production, when bean, maize, potato, rice and soybean were the main crops selected by the farmers, while it was negatively affected by the sex of the household head, when coffee and wheat were the crops chosen by the farmers. The results from Pearson's correlation analysis showed that food consumption was positively and significantly (p=0.00) correlated with family size, farm income, land size, and crop production. With reference to these findings, we recommend that the strategies to increase the crop yield and farm income and thus sustain food consumption and improve farmers' welfare should be enhanced.

Keywords: *crop yield, farm income, food consumption, farmers' welfare, Rwanda*.

Introduction

Crop production should be a primary source of rural development and a cornerstone of farmers' livelihood through the increased yield (Gollin et al., 2002) specifically in less developed economies (Jalan & Ravallion, 2002). It is expected to be a profitable enterprise for it to provide enough income to crop growers (Pender et al., 2004; Maniriho & Bizoza, 2013) and thus to contribute to positive transformation of farmers' welfare (Nyambose & Jumbe, 2013). However, the available resources (mainly land and labour) are not used for high productivity, and this constrains the agriculture to assume appropriately its role in economic development of mainly availing enough food to the population of a country and providing the surplus for exports (Johnston & Mellor, 1961).
In intent to move farmers to the improved level of livelihood, the Government of Rwanda has initiated different anti-poverty programs. As far as the agriculture sector is concerned, the Crop Intensification Program (CIP) was launched in September 2009 to modernise agriculture (MINECOFIN, 2012; Alinda & Abbott, 2012), complemented with "one cow per poor family program" whose main role was to enable the poor to access to protein, and to supply them sustainable organic fertilizers (Kato et al., 2011; Nilsson et al., 2019) that are important for erosion reduction, food security and climate change adaptation (Lal, 2004). Throughout the Economic Development and Poverty Reduction Strategy (EDPRS), agriculture is considered as cornerstone driver for long-term growth and appeals for public efforts in conjunction with

all other development partners to stimulate increasing use of improved inputs and to encourage them to participate in both implementation and monitoring (Government of Rwanda, 2007). Further, the National Decentralized Policy integrates agriculture development in the planning and management of the holistic development process by taking it as the priority area and empowering local populations for them to participate in the initiatives aiming at graduating them out of poverty (Bingen and Munyankusi, 2002).
Previous studies reported the positive effects of some socioeconomic development initiatives on household welfare in Rwanda. With reference to CIP, Maniriho and Bizoza (2013) showed that potato, wheat, maize, tomato, onion, and cabbage are profitable crops that are more likely to improve the farmer's welfare, including nutrition. For one cow per poor family program, Nilsson et al. (2019) found out that the program affected positively the crop production but not the per capita consumption.

Table 1. Comparison of potential yield to current yield of selected CIP crops in Rwanda.

No	Crop	Potential yield (Tones/ha)	Current yield (Tones/ha)	Gap (Tones/ha)
1	Maize	3.79	1.53	2.26
2	Bean	1.60	0.91	0.69
3	Rice	6.69	3.40	3.29
4	Wheat	3.17	1.33	1.84
5	Cassava	38.89	13.54	25.35
6	Potato	20	8.65	11.35
7	Soybean	2.25	0.51	1.74

Note: The information on the potential crop yields are obtained from the technical factsheets published by AFSR (*Appui à la Filière Semencière au Rwanda*) in 2007. The average for different varieties was computed except for the potato where the minimum is considered (the potato average potential crop yield is 29.69 Tones/ha). As for the current crop yield, we considered the statistics of Seasonal Agricultural Survey data, season 2018A, published by National Institute of Statistics of Rwanda. Further, CIP stands for Crop Intensification Program in Rwanda, and ha for hectare.

The current statistics show that the agriculture sector increased at the growth rate of 6% in 2018, while the whole economy increased at 8.6% (NISR, 2018a). Notwithstanding all these efforts and the agriculture development achievements, the malnutrition issues are still crucial among Rwandans especially the stunting cases (World Bank Group, 2018) at the countrywide rate of 35% (NISR, 2018b).
The purpose of this study is to assess the prospects of crop production and food consumption for farmers' welfare. It aims specifically to analyze the relationship between the crop choice and the welfare categories, to compare the mean food consumptions between the growers and non-growers of selected crops, and identify the determinants of crop production and factors influencing food consumption in Rwanda.

Materials and Methods

This study used secondary data collected through the Fifth Integrated Household Living Conditions (EICV 5) survey from October 2016 to October 2017. This is a national level survey conducted on a random sample of 14 580 households, sampled from 245 villages and 2 526 households in urban areas and 1 015 villages and 12 054 households in rural areas. It contained 10 cycles on the whole year to account for seasonality of household incomes and expenditures. Part of the purpose of EICV5 was to avail required statistics to enable the assessment of the anti-poverty initiatives undertaken by the Government of Rwanda (NISR, 2018b). The study variables are well described in Table 2.

Table 2. Descriptive Statistics of the study variables.

Variable	Obs	Mean	Std. Dev.	Min	Max
Age (years)	11801	46.94	15.58	14	105
Mar_status (1=married)	11801	.91	.28	0	1
Sex (1=female)	11801	.26	.44	0	1
Family size	11801	4.58	2.04	1	17
Production (FRW)	11801	393.13	1,250.81	1	58,000
Agri_income (FRW)	11801	5,515.69	47,371.66	0	2,730,000
Food cons. (FRW)	11801	742,000	513,000	8138.57	6,210,000
Land size (ares)	11801	58.64	256.47	.02	13,476
Labour (FRW)	11204	14,365.22	54,472.83	0	1,400,000
Chemical fertilizers (FRW)	11204	5,362.99	31,962.01	0	2,700,000
Organic fertilizers (FRW)	11204	1,590.95	10,888.66	0	575,000
Pesticides (FRW)	11204	1,774.81	14,120.99	0	700,000
Traditional seeds (FRW)	11204	6,312.17	17,169.83	0	700,000
Improved seeds (FRW)	11204	2,247.89	19,699.16	0	1,000,000
Poverty*	11801	2.50	.73	1	3

Note: * indicates that poverty is measuring the welfare categories (1=severely poor, 2=moderately poor, and 3=non poor). FRW stands for Rwandan francs. Food cons. means food consumption, and Mar_status stands for marital status.

For analyzing data, the Chi Square statistic was computed to test for the association between two categorical variables (Diener-West, 2008) using the formula 1.

(1) $\chi^2 = \sum \frac{(O-E)^2}{E}$,

Where χ^2 is the Chi Square statistic, O stands for the observed frequency, and E the expected frequency.

To test for the association between continuous variables, the Pearson Product-Moment Correlation coefficient "r_{xy}" was computed (Asuero et al., 2006; Hall, 2015). This is known as the ratio of the normalized covariance of two continuous variables "C_{xy}" to the square root of their variances "$\sqrt{C_{xx}C_{yy}}$", and well described by the formula 2. The pairwise correlations between food consumption, crop production, land size, farm income, family size and the age of the household head were computed.

(2)
$$r_{xy} = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i \{[(x_i - x)^2][(y_i - y)^2]\}}} = \frac{C_{xy}}{\sqrt{C_{xx}C_{yy}}} = \frac{C_{xy}}{\sigma_x \sigma_y}$$

The third method used to analyze data is the t test. The t statistic was used to compare food consumption between producers and non-producers of selected crops (maize, potato, bean, soybean, wheat, coffee and rice). The t statistic was computed using the formula 3.

(3) $t = \frac{M_1 - M_2}{\frac{S_1}{\sqrt{n_1}} + \frac{S_2}{\sqrt{n_2}}}$

Where $(M_1 - M_2)$ is the difference between the means of the two groups (the growers, and the non-growers of selected crops), S_1 and n_1 are the standard deviation and the size of one group, S_2 and n_2 are the standard deviation and the size of another group, respectively.

Finally, an econometric approach was used to identify the factors affecting crop production and food consumption among crop growers. Following Gujarati (2009) and Wooldridge (2013), the model to be estimated for this case study is described by the formula (4).

$$(4)\, Y_i = \beta_0 + \beta_2 \sum_{k=1}^{n} X_{ki} + e_i$$

where Y is a dependent variable (food consumption, or crop production), Xs are independent variables, e_i is a disturbance term, βs are parameters to be estimated. For identifying the determinants of crop production, two models were specified and estimated. One is the linear model that included both farm inputs and socioeconomic characteristics of farmers (following the application by Mpawenimana, 2005 as an example), while the other is a Cobb-Douglas production function that included exclusively the farm inputs (see Debertin, 2012 for details). As for the food consumption function, a linear model was chosen (see Gujarati, 2009). Seeing that crop production was among the determinants of food consumption, a Two-Stage Least Squares (2SLS) estimator was used to estimate the coefficients of this function and to account for endogeneity and simultaneity bias (Wooldridge, 2013).

Results and Discussion

This study attempted to compare the average food consumption between producers and non-producers of main crops grown in Rwanda, namely maize, potato, bean, soybean, wheat, coffee and rice. The results from the t test show that crops whose producers consume more food than the non-producers are the maize, potato, bean, soybean and rice, while the producers of wheat and coffee afford less food than the non-producers (see Table 3).

This could be due to the fact that the production system of coffee and wheat requires the farmers to incur huge amount of expenses, which appeals the household members to deprive themselves from some food items for sometimes. The table 4 summarizes the relationships between different crops and welfare categories. The results show the percentages of the crop producers who are severely poor, moderately poor and non-poor, respectively. For the maize for example, 11.01% of producers are severely poor, 20.64% are moderately poor, while 68.35% are not poor. The first three crops with high percentage of non-poor are rice, maize and soybean, with 72.43%, 68.35% and 67.49%, respectively. The Chi-square test (formula 1) indicates that the welfare of Table 3. Comparison of food consumption between producers and non-producers of different crops.

Crop grown	**Comparison of food consumption between producers and non-producers**						
	Maize	**Potato**	**Bean**	**Soybean**	**Wheat**	**Coffee**	**Rice**
Non-growers	617 297	695 665	640 848	725 631	740 894	745 280	735 999
Growers	799 781	782 997	750 755	785 122	770 575	716 409	877 743
Combined	742 317	742 317	742 317	742 317	742 317	742 317	742 317
Difference	-128 485	-87 331	-109 907	-59 491	-29 682	28 871	-141 744
t statistic	-13.65	-9.27	-6.21	-5.67	-1.34	1.86	-6.21
Prob > t	0.00	0.00	0.00	0.00	0.18	0.06	0.00

households is significantly associated with maize production (chi2=164.40; p-value=0.00), bean production (chi2=103.72; p-value=0.00), and potato (chi2=83.35; p-value=0.00), as well as soybean and rice. However, wheat (chi2=4.28; p-value=0.12) and coffee (chi2=0.57; p-value=0.75) are not significantly associated with household's welfare.

Table 4. Relationships between different crops and welfare categories.

Welfare categories	**Maize growers**	**Potato growers**	**Bean growers**	**Soybean growers**	**Wheat growers**	**Coffee growers**	**Rice growers**
Severely poor (%)	11.01	11.74	13.13	11.30	14.49	13.38	7.79
Moderately poor (%)	20.64	21.18	22.22	21.21	25.62	22.87	19.77
Non poor (%)	68.35	67.08	64.65	67.49	59.89	63.75	72.43
Total (%)	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Total (Numbers)	**6 522**	**6 304**	**10 895**	**3 310**	**566**	**1 211**	**526**
Pearson chi2	164.40	83.35	103.72	37.53	4.28	0.57	23.50
Prob > chi2	0.00	0.00	0.00	0.00	0.12	0.75	0.00

This enhances the idea that farmers should choose to grow the crops that contribute to the improvement of household welfare (Pender et al., 2004; Maniriho & Bizoza, 2013; Nyambose & Jumbe, 2013). This shows the positive effects of the crops selected for the implementation of the Crop Intensification Program (CIP) to the transformation of livelihoods in Rwanda, even though the issue of low productivity of these crops remains crucial (Table 1).
Concerning the econometric analysis (Table 5), the 2SLS estimates from Model 1 indicated that food consumption was positively influenced by the age and the marital status of the household head, as well as the household size, , while it was negatively affected by the sex of the household head. For the crop production, the log-log model (R2=0.39) was superior to the linear model (R2=0.36). The results revealed that the crop output increased with the increase in inputs (labour, chemical fertilizers, organic fertilizers, pesticides, and traditional seeds) in line with the existing literature in economics (Dwivedi, 2006; Schotter, 2009; Besanko et al., 2011; Debertin, 2012). The effect of improved seeds on crop production was unexpectedly negative. This could be due to the seeds and/or the farming practices that may not be adapted to the soils, or to climate variability. The results also show that the crop system uses intensively the organic fertilizers (given their elasticity of 0.38) followed by labour (elasticity=0.21). This implied that the use of chemical fertilizers should be enhanced if the increasing crop productivity is to be achieved. The table 5 shows the pairwise correlations of the key variables of the study, and table 6 summarizes the linear regression estimates of food consumption and crop production.

Table 5. Pairwise correlations of the continuous variables.

Variables	(1)	(2)	(3)	(4)	(5)	(6)
(1) Age	1.00					
(2) Food cons.	0.01	1.00				
	(0.53)					
(3) Family size	-0.03	0.47	1.00			
	(0.00)	(0.00)				
(4) Agri_income	0.02	0.14	0.07	1.00		
	(0.06)	(0.00)	(0.00)			
(5) Land size	0.04	0.13	0.07	0.08	1.00	
	(0.00)	(0.00)	(0.00)	(0.00)		
(6) Production	0.01	0.24	0.13	0.09	0.09	1.00
	(0.58)	(0.00)	(0.00)	(0.00)	(0.00)	

Note: The figures in the parentheses are the significance levels (p-values).

Table 6. Linear regression estimates of food consumption and crop production.

Model 1 Food consumption (2SLS)		Model 2 Crop production (OLS)		Model 3 Crop production (Ln) (OLS)	
Variables	Coefficients	Variables	Coefficients	Variables	Coefficients
Age	1199.81***	Labour	0.004***	Labour (Ln)	0.35**
Sex	-79400.00***	Land size	0.09**	Land size (Ln)	0.07
Mar_status	19199.52	Chem. fert.	0.01***	Chem. fert. (Ln)	0.14*
hhsize	90803.69***	Org. fertil.	0.001	Org. fertil. (Ln)	0.15
Crop prod [a]	274.51***	Pesticides	0.02***	Pesticides (Ln)	-0.06
--	--	Tradit. seeds	0.01***	Tradit. seeds (Ln)	0.21***
--	--	Improv. seeds	0.004***	Improv. seeds (Ln)	0.20
--	--	Age	2.35***	--	--
--	--	Sex	-6.16	--	--
--	--	Mar. status	-23.27	--	--
--	--	Hh size	34.11***	--	--
--	--	Farm income	-0.001***	--	--
--	--	Crops dummies	Yes [b]	--	--
--	--	Locat. factors	Yes [c]	Return to scale	1.06 [e]
Constant	165000.00***	Constant	-104.41***	Constant	1.66***
Obs.	11801	Obs.	11204	Obs.	148
R-squared	0.028	F-stat	23.86	F-stat	20.697
Chi2	2993.35	Prob > F	0.00	Prob > F	0.00
Prob > chi2	0.00	R-squared	0.35 [d]	R-squared	0.509 [d]

Note: *** p<0.01, ** p<0.05, * p<0.1. [a] Crop production was instrumented with land size, farm income, chemical and organic fertilizers, traditional and improved seeds, as well as pesticides. [b] Crop dummies (yes=1; selected crops: maize, bean, soybean, rice, wheat, potato, and coffee). [c] This study controlled the location by including province, district and clusts (villages) in the regression. [d] The R-square was significant as the study used cross-section data (see Wooldridge, 2002 for details). [e] The return to scale of 1.06 implied that the crop farming scored increasing returns to scale (see Debertin 2012 for details). Ln stands for natural logarithm, hhsize for household size, mar_status for marital status, chem. fert. for chemical fertilizers, org. fertil. for organic fertilizers, crop prod for crop production, tradit. for traditional, improve. for improved, and locat. lor locational (factors).

Besides, the return to scale of crop production was 1.06, which implied that the farming system scored increasing returns to scale. This means that the crop output increased more quickly than the inputs increase (Dwivedi, 2006; Schotter, 2009; Besanko et al., 2011; Debertin, 2012). The regression estimates were complemented with the pairwise correlations of continuous variables given in Table 6. These results showed that food consumption was positively and significantly correlated with farm income, land size, crop production and family size. As for the crop production, the results indicated that it was positively and significantly correlated with land size, farm income, family size and food consumption.

Conclusion and Recommendations

This study attempted to assess the way farmers' welfare is affected by crop production and food consumption in Rwanda. In collaboration with diverse development partners, the Government of Rwanda committed to boost crop yield so as to increase farm income and improve farmers' welfare. We used the 5th round of the Integrated household life conditions survey data collected from October 2016 to October 2017. The Chi-Square test, the Pearson's correlation coefficient, the t test, and the OLS methods were used for data analysis.
The results from linear regression analysis showed that the increase in agricultural inputs lead to a significant increase in crop output with increasing return to scale. The t test showed that the crop farmers who grew maize, potato, bean, soybean and rice had higher consumption than the producers of other crops (see Table 2). Besides, the chi-square test outcome indicated that the production of maize, potato, bean, soybean and rice were significantly associated with the welfare categories (see Table 3). The results of both t and chi-square tests implied that the production of maize, potato, bean, soybean and rice made the farmers better off than other crops. As for the correlation test (see Table 6), the results showed that food consumption was positively and significantly correlated with family size, farm income, and land size, but not significantly correlated with crop production. This complemented the positive effects of these variables on food consumption as revealed by 2SLS estimates of the Model 1.
From the OLS estimates of the Model 3, we concluded that the crop output was driven by the amount of labour, fertilizers, pesticides and seeds used. The most influential inputs were organic fertilizers, labour and pesticides and the agriculture scored increasing returns to scale. The results from econometric estimations and other statistical tests led to state that crop production was among the primary drivers of famers' welfare besides the farm income, the land size and the family size.
With reference to these findings, we recommend that the strategies to increase the crop yield and farm income and thus sustain food consumption and improve farmers' welfare should be enhanced.

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MONITORING AND EVALUATION SYSTEM IN AGRICULTURAL ADVISORY SERVICE

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Abstract

Monitoring and Evaluation in Agricultural Advisory Service provide the basis for organized learning from experience according the reliable information collected during and after the implementation of advisory programs. These processes allow improvement of the provision of services, responsible resource allocation as well as planning of advisory programs in accordance with agricultural policy as well as the needs of agricultural producers. The objective of the research is to determine the existing situation regarding monitoring and evaluation system. Research has been implemented on two levels. The first part of research is on the level of the Public Advisory Service of Republic of Srpska while the second part of the research has been conducted at the level of institutions and organizations that are partners of Public Advisory Service. Based on empirical research and data from different sources the goal is to create basic elements of monitoring and evaluation in agricultural advisory services that can be used in practice by employees in the Public Advisory Service of Republic of Srpska. Based on the analysis of the situation and the results of the research, it is concluded that for the implementation of the monitoring and evaluation process the most important commitment of the management of the public agricultural advisory service is to collect and analyse the data. It is also necessary to improve the human, material and technical capacities within the advisory service and also ensure the development of information systems as well as special resources, both human and material, for the implementation of monitoring and evaluation.

Keywords: *Monitoring and evaluation, Agricultural Advisory Service, Republic of Srpska.*

Introduction

To improve and optimise contribution of extension service to rural livelihoods it is essential to monitor and evaluate its achievements. High quality monitoring and evaluation based on reliable information about the outcomes and impacts of services are a foundation for structured learning from experience. Monitoring and evaluation are also essential to ensure that those supporting and undertaking interventions to improve advisory services are accountable to the direct clients of these services as well as to governments, farmer organisations, and others investing in improving extension service (Christopolos *et al*., 2012). The Global Consultation on Agricultural Extension observed that monitoring and evaluation are important yet frequently neglected functions in most organizations (FAO, 1990, p. 27). In the worldwide survey of national extension systems, it was found that only about one half of all national extension systems have some type of monitoring and evaluation (M & E) capacity. The consultation noted that in many cases the M & E units are weak and are limited to ad hoc studies. Frequently, these M & E units are abandoned when project funding terminates. In many organizations, monitoring and evaluation have a negative image because these units may concentrate on problems, exposing weaknesses and failures. Instead, monitoring and evaluation should be used in a positive manner to improve extension's performance and increase its efficiency.[9] In December 2014, the United Nations General Assembly adopted a Resolution entitled "Building capacity for the evaluation of development activities at the country level" (UNGA Resolution A/RES/69/237). Through this Resolution, member states agreed that evaluation was an important component of developmental processes and recognized national-level evaluation as a tool to help strengthen and support to developmental results.

Monitoring and evaluation (M&E) of development activities provides government officials, development managers, and civil society with better means for learning from past experience, improving service delivery, planning and allocating resources, and demonstrating results as part of accountability to key stakeholders.[10] Monitoring and evaluation can help organization extract relevant information from past and on-going activities that can be used as the basis for programmatic fine-tuning, reorientation and future planning. Without effective planning, monitoring and evaluation, it would be impossible to judge if work is going in the right direction, whether progress and success can be claimed, and how future efforts might be improved.[11]

Monitoring and evaluation in the world are a regular activity in implementing public policies, projects and programs to ensure responsible use of resources and achieving measurable and sustainable results. The Public Agricultural Advisory Service in the Republic of Srpska operates within Department for agriculture advisory service which is one of the five departments within the organizational structure of the Ministry of Agriculture, Forestry and Water Management of Republic of Srpska. This Department conducts its activities in the territory of 64 municipalities in Republika Srpska through the seven regional units and one coordinating office. There are 33 advisors employed in these regional units. The fact that 42377 agricultural farms were registered in Republic of Srpska in 2018 suggests is that all agricultural producers can not have a high quality access to advisory services, primarily because one advisor should oversee 1284 agricultural farms. Creating an appropriate information system for the recording of advisory work, needs of agricultural producers and the basic elements of the monitoring and evaluation plan or report, it is possible to collect information in a standardize and systematic way for the objective reviewed of the advisory work and to create the basis for lesson learned, improved personal and collective accountability.

Considering the limited human and material capacities of the Public for agricultural Advisory Service of the Republic of Srpska, the selection of the topic and the purpose of the present paper is to provide an overview for possibilities of achieved rational use of resources, and improved existing mode of work.

Material and Methods

The paper is based on an review of primary data collected by structured questionnaires carried out in 2018 and on empirical research and data from different sources. Primary data have been collected with two online survey questionnaires. These surveys provided information on the existing status in the area of advisory activities/services, and then on the relationship between advisors and partners who are in direct or indirect cooperation with the Public Agricultural Advisory Service of Republic of Srpska according to the monitoring and evaluation system. The first questionnaire was created for obtaining primary data from advisors who are

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employed in Public Agricultural Advisory Service of Republika Srpska. The questionnaire contained 25 closed-type questions aimed at identifying the existing situation regarding the records of advisory activities, then the relationship of advisors to the monitoring and evaluation system, and knowledge of the role and importance of monitoring and evaluation in advisory service. Second questionnaire contained 20 closed-type questions. Questionnaire was created for institutions and organizations that are in direct or indirect cooperation with Public Agriculture Advisory Service. The aim was to identify the importance and the role of the advisory service partners in the planning and implementation of advisory activities as well as the relation of the partners to the monitoring and evaluation process as well as the knowledge of the role of monitoring and evaluation in the planning process. The most responses were received by agricultural cooperatives (23%), then non-governmental sector (20%) and agricultural associations (17%). Also, the representatives of academic institutions (7%) and administrative bodies and organizations (13% and 3%), the local community (10%) and the private sector (7%) were interviewed. In addition to the questions specific to a particular group of respondents, several identical questions were defined in two questionnaires, in order to compare the attitudes of both groups of respondents. The comparison was made with regard to the attitudes of the respondents regarding the meaning of the monitoring and evaluation, the conduct and manner of keeping records of the activities carried out, the purpose of monitoring and evaluation, the importance of participation of individual actors in the monitoring and evaluation process, and the attitudes of respondents regarding education in the field of monitoring and evaluation .

Based on empirical research and data from different sources the optimal model of the monitoring and evaluation plan and reports were created.

Results and Discussion

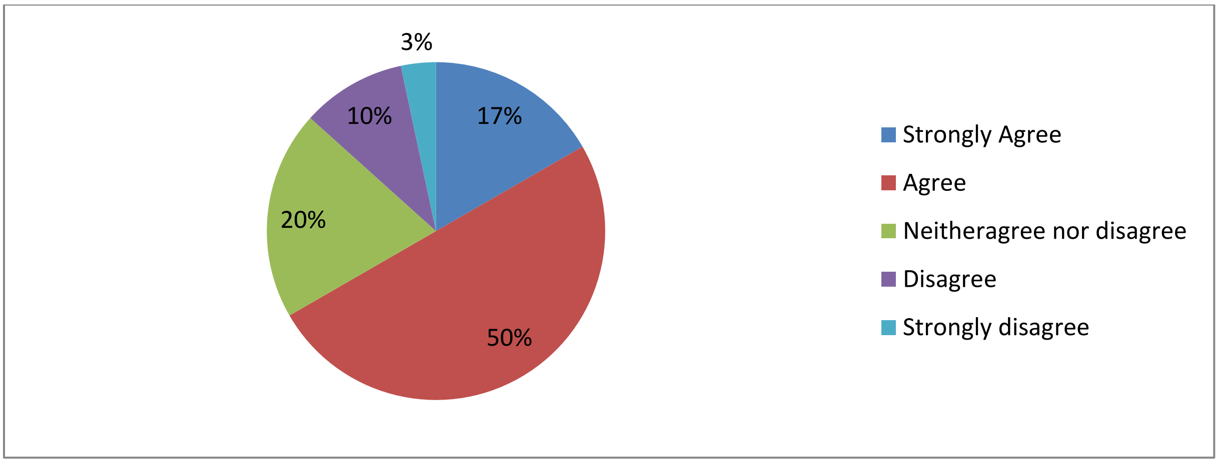
Obtained data provide insight into the importance and role of partners of public agricultural advisory services in the planning and implementation of advisory activities and process of monitoring and evaluation. Of the total number of surveyed partners, 90% are engaged in an institution/organization that cooperates with the Public Agricultural Advisory Service of Republic of Srpska. The analysis shows that 63% of the total number of respondents are beneficiaries of advisory services and 67% of them participated in the creation and implementation of advisory programs / projects / activities in cooperation with the Public Agricultural Advisory Service of the Republic of Srpska. The largest number of respondents (advisors and partners), 56% of them believe that the term monitoring refers to monitor the implementation of activities while 40% of them believe that the evaluation is a term describing the assessment of someone or something according to the established criteria. Most of respondents (70%) keep records of the activities that are being carried out, and activities are recorded in the planner/note books (41%) and in MS Word (30%).

Table 8 Methods of recording activities

Answers	Frequency of answers			Part %
	Advisors	Partners	Total	
MS Word	13	18	31	30
MS Excel	7	10	17	16
Planner/Notebook	23	20	43	41
System for recording activities	3	10	13	13

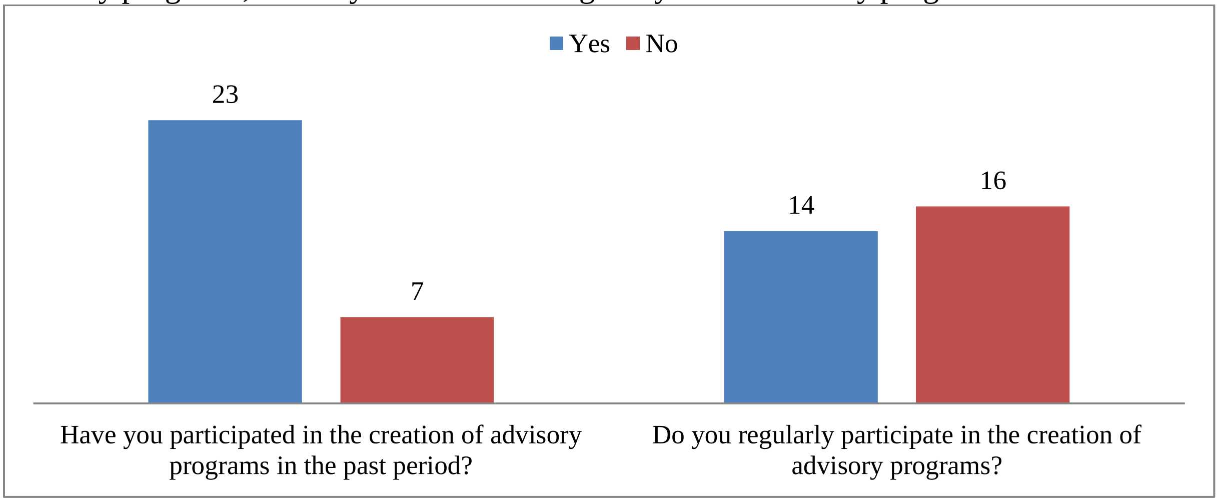
Most advisors (53%) believe that the existing record method only partially provides a clear overview and planning of future activities, and 40% of them believe that the existing method of evidence is quite appropriate.

Most advisors (60%) have had an insight into the opinion of agricultural producers on the basis of oral conversation with agricultural producers and 24% of them base their insight in producer's problems on their own estimates. Both groups of respondents agree that the purpose of monitoring and evaluation is to assess whether the activities are implemented according to plan, establish the value and importance of the specific activity, and helps to improve work with conclusions, lessons learned and recommendations and to allow the rational use of human and material resources. There is a significant degree of interest of the respondents (92%) for participation in trainings on monitoring and evaluation and in order to improve work in the future. Respondents believe that the greatest significance in the process of conducting monitoring and evaluation should have the management, employees and service beneficiaries while the least importance in the context of these processes should have external and internal evaluators. Claim that under the advisory services process of monitoring and evaluation should address to special unit which will dispose of the special human and material resources supports 67% of the advisors, 20% have a neutral attitude, while 13% disagree with the statement.



Graph 1. Attitudes about the existence of special unit for monitoring and evaluation

The largest number of advisors (64%) organizes their work within pre-defined advisory programs while 97% of institutions/organizations organize their work within pre-defined projects / programs. In the previous period 77% advisors participated in the creation of advisory programs, but only 47% advisors regularly create advisory programs.



Graph 2. The situation in the field of creating advisory programs

The monitoring process is carried out by 87% and the evaluation process is carried out by 77% surveyed institutions/organizations.
Based on the survey results and insight into the existing situation, activities within the public agriculture advisory services are planned according to the needs of agricultural producers without setting target values, results and impacts. When planning advisory programs, it is necessary to analyse the situation at first in order for these programs to respond to the needs of the target group and at the same time to enable agriculture policy to be implemented. The monitoring and evaluation process itself begins with the already conceived data collection system by pre-designing and determining the target values and impacts of the advisory programs. In order to create advisory programs and then monitor and evaluate these programs it is necessary to have defined basic elements of the logical framework. The basic elements of the logical framework of advisory programs are necessary to show the link between investment, activity, services (outputs) and effects, i.e. desired results or changes at individual and collective level. Based on the presented logical framework it is evident that monitoring mainly focused on investments, activities and results while evaluation is focused on outputs of programs.

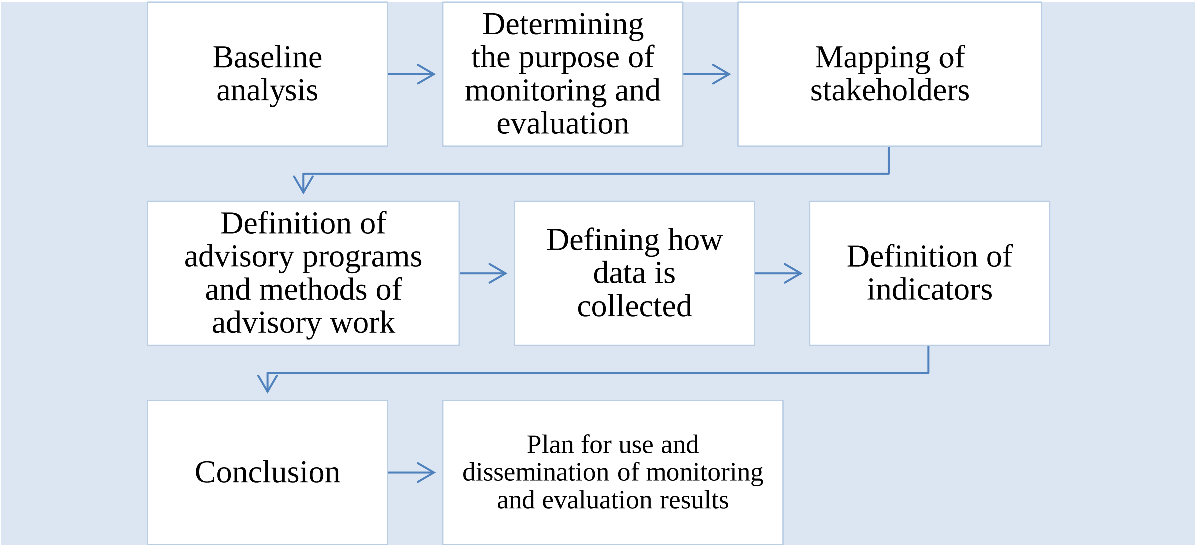
Table 9. Logical framework for monitoring and evaluation process in advisory service.[12]

Monitoring			Evaluation		
Inputs	Outcomes		Outputs		
Investments	Activities	Participation	Short Term	Medium Term	Long Term
What we invest?	What we do?	Who we reach?	What the medium term results are?	What the medium term results are?	What is ultimate impact(s)?
Staff; Volunteers; Time; Money; Equipment; Technology; Partners.	Conduct workshops, meetings; Training; Provide advisory; Work with mass media; Making field trials; organization of events;	Individual agricultural producers; Organized agricultural producers; Decision makers;	Learning: Awareness; Knowledge; Attitudes; Skills; Opinions; Aspirations; Motivation.	Action: Behaviour: Independence in work, application of new techniques and technology.	Conditions: Social; Economic; Environment.

Based on the research, the optimum model of the monitoring and evaluation plan is created. The model envisages a several steps in creation of an optimum MiE plan: first is a definition of the purpose of monitoring and evaluation, which then determines the stakeholders, the second is to define advisory program and advisory methods that will be used in the implementation of the program, the third is definition of the methods of collecting data as

[12] Adapted from: https://articles.extension.org/pages/70478/logic-models:-a-tool-for-program-planning-and-assessment

well as indicators and methods that will be used for their analysis and the person responsible for analysing and drawing conclusions. The final stage of this plan defines the plan for using the results.



Scheme 1. Proposal of model for monitoring and evaluation plan in agriculture advisory service[13]

The final monitoring and evaluation report is a written document that describes how the advisory program is monitored and evaluated. It presents the results, conclusions and recommendations of the monitoring and evaluation. The monitoring and evaluation report describes the advisory program, its purpose and advisory activities aimed at the implementation of the advisory programs. This report provides an answer as to whether the program is implemented in accordance with the plan and whether the changes that have been made are in accordance with the purpose of the program. The purpose of the report is to provide recommendations and conclusions to the management, advisers and other interested parties on how to improve future advisory programs or justify implementation, interruption or adjustment of advisory program to the current situation.

Conclusion

The monitoring and evaluation activities are a regular practice in implementation of public or development policies, that allows to determine the relevance, effectiveness and efficiency of such politics, projects and programs. Monitoring and evaluation serve to provide feedback as to whether advisory activities are related to the needs of the beneficiaries, whether satisfactory performance is achieved, and whether certain activities can be organized and implemented by other methods in a more efficient way.
Based on the research, it is noted that public agricultural activities carried out in Republic Srpska are performed using planner/notebooks and MS Word tools. Most advisors believe that the existing method of records allows a clear overview and planning of future activities, need assessment is performed via oral discussions with farmers and based on personal observation. Hense, there is a need to implement MiE system as integral part of Agriculture Advisory Service activities in order to obtain timely information for decision-making, planning of human and material resources and improving the provision of advisory services. Such a system should also alow measurement of a short-term, mid-term and long-term results of

[13] *Source: Author's elaboration based on the survey results.

implemented advisory activities. The MiE system should consist of an monitoring and evaluation plan and MiE report.

For the implementation of monitoring and evaluation system within Public Advisory Service it is necessary to have a commitment of management of advisory service to establish a special department in charge of monitoring and evaluation and to provide regular support to persons responsible for the management and maintenance of computer systems and networks. Insufficient numbers of human and material resources are also a limiting factor for the implementation of these processes.

Therefore, it can be concluded that the needs for monitoring and evaluation tools will became evident with the development of advisory service in the future in order to analyse the target groups and their needs, methods of advisory work and allocation of resources, all in order to improve efectivness of the advisory services on production productivity and economic benefits of the beneficiaries.

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THE SYMBIOSIS OF MODERN INFORMATION AND COMMUNICATION TECHNOLOGIES AND MARKETING STRATEGIES IN AGRICULTURE

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Abstract

Demands for quantity, quality and diversity of food products have never been higher. Satisfying consumers’ fast-changing demands and high expectation regarding food-related products is a big challenge for food producers and agriculture industry. Response to this challenge can be seen in smart and sustainable agriculture. Novel Information and Communication Technologies (ICTs) have brought immense improvements in a wide scope of domains and agriculture didn’t remain immune to the progress of modern technologies. The increased production and enhanced farming practices with the reduced negative influence on the environment and sustainable utilization of resources are some of the characteristics of agri-food industry of the 21st Century. However, the overall success of the agri-food sector is not possible without adequate marketing practices that will make a strong bond between food producers and consumers. The purpose of the paper is to examine the role and opportunities of ICTs use in agricultural marketing, and the significance of effective agricultural marketing in business development. The particular attention has been devoted to the digital marketing strategies applicable in agriculture and the benefits they bring. The presented research shows that the symbiosis of modern ICTs and marketing strategies in agriculture transformation will increase the satisfaction and loyalty of current customers, gain more customers, and boost sales and profits.

Keywords: *ICT, agriculture, marketing, customer.*

Introduction

With the increasing worldwide population, assuring required quantities of safe, quality, and affordable food is a key challenge of modern society. In order to achieve this goal, food processors and distributors work intensively to find novel methods for increasing the production and achieving sustainable agriculture and food industry (Maksimović et al., 2019). The novel Information and Communication Technologies (ICTs) have already transformed agriculture and food industry, through each phase of the agri-food supply chain (food production, packaging, processing, transportation, distribution, preparation, and marketing). Production automation, precision farming practices, remote and real-time monitoring and controlling, traceability, decision making, and forecasting, are some of the improvements brought by modern ICTs in the agri-food sector, that promise to make agriculture and food industry smarter and more sustainable than ever before.

In the 21st Century, agri-food industry sector has become consumer-centric and established on fast-changing consumers’ demands in the sense of diversity, quality, and quantity of food products (Maksimović et al., 2019). At the same time, the success of food producers and food products depends on food production and marketing system between agricultural producers and consumers (Islam, 2011). Agriculture and marketing strategies together have created a term “agricultural marketing” that involves all activities in the movement of agricultural inputs and products from the farms to the final consumers, and the effects of such activities on all players in the food supply chain (Ul-Rehman, et al., 2012). Marketing in the agri-food sector must be customer-oriented, hence providing all participants in the food supply chain with profit and satisfaction.

This paper presents an attempt to summarize the significance of novel ICTs and marketing strategies in realizing the smart and sustainable agri-food sector, that will substantially provide significant economic and social benefits. There is no doubt that technological innovations and marketing system improvement are key components for agricultural development.

The influence of ICTs on agriculture practices and marketing strategies

Technological achievements in recent years (sensors, robots, smartphones, automatic control, Cloud and Fog services, Wireless Sensor Networks (WSNs), Internet of Things (IoT), nanotechnology and many other emerging technologies) have completely revolutionized agri-food industry (Maksimović et al., 2019). The main benefits brought by novel ICTs in agriculture are: the automation of production, better management of cultivation factors, more efficient utilization of agrochemicals and water, precise, productive and sustainable farming practices, logistics, traceability, real-time and remote monitoring, decision making, forecasts, etc., therefore contributing to the fulfilment of the growing food requirements. Moving towards green technologies, environmental and human safety has been increased.

The IoT implementation in the agri-food sector enables the remote monitoring and real-time access to massive amounts of heterogeneous data collected from the farm to fork. A huge number of sensor nodes deployed throughout the entire food supply chain enable numerous real-time information related to food production, processing, transportation, and storage. These data must be gathered, processed and analyzed in a proper manner so that they can be useful for making predictions and decision making. Having the right information at the right time enable fast and timely reaction, associated to soil improvements, plant and animal breeding, agrochemicals control, disease diagnostic, food packaging, traceability, food safety, hence avoiding potential food contamination, spoilage, foodborne diseases, and increasing production, profit, energy and resource efficiency, and environmental protection. In other words, knowledge extraction and smart decisions lead to the realization of smart, efficient and sustainable agriculture and food sector.

Alongside the development of the agri-food sector influenced by novel technologies, the marketing system has also changed. IoT inclusion in marketing domain enables the constant company-customer connection, monitoring, analysis and prediction of consumer behaviour, delivery of personalized and targeted messages, products or services to customers, customers greater involvement, as well as automated loan eligibility computation, access to latest inventory data of agro inputs, logistics and storage service providers, provision of the up to date market information through the digital connection to the online domestic and international market place (Fig. 1) (JMR AgriNet, n.d.; Gavrilović and Maksimović, 2019). All these activities lead to a higher level of company and customer satisfaction.

Technology-driven marketing strategies play a key role in the production and consumption stimulation in agriculture and economic development. However, agricultural marketing should be considered as unique due to perishability and bulkiness of the food products. As such, agricultural marketing deserves special attention.

Figure 1. Connected agro-digital platform (JMR AgriNet, n.d.)

Agricultural marketing

The entire organization, as well as the entire marketing system, must adopt the marketing concept. An agricultural and food marketing system consists of interconnected sub-systems that are autonomous of one another, but which possess a defined common goal and perform actions necessary to profitably exploit opportunities in the marketplace (Crawford, 1997). Agricultural marketing consists of two major concepts:

- agriculture - producing agri-food products using natural factors, and
- marketing – business organization activities aimed to promote products and services to targeted customers.

Agricultural marketing is mainly defined as the complex of activities involved in the supply of farm inputs to the farmers and the flow of agri-food products from the farm producers to the consumers. Agricultural marketing system encompasses (Luanar, 2017):

- assessment of the requirement for farm-inputs and their provision,
- post-harvest treatment of farm products,
- execution of various actions needed in transferring farm products from the farm to processing industries and/or to end consumers,
- assessment of demand for farm products and public policies and plans pertaining to the pricing,
- handling, purchase, and sale of farm inputs and agricultural products and increasing the farmer profit.

Achieving maximum sales and profit requires an effective and profitable agriculture marketing plan. Development of such a marketing plan includes the following steps (Fig. 2) (Garcia, 2017):

- understanding the customers' needs and identifying the potential customers,
- offering something different and unique to customers,
- formation of experienced professionals' teams for each segment of the agriculture business,
- choosing the right advertising techniques,
- evaluation of implemented marketing techniques.

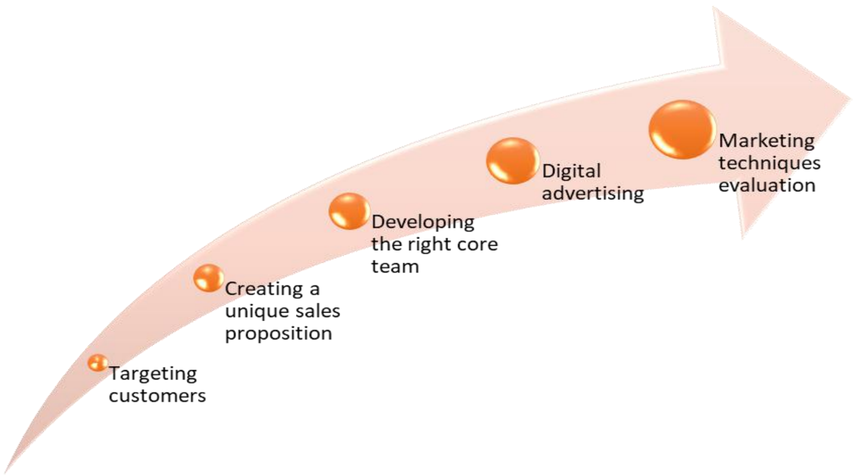

Figure 2. Marketing plan steps

Agricultural marketing is crucial for production and consumption stimulation, delivery of agri-food products in time, at the right place, in proper form and reasonable price. It also plays an immense role in agricultural and economic development through the optimization of resource usage and output management, farm profit increase, markets' widening, agro-based industries growth, price signals, inclusion and implementation of novel technologies, employment creation, addition to national income, improved living, creation-, form-, place-, time- and possession utilities (Luanar, 2017). The most substantial influence on the success of the agri-food sector have customers that can be attracted and maintained through the improved customer services and satisfaction and increased engagement. Prerequisites for achieving these goals are effective market surveys, market trending and continuous follow up (Kiruthiga et al., 2015).

Digital marketing strategies for agriculture

Nowadays, traditional agricultural marketing strategies are not adequate for modern society in which people are constantly connected to the web through smartphones, tablets, and computers. In order to extend to these viewers, traditional agribusiness marketing practices have evolved to the digital agribusiness marketing practices (Fig. 3) in which Internet is a most effective mean for the promotion of products and the provision of information.

Creating a name, symbol or design in agribusiness refers to a marketing branding practice which aim is to identify and differentiate an agri-food product from other similar products. Having a strong and internationally recognized brand is crucial for the success of any agri-food business.

The farmer and any agri-food related organization in digital marketing must have optimized and up to date website that will adopt the best practices of search engine optimization (SEO). On this way, the most interested users will be targeted and moved to these websites. The realization of such websites requires intensive work on certain tasks such as (WebFX, n.d.):

- The definition of title tags and keywords - a concise description of what can be found on a page enables users to move easily and faster, therefore finding easily what they are looking for.
- Posting blogs – regularly writing posts focused on readers keep visitors coming back to the website. Both short and long posts give impressions of serious business organizations and assist in building a better relationship with potential customers.
- The usage of graphics, images, and videos– visualizations related to the business attracts more visitors, make them stay longer on the website and potentially convert them to the customers.

- The usage of search engine marketing (SEM) –is the most effective way to grow the business and reach new customers. Using SEM for marketing and advertising enables more visits to websites and better brand recognition.

Figure 3. Digital marketing strategies (Shastri, 2017)

In the era of social networks, it is almost impossible that farmers or agriculture company's online strategy is not based on the popular social media platforms such as Facebook, Twitter, Pinterest, LinkedIn, and other platforms that are extremely effective for marketing purposes. Social media in agricultural marketing enables saving time and costs for getting information and is the most comfortable way for the farmers and companies to reach current and potential customers.

Email marketing is one of the best strategies to send the right information at the right time to current and potential customers. Sending the novel information and offers increases the brand awareness, remind customers of the business, and keep shoppers coming back for more.

Content marketing is one of the most powerful digital marketing strategies that is focused on the creation and distribution of timely, relevant and valuable content to current and potential customers in order to influence their behavior. In the agriculture industry, content marketing plays an immense role as it gives customers quality and valuable content and directly influences customer loyalty, what at the end lead to an increase in the company's revenue.

Mobile marketing services also play an important role in the agricultural business as they enable the instant connection with clients and customers (i.e. sending novel information, promotions and offers via SMS messages) without the participation of a middleman, what undoubtedly reduces the costs and increase the profit.

All presented digital marketing strategies bring numerous benefits in agriculture business as they enable the connection with the customers in an easier and faster manner, better understanding of their needs and preferences, sending customer-tailored messages, products, and services, improvement of the company-customer relationship, strengthening the company's brand, and boosting the sales and profit. However, the aim of any digital marketing strategy, alongside the development and delivery of personalized marketing messages, is to not be intrusive. Otherwise, they can lead to the opposite effects of the desired ones. Therefore, the evaluation of the implemented marketing techniques should be regularly performed.

Conclusion

Agriculture is one of the most important sectors that help boost economic growth. With the applications of ICTs, agriculture has become smarter and sustainable than ever before. At the same time, ICTs play a key role in the marketing domain of agribusiness. Digital marketing strategies have completely changed the way farmers and companies communicate with current and potential customers. Knowing what the customers want and need, and enabling them the right information, products, and services at the right time, at the proper spot and at the affordable prices is crucial for the success of agriculture business. The development of efficient and profitable digital agriculture marketing practice is not an easy task. It is a complex process that requires the intensive work of different professional teams throughout the entire agri-food sector. Effective and profitable agriculture marketing plan realized with the help of symbiotic approach of modern ICTs and marketing strategies will increase the customers' engagement, satisfaction, and loyalty, lead to stronger and internationally recognized brand, and boost sales and profits.

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MOBILE AND STATIONARY BEEKEEPING – SUCCESSFUL EXAMPLES

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Abstract

In the Republic of Croatia beekeeping is a traditional and esteemed agricultural activity. Due to different climatic zones (continental, mountainous and Mediterranean), Croatia has different conditions for the development of beekeeping, and this variety and richness of plant species allows the production of many types of honey. However, for most beekeepers in Croatia beekeeping is an additional activity. The honey production has increased significantly since the year 2010, with the highest recorded production in 2015 (11,477 tons). The number of beekeepers in the Republic of Croatia decreased by 42% in 2018 compared to 2015, while the number of beehives decreased by 34% in the same period. Only 4% of beekeepers have more than 150 beehives, while 42% have 1 to 30 beehives. Mobile beekeeping makes it possible to achieve higher honey yields per beehive, but additional equipment is also needed which increases production costs, estimated at EUR 1,474.53 a year (registration, maintenance of vehicles, fuel and work required when moving beehives). In case 1 (stationary production) 16 kg of honey was produced and income of 35.97 euros per beehive was generated, while in case 2 (mobile beekeeping) 42 kg of honey was produced and income of 100.45 euros per beehive was generated.

Keywords: *Honey, income, mobile beekeeping, stationary beekeeping*.

Introduction

The beekeeping has a long tradition in the Republic of Croatia and the first written document about beekeeping is a Vinodol Code from the year 1288. The beekeeping is an important agricultural branch which through pollination, honey production and other bee-products impact the total economic activity, rural development and sustainable ecological balance (Šakić Bobić *et al.*, 2018). The Republic of Croatia has three different climatic zones (continental, mountainous and Mediterranean) and different conditions for beekeeping development which enables production of many different honey types (Kezić *et al.*, 2014). Beekeeping production has a great economic significance which is reflected in the production of honey, pollen, propolis, royal jelly, wax and bee venom (Vojvodić and Bubalo, 2017). According to the European Commission data (2017) the EU is a second world largest honey producer (250,000 ton annually), behind China with about 17 million beehives. The EU is a great honey importer and the first supplier is China with a 40% share at European honey market. The honey production in Croatia increased since the year 2010, with the biggest recorded production for the year 2015 (11,477 ton). In the year 2017 there were 8,805 ton of honey produced, which is four times more than in 2010 (FAOSTAT, 2019). According to the data from beekeepers and apiary records for the National Beekeeping Program for 2020-2022, in the year 2018 the number of beekeepers decreased by 42%, while the number of beehives decreased by 34%. A significant number of beekeepers (41%) are small (up to 30 beehives), 55% have between 31 and 150 beehives, while only 4% have more than 150 beehives. Professional beekeepers have a total of 64,663 beehives and their share is 17.38% of the total number of beekeepers. In average they have 242 beehives. 90% of beekeepers that produce honey on conventional way are mobile beekeepers, as well as 95% of those who are organic beekeepers. Beekeepers with a smaller number of beehives usually use one of the forms of direct sales (at farm gate, at local markets) for their honey. Increasing the number of beehives increases the share of wholesale. About 3/4 beekeepers use direct sale channels, while 1/4 sells through the wholesale. Selling in retail chains and supermarkets covers large suppliers and honey packing companies (Ministry of Agriculture, 2019). In the beekeeping there are two beekeeping methods, stationary and mobile. Mobile beekeeping requires higher costs, but it is possible to achieve 20-40 kg of honey per beehive more than by stationary beekeeping (Abrol, 2010). Efficient beekeeping today requires 50 to 60 kg of honey per beehive, and mobile beekeeping is the only way to achieve high yields. Mobile beekeeping requires additional equipment (vehicle), and beekeepers need certain locations to move their beehives (Ćejvanović *et al.*, 2011). The research of Grgić *et al.* (2009) shows that moving beehives may take advantage of the opportunities and thus produce an average of 60 kg of honey per beehive. The aim of this paper is to see the economic success of beekeeping in a mobile and stationary mode.

Material and Methods

Primary data were obtained by interviewing the beekeepers/owners, mobile and stationary (case study). The interview was conducted in the year 2018, for the data of previous production year. The stationary beekeeping farm is in the City of Zagreb and has 60 beehives (type LR and Farar). It is engaged in beekeeping since 2012 and has 6 family farm members. The mobile beekeeping farm is in the Brodsko-Posavska County (municipality of Garčin) and has 70 beehives (type LR) that moves around the surrounding area, depending on the pasture conditions. The farm owns a van and has 3 family farm members. Calculation of indicators was made on the obtained primary data, which are processed in Excel. The questionnaire included questions about the social conditions of family farm (number of household members, qualifications, ...), technological questions (type of beehives, method of beekeeping, production of honey and other products, ...), and economic questions (selling prices, production costs).

Results and Discussion

Case 1: Stationary beekeeping

The farm produces three types of honey (acacia, flower and chestnut) sold at an average price of 7.37 EUR/kg. The farm equipment value is estimated at 8,732.57 EUR. A total of 60 beehives produce 960 kg of honey, or 16 kg of honey per beehive.

Table 1. Equipment value and annual depreciation, EUR/year.

Equipment	Purchase value, EUR	Depreciation rate (%)	Annual depreciation, EUR
Beehives	5,630.03	10	563.00
Honeybee colonies	2,412.87	25	603.22
Electric embedder for wax foundation	22.79	10	2.28
Honey extractor	268.10	10	26.81
Smoker	12.06	10	1.21
Wax extractor	251.34	10	25.13
Inox barrel	56.30	10	5.63
Other equipment	79.09	25	20.00
TOTAL	8,732.57		1,247.05

*Source: Car (2019).

Honey is usually sold at farm gate, local markets and fairs, which confirms the data from bee products market current state analysis made for National Beekeeping Program, that small beekeepers sell through the direct sales channels. In addition to honey, the farm produces propolis tincture (about 30 ml per beehive) sold at a price of EUR 2.68 per 20 ml. The owner pays pension insurance and estimates that one beehive requires 6 hours of work per year. Although this work is carried out by family farm members, it was calculated for 3,62 EUR/h as the average monthly net wage per hour of work in agricultural production for the year 2017 (Croatian Bureau of Statistic).

Table 2. Calculation for stationary beekeeping production, EUR.

	Total (60 beehives), EUR	Per beehive, EUR
Honey	7,077.75	117.96
Propolis	241.29	4.02
I. TOTAL INCOME	7,319.03	121.98
Feeding	804.29	13.40
Package	211.13	3.52
Wax foundation	965.15	16.09
Labor	1,302.95	21.72
Veterinary cost	268.10	4.47
Beehives maintenance	160.86	2.68
Depreciation	1,247.05	20.78
Pensioner's insurance	201.07	3.35
II. TOTAL COST	5,160.59	86.01
III. FINANCIAL RESULT (I-II)	2,158.45	35.97
Economy (I/II)	1.4	
Productivity (I/hours of work)	20.33	
Profitability (III/equipment purchase value)	25%	

*Source: Car (2019).

Calculated financial indicators show that the farm producing bee products in a stationary manner with 60 beehives operates positively and achieves a satisfactory level of business profitability, although the quantity of honey produced per beehive is relatively small (16 kg). Similar research by Grgić et al. (2009) indicates that the average honey production per beehive in stationary beekeeping is 18.4 kg, with variations from 11.1 to 26.8 kg, which confirms that selected beekeeping farm as a case study may represent a type of stationary beekeeping in Croatia.

Case 2: Mobile beekeeping

The farm produces four types of honey (rapeseed, acacia, chestnut and sunflower) and sells it at an average selling price of 5.36 EUR/kg on the doorstep, local markets and fairs. It achieves a lower selling price when compared to the farm from Zagreb, which confirms the data of the Croatian Beekeeping Association collected by the Ministry of Agriculture survey that the retail prices of honey produced in Slavonia are significantly lower than those achieved in Zagreb and surrounding. The farm owns a honey extractor kit, a wax extractor, a pollen trap, electric embedder for wax foundation, a smoker, a protective clothing and a van for honeybee's colonies transportation, and the assets purchase value is estimated at EUR 16,159.52, of which 42% for the vehicle's value. It is evident that mobile beekeeping requires higher investment than stationary production, but it also records higher honey production per beehive (42 kg).

Table 3. Equipment value and annual depreciation, EUR/year.

Equipment	Purchase value, EUR	Depreciation rate (%)	Annual depreciation, EUR
Beehives	4,222.52	10	422.25
Honeybee colonies	2,815.01	25	703.75
Honey extractor	1,340.48	10	134.05
Wax extractor	375.34	10	37.53
Pollen trap	609.92	10	60.99
Electric embedder for wax foundation	26.81	10	2.68
Smoker	13.40	10	1.34
Protective clothing	53.62	25	13.40
Vehicle	6,702.41	20	1,340.48
TOTAL	16,159.52		2,716.49

*Source: Car (2019).

In addition to honey, the farm produces pollen, about one kilogram per beehive, and sells it for 10.72 EUR/kg. Farm is operated by farm members (420 hours of work a year) and seasonal workforce (140 hours a year). Vehicle registration, maintenance, fuel and lubricant costs are estimated at 1,474.53 EUR/year.

Table 4. Calculation for stationary beekeeping production, EUR.

	Total (70 beehives), EUR	Per beehive, EUR
Honey	15,764.08	225.20
Pollen	750.67	10.72
I. TOTAL INCOME	16,514.75	235.92
Feeding	797.59	11.39
Package	591.15	8.44
Wax foundation	938.34	13.40
Labor – farm members	1,520.11	21.72
Labor – seasonal workforce	506.70	7.24
Veterinary cost	938.34	13.40
Vehicle cost	1,474.53	21.06
Depreciation	2,716.49	38.81
II. TOTAL COST	9,483.24	135.47
III. FINANCIAL RESULT (I-II)	7,031.50	100.45
Economy (I/II)	1.7	
Productivity (I/hours of work)	29.49	
Profitability (III/equipment purchase value)	44%	

*Source: Car (2019).

Based on the calculated financial indicators, the farm with mobile beekeeping, despite the higher investment cost of equipment, achieves a positive financial result. Although it has a lower selling price of honey, it is compensated with higher yields per beehive. The cost structure is dominated by the cost of human labor with a 21% share in total cost, which is correlated with the research of Grgić et al. (2009).

Beekeeping farms selected as case studies show main trends in assets investment, structure and amount of working capital, as well as production and economic performance typical for two analyzed forms of beekeeping. Farmers with stationary beekeeping invest less per unit of production (beehive) and products (kg of honey), and based on basic economic indicators, can be satisfied with beekeeping as "a profitable hobby". Especially since part of the working hours of family members can be devoted to other bee products except honey. In mobile beekeeping there are many unknown variables influencing the high yield of honey (duration, dates and location at which bees are transported) during the beekeeping year, which do not allow significantly better economic results.

Conclusions

The paper presents two successful examples of beekeeping production, one with stationary beekeeping and 60 beehives, other with mobile beekeeping and 70 beehives.

Stationary beekeeper produces 16 kg of honey per beehive and generates additional income through the production of propolis. Although it achieves relatively small honey yields per beehive, the farm operates positively and achieves a financial result of 35.97 EUR/beehive with efficiency 1.4, productivity 20.33 EUR/hour and profitability 25%.

The farm with mobile beekeeping produces 42 kg of honey per beehive, and as an additional product offers floral powder. This farm has additional costs for vehicles needed for beehives transportation, but also requires more human labour. Despite the higher costs, the farm operates successfully and achieves a financial result of 100.45 EUR/beehive with efficiency 1.7, productivity 29.49 EUR/hour and profitability 44%. Although the increased investment in mobile beekeeping is economically justified, some producers are not deciding to go on it because the stationary beekeeping with less risk achieves satisfactory financial results.

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THE ROLE OF AGRICULTURAL EXTENSION TOWARDS FACING CLIMATE CHANGE IN Al-GHARBIA GOVERNORATE, EGYPT

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Abstract

This study aims to identify the knowledge of extension agents at extension centers of the forms, causes and effects of climate change, and to identify the role of agricultural extension in addressing climate change. This study was conducted in all extension centers in Al-Gharbia governorate, Egypt, during December 2018. Data were collected from 68 respondents of agricultural extension agents affiliated to 17 extension centers; 51 respondents were males and 17 respondents were females, questioned through a personal interview questionnaire made especially for this purpose. Several statistical methods were used such as percentage and frequency tables. The most striking results of the study showed that 9.41% of the respondents had a high level of knowledge about the forms of climate change.76.47 % had a high level of knowledge about the causes of climate change, while 73.52 % had a high level of knowledge about the effects of such climate change. The most of respondent were aware of the role of agricultural extension in diminishing the causes of climate change and overcoming the effects of climate change. The effects of climate change on agricultural extension work were as follows: difficulties in implementing the action plan due to change in rain patterns, difficulties in running farms and applying new farming techniques, growing workload put on agricultural extension agents, and an increase in costs of training.

Keywords: *Agricultural extension, climate change, extension centers, Al-Gharbia governorate, Egypt.*

Introduction

The whole world today is interested in the global climate change due to their impacts on human existence. Climate change has serious future effects. El-Marsafawy (2007) defines it as "the total change in the earth's surface as a result of the gas emissions which, in turn, lead to global warming and the rise in the earth's surface temperature."

Climate change takes many forms; the most prominent are the increase in temperature, shortage of rainfall, windstorms and hurricanes, long periods of dryness and the failure to forecast the weather (Shankar, 2013). The reasons for these climate change can be natural or human. The latter can be divided into industrial reasons and agricultural ones. The total gas emissions from agriculture account for 15.7% of the total global warming gases resulting into climate change (Abu Hadeed, 2010).

As such, agriculture has a big share in causing climate change. It is affected by these changes as well. This appears in the decrease in crop yield and animal production; the increase in water consumption for irrigation, the increase in water levels on land, soil salt, the diminishing net farm revenue, the increase in diseases and plant insects, and the increase in the cotton yield (Abu Hadeed, 2010; EL-Marsfawy, 2009; Ifeanyi-obi et al., 2012; Nnadi et al., 2013; Al-Shaib et al. 2016; Abd Ella et al., 2018).

Agricultural extension has a vital role in facing climate change prior to their occurrence through informing farmers to amend their practices which lead to the increase in the greenhouse gases. It has also an essential role after the occurrence of climate change through

informing farmers of the practices which could diminish the impact of these changes, telling them of how to adapt with the changes and lessen their side effects on the agricultural sector (Saleh, 2009; Mustapha et al., 2012; Al-Shenawy et al., 2013; AL-Shaib et al., 2016; Abd Ella et al. 2018). In light of this, it seems necessary to identify the extent to which extension agents are aware of this climate change, and the role played by agriculture extension in facing and overcoming this phenomenon.
This study aims to identify the knowledge of extension agents at extension centers of the forms, causes and effects of climate change, their desire to participate in activities to overcome climate changes and to identify the role of agricultural extension in diminishing the causes of climate change and overcoming its effects. It also aims to identify the effects of climate change on agricultural extension work.

Material and Methods

This study has been conducted in all extension centers in Al-Gharbia governorate, Egypt, during December 2018. Data has been collected from 68 respondents of agricultural extension agents affiliated to 17 extension centers; 51 respondents are male and 17 respondents are female, through a personal interview questionnaire made especially for this purpose.
Research variables have been measured as follows:
Knowledge of the features of climate change has been measured through a tool containing 13 items. Participants have been asked about their knowledge of the features of climate change.
Knowledge of the reasons for climate change has been measured through a tool containing 14 items. Participants have been asked about their knowledge of the reasons for climate change.
Knowledge of the impact of climate change has been measured through a tool containing 14 items. Participants have been asked about their knowledge of the impact of climate change.
They have to choose one of three options (Yes, I do not know, and No), on a scale of three (1, 2 and 3 respectively). The real range has been divided into three groups: low knowledge, average knowledge and high knowledge.
Participants' desire to participate in the extension activities to face climate change, as well as their awareness of the role played by agricultural extension in facing them, has been studied, too.
Several statistical methods such as percentage and, frequency tables were used to analyze the data.

Results and Discussion

Knowledge of the features of climate changes:
Table 1 shows that 79.41% of the participants have a high level of knowledge of the features of climate change.

Table 1. Distribution of the participants' answers for the knowledge of the features of climate change

Knowledge of the features of climate change	Number	Percentage
Low level of knowledge	6	8.82
Average level of knowledge	8	11.77
High level of knowledge	54	79.41
Total	68	100

Source: Data collected and calculated from questionnaires.

Knowledge of the reasons for climate change

Table 2 shows that 76.47% of the respondents have a high level of knowledge of the reasons for climate change.

Table 2. Distribution of the respondents' answers for the knowledge of the reasons for climate change

Knowledge of the reasons for climate change	Number	Percentage
Low level of knowledge	9	13.24
Average level of knowledge	7	10.29
High level of knowledge	52	76.47
Total	68	100

Source: Data collected and calculated from questionnaires.

Knowledge of the impacts of climate change

Table 3 shows that 73.52% of the respondents have a high level of knowledge of the impacts of climate change.

Table 3. Distribution of the respondents' answers for the knowledge of the impacts of climate change

Knowledge of the impacts of climate changes	Number	Percentage
Low level of knowledge	9	13.24
Average level of knowledge	9	13.24
High level of knowledge	50	73.52
Total	68	100

Source: Data collected and calculated from questionnaires

The results of tables 1, 2 and 3 indicate that the respondents have high level of knowledge of the features, reasons for, and impact of climate change. This, in turn, reflects the fruit of the training they have already received in the field of climate change.

The desire to participate in extension activities to face climate change

Table 4 shows that 98.53% of the respondents have desire to participate in extension activities to face climate change. This is an indicator of their motivation and keen interest in achieving the goals of extension work to overcome this climate change, and diminish their negative impact on the agrarian sector.

Table 4. Distribution of the respondents' answers for the desire to participate in extension activities to face climate change

Desire to participate in extension activities	Number	Percentage
Have desire	67	13.24%
Do not have desire	1	13.24%
Total	68	100%

Source: Data collected and calculated from questionnaires.

The role of agricultural extension in diminishing the agricultural causes of climate change

Table 5 contains the respondents' answers to the role played by agricultural extension in diminishing the agricultural causes of climate change. Top on the list of the roles played by extension agents is informing farmers of the need to stop burning rice straw, and recycling it; 61 participants (89.1%) highlighted this role. Then, 60 participants (88.23%) have mentioned the role of the need not to increase the land allocated for growing rice. Finally, 58 participants

(85.29%) have pinned down the importance of informing about the environmental problems and pollution sources.

Table 5. Distribution of respondents' answers to the role played by agricultural extension to diminish the causes of climate change

The agricultural extension role	Frequency							
	Always	%	Sometimes	%	Rarely	%	Never	%
Less use of chemical fertilizers	54	79.41	13	19.12	1	1.47	-	-
Amending agricultural waste usage system	51	75	13	19.13	4	5.88	-	-
Not increasing the land for rice	60	88.23	7	10.29	1	1.47	-	-
Informing of environmental problems and pollution sources	58	85.29	10	14.71	-	-	-	-
Informing of the need not to overuse pesticides	52	76.47	15	22.06	1	1.47	-	-
Informing of the need to stop burning rice straw, and to reuse it	61	89.70	5	7.35	2	2.94	-	-
Increasing the culture of afforestation	52	76.47	15	22.06	6	8.82	-	-
Informing of the need to use organic fertilizers	47	69.12	15	22.06	6	8.82	-	-
Banning building on agrarian land	53	77.94	8	11.76	7	10.29	-	-
diffusing the integrated pest management methods	50	73.53	17	25	1	1.47	-	-

Source: Data collected and calculated from questionnaires.

Being aware of the role played by agricultural extension in overcoming the impacts of climate change on agriculture:
Table 6 shows the extension agents' answers to the role of agriculture extension in overcoming the impact of climate change in agriculture. Top on the list of the roles are using new or genetically modified species, lessening the land that consumes a lot of water, growing legumes and green manures. 60 participants (88.23%) reported equal ratios for such roles. Next, 86.76% of the participants referred to the need to diffusion short-term species that need the least amount of water, and using new and advanced irrigation and sewage systems.

Table 6. Distribution of respondents' answers to the role played by agricultural extension to overcome the impact of climate change on agriculture

The agricultural extension role	Frequency							
	Always	%	Sometimes	%	Rarely	%	Never	%
Coping and adapting with climate changes	51	75	14	20.59	2	2.94	1	1.47
Informing of lessening the dangers related to climate changes	53	77.94	12	17.65	3	4.41	-	-
Using new or genetically modified species	60	88.23	6	8.82	1	1.47	1	1.47
diffusing short-term	59	86.76	7	10.29	1	1.47	1	1.47

The agricultural extension role	Frequency							
	Always	%	Sometimes	%	Rarely	%	Never	%
species that need the least amount of water								
Lessening the crops that need a lot of water	60	88.23	4	5.88	4	5.88	-	-
Using new and advanced irrigation and sewage methods	59	86.76	6	8.82	3	4.41	-	-
Improving the use of pesticides and insecticides	56	82.35	10	14.71	2	2.94	-	-
Growing legumes and green manures	60	88.23	7	10.29	1	1.47	-	-
Informing of the change in growing schedules	58	85.29	10	14.71	-	-	-	-
Informing of the change in harvest schedules	54	79.41	9	13.23	5	7.35	-	

Source: Data collected and calculated from questionnaires.

The problems of climate change and their impact on the agricultural extension work
Table 7 indicates the respondents' answers to the problems of climate change and their impact on the agricultural extension work. These can be descending ordered as follows: the difficulty in implementing the work plan because of rainfall conditions (57.35%), the difficulty in implementing farm administration and the new farm technologies (55.88%), overload of the extension agents' work (52.94%), increase in training costs (51.47%), difficulty in training farmers in a timely manner (33.82%), difficulty in persuading farmers of increasing their investments in agriculture (30.88%), and lastly doubt in the extension advice (19.12%).

Table 7. Distribution of respondents' answers to the problems of climate change and their impact on the agricultural extension work

Problem	Agree		Partly agree		Disagree	
	Number	%	Number	%	Number	%
Difficulty in persuading farmers of investing their money in agriculture	21	30.88	27	39.71	20	29.41
Difficulty in implementing the work plan because of rainfall conditions	39	57.35	25	36.76	4	5.88
Difficulty in training farmers in a timely manner	23	33.82	23	23.82	22	32.35
Difficulty in implementing farm administration and new farm technologies	38	55.88	14	20.59	16	32.53
Doubt in extension advice	13	19.12	33	48.53	22	32.35
Overload of the extension agents' work	36	52.94	15	22.06	17	25
Increase in the training costs	35	51.47	14	20.59	19	27.94

Source: Data collected and calculated from questionnaires.

Conclusions

In light of the foregoing discussion, it is clear that agricultural sector affects the climate change phenomenon, and vice versa. It is also clear that extension agents in the extension centers have high level knowledge of the features, reasons and impact of the climate change on agriculture. This is clearly reflected on their desire to participate in agricultural extension activities to face this phenomenon. They are full aware of the role played by agricultural extension in this mission, and to what extent the sector can achieve this goal. Agricultural extension spares no effort in facing climate change, whether through diminishing their causes, or adapting with their impact through informing farmers and their families of the proper agricultural practices.

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RURAL WOMEN NEEDS ABOUT EXTENSION KNOWLEDGE REGARDING CLIMATE CHANGE PHENOMENON IN Al-GHARBIA GOVERNORATE, EGYPT

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Abstract

This study aims to identify the extension knowledge needs of rural women of the meaning, causes and effects of climate change phenomenon on agriculture, food and nutrition, public health, poultry raising, breeding and care of farm animals, and the field of protection and conservation of environmental resources. This study was conducted in some villages of Al-Gharbia Governorate, Egypt, from December 2018 to February 2019. Data were collected from 300 respondents, randomly selected multistage-clustered sample, through a personal interview questionnaire made especially for this purpose. Several statistical methods were used such as percentage and frequency tables. The most striking results of the study showed that 51.34% of respondents had a high extension knowledge need about the meaning of climate change phenomenon. 60.34% of respondents had a high extension knowledge need about the causes of the climate change phenomenon. 54.67%, 62%, 73.67%, 53.67%, 35.33%, 54.67% of respondents had a high extension knowledge need about the effects of climate change in order on; agriculture, food and nutrition, health, farm animal breeding, poultry, and the protection and maintenance of environmental resources, respectively.

Keywords: *Agricultural extension needs, climate change phenomenon, rural women, Al-Gharbia governorate, Egypt.*

Introduction

Climate change, more commonly known as global warming, is highly regarded by each country due to their impacts on human existence. Climate change has serious future effects and the reasons for these climate changes can be natural or human. The latter can be divided into industrial reasons and agricultural ones. The total gas emissions from agriculture account for 15.7% of the total global warming gases resulting into climate change (Abu Hadeed, 2010). So agriculture has a big share in causing climate change. It is affected by these changes as well. This appears in the decrease in crop yield and animal production; the increase in water consumption for irrigation, the increase in water levels on land, soil salt, the diminishing net farm revenue, the increase in diseases and plant insects, and the increase in the cotton yield (Abd Elhafez, 2007; Ifeanyi-obi et al., 2012; EL-Marsfawy, Nnadi et al., 2013; Al-Shaib et al. 2016; Abd Ella et al., 2018).

Agricultural extension has a vital role in facing climate changes prior to their occurrence through informing rural women to amend their practices which lead to the increase in the greenhouse gases. It has also an essential role after the occurrence of climate changes through informing rural women of the practices which could diminish the impact of these changes, telling them of how to adapt with the changes and lessen their side effects through training programs for rural women (Saleh, 2009; Keshta, 2012; Mustapha et al., 2012; El-Shenawy et al., 2013; AL-Shaib et al., 2016; Abd Ella et al. 2018).

The first step to create a good agricultural extension programs to increase rural women' awareness of climate change must be in accordance with the really extension needs of them (Yaghy, 1986). So, this study aims to identify the extension knowledge needs of rural women of the meaning, causes and effects of climate change phenomenon on agriculture, food and nutrition, public health, poultry raising, breeding and care of farm animals, and the field of protection and conservation of environmental resources.

Material and Methods

A multi-stage cluster sample of Gharbia villages was drawn yielding four sample villages. A sample of 75 rural women was selected from each sample village using the snowball technique; to be the total sample was 300 rural women. Data were collected from December 2018 to February 2019, through a personal interview questionnaire made especially for this purpose. Several statistical methods were used such as percentage and frequency tables.

The knowledge extension needs of respondents on the phenomenon of climate change were measured through several variables that measure their knowledge of aspects of the phenomenon as follows: the meaning of the phenomenon of climate change, the causes of climate change, the effect of Climate Change on the Study Areas, the effect of climate change on agriculture, the effect of climate change on food and nutrition, the effect of climate change on public health, the effect of climate change on poultry raising, the effect of climate change on breeding and care of farm animals, and the effect of climate change on the field of protection and conservation of environmental resources.

The knowledge extension needs were assessed as the degrees which complete perfect knowledge of each study variable. The total degree of knowledge extension needs according to the actual range of each study variable was divided into three levels: high knowledge extension needs, medium knowledge extension needs and low knowledge extension needs. Only the knowledge extension needs for the meaning of the phenomenon of climate change divided to two levels high and low; Knowledge needs for the meaning of the phenomenon of climate change: Were measured the rural women Knowledge needs of the concept of the phenomenon of climate change by asking the respondents about a specific concept of the phenomenon of climate change, and given answers values (2) to the correct answers; and that mean their knowledge needs about the concept of climate change phenomenon is low, So they do not need training of this aspect, and (1) those who did not know the answer; that mean their Knowledge needs of the concept of phenomenon is high, so they need training of this aspect. As shown in table (1).

Knowledge needs of rural women on the causes of climate change: were measured through a tool containing 12 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the causes of climate change and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 36 degrees. The real range has been divided into three groups: A low was less than 22, medium was from 22 to 29, and a high knowledge need was more than 29, so they need training of this aspect. As shown in table (2).

Knowledge Needs of the effect of Climate Change on the Study Areas:

Knowledge needs on the effect of climate change on agriculture: were measured through a tool containing 9 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on agriculture and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 27 degrees. The real range has been divided into three groups: A low was less than 15, medium was from 15 to 20, and a high knowledge need was more than 20, so they need training of this aspect. As shown in table (3).

Knowledge needs on the effect of climate change on food and nutrition: were measured through a tool containing 9 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on food and nutrition and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 27 degrees. The real range has been divided into three groups: A low was less than 16, medium was from 16 to 22, and a high knowledge need was more than 22, so they need training of this aspect. As shown in table (4).

Knowledge needs on the effect of climate change on public health were measured through a tool containing 9 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on public health and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 27 degrees. The real range has been divided into three groups: A low was less than 16, medium was from 16 to 22, and a high knowledge need was more than 22, so they need training of this aspect. As shown in table (5).

Knowledge needs on the effect of climate change on poultry raising were measured through a tool containing 10 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on poultry raising and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 30 degrees. The real range has been divided into three groups: A low was less than 17, medium was from 17 to 23, and a high knowledge need was more than 23, so they need training of this aspect. As shown in table (6).

Knowledge needs on the effect of climate change on breeding and care of farm animals: were measured through a tool containing 10 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on breeding and care of farm animals and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 36 degrees. The real range has been divided into three groups: A low was less than 17, medium was from 17 to 23, and a high knowledge need was more than 23, so they need training of this aspect. As shown in table (7).

Knowledge needs on the effect of climate change on the field of protection and conservation of environmental resources: were measured through a tool containing 5 items. Participants had asked about their knowledge of the all respondents were asked about their knowledge of the effect of climate change on the field of protection and conservation of environmental resources and a response was chosen from three responses: Yes, I do not know, and no, those responses had codes 3, 2, 1; respectively for the correct items and codes 1, 2, 3; to the Wrong items The answers obtained by each researcher were subtracted from the expected knowledge needs which 36 degrees. The real range has been divided into three groups: A low was less than 9, medium was from 9 to 12, and a high knowledge need was more than 12, so they need training of this aspect. As shown in table (8).

Several statistical methods such as percentage and, frequency tables were used to analyze the data.

Results and Discussion

The meaning of the phenomenon of climate change:

Table 1 show that 51.33% of the participants have a high level of the knowledge extension needs for the meaning of the phenomenon of climate change.

Table 1: the knowledge extension needs' levels for the meaning of the phenomenon of climate change

Level	N	%
High (1)	**154**	**51.33**
Low (2)	**146**	**48.67**
Total	**300**	**100**

Source: Data collected and calculated from questionnaires.

The causes of climate change:

Table 2 show that, 60.33% of the participants have a high level of the knowledge extension needs for the causes of climate change.

Table 2: the knowledge extension needs' levels for the causes of climate change

Level	N	%
High (more than 29)	181	60.33
Average (from 22 to 29)	102	34
Low(less than 22)	17	5.67
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 28.12, Standard deviation: 4.54

The effect of climate change on agriculture:

Table 3 show that, 54.67% of the participants have a high level of the knowledge extension needs for the effects of climate change phenomenon on agriculture.

Table 3: the knowledge extension needs' levels for the effect of climate change on agriculture

Level	N	%
High (more than 20)	164	54.67
Average (from 15 to 20)	77	25.66
Low (less than 15)	59	19.67
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 22.12, Standard deviation: 5.54

The effect of climate change on food and nutrition:

Table 4 show that 62% of the participants have a high level of the knowledge extension needs for effect of climate change on food and nutrition.

Table 4: the knowledge extension needs' levels for the effect of climate change on food and nutrition

Level	N	%
High (more than 22)	186	62
Average (from 16 to 22)	106	35.33
Low (less than 16)	8	2.67
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 21.38, Standard deviation: 3.16

<u>The effect of climate change on public health:</u>
Table5 show that 73.67% of the participants have a high level of the knowledge needs for effect of climate change on public health.

Table 5: the knowledge extension needs' levels for the effect of climate change on public health

Level	**N**	**%**
High (more than 22)	221	73.67
Average (from 16 to 22)	72	24
Low (less than 16)	7	2.33
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 21.1, Standard deviation: 3.46

<u>The effect of climate change on poultry raising:</u>
Table6 show that 53.67% of the participants have a high level of the knowledge needs for effect of climate change on poultry raising.

Table 6: the knowledge extension needs' levels for the effect of climate change on poultry raising

Level	**N**	**%**
High (more than 23)	161	53.67
Average (from 17 to 23)	83	27.66
Low (less than 17)	56	18.67
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 21.88, Standard deviation: 5.16

<u>The effect of climate change on breeding and care of farm animals:</u>
Table7 show that 35.33% of the participants have a high level of the knowledge needs for effect of climate change on breeding and care of farm animals.

Table 7: the knowledge extension needs' levels for the effect of climate change on breeding and care of farm animals

Level	**N**	**%**
High (more than 23)	106	35.33
Average (from 17 to 23)	122	40.67
Low (less than 17)	72	24
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 21.38, Standard deviation: 3.16

<u>The effect of climate change on the field of protection and conservation of environmental resources:</u>
Table8 show that 54.67% of the participants have a high level of the knowledge needs for effect of climate change on the field of protection and conservation of environmental resources.

Table 8: the knowledge extension needs' levels for the effect of climate change on the field of protection and conservation of environmental resources

Level	N	%
High (more than 12)	164	54.67
Average (from 9 to 12)	98	32.67
Low (less than 9)	38	12.66
Total	300	100

Source: Data collected and calculated from questionnaires. Mean: 11.38, Standard deviation: 3.16

The results of all the tables indicate that the respondents had a high level of knowledge extension needs of the meaning of climate change, climate change phenomenon' reasons, and its effects on agriculture, food and nutrition, public health, poultry raising, breeding & care of farm animals, and the field of protection and conservation of environmental resources. This, in turn, reflects how rural women need lots of agricultural extension interest to develop their knowledge about climate change phenomenon, and learn how to adapt with it and lessen the side effects of this phenomenon.

Conclusions

In light of the foregoing discussion, it is clear that rural women are in great need to develop their knowledge of climate change phenomenon. This will only happen through agricultural extension programs which increase rural women awareness about the effects of climate change phenomenon.

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ATTITUDE OF AGRICULTURAL EXTENSION AGENTS ABOUT ELECTRONIC AGRICULTURAL EXTENSION IN Al-GHARBIA GOVERNORATE, EGYPT

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Abstract

The main objective of the study was to identify the attitude of agricultural extension workers towards the applications of e-agriculture extension in Gharbia governorate. The size of the sample was determined by using the Krejcie & Morgan table. The sample encompassed 196 randomly selected participants according to the percentage of each category of agricultural extension workers in the overall. A questionnaire was distributed to the respondents during monthly meetings. Data were collected, and 192 cases were retrieved by 97.96%. Frequencies, percentages and T test were used for statistical analysis.The most important results were: Percentage of agricultural extension specialists 50.5% of sample size. About 80.7% of the respondents did not receive training courses in the field of e-agricultural extension. Only 39.6% of the respondents had computers connected to the Internet. 54.2% of respondents had a mobile phone with internet access. Half of the respondents had a low attitude towards e-agricultural extension. The most important problems of electronic agricultural extension were: lack of training of agricultural extension workers on e-agricultural extension, lack of Internet services in the work place, and lack of financial allocations to provide e-agricultural extension services.

Keywords: *E-agricultural extension application, ICT, VERCON, RADCON, Egypt.*

Introduction

The world now witnesses many economic, social and technological changes that have created a new reality of communication through information communication technology (ICT) applications. Now, the world is living the age of knowledge or ICT revolution. Information has become the power that can be used to increase the level of knowledge and a tool to influence the behavior of individuals in society (El-Baaly, 2011).

Agricultural extension is essential for achieving agricultural and rural development by exploiting all available resources and providing information and knowledge in all activities of rural life and changing rural knowledge, skills and attitudes, using various and different methods and means of guidance(Qamar,2005).

Traditional agricultural extension suffers from shortcomings in its methods and means of transferring agricultural knowledge, do not respond to the needs of the target audience, and lack effective mechanisms for interaction (Saleh, 2001; El-Baaly and El-Gohary, 2012, 2013).

Therefore, it was necessary to take advantage of the rapid developments in the field of information and communication technology based on the application and employment of computers and the Internet in the service of the agricultural extension sector and development through the provision of channels of communication wide and diverse as well as activating the role of information and communication technology, which provides the most appropriate communication environment for rural and agricultural development. The initiatives that employ ICT in agricultural extension should be seen as complementary to and not substitute for traditional extension services (Abdel Wahed, 2007, 2015).

The Agricultural Extension Service has recently introduced and applied ICT to overcome the difficulties faced by the agricultural extension system and the traditional extension methods and to increase their effectiveness. They are very powerful tools for learning rural people and providing them with the knowledge and skills they need to improve their living conditions. Development services, particularly in agricultural extension education, where ICTs constitute a fundamental change in the educational process (Qeshta, 2012).

Electronic extension is one of the forms of e-learning, so electronic extension can be defined as a new ICT-based extension system represented in computer technology and the Internet and made available to all users everywhere, all time, flexibly and easily (Qeshta, 2012; Abdel Wahed, 2015).

E-agricultural extension applications are a development in the communication channels in response to the ICT revolution. The Egyptian agricultural extension system has recently applied E-agricultural extension applications to overcome the difficulties faced by traditional extension methods and increase their efficiency. E-agricultural extension applications include: Agricultural Expert Systems, Virtual Extension and Research Communication Network (VERCON) http://www.vercon.sci.eg, Rural and Agricultural Development Communication Network (RADCON) http://www.radcon.sci.eg, Mobile Phone: M. Learning - M. Farming, and Social Media: Facebook https://www.facebook.com/Farmersuv-501445726675105/?fref=ts (El-Baaly,2018).

Although, e-agricultural extension applications improved agricultural extension service as an intelligent service, but diffusion of e-agricultural extension had not continued as expected. Therefore, the present study is intended to investigate the attitudes of agricultural extension workers towards E-agricultural extension applications.

The main objective of the study was to identify the attitudes of agricultural extension workers towards electronic extension in Al-Gharbia Governorate, Egypt by achieving the following sub-objective: (i) to identify the level of the attitudes of respondents towards E-agricultural extension applications (ii) To identify the differences between the attitudes of the respondents towards electronic agricultural extension when categorizing them on the basis of qualitative variables namely: job title, training in e-agricultural extension applications, availability of computer connected to the internet, availability of laptop connected to the Internet (iii) to identify the most important problems of e-agricultural extension applications from the point of view of the respondents and their proposals to solve them.

Research hypothesis:

To achieve the second research objective, the following research hypothesis was formulated: There are differences in the average scores of the attitude towards e-agriculture among respondents when classified according to: job title, training in e-agricultural extension applications, availability of a computer connected to the Internet, and availability of a laptop connected to the Internet. To test this hypothesis was formulated in its null hypothesis.

Material and Methods

There are 402 agricultural extension workers in Al-Gharbia Governorate. The size of the sample was determined by using the Krejcie & Morgan table (1970), 196 respondents were randomly selected. Data were collected from the respondents by interview questionnaire during the monthly meetings which were organized within 2018, and 192 cases were retrieved by 97.96% of whole sample.

A study of variables measured as follows:

<u>Job title:</u> There was a question about the job title. The responses were: Agricultural Extension Specialist, Agricultural extension agent, Responses were given (2, 1) in order.

<u>Obtained training in e-agricultural extension applications:</u> respondents were asked whether they received training courses in the field of e- agricultural extension applications or not, and the responses are: Yes or No and were given degrees (2.1), in order.

The availability of a computer connected to the Internet: It was expressed by the question of the availability of a computer connected to the Internet and whether or not the responses were: Yes or No and were given degrees (2.1), in order.
The availability of a mobile phone connected to the Internet: It was expressed by asking the question of the availability of a mobile phone connected to the Internet and the responses are: Yes or No and were given degrees (2, 1), in order.
The attitude of the respondents towards electronic agricultural extension: it is the main variable in the study. It has been measured through a tool containing 17 items. Participants have been asked about their response to each item was disagree, Partly agree, and agree, were given degrees 1, 2, 3 for positive items, and 3, 2, 1 were given for negative items, and the collection of degrees to express the researcher's attitudes towards electronic agricultural extension.
Problems of e-agricultural Extension: Each respondent was asked to identify the problems of e-agriculture according to his/her opinion..
Proposals to solve the problems of e-agricultural extension: each respondent was requested to identify appropriate solutions to overcome the problems of electronic agricultural extension.
Several statistical methods such as percentage, frequency and T- test were used to analyse the data.

Results and Discussion

A description of the study variables is presented hereinafter:
Job title:
Table 1 shows that 50.5% of the respondents are agricultural extension specialists and 49.5% of them are agricultural extension agent.

Table 1. Number and percentage distribution of respondents according to job title

Job title	Number	Percentage
agricultural extension specialists	97	50.5
agricultural extension agent	95	49.5
Total	192	100

Source: Data collected and calculated from questionnaires.

Obtain training in e- agricultural extension applications:
Table 2 shows that 80.7% of the respondents did not receive training courses e-agricultural extension applications. This may made negatively affect about their knowledge about e-agricultural extension applications, and its ability to provide an e-agricultural extension service as a smart service.

Table 2. Number and percentage distribution of respondents according to obtain training in e-agricultural extension applications

Obtain training in e-agricultural extension applications	Number	Percentage
Obtained training	37	19.3
Did not obtain training	155	80.7
Total	192	100

Source: Data collected and calculated from questionnaires.

This result indicates that, there are need to prepare intensive training programs for agricultural extension workers in the field of e-agricultural extension applications to improve their knowledge and experience, develop their skills and improve their abilities to benefit from the applications of e-agriculture to intelligent agricultural extension service.

The availability of a computer connected to the Internet:

Table 3 shows that only 55.2% of the respondents had computers. This percentage is not large in light of the call to activate the electronic agricultural extension.

Table 3. Number and percentage distribution of respondents according to computer availability

Availability of a computer	Number	Percentage
available	106	55.2
not available	86	44.8
Total	192	100

Source: Data collected and calculated from questionnaires.

Table 4 shows that 39.6% of the respondents had an internet computer. This is a small percentage despite the spread of Internet service in Al- Gharbia Governorate, Egypt. This may adversely affect the attitude towards e-agricultural extension applications.

Table 4. Number and percentage distribution of respondents by availability of computer connected to internet

computer connected to the Internet	Number	Percentage	Percentage of total sample
Available	76	71.7	39.6
Not available	30	28.3	10.6
The total number of respondents who have a computer	106	100	55.2

Source: Data collected and calculated from questionnaires.

The availability of a mobile phone connected to the Internet:

Table 5 shows that 94.3% of the respondents have a mobile phone. This is indicative of the spread of such technology, and that there is ease in the acquisition of mobile; perhaps for the price, ease of carrying and necessary in the communication of life in general.

Table 5. Number and percentage distribution of respondents according to their Mobile Phone Availability

Availability of a mobile phone	Number	Percentage
Available	181	94.3
Not available	11	5.7
Total	192	100

Source: Data collected and calculated from questionnaires.

Table 6 shows that 54.2% of the respondents have a mobile phone connected to the Internet; In other words, just over half of the respondents have the opportunity to deal with e-agriculture extension applications on the mobile phone connected to the Internet to obtain the agricultural extension information they need in their agricultural extension tasks and provide smart agricultural extension service.

Table 6. Number and percentage distribution of respondents by availability of Internet in Mobile Phone

Mobile connected to the Internet	Number	Percentage	Percentage of total sample
Available	104	57.5	54.2
Not available	77	42.5	40.1
Total number of respondents who have a mobile	181	100	94.3

Source: Data collected and calculated from questionnaire.

<u>The attitude of respondents towards e-agricultural extension:</u>
Table 7 shows responses of the respondents on the scale of the attitude towards e-agricultural extension, which indicated the weakness of attitudes towards e-agricultural extension, may be that because of weak awareness and training in e-agricultural extension field.

Table 7. Distribution of the responses of the respondents on the scale of attitude towards electronic agricultural extension

Items	Disagree		Partly agree		Agree	
	N	%	N	%	N	%
E-agricultural extension applications are complex and difficult.	57	29.7	31	16.1	104	54.2
Information obtained of e-agricultural extension applications is inaccurate.	41	21.4	50	16.2	101	52.6
Information obtained of e-agricultural extension applications is special.	118	61.5	39	20.3	35	18.2
Information obtained of e-agricultural extension applications is biased.	34	17.7	43	22.4	115	59.9
E-agricultural Extension applications are not a good source of agricultural information.	60	31.3	29	15.1	103	53.6
Information obtained of e-agricultural extension applications is uncertain results.	44	22.9	44	22.9	104	54.2
Information obtained of e-agricultural Extension applications is conflicting.	44	22.9	37	19.3	111	57.8
Encouragement the use of e-agricultural extension applications.	112	58.4	30	15.6	50	26
Information obtained of e-agricultural extension applications is applicable.	113	58.9	41	21.4	38	19.8
Information obtained of e-agricultural extension is consistent with other sources of agricultural information.	111	57.8	50	26	31	16.1
Information obtained of e-agricultural extension is credible.	110	57.3	49	25.5	33	17.2
E-agricultural extension applications are a waste of effort.	55	28.6	33	17.2	104	54.2
E-agricultural Extension applications are an effective method of communication between specialists.	105	54.7	37	19.3	50	26

Items	Disagree		Partly agree		Agree	
	N	%	N	%	N	%
E- agricultural Extension applications have become the best source of agricultural information.	112	58.4	51	26.6	29	15.1
E-agricultural extension applications are a waste of time.	58	30.2	27	14.1	107	55.7
E-Agricultural extension applications enable new agricultural information to be captured.	105	54.7	38	19.8	49	25.5
E-Agricultural Extension Applications inexpensive.	104	54.2	28	14.6	60	31.3

Source: Data collected and calculated from questionnaires.

In order to complete the view, the score of the items of the scale was collected to reflect the degree of attitude of each respondent. The actual range of the respondents was calculated. It was found to be 25 degrees; the lowest score was 17 and the highest score was 42. The actual range was divided into three sections as shown in Table 8; half of the respondents had a low positive attitude towards e-agricultural extension, while 5.2% of the respondents had an average positive response, and 44.8% of the respondents had a high positive attitude towards e-agricultural extension.

Table 8. Distribution of responses by respondents according to degree of attitude towards e-agricultural extension applications

Level of attitude toward e-agricultural extension	Number	Percentage
Low positive attitude from 17 to 25	96	50
Average positive attitude from 26 to 34	10	5.2
Positive attitude is high from 35 to 42	86	44.8
Total	192	100

Source: Data collected and calculated from questionnaires

The results of T-tests between the mean scores of the respondents' attitudes towards e-agricultural extension when classified according to independent variables qualitative:
Table 9 presents that there are significant differences in the attitude towards electronic agricultural extension applications among the respondents when they are classified on the basis of each of the qualitative independent variables namely: job title, training on e-agricultural extension applications, availability of computer connected to the Internet, availability of mobile phone connected to the Internet, Calculated (T) are 6.93, 3.66, 17.17 and 13.99 respectively, which are significant values at a significant level of 0.01. This result supports the research hypothesis.

Table 9. The results of T-tests of the differences between the mean scores of the respondents' attitudes towards e-agricultural extension when classified according to the independent variable categories

Independent variables		Mean scores of the respondents' attitudes towards e-agricultural extension	T value
Job title	Agricultural Extension Specialist	33.70	6.93 **
	Agricultural extension agent	23.03	
Training in e-agricultural extension applications	had training	34.65	3.66 **
	had not training	26.93	
Availability a computer connected to the Internet	available	39.85	17.17**
	unavailable	20.93	
Availability a mobile phone connected to the Internet	available	37.61	13.99**
	unavailable	20.64	

** Statistical significance at the level of 0.01

Problems of e-agriculture extension applications and proposed Solutions:
Table 10 shows that respondents identified ten problems of e-agricultural extension problems. Lack of training in the e-agricultural extension applications was in the top of the problems by 83.2% of responses, followed by lack of Internet services in the workplace of agricultural extension workers by 79.2% of respondents' answers.

Table 10. Respondents' answers about problems of e-agricultural extension applications

Problems	Frequencies	Percentages
Lack of training in e-agricultural extension applications.	160	83.2
Lack of Internet services in the work place of agricultural extension workers.	152	79.2
Lack of funding for electronic agricultural extension.	148	77
Continuous disruption of the Internet in the workplace of agricultural extension.	144	75
Lack of maintenance of computers.	138	71.8
E-agricultural extension applications are not updated regularly.	132	68.8
Lack of computers in the workplaces of agricultural extension.	128	66.6
High costs of e-agricultural extension applications.	108	56.2
Lack of e-agricultural extension applications.	100	52.1
Believes that e-agricultural extension is not important.	92	47.9

Source: Data collected and calculated from questionnaires.

Most of these problems appear to be related to the continued lack of funding for e-agricultural extension services after the initial pilot phases, which it are often funded by foreign projects or grants, and there are indicators confirming that success of e-agricultural extension applications as smart agricultural extension services in Egypt .
Proposals to solve the problems of e-agricultural extension from the point of view of respondents:

Table 11 that the respondents' suggestions solutions to problems of e-agricultural extension. The proposed solutions are arranged in descending order according to the answers of the respondents as follows: training agricultural extension workers on e-agricultural extension applications, connecting the internet service to the work place of the agricultural extension, providing periodic maintenance of electronic devices, updating information on e-agricultural extension applications, providing agricultural extension' workplaces with a sufficient number of computers, and other solutions.

Table 11. Respondents' suggestions solutions to problems of e-agricultural extension

Suggestions solutions	Frequencies	Percentages
Training agricultural extension workers on e-agricultural extension applications.	160	83.2
Connecting the Internet service to the work place of agricultural extension.	152	79.2
Providing periodic maintenance of electronic devices.	138	71.8
Updating information on e-agricultural extension applications.	132	68.8
Providing agricultural extension' workplaces with a sufficient number of computers.	128	66.6
Linking agricultural extension work to Internet.	77	40.1
Provide an incentive to encourage the use of e-agricultural extension applications.	74	38.5
Providing agricultural extension system with information technology experts.	74	38
Recruitment of young cadres able to use ICT in agricultural extension.	71	37
Diffusion awareness of the culture of electronic agricultural extension.	47	38.6

Source: Data collected and calculated from questionnaires.

Conclusions

The extension service can be provided through e-agricultural extension applications and can be offered in partnership and coordination between traditional agricultural extension and e-agricultural extension applications, and can only be provided through conventional agricultural extension according to the requirements of each extension situation and the circumstances of each extension agent.

There are several mechanisms to ensure that continue funding, whether by providing services entirely free of government funding or through various agricultural companies that invest in selling their products and marketing their services or paying the cost through the farmers' organizations that can be formed and Contractual farming with Agri-value chains , or directly pay farmers according to the number of their transactions specially when they use the most specialized services of high value, or adoption a combination of the previous methods.

Recommendations

Based on findings of the study there are practical/theoretical/political implications and originality/value, so it is possible to recommend:

(1) Preparation and implementation of training programs in the field of e-agricultural extension applications.

(2) Provide adequate financial allocations to activate the e-agricultural extension and ensure its continuity.

(3) Link agricultural extension services to an electronic database through smart cards for farmers.
(4) Providing advanced computers connected to the Internet in agricultural extension workplaces.
(5) Updating available agricultural information on e-agricultural extension applications.

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RURAL TOURISM AND SUSTAINABLE DEVELOPMENT : THE CASE OF TUNIS VILLAGE'S HANDICRAFTS, EGYPT"

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Abstract

Tunis Village, Fayoum governorate, Egypt, is considered a successful model in Egypt inmaking full use of handcraft in promoting the Village and achieving sustainable tourism development.This research aims at exploring the current status of Pottery handcraft in Tunis Village and assessing its role as a tool for tourism promotion and achieving sustainable development in the Village.Three segments were surveyed in July 2019; the first segment is Pottery crafters where forty Pottery crafters were interviewed, while the second segment are the Tourists where unstructured face to face interviews conducted with fifteen tourists, and finally a survey was directed to Tunis Village's Local residents (non-crafters) where thirty questionnaires were completed. The results revealed that allPottery crafters indicated that the profession helped them to provide better education and health care to their families, they stated also that they are feeling safe and social secure. Both craftersand tourists stated that the Potteryhandicraft is main motivationfor tourists to visit Tunis Village. The most important challenges facing the profession of handicrafts are high prices of raw materials, as well as the great effort and long time needed for the preparing and processing the raw materials till it become ready for shaping.Local residentsstated that Potteryworkshops have attracted the tourists to visit the village, the matter which enabled them to increase and diversify their source of income, as it has created many Tourism related jobs. The researchhas come up with a number of recommendations to sustain the model of Tunis Villagewhich managed to use the Handicrafts inachieving sustainable tourism development, among these recommendations;making some modifications in the furnaces to reduce the emissions generated during firing the products and holding special events such as exhibitions, fairs and festivals showcase Pottery handcraft products, especially in the low season in summer.

Keywords: *Sustainable development, Rural tourism, Tunis village,.Pottery handicrafts*

Introduction

Cultural industries now play a major role in tourism marketing and development. Many countries all over the world started to use handicrafts as the main core in tourism development and marketing, establish the tourism facilities near main handicrafts production centers. Some develop cultural itineraries that focus on showing tourists and visitors such valuable intangible heritage. Egypt is famous for its traditional handicrafts since the beginning of the history, many handcraft clusters are scattered everywhere along the country. Despite this fact, the Egyptian tourism in Egypt has been shortened to just one product which the leisure tourism which represents almost 96% out of the inbound tourism in Egypt the matter which means that Egypt didn't get benefited from the existence of such valuable cultural heritage.

Handicrafts definition and importance

There are different definitionsfor the term handicraft;maybe the most acceptable one is the one that has been adopted by UNESCO In 1977 at Manila during a syposthym Symposium on Crafts, at which Handicrafts are defined as:

"*a type of work produced by artisans, either completely by hand, or with the help of hand-tools or even mechanical means, as long as the direct manual contribution of the artisan remains the most substantial component of the finished product. These are produced without restriction in terms of quantity and using raw materials from sustainable resources. The special nature of artisan products derives from their distinctive features, which can be utilitarian, aesthetic, artistic, creative, culturally attached, decorative, functional, traditional, religiously and socially symbolic and significant."*
The handicrafts sector has attracted the global attention long time ago, due to its cultural and socio-economic importance; as from the cultural point of view Handicrafts are considered a valuable component of the intangible heritage of any society, it represents its culture, history and traditions (India-craft, 2013).
The craft sector alsohas a great Socio-Economic importance as it is considered a home-based industry that needs minimum spending and facilities, simple instruments and comparatively brief apprenticeship or training depending on the complexity of the craft. The matter which makes this sector one of the biggest and most important employers without the need for big investments.
The home –based nature of the craft sector has given the chance for both males and females to join itespecially in rural areas where the conservative social system put restrictions on women leaving their household even if there are searching for an income-earning activity.
The craft sector also -in most cases- is a clean, environmentally-friendly industry which uses the available raw materials in the environment.
These incredible features of handicraft sector have made it an excellent tool that can play a major role in promoting sustainable development, as one can notice that it covers the three main dimensions of sustainable development; the social, economic, environmental.
In this regard and for all what have been mentioned above that shows the significance of craft sector, many industrialized and developing countries have exerted great efforts to preserve, develop and promote this sector, the upcoming figures show to which extent these countries have managed to make full use of the craft sectore as a tool for achieving sustainable development.
In Italy, the craft sector employees around 1/5 of the total number of workers and exports from Italian crafts constitute 17% of its Gross Domestic Product In Thailand the total number of craftspeople is 2 million. In Morocco, The handicrafts exports are estimated at US$ 63 million. While In Tunisia the craft sector employs about 300,000 craftspeople (11% of the active population) and the sector accounts for 3.8% of the Gross Domestic Product. In Vietnam the craft sector is adding around US$ 109 million annually to the country's GDP, as the sector provides 1.5 job opportunities for locals most of them from rural Areas . (USAID, 2008; Ngo, Duc Anhm, 2005). In India the exports of handicrafts reached $3.2 billion in 2005-06. (Richard 2007)

Handicraft and Tourism

In general the craft and tourism sectors are connected to each other, as traditional handicraftsare considered among the authentic cultural products made by the indigenous people of the hosting destination that tourists eager to see and purchase, which means that the Handicrafts as one aspect of traditional indigenous culture can be used as a good tool for marketing the destination and attracting more tourists.
In this regard, many destinations all over the world have managed to make full use of Handicrafts as a cultural product in their national tourism promotional campaigns, to diversify their exhibited tourism products and increase the overall attractionsof the destination to be able toattract large number of tourists.The matter did not limit to using Handicraft in the promotional campaign, but also different destinations all over the world have developed a

number of tourism – related handicrafts itineraries that enable the tourists to see the process of making a certain craft or even to participate in the production of a certain product.
Tourism also is considered a very effective tool for handicraft marketing, according to WTO tourist spends around 20 – 80$ on average on Handicrafts product purchases, as they want a tangible item to take back home that would remind them about the destination they visited and the indigenous people they have seen and the memories they have made (Wicks, 2004).
According to the figures handicrafts are considered among the most important tourism sub-sectors; accommodation, food and drink, handicrafts and excursions, with regard to generating pro-poor income (ITC, 2010) as around 55%.

The case of Tunis Village

It is situated 95 km south west of Cairo; it is a Village inYoussef AL Sedik District in Fayoum governorate. Overlooking one of the natural protected Areas in Egypt known as Qaraoun Lake.
In the 1980s, Evelyne Porret, a Potter from Switzerland, decided to stay in TunisVillagewith her Husband, and then she started to establish a PotterySchool to teach children of the VillageHow to shape Pottery. She was the first potter in the Village;she managed to train many of the local children in the craft of Pottery. The first generation of her students after being trained in the Pottery School, they manage to establish their own Pottery workshops.
The establishment of Pottery School was the turning point of Tunis Villageas it was the main reason to turn the Village into a large tourist destination visited by many visitors interested in watching the Pottery products being made by the crafters.The matter which motivates some locals and investors to establish a number of Hotels and cafeterias and other tourism services to provide tourists and visitors with the services which they are looking for.Also some of the local have shifted from their traditional career as farmers into a tourism related professions like local tour guides, Safari drivers, Hotels employees …etc.

Research Question and Objectives

The current research is trying to answer the following question:
To what extent the Pottery craft has contributed in achieving sustainable tourism development in the village?
The main objectives of this research can be summed up in the following points:

1- To explore the current status of Pottery handcraft in Tunis Villageand define the main challenges which face this profession.
2- To assess to what extent the Pottery handcraft in Tunis Villagemanaged to improve the life of the local community by alleviating poverty, creating more job opportunities.
3- To assess to what extent the Pottery handcraft has become a tool for achieving sustainable tourism development.
4- To provide an action plan towards the preservation and development of Pottery handcraft in Tunis Village.

Material and Methods

The research was implemented by linking qualitative and quantitative data as well as qualitative observations.

Primary data

The information produced in this research is based on extensive fieldwork. Three surveys were conducted for the purpose of this research study. The first survey directed to the Pottery crafters in Tunis Village where a total of 40 questionnaires were distributed by the researchers. The qustionnairs is divided into thre parts; the first part is focusing on collecting general information about the crafter, the second part is focusing on collecting data about the

Socail and economic impact of the profession on the crafter himself/herself and on the Village in general, while the third part is focusing on the threats that may negatively affect the sustainability of Tunis Village. Unstructured face to face interviews were conducted with 15 tourists how were visiting Tunis Village during July 2019 to identify their level of satisfaction about Pottery craft and their recommendations to promote and develop such important craft. The selection of this segment was based on the willingness and availability of tourists to complete the questionnaire.
The third survey was directed to Tunis Village's Local residents (non- crafters) where a total of 30 questionnaires were completed. The main aim of this questioner is to explore their opinion about the Pottery craft and its impact on their social and economic life.

Secondary data

This research is based on comprehensive reading of many published references and other reliable internet sources which showed that Pottery handcraft is a very important unique selling point which resulted in attracting tourists, establishment of different hotels and cafeterias to provide tourists with accommodation, food and beverage.

Results and Discussion

Opinions of handcrafters, tourists, as well as local residents (non-crafters) were investigated to examine to what extent the Pottery craft has contributed in achieving sustainable tourism development in the village, the resultsare as follows:

PotteryHandcrafters

Among the crafters, results showed that 92.5% are males and only 7.5% are females; this is due to the conservative lifestyle in the rural areas long time ago which doesn't allow girls and women to learn this profession unlike these days where many families accept to teach their daughters the profession. The education level of crafters in Tunis Villageis a moderate in general: 20% of craftershad no formal education and they are illiterate, 32.5% of the farmers interviewed had obtained high school education, while only 2.5% had obtained bachelor degree, and 45% of them are young teenagers who still studying. Results revealed that 52.5% of Pottery crafters learnt the profession in "School of Pottery" that belongs to "Evelyne Porret" who early started the profession in Tunis since 1980, while 47.5% learnt it in the private workshops that have been established by the first generation of the Crafters who were trained by Evelyne . 100% of Pottery crafters indicated that the profession helped them to provide better education and health care to their families, they stated also that they are feeling safe and social secure by work in this profession. An 87.5% of crafters indicated that the profession achieves stability in their incomes, while 12.5% of them stated that it provide them with a partial stability in their incomes because sales volume is based more on seasonality as the sales usually go down during the summer season. As an average, Pottery crafters indicated that their income have been increased by 46.5% after working in the profession. A 100% of Pottery crafters are satisfied with work environment of the profession, while 87.5% of them agreed that the profession contributed to the reduction of unemployment in the village.
Pottery crafters stated that they take approximately 5 years as an average to learn the profession.

Table (1) Time to learn profession and percentage of income increase after profession

	N	Minimum	Maximum	Mean	Std. Deviation
Time to learn Pottery crafts' profession	40	0.5	15	4.91	2.78
Income increase % as working in profession	40	0	100	46.50	34.27

Source: field data, 2019

An 80% of crafters indicated that they receive support from government or private sector such as the training activities which provided by an Egyptian Italian project one of the UNDP development projects, moreover, the initiative of "Bank of Alexandria", where the bank provided electric wheels instead of manual one, the bank also sponsors the famous Handicraft festival of Tunis which take place in November each year.
A 100% of Pottery crafters confirmed that the Pottery industry in the Villageis what made Tunis its current form. "Ceramic products attracted tourism to Tunis, not vice versa". But then tourism has greatly contributed to the revival of the profession by providing buyers and increasing the demand for those products through domestic and foreign tourism.

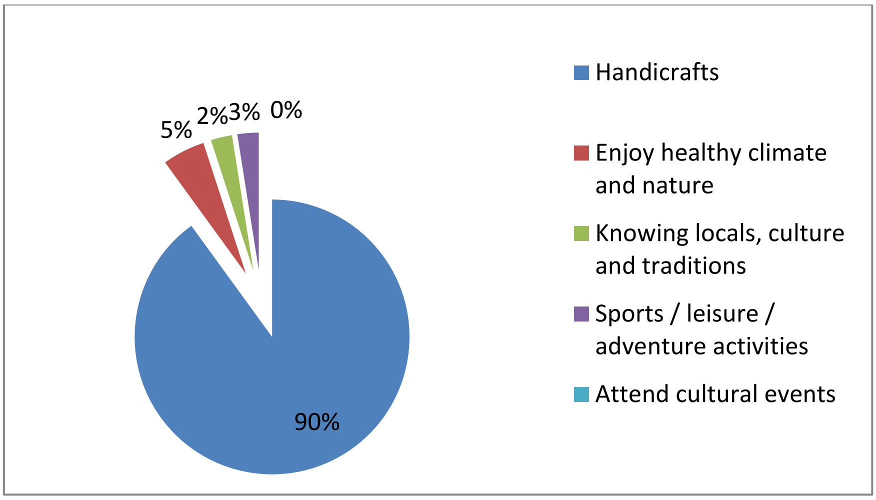
Table (2) socioeconomic Characteristics of Pottery crafters

Sample description		Frequency	%
Gender	Male	37	92.5
	Female	3	7.5
	Total	40	100
Education level	Still studying	18	45
	Illiterate	8	20
	High school	13	32.5
	Bachelor or more	1	2.5
	Total	40	100
Place of learning profession	School of Pottery	21	52.5
	In owner'sworkshop	19	47.5
	Total	40	100
Work before profession	Yes	20	50
	No	20	50
	Total	40	100
Adequate income from the profession	Yes	32	80.0
	No	8	20.0
	Total	40	100
The extent to which the profession achieves stability in income	Stable	35	87.5
	Partly stable	5	12.5
	Total	40	100
Receiving support from government or private sector	Yes	32	80
	No	8	20
	Total	40	100
Contribution of profession to the reduction of unemployment in Tunis village	Yes (to a large extent)	35	87.5
	To some extent (partly)	5	12.5
	total	40	100

Source: field data, 2019

Crafters were asked to list the primary categories of buyers for their products; they stated that around 60% as an average are local buyers, while 40% are tourists and wholesalers. Given their marketing outlets, they market around 72% of their products directly to the end user, while the rest are sold tointimidators.
Crafters indicated that the most important challenges facing the profession of handicrafts are the continuous rise in the prices of raw materials and the difficulties of obtaining it, as well as the great time wasted in preparing and processing the raw materials, which consumes the time

of the artist and reduces his/ her production in quantity and quality. Among the most important challenges is the absence of information about foreign markets and its requirements as well as the exploitation of traders, moreover, the inability to produce a big quantity because of using traditional production methods such as ovens and hand-held wheels.



Graph (1) The main reasons for tourists to visit and stay in Tunis Village
Source: field data, 2019

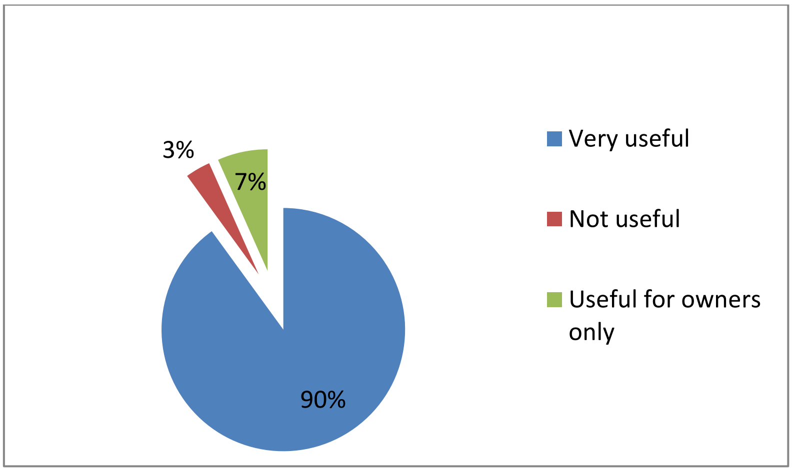
As it is shown in Graph(1), most of the crafters have agreed there are number of reasons that motivate the tourists to visit Tunis Village, but from their point of view the main motivation of the tourists to visit Tunis Village is to see Pottery School and workshops as well as to buy some Pottery products as souvenirs that would commemorate their visit to the village, they said that before the establishment of the Pottery school and other Pottery workshops Tunis Village was like any agricultural traditional EgyptianVillages and there was no tourism activity in the village, but after the establishment of the Pottery school and workshops the tourists started to feel motivated to visit the village, hotels and other tourism facilities and activities started to exist to fulfill the needs of the tourists.

Tourists/visitors (customers)

Unstructured face to face interviews were conducted with 15 tourists while they were visiting Tunis Village during July 2019, all of them have agreed that they have read about Tunis before visiting it, especially the story of the establishment of the Pottery school by the Swiss artist and engaging the locals and shifting the ordinary Egyptian Village into Eco-friendly touristic destination, and this is considered their main motivation for visiting Tunis village. They have shown a great level of satisfaction with the Pottery workshops and the final products. But the same time they have highlighted some issues that need to be dealt with in order to improve the business and sustain this great model, among these issues is the big gap in prices of the Pottery products in each workshop. Also they stated that it is important to train the crafters on how to diversify their final products to increase their customers as according to the respondents the Pottery products which are produced by the 13 workshops and the Pottery School are very similar to each other. Also they highlighted that establishment of an entity like NGO for example to help the crafter to overcome them challenges they face would positively affect the business and the Village as a whole.

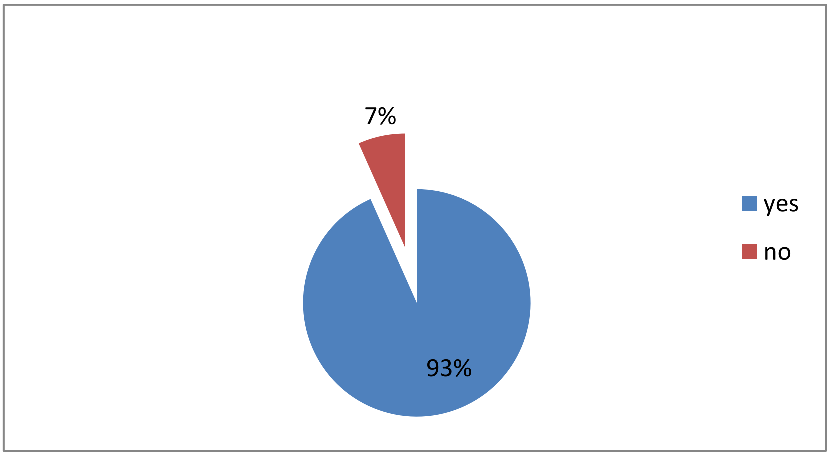
Local residents (non-crafters)

A 90% of local residentsinterviewed have a positive impression about Pottery workshops, while 7% of them think that these workshops are useful just for owners and who directly work in it, and a 3% of them think that it is not useful for the Village at all.



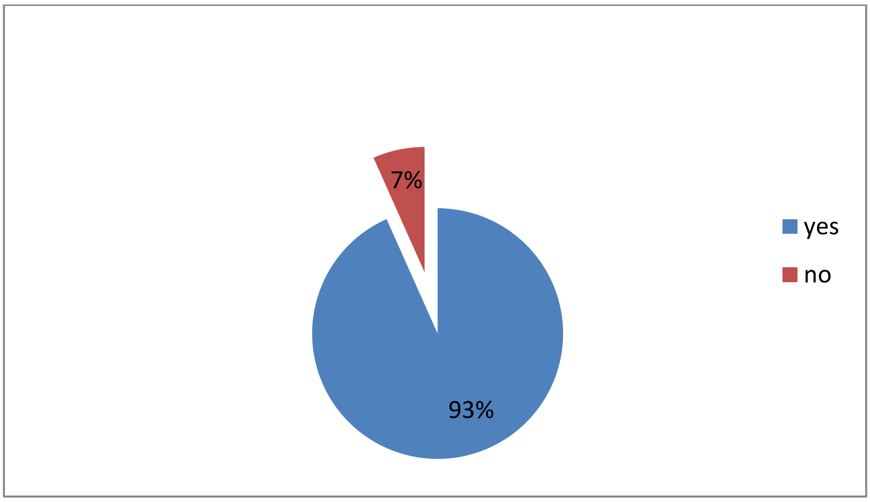
Graph (2) Impression of locals (non-crafters) about the pottery workshops
Source: field data, 2019

93% of locals (non-crafters) indicated that Pottery workshops benefit the Village in general. They think that it brought development to the whole Village through providing jobs opportunities in these workshops, hotels, and other tourism activities. It brought infrastructure which benefits all population at the village.



Graph (3) pottery workshops benefits in general to the Village
Source: field data, 2019

As it is shown in graph (4) 93% of the non-craftersrespondentshave mentioned that the existence of the Pottery School and workshops has attracted the tourists to visit the village,the matter which capacitates the non-craftersto increase and diversify their source of income, as it has created many Tourism – related jobs like tour operators, drivers, local tour guides, hotels owners and staff, coffee shops owner and staff…etc. Also they have mentioned that in each family in the Village at least there is a person who works in field Potteryhandicraft; all of these job opportunities have motivated their family members, who used to leave the Village to search for a job opportunity in Cairo, to stay at the Village and start their own business.



Graph (4)benefits of locals (non-crafters) in the Villagefrom pottery workshops
Source: field data, 2019

As a result of the increase of the tourism demand on the Village and their growing interest to taste the local healthy food, this fact has provide the females of the Village with a precious chance to fulfill the need of the tourists and earn a substantial amount of moneythrough the establishment of what so called a "community kitchen" in the Village where a group of the females meet to cock healthy local affordable and offer it to the tourist during their visit to the village.

Conclusion

The establishment of Pottery School was the turning point of Tunis Village as it was the main reason to turn the Village into a large tourist destination visited more than 120.000 tourists each year according to the village council, to watch the Pottery products being made by the crafters.The theoretical and the fieldwork have shown that Pottery handicraft has been playing a significant role in tourism promotion of the village; it also played a major role in achieving sustainable Development in the Village the matter which made Tunis Village Unique model which is totally different from any Craft Village in Egypt.
This piece of research proved that most of the local residents of the Village eitherPottery Crafters or non-Crafters are satisfied with the existence of Pottery workshops in the Village, they also feel satisfied with their income, this status has motivated the younger generations in the Villageto be motivated to learn the craft in order to start their own business.
A number of recommendations has been developed for ensuring the sustainability of this successful model, these recommendations include:

- Capacity building and technical Training workshops should be conducted to enhance the soft and technical skills of the Pottery crafters to assure better quality, pricing, packaging of final products.
- Holding special events such as exhibitions, fairs and festivals showcase Pottery handcraft products, especially in the low season (summer season) to motivate international and local tourists to visit the Village and push up sales during this season.
- Making some modifications in the furnaces to reduce the emissions generated during firing the products.
- An entilty (like NGO) should be established to to help the crafter to overcome them challenges they face especialy the procurement of the raw materials.

- The final products have to be diversified to expand the current market by addressing the demand a wide range of customers while taking into consideration the cultural idientity of the village
- Marketing the experience as well as the tangible products, throughorganizing tourism itineraries for tourists who are interested in how things are made or those who would like to take part in the production process as this will enhance the overall experience of the visitors and also will push up the sales of the tangible products.

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THE ROLE OF INNOVATION SUPPORT SERVICE PROVIDERS IN AGRICULTURAL INNOVATION MANAGEMENT

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Abstract

Innovations in agriculture tend to be slow because of the conservativeness of the agricultural sector. 'Sustainable Precision Farming through User-Centered Design', a research project of the author funded by the Co-operative Research Programme (CRP) of the Organisation for Economic Co-operation and Development (OECD), suggests that the adoption phase of innovation is a major reason for the slowness. Adoption could be better if the R&D process was more user-centred. In order to succeed in this challenge, the participation of end-users in innovation should be much more efficient. All actors in innovation should gain more skills in User-Centred Design (UCD). The adoption phase needs more support from advisory services or consultancies. This is because the potential benefits of innovations are not realized if the adoption is inefficient. A Horizon 2020-funded research project, 'AgriSpin – Space for Innovations in Agriculture', aimed to find efficient methods to enhance innovation. In AgriSpin, there were 15 partner organizations from 12 countries. The goal of AgriSpin was to systematically explore Innovation Support Service Providers (ISSPs) in agriculture and rural development across Europe. The overall goal was to help create a stimulating environment for innovations. The results point out the urgent need for effective Multi-Actor Approach (MAA) to support all phases of innovation. The paper promotes the role of ISSPs as actors in efficient agricultural innovation. The growing need of agricultural engineering consultancy is discussed. Needs for further research and development are outlined.

Keywords: *innovation management, agriculture, User-Centred Design, Multi-Actor Approach.*

Introduction

Joseph Schumpeter, who is regarded as one of the most important economists in the first half of twentieth century, defined innovations in early 1900's. Real innovations should produce significant benefits for their users. Furthermore, he claimed, innovation driven by entrepreneurs is the most important reason for economic growth (Schumpeter 1912; Opie 1983; McCraw 2010; Drejer 2004; Śledzik 2013).

Scumpeter divided the innovation process into four dimensions: invention, innovation, diffusion and imitation. He puts the dynamic entrepreneur in the middle of his analysis. The entrepreneur, drawing upon the discoveries of scientists and inventors, creates completely new opportunities for investment, growth and employment. The invention phase or the basic innovation has less impact, while the diffusion and imitation process have a much greater influence on the state of an economy (Śledzik 2013).

In other words, innovations never reach the real innovation level, where the benefits are realized, without efficient adoption (diffusion and imitation). When widely adopted, the better artifacts, methods or systems produce their best benefits for their users and the surrounding society as well. Only then they can be called real innovations.

In agricultural innovations, the role of users (entrepreneurs in Schumpeterian thinking) has traditionally been important. Farmers have developed and introduced the new or gradually advanced machines and production methods. However, as recently noticed by several researchers, the social process of trust building in novelties takes considerably long time

(Kaasinen 2005). Wider adoption of the new solutions happens only after a period of successful application (Li et al. 2008). In agriculture, this is evident for systems level innovations such as Precision Farming (PF) (Diekman & Batte 2010; Haapala 2013).
New technologies are often hard to learn, remember and use so that usability issues make the adoption slow (Nielsen 1996). Again, PF is a good example of this (Haapala & Nurkka 2006).
Who else than the farmers themselves supports the innovation process in agriculture? In history, the process of innovation adoption in agriculture has been supported mainly by local actors such as the innovators themselves and other farmers who have acted as early-adopters. In many countries the church has been active in delivering knowledge of e.g. new technological developments and plant varieties. Manufacturers and sellers of agricultural machinery have then taken the role of innovation supporters. Later on, special ISSPs (Innovation Support Service Providers) have entered the market. Advisory services have been established and universities have opened extension services to apply their scientific findings in practice (Russel 1966; Lehto 2010; Knierim et al. 2015; Jones & Garforth 2019).
Nowadays there is a multitude of ISSPs to choose from. Their owners are either private or governmental, and they act, respectively, according to profit or non-profit principle. Systems level thinking is growing so that the interaction of agricultural innovators with other branches of innovation and the surrounding society is growing. As the innovations are getting complicated, new specialized consultancies have emerged. Innovation Brokers are entering the market and special funding programmes are built to support rural innovation (Klerkx & Leeuwis 2009; Batterink et al. 2010; Knierim et al. 2015; Jones & Garforth 2019).
The objective of the paper is to focus on the adoption phase of innovation in agriculture. The possible remedies for the slow speed towards real innovation are discussed. Particularly the role of ISSPs is investigated. Conclusions are made on the needed actions.

Material and Methods

The paper is based on two research projects. The first one, 'Sustainable Precision Farming through User-Centred Design' (2011-2012), was funded by the CRP (Co-operative Research Programme) of OECD (Organisation for Economic Co-operation and Development). The author acted as a visiting researcher in Spain at IVIA (*Instituto Valenciano de Investigaciones Agrarias* – Valencian Institute of Agricultural Research). A web-based questionnaire was sent to over 200 selected recipients around the World using LinkedIn™. The recipients were members of EurAgEng (The European Society of Agricultural Engineers), FinAgEng (FinAgEng Maaseudun teknologia Ry - The Finnish Society of Agricultural Engineers), ASABE (the American Society of Agricultural and Biological Engineers), and CIGR (Commission Internationale du Génie Rural – International Commission of Agricultural Engineering). The respondents' competence profile was variable. They were strong in engineering, research and, most of them, also in practical use of technologies at the farm level. They were not experts in marketing or sales. As the questionnaire was challenging, it took almost an hour to answer it, only 41 complete replies were achieved. Thereafter 10 in-depth face-to-face interviews of selected experts were done. The interviews were made in Spain and Finland and the interviewees were originated from Spain, Israel, Denmark, and Finland. Webropol™ software was used for the questionnaire. The interviews were recorded and done as thematic (semi-structured) interviews (Hirsjärvi & Hurme 2001). The questionnaire and the interview had three main parts: assessing the current PF (Precision Farming) technology, the innovation in PF, and User-Centred Design (UCD) in designing PF technologies. The responders were asked to tell what they thought about UCD as a methodology to enhance innovation. The experts also prioritized the most important research and development topics of UCD in agricultural engineering. Finally, they rated the importance of related actions in research policy.

The second research project 'AgriSpin – Space for Innovations in Agriculture' (2015-2017), was funded by the Horizon 2020 research and innovation programme of European Union. In AgriSpin, there were 15 partner organizations from 12 European countries.
AgriSpin aimed to find efficient methods to enhance innovation. The goal was to explore ISSPs in agriculture and rural development. The overall goal was to help create a stimulating environment for innovations.
AgriSpin researchers applied and improved a Cross Visit Methodology that includes thorough analysis of innovation cases. In AgriSpin the analysis covered altogether 57 innovation cases in 15 countries in Europe. The Spiral of Innovation (Wielinga 2016, Fig. 1) was used to illustrate the cases and to communicate them to wider audience. For each case the research team arranged a Final Symposium where relevant stakeholders were informed about the findings. The stakeholders were challenged for developing the local innovation environment so that the speed of innovations in agriculture would be enhanced in the area.

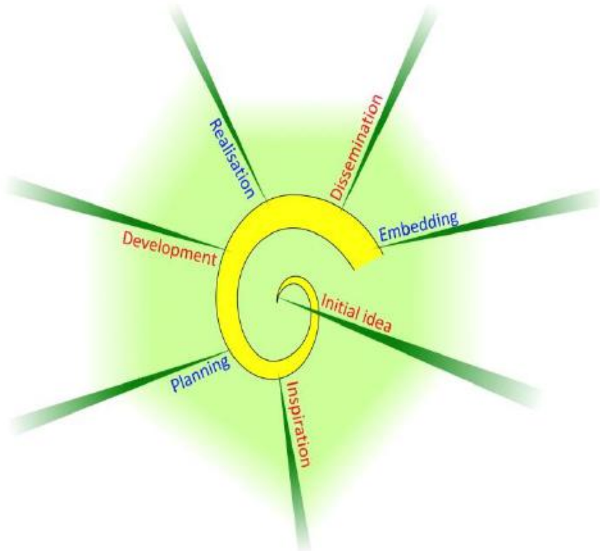

Fig. 1. Spiral of Innovations (Wielinga 2016).

Results and Discussion

The expert evaluations confirmed the proposed model for successful innovation (Haapala 2012, Fig. 2). According to the model, innovative technologies need first to be bought, and thereafter used in a proper manner so that their benefits are realized. Initially, in order to be bought, the buyers need to be convinced of the technical level of the technologies. The criteria of social and practical acceptability (as in Nielsen 1993) need to be fulfilled. User-Centred Design helps to create high-level, acceptable technologies and also helps the users to apply them. Thus, the use of UCD methodologies is seen as beneficial for the speed of innovation.
The experts agreed that the Schumpeterian real innovation model (Schumpeter 1912; Drejer 2004; McCraw 2010; Śledzik 2013) is required in agriculture. Innovative technologies and methods need to be spread and used by a wide user audience. According to expert evaluation, the most important obstacles for quick innovations are: lack of trust in new technology among farmers, bad usability of new technologies due to absence of User-Centred Design in the education of engineers and designers, omitting users in the innovation process. Thus, User-Centered Design (entrepreneur-driven in Schumpeterian theories) is considered as the key element for better acceptance of innovations.

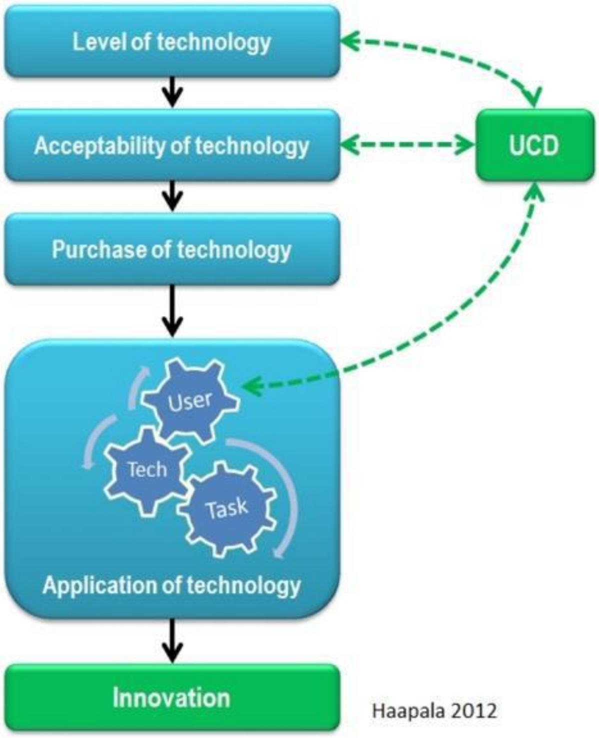

Fig. 2. Confirmed model of real innovation.

Most of the suggested topics for further research are concentrated around the user and his/her processes. Additionally, the elements of an effective user-centred R&D process, and the elements of highly acceptable solutions were prioritized (Fig. 3).

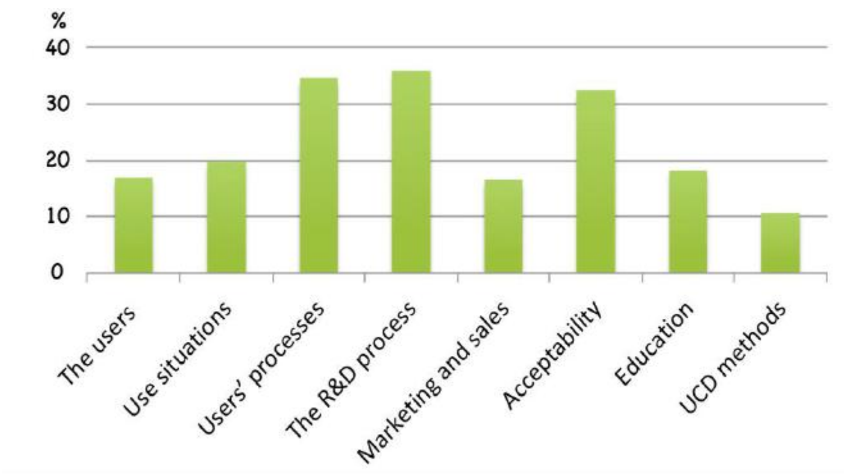

Fig. 3. Suggested topics for further studies.

The results of AgriSpin project were mainly methodological. The developed version of Cross Visit methodology (Wielinga 2016) enables the researchers, consultants, advisors and other actors to interact efficiently. The Spiral of Innovations was found to be an effective way to communicate. The Final Symposiums gave the participants a forum to reflect the findings immediately so that the final conclusions were accurate and the suggestions for the local actors were clear.
The main observations and suggestions from the 57 Cross Visits were:

- Besides economic and technological ones there were societal challenges limiting the acceptance of innovations in the cases;
- The successful cases had enjoyed multidisciplinary support for their innovation process;
- Free actors (independent, trustworthy and passionate individuals) had been important for the studied innovation cases.

Conclusions

Real innovation in agricultural engineering needs User-Centred Design (UCD) as a tool to make the solutions more acceptable for the users. The end-users decide to purchase new solutions or learn a new method or skill only if they consider their benefits high enough. Only realized benefits make the users return to buy again or to learn more.

The role of Innovation Support Service Providers (ISSPs) is crucial for efficient agricultural innovation. Supporting actions are needed: relevant services from advisory, research, consultation, authorities, and innovation funding. Relevant support speeds up innovation since it helps the innovators concentrate their efforts on the completion of the innovation process.

In order to be effective, the ISSPs need to have both in-depth knowledge and excellent co-operation skills in place. As the innovative solutions constantly get more complicated there is a growing need of specialized agricultural engineering consultancy. In addition, there is an urgent need for effective Multi-Actor Approach (MAA). The specialized advisors and consultants need to interact with each other, the end-users and other relevant stakeholders so that the MAA is realized and utilized to its full potential.

Innovation Brokers and Free Actors have a central role in future innovations, especially in the case of complicated systems level innovations, such as Precision Farming. To support the emergence of these services, funding needs to be available. Effective platforms for brokerage and operation of Free Actors need to be built.

Recruitment of Free Actors should be active and their role should be acknowledged as imperative for efficient innovation in agriculture.

The agricultural ISSPs should build better capacities for their professionals. Especially UCD and MAA methodologies should be learnt and practiced. These methods should be used throughout the innovation-supporting actions to make sure that the needed supporting elements are present in each phase of the innovation process. The available services should be easy to approach and preferably also physically near to the innovators.

Agricultural engineering professionals have the opportunity to take a leading role in the implementation of the needed ISSPs, in close co-operation with biological and economic specialists and related authorities and funders. Finally, close co-operation with innovative companies, entrepreneurs and end-users would be of great importance.

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WHY INVEST ON INNOVATIVE PRODUCTION? A QUALITATIVE EVALUATION OF THE EMERGING AVOCADO SECTOR IN CRETE, GREECE

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Abstract

Avocado is actually one of the most lucrative agricultural activities worldwide. Demand is rising rapidly relating to healthy diets and social status, while the increasing supply does not seem able to cope with it. Chania area in the Greek island of Crete has ideal climate and soil conditions to produce high quality avocados. As a result, orange trees are being eradicated and replaced by avocados. The purpose of this study is to provide a qualitative assessment of avocado production in Crete, in order to demonstrate under which prerequisites its growing trends will lead to overall sustainability (economic, social, environmental). The system is assessed by means of a SWOT analysis integrating considerations of actors across the value chain. The key finding is that the system requires synergies with other sectors but also close collaboration among stakeholders in order to achieve overall sustainability in the long term. Strategic design and collective actions are required towards this end.

Keywords: *Innovative production; Qualitative evaluation; Avocado.*

Introduction

Avocado production is a very dynamic and fast growing industry worldwide. In 2016, world production was 5.68 million ton., showing an overall increase of 24.6% from 2012 onwards. It is estimated that production will amount to 6.42 million ton. by 2020, with a respective estimated value increase by about $10 billion, from $13 billion in 2017 to $23 billion in 2027 (www.producereport.com).

Avocado has particular requirements for light soils, mild temperatures without large fluctuations and high quality irrigation water. For this reason, production is only feasible in specific areas globally. According to freshplaza.com, Latin America is the largest avocado producer in the world, valued at around $5.5 billion annually, with main centers in Mexico, the Dominican Republic, Chile and Guatemala. Production in North America is also significant in specific US states, such as California and Florida. In Africa, there has been systematic avocado production since the 1960s, particularly in Kenya, while on the Asian Pacific, New Zealand and China play an important role. For Europe and the Mediterranean, Israel is the most important producer (8.000ha), followed by Spain, which has elaborated high-level research regarding varieties and cultivation methods. The dominant avocado variety actually is Hass and represents more than 50% of production.

Global production is increasing every year, seeking to cover the rapidly increasing consumption. The incorporation of avocados into healthy diet programs, strong advertising and promotion, and generally the prevalence of a favorable consumption pattern related to lifestyles, contribute to this rising demand. The main market for avocados actually is Mexico, which hardly produces enough avocado to supply internal markets and cover its existing trade agreements. The USA is the second largest consumer, as in 2016, per capita consumption was about 3.5 kg of avocados per year, having doubled from 2006 to 2015. However, USA only produces a small portion of its needs and is dependent on imports from Mexico and other South American countries. This is largely the case for Asian countries as well, such as China and New Zealand, where production is mainly oriented to internal markets rather than international trade. In the EU, per capita consumption exceeded the threshold of 1kg per year just in 2017 and is constantly increasing (www.linkedin.com). Although European marketing channels are among the best organized worldwide, European production struggles to pace up with increasing demand and actually the EU is heavily dependent on imports. For instance, its main producer - Spain - has significant internal consumption and its production is mainly directed to the internal market, with only few exports, while in some periods massive quantities are imported to cover seasonal deficiencies. As a result, EU imports in 2016 exceeded 450 th.ton., with 90% originating from developing countries (Peru, Chile, South Africa, Kenya and Mexico) and the remainder being mainly covered by Israel (www.cbs.nl).

Due to its vast global potential, avocado production is associated with socio-economic changes and environmental pressures (Darnton, 2017). Speculation about short-term profits is common and often leads to adverse mid-term effects. Academic research on such issues is limited, however a review of relevant reports shows that avocado production is faced with increasing socio-economic problems, especially in developing countries. Recently, social instability, violence and other related issues were reported in the top avocado - producing country - Mexico (https://elpais.com/). Furthermore, avocado production is subject to numerous natural and market-related factors which increase fluctuations and bear increased risks (www.agroinsurance.com). For instance, the high income elasticity of avocado production shows that the production system is likely to be vulnerable to changes in international markets and the trade agreements with the EU and USA create serious commercial dependencies, despite being important sources of revenues for these countries. Another issue of importance is the increase in land prices, which limits the socioeconomic viability of avocado. It has been reported that land prices in Spain are increasing rapidly due to avocado, even rendering production unprofitable in the short run (www.freshplaza.com). According to Darnton (2017), social problems also arise relative to limited labor supply during intensive harvesting period, as the job is not at all alluring for occasional workers. In addition, extensive environmental problems, such as desertification and land degradation, have been reported in areas with intensive avocado monoculture, such as Chile and Mexico (phys.org, www.theguardian.com).

In this context, there are numerous opportunities for producers in global avocado markets. The Regional Unit of Chania in the Greek island of Crete, has the necessary soil and climate conditions that allow avocado production. Avocado plantations are being installed in the area for decades and the sector was quickly established as an industry with economic importance. However, responding to international developments, avocado is expanding in the area - in full production and/or new ones - much more rapidly than before. This implies a substantial change in the production pattern, in which citrus fruits - in particular oranges - prevailed until recently, also playing a very important role in socioeconomic development. Indeed, the structure of orange varieties permits production almost throughout the year, in contrast to the avocado production system, which exhibits significant fluctuations between years (alternate bearing) and seasonally (no production from June to October), and this created a credible and well-functioning value chain. Nonetheless, low producer prices for oranges have now led to a decrease and - combined with frequent disease outbreaks - to gradual substitution by avocado. Under this situation, marketing infrastructure is under-utilized and markets traditionally fed with Cretan oranges have already turned to other areas in Greece or abroad.

The organization of the avocado production system and of the respective value chain in Chania is endowed with significant elements of innovation and should be considered as such. The expansion of the activity in the area should be accompanied by targeted interventions that limit the environmental and socioeconomic impact of the transition while simultaneously securing long-term benefits for stakeholders. Therefore, strategic design is essential to accommodate endeavors to establish a new lucrative activity with development potential across the value chain.

The purpose of this paper is to provide insights regarding the proper interventions to render avocado production in Chania a viable agricultural activity with positive socioeconomic impact. For this reason, the paper presents a SWOT analysis of the sector, based on qualitative data from a survey of actors involved in the avocado sector in the area. The analysis seeks to demonstrate how interconnected internal and external factors should be considered simultaneously, in order to design integrated strategies at different temporal levels.

Material and Methods

The paper is based on a primary questionnaire survey which took place in Chania area in 2018-2019. Since data availability regarding the structure of avocado production in Greece (and in Chania) is scarce and not up-to-date, a probability sampling approach was not considered. A qualitative approach was thus preferred in order to collect information from a variety of actors across the avocado value chain or from other related stakeholders. Therefore, a questionnaire was designed in order to collect qualitative information regarding (a) the evolution and development of avocado production in Chania; (b) the history of the businesses (farms/nurseries/manufacturers) and their decision to get involved in the sector; (c) their main sources of information and advisory services available to farmers; (d) the problems that actors encounter in avocado production and marketing; (e) their plans for the future in avocado/orange production and marketing.

The questionnaire was addressed to actors in the avocado value chain in the area, including 20 avocado producers, 6 fruit industries, 1 nursery owner, 1 researcher, 2 representatives from local state institutions and four persons belonging to more than one categories (researcher/producer/nursery owner). The qualitative survey data were combined with other information found in relevant publications - although literature regarding the avocado sector in Greece is very scarce – or through informal discussions with other local stakeholders.

This information was systematically evaluated and was used in a SWOT analysis of the avocado sector in Chania. SWOT analysis (Strengths – Weaknesses – Opportunities – Threats) is one of the best-known strategic planning tools, widely used to analyze the internal and external environment of businesses, industries and markets (Hill and Westbrook, 1997; Jyothi et al., 2008). SWOT analysis captures aspects of the status-quo situation and examines the strengths (comparative advantages) and weaknesses associated with the internal environment as well as opportunities and threats in the external environment, that will need to be considered in future interventions, thus facilitating strategic planning.

Results and Discussion

Table 1 presents the SWOT analysis of the avocado sector in Chania. It is evident that there are complex issues in the internal and external environment that need to be considered simultaneously in order to understand the dynamics of the sector. Therefore, based on the SWOT analysis, several key points emerge.

Chania area has favourable soil and climate conditions for the production of high quality avocado. This has been shown since decades, when the cultivation started in specific parts of the Region. Since avocado is well adapted in Chania, it requires minimum agrochemical inputs and valorizes existing irrigation infrastructure. Nonetheless, the survey showed that only few farmers comply with 'best' production methods and that there is a wide variety of practices in fertilization (excessive use of inorganic nitrogen), weed management and, especially, in harvesting. Actually, several varieties are being successfully installed (mainly Hass varieties but also Pinkerton and Ettinger) together with existing Fuerte and Zutano plantations and this can accommodate a successful future pattern, by prolonging the production period, which will cover demand in markets almost throughout the year. This can be an important advantage, however, if producers have access to high-quality propagation material, which will demonstrate high adaptability and endurance to local conditions- (eg salinity, adaptability to various soil types etc.). This would also contribute to tackling disasters from extreme weather events, such as heavy rain and strong winds.

Table 1. SWOT analysis of avocado production in Chania, Crete, Greece

Strengths	**Weaknesses**
• Favourable soil and climate conditions • Important production and marketing infrastructure – "Cretan product" • Choice of varieties which permit a wider period of production and sales • Possibility to ensure high farm incomes • Low requirements in agrochemicals and crop interventions • Availability of farm labour	• Informal production and sales • Lack of central organization and strategic planning and low levers of cooperation • Low adoption of 'best' production practices and high variability • Low levels of scientific knowledge, lack of advisory services and information • Lack of integrated "Cretan avocado" identity
Opportunities	**Threats**
• Increasing demand – Emerging markets • Low proportion of European/global production • Multiple possibilities for certification • Niche markets – Alternative markets • Synergies with other activities and networks (e.g. tourism) • Contractual production • Research and development structures	• Competition from other European and Mediterranean countries • Higher national market prices that international prices – Low competitiveness • Low penetration in niche markets • No well-adapted propagation material • Climate change – Extreme weather events • Decline in orange production

A major issue is the high percentage of informal avocado production and sales. Orange trees are being replaced by avocados rapidly, without, in many occasions, this being formally declared to competent control bodies. Undeclared production is sold through informal channels, reaching the consumer without identity and, in some cases, with significantly lower quality than the branded product, at almost the same price. It is evident that all actors involved in informal value chains gain higher profits, however unfair trading practices will be detrimental for the whole system in the mid- and long-term, compromising the reputation of the Cretan product in markets. A side-effect of informal production is the increase of stealing avocados from plantations, although this phenomenon is not a problem only for Crete.

One of the main effects of informal production is the lack of strategic planning in avocado but also in the whole primary sector of the area. Any plan and proposal for avocado should be followed by adjustments to tackle this issue, which distorts the 'true' image of the sector and results in marketing problems, lack of product identification and irregural harvesting periods reflected in lower product quality. This is combined with a relatively low level of knowledge and of targeted scientific research on avocado production under the specific conditions of Chania, although the area has structures that could contribute to the improvement of knowledge. Cutting-edge research topics would include new varieties, diseases, other enemies and propagation material. In order to achieve this, a basic prerequisite is extroversion and close interconnection with farmers and businesses across the value chain. Nonetheless, farmers are skeptical concerning the role of research, institutions and the processing sector. The latter comprises well-organized and dynamic companies, but lack of trust does not allow to reach important markets abroad, which leads to lost opportunities. In this context, the lack of qualified advisory managers threatens the development of the system. There is a relatively low level of information among producers, asymmetric information among actors and – more

than often – false, exaggerated or deceitful information, yielding high expectations which avocado production in the area cannot uptake.
Avocado production can be a lucrative activity for farmers, as it combines important advantages: growing international demand due to the prevalence of a new consumption pattern for avocado, deficit in the national market and high product quality (under specific conditions). Actually, local producer prices are even 50% higher than international prices. In addition, Regional production is still low and even if it increases significantly it will only stand for a very small part of global production, which makes its marketing relatively easy through short or mainstream supply chains to markets such as Germany, Netherlands and, of course, Greece. The degree to which farmers will profit from these favourable conditions in the long run depends on how the value chain will adapt to accommodate the increasing supply. For instance, the low production volume also leads to losses of significant opportunities in international markets, which require the regular timely provision of high product quantities, unavailable under the current conditions in the production system. For a future increase in production, especially to the extent that the Greek market may become saturated, the sector should be expected to conform to international standards or else will not be able to reach competitive markets. A solution here could definitely be provided through contractual farming, which not only constitutes a way to increase overall market performance, but will also reduce risk and uncertainty for farmers and other actors in the value chain. The economic viability of avocado production is determined by other equally important parameters such as (a) low requirements for fixed and variable capital, (b) high importance of family labor and (c) availability of product at times when international supply is low, through the selection of suitable varieties.
By looking at the weaknesses - and even at some strengths - of Cretan avocado sector, it can be argued that the system has neglected its comparative advantages, sometimes presenting a non-coherent image in markets. The low level of production has so far exacerbated this problem, as there was no need to promote the product collectively or incorporate it into existing networks, value chains and quality schemes (eg "Cretan product", olive oil, tourism etc): avocados would 'sell themselves' effectively. Nevertheless, in the new situation which is anticipated, the standardization and certification of Cretan avocado combined with its high quality could be proven vital. In particular, certification of the production practices, with an emphasis on the agro-ecological character of the product and on its origin (PDO, PGI), could diversify the local product from the avocados of competing countries. In addition, certification of the product will be the "key" for Cretan avocado to access niche markets. Indeed, such markets are ideal for Cretan avocado as they (a) usually demand premium quality characteristics, (b) require relatively small quantities, (c) are interested in origin and production practices. Nonetheless, under the current conditions, the production system cannot approach such markets and this is highly due to the informal production and the lack of certification. This is a serious problem in a highly competitive market with rapidly growing production in competing countries such as Spain and Israel, with tradition, high level of innovation and availability of scientific knowledge and highly skilled staff.
Chania area has a healthy economic environment that attracts human resources. Labor availability is, therefore, an important asset for successful avocado production, as the sector is labor intensive. This is especially crucial for early varieties, as the harvesting period coincides with olives and some varieties of oranges. However, workers are rarely specialized or trained resulting in problems in fruit quality. Another related issue is undeclared labor, as almost all workers in avocado production are migrants who do not generally want to be declared officially. Apart from the obvious consequences, this issue is a problem for producers, as they cannot declare labor costs as expenses and should thus incur higher taxes. Increased production costs are a serious threat to the long-term viability of the sector, considering that

producer prices in the following period should adapt to international, if local produce is to access international markets.
Last but not least, special reference should be made to orange production. Although is a traditional activity with important infrastructure in the area and despite the fact that Cretan oranges are well-respected in markets, there was consensus that the sector is not profitable under current conditions and that this is largely due to lower demand in the internal market. However, orange production should be continued in Chania to reduce the risk and uncertainty associated with avocados, with interventions mainly related to the sale of the product and promotion, in order to redeem the market share of past decades. Existing technical knowledge, PDO certification, marketing networks and recognizable brand name are also important elements to support a promotion campaign for orange.

Conclusions

The avocado sector in Chania is fast developing, following an irrational pattern: selecting inappropriate soils and areas, planting of not well-adapted propagatin material, varying cultivation practices, lack of cooperation across the value chain. Under this form, avocado does not have the potential to support the local rural economy in the long run. The system requires to invest on quality and to develop an integrated strategic plan to approach niche markets in order to achieve higher prices, develop stable, trust-based trade relationships and reduce uncertainty. In the internal level, cooperation and coordinated activities are necessary to support sustainable development, in the form of the well-organized orange production system. It should be noted, however, that the expansion of avocado could bring environmental problems related to pressures on water resources, as relevant examples have already been identified abroad (eg Chile, Mexico) and have led to desertification.

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IMPLEMENTATION OF THE SUSTAINABLE DEVELOPMENT GOALS IN THE MEDITERRANEAN: INSTITUTIONAL AND GOVERNANCE ARRANGEMENTS

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Abstract

A robust institutional framework is necessary to turn into reality the ambition of the 2030 Agenda for Sustainable Development, which encompasses the Sustainable Development Goals (SDGs). This review paper analyses the institutional arrangements and governance mechanisms adopted in the Mediterranean countries for the implementation of the 2030 Agenda. It is mainly based on an analysis of the Voluntary National Reviews submitted by Mediterranean countries to the United Nations' High-Level Political Forum on Sustainable Development (HLPF) from 2016 to 2019. Basically, a variety of institutional arrangements have been used by Mediterranean countries with the aim of ensuring an effective and operational inter-ministerial coordination (cf. "whole-of-government" approach). In this respect, there are two types of institutional mechanisms: coordinating mechanisms at the highest government level (e.g. national committee under prime minister's or president's office) and implementation coordination by specific ministries (e.g. Ministry of Development in Turkey, Ministry of Foreign Affairs in Cyprus and Morocco, Ministry of Tourism and Sustainable Development in Montenegro). Some countries opted for the establishment of new structures (e.g. National committee for monitoring the implementation of SDGs in Egypt), while others use the institutional arrangements put in place for the Millennium Development Goals (MDGs). Apart from inter-ministerial coordination, these institutional arrangements also aim to ensure the "localisation" of the SDGs and their implementation at sub-national level (e.g. regional, local), increase the involvement of the legislative power and parliaments in the process, and improve the inclusiveness of and ownership by a wide range of stakeholders (e.g. civil society, private sector, academia). The achievement of the SDGs implies going beyond fragmented, hierarchical and "siloed" processes and cultures and working across institutional boundaries. Finally, this review provides evidence of good practices and helps to promote mutual learning among Mediterranean countries.

Keywords: *Sustainable Development Goals, Mediterranean region, institutional architecture, governance, coordination*.

Introduction

At the United Nations' Sustainable Development Summit of September 2015, Member States adopted the 2030 Agenda for Sustainable Development, with a set of 17 Sustainable Development Goals (SDGs) at its core (United Nations, 2015). The 2030 Agenda sets out ambitious and transformative goals to respond to the complex challenges facing humanity. The 2030 Agenda and the SDGs provide a universal framework for action to achieve sustainable development but recognize differences among countries in terms of challenges and resources (Fukuda-Parr et al., 2018). While the Millennium Development Goals (MDGs) targeted only developing countries, the SDGs are universal i.e. for both developed and developing countries. The wide-ranging and broad scope of the 2030 Agenda implies that to achieve the SDGs there is a need to strengthen linkages and coordination both horizontally, among ministries/sectors, and vertically, among government levels. Moreover, the 2030 Agenda commits stakeholders to work together to promote social development, environmental protection and inclusive economic growth. Indeed, the achievement of the 2030 Agenda and the SDGs requires substantial and sustained commitment and cooperation of various stakeholders (United Nations, 2015). Therefore, establishing and/or strengthening existing governance and institutional frameworks is fundamental for the implementation of the 2030 Agenda. Indeed, even many developing countries need to strengthen and expand existing institutional mechanisms used for the implementation of the MDGs to suit the coordination and monitoring requirements of the 2030 Agenda, which is much broader and comprehensive than the MDGs (UN-DESA, 2016).

This review paper analyses governance mechanisms and institutional arrangements adopted in the Mediterranean countries for implementing the SDGs. The paper addresses several issues: type and form of the adopted governance mechanism; horizontal coordination mechanisms between the established high-level coordination unit and other public institutions; support of the established high-level institution by technical bodies; mechanisms to ensure the legislative and parliamentarian follow-up of the SDGs; processes of vertical institutional coordination and "localisation" of the 2030 Agenda.

Material and Methods

The geographical coverage of the review is similar to that of the Mediterranean Strategy for Sustainable Development (UNEP/MAP, 2005, 2016) including 11 Northern Mediterranean Countries (Albania, Bosnia and Herzegovina, Croatia, Cyprus, France, Greece, Italy, Malta, Montenegro, Slovenia and Spain) and 10 Southern and Eastern Mediterranean Countries (Algeria, Egypt, Israel, Lebanon, Libya, Morocco, Palestine, Syria, Tunisia and Turkey).

The paper is based on a desk review of recent academic and expert literature from different sources. The main sources of information were the Sustainable Development Knowledge Platform and the Sustainable Development Solutions Network. From the former, data were mainly collected from the Voluntary National Reviews (VNRs) of Mediterranean countries and the syntheses of VNRs submitted in 2016 (UN-DESA, 2016), 2017 (UN-DESA, 2017) and 2018 (UN-DESA, 2018). From the latter, data were collected mainly from the SDG Index and Dashboards (Sachs et al., 2018; Sustainable Development Goals Center for Africa & Sustainable Development Solutions Network, 2018). Moreover, a desk review of scholarly literature (e.g. Allen et al., 2018) was carried out. The VNRs are a central element of the follow-up and review mechanisms of the 2030 Agenda (Fukuda-Parr et al., 2018; United Nations, 2015). Out of the 21 Mediterranean countries, five (Egypt, France, Montenegro, Morocco, Turkey) submitted their VNRs in 2016, three (Cyprus, Italy, Slovenia) in 2017, six (Albania, Greece, Lebanon, Malta, Spain, Palestine) in 2018, and five (Algeria, Bosnia and Herzegovina, Croatia, Israel, Tunisia) in 2019, while no VNRs are planned by Libya and Syria, due to unrest situations in both countries. Meanwhile, Egypt (2016, 2018), France (2016, 2019) and Turkey (2016, 2019) submitted twice their VNRs.

The main limitation of the present study is that the scope of the analysis is mainly limited to the content of the VNRs submitted by Mediterranean countries in the period 2016-2019 and, thus, reflects official opinions of these countries without much further cross-validation. It should also be highlighted that VNRs submitted in 2017, 2018 and 2019 provide more details than those submitted in 2016 (first year of SDGs implementation), which implies that comparison might has been biased towards countries submitting recently their VNRs.

Results and Discussion

Most of countries have adopted a *three-tier implementation approach* to achieve coordination and coherence in the landing of the SDGs (UN-DESA, 2018):

- The *first tier* is typically concerned with strategic national priorities and policy direction, and the integration of global frameworks and commitments (UN-DESA, 2018). In general, countries have some high-level entity or mechanism – which in most cases already existed prior to the 2030 Agenda adoption – that makes political decisions on the country's priorities, vision and development frameworks through which to implement the 2030 Agenda (UN-DESA, 2017).
- The *second tier* typically carries out coordination among government agencies and line ministries and addresses strengthening their capacity to implement the SDGs (UN-DESA, 2018). The first two tiers represent a decision-making level that defines how the country can achieve coherence in the implementation of the 2030 Agenda and they are usually coordinated by the offices of the president/prime minister (PM), or a coordinating ministry/body that is directly responsible to the president/PM or a high-level council (UN-DESA, 2017).
- The *third tier* deals with coordination of technical work, thus involving both government and non-state stakeholders (UN-DESA, 2018). This tier has, often, working groups to deal with sectoral issues where experts provide evidence and advice on different implementation areas and aspects to inform the decision-making tiers (UN-DESA, 2017).

As in other countries and regions (Anonymous, 2018c; UN-DESA, 2016, 2017, 2018), a variety of institutional arrangements have been used by the Mediterranean countries for the implementation of the 2030 Agenda. The utmost aim of these arrangements is to ensure an operational inter-ministerial coordination (cf. "whole-of-government" approach) as well as an active involvement of a wide range of stakeholders (cf. "whole-of-society" approach). The emphasis on integration and coordination is an important feature of many of the institutional mechanisms and frameworks (UN-DESA, 2016, 2018) and high-level political support is highlighted as important to coordinate and mobilize public institutions (UN-DESA, 2016). Therefore, coordination mechanisms are often connected to and under the supervision of the offices of presidents or prime ministers (Albania, Egypt, France, Greece, Italy, Israel, Lebanon, Palestine, Slovenia, Spain) (Anonymous, 2016b, 2017, 2018c, 2019a; General Secretariat of the Government – Greece, 2018; Italian Ministry for the Environment, Land and Sea, 2017; Ministry of Planning Monitoring and Administrative Reform - Egypt, 2018; State of Palestine, 2018; UN-DESA, 2018). In some countries, the inter-ministerial committees on the SDGs are chaired by the PM, led jointly by two ministries, or by one ministry (Anonymous, 2018c; UN-DESA, 2018). In other countries, specific ministries were designated to coordinate the 2030 Agenda implementation (UN-DESA, 2016, 2018).

Most institutional arrangements and frameworks involve numerous relevant institutions and ministries, including those addressing economic growth, social and employment sectors, environment/natural resource management (UN-DESA, 2016, 2018). *Egypt* established in 2015 a new entity, the National Committee for Monitoring the Implementation of the Sustainable Development Goals, to coordinate and follow up the implementation of the SDGs (Anonymous, 2016a; Ministry of Planning Monitoring and Administrative Reform - Egypt, 2018). The Committee is under the direct supervision of the PM and is composed of representatives of 17 ministries and state entities (Anonymous, 2016a). In *Lebanon*, the Council of Ministers established in 2017 a National committee to oversee and guide the roll-out of the SDGs. The committee is chaired by the PM and involves more than 50 director-general officials (Anonymous, 2018a; UN-DESA, 2018). In *Greece*, the General Secretariat of the Government (GSG) – especially the Office of Coordination, Institutional International and European Affairs – plays a central role in the inter-ministerial coordination and governance of the implementation of the SDGs. The GSG coordinates the activities of the Inter-Ministerial Coordination Network for the SDGs (established in December 2016 and including all line ministries), the Economic and Social Committee of Greece (a national multi-stakeholder consultation platform), Hellenic Parliament as well as the Hellenic Statistical Authority (General Secretariat of the Government – Greece, 2018; UN-DESA, 2018). In *Albania*, the Inter-Ministerial Committee on the SDGs, chaired by the deputy PM, ensures coordination and leadership of the process (Council of Ministers - Republic of Albania, 2018). *Croatia* established the National Council for Sustainable Development for implementing the SDGs (Anonymous, 2019b). In *Italy*, the Presidency of the Council of Ministers took the lead in coordinating and managing the National Sustainable Development Strategy 2017-2030 (NSDS), that is aligned with the SDGs (Italian Ministry for the Environment, Land and Sea, 2017). *Malta* set up the Focal Point Network – under the Ministry for the Environment, Sustainable Development and Climate Change – that involves a senior representative from each line ministry and meets periodically (Anonymous, 2018b). *Palestine* established a National SDG Team, under the overview of the PM's office, to lead the implementation of the SDGs (State of Palestine, 2018). In *Slovenia*, the Permanent Inter-Ministerial Working Group on Development Policies – coordinated by the Government Office for Development and European Cohesion Policy – assumes a leading role in SDGs coordination mechanism. It was established in 2017 and is composed of two representatives from each ministry that also serve as focal points at their ministries (Anonymous, 2017). The Government of *Spain* created the figure of a High Commissioner for the 2030 Agenda, who will report directly to the President of the Government (i.e. PM). It also created a High-Level Group (HLG) for inter-ministerial coordination where all levels of government (e.g. ministries, Autonomous Communities, local governments) are represented (Gobierno de España, 2018). *Tunisia* created a National technical committee on the 2030 Agenda that is led by the Ministry of Development, Investment and International Cooperation (MDICI), and the Ministry of Foreign Affairs (Anonymous, 2019c). In *Turkey*, the National Sustainable Development Commission (NSDC) – established in 2004 under the Ministry of Development – serves as the central political body in the follow-up of the SDGs (Ministry of Development – Turkey, 2016).

Countries are mandating different *ministries* as overall coordinators of the SDGs implementation. The importance of leadership by a government ministry with sufficient political power (e.g. planning and sectoral lead ministries) as well as some degree of control over financial resources (cf. finance ministries) is highlighted (UN-DESA, 2016). Examples in Mediterranean countries include key ministries such as the Ministry of Development (Turkey), Ministry of the Environment, Energy and Sea (France), Ministry of Foreign Affairs and Cooperation (Morocco), Ministry of Foreign Affairs and Trade Promotion (Malta), Ministry of Tourism and Sustainable Development (Montenegro). In *Lebanon*, the "4Ps" thematic groups of the National committee on SDGs are coordinated by different ministries and state institutions; Ministry of Public Health for "People", Ministry of Environment for "Planet", Ministry of Economy and Trade for "Prosperity", Office of Minister of State for Administrative Reforms for "Peace" (Anonymous, 2018a). In *Cyprus*, while the implementation of the whole 2030 Agenda is under the coordination of the Ministry of Foreign Affairs; a relevant line ministry has been assigned as coordinator for each of the 17 SDGs (Ministry of Foreign Affairs - Cyprus, 2017). For promoting SDG ownership within the government, some countries established specialized *SDG units* or *focal points* in line ministries (Anonymous, 2018c). In *Egypt*, the national SDGs committee is complemented by sustainable development units and working groups within line ministries (Anonymous, 2016a; Ministry of Planning Monitoring and Administrative Reform - Egypt, 2018).

High level committees and institutions are often supported by *technical bodies*. Several countries (e.g. Croatia, Egypt, Italy, Morocco, Turkey) reported that their statistical offices are members of the coordination mechanisms or provide them with technical support especially regarding monitoring. Technical and expert committees as well as advisory boards

and panels have been set up in some countries to integrate the SDGs into the mainstream agenda. In *France*, a multidisciplinary committee of international experts was set up to capture the systemic nature of the SDGs in the development of the next national action plan (Anonymous, 2016b). In *Turkey,* a task force of experts, within the Ministry of Development, has been assigned to integrate the SDGs into national development plans (Ministry of Development – Turkey, 2016; UN-DESA, 2016). In *Albania*, technical support to the Inter-Ministerial Committee on the SDGs is provided by the SDG Inter-Ministerial Technical Working Group and the UN agencies (Council of Ministers - Republic of Albania, 2018).
Governance arrangements such as inter-ministerial committees provide coordination and leadership, sometimes drawing on *non-state stakeholders* (Anonymous, 2016b, 2017, 2018c, 2019a, 2019c; General Secretariat of the Government – Greece, 2018; Gobierno de España, 2018; Italian Ministry for the Environment, Land and Sea, 2017; Ministry of Development – Turkey, 2016; Ministry of Foreign Affairs and Cooperation – Morocco, 2016; State of Palestine, 2018; UN-DESA, 2018). Apart from line ministries, national committees on the SDGs also include other authorities and bodies and, sometimes, representatives of the civil society and private sector. For instance, in *Egypt*, the Central Authority for Public Mobilization and Statistics, the National Council for Motherhood and Childhood, and the National Council for Women are also members of the National committee on the SDGs (Anonymous, 2016a; UN-DESA, 2016). In *Lebanon*, the National committee to oversee the roll-out of the SDGs includes representatives of the private sector and civil society to promote a participatory, open and inclusive approach (Anonymous, 2018a; UN-DESA, 2018). In *Albania*, the Inter-Ministerial Committee on the SDGs involves, besides key government institutions, stakeholders from the civil society, business community, academia and international organisations (Council of Ministers - Republic of Albania, 2018). In *France*, the civil society, private sector and general public as well as their representative bodies (e.g. National Council for Ecological Transition; National Council for Development and International Solidarity; Economic, Social and Environmental Council) are actively involved in the implementation of the SDGs (Anonymous, 2016b). The National SDG Team in *Palestine* is supported by 12 working groups – each tasked with one or different SDGs – that involve different stakeholders (civil society, private sector, workers' union, women organizations, youth organizations, academic and research institutions) (State of Palestine, 2018).
In the context of decentralisation, it is widely recognised that effective *vertical integration* is needed to facilitate the "*localisation*" of the 2030 Agenda (UN-DESA, 2017, 2018). Many countries established structures that link national with sub-national (regional, cantonal, local) governments to strengthen their capacity to implement the SDGs (UN-DESA, 2017, 2018). Such structures have an added value in countries with a federal system (UN-DESA, 2017) e.g. Bosnia and Herzegovina that consists of the Federation of Bosnia and Herzegovina, Republika Srpska and Brčko District. Indeed, a number of strategies and mechanisms are being undertaken by national governments to connect, engage and build capacity of local and sub-national governments (Anonymous, 2018c; UN-DESA, 2016). In *Spain*, sub-national governments (e.g. 17 Autonomous Communities / regional administrations, Federation of Municipalities and Provinces of Spain) are actively participating in national decision-making and decentralised SDGs implementation. For instance, the government of the Autonomous Community of Andalusia adopted the Andalusian Sustainable Development Strategy 2030 (EADS 2030) (Gobierno de España, 2018; UN-DESA, 2018).
Countries also report the involvement of *parliaments* through actions such as establishing working groups for the legislative follow-up of the SDGs (Anonymous, 2018c), so that they can exert their vital legislative and oversight functions, including budget adoption (UN-DESA, 2016, 2017, 2018). Some parliaments are represented in the institutional and

coordination frameworks to implement the 2030 Agenda and/or have the SDGs integrated in their activities (UN-DESA, 2016). Many Mediterranean countries (e.g. Albania, Cyprus, Egypt, Greece, Montenegro, Slovenia, Spain) emphasized the importance of engaging parliaments and members of parliaments (MPs) in the whole implementation process. The parliament reviews reports on the SDGs in *Montenegro* (Ministry of Sustainable Development and Tourism – Montenegro, 2016; UN-DESA, 2016). In *Lebanon*, the SDG parliamentary committee mapped existing legislation against the 2030 Agenda and ensures follow-up of progress towards the SDGs (Anonymous, 2018a; UN-DESA, 2018). In *Cyprus*, the House of Representatives' Committee for the Environment monitors and review Cyprus's progress in SDGs achievement (Ministry of Foreign Affairs - Cyprus, 2017). In *Spain*, the Joint Parliamentary Committee for the 2030 Agenda (with members from the Senate and the Congress of Deputies) monitors the implementation of the SDGs. The Action Plan for the Implementation of the 2030 Agenda in Spain foresees holding an annual plenary session to monitor progress made on the 2030 Agenda (Gobierno de España, 2018).
Institutional frameworks are among the major *challenges* for the implementation of the 2030 Agenda (Allen et al., 2018; UN-DESA, 2016, 2017, 2018). Indeed, challenges include insufficient coordination and communication between ministries and agencies dealing with sustainable development; and fragmentation of responsibilities and mandates for SDGs implementation (UN-DESA, 2016). *Turkey* highlighted that many institutions still have 'siloed' processes and hierarchical cultures, noting that coordinating efforts across institutional boundaries and sectors is a real challenge (Ministry of Development – Turkey, 2016). *Montenegro* (Ministry of Sustainable Development and Tourism – Montenegro, 2016) emphasized that "*Institutional coherence and alignment of sectorial policies and responses should be interwoven into the everyday activities of competent bodies and institutions at the local and national level, as well as of the entities outside public administration system*" (p. 127). Countries also noted insufficient harmony and coherence between national and sub-national (regional, provincial, local) levels of government and, generally, lack of capacity at local government level (UN-DESA, 2016). Also *Albania* identified the strengthening of the institutional capacities of all key stakeholders engaged in the SDG implementation process as one of the main challenges to be addressed (Council of Ministers - Republic of Albania, 2018). Meanwhile, *Croatia* highlighted the need to further develop and improve the institutional setup with well-defined organization, coordination and control structure (Anonymous, 2019b).

Conclusions

This paper undertook an analysis of the Voluntary National Reviews submitted by the Mediterranean countries in the period 2016-2019 to highlight governance mechanisms and institutional arrangements put in place to ensure a smooth and effective implementation of the 2030 Agenda for Sustainable Development. The analysis aimed to pinpoint key aspects that may require special attention and to highlight good practices and valuable experiences that could be utilized to promote mutual learning and share learned lessons in the Mediterranean region and beyond. In general, substantial progress was reported by Mediterranean countries in strengthening existing and/or establishing new institutional and governance frameworks. However, while achieving the 2030 Agenda requires governments to work across policy areas, institutional set-up still presents a major challenge in the implementation of the SDGs for many Mediterranean countries. Therefore, institutions in several Mediterranean countries need further strengthening to be able to formulate coherent governance frameworks that allow for different government agencies and bodies, especially ministries, as well as the different levels of governments (central/national, regional, provincial, local) to work together to ensure an effective and efficient implementation of the SDGs. It is clear that the success of the SDGs

– and the transformative agenda inspiring them – will depend on institutions overcoming 'siloed', hierarchical and fragmented processes and cultures and working across institutional boundaries in close collaboration with the civil society, private sector and academia.

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CONTRIBUTION OF GRASSROOTS INITIATIVES TO SUSTAINABLE URBAN FOOD SYSTEMS: THE CASE OF A CAMPUS GARDEN IN MUENSTER, GERMANY

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Abstract

Cities depend on a constant food supply from the outside. Current challenges (e.g. climate change, soil depletion, biodiversity loss) and their interrelatedness with modern, industrialised food production may compromise future food security for millions of urban dwellers. Evidence shows that grassroots initiatives can foster transition towards resilient and sustainable urban food systems. This research contributes to the literature on grassroots initiatives and their role in sustainability transitions by analysing the urban gardening project campus garden GrüneBeete e.V. in Muenster. Semi-structured interviews with key informants from the initiative served as a basis for the analysis that was conducted according to an integrated sustainability transition framework. The analysis was performed along the three elements of the Multi-Level Perspective, i.e. niche, regime and landscape. Potential leverage points for transition, sustainability dimensions the initiative touches and its impacts were also studied. Special attention was devoted to social practices in educational and communal spheres. Urban gardening can be considered as a radical niche that aims to create an alternative urban food system. However, the analysed initiative has developed anchoring mechanisms with the regime institutions. Therefore, campus garden GrüneBeete e.V. is rather a symbiotic niche that, nevertheless, has the potential to induce a cumulative, incremental transformation in the regime. The main levers of change used by the initiative are raising awareness and spreading knowledge. In doing so, the campus garden impacts different dimensions of sustainable food systems (environmental, human, social, cultural, political, economic/financial). While the initiative is still small, it has a high upscaling potential given the favourable institutional and political landscape in Muenster and Germany at large. Urban gardening can be identified as a growing, but rather regime-compatible niche, one main strength being its potential impact on society's beliefs and value systems, and fuelling sustainability transitions through influencing social practices.

Keywords: *urban gardening, sustainability transitions, urban food systems, grassroots initiatives, food security*.

Introduction

For the first time in human history, more people are living in cities than in rural areas (Stierand, 2013). Activities in cities worldwide, directly and indirectly, are the most powerful drivers for global environmental problems, e.g. climate change, soil depletion, and biodiversity loss (WGBU, 2016). Modern agriculture and industrialised food production play a major role in these developments (cf. Clark & Tilman, 2017; FAO, 2019; WGBU, 2016). Cities depend highly on global markets to fulfil their demands, which – along with the aforementioned environmental changes – entail a growing vulnerability and unpredictability with regard to food security (Olsson, 2018). Other challenges play a role in this as well, like fair distribution and access to food, unhealthy diets, or the loss of nutritious value due to the way we prepare and process food. To combat all these problems, our food systems will have to change.

As complex systems are prone to inertia (Unruh, 2000; Walker, 2000), the necessary change is often difficult to achieve. Therefore, the body of research on sustainability transitions has been growing fast over the last decade (STRN, 2010; STRN, 2019). With the above-mentioned problems being closely intertwined with societal challenges, changes are particularly necessary in key areas of human activity (STRN, 2010). One topic of interest has been the role of civil society in driving those changes, and that of grassroots innovations in particular (Gernert, et al., 2018; Smith, et al., 2014; Seyfang & Smith, 2007). As their potential has not yet been sufficiently studied, it is still a relevant research object in this field (STRN, 2019).
Prior research has been conducted on grassroots initiatives in the foodshed of Muenster, Germany, in the research project "Sustainability Transitions and Food Security" of the Muenster University of Applied Sciences (MUAS) and the University of Natural Resources and Life Sciences, Vienna. Local initiatives have been mapped and their role in the transition towards a sustainable urban food system has been studied in this project (cf. Gernert, 2018). One of these initiatives is the campus garden GrüneBeete e.V.
The campus garden, situated on the grounds of MUAS, started as a student project at its Department of Food – Nutrition – Facilities in 2013, but is open to everyone interested. The registered association GrüneBeete e.V. was founded in 2014. Seeing urban gardening as a growing niche with transformative potential (WGBU, 2016), a case study of GrüneBeete e.V., an urban gardening project in Muenster, has been conducted in this research, the primary research question being: How does urban gardening contribute to the transition towards a sustainable urban food system?

Materials and Methods

To be regarded as relevant to the superordinate research project, initiatives had to fulfil four predefined criteria: they had to have voluntary initiative character (i.e. a group with two or more participants as opposed to single person or commercial actors), to be active within the foodshed of Muenster (i.e. located in or around the city), to have a focus on food and/or agriculture, and to be in existence for at least two years. GrüneBeete e.V. was one of six initiatives meeting the criteria, and the only urban gardening project among them.
The initiative was further examined using desk research and a first qualitative, semi-structured interview with key informants. This qualitative research method was chosen based on a reflexive, constructivist research paradigm which draws on the presumption that reality is individually and socially constructed rather than objective (Göpel, 2016; Kornmeier, 2007). An interview guide based on "W" questions was prepared and used as orientation in order to structure the interview. The interview took place on 7th November 2017 after one of the weekly official initiative meetings and lasted for about an hour. Two interviewers and two initiative members were present. One of the interviewees has been a member of the association since 2014 (i.e. since it was founded) and is involved in project organisation and as a tutor for classes in cooperation with MUAS, the other joined more recently in 2017.
Building on prior information, another semi-structured expert interview was conducted. The interview with two members of the initiative and registered association GrüneBeete e.V. took place on 15th May 2019 and lasted one and a half hours. One of the interviewees was involved in the managing committee and in creating a cookbook for the garden and actively represents it during the Muenster Sustainability Days and at the currently forming food policy council in Muenster. They joined the initiative in 2015 and 2016.
An interview guide that is based on the "Beacons of Hope"[14] checklist and questionnaire was developed for an earlier case study of one of the selected initiatives and served as a basis for

[14] The "Beacons of Hope" project at the University of Natural Resources and Life Sciences in Vienna was commissioned by the Biovision Foundation and funded by the Global Alliance for the Future of Food.

the interview. The collected data was therefore coded and portrayed according to an integrated analytical framework for analysing food system sustainability transitions that was developed in the "Beacons of Hope" project and further explained by El Bilali and Probst (2017). The framework uses the three elements of the Multi-Level Perspective, i.e. niche, regime, and landscape, as a basis. In addition, it incorporates elements of other relevant transition frameworks (e.g. Transition Management, Strategic Niche Management) to analyse food systems distinctly and assess the type of transition path taken as well as its transformative potential. To identify levers of change, niche-regime and regime-regime interactions are examined, studying how the niche anchors in the current regime and how it gains legitimisation. As for transition pathways, El Bilali & Probst refer to the classification of Geels & Schot (2007) into transformation, technological substitution, reconfiguration, de-alignment and re-alignment. To assess the transition impacts an initiative has, the desirable system state has to be defined as well as the initiative's effect on the different sustainability dimensions (environmental, human, social, cultural, political, financial and physical).

Results and Discussion

The campus garden GrüneBeete e.V. was founded in 2012 by two master degree students of the MUAS department of Food – Nutrition – Facilities as a semester project. For this reason, the land necessary for maintaining the garden is made available by the University. Over time, the project expanded, and to facilitate organisational tasks and ensure continuity for the project, the registered association was founded in 2014. During the spring-summer months, the initiative meets twice a week for gardening activities, and throughout the year it participates in city events. Despite its close connection to MUAS, the initiative emphasises its autonomy and independence from institutions to facilitate self-organisation and empowerment. It is not the initiative's vision for the garden to grow much further, but to spread the idea and set an example for alternative concepts while increasing quality of life for the individual. A main focus lies on the community aspect, and participation in urban development processes plays a role as well.

As a *niche*, the campus garden presents an alternative to incumbent *regime* structures with its outsourced food production in cities and the consequential alienation of citizens from nature and the way food is grown. In the Muenster region, agriculture is aligned to cater to global markets instead of regionality, with only 6% of agricultural produce being distributed via direct marketing (Lammers & Becker, 2014). Producers and consumers have become anonymous, and the interviewees deplore that food is something bought at the supermarket that has to be cheap, fast and convenient. By contrast, the community aspect and spatial proximity of the initiative create trust, which in turn is the basis for relatedness (Stierand, 2013). It offers room for exchange regarding experiences, knowledge, and cultural aspects, and also provides opportunities to integrate knowledge from the outside (i.e. through workshops, literature and seminars). Instead of conventional farming methods that are predominant in the region and modern agriculture in general, the gardening activities are based on organic farming principles (i.e. health, ecology, fairness, care) and value cycles.

While it does not have a direct influence on rules and institutions, the initiative fulfils three of the four criteria for alternative food systems (cf. El Bilali, et al., 2017): it reduces the distance between producer and consumer by merging the two roles in a local, small-scaled endeavour (space attribute); it gives food time to grow and be prepared with care (time attribute) and relies on organic and circular farming techniques (integration). Furthermore, in contrast to usual regime rules, none of the members own a patch in the garden, but everything belongs to everybody and everybody is responsible. As part of a national urban gardening movement, GrüneBeete e.V. contributes to pioneering activities that aim to spread the idea within an existing regime while the status quo is still relatively unaltered.

With regard to *landscape*, mega trends like individualisation and urbanisation are shaping today's society (WGBU, 2016), while at the same time, global threats like climate change and biodiversity loss have been entering the public debate. Recent developments (e.g. school strikes for the climate or the remarkably hot, dry summer) have fuelled this trend even further, especially in Muenster as winner of the German Sustainability Award in 2018. Where these developments are answered with a growing need for community and togetherness as well as a sensitisation towards environmental problems, landscape pressures arise. With individualisation as another mega trend, the DIY[15]-movement as a counter-trend draws people to urban gardening movements as well.
When asked about potential *levers of change*, the initiative members' top answer was raising awareness and spreading knowledge. However, the translation of knowledge into actual practices remains a main factor in unsustainable lifestyles. Ecological experiences play a crucial role in this (Frantzeskaki, et al., 2017). Therefore, appreciation for food and nature is increased through hands-on experiences in the garden, and the reproduction of routines that keeps incumbent social practices alive might thus be disrupted. The initiative seeks to incorporate experiential learning into formalised education as well, e.g. by collaborating with MUAS or by helping to set up gardens in primary and secondary schools in Muenster. The city of Muenster actively seeks the initiative's help in these endeavours. The garden is defined in its Articles of Association as a promotor of education, offering skilled support, workshops, and collaborations with Muenster's universities. According to Meadows (1999), one of the mightiest leverage points is changing the underlying paradigm inherent in the system. Social practices are closely interrelated with this paradigm, and initiatives like GrüneBeete e.V., through influencing these practices and the underlying views and values, might potentially fuel a paradigm shift, paving the way for more sustainable food systems.
The main form of *anchoring* for the initiative is the creation of networks. It puts a great emphasis on collaborations, and while the garden benefits from the support it gets from the city of Muenster and other local actors, it is at the same time a source of information and a potential partner for questions that arise in the context of other gardening projects, creating interdependencies. The initiative gains legitimacy through its status as a registered association (i.e. "e.V.", *eingetragener Verein*). Because of its close connection to the MUAS, the initiative often gets inquiries for research projects, and it is also part of a greater network of urban gardening initiatives ("*die anstiftung*") that supports research into the topic.
It is assumed that incremental change will not suffice to cope with sustainability challenges in urban food systems (e.g. Gernert, et al., 2018). Indeed, for the activities mentioned above, a certain degree of conformity and formalisation is needed. However, even as a symbiotic niche, urban gardening projects like GrüneBeete e.V. might change regime insiders' perceptions, resulting in new regimes through cumulative adjustments (cf. transformation path according to Geels & Schot, 2007). Additionally, the adoption of niche innovations might trigger further adjustments within the regime and the integration of further niche innovations (cf. reconfiguration pathway, Geels & Schot, 2007).
As is the case for the campus garden, collaborations with institutions constitute the predominant context for grassroots initiatives in Northern Europe (Sanna, 2018). This regime compatibility might render a niche less radical, resulting in incremental rather than transformative change. However, it is important to keep in mind the bigger picture: Urban gardening projects are just one of many niche innovations that act in concert to reach the same goals. It might not be able to feed the urban population, but it promotes a form of direct contact of people with nature and food production that has long been lost. The community aspect of communal gardens may strengthen the individual's identification with the local area,

[15] Do It Yourself.

and in combination with participative, democratic structures, contribute to forming a sense of responsibility. Additionally, it adds to individual (and consequently communal) wellbeing. This creates a context that facilitates learning and personal reflection. Together with the initiative's self-conception as a place of social learning, consumption practices of its members might become more sustainable, especially in initiatives like GrüneBeete e.V., which explicitly focuses on sustainable gardening techniques. Consumption practices play a crucial part in the transition to sustainable food systems, seeing as feeding the world on organic agriculture seems possible provided that consumption patterns change (Muller, et al., 2017).
Despite scholars identifying contradictions in regime institutions – which are embedded into the incumbent regime paradigms – collaborating with initiatives that follow a de-growth and sustainability transition narrative (Sanna, 2018), collaborating with MUAS has contributed to the initiative's stability without compromising its autonomy. This leaves room for debate over how reasonable it is to categorically deny grassroots initiatives their transformative potential when working symbiotically with the regime, and if a niche needs to be radical to have an impact. Smith et al. (2014) point out that this kind of dismissal might be premature, considering that each response to challenges by grassroots movements "create forms of knowledge of considerable social value in debates about innovation policy." Moreover, it can be argued that the initiatives need not necessarily demand radical system change, as "gradual steps of modernization might very well carry the potential for a fundamental transformation as well" (Adloff & Neckel, 2019, p. 8). This can constitute a valuable contribution to public discourse, and the more initiatives sow the seeds, the closer a society may get to reaching a critical mass.
Ultimately, the desirable system state the initiative strives for is a sustainable urban food system for the city of Muenster which takes ecological, social and economic factors into account. The impact an initiative may have in fostering such a transition increases the more dimensions are driven towards sustainability (El Bilali & Probst, 2017). GrüneBeete e.V. acts in the *environmental* dimension by making use of ecological farming methods and aiming at circular value production, and it increases *resilience capacity* by promoting biodiversity. The initiative's focus on education and development of knowledge and skills individually and as a community plays a role in the *human/creative* dimension. The *social/equity* dimension is influenced by the garden's communal, democratic structure and its aim to provide people in the city with access to a garden. The garden puts a great emphasis on the *cultural* aspect: It aims to conserve old cultivars and gardening techniques, and it promotes cultural exchange, e.g. in the form of a gardening project with refugees. While the initiative itself is not *politically* active, there have been group trips to demonstrations supporting sustainable agricultural policies. At the *financial* level, there is a direct effect on the members' disposable income as the harvest adds to their food supply, and regional value generation is promoted. As the initiative does not have any economic aims, it mostly operates in the social and ecological sustainability dimensions and combines the two by creating a meeting place and location for social learning that focuses on sustainable gardening and facilitating self-supply. Thus, many of the sustainability dimensions are touched by the initiative, implying a certain transformative potential towards a sustainable urban food system.

Conclusion

The results of the case study show that GrüneBeete e.V. does not try to overthrow the current system, but rather to show alternatives to regime structures while simultaneously raising awareness and stimulating behavioural change. It does so by focusing on social aspects such as learning and education, community, and participation. Urban gardening might only be one of many niche innovations and a puzzle piece in inducing a food system transition, but it

offers people a safe space where they can meet like-minded people and where shared values and opinions form that can facilitate an overall system change.
The city of Münster, being the winner of the German Sustainability Award of 2018 and a city with high levels of education, offers very favourable conditions for sustainable innovations. This needs to be taken into consideration when assessing local sustainable grassroots initiatives and the applicability of their structures to other cities. The role of authorities and policy-makers in supporting niche developments therefore deserves more attention. Further research might also be needed to find out to what extent the initiative (and other urban gardening projects) attracts members who already follow sustainable consumption patterns, if these patterns become even more sustainable, and if the initiative can, in reality, contribute to the formation of sustainable consumption patterns in people who have previously followed unsustainable "mainstream" patterns.
Taking into account the impact practices can have on sustainability transitions and assuming that the economic growth paradigm is the root of unsustainable practices, urban gardening – particularly if it continues to spread – can make a considerable contribution by providing regular first-hand experiences and influencing the urban population's values and beliefs. And indeed, the concept of urban gardening is spreading: one main topic in the German Garden at the International Horticultural Exhibition 2019 in Beijing is how urban gardening can contribute to the sustainable development of metropolitan areas.

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AGRIBUSINESSES AND RURAL DEVELOPMENT: STATISTICAL EVIDENCES OF POTENTIALS TO GROW NIGERIAN ECONOMY

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Abstract

The Nigerian rural economy is driven by 70% of rural subsistent smallholder farmers who produce 90% of Nigeria's food. These proportions of the population are engaged in various agricultural related businesses that sustain their rural households. The persistent decrease in the world crude oil prices has squeezed the Nigerian economy with an estimated population of 182 million people. This situation calls for attention to agriculture to revive the crumbling economy. The agricultural sector in Nigeria contributes 23% of the GDP and employs about 75% of the working population directly or indirectly. Most of these people are engaged in agricultural production, processing, warehousing, marketing and distribution and different agricultural enterprises. Agribusiness therefore becomes a sure way for economic recovery. A robust investment in agribusinesses in Nigeria will enhance job creation, provide income, reduce poverty, improve GDP and improve the livelihood of the rural population. This article made a review of the potentials of agribusinesses in growing Nigeria economy. The research adopted a desktop approach making statistical presentations and analysis of the present agribusiness situations in Nigeria. Documents from the National Bureau of Statistics (NBS) were used as secondary sources of data. The review made policy recommendations based on statistical evidences as reviewed.

Keywords: *Agribusiness, Rural Development, Rural Population Potential, Evidences, Nigeria.*

Introduction

Nigeria with an estimated population of more than 160 million people has the largest population in Africa and accounts for 47 percent of West Africa's total population (World Bank, 2012). According to Ahmed *et al.* (2015), the Nigerian economy is undoubtedly dominated by crude oil sector with regards to revenue generation and foreign exchange, but agriculture holds the key to sustainable development of the country with respect to provision of employment opportunities, the provision of raw materials for agro-industries, a source of income for rural families, and perhaps most importantly, provision of food for the population. The dwindling Nigerian economy could leverage on the potentials of agribusiness enterprises and subsectors to attain growth through sustained employment, income generation, provision of raw materials for infant industries and efficient marketing network. The surging population of youths in Nigeria is a great potential if engaged in the agribusiness. According to Tersoo (2013), the centrality of agribusiness in growing Nigerian economy cannot be overemphasized. According to Ekpo and Umoh (2013), agribusiness contribution to the economy of Nigeria decreased to 14% in 1980-88 and increased to 18.2% in 1991-98. This increase could be attributed to government policy to diversify the Nigerian economy and break the dependence on crude oil production. Despite the huge government financial investment in the production of crude oil and income generated through the export of crude oil, unemployment, hunger and poverty are on the increase. This situation requires a proactive action to improve the agribusiness sector in other to grow the economy. The ongoing Agricultural Transformation Agenda (ATA) of the Federal government could be leveraged upon to develop the agribusiness sector. The study therefore was carried out to examine estimated area of cultivated land for various agribusiness enterprises and production output, ascertain the contribution of agribusiness enterprise to Nigerian GDP and examine income generated from selected agribusiness enterprises in Nigeria.

Material and Methods

The study carried out in Nigeria made use of secondary data obtained from National Bureau of Statistics (NBS) statistical bulletin on agricultural land use, agricultural business enterprises and production output and contribution of agribusiness enterprises to National GDP from 2010 - 2018. The review presented obtained secondary data through the use of descriptive statistical tools such as tables, frequency and graph. The study adopted a desktop research approach in reviewing relevant documents and official data facts in agribusiness potentials in growing the Nigerian economy. Nigeria has an estimated population of 182 million with a population growth rate of 2.7% per annum (NPC, 2006). It has an annual rainfall of 400 to 600 mm. Agriculture is one of the major occupations of the majority of the people. The climate is tropical with distinct rainy season and dry season.

Agribusiness Concept

In Nigeria, agribusiness is the business of agriculture involved in the production, processing, and distribution of food and other agricultural products including the financial and other institutions that fund or assist or contribute to these activities. These activities include farm production, storage, processing, marketing, distribution and transportation of farm commodities and products to the end users (Consumers). The agribusiness concept seek to change the traditional production and sale of the products to identifying business opportunities that exist in the value change of each agricultural enterprise and subsectors that enhance income generation and employment of the individuals in the social system.

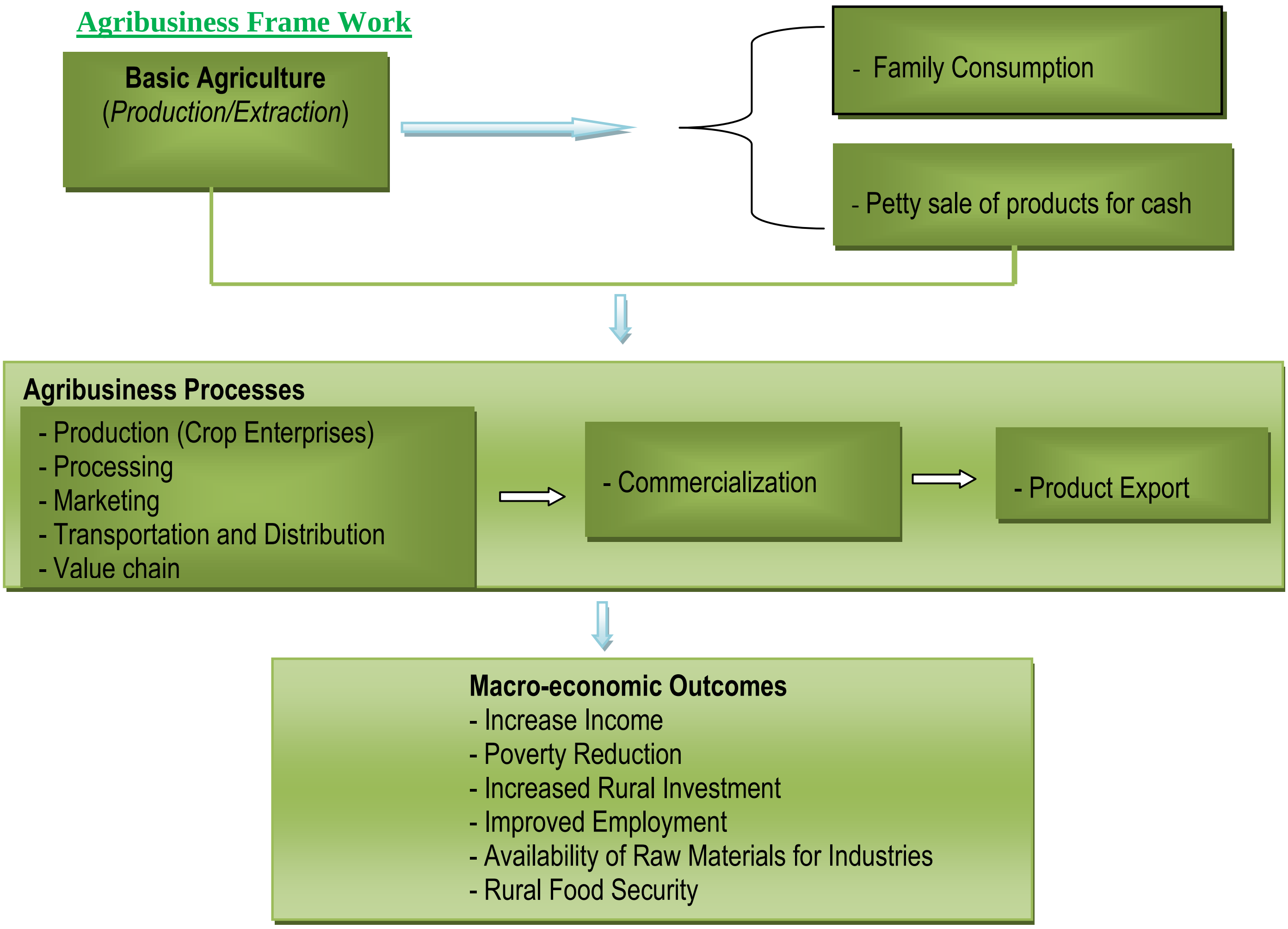
The Concept of Rural Development

Development is a concept that is not limited to certain specific activities but recognizes the existence of change in various aspect of human life. All societies, rural and urban, are changing all the time. These changes affect the society's social norms and values, institutions, methods of production, attitudes of the people and the way in which it distributes its resources. Development refers to some form of action or intervention to influence the entire process of social system. When these interventions or actions happen in the rural area, it is referred to as rural development. It is a dynamic concept which suggests a change in, or a movement away from, a previous situation. In the contest of agribusiness process, agricultural production in the rural society is expected to be infused with business ideas that will change the fortunes of the rural farmers.

Agribusinesses and Rural Development in Nigeria

Agriculture is the basis of the livelihood of most rural families. Agribusiness therefore is expected to engineer the development in the rural society especially in the areas of economic development (production and income generation), social development (provision of social amenities) and human development (capacity building). According to Onwumere and Onwusiribe (2013), agribusiness comprises businesses that either supply farm inputs or are involved in the marketing of farm products through warehousing, processing, wholesaling and retailing. This implies that agribusiness facilitates production and supply to meet the demand in the rural society. The synergy between agribusiness and rural development is captured by Todaro and Smith (2011) in their assertion that rural development, though dependent primarily on small-farmer agricultural progress, implies much more. Rural development through agribusiness process encompasses (a) efforts to raise farm and non-farm rural real incomes through job creation, (b) decrease inequality in the distribution of rural incomes and (d) the capacity of the rural sector to sustain and accelerate the pace of improvements over time.

Agribusiness Frame Work



Adapted from Ikenwa *et.al* (2017) with adjustments

The framework outlined the transition from subsistence agricultural production (family consumption) to agricultural business model including crop production (various crop enterprises and subsectors), processing, marketing, transportation and distribution and value chain. The business model will ensure efficiency in production, labour productivity, maximum input utilization, employment, income generation and food security. According to Tersoo (2013), there is a synergy in the agribusiness – rural development nexus through inputs supplies, processing and distribution.

Results and Discussion

Agricultural production in Nigeria is anchored on the efficient utilization of land. Most often, intercropping is the commonest farm practice to ensure land use efficiency. Agribusinesses in Nigeria depend on production output of the various crop enterprises in relation to hectare of land used.

Table 1: Estimated Area of Cultivated Land for various Agribusiness Enterprises and Production Output

Agribusiness Enterprises	State	Land Area (Ha)	Production Output (Metric tons)
Beniseed	4	5, 900	13, 260
Tea	18	339, 590	176, 870
Cashew	21	163, 590	87, 970
Cassava	36	6, 669, 000	60, 529, 860
Cocoa	7	1, 158, 390	323, 620
Coffee	11	47, 410	31, 490
Cotton	18	406, 160	195, 100
Garlic	13	42, 960	179, 060
Ginger	7	38, 000	174, 630
Groundnut	31	2, 566, 390	3, 487, 330
Gum Arabic	9	17, 070	13, 750
Kolanut	18	213,700	159, 460
Maize	36	5, 905, 910	10, 532, 340
Oil Palm	24	1, 897, 030	1, 519, 220
Rice	36	4, 101, 180	10, 516, 560
Rubber	9	87, 970	66, 800
Yam	27	5, 347, 480	48, 982, 620
National	**36**	**29, 007, 730**	**136, 989, 940**

Source: National Bureau of Statistics (2017)

Available statistics reveals that the agriculture sector has the highest land investment for production. Nigeria is blessed with favourable environment and climate to grow crops, rear animal and fish in all the states of the country and provide food all year round. Table one shows investment in land area and production output for various crops. At the National level, area of land cultivated for various crops is about 29 million hectares with production output of about 136 million metric tons every year. The production of these crops involves various value chain activities that provide employment for rural and urban populace in the country.

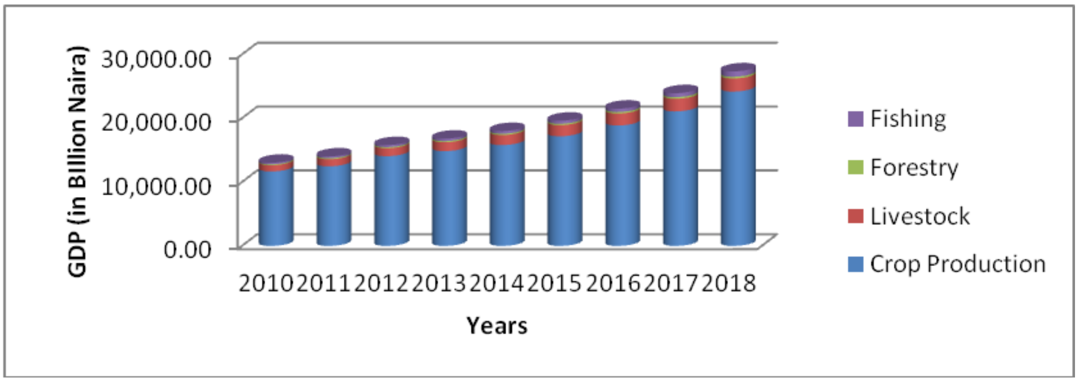


Fig 1: Agribusiness Sector Contribution in (₦'Billion)
Source: Author's Computation with data from National Bureau of Statistics (NBS, 2018)

The contributions of agriculture to the Gross Domestic Product (GDP) of Nigeria cannot be under estimated. Fig.1 reveals that from 2010 to2018, the contribution of selected agriculture sector increases on a yearly basis. In 2018, the sectors contributed about ₦27 Billion Naira to the Nations GDP. This contribution is enormous and marks agriculture business as a veritable option with the potential of growing the Nigerian economy.

Table 2: Estimated Income Generated from Selected Agribusiness Enterprises

Agribusiness Enterprises	**Output(Kg)**	**Average Price (₦)/Kg**	**Total (₦)**	**Average Price ($)/KG**	**Total ($)**
Beniseed	13,260,000	291.76	3,868,737,600	0.810444	10,009,826
Cashew	87,970,000	88.68	7,801,179,600	0.246333	21,669,943
Cassava	60,529,860,000	52.83	3,197,792,503,800	0.14675	8,882,756,995
Cocoa Seed	323,620,000	830.00	268,604,600,000	2.305556	746,123,888
Cotton Seed	195,100,000	65.02	12,685,402,000	0.180611	35,237,227
Garlic	179,060,000	184.08	32,961,364,800	0.511333	91,559,346
Ginger	174,630,000	143.97	25,141,481,100	0.399917	69,837,447
Groundnut	3,487,330,000	95.05	331,470,716,500	0.264028	920,751,990
Kolanut	159,460,000	189.83	30,270,291,800	0.527306	84,084,143
Palm Oil	1,519,220,000	427.48	649,436,165,600	1.187444	1,803,989,348
Rice	10,516,560,000	105.42	1,108,655,755,200	0.292833	3,079,627,153
Yam	48,982,620,000	101.79	4,985,940,889,800	0.28275	13,849,835,805
Total			**10,687,060,926,200**		**29,686,280,350**

Source: Author's Computation with data from National Bureau of Statistics (NBS, 2017)

Table 2 indicates the Naira worth of income generated through different agribusiness enterprises in Nigeria. These enterprises generated about ₦10 trillion amounting to $29 billion in 2017. This indicates that with well articulated investment in agribusiness enterprises and other agricultural subsectors, the Nigerian economy will no longer depend on crude oil to grow her GDP and the National income. The income generated with good governance and political will, can finance the National budget and provide infrastructure for development

Agricultural Businesses in Nigeria – The Constraints

The potentials of agribusinesses in growing the Nigerian economy face a lot of challenges and drawbacks. The following are considered as some of the constraints of agribusiness development in Nigeria; land use policy, poor budgetary allocation, inadequate application of modern technologies, poor agricultural infrastructure and inadequate incentive for youths to participate in agriculture.

Conclusion and Recommendation

The potentials of agribusiness in growing the Nigerian economy are enormous if optimally harnessed. Agribusiness impacts on the GDP of the economy positively. Agribusiness investment creates income, generates employment, improve food security status and reduce the level of poverty in the country.

Based on the reviews made and the statistics projected, the following recommendations are made; government should improve budgetary allocation to the federal ministry of Agriculture and Rural Development, the Land Use Act of 1978 that empowered the Governors and Local Government Authority to grant customary rights of occupancy to any person or organization for agricultural, residential and other purposes should be enforced and the agribusiness sector should be mechanized, commercialized and industrialized to enhance its contribution to the economy.

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CONSUMER BEHAVIOUR AT THE LOCAL FOOD MARKET

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Abstract

Consumer behaviour at the food market is determined by several factors which can be classified as external and internal. Literature review points out that the most important factors are economic components, however economic, social and environmental circumstances cause the emergence of new factors affecting consumers' decisions. The following can be listed among them: consumer ecological awareness, ethical food production, production in line with traditional methods, paying special attention to the quality of food processing, place and manner of food production, place of purchasing food or distribution channels. The paper presents the results of the surveys carried out in 2018 among 371 residents of the Małopolska Province, the aim of which was to determine the attitudes and behaviours of consumers at the food market with special regard to local products. Based on the conducted studies it has been determined that about 1/3 of surveyed respondents prefer buying food on local markets. A third of the contents found in the food products baskets come from local producers. The respondents were also surveyed in terms of reading labels on food products, and it was found that while percentage of such responses is quite high among women (63%), it is very low among men (18%). Four out of five respondents always or sometimes check the price of the products before purchasing them, thus this factor strongly impacts their decisions. The interest of consumers in local food and ability of local producers to meet this demand in the near future will build opportunities to strengthen as well as expand small farms and small food businesses.

Keywords: *consumption, local production, consumer behaviour*.

Introduction

The purchasing behaviour of consumers on the food market has been the source of interest to many scientists (Kwasek, 2015; Szwacka, 2007). These behaviours emerge as part of the elementary cycle of life as they lead to satisfying staple human needs for sustaining life. Typically, the needs are characterised by their renewability, limitlessness, complementarity, substitutability and diversified intensity (Rudnicki, 1996). The complexity of consumer behaviour on the food market results from the diversity of possibilities to satisfy physiological needs, however, the most important action taken on the market of satisfying hunger and thirst is the act of purchase (Sojkin, 2012).

Food consumption impacts our health, as well as affects the ability to live as part of society and the ability to work. The theory of consumption points to consumer behaviour as being divided into a few fundamental areas. The first one is purchasing products, leading to the maximization of marginal utility, i.e. the satisfaction of the user with consumption. A decrease in the price of a product affects the increase in its consumption, an increase in the price causes a drop in consumption. Finally, an increase in real income leads directly to an increase in consumption (Kowalska et al., 2016).

The purchase of a specific good is determined by many factors, which, in the simplest terms, can be divided into internal and external. The latter include geographical, demographic, economic, sociological and situational factors. Internal factors include those of a psychological nature, e.g. age, lifestyle, values, motivations, etc. (Bojkovska et al., 2014). The purchasing process, thus, is impacted by the market situation (price, surplus or shortage of a given good), the economic situation of the purchaser and sociological factors (Szczepański, 1981). Consumer purchasing behaviour on the food market is to a large extent determined by their demographic and economic profile. Different factors will influence the choices of teenagers at the food market, and different will impact the actions of older people (Chrzan, 2018). Family status, household size and the amount of income are only some of the elements that determine the decisions to buy food. The shopping basket of a family with two children will probably differ significantly from the shopping basket of an elderly person, if only because each of these persons has a different demand for nutrients and nutritional substances (Oczachowska, 2010).

Although well known, the food selection criteria are still evolving with changing consumer awareness and views. And although most buyers are guided by the economic criterion while completing the act of purchase (Łuczka-Bakuła, 2011), there are new factors emerging that impact their market behaviour. These include, for example, the environmental awareness of consumers, the production of a given good in line with ethical requirements, certificates proving that a given product is a traditional product (Barska, 2018), or paying particular attention to the extent to which the food has been processed, the place and manner of its production and the place of its purchase as well as the distribution channel through which the food product is being delivered. Consumer ethnocentrism and tendency to buy domestic products (Szromnik et al., 2013), in particular local products, are becoming more and more common among buyers. Manufacturers are also aware of this trend, using names that are closely associated with a particular geographical region or area to designate products. Therefore, it is an excellent tool for promoting local and regional producers (Kowalczyk, 2016). Food manufacturing intended for local markets is usually small-scale, but features a great deal of confidence that consumers have in the manufacturers (farmers and processors). Short supply chains are increasingly being promoted, pointing out that "*local tastes better*" (www.euractiv.pl), thus convincing purchasers to become local patriots.

The main objective of the paper is to present selected aspects of the food purchasing process in the households of the Małopolska Province residents. The study focused on establishing sites the surveyed consumers buy their food products from, the places of origin of the purchased food (from local, domestic or foreign manufacturers), whether the price of food products impacts the respondents' purchasing decisions and whether the respondents are aware of the quality of food they consume and if they read the information on the label of the products they purchase.

Material and methods

The study involved the use of primary and secondary data. Primary data was obtained using a paper questionnaire. The interviewer read the questions and answers and marked the responses selected by the respondents. The method of non-probability sampling has been selected for the study. The survey involved 371 respondents representing households located in the Małopolska province. The study was conducted in 2018. The questionnaire consisted of 31 questions, of which 30 were multiple-choice and one was open-ended. In the preparation of this study only a part of the data obtained during the research process was used. The questions dealt with issues directly related to food consumption, beginning with the place of purchasing food to the amount of food consumed and its form. The source of secondary data is subject matter literature. The obtained results were prepared in a descriptive and tabular form.

The majority of the respondents, 61.2%, were female. The largest number of respondents included people aged 20-31 (29.7%), followed by people aged 32-45 (28.7%) and 46-57

(24.6%). The smallest group of the respondents included people aged 50 and over (17.0%). Approximately 39.0% of the respondents have a university degree, while almost 28.2% of respondents declared completing secondary school education. Among the respondents, 27.9% declared completing basic vocational education. Only 4.9% of all respondents have only completed elementary education. In terms of the number of the household members, the largest group of respondents were people living in five-person or larger families - 24.4% of the sample. About 23.6% of respondents lived in households including three people and 23.3% came from four-person households. Every fifth respondent represented two-person households, while people living alone constituted 9.0% of the total number of respondents. As many as 42.1% of the respondents declared that their families do not have any children at the moment. As regards the monthly income of all household members, the most numerous group of respondents included the wealthiest, with monthly income per household above PLN 6,000 (24.6%). Respondents with a monthly family income up to PLN 3000 constituted 18.7% of the entire surveyed group.

Results and discussion

Table 1 shows consumer preferences (division of the study group with regard to sex) in terms of places for purchasing food products. The first choice of both male and female respondents is a big-box store (supermarket) (approx. 80%), and the second a small shop in the neighbourhood (approx. 40%). The respondents rarely shop on-line. Women are much more prone to do their grocery shopping on the marketplace (38.3% of the women surveyed compared to 25.7% of the men surveyed).

Table 1. Consumer preferences in regard to sites for purchasing food and grocery products*

Specification	FEMALE		MALE		TOTAL	
	%	Number	%	Number	%	Number
Small shop in the neighbourhood	44.9	102	40.3	58	43.4	161
Big-box store (supermarket)	82.4	187	79.9	115	81.4	302
On-line	6.2	14	6.9	10	6.5	24
Marketplace	38.3	87	25.7	37	33.4	124
Other	3.1	7	0.0	0	1.9	7

Source: Own study based on surveys, * answers do not add up to 100, as it was possible to provide more than one answer.

Preferences regarding the place of purchase do not necessarily have to correspond to the actual place of purchase. Thus the questionnaire asked the respondents to determine, in percentage terms, the sources of food consumed in the household, with the total consumption being a 100%. Table 2 presents the results obtained from the study.

The food consumed by the respondents in the most part comes from the big-box store. Approximately 23.4% of the food is purchased at the small neighbourhood shop. Part of the grocery shopping is done at the marketplace (14.5%), another part is home-made or gifts from the family - 5.5% and 4.1% respectively. As far as the source of food consumed in the households surveyed is concerned, there was no definite difference of responses in regard to the sex of the respondents.

Table 2. Source of food consumed in the households of respondents [%]

Specification	FEMALE	MALE	TOTAL
Small shop in the neighbourhood	21.2	26.8	23.4
Big-box store (supermarket)	52.0	53.7	52.7
Market	15.5	12.8	14.5
Gifts from the family	4.9	3.1	4.1
Home-made	6.5	3.6	5.5
Total	100.0	100.0	100.0

Source: own work on the basis of research.

Respondents also defined the extent to which the food they consume comes from local, domestic or foreign producers (Table 3). The studied group purchases around 1/5 of the food that has been imported. It is also great news that the basket of the surveyed consumers includes nearly 80% of domestic (47.4%) and local (32%) manufacturers. The surveyed women are less inclined than men to buy local products over the domestic ones, thus the men have shown a little more local ethnocentrism. It is also great to see less than 1% of products of unknown origin in the shopping baskets of the respondents.

Table 3. The share of local, domestic and foreign food products in the grocery basket of respondents [%]

Specification	FEMALE	MALE	TOTAL
Local manufacturers	30.8	34.0	32.0
Domestic manufacturers	48.2	46.0	47.4
Imported food	20.0	19.7	19.8
I don't know	1.0	0.3	0.8
Total	100.0	100.0	100.0

Source: own work on the basis of research.

Nowadays, the product packaging and information one can find there is the first direct message that may impact the buyer and their decision whether to purchase the product or not (Nieżurawski et al., 2015). The majority of packaging have an integral label which presents the first impression in terms of its quality (Krasnowska et al., 2011). Respondents surveyed in the Małopolska Province were asked whether they read the information on the label when shopping. 50% of the respondents familiarize themselves with the ingredients found on the label (table 4). 18.9% of respondents do it all the time and 31.5% read it sometimes. Among the respondents, only 14.1% of women never read labels before buying a product, while among men this share is much higher and amounts to 42.3%.

Table 4. Selected habits of respondents during purchasing food products.

Specification	FEMALE		MALE		TOTAL	
	%	Number	%	Number	%	Number
Do you read the label on a food product before purchasing it?						
Yes, I always do	23.3	53	11.1	16	18.9	70
Sometimes	40.5	92	17.4	25	31.5	117
Rarely	22.1	50	29.2	42	24.8	92
No	14.1	32	42.3	61	24.8	92
When shopping do you consider the price of the product you are purchasing?						

Yes, I always do	49.8	113	32.0	46	42.9	159
Sometimes	42.7	97	41.6	60	42.3	157
Rarely	2.6	6	13.2	19	6.7	25
I don't limit my expenses on food	4.9	11	13.2	19	8.1	30

Source: own work based on the study.

Papers analysing consumer purchasing behaviour at the food market point to the significance of the price - the purchase decisions of Polish consumers are impacted by the amount they need to pay for the product (Kowalczuk and Maciąg, 2017; Michałowska 2010). Buyers are willing to spend more money on a food product if the product they purchase is of satisfactory quality for them. Respondents were asked a question: do you take into account the price of the goods you buy? In the study group over 90% of the surveyed women agreed with the statement that they take into account the price of the product they are purchasing, with 49.8% doing it always, and not much less, 42.7%, doing it sometimes. However, the men surveyed much more often than women claimed that they rarely consider the price of the product and that they do not put limits on their expenses on food (Table 4).

Conclusions

In view of the data provided by the literature on the consumer behaviour at the food market in Poland we can see the origins of ethnocentrism are emerging, which can also be seen as a way of supporting rural areas and agriculture. Food purchased directly from food manufacturers enjoys particular consumer confidence. Only small farms can afford to sell on a small scale, directly by the farmer. Selling this way is also an opportunity for the smallest players on the market and gives them a chance to deliver niche products which are often in great demand.

These trends are at least in part confirmed by the completed study. Respondents prefer a big-box store (supermarket) as their shopping destination, but when buying food, about 1/3 of them (more often women than men) prefer to shop at marketplaces. These sites often offer grocery products of local manufacturers (farmers) and processors.

The research implies that almost 1/3 of the food purchased by respondents comes from local producers, and the fact that only about 20% of the respondents put imported products in their shopping basket is good news.

Polish consumers are increasingly gaining awareness, they wish to obtain as much information as possible about the food they buy and consume. They are interested in the information found in the product label, as well as in the price of the good. The study points out that only 14% of female respondents do not read labels before buying a product. The percentage of these responses is, unfortunately, three times higher among the men surveyed. The purchasing decisions of the respondents are strongly impacted by the price of a food product, as four out of five respondents always or sometimes check its amount before buying a food product.

Contemporary consumers more and more often consider the general quality of the food purchased, which is, for instance, identified with the place and manner of its manufacturing as well as the extent to which it is processed. This situation is a great opportunity for local food manufacturers, who can take advantage of the gap that is being created for them and ensure the presence of the well-known, well-established and traditionally prepared regional products on the local market.

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ECONOMIC ASPECTS OF HAZELNUT PRODUCTION IN REPUBLIC OF SERBIA

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Abstract

Areas under hazelnut made 1.8% of total areas under fruits in Republic of Serbia in 2017 and significant increase was recorded in comparison with previous period (7.9% relative to 2016 and 19.1% in comparison with 2015). In this work, costs and achieved results in hazelnut production are analyzed on the agricultural holding from Republic of Serbia which is primarily involved in crop production. The main aim of the research is to determine profitability level in hazelnut production. Average hazelnut production in the world, from 2008 to 2017, was 863,847 tons. Turkey predominates with 66.3% share in world production, then follow Italy with 12.3% and Azerbaijan with 3.8%. Hazelnut production demands high labor share primarily because of the fruit collection and crunch the hazelnut. Because of that, in the cost price structure labor cost are dominant with 37.7%, followed by depreciation of equipment costs with 34.6%. Material costs made 12.1% of total production costs. Coefficient of economy is 2.23 and profitability rate is 55.1%. Investment in raising the plant is payed for 9 years and 259 days, which is relatively long period of time but it is important to emphasize that the period of profitable orchard exploitation can be more than 50 years. Also, investment in modern equipment can additionally intensify hazelnut production which represents good way for increasing earnings of agricultural holdings, with multiple positive effects on increasing export, rural and whole agrarian sector development in Serbia.

Keywords: *Hazelnut, profitability, coefficient of economy, agricultural holding.*

Introduction

The hazelnut is significant fruit species which fruits are extremely appreciated on domestic and foreign market. Hazel bush originated from East Asia, where from it has spread to the west towards Southern Europe and North Africa. It is one of the oldest useful plants known to man since the "Stone Age".

The nut of the hazel have about 60% fats, 14% of proteins and about 7% of carbohydrates. It is an excellent source of vitamin E and vitamin B6 (*Zhao et al., 2017*). Hazelnut can be consumed underdone, roasted and dried and it also can be used as a raw material in confectionery industry. The nut of the hazel can be also used in precise and airplane industry, painting and perfumery. Tannin, obtained from the hazel crust and leaf, is used in medicine. Shell, which is an nus product obtained in hazelnut production, is characterized by high calory value and it is more favorable heating material than other solid fuels.

Average hazelnut production in the world, from 2008 to 2017, was 863,847 tons. Asia is the continent which dominates in the world production with 78.9% share, followed by Europe with 16.2% and North America with 3.9%. Turkey with 66.3% is the country with the highest share in world production, while Italy with 12.3% and Azerbaijan with 3.8% are the second and third world producers of hazelnut (*FAOSTAT, 2019*).

Republic of Serbia (RS) participates with 0.5% in the hazelnut world production and with 3.0% in european production. Areas under hazelnut made 1.8% of total areas under fruits in RS in 2017 and they increased for 7.9% compared to last year, while the increase was even more in comparison to 2015, 19.1% (*RSO, 2019*).

Hazel bush can be grown on different types of land, from neutral to acidic. Irrigation is necessary more times during the vegetation period. It can be grown without regular protection, but when is grown in plantation conditions it is necessary to protect hazelnut from various parasites, harmful insects and diseases. RS has favorable natural conditions for raising hazelnut plantations.

The paper primarily considers economic aspects of hazelnut production on the agricultural holding (AH) from AP Vojvodina[16]. The main aim of the research is to determine and evaluate profitability level in this production.

In the first part of the paper, cost structure and cost price in hazelnut production is considered, while in the second part economic analysis and evaluation of justification of investment in raising hazelnut plantation are performed.

Material and Methods

The material basis of the research are data from AH which is primarily involved in crop production. Also, data from publications of Republical Statistical Office (RSO) and Food and Agriculture Organization of the United Nations (FAOSTAT) were used.

Cost-effective analysis of hazelnut production is based on analytical calculation of primary costs and achieved results in this production (*Lukač Bulatović et al, 2017*). These calculations are the basis for developing an investment plan and deadline of the return of the investment in raising the plant (*Miljatović and Vukoje, 2019*).

Investment in raising the plant was calculated according to the following formula (*Andrić, 1991*):

$$Vm = A + (a_1 + a_2 + ... + a_m)$$

A – investment which was made once during the raising the plant,
a – cost of fertilization, care and protection in some years during its raising,
m – number of years of raising the plant.

Costs in calculation are divided into fixed and variable, whereby the year when was reached full hazelnut yield (the tenth year from raising the plant) was taken as a representative year. Cost price was calculated according to "*the old method*", which means that from total production costs, value of nus product was taken away and the rest of the value presents production costs of main product (hazelnut – the core) (*Vukoje et al., 2015*).

Success indicators, such as profit, coefficent of economy and profitability rate, were calculated. Also, other research methods, such as interview, observation method, "desk research" method etc, were used.

All calculations are based on real market prices of inputs and products from 2018 expressed in euros. Average exchange rate from 2018 was used (€ 1 = RSD 118.27) (*NBS, 2019*).

Results and Discussion

In order to make hazelnut production successful, it is necessary to consider numerous factors which have influence on the economics of production. The economics of production, as production value and production costs ratio, depends on the market price and achieved yield in hazelnut production, from one side, and purchase price (cost price) and quantity of inputs which are spent in the production, from the other side.

In the cost structure of hazelnut production, labor costs are dominant with 37.7% share, because of the collecting hazelnut and its processing (*tab. 1*). If these jobs were completely mechanized, labor costs share in the cost structure would be significantly reduced.

Material costs made 12.1% of total hazelnut production costs. Therefrom, pesticides with 7.0% presents the most important item. Fertilizer and fuel costs have significantly lower share in the total costs (2.7% and 1.7% respectively).

[16] AP Vojvodina (APV) is autonomous province of Serbia, located in the northern part of the country.

Table 1. Calculation of the cost price in hazelnut production (per ha)

No.	C O S T S				Amount (€/ha)
1	Fertilizer				80.2
2	Pesticides				210.6
3	Fuel				51.1
4	Electrical energy				8.2
5	Other material				14.1
I)	Material costs (1 to 5)				364.1
6	Direct services				235.8
	- *Insurance*				169.1
	- *Consulting*				66.7
7	Labor costs				1,135.7
A)	VARIABLE COSTS (1 to 7)				1,735.6
8	Fixed costs				1,273.1
	- *Depreciation*				1,239.3
	- *Overheads*				33.8
B)	TOTAL COSTS (A + 8)				3,008.7
No.	ACHIEVED RESULTS	Production		Price (€/kg)	Amount (€/ha)
		Unit	Total		
9	Hazelnut	kg	1,288.0	5.07	6,534.2
10	Shell	kg	1,512.0	0.08	127.8
11	Subsidies				44.0
C)	VALUE OF PRODUCTION (9 to 11)				6,706.0
D)	PROFIT (C - B)				3,697.3
E)	COST PRICE (€/kg)				2.24

*Source: Author's calculation based on data from agricultural holding

Depreciation of equipment costs have high share (34.6%) in total costs because of the high depreciation base, which is purchase value of equipment used in this production. If household wants to reduce share of these costs, universal mechanization should be used. On that way, depreciation costs would be proportionally distributed on several production lines according to the appropriate allocation key.

Raising an orchard in own direction implies its valuation according to the cost price (costs of its raising). In that case it is necessary to calculate orchard depreciation at a 2% rate if is known that exploitation period is 50 years. Costs of orchard depreciation, calculated on that way, made 5.4% of total hazelnut production costs.

Insurance costs could have significant participation in the cost structure if the crop is provided from harmful events (hail, cold, flood, high temeratures etc). It is necessary to emphasize that the state subsidizes 40%-45% of insurance premium, which affects on the total production costs reduction. On the observed AH insurance costs are 5.6% of total costs (*tab. 1*).

Retail price of hazelnut (core) on the domestic market reches 8.5 €/kg (*STIPS, 2019*). AH sells hazelnut at 5.07 €/kg, and nus product (hazelnut shell) at 0.08 €/kg.

Average yield of hazelnut with shell in the world in a ten-year period (2008-2017) ranged from 1.1 t/ha to 1.8 t/ha. In RS average yield ranged from 1.3 t/ha to 1.5 t/ha (*RSO, 2019*). Hazel bush starts to give some yield in the fourth year. Yield rises from year to year and full

gender hazel bush gives in the tenth year of orchard exploitation period, which is over 50 years (*Novković et al., 2017*).
Subsidies aren’t so significant item in the income structure of hazelnut production. Namely, AH uses the basic incentives in plant production for 33.82 €/ha, and subsidy for diesel fuel for 10.15 €/ha (*Official Gazzete of RS, 2016*).
Coefficient of economy in hazelnut production is 2.23 which means that observed production reaches € 2.23 value of production on € 1 production costs. Profitability rate, as achieved profit and value of production ratio, is 55.1%.
In order to calculate investment in raising the plant all investments which are made once during the raising orchard are taken into consideration (analysis and soil preparation, seedlings, planting, irrigation system, fence). Costs of fertilization, care and protection till year in which revenues are higher than these costs, made investment in raising the plant also (*Andrić, 1991*). In hazelnut production that is the sixth year. Investment in raising the plant, if the investment in equipment is excluded, is about 8,100 €/ha (*tab. 2*).

Table 2. Investment in raising hazelnut plantation (per ha)

No	Investment	Value €/ha
1	Analysis and soil preparation	318.7
2	Seedlings	1,902.4
3	Irrigation system	2,113.8
4	Fence	1,500.0
5	Cost of fertilization, care and protection in the first 5 years	2,273.7
	Total	**8,108.6**

*Source: Author’s calculation

Hazelnut production in not possible without adequate equipment (tractor, rotary mower, atomizer, fertilizer spreader, equipment for collecting and processing hazelnut). If AH sells hazelnut with shell, investment in equipment is lower for hazelnut crunch purchase value. But, in that case, market price will be significantly lower than the price for hazelnut core, ranged from 1.8 €/kg to 2.5 €/kg.
Total investment in raising the plant and required equipment on the observed AH (3 hectares surface area), is about € 49,400. Investment is payed for 9 years and 259 days (*tab. 3*).

Table 3. Return time of investment

Year	Current value of net inflows (€)	The rest of the long-term investment (€)
-		-49,366.9
2019	-1,699.1	-51,066.0
2020	-1,973.9	-53,039.8
2021	-2,248.7	-55,288.5
2022	-1,885.0	-57,173.5
2023	-1,339.5	-58,513.1
2024	781.8	-57,731.3
2025	4,840.9	-52,890.5
2026	8,949.8	-43,940.7
2027	11,525.6	-32,415.0
2028	45,688.2	13,273.1

*Source: Author’s calculation

Relatively long deadline of the return of the investment is the main obstacle to potential producers to invest their money. However, observed in the long run, farms which are primary involved in crop production can resolve that problem by organizing hazelnut production as their second, additional, job. Profit, which they can expect in this production, is 3,697.3 €/ha per year (*tab. 1*). This can be coinsidered as good result, if is known that plum production, which is with the apple most widespread fruit species in APV, gives 1,008.6 €/ha year profit (*Vukoje and Miljatović, 2018*).

Conclusion

Average hazelnut production in the world during the observed period was about 865 thousand tons. Areas under hazelnut in Republic of Serbia are recording increase from year to year. This is explained by favorable natural conditions for hazelnut production, from one side, and quite stable and constant market price, from the other side.

Research has shown that this production, generally observed, can provide satisfactory level of earnings on the investment. Current market price is about 5 €/kg and average yield, which can be expected in full gender year on the observed area, ranged from 3 t/ha to 3.5 t/ha. Cost price of the main product (hazelnut core) is 2.24 €/kg and it is significantly lower than the current market price.

In the production costs structure labor costs predominate with 37.7% share and depreciation of equipment costs with 34.6%. It is possible to reduce these costs with reducing the need for labor force by automation of the production proces and by using universal mechanization, but that requests signifacant investment increase.

The deadline of the return of investment raising the plant is relatively long, 9 years and 259 days. However, long period of orchard exploitation (over 50 years), achieved profit in full gender year (3,697.3 €/ha), high coefficient of economy (2.23) and profitability rate (55.1%) have influence on the positive overall rating of the investment.

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COMPARATIVE ANALYSIS OF ONION PRICES

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Abstract

This paper analyzes the prices of onion. The main goal of this study is to use quantitative analysis to find out the trends in onion price movements and to make a comparative analysis of onion prices in Serbia, Macedonia and Entity of Republic of Srpska (Bosnia and Herzegovina). The onion prices are analyzed for the period 2012-17. The prices in the study are based on the average annual prices. The quantitative analysis was performed by using the method of descriptive statistics and the average annual change rate to determine the trends in changes for the analyzed period. The average annual price of onion in Serbia was 182.40euro/t. The price fluctuated in the interval between 159 and 208euro/t. The price variation in the observed period was low (CV=10.42%). The price of onion in Serbia showed a slightly negative trend, almost stagnation. The annual change rate of onion prices in Serbia in the analyzed period was -0.76%. In Macedonia, the average annual price of onion was 304.07euro/t. The price varied in the interval between 259 and 392euro/ton. The price variation in the observed period was medium (CV=16.90%). The price of onion in Macedonia showed a very slight negative trend, almost stagnation. The annual change rate in the analyzed period was -0.05%. In the entity of the Republic of Srpska, the average annual price of onion was 276.07euro/t. The price ranged between 195 and 364euro/ton in the analyzed period. The price of onion in the Republic of Srpska showed a negative trend. The average annual change rate in the analyzed period was -3.55%. The lowest price of onion was in Serbia. In the Republic of Srpska, the price of onion was 51% higher, while the price in Macedonia was 10% higher than in the Republic of Srpska, and 67% higher than in Serbia. The prices of onion show a negative trend in all analyzed countries (entities).

Keywords: *onion, price, Serbia, North Macedonia, Republic of Srpska*

Introduction

Vegetable production is one of the most intensive branches of plant production, and along with grain production, it is one of the most intensive branches of arable land production. This is confirmed both by the yields produced per unit of area, i.e. the amount of organic matter produced annually per unit of area, and by achieved economic effects. Bearing in mind the importance that this branch of agriculture has in both the production and economic sense for producers as well as for agriculture as a whole, it is justified to expect its further development. The subject of the research in this paper is a comparative analysis of changes in the sale prices of onion in Serbia, the Republic of Northern Macedonia and the Republic of Srpska. The prices were analyzed for a six-year period 2012-2017.

There are numerous examples of applying quantitative methods (statistical methods and methods of operational research) and qualitative methods in analysing, modelling, forecasting and planning of production and economic characteristics of agricultural products and inputs in agriculture.

Mutavdžić et al. 2011 determined the trends in the development of vegetable production and concluded that in the period 2001-2010 vegetable production in Serbia had the following characteristics: the areas under certain vegetables including tomato, pea, cabbage, kale, pepper, carrot and cucumber increased, whereas the areas under potato, onion, melon, watermelon and garlic decreased compared to the previous decade (1991-2000); the average yield of all the analyzed vegetable crops increased (except for beans); the total vegetable production in Serbia rose significantly, primarily as a consequence of production intensification i.e. higher yields. Novković et al. 2014 used the method of descriptive statistics to conduct the analysis of the main production parameters in the production of important vegetable crops for a long-term period in Vojvodina. Lazić 2014 used the descriptive, statistical method to analyze the production and evaluate the development of vegetable production in the countries that are leading vegetable producers in the European Union. The quantitative data on the vegetable production indicated the following 13 countries as the leading vegetable producers in the EU: Spain, France, Germany, the Netherlands, Portugal, Hungary, the United Kingdom, Greece, Italy, Belgium, Poland, Bulgaria and Romania. Based on the defined trends, it was possible to forecast reduction in the areas under vegetables in Poland, Italy, Greece, Bulgaria, Hungary and Belgium, and increase in the Netherlands, Spain and Portugal. The quantitative analysis pointed to the development of cropping practices in vegetable production, since out of the 42 studied cases. Novković et al. 2006 performed a time series analysis of wheat / mineral fertilizers price parities, and used the ARIMA model to forecast the price parity trends in the following period. Mutavdžić et al. 2007 analyzed the tendencies and forecast the movements in price parities of fattening pigs and commercial maize. Mutavdžić et al. 2010 focused on forecasting the price parities of the main field crops based on time series analysis and application of the ARIMA model. Mutavdžić et al. 2017 analyzed quarterly movements of wheat and maize sale prices in Serbia and the Republic of Srpska for the period 2010-15. By applying the method of ratio to the overall quarterly average, the results showed that the prices of grains in the Republic of Srpska are higher. There are also a large number of papers dealing with the prices of vegetable crops through analyses and forecasts of certain vegetable prices. Ivanišević et al. 2015 analyzed the movements of tomato prices in Serbia using the method of descriptive statistics, followed by forecasting its value for the future period based on time series analysis. Novković et al. 2016 performed the analysis and forecast of cabbage prices in Serbia. Novković, Mutavdžić 2016 performed the analysis of bean prices in Serbia by means of descriptive statistics. On the basis of these results, an adequate ARIMA model was applied to forecast the movements of bean prices for the following period.

The aim of the research is to analyze and forecast the trends in onion prices in these countries/entity, as well as to conduct a comparative analysis in order to examine the comparative economic advantages. On the basis of the results of the comparative analysis, it is possible to draw conclusions on the prospects of onion production, as well as the causes of positive or negative trends in the previous period.

Material and Methods

The research methods applied in this paper are quantitative research methods. The quantitative analysis included the absolute prices of onion expressed in euro per ton in Serbia, Northern Macedonia and the Republic of Srpska. The analysis was applied for the period 2012-17.

The average annual prices of onion were first converted into a common indicator, euro per ton, to enable comparison and to reduce the impact of inflation in certain countries. Conversion of the prices into euro was carried out according to the average annual exchange rate of euro based on the data of the National Bank of Serbia and corresponding institutions in the Republic of Northern Macedonia and the Republic of Srpska.

The data were processed using standard statistical instruments of descriptive statistics: arithmetic mean – average value of the studied phenomena ($\overline{X}$) and a rate of change (r). The rate of change was calculated directly from the absolute values of the time series using the following expression:

$$r = (G - 1); \qquad G = \left(\frac{Y_n}{Y_1}\right)^{\frac{1}{n-1}}$$

Where in:
r = rate of annual change; G = constant relative change in a phenomenon; Y_1 = the absolute value of the first member of the time series; Y_n = the absolute value of the last member of the time series; n = the number of the time series members, i.e. the number of years

The analysis was based on the official published data of the Republic Statistical Office of the Republic of Serbia, Republic of Northern Macedonia and Republic of Srpska.
Apart from individual analyses of onion prices, the paper also includes a comparative analysis conducted for these republics. The comparison was carried out using the index method. If in comparing the trends the observed phenomenon did not move in the same direction in different republics, a qualitative comparative analysis was performed instead of calculating the indices.

Results and Discussion

The average annual sale price of onion in the Republic of Serbia in the analyzed period was 182 euro/t. The lowest price was in the last year (2017), when it amounted to 159 euro/t, while the highest price was 208 euro/t in 2013. The price was relatively stable, which is confirmed by the coefficient of variation of 10%. The onion price had a tendency of a slight decrease in the analyzed period, at an average annual rate of -0.76%.
Figure 1 shows the average annual prices of onion in the Republic of Serbia for the period 2012-17.

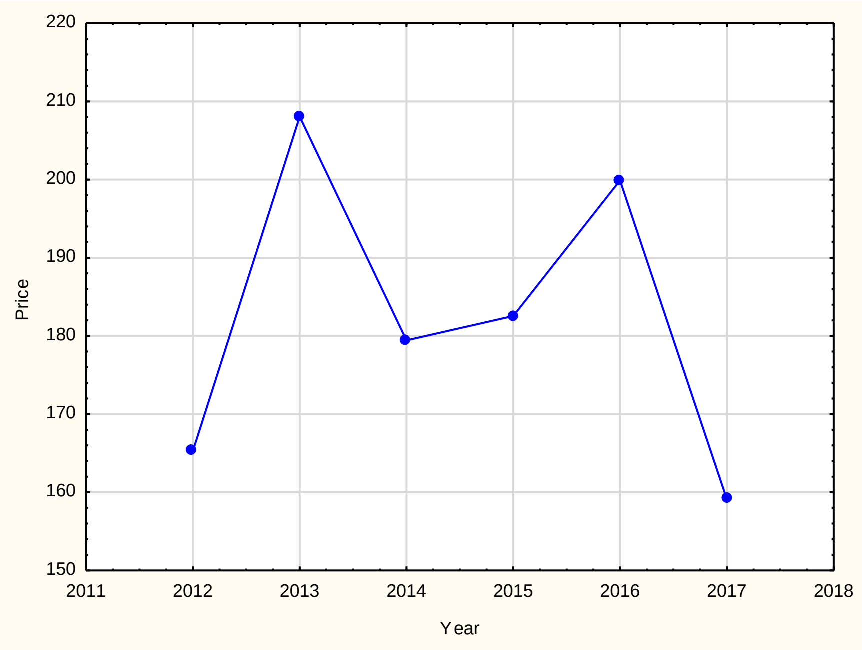

Figure 1: Average annual prices of onion in the Republic of Serbia for the period 2012-17
In the Republic of Northern Macedonia, the average annual sale price of onion in the analyzed period amounted to 304 euro/t. The lowest value of the price of 259 euro/t was in 2017 (as in

Serbia), while the highest value of 392 euro/t was realized in 2013 (also in the same year as in Serbia). The onion price showed moderate oscillations in the analyzed period. The coefficient of variation was 17%. The price had a tendency of a very slight decrease, almost stagnation, since the average annual change rate was -0.05%. Figure 2 presents the average annual prices of onion in the Republic of Northern Macedonia.

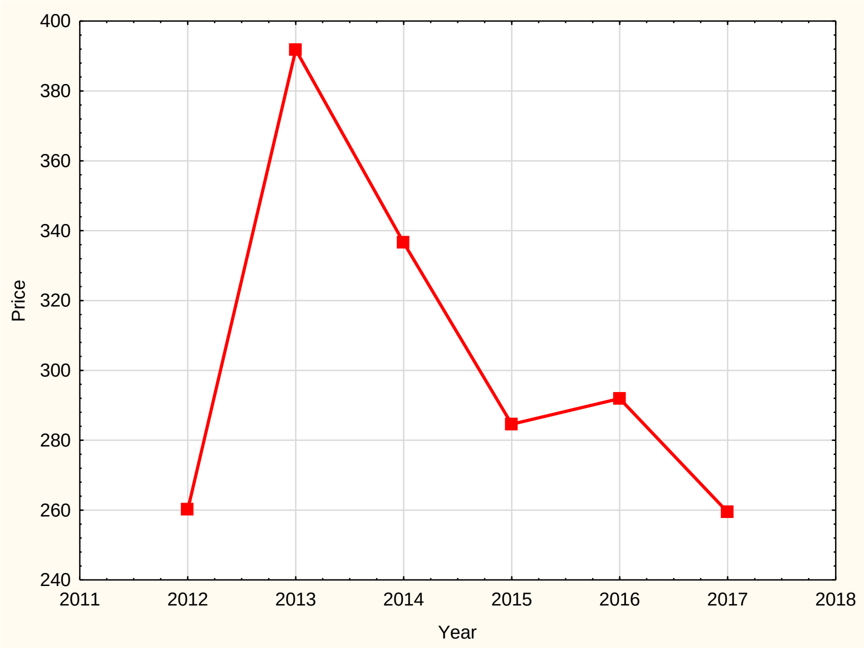

Figure 2: Average annual prices of onion in the Republic of Northern Macedonia for the period 2012-17

The average annual sale price of onion in the Republic of Srpska in the analyzed period was 276 euro/t. The lowest values of the price were recorded in 2016, when it amounted to 159 euro/t, and the highest price of 364 euro/t was in 2013 (in the same year when the highest prices were recorded in the other two observed countries). The price was unstable, which is confirmed by a high coefficient of variation of 21%. The price of onion had a marked declining trend in the analyzed period, at an average annual rate of -3.55%.

Figure 3 shows the average annual prices of onion in the Republic of Srpska for the period 2012-17.

The comparative descriptive analysis of the onion prices has shown the following:

- The Republic of Serbia had the lowest average price of onion. The onion price in the Republic of Srpska was 51% higher than in Serbia, and 10% lower than in the Republic of Northern Macedonia. Northern Macedonia had the highest price of onion, which was 67% higher than in the Republic of Serbia;
- The most stable price was in the Republic of Serbia (the lowest coefficient of variation), followed by the Republic of Northern Macedonia (62% higher than in Serbia), while the most variable price of onion was in the Republic of Srpska, where the coefficient of variation was 105% higher than in Serbia, and 27% higher than in Northern Macedonia.
- The trends of price decrease were recorded in all three observed countries (entities). The most pronounced decreasing trend was in the Republika Srpska. This trend was significantly weaker in the Republic of Serbia, while the price of onion in the Republic of Northern Macedonia practically stagnated.
- It is also important to note that the highest price was reached in the same year (2013) in all three countries.

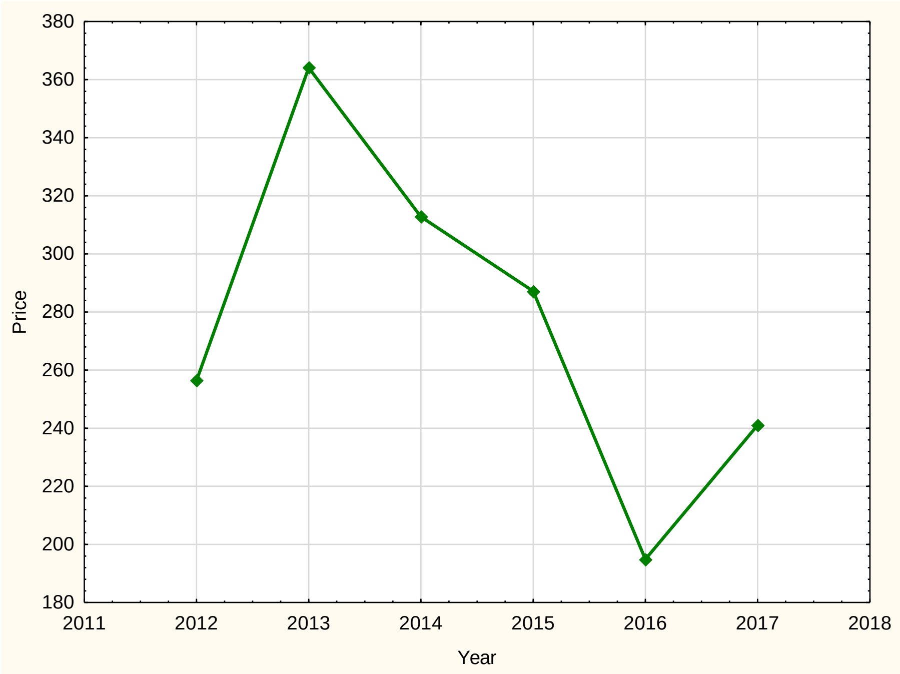

Figure 3: Average annual prices of onion in the Republic of Srpska (2012-17)

Table 1 presents the values of the descriptive statistics for all three analyzed republics. For the purpose of comparison.

Table 1: Descriptive statistics of onion prices in the period 2012-17

Territories	Average	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Lowest	Highest		
Serbia	182.40	159.14	208.06	10.42	-0.76
Northern Macedonia	304.07	259.43	391.81	16.90	-0.05
Republic of Srpska	276.07	194.87	364.10	21.39	-3.55

Conclusion

The results of the study showed that there are significant variations in the prices of onion in different parts of the Western Balkans despite similar natural conditions for production and similar standards of living. It is logical that the price of onion is the lowest in the Republic of Serbia, as it has the lowest standard of living and the lowest level of economic development. However, it is not logical that there are such big differences in prices, which are larger compared to the differences in the standards of living and the levels of economic development.

The Republic of Northern Macedonia had the highest onion prices, which can be accounted for by the existence of a favorable market for vegetables in Kosovo and Metohija. Namely, large quantities of vegetables are placed in the territory of Kosovo and Metohija, which has significant effects on the increase in vegetable prices in general.

The Republic of Srpska has the highest rate of decline in onion prices, so in the future it is expected that these prices will be competitive with the prices in the Republic of Serbia.

Also, it is evident that the mechanism of supply and demand works for the prices of onion. The highest prices were achieved in all three republics in the same year of 2013, which was a very favorable year for onion production.

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FARM PRODUCTION EFFICIENCY IN SERBIA

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Abstract

Process of farm structure changes in Serbia has sped up. Significant decrease of farm number followed by increase of average resources motivates farmers to re-examine their production types. Farm production efficiency becomes more important to understand how they adjust production strategies. The main goal of this paper is to investigate the technical efficiency farms in the regions of Serbia during 2017. All data for 1420 farms used in research originates from Farm Account Data Network (FADN) database. The efficiency analysis is performed on the basis of two criteria: region and production type. In order to determine technical efficiency, input-oriented Data Envelopment Analysis (DEA) with variable return to scale (VRS) was used. The results reveal that farms in region Serbia North are in average more efficient than farms in Serbia South. Also, among production types in plant production higher average efficiency scored farms oriented on horticulture indoor and outdoor, as well as fruit producers. In livestock production types higher average technical efficiency is reached by farms focused on poultry and pig production

Keywords: *Farm, Efficiency, DEA, FADN, Serbia.*

Introduction

Agriculture stands for an important part of the Serbian economy. It create 16% of working places and 6% of GDP (Statistic Office, 2019). Structural changes in agriculture speed up recently. Number of farms decreased for 10.6% in period 2012-2018, and average size of farm, measured in hectares of utilized agricultural land increased to 6.1 hectares, up to 12% (Census of agriculture 2012, Farm structure survey 2018). Family farms dominates in farm structure by 99,7%. Farm structure changes attract attention for technical efficiency research by farm types and by regions.

Material and Methods

Material basis for the paper is Serbian FADN database for 2017. It includes data for 1,420 farms across Republic of Serbia, as representative sample of farm production. Farmers in Republic of Serbia don't have obligation and also practice to keep records of farm business. In past it complicates research of any economic aspects of farm business. Today, well established FADN database enables analysis of production and economic aspects of agriculture on farm level.

Farrell established the modern approach to measuring economic efficiency and its decomposition to technical and allocative efficiency in 1957. Two decades later on the basis of this approach, Charnes et al. (1978) built the first data envelopment analysis (DEA) for estimating the relative efficiency of individual organizations.

In order to assess the technical efficiency of farms, the input-oriented DEA method with variable return to scale (VRS) was used, developed by Banker et al. (1984). According to Coelli et al. (2005) the DEA method variable return to scale (VRS) has advantages over a model with a constant return to scale (CRS) in conditions where imperfect competition exists. This cause a situation in which companies do not operate at the optimum level (size). The advantage of the DEA method in relation to stochastic methods is that it enables the determination of the relative efficiency of each individual unit of observation (Decision Making Unit – DMU) in relation to all other. In this paper DEAP Version 2.1 was used (developed by Tim Coelli). This program is created to construct DEA frontiers for the calculation of technical and cost efficiencies and also for the calculation of Malmquist TFP Indices.

Results and Discussion

The Farrell's study (1957) divided the concept of economic efficiency at technical and allocative efficiency. The focus of this work is on technical efficiency of farms. The analysis of technical efficiency assumes that the evaluated units are doing things right, as determined by the relationship between the consumed inputs and the produces outputs (Kočišová, 2015). There are numerous methods used to calculate efficiency. One such model is nonparametric *Data Envelopment Analysis* (DEA). DEA is a mathematical method based on a linear programming, which is the most often applied to data on a sample of firms (at one point in time) and provide measures of relative efficiency among those firms (Coelli et al., 2005). In relation to the parametric approach for measuring the efficiency (the most commonly used methods in the literature is *the Stochastic Frontier Analysis*-SFA), DEA method does not require a priori specific function of production which is one of the advantages of this model (Madau, 2015; Coelli et al., 2005). Also, the ability to use multiple inputs and multiple outputs in the analysis allows defining best practices for each unit of observation (Mussa et al., 2012). DEA method has been used in many studies and applied in many areas, including in agriculture.

Because this study used FADN databases, experience of researchers from various countries of the European Union, who applied this method to the FADN, are extremely important. It is notable that, depending on the aim of research, this method can be applied to different levels of research. Thus Tóth et al. (2015), Baležentis et al. (2013), Bojnec et al. (2008) have studied the efficiency of farms in Hungary, Lithuania and Slovenia, respectively, in order to define the difference between the individual and corporate farms, differences farming types or production branches. Also, DEA can be applied for comparison between the regions or countries (Table 1).

Kočišová K. (2015) investigated and compared the relative technical efficiency of the agricultural production in the European countries at the national level. The efficiency of the EU agricultural sector has changed over the past few years, and in both models it could be seen that the efficiency has generally decreased over time. Błażejczyk-Majka & Kala (2015) had their own research on the level of the region in the EU, investigating technical efficiency concerning the agricultural production in the USA and the selected regions of the European Union. Fogarasi & Latruffe (2009) investigated the difference in technical efficiency and potential technology gap between French and Hungarian dairy farms during 2001-2006. Results indicate that French farms have a more optimal scale of production than Hungarian farms, but Hungarian farms make better use of the technology.

The use of the FADN database to research the efficiency of different production types is widely accepted in the literature. Tóth & Takács (2015) used FADN data based on legal forms, farm size and type of farming in Hungary. The results have shown that the technical efficiency was the highest in the biggest farms (11–14 SO), in the pig breeding and in the dairy farms. Also, correct the technical efficiency was the highest by the corporate farms and by those farms who deal with animal husbandry. The technical efficiency was very low by arable crops production and by the individual farms. This research points to the importance of small family farms and their role in employment in the agricultural sector in Hungary especially in rural areas. Small family farms usually cultivate only few hectares but their importance in supplementing the family incomes or generating modest surplus is unquestionable**.** Baležentis & Kriščiukaitienė (2013) investigated efficiency across different farming types in Lithuania. The results had shown that mixed crop and mixed livestock (mainly grazing) farming was peculiar with the highest technical efficiency. Bojnec & Latruffe (2008) also used FADN sample for 13 farm business branches in Slovenia in the period 1994-2003. Their results had shown that five farm branches (crop, dairy, livestock using own feed, fruit, and forestry) are fully efficient with respect to all four analyzed efficiency measures (technical, scale, allocative and economic efficiencies), suggesting that these specializations have the best chance to compete on the European and world markets.

Table 1 An overview of studies that use DEA method on FADN database

Authors	*Countries included in the study*	*Level and subject of research*	*Inputs*	*Outputs*
Kočišová K. (2015)	EU-27	*national level:* comparison of EU countries	Total labour input (AWU), total utilized agricultural area (ha), total assets (EURO)	Total output crops and crop production (EURO), total output livestock and livestock products (EURO)
Tóth O., & Takács I. (2015)	Hungary	*farm level:* comparison of individual and corporate farms in the sample from 1,850 farm	Total utilized agricultural area (ha), agricultural employment (AWU), material costs (HUF), depreciation of fixed assets (HUF), livestock (LU)	Gross value of production minus the value of subsidies (HUF)
Fogarasi J., & Latruffe, L. (2009)	France and Hungary	*national level:* comparison of France (6,549 farms) and Hungary (574 farms)	Total utilized agricultural area (ha), total labour input (AWU), capital (EURO), intermediate consumption (EURO), livestock units, milk output per livestock unit, livestock units per agricultural utilized land	Milk output (liters), other outputs (EURO)
Baležentis, T., & Kriščiukaitienė, I. (2013)	Lithuania	*farm level:* comparisons of different farming types	Total labour input (AWU), total utilized agricultural area (ha), total assets (Lt), intermediate consumption (Lt)	Total output (Lt)
Bojnec, Š., & Latruffe, L. (2008)	Slovenia	*farm level:* 130 observations in 13 farm productions branches	Total labour input (AWU), total utilized agricultural area (ha), total assets (SIT), total intermediate consumption (SIT)	Total output crops and crop production, total output livestock and livestock products and other output (SIT)
Błażejczyk-Majka, L., & Kala, R. (2015)	34 regions of the EU in 5 countries	*regional level:* estimates of technical efficiency in regions of EU and USA	Total labour input (AWU), total utilized agricultural area (ha), material costs (EURO)	Total output (EURO)

Farms production in Serbia is very diversified. Only farms with pig and poultry production increased specialisation to some degree, where in several cases farms do not produce feed, using feed industry as only source. In situation where most of farms have multi products it is not easy to find single output. Usually, in such situation money value of all farm products serve as measure of unique output (Table 1). Additionally, in this research value of production is corrected for subsidies and taxes. Main reason for that step is negative balance between subsidies and tax, that occurred in some cases. Value of farm production corrected for subsidies and taxes represent unique result of farm activities in one year, and serve as source for covering all incurred costs of inputs.

Table 1. Descriptive statistics for variables of 1420 DMU, used in DEA method.

Variable	**Unit**	**Average**	**Standard deviation**	**Minimum**	**Maximum**
Value of production + subsidies -taxes	RSD	6.376.101	10.405.680	222.965	152.229.280
Total intermediate consumption	RSD	2.941.988	5.326.118	50.000	115.924.000
Labour input	hour /year	3.896	3.381	170	64.800
Total fixed assets	RSD	19.383.424	46.348.068	647.250	1.508.909.000
Total utilised agricultural area	ha	29	46	0	549

Source: FADN database 2017.

On input side variables for chosen DEA model includes: total intermediate consumption, labour input, total fixed assets and total utilised area. Total intermediate consumption covers total specific crop and livestock costs and overheads arising from production in the accounting year. Labour input is embraced in total number of working hours on farm per year. Total fixed assets includes value of very diversified quantity and quality of fixed resources on farms. Last input variable is total utilised agricultural area, including owned and rented land expressed in hectares.

Table 2. Correlation analysis of input and output variables for 1420 DMU.

	Value of production + subsidies - taxes	Total intermediate consumption	Labour input	Total fixed assets	Total utilised agricultural area
Value of production + subsidies - taxes	1				
Total intermediate consumption	0,817	1			
Labour input	0,410	0,338	1		
Total fixed assets	0,323	0,250	0,124	1	
Total utilised agricultural area	0,651	0,545	0,185	0,351	1

Source: Estimate based on variables from FADN database

Table 2. provide correlation analysis of input and output variables. The strongest positive correlation exist in relation of output variable and total intermediate consumption. This is expectable since first prerequisite for higher farm output is increase of intermediate consumption. Significant correlation is estimated in relation total utilised agricultural area and output variable and between total utilised agricultural area end total intermediate consumption. Labour input and total fixed assets are not correlated with output and other two input variables.

Table 3. Technical efficiency scores by DEA method of farms in North and South Serbia regions for 2017.

	Serbia North				Serbia South			
	DMU	**CRS**	**VRS**	**Scale effic.**	**DMU**	**CRS**	**VRS**	**Scale effic.**
Field crops	297	0,227	0,374	0,617	143	0,172	0,310	0,570
Fruit production	30	0,327	0,480	0,664	66	0,258	0,408	0,629
Horticulture outdoor	9	0,372	0,717	0,566	22	0,311	0,390	0,750
Horticulture indoor	6	0,448	0,778	0,578	9	0,327	0,479	0,758
Vineyards	3	0,138	0,421	0,347	9	0,235	0,305	0,762
Mixed crops and livestock	78	0,235	0,337	0,699	224	0,174	0,333	0,543
Dairying	53	0,249	0,342	0,743	226	0,162	0,278	0,605
Livestock production - grazing livestock	24	0,203	0,325	0,652	151	0,148	0,294	0,538
Poultry	12	0,715	0,900	0,778	26	0,519	0,721	0,742
Pigs production	8	0,280	0,497	0,594	24	0,401	0,532	0,735
Total	520	0,252	0,394	0,647	900	0,194	0,333	0,589

Source: Estimate based on DEAP software and FADN database

Estimated technical efficiency for 10 farm types in two Serbian regions, during 2017 showed very diversified results. All farm types in North region, except those specialised in pig production, are more technically efficient than farms in South. The most efficient type of farms in both regions is poultry. In other words, for production one unit of output those farms use the smallest amount of inputs. Among farm types focused on plant production the most technically efficient are those in horticulture business, followed by fruit producing farms. In the group of livestock types of farms the best results showed poultry and pig producing farms. On opposite side, the lowest technical efficiency is scored by dairy farms in South region.

In region Serbia North the most presented type is field crops farms. Farmers in region Serbia South are more oriented on livestock types of farming: mixed crop and livestock, dairying, and livestock production - grazing livestock. In both regions most numerous types of farming scored technical efficiency below level of average efficiency for all farms.

Scale efficiency in all farm types is lower than 1, indicating another way to increase efficiency trough adjusting size of business. In case of 96.9% farms it means increase of resources, while only 1.7% have to decrease its resources on more optimal level. Only 1.4% farms operate on optimum level of resources.

Conclusions

This paper examined technical efficiency of 10 farm types in two regions of Serbia during 2017 accounting year. The results indicated very diversified levels of farm efficiency. It varieties not only among farm types, but through regions. Farms in region Serbia North expectably encountered higher level of efficiency, mainly because better resource structure. The most efficient type of farming were poultry farms, as most specialised one. Extremely dry production season in second half of 2017 significantly lowered results in corn, and soybean production, that complicate production on livestock farms with own production of feed. That can be main source of low technical efficiency of diversified livestock farm types in bouth regions of Serbia.

As improvement, for future research analysis period could be expanding on several years. With inclusion farm data from more years research result can overcome problem with harsh production condition in some years. It is important, since dry or too wet conditions don't decrease output on all farm types in same manner.

Acknowledgments

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THE OPERATIVE PROFIT MARGIN IN RETAIL FOOD

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Abstract
Both theoretical and practical importance have recently been attached to an analysis of the operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of the long-term performance of companies. In the integrated financial reporting it is presented through various indicators based on it. In view of this, we have made a comparative analysis of the operating profit margin and its impact on the performance of food trade companies in Serbia and comparable countries. Under the influence of different factors, the dynamics of the size of the operating profit margin of food trading companies in Serbia varies from comparable global food retailers in various countries. The EBITDA margin of the leading food trading companies in Serbia is lower than the in analyzed comparable food retail trade companies. It points to the conclusion that it is necessary to efficiently manage revenues, costs, profit, assets, and financial structure in order to improve the performance of food trading companies in Serbia in the future**.** The general conclusion is that it shows a growth tendency and is, nevertheless, lower in comparison to food trading companies from countries of a developed market economy. In order to increase the operating profit margin, as a measure of long-term performance, it is necessary to manage the financial structure of the food trading companies in Serbia as effectively as possible. (JEL codes: L81, M31, M41, O32)

Keywords: *net profit, interest, tax, depreciation, amortization.*

Introduction
Considerable theoretical and practical attention has been paid in recent years to analyzing the operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of the long-term performance of companies. On the basis of it, special indicators of long-term performance of companies have been developed. They are comparatively analyzed by individual companies (from the same and different sectors) and based on this –their long-term business success is recognized. Bearing this in mind, the subject of research in this paper is a comparative analysis of the operational profit margin of food retail enterprises in Serbia and comparable foreign retailers. The aim of this research is to thoroughly investigate the problems of the operating profit margin as one of the determinants of the long-term performance of food trading companies in Serbia and, on that basis, to propose the measures for its improvement in the future. This gap is to a certain extent filled with this paper, in what we find its scientific and professional contribution. The basic hypothesis of research in this paper is that the operating profit margin is a significant measure and determinant of the long-term performance of food trading companies. For these reasons it is necessary to investigate it more extensively on the example of food trade companies in Serbia, particularly the dynamics and factors of its size. In this paper, we will explore the dynamics of the size of EBITDA margin of well-known global food retailers, such as Wal-Mart, Tesco and Ahold Delhaize, in order to make comparisons of the EBITDA margin with Serbian trading companies. This provides the basis for proposing adequate measures to increase the size of the EBITDA margin, as a measure of long-term performance of food trading companies in Serbia. The EBITDA margin of the analyzed leading food trading

companies in Serbia (Ahold Delhaize Serbia, Mercator-S and IDEA) is lower than that of analyzed comparable food retail (primarily food) trade companies from the developed market economies. Overall, more efficient management of the financial structure of capital, sales revenues, costs of goods sold (including operating costs, interest) and profit can significantly influence the increase in the EBITDA margin as a measure of the long-term performance of food trading companies in Serbia. This will definitely have a positive impact on the dynamics of the size and efficiency of investments, as a key factor in the performance of food trade companies in Serbia.

Material and methods

For the needs of the research of the treated problems in this paper, empirical data from the Agency for Business Registers of the Republic of Serbia were used. They are completely comparable to the same type of other global food retailers 'data and, in this sense, there are almost no restrictions on the obtained research results in this paper due to the fact that we used empirical data from their publicly disclosed financial statements in this study. With the defined aim and research hypothesis, the basic methodology in this paper is the comparative analysis and application of the relevant statistical analysis. Also, to a certain extent, the historical and normative methodology was applied in researching the treated problem in this paper. The operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of performance has been used since the mid-1980s, especially since the 1990s in all companies, including wholesale and retail (Levy, 2014). There is extensive literature written on the subject of general problem of measuring the significance of gross operating margin in financial reporting for the needs of more efficient company management (Sui, 2017). However, a number of published papers dedicated to the specificities of gross operating margin analysis in commercial enterprises (food retailers) is significantly lower (Berman, 2013; Corona, 2014; Špička, 2016; Tan, 2016; Calva, 2017; Carstea et al, 2017; Ko et al., 2017; Hoe, 2017; Manini, 2017). This particularly applies to literature in Serbia (Lukic, 2017a, b; Lukic, 2018) – as far as we know, there is no complete work that has been published so far on the issue of the importance of measuring and analyzing gross operational margin in Serbia's trade companies (food retailers). As a measure of long-term performance of (food) retailers, the operating profit margin has its advantages and disadvantages. It is considered that during the usage of this criterion retailers are focused on the performance of fundamental business rather than on financial decision-making related to depreciation of fixed assets, interest and financial structure (lending instead of increasing equity by selling shares) (Levy, 2014). In view of this, it provides bankers, investors, creditors, fiscal authorities and others an insight into the long-term potential options for collecting their retailers' claims. More and more financial analysts are aware of certain problems of interpreting the EBITDA margin, and in order to overcome them, the model of economic additional value (the so-called EVA model) is recommended. Nevertheless, it should also be noted that the very model of economic value addition has its own weaknesses, which primarily relate to subjective assumptions regarding the calculation of capital costs. In conclusion, it is necessary to use both models (EBITDA margin, EVA model) concurrently when assessing the long-term performance of retailers. We are well aware of the fact that in recent years many global retailers, and what we consider quite right, also regularly report on the economic value added (for example, METRO group and others) in the context of integrated financial reporting. In this way, the problem of interpreting the EBITDA margin is partially mitigated. Due to the specifics of the nature itself, way of doing business and the applied financial management strategy, the dynamics of the size of the operating profit margin varies by individual types of trade (wholesale and retail), retail companies and countries in which they operate, retail chains (types of shops) and product categories. This is scientifically proven by the empirical analysis of EBITDA-size dynamics of retailers 'margin which has been carried out from different perspectives. Model of calculating the EBITDA margin, it is determined in the following way: EBITDA = Revenue - Expenses (excluding interest, taxes, depreciation and amortization), i.e. EBITDA = Net profit + Interest + Tax + Depreciation + Amortization. From this last formula it follows that: Net profit = EBITDA - (Interest + Tax + Depreciation + Amortization). For illustration purposes Table 1 shows the model for calculating the EBITDA margin in the global retailer Wal-Mart. Therefore, it is consistent with the model shown above.

Table 1. Model of calculating the EBITDA margin at Wal-Mart (USD $ million)

	January 31, 2018	January 31, 2017
Net income	9,862	13,643
Add: Net income attributable to non-controlling interest Less: Income from discontinued operations, net of income tax Add: Income tax expense	661 - 4,600	650 - 6,204
Earnings before tax (EBT)	15,123	20,497
Add: Interest expense, debt, capital lease and financing obligations	2,330	2,367
Earnings before interest and tax (EBIT)	17,453	22,864
Add: Depreciation and amortization	10,529	10,080
Earnings before interest, tax, depreciation and amortization(EBITDA)	27,982	32,944

Source: https: // www.stock-analysis-on.net, (May 10, 2018)

Results and discussion

The value multiplier is determined as follows:
Enterprise Value / EBITDA = (Market Value of Equity + Value of Debt-Cash) / EBITDA.
It shows how the market values the (retail) firm in accordance with the ability to generate operational profits (Enterprise value/EBITDA (EV/EBITDA). As an illustration, Table 2 shows a value multiplier on the example of a food value chain in the US.

Table 2. Food value chain value multiplier in US, January 5, 2018

	EV/EBITDA
Farm/Agriculture	13.07
Food processing	13.01
Food wholesale	10.43
Retail (grocery and food)	8.40
Restaurant/Dining	12.69

Note: Enterprise Value / EBITDA = (Market Value of Equity + Value of Debt-Cash) / EBITDA.
Source: Enterprise Value Multiples by Sector (US), January 5, 2018, http://pages.stern.nyu.edu/~adamodar/New_Home_Page/datafile/vebitda.html, (May 10, 2018)

The data in a given table show that the value multiplier is different for some members of the food value chain in the US. Thus, for example, it is significantly higher for farming/agriculture (13.07) than for retail (8.40). This is partly due to differences in the very nature of their business. In order to make in-depth analysis of the EBITDA margins in the food retail sector, Table 3 shows a value multiplier of the Wal-Mart retailer and its competitors for 2017 and 2018.

Table 3. Value multiplier,Wal-Mart (January 31, 2017 and 2018)

Wal-Mart Inc., EV / EBITDA calculation	January 31, 2018	January 31, 2017	January 31, 2016	January 31, 2015	January 31, 2014	January 31, 2013
Enterprise value (EV), (USD $ million)	305,207	260,427	260,724	306,165	300,184	297,926
Earnings before interest, tax, depreciation and amortization (EBITDA), (USD $ million)	27,982	32,944	33,640	36,433	35,861	36,489
Ratio EV / EBITDA	10.91	7.91	7.75	8.40	8.37	8.16
Benchmarking EV / EBITDA competition						
Amazon.com Inc.	-	43.74	31.40	33.08	33.45	41.58
Costco Wholesale Corp.	-	12.63	13.27	13.49	12.08	12.30
eBay Inc.	-	14.50	8.04	8.92	12.34	12.88
Home Depot Inc.	13.46	12.90	13.23	12.90	11.11	11.74
Lowe's Cos.Inc.	11.24	11.43	12.18	12.79	10.47	9.60
Netflix Inc.	-	18.03	11.80	10.59	8.22	9.62
Target Corp.	6.96	5.59	7.47	9.21	7.85	8.09
TJX Cos.Inc.	-	11.16	11.83	11.14	10.70	9.10
EV / EBITDA, Sector						
General retailers	-	15.92	12.72	12.34	10.93	10.95
EV / EBITDA, Industry						
Customer service	-	12.26	11.49	10.91	10.77	10.30

Source: https: // www.stock-analysis-on.net, (May 28, 2018)

The data in the given table show that the value multiplier differs between some food retailers. Thus, for example, on January 31, 2018, in Target Corp. it was 6.96 and in Wal-Mart 10.91, respectively. The Wal-Mart value multiplier is lower than the average of the sector and industry. These differences are certainly the result of the implementation of different financial management strategies (lending versus the increase in equity by selling shares). Earnings before interest, taxes, depreciation and amortization differ among individual food retail companies. Table 4 illustrates the dynamics of the EBITDA margin of the global retailer Wal-Mart for the period 2008 - 2017.

Table 4. Dynamics of EBITDA margin of Wal-Mart, 2008 – 2017

End of period	WMT
January 2008	NA
January 2009	7.3%
January 2010	7.6%
January 2011	7.9%
January 2012	7.7%
January 2013	7.7%
January 2014	7.5%
January 2015	7.5%
January 2016	7.0%
October 2016	6.8%
January 2017	6.8%
October 2017	6.6%

Source: https://finbox.io/WMT/explorer/ebitda_margin, (May 10, 2018)

Recently, the EBITDA margin has decreased in Wal-Mart compared to the previous period. Compared to some competitors it is larger and compared to others – smaller (for example, Target Corporation 9.9%) (Table 5). This is, partly, the result of the very nature of the industry operations of its own, sector, company size and business operations model (i.e. the applied financial strategy of the business).

Table 5. EBITDA margin of Wal-Mart and its competitors, 2017

Company	EBITDA margin
Spartan Nash Company (SPTN)	-0.3%
Smart & Final Stores, Inc. (SFS)	3.1%
Kroger Company (The) (KR)	4.5%
CompanhiaBrasileira de Distribuicao (CBD)	6.1%
Casey's General Stores, Inc. (CASY)	6.2%
Best Buy Co., Inc. (BBY)	6.2%
CVS Health Corporation (CVS)	6.6%
Wal-Mart Stores, Inc. (WMT)	6.6%
Target Corporation (TGT)	9.9%
Consumer Staples (SECTOR:STPL)	12.5%
Procter & Gamble Company (The) (PG)	25.6%
#ERROR! (CNCO)	N

Source: https://finbox.io/WMT/explorer/ebitda_margin, (May 11, 2018)

Table 6 shows the dynamics of EBITDA margin of Tesco for the period 2014 - 2018.

Table 6. EBITDA margin of Tesco, 2014 – 2018

Fiscal year March-February. All values are expressed in millions of pounds (GBP)	2018	2017	2016	2015	2014
Sales/Revenue	57,491	55,917	53,933	56,925	63,557
EBITDA	2,957	2,581	2,202	(1,733)	4,757
EBITDA growth	14.57%	17.21%	227.06%	-136.43%	-
EBITDA margin	5.14%	-	-	-	-
EBIT	1,663	1,284	-	-	3,225

Source: https://quotes.wsj.com/UK/XLON/TSCO/financials/annual/income-statement, (May 11, 2018)

The data in the given table show that the share of EBITDA margin in revenues is lower in Tesco (5.14%) than in Wal-Mart (6.6%). This is partly a consequence of a different model of doing financial operations. Table 7 shows the EBITDA margin of Ahold Delhaize, which operates in Serbia as Delhaize Serbia.

Table 7. EBITDA margin of Ahold Delhaize

	12/16A	12/17E	12/18E	12/19E
Revenue (€ million)	63,093	63,943	65,348	66,920
EBITDA (€ million)	4,142	4,267	4,507	4,836
EBIT (€ million)	2,420	2,386	2,638	2,923
EBIT growth (%)	7.9	(1.4)	10.6	10.8
EBITDA margin (%)	6.6	6.7	6.9	7.2
EBIT margin (%)	3.8	3.7	4.0	4.4
EV/EBITDA (x)	6.7	6.4	6.0	5.5
EV/EBIT (x)	11.4	11.4	10.3	9.2

Source:https://research-doc.credit-suisse.com/docView?language=ENG&format=PDF&sourceid=emgpm&document_id=1077229781&serialid=7%2F%2FS9ldDW4ewIdMX6A26zlMtYs6VxLxiTmpgD2zQdGM%3D, (May 22, 2018)

In Ahold Delhaize, the EBITDA margin is higher than at Tesco (5.14%) and is approximately the same as with Wal-Mart (6.6%). In the future, there is an estimated growing trend. The EBITDA margin is certainly different among observed countries in which Ahold Delhaize operates. Ahold Delhaize's operating profit margin, observed by individual countries in which it operates, is significantly higher in the US and the Netherlands than in Belgium and Central and Southeastern Europe (to which the Delhaize Serbia belongs). These differences are the result of different general business conditions and applied (financial) business strategies.

Table 8 shows the EBITDA margin of the Russian company X5 Retail Group for the period 2012-2016.

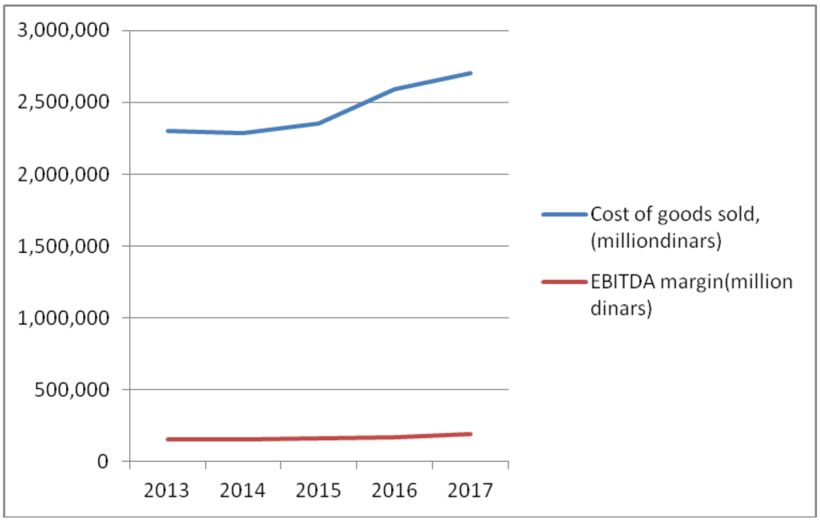
Table 8. Dynamics of EBITDA margin of the company X5 Retail Group, 2012-2016

	EBITDA Margin (Rub bn)	EBITDA margin, %
2012	35,1	7.1%
2013	38,4	7.2%
2014	46,4	7.3%
2015	59,4	7.3%
2016	79,5	7.7%
2016/2015	33.8%	

Source: Q1 2017 Financial Results, X5 Retail Group, Moscow, Russian Federation 27 March 2017, https://www.x5.ru/en/Documents/X5-Q1-2017-Financial-results.pdf, (May 12, 2018

The data in the given table clearly show that the EBITDA margin of the company X5 Retail Group is higher than in Wal-Mart, Tesco and Ahold Delhaize. In other words, its profitability measured by cash flows from operations (using EBITDA margin) is slightly better than the observed retail companies.

In the analyzed period, the costs of goods sold by trade companies in Serbia have slightly increased dynamically until 2015 and from that year up to 2017 much faster, as can be seen from Figure 1. The annual growth rate of the costs of goods sold (3.3%) is thus lower than the annual growth rate of the EBITDA margin (4.99%).To sum up, the return on investments in Serbia's trade companies increased to some extent. In order to make anin-depth analysis of long-term trade performance in Serbia measured by the EBITDA margin, we will show the respective margin for three significant trade companies in Serbia for 2016 (Table 9). Based on the data presented in the given table, we can also conclude that the EBITDA margin of the leading (food) trading companies in Serbia is lower than the analyzed comparable food retail trade companies from the developed market economies.



Source: Business Registers Agency.

Figure 1. Cost dynamics of goods sold and EBITDA margin of trade companies in Serbia

Note: Figure illustrated by the author.

The regression analysis show that the costs of goods sold, as a specific expression of investment, significantly affect the EBITDA margin (Pearson Correlation, 927, Sig. (1-tailed), 012 p <0.05). (The regression equation is: Y = -46285.142 + 0.087 X, where: Y = EBITDA margin, and X = costs of goods sold.) The method of financing working capital (especially inventories) is a significant determinant of the EBITDA margin of trading companies. In this context, we will examine the impact of interest costs on the costs of goods sold of the commercial enterprises in Serbia. The results of the correlation analysis show that there is a negative (statistically significant) strong correlation between the costs of sold goods and interest as a component of the EBITDA margin of trading enterprises in Serbia – this is quite logical – given their character.

Table 9. EBITDA margin of significant trade companies in Serbia, 2016

	EBITDA margin, (million dinars)	EBITDA margin, (% from sales)
Ahold Delhaize Serbia	3,719	4.3%
Mercator-S	3,081	2.9%
IDEA	117	3.99%

Note: Author's calculation.
Source: Business Registers Agency, Belgrade.

Conclusion

Due to the growing EBITDA margin trend and based on the analysis conducted in this paper, we can conclude that the long-term performance of trade companies in Serbia has recently improved. The average operating profit margin (EBITDA) of trading companies in Serbia expressed as percentage of revenue is slightly higher than 5% (Mean 5.1840). However, it is lower compared to the US, Canada, Europe, the Netherlands, Belgium, Central and Southeast Europe, Germany and Russia. More efficient management of the financial structure of capital (financial leverage = assets / capital) can influence the improvement of the return on sales measured by the relationship between the EBITDA margin and total revenues. Costs of goods sold, as a specific expression of the size of investments, significantly affect the EBITDA margin (Pearson Correlation, 927, Sig. (1-tailed), 012 p <0.05). There is negative (but statistically significant) strong correlation between the costs of goods sold and interest as a component of the EBITDA margin, which is quite logical given their character. The EBITDA margin of the analyzed leading trading companies in Serbia (Ahold Delhaize Serbia, Mercator-S and IDEA) is lower than that of analyzed comparable retail (primarily food) trade companies from the developed market economies. Overall, more efficient management of the financial structure of capital, sales revenues, costs of goods sold (including operating costs, interest) and profit can significantly influence the increase in the EBITDA margin as a measure of the long-term performance of trading companies in Serbia. This will definitely have a positive impact on the dynamics of the size and efficiency of investments, as a key factor in the performance of trade companies in Serbia.

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CROP PRODUCTION IN THE COUNTRIES OF THE WESTERN BALKANS

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Abstract

Crop production is one of the most important branches of agriculture and represents the raw material base for other agricultural production. Areas under basic crops have a dominant share in the structure of sown areas, and the value of realized yields significantly participatesin the GDP of the observed countries. The agrarian budget is an integral part of the total budget of each state and its size speaks about the importance of agriculture in the observed country. The authors analyzed achieved yields for the countries of the Western Balkans, candidates for EU members in all phases. Data for the Republic of Serbia, Bosnia and Herzegovina, North Macedonia and Albania are compared for the production of wheat, maize, rye, barley and sunflower. On the basis of the obtained results, production can be estimated in the next few years for each country in the region. Although, countries with different sizes and different areas for crop production are suitable for comparison of areas and yields over a longer period of time and allows noticeable changes in the structure of production, progress in the yields of individual production, etc. The obtained results enable the assessment of the market supply and self-sufficiency of each country in the near future as well as the potential for export. Adjustment of agrarian policy through the introduction of subsidies and reliefs, as well as the forcing of certain production, allows negative trends to be stopped and the dependence on imports decreases. This is especially important for strategic agricultural products.

Keywords: *Wheat, Maize, Barley, Rye, Sunflower, Western Balkan.*

Introduction

Agricultural land is a non-recoverable resource. In the world, especially in the Western Balkan region, there is a clear trend of decreasing agricultural land in favor of residential and industrial land. If it is kept in mind constantly expanding human populations, then the lack of agricultural area must compensate by higher yields per unit area. Total production must meet the needs of the population, at least. Four countries, which are in various stages of negotiations on EU membership, have been selected for the analysis of production. Production data for individual crop are the basis for negotiating about product quotas for each country and represent a part of the chapter on agriculture in the negotiations. Particularly, it is necessary that individual countries have to negotiate with the positions of net importers of some products because the existing production does not meet the needs of the domestic market.

Material and Methods

The conducted survey is based on the statistical data of each country's production compared to the data collected by different statistical organization such as Statistical bureau of each country, FAO, Eurostat, IndexMundi, Knoema, etc. Data for the Republic of Serbia, Bosnia and Herzegovina, North Macedonia and Albania are compared for the production of wheat, maize, rye, barley and sunflower for last decade, at least. Montenegro was omitted from the survey because the data collected were estimates and production was not significant.

Data are systematized for each country and production separately with the trend of production shown over the next two years.

The charts show data for all years for which data were collected, mainly the period 1990-2019.

Results and Discussion

Serbia, on approximately 500 thousand hectares produces annually around 2.3 million tons of wheat. Production ranged from 1.65 million ton (2010) to 3.4 million ton (2018). There is a noticeable upward trend in production and Serbia is a net exporter of wheat, with an average of over one million tons (Figure 1). Maize production has high oscillations because it is produced mainly under non-irrigation conditions, so in dry years the yield is significantly lower. The smallest production was 3.7 million tons (2012) and the largest 7.7 million tons (2014) with a slight upward trend (Figure 2). Rye production in Serbia has been declining since 2006 and has stabilized at around 10,000 tons on the last 7 years (Figure 3). Barley, with minor fluctuations in production, shows a steady increase with the highest production of 470,000t (2018) (Figure 4). Sunflower, as one of the most important oilseeds, had its smallest production in 2007 (486,000t) and the largest in 2018 (1.1million tons). There is a significant upward trend in production (Figure 5).

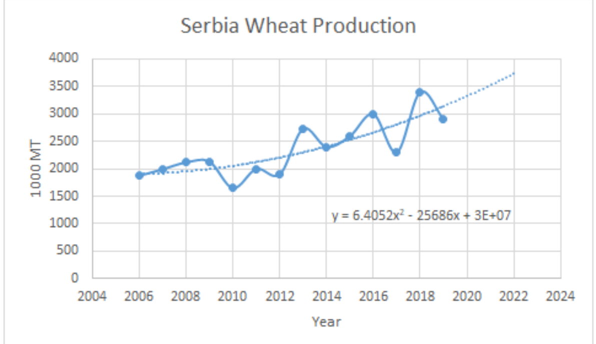

Figure 1: Production of Wheat in Serbia

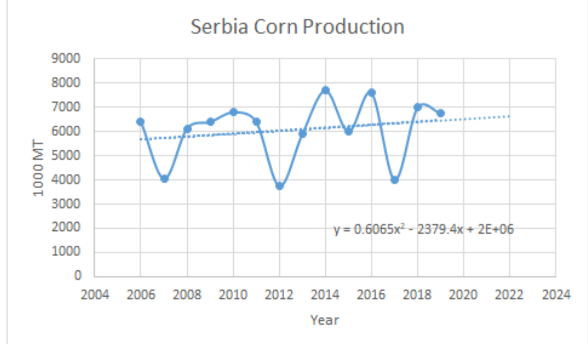

Figure 2: Production of Maize in Serbia

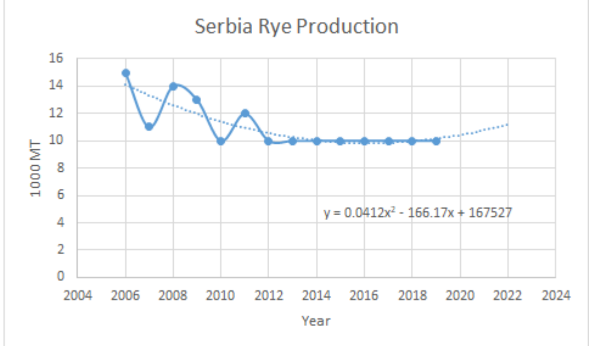

Figure 3: Production of Rye in Serbia

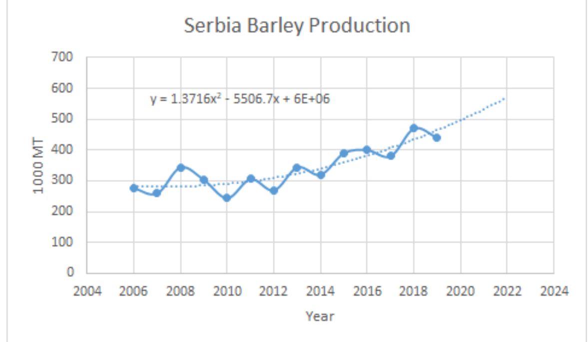

Figure 4: Production of Barley in Serbia

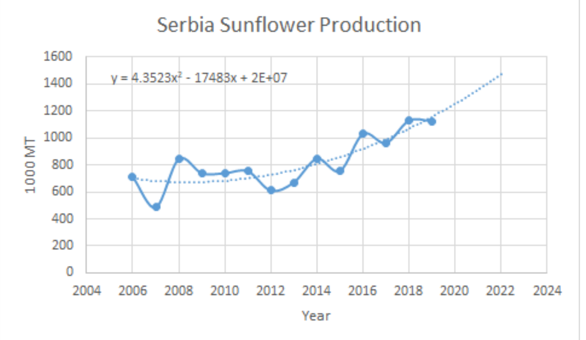

Figure 5: Production of Sunflower in Serbia

Source:
https://www.indexmundi.com/agriculture/?country=rs&commodity=wheat&graph=production
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https://www.indexmundi.com/agriculture/?country=rs&commodity=rye&graph=production
https://www.indexmundi.com/agriculture/?country=rs&commodity=barley&graph=production
https://www.indexmundi.com/agriculture/?country=rs&commodity=sunflowerseed-meal&graph=production
https://data.stat.gov.rs/Home/Result/130102?languageCode=sr-Cyrl

Bosnia and Herzegovina, comparing with Serbia, has significantly less arable land under the analyzed crops and it's a net importer of these crops. Nevertheless, it has significant production, on a global scale, of certain crops as rye, where it ranks 12th in the world. Wheat production ranged from 145,000t (2010) to 341,000t (1998). In the last 4 years, it has stabilized at around 300,000t (Figure 6). Maize production is characterized by higher oscillations, from 500,000t to almost 1.2 million tons (2016). For the last 7 years, the average production is just over 800,000t with pronounced annual fluctuations (Figure 7). Rye is symbolically represented in production from 7000t (2010) to 14000t (2019). There is a slight increase in growth but the produced amounts are symbolic (Figure 8). Barley production in the last 15 years has ranged from 48,000t (2014) to 79,000t (2018) with a trend of stagnation at around 65,000t (Figure 9).

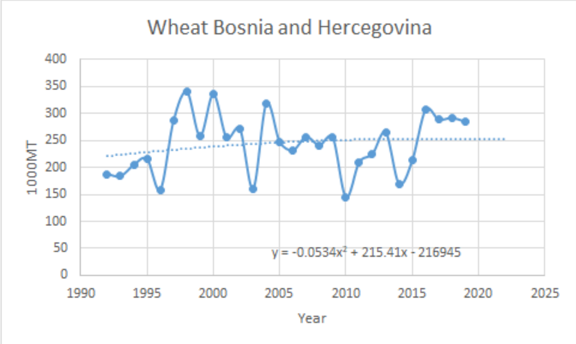

Figure 6: Production of Wheat in Bosnia and Herzegovina

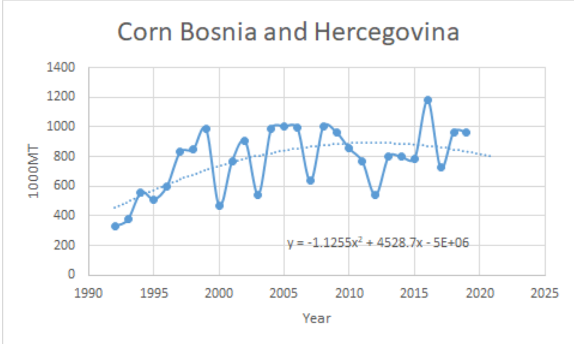

Figure 7: Production of Maize in Bosnia and Herzegovina

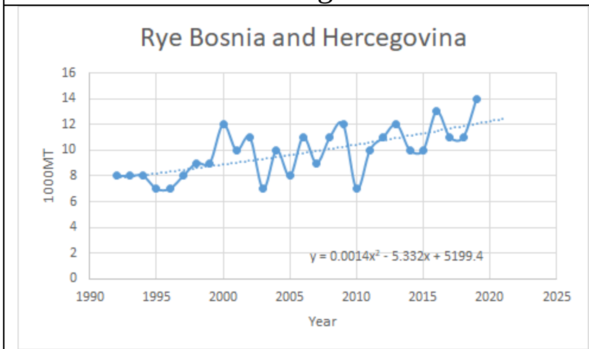

Figure 8: Production of Rye in Bosnia and Herzegovina

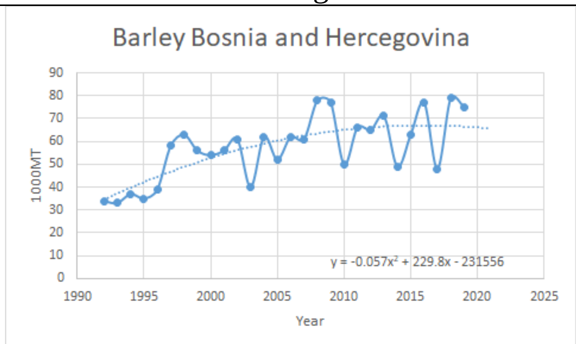

Figure 9: Production of Barley in Bosnia and Herzegovina

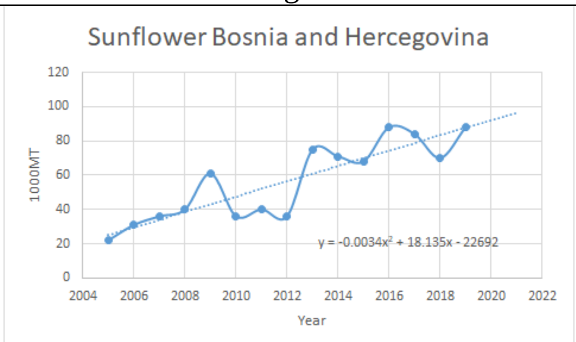

Figure 10: Production of Sunflower in Bosnia and Herzegovina

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https://www.indexmundi.com/agriculture/?country=ba&commodity=barley&graph=production
https://www.indexmundi.com/agriculture/?country=ba&commodity=sunflowerseed-meal&graph=production
http://fzs.ba/index.php/publikacije/godisnji-bilteni/poljoprivreda-i-ribarstvo/

North Macedonia has excellent conditions for producing the analyzed crops, but the areas are relatively small. Wheat production is represented in insufficient quantities for the needs of the population, so imports are necessary. Production ranged from 381,000t (1995) with a steady decline to 200,000t (2011). Although annual production fluctuations are around 100,000t, there is a noticeable trend of production decline (Figure 11). Maize production has seen a significant downward trend, especially in the last 7 years, when it dropped from 130,000t (average for 20 years, from 1992 to 2011) to 30000t (2012-2017). The only rise in production was in 2018 (Figure 12). Rye production has stabilized at an average of 9000t over the last 20 years and there is a noticeable trend of production stagnation (Figure 13). Barley production in the observed period was in line with annual fluctuations and ranged from 80,000t to 163,000t. A slight downward trend in production has been observed in the last 27 years (Figure 14). Sunflower quantities ranged from 74000t (1990) to 8000t (2007). If one considers only the last 20 years, a slight upward trend in production can be observed, but at a level below 20000t (Figure 15).

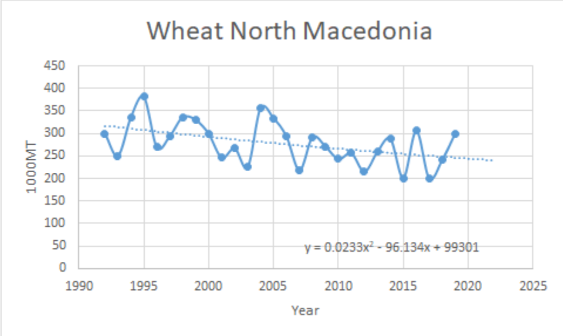

Figure 11: Production of Wheat in North Macedonia

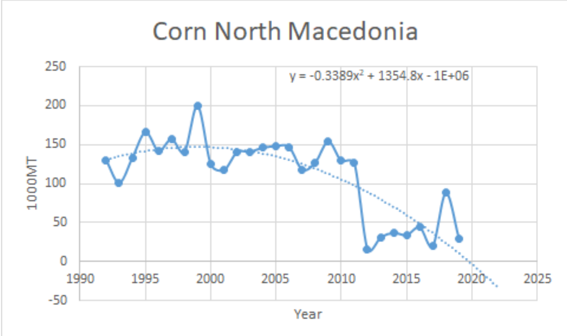

Figure 12: Production of Maize in North Macedonia

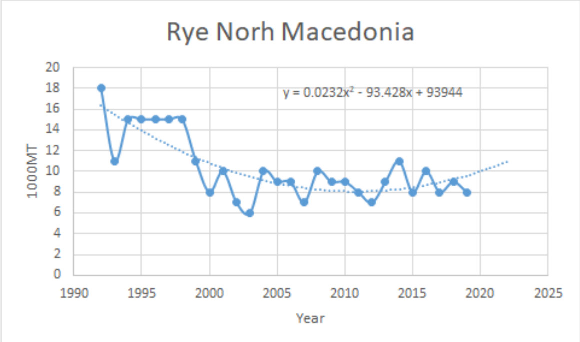

Figure 13: Production of Rye in North Macedonia

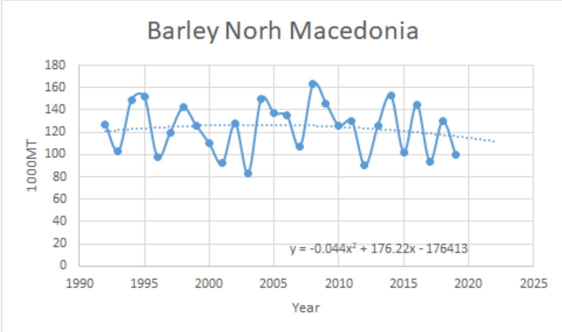

Figure 14: Production of Barley in North Macedonia

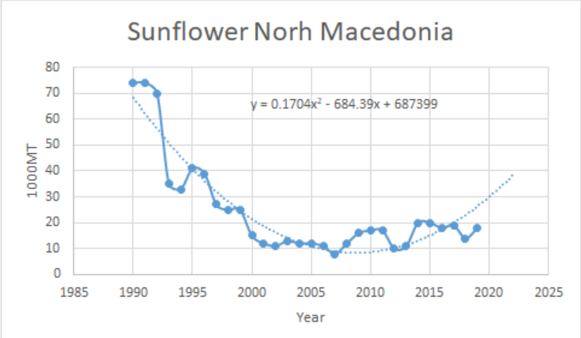

Figure 15: Production of Sunflower in North Macedonia

Source:
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https://www.indexmundi.com/agriculture/?country=mk&commodity=barley&graph=production
https://www.indexmundi.com/agriculture/?country=mk&commodity=sunflowerseed-meal&graph=production
http://www.stat.gov.mk/OblastOpsto.aspx?id=33
http://www.factfish.com/country/macedonia

Wheat production in Albania ranged from 615,000t (1992) to 231,000t (2006). In the last 20 years, production have been on an average of about 300,000t with a trend of stagnation

(Figure 16). Maize production ranged from 150,000t (1993) to 381,000t (2017) with a significant upward trend, especially in the last 10 years (Figure 17). Rye production is under-represented and since 2000 has settled at around 3000t (Figure 18). Barley was a significant product until 2000 (42,000 tons in 1990) but declined in 2000 (2000t). After 2000, a slight upward trend can be observed up to 9000t (2016 and 2017) (Figure 19). Sunflower production has been steadily declining, highest production was recorded in 2011 (3045t) and the smallest in 2018(846t) (Figure 20).

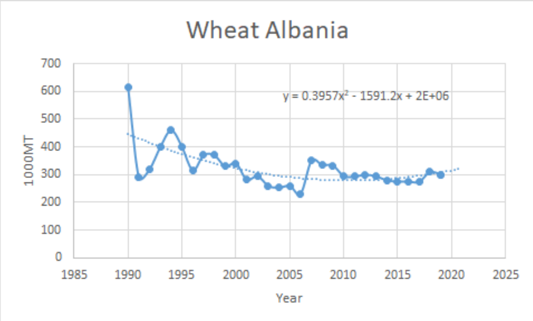

Figure 16: Production of Wheat in Albania

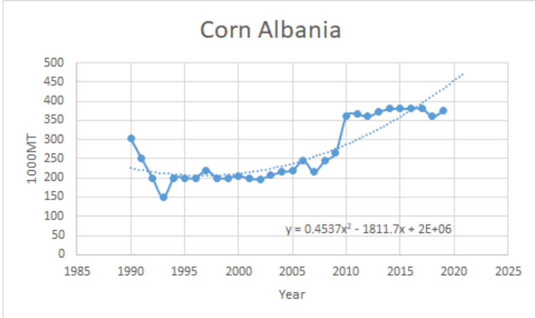

Figure 17: Production of Maize in Albania

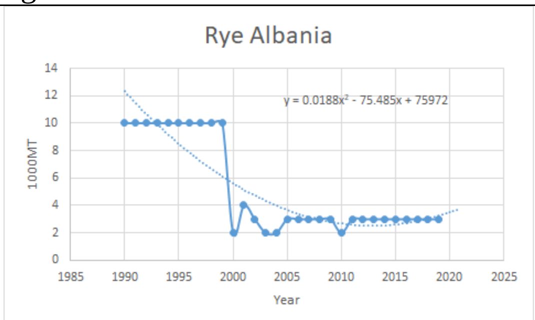

Figure 18: Production of Rye in Albania

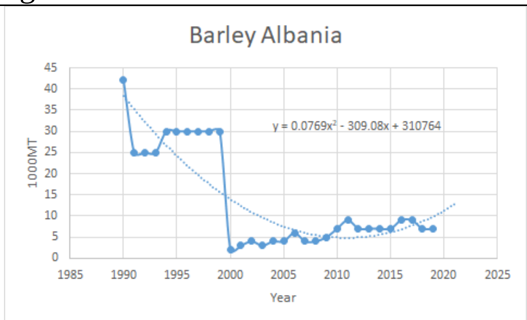

Figure 19: Production of Barley in Albania

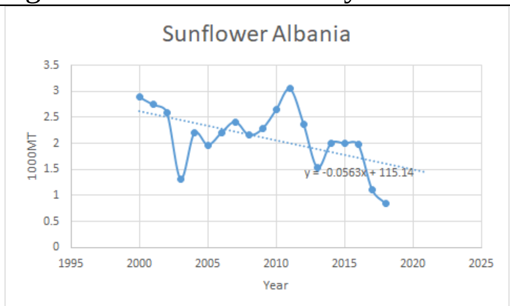

Figure 20: Production of Sunflower in Albania

Source:
https://www.indexmundi.com/agriculture/?country=al&commodity=wheat&graph=production
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https://www.indexmundi.com/agriculture/?country=al&commodity=barley&graph=production
http://www.fao.org/faostat/en/#data/QC
https://knoema.com/atlas/Albania/Crop-production-index

Conclusions

Wheat production, as a highly represented cereal in the four analyzed countries, has a trend of production growth, primarily due to the increase in production in Serbia. By world production of wheat in 2019, Serbia take the 24th place with 2.9 million tons, North Macedonia take the 45thplace with 300,000t, Albania is on 46th with 300,000t and Bosnia and Herzegovina takesthe 47thplace with 285,000t.

The amount of maize produced in the analyzed four countries shows that it is the most represented production in Serbia, Bosnia and Herzegovina and Albania. They occupy the following positions worldwide: Serbia 17th place with 6,750,000t, Bosnia and Herzegovina 50th place with 965,000t, Albania 68th place with 375,000t and North Macedonia 82th with 130,000t.

Rye production in Bosnia and Herzegovina is the largest of the fouranalyzed countries and the following countries are ranked globally: Bosnia and Herzegovina 12th place with 14000t, Serbia14th with 10000t, North Macedonia 15thplace with 8000t and Albania 19thplace with 3000t.
Barley is the most represented in Serbia and production is around 440,000t, so Serbia is on the world producer list on 24th place with 440,000t, North Macedonia 39th place with 100,000t, Bosnia and Herzegovina takes 42th place with 75000t and Albania takes 58th place with 7000t. Significant quantities of sunflower are produced in Serbia alone (around 1.1 million tons). From the data collected, it can be concluded that all four countries are trying to satisfy the domestic market with their production. The trend of increasing production is noticeable. Some countries are also significant exporters and are beginning to negotiate production quotas with the EU Commission from better positions. By opening the market and adopting new rules, agricultural production will be under new conditions, which will certainly affect the structure of production as well as the quantity produced.

Acknowledgments

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STRATEGIC APPROACH TO LAND CONSOLIDATION

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Abstract

Land consolidation is defined and considered as the process through which the fragmented parcels and land ownership are grouped into more functional shapes. Consequently it contributes to reduction of agricultural production costs, increment of agricultural production and consequently increases the competitive potential of agricultural production in certain country. The development of agricultural production is one of more significant aims in contemporary society because of rising demand for food. On the one side the demand for food is expecting to rise, while on the other side the land could be considered as non-renewable and limited resource. What more, the food industry is nowadays treated as commercial issue what additionally increase the complexity of food security problem in the future. Bearing in mind that food is mostly produced by agriculture immediately follows the very big importance of agricultural land. Land consolidation as a process of grouping fragmented land ownership is not only mechanical approach in that direction, actually it allows the improvement of land involved functionality. The functionality of land included in land consolidation process is reflected in increasing efficiency of agricultural production obtained by improvement of road and channel network, regularization of parcels shapes and their grouping. Considered on the level of the country the land consolidation could influence the increase of gross domestic products, food safety and instigate sustainable economic development. In that sense, decision of providing land consolidation on the level of the country is strategic decision which shall be treated as investment in agricultural land preservation and long term development factor. This paper aims to investigate the strategic aspects of the land consolidation.

Keywords: *Land consolidation, strategy, agriculture, economy, sustainable development*

Introduction

Land consolidation is originated by the idea to group fragmented parcels aiming to increase efficiency of agricultural land utilization. Development of basic idea of land consolidation has led to the wide spectra of its utilization and common contributions to the society. The economic, social and environmental analysis of land consolidation showed that all of these parameters were better in the area where land consolidation was provided related to areas where it was not (Crecente *et al.*, 2002). Some negative influence were noticed to the environment because of intensification of mechanization, fertilisers and pesticides utilization but the parcels rationalization is not the main cause of this, but this negative influence is driven by market process. In Japan the effects of land consolidation were researched from aspect of erosion process and the conclusion is that the number of erosion events per hectare in consolidated paddy fields was smaller than in non-consolidated paddy fields (Mihara, 1996). Generally, land consolidation is a tool for ensuring the effective and rational cultivation of farmland and depending on the political, socio-economic and environmental demands to the particular countries or regions places stress on various aspects of it as follows (Sklenička *et al.*, 2009):

- Land reclamation;
- Nature and landscape conservation and
- Social and economic development of countryside.

Interesting approach is to consider land consolidation as the rearrangement of land areas according to developing agricultural technology (Cay & Uyan, 2013). This approach is quite interesting because it suggests that land consolidation should follow the agricultural technology. That means, bearing in mind that agricultural technology is developing permanently, that land consolidation also shall be developed and provided continuously. The measurable effects of land consolidation is increase of arable area from 3% to the 15% obtained by plot amalgamation, the exploitation of unutilized land and reclamation of different kind of devastated lands (Yu *et al*., 2010).
On the level of general aims which shall be obtained by land consolidation it is possible to define them in different ways including increasing competitive approach of countries. In Spain, for example, the goal of land consolidation policies is more connected with productivity than in other European countries' policies (Crecente *et al.*, 2002). The basic objective in Spain is to increase farmers' income by changing the parcel structure and infrastructure and is understood as a sectoral planning tool. In other European countries the policy is connected with spatial physical planning, improvement to landscape and natural conditions. Possibility of land consolidation to response to the wide spectra of general goals supports the idea that land consolidation is a powerful tool for achieving different effects and almost all of them positive. In the developed forms the land consolidation could be utilized for sustainable development of agricultural production.
The arable land is limited and (could be considered) non-renewable resource. According to (FAO, 2010) the global land area is $13.2*10^9$ ha of which the 12% ($1.6*10^9$ ha) is currently in use for cultivation of agricultural crops, 28% is under forest ($3.7*10^9$ ha) and 35% ($4.6*10^9$ ha) comprises grasslands and woodland ecosystems. Low-income countries cover about 22% of land area. The global area of cultivated land has grown by a net $159*10^6$ ha since 1961. This is the difference between area of land newly brought into cultivation, "*while over the same period previously cultivated land have come out of production*" (FAO, 2010). At the same time the irrigated area of land increased more than double and the number of hectares needed to feed one person has reduced from 0.45 to 0.22 ha per person. This reduction is mostly consequence of irrigation system development. Yet, it seems that it is enough arable land for cultivation crops some warning facts and voices are raising. For example: China needs a minimum of $120*10^6$ ha of arable land to feed its people and even though the $135*10^6$ ha is classed as planted with corps it could be not enough to supply the future generations with enough food (Kong, 2014). Sustainable land use is recognized in literature and practice. "*Maintenance of the productive potential of land resources, and checking of land degradation, is fundamental element of sustainable land use*" (Pieri, 1996). Land degradation takes different forms reducing chemical, physical and biological soil properties (Stenberg, B. 1999). Among different forms of land degradation in literature especially are stressed soil erosion (Lal, 1990) and desertification (Puigdefábregas & Mendizabal 1998). Extreme land ownership fragmentation is recognized as a new form of land degradation (Sklenička *et al.*, 2014).
Summarising the significance of arable land (its existential and economic importance for society), its limited amount and increasing need for food and degradation processes which reduce its productive potential immediately follows that every reasonable action shall be provided in order to preserve and develop it. The land consolidation seems to be one of most efficient tools for achieving this goal. Bearing in mind that land consolidation is a tool which provide realization of different sets of goals connected with land resources and cause positive effects on arable land it shall be provided continuously in order to ensure sustainable utilization of land resources.
In this paper the measurable economic effects of land consolidation is considered. Starting from basic principles of strategic approach (forecasting, goals definition and quantification of

return on investment) three different scenarios has been investigated. The scenarios are based on the assumptions that need for food will increase with time and that arable land will be the main resource for food production. The economic aspect is based on the assumption that land consolidation increases the arable area from 3% to 15% (Yu *et al*., 2010). This result is harmonized with the logic that rearrangement of shape and position of parcels could put into function the unreachable parts of them.

Material and Methods

In this paper general economic model is formed to estimate the economic justification of investment in land consolidation. The model is based on the analysis of available statistical data for certain country where the following parameters are included:

- The gross domestic product (GDP) in money units;
- The area of arable land in hectares [ha];
- The costs of land consolidation per hectare in money units;
- The yield of crops per hectare in kg or tones;
- The prices of crops per kg or per ton in money units denoted by $\boldsymbol{p_C}$ and
- Contribution of land consolidation to GDP denoted by $\boldsymbol{d_{KBDP}}$.

The data shall be obtained from official statistics. The economic model was developed in order to obtain results about return on investments in land consolidation (ROI) for making rational economic decisions about land consolidation realization.

Economic model is formed as follows:

$$C_{LCGDP} = AP * C_{LCap} \qquad (1)$$

where:

- C_{LCGDP} – contribution of land consolidation to GDP;
- AP – contribution of agricultural production to GDP and
- C_{LCap} – contribution of land consolidation to agricultural production.

$$\boldsymbol{G_{LCGDP} = C_{LCGDP} * GDP} \qquad (\mathbf{2})$$

where:

- $\boldsymbol{G_{LCGDP}}$ **– contribution of land consolidation to GDP in amount of money;**
- $\boldsymbol{C_{LCGDP}}$ **– relative contribution of land consolidation to GDP and**
- $\boldsymbol{GDP}$ **– Gross Domestic Product.**

$$t = \frac{C_{LC}}{G_{LCGDP}} \qquad (3)$$

where:

- t – period of time in which the investments in land consolidation will return (ROI);
- C_{LC} – land consolidation costs and
- G_{LCGDP} – contribution of land consolidation to GDP in amount of money

$$C_{LC} = A * LC_c \qquad (4)$$

where:

- A – area for land consolidation in hectares and
- LC_c – land consolidation costs per hectare.

The proposed model is based only on the increment of arable land obtained through process of land consolidation and it neglects the other positive effects of land consolidation. This model is chosen in order to test the return on investments based only on this parameter because it was proven in practice while the other parameters (societal, environmental and land protection) are not easy for measurements. Also the assumption is that, under the same conditions, the increase of arable land cause increase of agricultural production. In the case that this model shows the acceptable return on investments then it is possible to make strategic decision to initiate land consolidation process. This decision for land consolidation for one country has strategic characteristic because it engages the significant and rare resources (knowledge, know-how, finance) in order to be successful. Also, those resources are engaged in period of time needed for land consolidation project realization.

Results and Discussion

For case study the data for the Republic of Serbia are taken. According to Statistic Office of the Republic of Serbia the plant production participate in GDP by approximately 5% and there exists $3.3*10^6$ hectares of arable land. According to World Bank the GDP in Serbia was $50.5*10^9$\$ in the year 2018. The contribution of land consolidation according to (Yu et al., 2010) is in increase of arable land between 3% and 15% which corresponds with results from practice in Serbia. This parameter is used to measure the contribution of land consolidation to the agricultural production in three scenarios. The pessimistic scenario is taken as a smallest increase of arable land in amount of 3%; the average (realistic scenario is taken as an average increase of arable land in amount of 9% and the optimistic scenario is taken as maximal increase of arable land in amount of 15%. The cost of land consolidation per hectare (according authors insight in the prices) in Serbia amounts about 200$.The obtained results are given in table 1.

Table 1. Period of ROI for land consolidation (Case study the Republic of Serbia)

Scenario	Pessimistic 3%	Average 9%	Optimistic 15%
C_{LCGDP} [%]	0.15	0.45	0.75
G_{LCGDP} [$]	7.58E+07	2.27E+08	3.79E+08
LC_c [$]	6.60E+08	6.60E+08	6.60E+08
t of ROI [year]	8.7	2.9	1.7

The obtained results showed that even in case of pessimistic scenario the ROI in land consolidation exists and the period of time is approximately 9 years. The other scenarios are proportionately better reaching ROI in approximately 3 years for realistic (average) and approximately 2 years for optimistic scenario. The short period of ROI seem to be the consequence of very low prices of land consolidation in the Republic of Serbia. This could be treated as good chance for providing land consolidation in this period because the prices could rise in the future. In this paper the maximal prices were utilized because in practice the prices could be even smaller.

This model shows that additional benefits obtained by land consolidation only could improve sustainability of land use for agricultural production. Additional benefits of land consolidation as improved road and channel networks shall result with improved efficiency of land use and consequently reduction of area of arable land needed for feed one person per year. For countries which have more arable land than necessary for providing food security it could be the chance to export surplus of agricultural production.

Conclusions

The arable land is and it will be in the future the main source of food for humanity. Its limitation and non-renewability in the conditions of intensive use and many processes of degradation force the research for its protection and preservation. Existing knowledge points the land consolidation as a most efficient tool for achieving that primary goals connected with arable land.

Secondary goals of land use are the economic effects of agricultural production. The countries with agricultural land have two choices depending on the amounts of their agricultural land. If they possess enough agricultural to only provide food security (or if they lack with agricultural land) for own population should preserve its land resources and increase the efficiency of its use. If they possess more agricultural land than needed for providing food security for own population also shall preserve it but they have a strategic chance to increase their export of agricultural products.

The economic aspects of land consolidation predominantly are in the domain of efficiency (reducing costs and increasing productivity) of agricultural production. Costs reduction are obtained by optimization of parcels size and shape, optimized road networks which reduces costs of mechanization utilization, while the increase of agricultural production is consequence of optimized irrigation systems.

Societal and environmental benefits are difficult for measurements but some research showed that those parameters are better in the regions where the land consolidation was provided related to regions where it was not.

Case study for the Republic of Serbia, provided by model established in this paper, showed that the return on investment is very short even in the pessimistic scenario of arable land increment obtained by land consolidation. In the realistic (average) and optimistic scenarios the return on investment is very short. This is the consequence of relatively low prices of land consolidation per hectare.

Summarizing the justification decision of land consolidation realization, it is possible to conclude that its realization is justified for every country because it is existential resource and also chance for economic development, which also could be paid off only thorough increase the area of arable land.

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AGRICULTURAL TRAINING CHALLENGES FACED BY SMALLHOLDER FARMERS IN THE AMATHOLE DISTRICT, EASTERN CAPE IN SOUTH AFRICA

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Abstract

Agricultural training plays a strategic role in improving the competitiveness and the productivity of the agricultural sector. This study aimed at identifying the agricultural training challenges faced by smallholder farmers in the Amathole District. Representative sample consisting of 37 smallholder farmers with 801 beneficiaries was taken. A number of smallholder farmers in seven local municipalities were visited: Nxuba (8.1%), Ngqushwa (5.4%), Amahlathi (5.4%), Mnquma (21.6%), Mbashe (54.1%), Buffalo City (2.7%) and Great Kei (2.7%). Quantitative and qualitative design was used with a detailed questionnaire written in English. Stakeholder's discussion and field observations were also part of the data collected. A purposive sampling technique was used to select thirty-seven smallholder farmers. Data was coded, captured and analyzed with the software Statistical Packaged for Social Sciences (SPSS version 24). Descriptive and Univariate analysis were conducted. Results identified the following agricultural training challenges: Soil Preparation, Seed Sowing, Pests and Diseases, Marketing, Harvesting, Transplanting and Post-Harvest Storage. The Univariate analysis showed a high level of positive association among Pest and Diseases (Dependent variable) and the following Independent variables: Water source, Crops planted, Land size, Education and Farming experience. The model fit was predicted by the r^2 at 0.937 (94%) and in general, the higher the r^2, the better the model fits the data and the better interaction between dependent and independent variables. It is thus concluded and recommended that the transfer of agricultural knowledge to support smallholder farmers should be a priority for the government especially the seven training challenges identified by smallholder farmers.

Keywords: *Smallholder Farmers, Agricultural Training, Amathole District, Limpopo Province, South Africa.*

Introduction

Training and education plays an important role in smallholder farmer development and research showed that smallholder farmers can benefit from training in agricultural techniques, business management and marketing skills (World Bank 2013; Danida 2004; Maponya *et al.*, 2015). Failure to address some of these training needs has led to constrained agricultural growth in some districts in South Africa (Maponya *et al.*, 2014; Maponya *et al.*, 2015). It must also be noted and acknowledged that only training that accurately addresses the current needs of smallholder farmers and takes into account their different production activities and challenges offers serious prospects for raising productivity, knowledge, skills and improving their livelihoods. According to Maponya *et al.* (2014), designing this kind of trainings is a complex challenge, and it requires detailed local knowledge and a proper understanding of the challenges faced by smallholder farmers. This will go a long way in designing programmes that effectively target smallholder farmers' training needs.

Amathole district is the second largest economy in the Eastern Cape Province, providing 27% of value added (ECDC, 2014). Agriculture provides only 8% of formal employment but varies greatly within local municipalities. Agriculture in the ex-homelands is mostly small-scale crop farming and open grazed livestock. Farming is for subsistence rather than commercial sale, although some black commercial farmers are present (ECDC, 2014). The coastal belt south of East London is the center of the pineapple farming industry, with citrus, horticulture and livestock also farmed. Significant forestry plantations are sited in both the Amatola Mountains and around Butterworth. According to ECDC (2014), the areas around Peddie and beyond Alice are excellent cattle farming areas. The agriculture sector is expected to grow fastest at an average of 3.36% annually from R 393 million in Amathole District Municipality to R 463 million in 2021 (ECDC, 2014).

The Agricultural Research Council (ARC) was involved in an agricultural project in the Amathole district in Eastern Province, where smallholder farmers were identified, assessed and trained in vegetable production. Research was conducted with the overall aim of training smallholder farmers in agricultural production and business management skills. The objectives were: (1) to describe the socio-economic characteristics of selected agricultural farmers in the Amathole District, (2) to identify and determine training needs faced by smallholder farmers in the Amathole District.

Materials and Methods

The research used quantitative and qualitative methods as a detailed questionnaire written in English was developed for the data collection. The questionnaire used both open and closed ended questions. Data collection included stakeholder discussions and field observations. As part of standard protocol for conducting the study, meetings were held with all stakeholders in the Amathole district namely: (1) Local municipalities, (2) Department of Agriculture, Forestry and Fisheries (DAFF), (3) Department of Rural Development and Land Reform (DRDLR), (4) Local economic agencies, and (5) Local farmers. The aim of the meetings was to introduce and explain the intended project. A purposive sampling technique was used to select 37 agricultural farms. The sampling was used to assess uniformity and homogenous characteristics like infrastructure needs, skills availability, production challenges, agricultural training needs, and water source needs, educational level, land acquisition, size of land, farming experience, source of water, inputs and implements used. Agricultural farms visited in the Amathole district were prioritized based on the agriculture potential of the area namely project type, numbers of projects around an area, size of the land, chances of extending production, water availability, commitment of members to their projects, internal conflicts, working material and infrastructure. As shown in Figure 1, Amathole District had seven local municipalities. Data was coded, captured and analyzed with the software Statistical Packaged for Social Sciences (SPSS version 24). Descriptive and Univariate analysis were conducted.

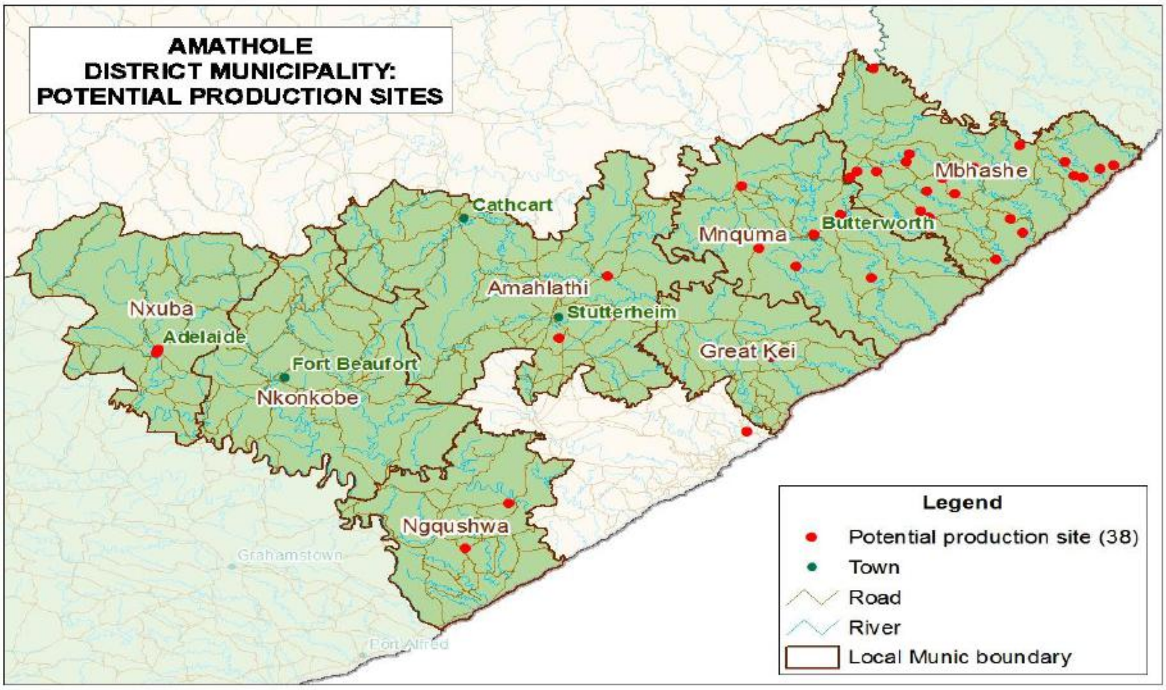

Figure 1. Amathole District with seven local municipalities.

The following econometric model was used to determine association of variables (Greene, 1993):

Wi = _ + _Xi + _i (1)

Where: Wi is the dependent variable value for person i, Xi is the independent variable value for person i,_ and _ are parameter values, _i is the random error term. The parameter _ is called the intercept or the value of W when X = 0. The parameter _ is called the slope or the change in W when X increases by one.

The sample data variables predicted 94% for McFadden (measure for multinomial and ordered logit) variation in the dependent variable was explained by the independent variables. Prediction accuracy were assessed based on the coefficient of determination (R-squared). The coefficient of determination R-squared was used to explain the total proportion of variance in the dependent variable explained by the independent variable. The R-squared removes the influence of the independent variable not accounted for in the constructs. R-squared is always between 0 and 100%. In general, the higher the R-squared, the better the model fits the data.

Results and Discussion

A total of 37 farms in seven local municipalities were visited and spread as follows: Nxuba (8.1%), Ngqushwa (5.4%), Amahlathi (5.4%), Mnquma (21.6%), Mbashe (54.1%), Buffalo city (2.7%) and Great Kei (2.7%). The farms visited had a total of 801 beneficiaries and spread across local municipalities: Nxuba (30), Ngqushwa (30), Amahlathi (20), Mnquma (29), Mbashe (676), Buffalo city (6) and Great Kei (9).

The majority of farmers interviewed were female. According to Table 1, twenty women were interviewed as compared to seventeen females. In terms of educational attainment (Table 1), 5.4% had incomplete primary education, 21.6% had completed primary education, 32.4% had incomplete secondary education, 27% of farm respondents had completed secondary education, 2.7% had incomplete tertiary education and 10.8% had completed tertiary education. These results indicated that educational level of the selected farmers is generally adequate to enable interpretation and understanding of basic farming activities. According to (Maponya *et al.*, 2016) training and education plays an important role in smallholder farmer development and failure to address some of the training needs has led to constrained agricultural growth in some districts in South Africa (Maponya *et al.*, 2014; Maponya *et al.*, 2015). As indicated in Table 1, only twenty-two farmers received agricultural training and whereas fifteen of farmers did not receive any training. The majority of farmers (25) were farming fulltime while only twelve were farming fulltime and working part time. Results on land acquisition (Table 1) indicated that the majority of farmers got land through inheritance (19) while others received land through the following: leased (10), own finance (6) and Land Redistribution for Agricultural Development (LRAD) (2). The age distribution of farm respondents indicated that the majority were in the age group of >51 (35.1 %). As indicated in Table 1, youth involvement is very low (10.8 %), 36 – 45 (21.6%) while 46 – 50 had 32.4%.

Table 1: Amathole District Smallholder Farmers Selected Socio-Economic Characteristics.

Variables	Farmers	% of Farmers
Gender		
Female	20	54.1
Male	17	45.9
Total	**37**	**100**
Level of Education		
Primary Education Incomplete	2	5.4
Primary Education Completed	8	21.6
Secondary Education Incomplete	12	32.4
Secondary Education Completed	10	27
Tertiary Education Incomplete	1	2.7
Tertiary Education Completed	4	10.8
Total	**37**	**100**
Employment Status		
Farming Fulltime	25	67.6
Farming Fulltime & Working Part-time	12	32.4
Total	**37**	**100**
Age		
18 - 35	4	10.8
36 - 45	8	21.6
46 - 50	12	32.4
>51	13	35.1
Total	**37**	**100**
Agricultural Training		
Yes	22	59.5
No	15	40.5
Total	**37**	**100**

Land Acquisition		
Own Finance	6	16.2
LRAD	2	5.4
Lease	10	27
Inheritance	19	51.35
Total	**37**	**100**

The results from Table 2 should be interpreted as follows: 1st – 3rd training challenges = Most challenging; 4th – 5th training challenges = Moderate challenging, and 6th – 7th training challenges = Least challenges. The results showed that 45.9 percent of farmers recognised soil preparation as a 1st training challenge and 2.7 percent of farmers recognised soil preparation as a 2nd training challenge. Table 2 also indicated that 16.2 percent, 5.4 percent and 8.1 percent of farmers indicated soil preparation as a 3rd, 4th and 5th training challenge respectively. Only 2.7 percent and 18.9 percent of farmers recognised soil preparation as a 6th and 7th training challenge. It is very clear that attention should be given to soil preparation as indicated by the 1st, 2nd and 3rd training challenges.
The results indicated that 27 percent of farmers recognised seed sowing as a 1st training challenge and 13.5 percent of farms recognised seed sowing as a 2nd training challenge. Table 4 showed that 5.4 percent, 21.6 percent and 2.7 percent of farmers accepted seed sowing as 4th, 5th and 6th training challenge respectively. Only 24.3 percent of farmers recognised seed sowing as a 7th training challenge. It is very clear that attention should be given to seed sowing as indicated by the 1st, 2nd and 3rd training challenges. The results showed that 21.6 percent of farmers recognised transplanting as a 1st training challenge, 8.1 percent of farmers recognised transplanting as a 2nd training challenge and 5.4 percent of farmers recognised transplanting as a 3rd training challenge. Table 5 showed that 13.5 percent, 21.6 percent and 20.7 percent of farmers indicated transplanting as a 4th, 5th and 6th training challenge respectively. Only 20.7 percent of farmers recognised transplanting as a 7th training challenge
The results showed that 51.4 percent of farmers recognised pest and diseases as a 1st training challenge and 21.6 percent of farmers recognised pest and diseases as a 2nd training challenge. Table 6 showed that 18.9 percent, 8.1 percent and 8.1 percent of farmers indicated pest and diseases as the 4th, 5th and 6th training challenge respectively. Only 18.9 percent of farmers recognised pests and diseases as a 7th training challenge. Almost 76 percent of farmers emphasised pests and diseases as the most important training challenge as indicated by 1st, 2nd, 3rd training challenges. This is a worrying situation and a clear indication that the management of pests, diseases and weeds is still very challenging to many smallholder farmers in Amathole District. The results showed that 10.8 percent of farmers recognised harvesting as a 1st training challenge, 5.4 percent of farmers recognised harvesting as a 2nd training challenge and 16.2 percent of farmers recognised harvesting as a 3rd training challenge. Table 2 showed that 32.4 percent and 8.1 percent of farmers indicated harvesting as the 5th and 6th training challenge respectively. Only 27 percent of farmers recognised harvesting as a 7th training challenge. The results seem to indicate that smallholder farmers are comfortable with harvesting.
The results indicated that 35.1 percent of farmers recognised post – harvest handling as a 1st training challenge, 8.1 percent of farmers recognised post – harvest handling as a 2nd training challenge and 2.7 percent of farmers recognised post-harvest handling as a 3rd training challenge. Table 2 showed that 18.9 percent and 8.1 percent of farmers indicated post-harvest handling as the 5th and 6th training challenge respectively. Only 18.9 percent of farmers recognised post-harvest handling as a 7th training challenge. Post-harvest handling and storage is one of the key constraints faced by majority of the smallholder farmers across South Africa

(Maponya and Mpandeli, 2015) and is thus critical that smallholder farmers in Amathole District should be bettered with postharvest handling skills and knowledge.
The results showed that 56.8 percent of farmers recognised marketing as a 1^{st} training challenge, 10.8 percent of farmers recognised marketing as a 2^{nd} training challenge and 5.4 percent of farmers recognised marketing as a 3^{rd} training challenge. Table 2 showed that 2.7 percent, 13.5 percent and 2.7 percent of farmers indicated marketing as the 4^{th}, 5^{th} and 6^{th} training challenge respectively. Only 8.1 percent of farmers recognised marketing as a 7^{th} training challenge. It is very clear that attention should be given to marketing, indicated as the 1^{st}, 2^{nd} and 3^{rd} training challenges.

Table 2 : Amathole District Smallholder Farmers Agricultural Training Challenges.

Soil Preparation as a Training Challenge.			**Seed Sowing as a Training Challenge**			**Transplanting as a Training Challenge**			**Marketing as a Training Challenge**		
Training Challenges	No: Farmers	%	Training Challenges	No: Farmers	%	Training Challenges	No: Farmers	%	Training Challenges	No: Farmers	%
1st Challenge	17	45.9	1st Challenge	10	27	1st Challenge	8	21.6	1st Challenge	21	56.8
2st Challenge	1	2.7	2st Challenge	5	13.5	2st Challenge	3	8.1	2st Challenge	4	10.8
3st Challenge	6	16.2	3st Challenge	2	5.4	3st Challenge	2	5.4	3st Challenge	2	5.4
4st Challenge	2	5.4	4st Challenge	2	5.4	4st Challenge	5	13.5	4st Challenge	1	2.7
5st Challenge	3	8.1	5st Challenge	8	21.6	5st Challenge	8	21.6	5st Challenge	5	13.5
6st Challenge	1	2.7	6st Challenge	1	2.7	6st Challenge			6st Challenge	1	2.7
7st Challenge	7	18.9	7st Challenge	9	24.3	7st Challenge	11	20.7	7st Challenge	3	8.1
Total	37	100	**Total**	37	100	**Total**	37	100	**Total**	37	100
Pest and Diseases as a Training Challenge.			**Harvesting as a Training Challenge.**			**Postharvest Handling/Storage as a Training Challenge.**					
Training Challenges	No: Farmers	%	Training Challenges	No: Farmers	%	Training Challenges	No: Farmers	%			
1st Challenge	19	51.4	1st Challenge	4	10.8	1st Challenge	13	35.1			
2st Challenge	8	21.6	2st Challenge	2	5.4	2st Challenge	3	8.1			
3st Challenge	1	2.7	3st Challenge	6	16.2	3st Challenge	1	2.7			

4^{st} Challenge	1	2.7	4^{st} Challenge			4^{st} Challenge	3	8.1
5^{st} Challenge	6	16.2	5^{st} Challenge	12	32.4	5^{st} Challenge	7	18.9
6^{st} Challenge			6^{st} Challenge	3	8.1	6^{st} Challenge	3	8.1
7^{st} Challenge	2	5.4	7^{st} Challenge	10	27	7^{st} Challenge	7	18.9
Total	37	100	**Total**	37	100	**Total**	37	100

Lack of knowledge and agricultural inputs are the major casual factors of training challenges as indicated in Table 3. Results showed that 64.9 percent of farms had no knowledge and inputs to participate in sustainable agricultural production. The same trend was observed in the Mopani District in Limpopo Province, whereby majority of smallholder farmers indicated a lack of knowledge and inputs as casual factors of training challenge (Maponya *et al.*, 2016).

Table 3. Casual factors of smallholder farmers training challenges in Amathole District.

Casual Factors	Number of Farmers	Percentages
Lack of Knowledge	5	13.5
Lack of Inputs	1	2.7
Lack of Knowledge, Inputs	24	64.9
Lack of Knowledge, Inputs, Workers	5	13.5
Lack of Knowledge, Group Conflicts	2	5.4
Total	**37**	**100**

As indicated in Table 4, the odds of farmer's land size, water source, crops planted, education and farming experience were more than 1. This clearly indicated a positive association with pest and diseases as a training challenge. It was not surprising to realise a positive association among water source, crops planted and land size as pest and diseases are mostly transmitted from water sources especially polluted water. Moreover, this pests and diseases challenge can affect any crops planted in any land size. This required an educated and more experienced smallholder farmer to deal decisively with pests and diseases on their farms. According to ARC (2014), many smallholder farmers lack knowledge on the cycles of specific pests, diseases and weeds and find it difficult to distinguish their specific characteristics. As a result, they cannot apply suitable preventive measures nor implement proper control measures. To avoid major crop losses, smallholder farmers must be well trained and monitored to implement affordable and effective measures against pests and diseases (Maponya et al., 2016).

Table 4. Univariate analysis among factors contributing to pest and diseases as a training challenge in the Amathole District.

Variables	**OR and 95% CI**
Water Source	1.499[0.31–21.1]1
Crops Planted	1.19[10.5–77.9]1
Land Size	1.01[0.04-0.57]1
Education	1.35[0.10 – 2.99]1
Farming Experience	1.78[1.11 – 11.7]1

(N = 37); OR = Odds Ratio; 95%CI = 95% Confidence Intervals; 1< = No Association; 1> = Association.

Conclusion

The study identified seven training challenges faced by smallholder farmers in Amathole District, namely: Soil preparation, Seed sowing, Harvesting, Transplanting, Pests and Diseases, Post-harvest handling and Marketing. Based on the identified training challenges, it can be concluded that smallholder farmer experienced a lack of training in Amathole District. It is thus concluded and recommended that the transfer of agricultural knowledge to support smallholder farmers should be a priority for the government especially the seven training challenges identified by smallholder farmers. Smallholder farmers should therefore, be trained in line with the seven training challenges identified by the study. To accomplish these, smallholder farmers should have access to research and training institutions, for example, the Agricultural Research Council, to improve their skills and knowledge. This in turn will lead to higher yields, increased income, increased knowledge and skills, food security and resilience to a changing climate.

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SOCIAL INNOVATIONS IN MOUNTAIN REGIONS

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Abstract

Two case studies developed in the framework of the Horizon 2020 project *Social Innovation in Marginalised Rural Areas (SIMRA)* are presented: (a) The Val Lumnezia, a lateral valley in Grisons in the Alps. This Romansh-speaking valley was particularly isolated and characterised by ongoing emigration. An initiative of young craftsmen/entrepreneurs in the 1980s led to a structural change from purely agricultural production to agro-tourist specialisation. They were not guided by a strategy of mass tourism, but were among the first to work together with environmental organisations for environmentally compatible tourism. (b) Val de Travers, a lateral valley in the canton of Neuchâtel in the Swiss Jura, specialised in the watch and micro-industry. The crisis in the watchmaking industry and an increasingly visible division between mountain areas and the littoral plain led to the initiative to develop a mobility and agglomeration concept for the entire canton. They involve both municipalities and local companies in a participatory manner and follow an integrated approach of the local economy (agro-tourism, manufacturing). Social innovation is always triggered by a crisis in the regional trajectory. Such upheavals are often associated with the generational change of the most-important decision-makers. Although civil society initiatives play an important role, public and private institutions must not be neglected and it is the interaction between all three groups of actors that decides on the trajectory of a social innovation.

Keywords: *Mountain regions, Social innovation, spatial disparities, spatial justice, cohesive societies.*

Introduction

Social Innovation has become an important topic also for mountain regions. In the context of global metropolisation, mountain regions undergo a profound transformation where settlements at low and very high altitudes have good chances in the valorization of their landscapes while the intermediate areas risk decline (Perlik, 2019). *Social innovation* has become a popular term but various research communities use the notion in a different manner. Broadly speaking, two different schools can be distinguished in the international debate on social innovations (SI): an Anglo-Saxon school and a continental or Francophone school. The first emphasizes an entrepreneurial understanding, which is ultimately an economic innovation linked to a field of social issues (example: the activities of Muhammad Yunus and the Grameen Bank in Bangladesh), while the continental/French-speaking understanding follows an institutional approach (e.g. Andrew and Klein, 2010; Fourny, 2018; Moulaert et al., 2013; Salathé-Beaulieu et al., 2019). With regard to the depth of the intervention, this means: The first is mainly affirmative-adaptive, the second transformative.

The intervention presents two case studies on social innovation developed as part of the Horizon 2020 project "Social Innovation in Marginalized Rural Areas" (SIMRA). SIMRA attempts to analyse the impact of social innovation using quantitative and qualitative methods and, in particular, to highlight the important role of civil society as an innovator. These methods cover a wide range, from social network analyses to comparative analysis of different regional policies. The intervention is based on 211 cases from the SIMRA database, some dozen cases of in-depth studies at European level, including two case studies from the Swiss mountain region.

In the context of European peripheries, SIMRA defines social innovation as follows: "*The reconfiguring of social practices in response to societal challenges which seek to enhance the outcomes on societal well-being and necessarily include the engagement of civil society actors*." This definition of SIMRA is a compromise between the two schools on SI.

Two Swiss case studies

The Val Lumnezia is a lateral valley of the Anterior Rhine in the canton of Grisons, in the Alps. This Romansh-speaking valley was particularly isolated and marked by emigration. Thanks to an initiative of young craftsmen/entrepreneurs in the 1980s, the region has undergone a structural change from purely agricultural production to agrotourism specialisation. The innovators were not guided by a mass tourism strategy, but were among the first to cooperate with environmental NGOs for environmentally friendly tourism. The Val de Travers (canton of Neuchâtel) in the Swiss Jura is specialized in watchmaking and micro-industry. The crisis in the watch industry and the increasingly visible division between the mountainous and lakeside areas have led the cantonal administration to develop a concept of mobility and agglomeration-building for the entire canton, integrating local municipalities and companies into an institutionalised form. The two case studies were part of the SIMRA project design and included quantitative evaluation of detailed, guided questionnaires as well as qualitative semi-structured interviews. The fieldwork took place in the summer of 2018; the repondants were local actors involved in local initiatives which we had selected as typical examples of social innovation in mountain regions. The interview partners were typologized according their different roles in the process. The reports on methodology and results will be (and are already partly) published on the SIMRA website (see below). First journal papers have been already published (e.g. Melnykowych et al, 2018; Perlik, 2018).

Results of Swiss case studies

First, both examples show that social innovations in marginalised areas can trigger new development dynamics that contribute to reducing regional disparities, at least in the short term. However, social innovations often remain linked to certain actors or constellations of actors and lose their significance as soon as these constellations change. Social innovations therefore have a life cycle ranging from the crisis as a triggering event to its end. Long-term sustainable territorial development is therefore not guaranteed. This remains linked to favourable framework conditions for territorial balance, which should be guaranteed by institutions at national and supranational level.

Although social innovation is very often seen in relation to civil society initiatives, Switzerland's two examples show that the role of existing public administration institutions and private sector actors should not be neglected under any circumstances, provided that they are capable of change. Social innovation is always triggered by the reaction to the impact of a major crisis, which has developed between social actors before, with specific consequences in regions with low population density. These upheavals are often associated with personalities of individuals and a generational change in political or economic decision-makers. In the case of Lumnezia, it is the return of a new generation of professionals (craftsmen, entrepreneurs) trained outside the valley. In the case of Val de Travers, it was a new generation of heads of public services at the cantonal level whose ideas met at the time perfectly the changing requirements of the Swiss Confederation's New Regional Policy (NRP), just being developed. This concordance allowed a creative process for the implementation of new regional development concepts. Both case studies show the importance of changing constellations between political institutions, private sector actors and new civil society actors (Marini Govigli et al., 2019b).

Trajectories of social innovations

Due to the definition of SIMRA which has not established a normative model for "societal well-being" and because long-term success cannot be monitored in the project, trajectories cannot be measured by their success (e.g. regional growth). That, what is feasible to analyse: based on theoretical approaches, it is possible to select key factors recognized for divergent regional development. In a second step, the elaborated trajectories can be checked with the aid of the SIMRA database. Therefore, we have selected the following key factors:

Origin of the main actors

It is important to know whether the SI is triggered by the local population or whether the dominant actors are newcomers from outside from an urban context. The different motivations determine to what extent peripheral mountain areas base their ideas and strategies on an urban context or whether they search rather alternatives based on their local knowhow.

Origin of knowledge

Tacit knowledge offers a unique selling point and makes it difficult to copy regional innovations elsewhere. On the other hand external knowledge gives new inputs and might prevent paralysis, isolationism and regional decline through depopulation.

Type of innovators

Innovators may come from the public sector, the private sector, or they are new actors, not yet organized before (civil society). A combination of all three is also possible.

Depth of intervention

SI in peripheral regions are mainly adaptive. Not fundamentally new, but new in a specific context. As from adaptive character, these SI do not change the territorial hierarchies. Due to their low population density, peripheral areas in general have little potential for transformative SI, i.e. deeper change. There, where peripheral areas produce transformative SI, they may also stimulate the sustainable development of urban areas.

Dissemination of SI

In particular, the Anglo-Saxon school, which interprets the SI as the creation of social enterprises, sees the success of an innovation through its dissemination in other regions. But also innovations in institutional practices (such as participatory spatial planning) can also be disseminated, even though this dissemination is rarely measurable.

Benign or malign evolution

Since SIMRA is mainly interested in the positive effects of SI, its database does not contain any examples that have turned bad. However there exist certainly cases where the region is weaker than before after an SI initiative. These cases are rarely named as SI, so they escape analysis.

Preliminary results of trajectory analysis

In 173 cases (of 211 records of SI in SIMRA), the local actors are the initiators; in 37 cases they come from outside. In the majority of cases, they are based on local (105) or local and external (99) knowledge. External actors who exclusively use external knowledge appear only rarely in the database (6), but these cases may occur in reality even more often: for example, the neo-rural movement since the 1970s or the establishment of alternative tourism hostels run collectively by young urban people may be part of it (Kluvánková et al., 2019, forthcoming).

The depth of the intervention is generally adaptive: it is an adaptation of regional development strategies under the conditions of increased competition. However, this factor has not yet been definitively assessed; in addition, there are still often disagreements about classification. As an example for transformative SI can be considered initiatives for the integration of refugees in mountain regions (e.g. Borzaga and Galera, 2016; Perlik and Membretti, 2018). These are innovative today because since the end of the Second World War, the welcoming experience tends to become forgotten and isolation has become more and more the rule. The reception of people in poverty is seen as a transformative innovation because it resists the nationalist and regionalist tendencies that currently prevail.

The assessment of the dissemination factor is not yet complete due to the heterogeneity of cases. It is becoming more and more obvious that this factor only makes sense for social enterprises because only in cases like this do we have information about the dissemination.

There are no malign or toxic initiatives in our data collection. Rather, there are many examples of stabilizing regional decline. Although they did not reverse the fragile situation, their absence would have considerably worsened the position of the peripheral regions within the territory.

Discussion and conclusions

The trajectories were developed on the basis of theoretical approaches and the existing data base on social innovations. They cannot be used to measure the success of initiatives.

The term "trajectory" refers to the development of initiatives and general future options resulting from these initiatives. These options can be interpreted as follows:

- In most cases, local actors try to stabilize existing local structures based on their local knowledge but with the aim of capturing external expertise.
- If external actors trigger the SI, they rely on local knowledge. There are only a few cases where this is not the case. But even though we have not found in our database, cases in which external actors rely mainly on their own (external) knowledge, these cases exist.
- While local actors rely heavily on local knowledge, they give a signal for the pursuit of decentralised regional development with its own specificities and disadvantages. In the case of transformative SIs, this could also mean impulses for urban areas. If SI based on local knowledge does not succeed, there is a risk of decoupling between rural and urban areas.
- If actors rely heavily on external knowledge, they can bridge the gap between urban centres and peripheries. This allows them to cooperate better with urban areas, but they must ensure that they can defend their own interests against urban agglomerations.

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THE ECONOMIC EFFICIENCY OF THRIFT AND CREDIT COOPERATIVES IN NORTHEAST OF THAILAND

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Abstract

Thrift and Credit Cooperatives (TCCs) in Thailand are the biggest operation in terms of business volume. Their members are people having the same career and the same community. Their main purpose is to promote savings and provide loan for members. These research objectives were to 1) study the operation of TCCs in the northeast of Thailand, and 2) analyze the economic efficiency of TCCs in the northeast of Thailand. The study population was divided into 2 groups 1) TCCs members in the northeast of Thailand which comprised of 1,230 individuals and 2) TCCs committee and management staff of 9 TCCs. Purposive sampling technique was applied as the sampling technique. It turned out with the 400 samples and 18 samples of committee and management staff. Primary data were collected by questionnaires and focused group. The secondary data were collected from the database of Cooperatives auditing department. Ministry of Agriculture and Cooperatives. Data analysis was composed of descriptive statistics, Data Envelopment Analysis Model (DEA Model) and content analysis. The research found that 1) from last 20 years, all of 9 TCCs have operated their business with their good shape in terms of financial status which were assets, debts, capitals, income, expenditure, and profit. Most of the time, 2 main businesses of TCCs are saving and lending for members, 2) 6 out of 9 TCCs acquired the economic efficiency while 3 of them faced with economic inefficiency. According to the research results, TCCs whose face with economic inefficiency could have their own business strategies to improve themselves to achieve economic efficiency. Economic efficiency of TCCs also made members confidently and trustworthy.

Keywords: *Economic efficiency, thrift and credit cooperatives, northeast of Thailand*

Introduction

Cooperatives in Thailand, like in all developing countries, have been initiated by the government since 1915 with the prime aim of using as a means to improve the livelihood of small farmers. This is due to the increasing indebtedness problem resulting from farmers who were suffering from the shifting of the self-sufficient economy to trade economy. The natural disaster such as drought and flood even added further to create more chronic and severe indebtedness to the farmers. Consequently, they lost their farmland and becoming laborers and thus leaving their debts unpaid (Cooperative Promotion Department, 2019). The first cooperative in Thailand named Wat Chan Cooperative Unlimited Liability was established by the government on February 26, 1916, in Phitsanulok, following the Raiffeisen credit cooperative type with a single purpose of providing farm credit and being organized as a small village credit cooperative to help the severely indebted farmers. The success of this cooperative type in preventing many farmers' land from being foreclosed by the moneylenders led to the increasing number of small village credit cooperatives all over the country. The small credit cooperatives had prevailed in the country until 1938 other cooperative types then established in responding to the people's needs (Cooperative Promotion Department, 2019).

In 1966, the government-cum-credit cooperative-owned Bank for Cooperatives was reorganized to the "Bank for Agriculture and Agricultural Cooperatives" a state enterprise, functioning as a financial center of agricultural cooperatives including lending directly to individual farmers (Cooperative Promotion Department, 2019). In 1968 with the objective to strengthen the cooperative movement, the Government enacted the Cooperative Act, 1968, which allowed the establishment of the Cooperative League of Thailand, functioning as the apex organization of the cooperative movement. The said Cooperative Act also allowed for the amalgamation program which combined the neighboring small village credit cooperatives, paddy and marketing cooperatives, land improvement and land settlement cooperatives into a large scale cooperative at district level performing multipurpose functions and was officially categorized as agricultural cooperatives.

At present, the cooperatives in Thailand are officially categorized to seven (7) types, namely: 1). Agricultural Cooperative, 2). Land Settlement Cooperative, 3). Fisheries Cooperative, 4). Consumer Cooperative, 5). saving and Credit Cooperative, 6). Service Cooperative, and 7). Credit Union Cooperatives.

Thrift and Credit Cooperative (TCC) is one of seven types in Thailand. In this research, the researcher pays attention to TCC which could be explained as below:

Thrift and Credit Cooperatives are those whose members are people having the same occupation or living in the same community. Their main purpose is to promote savings among members and provide loans for productive investment.

Thrift and Credit Cooperative Objective, being a financial institution, the specific objectives of Thrift and Credit Cooperatives are aimed to: 1) Encourage thrift among members. To encourage the saving habit, the cooperative currently offers two types of savings and 2) Provide loan services to members. Members' shares and deposits comprise a significant part of the loan funds made available to members with interest charged usually at rates lower than that of the prevailing market rates. In general, thrift and Credit Cooperatives raise capital funds through shares, deposits, reserve funds, loan funds, and support fund or donations. In its essence, a cooperative belongs to its members. Effective and efficient management of the cooperative is ensured when members exercise their rights and responsibilities properly. The most important responsibility of the individual member in attendance at the annual general assembly (AGA). The AGA gives him/her the opportunity to protect membership rights, a fair sharing of benefits and monitor cooperative operations. It provides also a forum to determine general policies, elect committee members and assign tasks to further benefit all the members. Within the framework of cooperative principles, laws and regulations and procedures, members must discuss problems together, share ideas and exercise the right to vote on committees and meeting resolutions.

Thrift and credit cooperatives (TCCs) play a key role in Thailand. In this study, the researcher intensively aimed at the role of thrift and credit cooperatives operational efficiency in the northeast of Thailand. The study area was focused on Kalasin province. In this study, the researcher set up 2 research objectives as 1) to study the operation of Thrift and Credit Cooperatives (TCCs) operation in Kalasin province, the northeast of Thailand and 2).To analyze the economic efficiency of TCCs in Kalasin province, the northeast of Thailand.

Material and Methods

The study population was the total numbers of saving and credit cooperatives in 1,230 TCCs members in Kalasin province, the northeast of Thailand which were 1,230 individuals. Samples were collected from 1,230 thrift and credit cooperatives' members. Simple random sampling was applied as the sampling technique. It came up with 400 saving cooperative members selected from 9 TCCs members as the sample size. Along with 18 samples of committee and management staff.

In this study, there were both primary and secondary data were collected. The primary data were collected by questionnaire as the data collection tool. The content validity and reliability were tested. The index of item-objective congruence was equal to 0.725 while the conbrach's alpha was equal to 0.95. Focus group was also applied as a primary data collection tool. Secondary data were collected from the database of Cooperatives auditing department. Ministry of Agriculture and Cooperatives, the Royal Thai Government.

Descriptive statistics was applied as data analysis such as arithmetic mean and standard deviation. The operational efficiency of thrift and credit cooperatives was calculated by Data Envelopment Analysis Model (DEA Model) as well as content analysis. Data envelopment analysis (DEA) is a nonparametric method in operations research and economics for the estimation of production frontiers. It is used to empirically measure productive efficiency of decision-making units (DMUs). Although DEA has a strong link to production theory in economics, the tool is also used for benchmarking in operations management, where a set of measures is selected to benchmark the performance of manufacturing and service operations. In benchmarking, the efficient DMUs, as defined by DEA, may not necessarily form a "production frontier", but rather lead to a "best-practice frontier" (Cook, Tone and Zhu, 2014). DEA is referred to as "balanced benchmarking" by Sherman and Zhu (2013).

Data envelopment analysis or DEA is a linear programming technique developed in the work of Charnes, Cooper and Rhodes (1978). It is a non-parametric technique used in the estimation of production functions and has been used extensively to estimate measures of technical efficiency in a range of industries (Cooper, Seiford and Tone, 2000). Like the stochastic production frontiers, DEA estimates the maximum potential output for a given set of inputs and has primarily been used in the estimation of efficiency. However, again like the SPF approach, DEA also can be used to estimate capacity utilization (Färe, Grosskopf and Lovell, 1994). The Färe, Grosskpof and Lovell approach, however, seeks to determine capacity output, conditional on the fixed input binding production. This is the weak concept of capacity output offered by Coelli, Grifell-Tatje and Perelman (2001). The strong concept includes the weak concept, while the weak concept does not include the strong concept of capacity output. In addition, the weak concept avoids problems caused by particular functional forms and decreasing returns to scale---the Cobb-Douglas production function, which does not have an absolute mathematical maximum. Seiford and Thrall (1990) describe DEA in terms of floating a piece-wise linear surface to rest on top of the observations (i.e. envelop the data). More specifically, the key constructs of a DEA model are the envelopment surface and the efficient projection path to the envelopment surface (Charnes et al., 1995). The projection path to the envelope surface is determined by whether the model is output-oriented or input-oriented. The choice of input- or output-oriented models depends upon the production process characterizing the firm such as minimize the use of inputs to produce a given level of output or maximize the level of output given levels of the inputs. For the purpose of estimating capacity in fisheries, only the output-oriented DEA measures have been empirically estimated.

Results and Discussion

According to the research objectives, the researcher would present the research results following the research objectives as below:

1) The situation of Thrift and credit cooperatives (TCCs) operation in Kalasin province, the northeast of Thailand. The 9- study thrift and credit cooperatives limited in kalasin province shown that the maximum value of assets, debts and capital were 427,326,522.80 $, 261,581,841.17 $, and 165,744,681.64 $ respectively. Kalasin Establishment Thrift and Credit Cooperative Ltd had the maximum value of assets among others while Kalasin Teacher's saving and Credit Cooperative Ltd. had the maximum value of debts and capital. The maximum value of assets, debts and capital were 15,810.84 $, 189,734.38 $, and 84,104.67$ respectively. Non Sung Community Saving and Credit Cooperative Ltd had the minimum value of assets and debts while Kalasin Private School

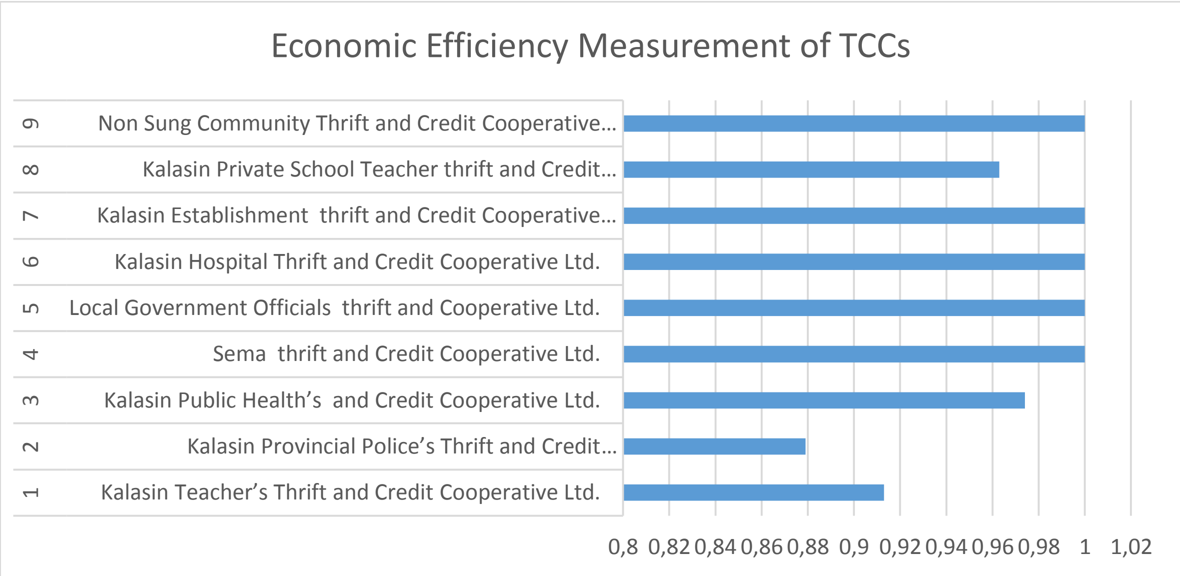
Teacher Saving and Credit Cooperative Ltd. had the minimum value of capital. The average value of assets, debts, and capital were 71,791,521.64 $, 43,230,871.46 $ and 28,599,299.86 $ respectively. The standard deviation of assets, debts and capital were 71,791,521.64 $, 43,230,871.46 $, and 28,599,299.86 $ respectively.

The 9- study saving and credit cooperatives limited in Kalasin province shown that the maximum value of income, expenditure, profit (loss), operational capital were 30,176,539.12 $, 22,322,328.04 $, 2,980,634.23 $, and 124,296,219.77 $ respectively. Kalasin Teacher's thrift and Credit Cooperative Ltd had the maximum value of income, expenditure, and operational capital while Kalasin Public Health's thrift and Credit Cooperative Ltd. had the maximum value of profit. The minimum value of income, expenditure, profit (loss), operational capital were 13,610.97 $, 17,767.40 $, 9,959.64 $, and 15,810.84 $ respectively. Non Sung Community Thrift and Credit Cooperative Ltd. had the minimum value of income and operational capital while Kalasin Private School Teacher Thrift and Credit Cooperative Ltd. had the minimum value of expenditure and profit. The average value of income, expenditure, profit (loss), operational capital were 30,176,539.12 $, 22,322,328.04 $, 1,793,605.02 $, and 124,296,219.77 $ respectively. The standard deviation of income, expenditure, profit (loss), operational capital were 3,007,623.33 $, 837,921.27 $, 2,169,702.06 $, and 39,421,067.41 $ respectively.

2.) Measurement the Economic Efficiency (EE) of Thrift and Credit Cooperatives in Kalasin Province. The overall economic efficiency score of TCCs in Kalasin province showed up in the 0.985. This meant that all TCCs in Kalasin acquired very high efficiency shown in Figure 1.

There were 9 thrift and credit cooperatives limited were measured operational efficiency (OE) applying the Data Envelopment Analysis Model (DEA Model). The results turned out that there 6 thrift and credit cooperatives limited met the standard score of EE which was 1.00. These were 1) Kalasin Public Health's Thrift and Credit Cooperative Ltd., 2) Sema Thrift and Credit Cooperative Ltd., 3) Local Government Officials Thrift and Cooperative Ltd., 4) Kalasin Hospital Thrift and Credit Cooperative Ltd., 5) Kalasin Establishment Thrift and Credit Cooperative Ltd., and 6) Non Sung Community Thrift and Credit Cooperative Ltd. Nevertheless, there were 3 out of 9 thrift and credit cooperatives limited acquired the standard score less than 1.00. This meant that these thrift and credit cooperatives had their operational performance under the standard.

Figure 1. Economic efficiency measurement of Thrift and credit cooperatives limited in the northeast of Thailand



Conclusions

Thrift and credit cooperatives (TCCs) limited in the northeast of Thailand played the crucial role in the rural areas in Thailand not only in the northeast of Thailand but also all over rural areas in Thailand. The role of TCCs Economic Efficiency in northeastern Thailand. According to the research results, there were 6 out of 7 of TCCs met the requirement of operational efficiency. This means that the operational efficiency score was less than 1.00. Since TCCs in the provincial area including in the study area perform as microfinance function. They were a provision of microloans to the middle and low-income class in Kalasin province, the northeast of Thailand and small business lacking access to banking and service. TCCs is the local microfinance institution in the local area which should be based upon the noble intention of local people. TCCs has still been the lifeblood in the local economy. In accordance with Begajo, T.M. (2018) who studies the role of thrift and credit cooperative in improving rural micro-financing the case of Bench Maji, Kaffa, Shaka Zones, Ethiopia. In addition, commodity developer had long understood the importance of local participation in the events and processes that shape community effective, democratic, and people-and-place-centered development strategies had the potential to achieve such participation (Majee, W. and Hoyt, A., 2011).

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ROLE OF IMPROVED PRODUCTION TECHNOLOGIES IN ENHANCING FARMERS PRODUCTIVITY IN OILSEED BRASSICAS IN INDO-GANGETIC PLAINS OF INDIA

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Abstract

Rapeseed mustard is an important oilseed crop of world with area of 36.53 million hectare average productivity of 2.05 tonnes per hectare and global production of 74.91 million matric tones. India is a global player in edible oil arena, being the 2nd largest importer, and 3rd largest consumer of edible oil as well as 4th largest oilseed producer. *Brassica rapa* (L.) and *Brassica juncea* (L.) (Czern &Coss) are the major cultivated oeliferous *Brassicas.* Its cultivation is facing some major constraints like non availability of quality seeds, poor soil healthnot being grown as sole crop, effect of major biotic and abiotic stresses and slower adoption of improved production technologies.Front line demonstrations (FLDs) being conducted under All India Coordinated Improvement Project on Rapeseed & mustard Research are playing an important role in mitigating the major constraints in rapeseed-mustard productivity. A large number of FLDs were carried out in farmer's field involving seven districts of eastern Uttar Pradesh region (Varanasi, Mirzapur, Chandauli, Sonbhadra, Ghazipur, Deoria and Jaunpur)for over three consecutive years i.e. during *(Rabi)* winter seasons of2016-17, 2017-18 and. 2018-19. Studies were carried out on overall effect ofuse of two improved varieties Giriraj (IJ-31) and NRCHB-101 over local variety 'Varuna' as well as the improved practices also including line sowing, proper weeding, spacing, Sulphur application along with the recommended dose of NPK, integrated disease and pest management and mechanized row sowing. The results exhibited remarkable increase in yield as well as NMR (Net Monetary Return) percentage by following improved Practices (IP) over Farmers Practices (FP).

Keywords: *Rapeseed-mustard, improved production technologies, NMR.*

Introduction

India is the 4th largest edible oil economy in the world and contributes about 10 per cent of the world oilseeds production, 6-7% of the global production of vegetable oil, and nearly 7 percent of protein meal. The major oilseeds production statistics of the World for the year 2016-17 includes a production of 549.98 million metric tons from an area of 234.57 million Hectare with the productivity of 2.34 metric tons per hectare. India ranks second in terms of area, i.e. 33.83 million Hectares but third in both production and productivity being 36.32 million metric tons and 1.07 metric tons per hectare, respectively. Rapeseed-Mustard is the second most important oilseed crop of India and plays a significant role in Indian economy by contributing about 27.8 % of the total oilseed production. Considering the World scenario, the important Rapeseed-mustard growing countries includes India, China, Canada, France, Poland, Pakistan etc. where India ranks third both in area and production of this crop. The average global area, production and productivity of rapeseed-mustard was recorded to be 35.37 million ha, 74.00 million tonnes and 2090 kg/ha, respectively in 2017-18 (USDA Circular series WAP 3-19, March 2019). In India, the average area of rapeseed-mustard was 6.02 million hectares with 7.92 million tonnes production and 1304 kg/hectare productivity during 2016-17 (Directorate of economics & Statistics, Govt. of India). The major mustard producing states are Rajasthan, Madhya Pradesh, Uttar Pradesh, Haryana, West Bengal, Gujarat, Jharkhand and Assam. Almost half (48.12%) of Rapeseed and Mustard is produced by only Rajasthan. Rapeseed-mustard is the second most important oilseed crop mainly grown in Rajasthan, Madhya Pradesh, Haryana, Uttar Pradesh, West Bengal and Gujarat. Rapeseed-mustard tops the list in case of vegetable oil with total contribution of 31% followed by soybean (26%). The combined area of *Kharif* and *Rabi* oilseeds during 2016-17 as per Government of India estimates is 282.22 lakh hectares and production of key nine oilseeds crops for the current year 2016-17 is estimated at 269.31 lakh tonnes compared with 203.41 lakh tonnes last year, showing 32 per cent rise.

The demand for vegetable oil in India in 2015-16 was around 235 million tonnes. This demand is met from domestic sources and imports. The domestic sources of vegetable oil are of two types viz. primary and secondary. Primary sources include Groundnut, Rapeseed (Mustard), Soybean, Sunflower, Sesamum, Niger seeds, Safflower, Castor and Linseed. Secondary sources include Coconut, Cottonseed, Rice bran, Solvent Extracted Oils and oils from tree and forest origin. Primary sources cover around 60MT of the total demand, while secondary cover 29 MT, thus making domestic supply to meet around 89MT of the total domestic demand. Of this, around 5MT is exported or used in industries. The Net domestic availability of Edible Oils thus remains around 86.37 MT and rest is met through imports. In 2015-16, India imported 148.20 million tons of edible oils. Thus, India has an alarming level of import dependency on oil. This makes it clear that a huge demand-supply gap exists and India is number one edible oil importer in the world. Since the productivity of India is way far behind the World's average and also with that of the country with highest productivity.

The name of Rapeseed mustard is used for oilseed Brassica, *Eruca* and *Sinapsis* which comes under the family Cruciferae or Brassicaceae. The oil content of the mustard seeds ranges from 38- 46% which is used as a cooking medium in Indian cuisine. Mustard oil possesses one of the best fatty acids profile, (low saturated fatty acids (8%), high monounsaturated fatty acids (70%) and alpha linolenic acid (10%). The Rapeseed-mustard cultivation is mainly rainfed and Oilseed Brassica (OSB) is a high nutrient demanding crop but mostly cultivated by small and marginal farmers in nutrient deficient soil of semi-arid regions. Continuous cultivation of OSB in such regions has resulted in stagnation of yield, increased cost of production and decline in factor productivity. This accounts for the main reason behind the low productivity. Poor soil health and lack of adequate quantities of soil macro and micro nutrients are also an important factor contributing to the same. The main reasons are insect-pest infection, disease incidence and poor technology transfer etc. Another important reason which cannot be ignored in the changing climate scenario is non availability of upgraded technology by scientists so that technologies could be transferred to farmer's field successfully. The adoption of recommended practices by the farmers is very low.

Under the scheme of Frontline demonstrations and other related activities of oilseeds funded under NMOOP by DAC&FW, Ministry of Agriculture and farmers' welfare, Govt. of India, over many years has been conducting FLDs under irrigated as well as rainfed conditions on Rapeseed-mustard. The Objectives of the FLDs was to find out gap between potential yield, demonstration yield, extension gap, and technology index.

Materials and methods

The FLDs were conducted for two varieties of Rapeseed-mustard viz. Giriraj and NRCHB-101 in the fields of selected farmers in Varanasi, Mirzapur, Chandauli, Sonbhadra, Ghazipur, Deoria and Jaunpur district over three consecutive years i.e. during winters of2016-17, 2017-18 and 2018-19. Studies were carried out on overall effect of use of two improved varieties Giriraj (IJ-31) and NRCHB-101 over local variety 'Varuna' as well as the improved practices also including line sowing, proper weeding, spacing, sulphur application along with the recommended dose of NPK, integrated disease and pest management and mechanized row sowing.The sowing was done in the first fortnight of October to first fort night of November.

Seeds and basic inputs were provided to the farmers through front line demonstration project. The farmers were guided and the crops were monitored by scientists from Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in every stage of the crop development. The yield data per hectare were collected from both the demonstration and farmers practice by random crop cutting method. Table 1 shows the summary of the FLDs Conducted During 2016-19 and net monetary return and benefit cost ratio was calculated using formula:

Net Return = Gross return - cost of cultivation;

Cost of all inputs was cumulated as **cost of cultivation**;

Yield increase over farmers practices = $\frac{\text{Yield under improved technology}}{\text{Yield under farmer's practices}} \times 100$

Benefit cost ratio = Gross return / cost of cultivation.

Table 2 shows the particulars of materials and methodology followed in Farmers practice and Improved practices.

Table 1. Summary of the FLDs Conducted During 2016-19

Particulars	2016-17	2017-18	2018-19
Name of crop	Rapeseed-Mustard	Rapeseed-Mustard	Rapeseed-Mustard
Type of FLDs with numbers	Whole Package (30)	Whole Package (80)	Whole Package (100)
Name of varieties	Giriraj and NRCHB-101	Giriraj and NRCHB-101	Giriraj and NRCHB-101
Situation:	Irrigated Normal sown	Irrigated, Normal sown	Irrigated, Normal sown
Name of the districts covered	Varanasi, Mirzapur, Chandauli, Sonbhadra, Ghazipur, Deoria and Jaunpur	Varanasi, Mirzapur, Chandauli, Sonbhadra, Ghazipur, Deoria and Jaunpur	Varanasi, Mirzapur, Chandauli, Sonbhadra, Ghazipur, Deoria and Jaunpur
Date of sowing Range:	15/10/2016-10/11/2016	15/10/2017-10/11/2017	15/10/2018-10/11/2018
Date of harvesting	20/02/2017-10/3/2017	20/02/2018-10/3/2018	20/02/2019-10/3/2019

Table 2.Particulars of materials and methodology followed in Farmers practice and Improved practices

S.N	Particulars	Farmers Practice	Improved practices
1	Field preparation	1 deep ploughing followed by planking	One deep ploughing followed by 2-3 ploughing by harrowing planking
2	Variety	Varuna	Giriraj and NRCHB-101 (Improved varieties)
3	Seed rate	Not fixed	5 kg/ha
4	Seed treatment	No treatment given	1 kg seed with 2.5gms Thirum
5	Method of sowing	Broadcasting	Line sowing with spacing of 30cm x 15cm
6	Date of sowing	As and when field is available	Irrigated : 15^{th} October to 10^{th} November
7	Fertilizer dose	No fixed dose	Irrigated: 120,60,60,40; N,P_2O_5,K_2O and S
8	Weeding	Not done	One before 1^{st} irrigation and 2^{nd} after 1^{st} irrigation Pendimethalin 35EC @1 Kg a.i. in 800-1000 lit water per hectare as pre-emergence followed by hand weeding
9	Thinning	Not done	After 15-20 DAS
10	Irrigation	One pre-sowing irrigation and one irrigation after 30-35 DAS	Pre-sowing irrigation, and 2 irrigation one after 30-35 DAS and if there is no effective rainfall then 55-65 DAS
11	Disease and pest control	Not managed	As per need of the crops
12	Harvesting	At complete maturity	When 75% plants show physiological maturity

Results and discussion

Front line demonstrations (FLDs) being conducted under All India Coordinated Improvement Project on Rapeseed & mustard Research play an important role in mitigating the major constraints in rapeseed-mustard productivity. From Table 3 it is revealed that even though cost of cultivation from improved practice (25928.3Rs/ha) was more than the farmers practice (21372 Rs/ha) the net monetary return from the improved practice (47508 Rs/ha) was more than the farmers practice (36867.3 Rs/ha). The seed yield was more when improved practices were followed (20.9 q/ha) compared to the seed yield from farmers practice (16.3 q/ha). The results exhibited remarkable increase in yield as well as NMR (Net Monetary Return) percentage by following improved Practices (IP) over Farmers Practices (FP). Percentage yield increase over farmers practice (YIOFP) was significant and Benefit-Cost ratio (BC ratio) of improved practice (2.9) was found to be more than farmers practice (2.57). Increase in Net Monetary Return means there is increase in farmers' income henceforth apprehension in adoption of new technologies can be overcome. Positive effect of front line demonstration on Production, Profitability, and social impact on mustard cultivation was seen in the average status of mustard production in different districts of Uttar Pradesh.

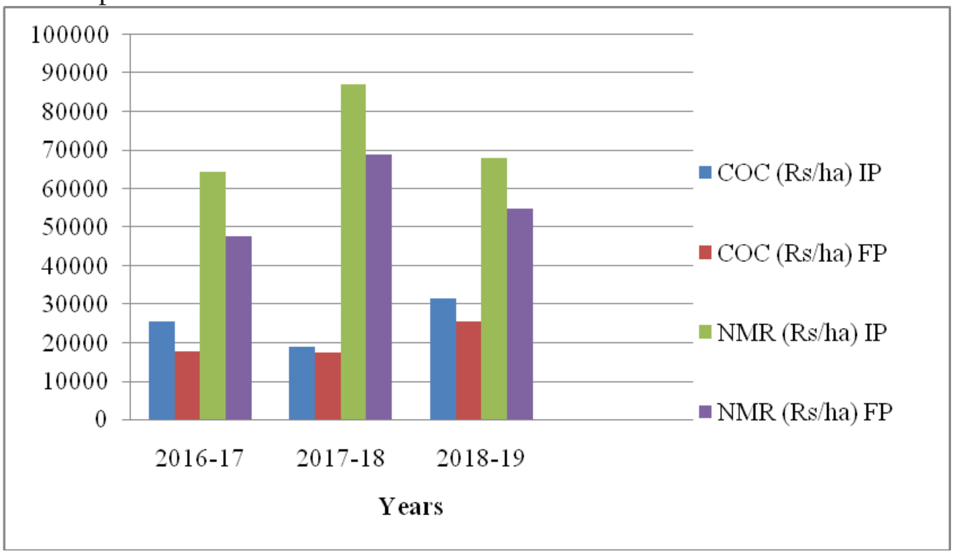

Figure 1: Cost of cultivation and Net monetary return over the years

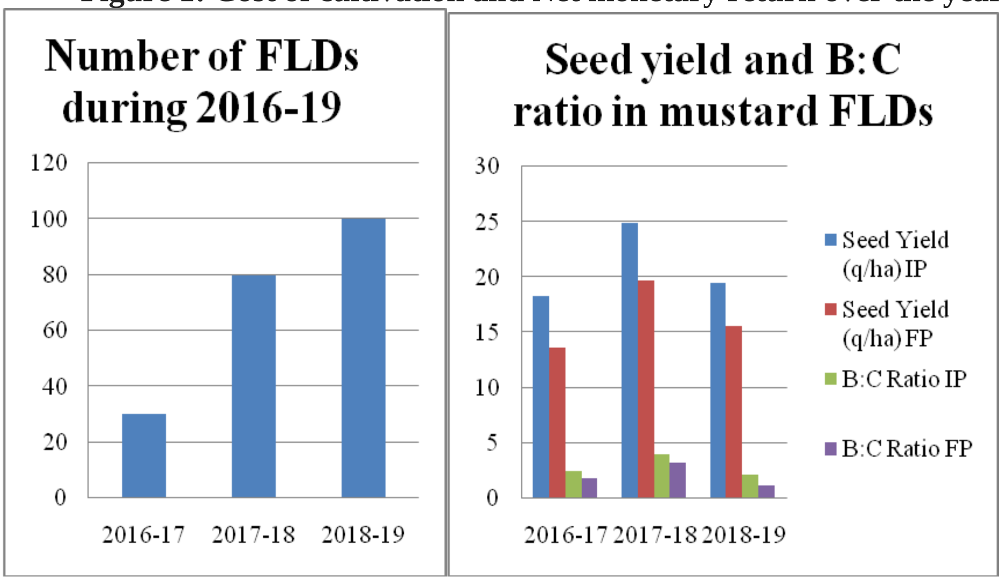

Figure 2. Numbers of Front Line Demonstration conducted Seed yield and Cost Benefit ratio during the period 2016 to 2019

Year	No. of FLDs	Variety		Seed Yield (q/ha)		COC (Rs/ha)		YIOFP (%)	GMR (Rs/ha)		B:C Ratio	
		IP	FP	IP	FP	IP	FP		IP	FP	IP	FP
2016-17	30	Giriraj and NRCHB-101	Varuna	18.35	13.60	25453	20767	34.92	64225	47600	2.51	2.29
2017-18	80	NRCHB-101, Giriraj,	Varuna,	24.86	19.70	21886	17885	26.19	87010	68950	3.96	3.28
2018-19	100	Giriraj, NRCHB-101	Kranti,	19.45	15.62	30446	25466	24.51	68075	54670	2.23	2.14
Average				**20.9**	**16.3**	**25928.3**	**21372**	**28.54**	**73103**	**57073**	**2.9**	**2.57**

Legend: IP: Improved practices, FP: Farmer's practices, COC: Cost of Cultivation, YIOFP: Yield increase over farmers practice, NMR: Net monitory return

Table 3. Results of Front Line Demonstrations conducted during 2016-19

Plate 1: Farmers field conducting the mustard FLDs.

Conclusion

The gap between the import and export can only be decreased only if there in increase in production and productivity. Increase in production and productivity can be achieved by adoption of new technologies and proper package of practices. Farmers should switch to cultivation of newly released, high-yielding, improved varieties with special characteristics such as tolerance to biotic and abiotic stresses in place of poor performing traditional varieties. This way they can get higher returns from same input area. One of the most important non-monetary input which is often ignored is sowing time: timely sowing of mustard results in higher yields due to escape from aphid attack, diseases and high temperature during siliqua development stage. Use of gypsum as a source for sulfur is also recommended since sulfur is one of the important constituents of mustard oil. The FLDs conducted over years suggest that if farmers adopt new technologies such as farm mechanization, line sowing with proper spacing, ensuring water availability at critical stages, integrated nutrient management, use of weedicides, integrated disease-pest management, there would be remarkable increase in the monetary return which ultimately helps to increase their income henceforth contributing toward growth in countries GDP and attaining self-sufficiency in oilseed production.

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THE WINE ROUTES IN SICILY AS A TOOL FOR RURAL DEVELOPMENT: AN EXPLORATORY ANALYSIS

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Abstract

"Food and Wine Tourism" is framed inside wider context of promoting and developing rural areas of a territory. The Wine Routes (alias wine roads) (WR) should allow the promotion of wine tourism and the enhancement of the rural territory, offering a particular "integrated system of tourism supply" that winds along with a specific territory and operates as a "center of multi-services". The Sicilian Wine Roads (SWR) have been established in 1999 to pursue these aims and to offer wine producers a different opportunity to diversify their offer. The Wine Roads (WR) are routes along the wine-growing territories that offer to tourists and visitors the opportunity to take advantage of different complementarian services to the wine, proposing a form of experiential tourism with powerful emotional involvement, as it combines gustative elements with naturalistic (e.g. wine landscape), recreational (e.g. socializing) and cultural ones. This study has the aim to know the current situation of the SWR, identifying their contribution to the Sicilian rural territory, highlighting strengths, weaknesses and treats that still exist. The results have highlighted that "Enjoy the experience of visiting the winery" was the main motivation for tourists, nevertheless, visitors appeared more experienced than in the past and aware of what wine tourism is.

Keywords: *Wine tourism, Sustainable toursim, Experiential Tourism, Motivational study, Rural Tourism.*

Introduction

Definitions of wine tourism abound as the discipline continue to evolve. The Wine Routes (alias wine roads) (WRs) should allow the promotion of wine tourism and the enhancement of the rural territory, offering a particular "integrated system of tourism supply" that winds along with a specific territory and operates as a "center of multi-services". In turn, these routes have been further promoted through the development of gastronomy and the relationship between culture, cuisine, and wine. In this sense, we can speak of a wine escape with the presence of vineyards, wine production activity and the wineries where the wine is produced and stored (Alebaki & Iakovidou, 2010; 2011). The "Wine Route", which combines landscape elements with experimentation and innovation activities, is a distinctive element and it becomes an extraordinary multiplier of value also for other regional products, as well as for the tourism system as a whole. According to other literature, it must be recognized that wine tourism involves both destination planning and marketing strategies (Getz, Dowling, Carlsen, & Anderson, 1999). In fact, the WRs promote a virtuous exchange between corporate interests and territorial policies and targets, aimed at enhancing the value of the territory, well aware that all this has a positive impact on the quality perception of the product by consumers.
The potential for wine tourism is enormous (Byrd et al., 2016), but the interesting issue is to focus on who are the stakeholders in the different wine territories and who stands to gain most, whether and when the effective potential will be realized.
In Europe, the first important studies on wine routes date back to 20 years needle. In one of their paper, Hall and Mitchell (2000) examined wine tourism in different regions publishing a

study on wine tourism on the Mediterranean area;in this study they argued that wine has always been part of the Mediterranean culture and it is an essential element of the so-called "Mediterranean Diet", which was awarded the designation of Intangible Cultural Heritage of Humanity in 2009 (Altamore et al., 2018; Lopez-Guzman 2014). Wine tourism in Europe has primarily been developed through the creation of WRs, with several countries implementing official wine routes (e. g., Greece, Italy, Portugal and Spain). However, in a continent like Europe with such a rich cultural heritage, wine routes are designed to be more than just an opportunity to taste fine wines (accompanied by the local cuisine). Indeed, these routes allow travelers to experience the social, cultural and environmental attributes that lend a distinctive character to each wine route, which although similar in terms of content, are each endowed with their own regional or geographical identity (Bruwer, 2003).

In Italy, the Wine Routes were regulated by a law since 1999, that financed also the birth of more than 179 territorial networks; unfortunately, only about 20 are fully functional and able to generate income while most of them are merely administrative decrees (Colombini, 2015). In the absence of an Italian Ministry of Tourism, wine tourism is regulated by the regions, but wine tourism and the wine routes have always fallen between two areas: agriculture and tourism with the result that neither of them is efficiently managed. The flow of tourists in Italy is observed without any scientific attention to the relationship between tourism in general and oenotourism. The data available for evaluating business generated by wine tourism is very vague and there are no in-depth studies (Colombini, 2015). All the experts of this sector refer to the Osservatorio del Turismo del Vino, drafted by Censis Servizi for the Associazione Città del Vino, which indicates a turnover of 3 to 5 billion of Euros.

Sicily, is one of the most important Italian wine producing regions, and also a particularly popular tourist destination, whose wineries receive about 500,000 visitors annually (Movimento Turismo del Vino, 2018; Associazione nazionale Città del Vino, 2016). The Wine Routes in Sicily propose an authentic and unreplicable identity: the Sicilian Identity alias the "Sicilianity" as a positive value. From a sociological point of view, the Sicilian WRs, are an "integrated system of tourism supply", they represent a paradigmatic case of "social capital" within a defined territory in which investments, experiences and politics must be shared in order to have a united territory wherein public and private subjects work together got the development of a territorial network of growth. The oeno-food-tourism itineraries have represented, therefore, in the last ten years, an important opportunity to trigger processes of participation in development projects of marked territorial importance in Italy. In Sicily, the Federation "Strade del Vino e dei Sapori" ("Wine and Food Routes") was founded few years ago to cluster most part of the existing Wine Routes with the aim to promote Sicilian excellence food products both in Italy and around the world, through an integrated tourist offer able to combine culture, nature, wine, and food. The members of the Federation are the Wine Routes and Wine cellars that aim to enhance the quality and typicality of their products closely linked to the territory of origin as well as "bed and breakfast", hotels, resorts, restaurants and also Municipalities that works for the valorization of their territory. Following recent studies that investigated motivations of wine tourists (Bruwer et al., 2018) and our previous studies (Chironi and Ingrassia, 2008), in this paper we present an investigation on the actual *profile* and motivations of the wine tourist in the Sicilian region of Italy. We wanted to know the role of the 12 Sicilian Wine Routes for the development of wine tourism. The objective was to know the current situation of the Wine Routes in Sicily, identifying the elements that characterize their strategic strength in promoting rural areas, but also their criticality. More particularly, we investigated the motivational factors that drive visitors-tourists to visit the cellars of the Wine Routes highlighting the current characteristics of wine tourism in Sicily.

Material and Methods

For this survey, the reference population is made up of the wineries associated with the Sicilian Wine Roads in 2018. The producers or managers of Marketing, Reception, etc. of the cellars contacted were asked, in advance, whether they were performing reception activities of tourists or visitors, even if not systematically, and whether they were willing to participate in the investigation. Only the wine cellars associated with the Wine Roads where the activity of receiving wine tourists or visitors was carried out and whose managers declared their willingness to collaborate in the investigation, which turned out to be equal to N=65 (census), were identified.

Questionnaire type

In each of the cellars surveyed (N=65), the owners or persons responsible for marketing, communication were interviewed in order to know the type of reception and the level of services offered, the target audience of visitors and the characteristics of the visit to the cellar. Moreover, for the study of motivational factors that lead a visitor/tourist to visit a cellar in Sicily, the interviewees were asked to give an order of preference to the 17 qualitative variables previously identified. Interviews were carried out using a specially structured questionnaire with closed questions, which was given by telephone interview or even sent by email if the interviewees had asked to fill it in at a different time. In addition to a first series of questions about the characteristics of the wine cellar and the visits, the questionnaire contained a list of 17 reasons (variable) for the motivational survey, which should be sorted in order of preference using a scale from 1 to 17 (ranging from 17 points).

For interviews with experts, a "question route" was used, usually structured in the event that qualitative surveys are carried out through Focus Group (Chironi et al., 2017; Ingrassia et al., 2017; Ingrassia et al., 2016) in order to maintain the same course of the discussion, while acquiring, from time to time, the different opinions of the interviews.

Factor Analysis

Factor Analysis (AF) and Principal Component Analysis (ACP) are two techniques used when the researcher's interest is to identify a smaller number of factors underlying a large number of observed variables (Bhunia, 2013). The purpose of the Factor Analysis is not to perfectly reproduce variance, as is the case in the Principal Component Analysis, but rather to simplify the correlation matrix so that it can be explained in terms of a few underlying factors (De Lillo et al., 2007). Therefore the components are real size, the factors are hypothetical dimensions, which are estimated from the observed variables (De Lillo et al., 2007). In this study, we are interested in highlighting the main factors that explain the phenomenon of wine tourism along the Strade del Vino Siciliane, and that summarize the different motivations that drive tourists/visitors to visit the wineries associated with the Roads. Therefore, in this case, the Exploratory Factor Analysis is able to better reveal the underlying dimensions of all the variables considered, since there is no pre-determined expectation as to which and how many factors will be extracted and, even if expectations are expected, these do not in any way influence the analysis (Ingrassia et al., 2016; Stevens, 2012). Following an in-depth preliminary study, the following seventeen motivational variables have been identified.It was not necessary to standardize the data beforehand, because the variables have the same units of measurement, that is, in this case, the values of the scale from 1 to 17 points, so we are imposing the same contribution of the original variables. Despite the fact that this study was carried out on a population, and not on a sample, it was, however, necessary to measure the goodness of the collected data; therefore, as usual in the case of Factor Analysis, the Kaiser-Meyer-Olkin Test (KMO) was applied, which suggests accepting index values at least equal to 0.7 or higher (De Lillo et al., 2007). Rotation is not always possible, but when it can be done, it redistributes the variance individually explained by each factor. The orthogonal rotation must be used under the hypothesis that the underlying factors are not correlated with each other, and, in this case, this hypothesis is acceptable because there is no theoretical reason to assume, in the beginning, that the factors can be correlated. We have therefore turned the factors using the orthogonal rotation technique called Varimax, which is the most widely used rotation technique in the literature (Landau and Everitt, 2004). The statistical software SPSS v. 21 has been used for processing.

Results and discussion

The profile of the tourist/visitor

One-third of the tourists/visitors to the cellars are of foreign origin, with German and American tourists prevalently, while two-thirds are Italian visitors. Visitors go in groups to some particular cellars, that is to say to those that have established a partnership with some tour operators or travel agencies over time, or that have a real activity of tourist reception in addition to the cellar, also made up of real resorts of high quality, thus welcoming tourists from Italy, Europe and the USA.

Usually, wine tourists who come to Sicily are of medium age, between 41 and 50 years old, graduates or graduates, carry out the profession of employees or freelance professionals, love to move in groups and visit the cellars as part of an organized trip. They can choose to make a "do-it-by-yourself" visit with the aim of spending a different day or weekend, but they always choose to move with other people. They are not always connoisseurs of wine, but they are still looking for an element in wine that makes them attractive to other factors that may be of different types and that complete the overall tourist "demand". Most tourists who travel along the Sicilian Wine Routes, to visit the cellars and taste wine, makes an organized trip. A large number of visitors make a simple one-day visit, usually on weekends, usually without a travel agency, but following a do-it-yourself organization, or through an association (of experts in the field or other), a club, a club, an institution or a club to which they belong. The interviewed visitors declared that the main characteristic to chose a wine cellar was the quality of the services offered (reception, cellar guides), hospitality and entertainment of visitors.

Results of the Factor Analysis

Table 1 shows the descriptive statistics relating to the motivational variables evaluated by the interviewees.

Tab.1 - Descriptivestatistics

Variables	Average scores	Std. Deviation
Experience the atmosphere of wine tasting in the cellar	2.45	1.368
Because they know the cellar and the producer directly	5.89	3.940
Know new wines and cellars	4.94	3.594
Get to know this particular cellar	3.50	2.690
They have heard a lot about the cellars and wines of "this area" and I want to know them personally	3.86	3.012
Buy wines in the cellar after tasting them to experience a different sensation from the one you try to buy it in a wine shop	6.14	3.023
Tasting wines and eating typical local foods	6.52	2.612
Have exchanges of ideas with the producer on labels and types of wine	7.94	2.612
Have exchanges of ideas with the producer on wine prices	10.27	1.810
Stay in company with other wine lovers	8.89	2.168
Pleasant aggregative moment with friends and/or relatives	11.11	3.107

Visit the beauty and landscape of the area and taste local products	9.63	2.186
For pure pleasure	12.59	2.029
For Hobby	14.70	1.508
For Business. job opportunities. conventions. etc.	14.73	1.504
It is a pleasant way to spend the weekend	15.08	2.318
Because the cellar is part of a Wine Route and the wine and food route is integrated with the territory.	14.02	3.124

Table 2 shows the total variance explained by the extracted factors and the cumulative variance. In addition, the graph of decreasing auto-values (Scree Plot Figure 14) also shows the identification of the number of useful factors, which in this case were six factors (KMO test equal to 0.871).

Table 2. Initial values of all components, unrotated and rotated factor weights, explained variance (total and cumulative of the six extracted components)

Factors	**Initialvalues**			**Unrotated factor weights**			**Weights of rotatedfactors**		
	Total	% of variance	% of cumulated variance	Total	% of variance	% of cumulated variance	Total	% of variance	% of cumulated variance
1	3.697	21.747	21.747	3.697	21.747	21.747	3.240	19.058	19.058
2	3.121	18.360	40.106	3.121	18.360	40.106	2.756	16.212	35.269
3	2.093	12.313	52.419	2.093	12.313	52.419	2.052	12.071	47.340
4	1.911	11.242	63.662	1.911	11.242	63.662	1.980	11.649	58.989
5	1.426	8.390	72.052	1.426	8.390	72.052	1.773	10.429	69.418
6	1.027	6.041	78.093	1.027	6.041	78.093	1.475	8.674	78.093
7	.840	4.939	83.032						
8	.692	4.070	87.102						
9	.605	3.560	90.662						
10	.429	2.526	93.188						
11	.343	2.020	95.208						
12	.257	1.513	96.720						
13	.240	1.411	98.131						
14	.135	.797	98.928						
15	.094	.556	99.484						
16	.081	.474	99.958						
17	.007	.042	100.000						

The Analysis showed that the first six identified factors highlight the following dimensions:

1) Aggregative

In this case, the first Factor (or component) highlights the highest factor coefficients for the variables *Pleasant aggregative moment with friends and relatives*, *Knowing new wines and cellars* and *Ashobby*, inversely related to *For business occasions*, and *Because they know directly the cellar and the producer*, and for this reason highlights the choice of spending free time in the company of other people known to discover wines and cellars of the territory.

2) Learning

This construct is characterized by variables, *Having exchanges of ideas with the producer on wine prices* and *For pure pleasure*, inversely correlated with *Staying in company with other wine lovers and tasting wines and eating typical local foods*, and highlights the consolidation of a trend that has been going on for several years now, namely the desire to visit the cellars to get closer to the world of wine, learn and know.

3) Oenotourism

The construct is characterized by variables *It is a pleasant way to spend the weekend, Visit the beauties and landscape of the area and taste local products* and *Live the atmosphere of tasting in the cellar* and for this reason, there is a strong tourist connotation in which it emerges the desire to know the territory under all aspects, including that of wine and food.

Factors 4,5 and 6 are those that most represent the size of an experienced and aware wine tourist, in fact, the variables outline the profile of a visitor who is increasingly interested in wines, experience, and atmosphere of tasting in the cellar and to personally know the territories of the wines consumed.

4) Pleasure/leisure

This dimension is characterized by variables, *For pure pleasure* and *Live the atmosphere of tasting in the cellar* and, therefore, it allows to identify the profile of a visitor who goes to a cellar for the pure pleasure of living the atmosphere of tasting, that he/she knows, and and so, the memory of this positive experience will push visitors to return to the same cellar or to a new one, to repeat the experience.

5) New experience

In this dimension the driving variableare, *Having exchanges of ideas with the producer on labels and types of wine*, inversely correlated with the variable *Buying wines in the cellar after having tasted them* to experience a different situation with respect to that of buying the wine in a wine shop.

6) Territory

The sixth and last factor is characterized by the variable *They have heard a lot about the wine cellars and wines of this area and want to know them personally* inversely correlated with *Because the cellar is inserted in a Wine Route* and it is possible to experience a food and wine route integrated with the territory.This dimension characterizes awine tourist who chooses to move, often autonomously, to discover the territory, along routes and roads.

Results of the Experts' interviews

According to the experts, there is still a very wide gap between the expectations of wine cellars associated with the Roads and the activities that the Wine Roads carry out for the purposes for which they are responsible. At present, the routes along the Wine Roads are still uncomfortable, difficult to identify, difficult to follow and without any form of signs or information signage. It is possible to affirm that the problem lies mainly within the Road Wine Associations which, for reasons of internal organization, are unable to operate as they should.

Short discussion

The analysis of the results showed that, certainly, all the dimensions extracted are characterized by a strong hedonic connotation. In fact, in each of these Factors, there are always one or more variables linked to the dimension of "Leasure/Pleasure" and the pleasantness that links the view to the tasting in the cellar.In addition, the results show that the main factors that lead visitors to visit the wine cellars associated with the Sicilian Wine Routes are not closely linked to the fact that the cellars belong to a Wine Route, but are instead the result of ongoing cultural and social phenomena, which show two main motivational lines, the first linked to wine and food tourism in general and the second more closely linked to pure tourism, characterized by tourists/visits. Finally, it is important to note that the fact that the cellars belonged to the WRs was not one of the important reasons that influenced the visitor in choosing the cellar. This confirms the results of the interviews with qualified persons who pointed out that the WRs are difficult routes to identify by visitors due to structural and infrastructural deficiencies and that the visitor becomes aware of the WR in which the cellar is located only after having arrived there. The critical element of the WRs that emerged from this study lies in the fact that today the WRs in Sicily have become only tools on the part of the associates, aimed at intercepting contributions or funding to carry out promotion and territorial communication activities, provided for by the Regional RDP measures, but not specifically aimed at what was the original purpose of the Legislator who designed and established the Wine Roads.

Conclusions

The main results of the research show that the tourists are highly satisfiedwith thewinery visitwhile highlighting the relationshipbetweenwine, local cuisine, and the growing interest of travelers in everything related to wine culture. Results of the Factor Analysis show the main factors that optimally explain the phenomenon of wine tourism in Sicily and highlighted the main motivations that drive visitors to visit the Wine Routes wineries that are: aggregation of wine lovers, learning of new wines, tourism, pleasure of a leasure experience, new experience, visit the territory. The study revealed that unfortunately the Wine Roads in Sicily are still less effective than those in other Italian zones or foreign Countries, because of many reasons linked to the poor interst of wineries and stakeholders to work for a common project of valorization of wines and territory. In order to improve the effectiveness of the Wine Routes in Sicily it would be necessary to detach oneself from the interests purely linked to the economic aspect (financing for communication activities) and return the lost identity of these routes, repositioning them as the Legislator had thought, i.e. as a tool for the valorisation and integration of rural territories around the Wine as a central element of the System. The main contribution of this study is the possibility to replicate the method applied in other similar cases. In addition, Public Bodies and Local Administrations can implement effective actions to improve structures and infrastructures. Cohesion around a shared project can be the best way to improve the Wine Routes in Sicily. A SWOT Analysis might be useful to complete this study.

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MULTIFUNCTIONALITY, A STRATEGIC PILLAR FOR AGRICULTURE IN DEVELOPING COUNTRIES: AN EVIDENCE FROM NEPAL

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Abstract

The approach multifunctionality of agriculture in the developed countries is applied as a means of rural revitalization and economic circulation. Although farm diversification and diversification in the developing countries is much more crucial, in most of the rural areas of the developing countries, it is not performed, and it is delayed. In this regard, the main objective of this study was to explore the potentialities of the multifunctionality of agriculture to support the sustainable rural development in the framework of the sustainable development goals (SDGs 2016-2030) in the developing countries. The empirical study was conducted in two of the rural districts of Nepal called Nuwakot and Rasuwa. The authors intentionally selected these districts because it is one separate block at the northern part of the capital city Kathmandu and connected to China. Further, even though it is located in the nearby capital city, and the rural villages have high potentials for tourism activities, the farmers are still focusing on subsistence farming without getting benefits from tourism. Based on the macro data of Nepal government and field visit, we conducted a case study. The result explored that this block needed the external supports in terms of knowledge, technology, and more importantly, supportive policy. The federal and central government should give support to the farmers so that they can implement multifunctionality in their farms, which leads to sustainable rural development.

Keywords: *multifunctionality, circular economy, SDGs 2016-2030, sustainable rural development, Nepal*.

Introduction

Multifunctionality of agriculture is a hot issue for rural development, and many of the industrial countries practiced it (Ohe, 2001; Ciani *et al*., 2012; Ragkos *et al*., 2015). Recently, the European Union (EU) developed the concept of territory development through the multifunctionality of agriculture (Todorova and Ikova, 2014; Lehman *et al.,* 2009) which means not the only locality of the rural areas but also the areas of cities will prioritize where agriculture is the main occupation. Indeed, the small farmers not only in the developing countries but also in the developed countries are facing many problems like selling their products and the low price even if they could sell the products and which ultimately pushes them towards poverty. Therefore, the farm diversification to generate additional income for the farmers is necessary both in developed and developing countries.

The condition of farmers in developing countries is more serious than in developed countries. It is due to no or very low government support to the farmers (Yu *et al*., 2019), lack of adequate infrastructures and subsidies on the agricultural inputs, traditional farming system, conventional types of seeds used in the farms. These problems are causing low agricultural products and low farm income for the farmers. However, these problems can be utilized as the identity of that particular area and start the multifunctionality. If it is properly promoted, different tourism activities can be launched in that area, and income for the farmers will be automatically generated, and territory development will be implemented.

In this regard, this study aims to explore the possibility of multifunctionality as a pillar for the economic diversification and economic circulation in the developing countries. For this purpose, the authors decided to conduct a case study in Nepal because it is one of the developing countries, and this country can almost represent the characteristics of the other developing countries. Also, the farm diversification in Nepal is more necessary and possible because Nepal recently received the new constitution and got a stable government through the election. The elected government declared "Visit Nepal Year 2020" to double the current number of foreign tourist arrivals at the country and the total number of visitors is expected to cross 2 million in the year 2020. At this moment, exploring the multifunctional activities in the country, first of all, help to diversify the farm income and economic circulations in the rural areas, secondly, it provides additional tourism destinations for the visitors. Ultimately, multifunctionality of agriculture leads to poverty alleviation and SDGs implementation in the developing countries.

Literature review

The concept of multifunctional agriculture emerged in the last decade of the twentieth century in developed countries where the economic importance of agriculture was negligible, and the community was increasingly concerned with the quality of consumed food and the surrounding environment (Todorova and Ikova, 2014). In the meantime, several policies to support the multifunctionality of agriculture, e.g. agri-environmental schemes, started to be implemented in Europe (Lehman *et al*., 2009). Multifunctionality or multifunctional agriculture are terms used to indicate generally that agriculture can produce various non-commodity outputs in addition to food. It is associated with particular characteristics of the agricultural production process and its outputs:

- the existence of multiple commodities and non-commodity outputs that are jointly produced by agriculture; and that
- some of the non-commodity outputs may exhibit the characteristics of externalities or public goods, such that markets for these goods function poorly or are non-existent (OECD, 2003).

Multifunctional agriculture generates relatively much employment per additional unit of output; that is, the employment/production rate is higher in multifunctional agriculture than in primary agriculture (Heringa *et al.,* 2013). Multifunctional activities in many rural areas are providing positive benefits to the farmers (Ohe, 2007). For instance, pluriactivities in the hamlets of Japan helped to prevent the farmland from being abandoned (Ohe, 2001), it affected a range of social, economic and environmental aspects of life on the island of Cyprus (Ragkos *et al.,* 2015). Similarly, the total value of agricultural multifunctionality in Yangtze River Delta in China had increased by 23.2%, which was mainly attributed to a significant increase in food provision and cultural leisure values (Yu *et al*., 2019).

Due to the importance of multifunctional agriculture, in many countries, the multifunctionality of agriculture has been promoted. From the analysis of the territorial system of Antica Volcej in Italy, it has emerged an excellent propensity at diversification. Indeed almost 70% of the firms had at least one added activity to the classic production (Borrelli, 2016). As multifunctional activities contribute positively to the farmer, therefore, Thai citizens are willing to pay for changes toward multifunctional agriculture, as expressed in an increase of their monthly food expenses (Sangkapitux *et al*., 2017). Among the attributes of agri-environmental practices, "*organic agriculture in combination with agro-biodiversity conservation*" garnered the highest preference, followed by "*organic farming as single practice*" and "*Good Agricultural Practice combined with agro-biodiversity conservation*" was suggested for being promoted by the Thai citizens (Sangkapitux *et al*., 2017).

As the importance of the multifunctional agriculture is understood, Yu *et al*. (2019) suggested that the future land use policy should focus on both urban control and promoting agricultural multifunctionality to foster the sustainable development of agriculture in metropolitan agglomerations in China. Ongoing transformation practices, such as land consolidation, should aim to improve the biophysical and socio-economic functions of farmland (Yu *et al*., 2019).
Based on the presented literature, we can claim that multifunctional agriculture is important from different perspectives and it should be introduced in developing countries too. The concept of multifunctional agriculture should also be promoted in Nepal. Thus, we search for its possibilities in Nepal because of the no research on this topic.

Material and methods

Study area
As the central theme of this study is to explore the possibilities of multifunctionality of agriculture in the rural areas to diversify the economic activities in the developing countries. We chose Nepal because it is one of the developing countries, more than 2/3 of the total population are working in the agricultural sector however this sector is contributing less than 27% in the national GDP (MOF, 2018). Thus, the farm income for the Nepalese farmers should be increased, and diversifying farm activities is one of the ways of income generation for the farmers.
In this study, we mainly focused on two districts of Nepal called Nuwakot and Rasuwa; these districts are located on the northern side of the capital city Kathmandu and becoming a separate block. Nuwakot is the neighboring district of the capital city, and Rasuwa has a border with China, and these two districts are crossed by the Pasang Lhamu highway which is proposed as the parts of OBOR (One-Belt-One-Road) initiatives of China government (Bhatta, Itagaki and Ohe, 2019). Similarly, Nuwakot district is connected by Pushpalal Lok Marga (a highway, which connects east and west of the country focusing on the hilly regions). A proposed inter-country railway also goes from these districts. Thus, shortly, this area is going to be a transportation hub.
Further, these districts are famous not only for nature as well as adventure tourism of Langtang National Park and Singla trekking trails but also renowned for the religious attractions like holy lake Gosaikunda, and other 107 sacred lakes of Rasuwa district and Bhairabi Temple, Devighat Temple, Dupcheswor, etc. in Nuwakot district are the representative examples. There are many more popular religious as well as cultural and historical places in these districts. Also, the habitat for the red panda in Lang Tang National Park attracts the visitors. These attractions and accessibilities can be the potential sources of tourists in the diversified farm activities after its establishment in the rural villages of both districts.

Data and analytical methodology
For the completion of this study, two types of data are administered. The table survey and the secondary data was collected from the different websites of Nepal Government's authorized bodies such as the central bureau of statistics (CBS), Ministry of Finance (MOF), and Nepal Rastra Bank. The micro-level data were collected directly from the field survey in August 2017. A total of 64 farmers fully participated in the field survey. By the help of the village representatives, farmers were invited in a specific place for the study purpose, then a small lecture on agritourism development in the village was given to the farmers and individual farmers were interviewed. As this study is mainly qualitative, we applied mostly the descriptive analysis of the data and conducted a case study of the village based on the primary and secondary data collection.

Results and Discussion

Both Nuwakot and Rasuwa districts have a significant number of people working in the agricultural sector (Table 1). In Nuwakot, 97% of the household's primary source of income is agriculture, whereas, in Rasuwa, it is around 91%. This data clarifies that most of all the people of the village are somehow depending on agriculture, and their income level is not good enough. As they are farmers, they focus more on farming, and education was not their priority, which resulted in the lower literacy rate in both districts. Around 40% of the people are illiterate, and those who are literate have only some primary level of education. Female educational level is much lower compared to the male in both districts. Similarly, the number of female-headed households is only around one-fifth in both districts.

Table 1: Statistics of the two districts.

Item	Nuwakot	Rasuwa
Total farming population	264498 (person) 95.32% (percent) 53984 (holdings)	43798 (person) 87.30% (percent) 8504 (holdings)
Household whose main income is from agriculture	97.1%	91.30%
Literacy rate	59.8% (M=68.0%, F=52.4%)	59.80% (M=67.95, F=52.41)
Female-headed household	21%	22%
Household head is a farmer	51821 (95.98%)	8168 (96.04%)
Agricultural land area	32992 Hectares	4557 Hectares
Total forest area	43%	31.4%
Evergreen snow area	-	16.6%

Source: Central Bureau of Statistics - Nepal (2017).

The role the household head plays is essential for the multifunctional activity in agriculture. More than 95% of the household heads of both districts are farmers, and their main source of income is farming. Thus, we can assume that the farmers are willing to develop the multifunctionality of agriculture to increase their income. Based on the result of the previous study conducted by Bhatta and Ohe (2019), we can say that the farmers of the rural areas will be willing for the multifunctionality of agriculture because the authors presented that farmers of rural Nepal where agritourism does not exist were willing to establish agritourism in their ordinary farms.

Both districts have plenty of natural attractions, especially mountains and jungles. These jungles can be utilized for the major attractions in the villages. The buffer zone of Chitwan National Park is used for the jungle safari, Tharu cultural trail to the local communities is developed which increased the numbers of tourists in the nearby villages (Poudel, 2014). Similarly, the jungles and buffer zone of Lang Tang National Park can be used as a complementary tourist attraction for the tourists.

Our survey (Table 2) showed that at least one person from 45.3% of households is either working in foreign countries currently or had worked in those countries for more than one year as a temporary worker. The experience of the migrant returnee can be employed for the multifunctionality purpose because a study conducted by Bhatta and Ohe (2019) statistically proved that those who worked in the foreign countries more than one year are more likely to start agritourism in their home village. Working as a tourist supporter was also an additional supportive factor for the diversification of the farm activities as our survey shows that 87.5% of the farmers have experience working as a tourist's supporter. That means the farmers are already aware of the tourism environment, and they also know the pros and cons of tourism.

Only 54% of the farmers said they have enough farm products to sustain their life, which means the remaining 46% of the farmers need some extra income to run their daily life.

Table 2: Attributes of farmers.

Item	Percentage	Sample size
At least one person worked in a foreign country	45.3%	64
Experience working as a tourist supporter	87.5%	64
Sufficiency of farm products	54.0%	64
Interested into new jobs	50.0%	64
※Regular student	41.6%	334
Handicrafts	50.0%	64

Source: Field survey Aug. 2017.
Notes: ※includes the information of the family members of the respondents and sample size only for this variable is 334.

On the other hand, 50% of farmers said that they want to challenge new job, farmers who are willing to move towards new jobs can be employed in the multifunctionality of the farms. As a supplementary product of the farm, 50% of the respondents said that they are still producing some kinds of handicrafts in their homes. If we can monetize them, the young generation will also be attracted and contribute to sustainable development. Moreover, 41.6% of the total sample are regular students. Based on this data, we can expect that if the farms start generating money, the new generation will also be attracted to farm activities.

Table 3: SWOT analysis for the possibility of multifunctionality in the northern block.

Strengths • Plenty of attractions (natural, cultural, historical and religious) • Significant numbers of people are in agriculture • Some areas are performing specialized farming like rainbow trout fish farming, goat, yak farming and so on • Forest area and evergreen snow-covered landscape, Lang Tang National Park, evergreen snow-covered mountain range view	**Weaknesses** • Poor infrastructure • Small landholdings • Lack of coordination between the government bodies • Low educational level of the farmers
Opportunities • Connected to China and the capital city • Crossed by the national highway and proposed train league between Nepal and Japan • 3rd popular tourist destination in Nepal including two popular trekking trails and one cultural trail • Good geography (from the plain land to the mountain 7270m) • Ongoing transportation network development projects	**Threats** • Young people do not like to work in agriculture • Easy to go to foreign countries especially for the temporary job and payment is comparatively high • Fear of losing culture and tradition

Source: Authors elaborations based on data collection.

Conclusions

In this study, the authors first defined why multifunctionality of agriculture is a crucial pillar for developing countries, and later on presented how farm diversification is possible in one of the rural blocks of Nepal. The authors gave a case study based on both primary and secondary data and explored that the rural villages have the potential for multifunctional agriculture.

Also, they defined SWOT for the multifunctionality of farms in the villages and its possibilities.
This study explored that the rural block of Nepal has possibilities of multifunctionality of agriculture. The authors can claim that numbers of household heads working in agriculture is an opportunity. Similarly, farmers who are willing to challenge to start new works can be another opportunity. The available attractions (natural, religious, cultural, historical) support the additional activities in and around the territory and should be promoted from the tourism point of view.
Based on the result of this study, the authors suggest the following implications for the policymakers. For the multifunctionality of agriculture, the adequate infrastructure development, capacity building of the farmers should be done. More precisely, view towers in a different destination increase the numbers of visitors in the rural areas. The additional supportive activities can be developed in the nearby jungles, and the trekking and hiking in the snow-covered areas can be employed. The different villages can be connected in a single trail, and the agritourism trail can be developed where tourists can enjoy many different cultures and traditions of the diverse communities. For instance, the red panda trail, agritourism trekking trails, the multi-ethnic cultural trail can be developed by joining multiple villages in the single package according to their interests. This study deals only with the macro data, the micro-level survey, and the capacity of the farmers on hosting tourist and implementing multifunctionality should be the next step of the study.

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THE ECONOMIC AND SOCIAL EFFECTS OF LIGHTING DEVELOPMENT IN THE FIELD OF FISHING ON THE PRODUCTIVITY OF FISH WEALTH IN THE GAZA STRIP

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Abstract

The present study aimed to identify the impact of the introduction of modern innovations of marine lighting in the Gaza Strip on the socio-economic aspects of the fishing sector. The study used the analytical descriptive method to identify the effects of marine lighting on the fishing sector. The study relied on the secondary and primary data collection, where the method of literature review related to the research subject was used to form a theoretical framework for the subject of the study. Moreover, the questionnaire was used as a tool to collect primary data: over the study population of 300 fishermen in Gaza city, 120 questionnaires were distributed and 85 valid forms were retrieved for analysis purposes. The most important result was the presence of a significant effect between the aspects of development in the field of marine lighting in the Gaza Strip, the size of fisheries production, and the cost of marine production. Moreover, marine lighting resulted in the development of different aspects of marine management, including the volume of fisheries production in the Gaza Strip of 0.50: the expansion in the field of marine luminescence and double use would contribute to increases in the volume of fish production by 50%. In addition, the expansion of marine lighting and the doubling of its use would contribute to the reduction of marine production costs by 65%.

Keywords: *fishing sector, marine lighting, development, Gaza Strip, production*

Introduction

Fishing is one of the most important activities within the Palestinian agricultural sector. Despite the limited fishing area and the low availability of land for aquaculture, fish stocks contribute to the production of food of the Palestinian people, as well as the absorption of a part of the labor force in the agricultural sector. Workers in the fishing industry in the Gaza Strip account for about 36 thousand people (Shawa, 2017, p. 31).

Since 2001, the Gaza Strip has suffered from a shortage of local production of fish, which has led to the inability to meet the local needs of fish and thus resulting in the decrease of local consumption of fish for the citizens of the Gaza Strip. The shortage was accompanied by a decrease in the Gaza Strip per capita consumption of fresh approximately 4.1 kg per year for 2016. To compare with per capita global fish consumption, the Food and Agriculture Organization of the United Nations (FAO) recommended that the individual should consume 13 kg per year of fish as a minimum, indicating a deficit in fish production accompanied by a decrease in the per capita share of fresh fish in Gaza Strip. Gaza Strip produced 3305 tons of fresh fish in 2016, while Gaza Strip imports amounted to 3998 tons of fresh and frozen fish to cover the deficit in local production (Shawa, 2017, p. 5).

The low volume of local fish production in the Gaza Strip is due to a combination of internal and external factors that directly affect harvesting. Perhaps the most important external factor is the limited area for fishing allowed by the Israeli occupation, which reaches up to 9 miles from the coastline. As for the internal factors, they are concentrated in the weakness of the capabilities and equipment necessary to carry out the fishing process on the one hand, and the lack of fishermen to the advanced experiences that allow them to practice the profession of hunting professionally on the other (Salman, 2017, https: //www.alaraby.co.uk). As the work of the fishing sector requires the fulfillment of a range of important aspects that are not applicable to the fishing sector in the Gaza Strip. The most important health examination procedures and certificates of competence and vocational training and others (International Labor Conference, 2004, p. 42). In view of this difficulty experienced by fishermen in Gaza Strip, a number of them have emerged as important innovations and innovators in the fishing sector. These fishermen have overcome many obstacles in the fishing process and in some cases have increased the productivity of this important sector and contributed to increasing their contribution to agricultural production in the Gaza Strip.

In this context, the process of developing novel or more distributed uses of the central lighting for marine fishing through the introduction of generators on the central ships and supply these ships with LED lamps is an important process to evaluate the technique consists in the distribution of light on small ships and boats that have become operational in the vicinity of the central energy source. This technology has been the most important alternative to the traditional techniques that were previously adopted and based on the use of small lamps working with kerosene and cooking gas. These comburents, however, are very expensive and limited the productivity of fish in the Gaza Strip (Nizar Ayyash, interviewed by Doaa Hussein, September 9, 2018). This study is considered important because it diagnoses the aspects of innovation and development in marine fishing supplies in the Gaza Strip and it shows their significant impact on the development of various maritime work within this sector.

Materials and methods

1. Scope of study

2.1 Spatial Limit

The present study was carried out on the State of Palestine. The Gaza Strip was targeted as one of the important components of the State of Palestine because it has a seacoast overlooking the Mediterranean Sea that distinguishes it from the rest of the Palestinian areas. The focus was on targeting Gaza city as one of the most important components of the Gaza Strip. The current research focused on the agricultural sector as one of the main productive sectors of Gaza Strip.

2.2 Time Limit

The present study targets the fishing sector in Gaza City during the year 2018.

2.3 Human Limit

Staff in the marine fishing sector have been the main component of the current study community, and technical professionals in the fishing profession have been targeted as the direct element engaged in central lighting technology.

2. Activity description

The research activity focuses on exploring the areas of innovation and development in the fishing sector, where the fisheries sector represents the most important branches of the Palestinian agricultural sector. The Palestinian agriculture sector faces a number of challenges related to the introduction of raw materials through the problems of agricultural infrastructure. Studies indicate that the Palestinian agricultural sector, specifically in the Gaza Strip, suffers from many structural weaknesses that led to a decline in the productivity of this sector. The contribution of fishing to the GDP decreased from 32% in the year 1994 to about 6% in 2017. These difficulties were associated with the fishing sector, which is an important component of agricultural food production. The fishing productivity is the focus of this research activity which monitors aspects of innovation for this branch of the agricultural sector. Monitoring the impact of the development of lighting on the productivity levels in the field of fisheries in the

Gaza Strip, as developed in this study, allows to achieve a number of sub-objectives, namely, this study allows to:

- Explain the reality of fish production in the Gaza Strip before the introduction of lighting technology and after the real use of this innovation.
- Understand the impact of the development in the field of lighting on the cost of marine products in the Gaza Strip.
- Identify the effects of development in the field of marine luminance on the social aspects of workers in the fishing profession in the Gaza Strip.
- Demonstrate the main implications of the use of creative thinking among fishermen in the field of marine lighting for fishing in the Gaza Strip.
- Provide a set of recommendations to the relevant parties in the fishing sector to develop local innovations and activate them in the fields of fishing in the Gaza Strip.

The Researcher relied on the exploratory approach and used the analytical descriptive approach to construct a clear vision about the effects of innovation and development in the field of sea fishing.

The dimensions of the study tool were limited to four different dimensions to study the effect of innovation in the field of marine lighting and its reflection on the reality of the fishing profession in the Gaza Strip. These dimensions included the following:

The first: Dimension of marine luminescence

This dimension includes the monitoring of the development aspects of the marine lighting used in the fishing process in the Gaza Strip. This dimension included a set of specialized questions that monitor the nature of the development process introduced by the fishermen in the use of electricity generation techniques necessary for the fishing process and the feasibility of this development and their professional preferences in this scope.

Second dimension: The productivity of fish fresh

This dimension includes monitoring the impact of the use of marine luminaires developed on the volume of fisheries production in the Gaza Strip. This dimension included determining the nature of the opinions of marine fishing professionals about the contribution of lightening in increasing the size of marine fishing and its reflection on the volume of fish production in the Gaza Strip.

Third dimension: Marine production cost rates

This dimension includes an objective assessment of the contribution of the development in the field of marine lighting to the cost of marine fishing. This dimension included a number of specialized questions related to the size of the cost of marine fishing, especially in relation to the lighting and fuel used before the introduction of new development techniques and the current reality after using innovative techniques in the field of lighting for fishing.

Fourth Dimension: Maritime Management

This dimension is an objective evaluation of the impact of marine research in marine management. This dimension includes a set of special questions that monitor these effects on improving the flow of fishing business, the extent and distribution of fishing activities, and the extent to which this technology contributed to Introducing administrative concepts in the fishing profession based on concepts of job skills to a central source of lighting that can be worked in its surroundings by various teams of fishermen.

These dimensions were based on the interrelationship between the various activities of innovation and their impact on the economic and social environment within the Gaza Strip. In addition, one of the most important aspects of innovation and development in the field of fishing in the Gaza Strip is the impact of this innovation on other economic sectors. For example, the sector involved in the development of the marine luminaires, that does not represent an important effect for other countries but is relevant in the Gaza strip where vast unemployment is a critical issue. However in the Gaza Strip environment, which lacks the means and equipment necessary for fishing as a direct result of the Israeli occupation of the Palestinian territories and the siege imposed on the Gaza Strip from the outside world, this development is an important innovation from the point of view of workers in the fishing sector and those responsible for organizing this important sector within the Gaza Strip. In order to determine the nature of this innovation and development in the field of marine lighting, the candidate relied on the use of the questionnaire as a tool to analyze the detailed aspects related to it, and to identify the direct and indirect effects of this innovation on the economic and social aspects of workers in the fishing profession. The candidate has tested the validity and reliability of the approach (questionnaire). The results indicate that the questionnaire has a high capability to describe measure or evaluate a target issue, and the high degree of honesty indicates the absence of methodological errors in the method. The questionnaire reflects the real concept and allows measuring it on an appropriate scale. While the stability means to what degree can rely on the study tool to ensure the same results in the repeated applications. If the use of another researcher for the same study tool under the same conditions will achieve the same results, indicates, for instance, a high degree of stability and repeatability of the approach (Jonathan Weiner, 2007). After conducting the tests for the honesty of internal consistency, which reflects the size of the correlation between the paragraphs (question questions) for each of the different dimensions of the questionnaire form (marine light, fisheries productivity, marine production cost rates, marine management). Questions are checked for availability by calculating correlation coefficients between the score of each paragraph and the total score of the dimension to which it belongs. This test found the internal consistency between the paragraphs and axes belong to them at the level of statistical significance 0.05, except for the sixth paragraph of the first axis and the first paragraph of the third axis where they were excluded from the analysis. Table (1) shows the result of this test used to measure the degree of stability (Jonathan Weiner, 2007). By calculating the correlation coefficients between the score of each paragraph and the total score of the dimension to which it belongs, allowed to test for the internal consistency between the paragraphs and axes that Belong to them at the level of statistical significance 0.05. Results indicate that the correlation coefficient was significant for all paragraph, except for the sixth paragraph of the first axis and the first paragraph of the third axis that, therefore, were excluded from the analysis. Table (1) shows the result of this test.

Table 1. The validity coefficients illustrate the internal consistency of the Dimension study

First dimension: central lighting					
Paragraph	Coefficient of correlation	Level of significance	Paragraph	Coefficient of correlation	Level of significance
A1	*0.752	0.000	A7	*0.248	0.022
A2	*0.474	0.000	A8	*0.739	0.000
A3	*0.680	0.000	A9	*0.354	0.000
A4	*0.700	0.000	A10	*0.604	0.000
A5	*0.790	0.000	A11	*0.493	0.000
A6	0.171	0.117			
Second dimension: The productivity of fisheries			Third dimension: Marine production cost rates		
Paragraph	Coefficient of correlation	Level of significance	Paragraph	Coefficient of correlation	Level of significance
B1	*0.494	0.000	C1	0.202	0.065
B2	*0.779	0.000	C2	0.692*	0.000
B3	*0.609	0.000	C3	0.644*	0.000
B4	*0.658	0.000	C4	0.749*	0.000
B5	*0.718	0.000	C5	0.605*	0.000

Fourth Dimension: Maritime Management		
Paragraph	Coefficient of correlation	Level of significance
C1	0.826*	0.000
C2	0.826*	0.000
C3	0.779*	0.000
C4	0.795*	0.000
C5	0.770*	0.000

*significance at the level of 0.05

The stability of the study instrument (the questionnaire), was measured by the Alpha Cronbach and Jattman indices for the midterm segmentation that was calculated and reported in Table 2. The values of the Alkronbach coefficient ranged between the dimension of the study (0.481 - 0.858). This indicates that there is a good degree of consistency in the data collected from the sample. It is, therefore, possible to rely on it, analyze it, interpret its results and disseminate it to the study community.

Table 2. Results of tests of the stability of the study Dimension

Dimension		Number of items	Cornbrash's Alpha	Split half Reliability
	First dimension: central lighting	11	0.759	0.868
	Second dimension: the productivity of fisheries	5	0.661	0.569
	Third dimension: Marine production cost rates	5	0.481	0.621
	Fourth Dimension: Maritime Management	5	0.858	0.885

As for the statistical description of the individuals of the sample of the study based on personal data, the results showed that most of the workers in the sector of fishing in the category non holders of university degrees, as 74.8% have obtained degrees below the secondary certificate, While the proportion of educated class of holders of scientific degrees working in the profession of fishing 12%. It is noted that the sample members of the study of workers in the profession of marine fishing are the owners of over ten years' experience, where this group accounted for 46% of the study sample. In addition, most of the workers in the fishing profession under the age of 40 years, which accounted for 59.9% of the sample which is associated with the nature of maritime work, which requires a high physical effort compared to other occupations, Finally, most of the study sample consisted of married family members as the table (3).

Table 3. The statistical description of the sample of the study according to personal information

Demographic data		Total (N=85, P= 100%)
Age	Less than 30 years	(39, 45.9%)
	From 30 years to less than 40 years	(20, 23.5%)
	From 40 years to less than 50 years	(11, 12.9%)
	50 years and over	(15, 17.6%)

Marital status	Married	(60, 70.6%)
	Single	(21, 24.7%)
	Widowed	(3, 3.5%)
	Absolute	(1, 1.2%)
Years of Experience	1 to less than 5 years	(17, 20%)
	5 years - less than 10 years	(22, 25.9%)
	10 years - less than 15 years	(15, 17.6%)
	15 years and over	(31, 36.5%)
Qualification	Diploma and less	(9, 10.6%)
	BA	(1, 1.2%)
	Postgraduate	(1, 1.2%)
	Other *	(74, 87.1%)

* Other educational qualifications (elementary, preparatory, public high school)

Results of analysis of axes of the study tool

The results of the analysis of the axes and the dimensions of the questionnaire indicate that there is a positive trend in the respondents' opinions on the importance of development in the field of central lighting and its various implications on both the productivity of the fishery and its associated costs. Moreover, respondents indicate that central lighting contributes to the development of marine management practices associated with the more benefits obtained by the innovation. Table (4) Explains this.

Table 4. Trends of Respondents on Question Areas

Dimension	Number of items	M	SD	RII	T	Rank
First dimension: Central lighting	10	4.04	0.52	80.8%	*18.4	4
Second dimension: The productivity of fisheries	5	4.41	0.48	88.3%	*27.0	1
Third dimension: Marine production cost rates	4	4.37	0.53	87.4%	*23.5	2
Fourth Dimension: Maritime Management	5	4.28	0.66	85.6%	*17.7	3

Hint: M=Mean of answers, RII=Relative Importance Index ((Mean/5) *100%), SD=Standard Deviation., T = statistics of one sample T-test about (3), and *sig = significance of the test

Table 4. Shows that 80.8% of the participants show positive tendencies towards adopting the areas of development in the marine lighting due to a number of considerations related to the contribution of this technology including the belief that this technology will simplify the work procedures and increase the economic return. The results showed that 88.3% of the workers in the field of marine fishing believe that the introduction of lighting technology central will contributed to increasing the productivity of fish wealth, and 87.4% of fishermen believe that the development and use of central lighting will contribute to the reduction of costs of marine production. Finally, 85.6% of the workers in the marine fishing profession believes that the use of central lighting has contributed to the development of aspects of marine management.

Testing hypotheses

The effect of the independent variable (central light) on the dependent variables (fish production, marine production costs, and marine management) was studied using the simple linear regression model. The null hypothesis is statistically tested (H), which assumes no significant effect on the alternative hypothesis (H1). The test result is judged based on the value of the measured significance level of the test (Sig) where the null hypothesis is rejected and the hypothesis is validated if the Sig is less than 0.05. It is said that the test with the null hypothesis is accepted if the value of Sig is higher than 0.05 and Nesting when there is no statistically significant effect. Table (5) shows the results of the estimation of the simple linear regression model.

Table 5. Results of estimation of the simple linear regression model

Independent variable "Central light"								
#	Dependent variables	Pearson the correlation coefficient (r)	Fixed limit (a)	Regression coefficient (B)	Coefficient of determination (R2)	The quality of the model at level 0.05		
						F	Level of significance (Sig.)	The result
1	Fisheries productivity	0.54	2.357	0.508	0.307	36.82	0.000	Significant
2	Marine production cost rates	-0.639	1.732	-0.655	0.409	57.39	0.000	Significant
3	Maritime Administration	0.587	1.307	0.735	0.345	43.12	0.000	Significant

* Significant at level 0.05

The first main hypothesis

The first main hypothesis is that "There is a statistically significant effect at the level of significance ($\alpha \leq 0.05$) in the central lighting on the production of fish wealth in the Gaza Strip,"

The results of the analysis of regression coefficients indicate that the doubling of the use of central lighting technology from the current situation will contribute to an increase in the productivity of fish stocks by 50.8%. Results indicate that there is a linear correlation between the central lighting and the productivity of fish wealth in the Gaza Strip with a correlation coefficient of 0.554. It is important to note here that the level of significance of the F-Test was 0.000, which is less than 0.05. A simple linear regression that measures the effect of the central luminance, thus, indicate a significant effect on the fish wealth due to the central light variable at the level of significance 0.05.

This finding confirms the validity of the first hypothesis which states that "there is a significant statistical effect at the level of significance ($\alpha \leq 0.05$) in the central lighting on the production of fish wealth in the Gaza Strip,”

The second main hypothesis

The second main hypothesis is that "There is a statistically significant effect at the level of significance ($v \leq 0.05$) in central illumination on the cost of marine products in the Gaza Strip,”

Table 5 shows a positive effect of the development process in the field of marine luminance on the reduction of the costs of marine products in the Gaza Strip. The results of the analysis of regression coefficients indicate that the doubling of the use of the central lighting technology from the current situation will contribute to reducing the costs of marine production by 65.5%, there is a linear correlation between the central lighting and marine production costs in the sector (correlation coefficient 0.639). It is important to note here that the level of significance of the F-Test was 0.000: this indicates the significance at the mean level of 0.05 of the simple linear regression model, which measures the effect of the central illumination on marine production cost rates. This finding confirms the validity of the second hypothesis which states that "There is a statistically significant effect at the level of significance ($\alpha \leq 0.05$) in the central illumination on the cost of marine products in the Gaza Strip."

The third main hypothesis

The third main hypothesis is that "There is a statistically significant effect at the level of significance ($\alpha \leq 0.05$) in the central illumination of the marine administration in the Gaza Strip," The results of the analysis of regression coefficients indicate that the introduction of central lighting technology has contributed to the development of the management practices of the marine fishing sector. As the doubling of the use of this purification from the current situation will contribute to the positive increase of the administrative practices related to maritime work, which will contribute to the development of these practices by 73.5%. There is a linear correlation (Pearson coefficient 0.587) between the central lighting and marine management in the Gaza Strip. It is important to note here that the level of significance of the test (F-Test) was (0.000) which is less than the level of 0.05, and this indicates the significance of the simple linear regression model.

This finding confirms the validity of the third hypothesis which states "There is a statistically significant effect at the level of significance ($\alpha \leq 0.05$) in the central illumination of the marine light in the Gaza Strip,"

Conclusion

Gaza Strip suffers from a lack of local production in fish, which has led to the inability to meet the local needs of fish; this developed into a reduction in the consumption of domestic fish production for the citizens of the Gaza Strip, accompanied by a decrease in per capita consumption of fresh fish meat per year compared to per capita world level. The annual per capita fish population in the Gaza Strip is about 4.1 kg for the year 2016. The Food and Agriculture Organization of the United Nations (FAO) recommended that the individual should consume at least 13 kg per year of fish.

There are a number of the main reasons for the decrease in the size of the local production of fish in the Gaza Strip, which include the limited area of fishing allowed by the Israeli occupation (limited to 9 miles from the coast), as well as the poor capabilities and equipment to carry out professional fishing.

The results showed a positive trend in the respondents' opinions on the importance of development in the field of central lighting and its various implications on both fish productivity and related costs, as well as its contribution to the development of marine management practices associated with this development.

The development of marine luminance is one of the important innovations at the level of the Gaza Strip, which contributed to the increase of the catch of fresh fish by 50% Where the quantities of production increased from 1,252,353 kg in 2010 to 2,504,706kg in 2015, according to statistics issued by the General Directorate of Fisheries in the Ministry of Agriculture in the Gaza Strip. The development of marine lighting techniques has contributed to the economic empowerment of marine fishing professionals in the Gaza Strip by increasing their income rates by between 30-50%, which supports the overall sustainability of work in the maritime sector. There is a significant statistical significance for innovation and development in the field of marine lighting on the volume of fisheries production in the Gaza Strip, as doubling the use of lighting technology from the current situation is believed to contribute to raising the productivity of fisheries by an average of 50.8%.

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FINANCING NON-AGRICULTURAL ACTIVITIES IN THE RURAL REGIONS OF ROMANIA: A REAL SOLUTION FOR RURAL DEVELOPMENT AT THE BEGINNING OF THE SECOND DECADE OF EU MEMBERSHIP?

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Abstract

The current paper intends to present the non-agricultural rural economy from Romania. Following the end of the centralized communist economy, Romania still lacks a private sector strong enough in order to ensure production and services at an appropriate level. That is why the financing of non-agricultural activities through National Rural Development Programme (NRDP) was one of the requested financing lines by the beneficiaries. For that purpose, the present paper intends to present the situation of the European funds available for the financing of non-agricultural activities for the 2014 – 2020 financial period using the publicly available information. The financing line analysed is the Sub-measure 6.4. Investments in the creation and development of non-agricultural activities. The paper would use a quantitative method in order to emphasize the interest of potential beneficiaries toward this type of activities. That would provide enough empirical evidences concerning the interest of potential beneficiaries of the financing.

Keywords: *NRDP, non-agricultural activities, financing, Sub-measure 6.4.*

Introduction

Traditionally the agriculture has formed the mainstay of rural economic activities, but this has changed in the second half of the twentieth century toward a much more balanced and diversified economy as more and more labour was driven out of the agricultural sector into employment in alternative sectors (Smit et al., 2015). Rural economic activities in Romania are very little diversified, the economy being dominated and dependent on agricultural activities. Non-agricultural activities are relatively under-represented, being dominated by the primary sector, mainly related to the exploitation and processing of natural resources (Gavrilescu, 2006). As far as the business environment is concerned, it is poorly represented in rural areas. Generally it can be noticed that after the destruction of the local economy specific to the communist regime, it has not emerged a strong enough private sector in order to take over the labour force and to ensure production or services at an appropriate level (Stănică, 2016).

The development of the non-agricultural enterprises sector in rural areas is a priority for Romania, as this could reduce the rural under employment, improve the use of local resources, increase the incomes in rural areas, and ensure a higher standard of living for the rural population (Kerekes et al., 2010). Rural development in Romania has been and is one of the most debated topics since the pre-accession period in the context of the Common Agricultural Policy (CAP), namely of the support that our country had benefited and still benefits through the 2nd Pillar, that of rural development, support related to the National Rural Development Programmes (NRDP) funded through the European Agricultural Fund for Rural Development (EAFRD). These ambitious programmes of high complexity, mainly financed by the EU, aim at stimulating the development and the territorial cohesion. Referring to the least developed regions in Romania, it will be rather an effort to stop the potential growth of disparities (Dachin, 2008).

The rural areas development in Romania and the other European countries can only be achieved on sustainable principles, in agreement to new challenges of contemporary civilization. This development pattern needs, however, adequate public policies, based on reality knowledge and adequate implementation (Burja and Burja, 2014). For developed communes that are economically and socially connected to nearby cities, the problem of dependence on agricultural activities seems to be overcome, many of which already having diversified economic activities, and the trend for the next period is to accelerate these processes (Mihalache, 2013). The financing of non-agricultural activities within the NRDP 2007-2013 and 2014-2020 by the related measures and sub-measures was one of the most requested directions by the potential beneficiaries of these types of funds, even though between the two programs had appeared a series of changes concerning the type of activities considered eligible for funding, respectively of the non-reimbursable support.

In the 2007-13 financial exercise, according to a scientific paper (Mack et al., 2018), it is found that: public support for business creation in non-agricultural activities (e.g., the public encouragement of tourism activities) has the strongest positive effect on rural vitality of all analysed measures. A healthy and diversified rural economy offers job opportunities outside of farms as well as social, economic and cultural services that attract and retain people in rural areas (Kerekes et al., 2010). In Romanian rural areas, entrepreneurship still encounters a large number of vulnerabilities and there is an important need to create the means for the rural entrepreneurs to have access to finance, professional advices and support and to benefit from a better and modernized infrastructure (Dan et al., 2017). The lack of a realistic vision of the future of the Romanian village, of the new occupations and qualifications required in the process of diversification of activities - to establish a multifunctional rural development - is becoming more acute (Dona, 2010).

Taking into account the aforementioned, the paper presents the analysis of the situation of the European funds related to the European Agricultural Fund for Rural Development (EAFRD) available for non-agricultural activities in the multiannual financial framework 2014-2020 up to the present moment. The analysis was made to highlight both the interest of rural applicants for starting a non-agricultural activity and to diversify their activity at the level of agricultural exploitation, depending on the total value allocated to Sub-measure 6.4 and the possibility of financing these activities under NRDP 2014-2020, as the main source of European non-reimbursable financing.

Material and Methods

The aim of the present paper is to present the situation of the European funds related to the sub-measure 6.4 *Investments in the creation and development of non-agricultural activities*, a sub-measure financing both the creation and the development of non-agricultural activities in the rural area, in the 2014-2020 financial exercise, funds from which Romania benefits through the Pillar 2 of CAP that of Rural Development.

Its elaboration was based on the official information provided by the Agency for Rural Investment Financing (AFIR) and the Ministry of Agriculture and Rural Development (MADR) through the documents available online on the websites of the two institutions. At the same time, these information were corroborated with the information related to the National Program for Rural Development 2014-2020 (NRDP - version IX, January 2019), regarding the sub-measures related to the financing of non-agricultural activities, namely of the value allocated within the framework of the entire financial exercise and of the non-refundable support as its intensity, in the rural areas within the context of the CAP and of Romania's integration in the EU.

As indicators used in the study, we mention the following:

– The value of the funds allocated for financing the non-agricultural activities;

- The number of application sessions for the projects for the 2014-2020 multiannual financial framework;
- The number of financing applications submitted, the number of financing applications accepted for funding;
- The number of signed contracts, the number of finalised contracts;
- The share of projects related to the investments in the modernization or the establishment of agro-touristic boarding houses.

The method used in the present study is a quantitative one and it was intended to emphasize first of all the interest of the potential beneficiaries towards this type of activities, namely the development of non-agricultural activities in rural areas. At the same time was followed the analysis of the number of projects that were contracted and also finalized in the timeframe analysed, in order to highlight the real interest of the applicants who became beneficiaries of European non-reimbursable funds for the diversification of activities at the level of the agricultural exploitation. At the same time was done a literature review concerning the speciality literature on non-agricultural activities and the importance of this type of activities in the real evolution of rural area both in Romania as an EU Member State and at EU level.

Results and Discussion

The sub-measure that was analysed in the present study, the sub-measure related to NRDP 2014-2020 that financed the non-agricultural activities is Sub-measure 6.4 *Investments in the creation and development of non-agricultural activities*. Sub-measure 6.4 aims (AFIR, 2017): Stimulating the business environment in the rural areas;

- Increasing the number of non-agricultural activities carried out in rural areas;
- Development of activities of the rural population;
- Reducing the differences between rural and urban areas;
- Diversification of economic activities of farmers or members of agricultural households by practicing non-agricultural activities in order to increase incomes and create alternative occupations.

According to the guidelines in the Applicant's Guide, concerning the areas of diversification, we mention as eligible the activities of production, handicraft, tourism, services providing, the production of pellet and briquettes of biomass (AFIR, p.4).

One of the main changes in the current financial exercise for this type of sub-measure is the one regarding the financing of the types of tourism activities compared to the previous financial exercises, i.e. the strict financing of the agro-touristic accommodation services, thus excluding the financing of the establishment of the tourist boarding houses, and this agro-tourism activity is seen as a diversification of the agricultural activity at the level of the exploitation. The emphasis is placed on the possibility of diversifying the sources of income of farmers, and also on the possibility that the young people studying in this field will return to rural areas to take care of this kind of activity, and then to take over the family's holding, thus bringing an added value both to the activity carried out and to the community in which they live. The aim of the sub-measures that finance the non-agricultural activities in rural areas is also to repopulate these areas, to make them attractive for young people studying in the cities.

Taking into account the above, the fact that the financial demand of sM6.4 was a very high one, supports the need to advocate further for the development and the diversification of activities in the rural areas in order to talk about rural development as an EU member state.

As a result of the analysis we made concerning sM6.4, it transpired that from the moment of opening the financing under the NRDP 2014-2020, there were 3 sessions for receiving projects for sM.6.4, sessions whose assigned values had reached the threshold allocated per total under the program, as shown in table 1.

Table 1. Sessions for submission of applications for funding sM.6.4 in the NRDP 2014-2020 and the total value of the projects submitted for financing

No.	Session No.	Allocated value (euro)	Value per session/ Total value %
1	sM 6.4 – 01/15 – 30.10.2015	57.214.935	32
2	sM 6.4 – 02/16 – 30.11.2016	85.000.000	48
3	sM 6.4 – 01/17– 31.07.2017	35.000.000	20
4	Total value concerning sM 6.4 in accordance with the announcements of project demands	177.214.935	100
5	Total value allocated in accordance with NRDP 2014-2020	166.503.969	106,43
6	Total value of submitted projects	423.635.360	254
7	Total value of submitted projects – total value allocated	257.131.391	154

Source: Own calculations after Archives of Projects Submission Session under the NRDP sub-measures 2014-2020, www.afir.info

From the above mentioned it is noted the interest of the potential beneficiaries regarding the diversification of the activity carried out at the farm level by requesting funds for non-agricultural activities, the funds available for the entire period being requested during the three submission sessions carried out between October 2015 and July 2017. It can be seen from Table 1 that the total value of the projects submitted for financing is 154% higher than the amount allocated for the whole financial exercise.

Table 2. The situation of the projects submitted, selected, contracted, finalised and cancelled at 11.04.2019 related to Sub-measure 6.4

Date of the implementation stage	Submitted projects		Selected projects		No. of selected projects / No. of submitted projects %	Projects under contract							% cancelled projects / projects under contract
						Projects under contract (ongoing and finalised)		*Finalised projects*		*Finalised projects out of projects under contract %*	Cancelled projects		
	No.	Value Euro	No.	Value euro		No.	Value Euro	*No*	*Value Euro*		No.	Value Euro	
11.04.2019	2.512	423.635.360	985	162.489.275	39	877	145.575.603	*224*	*28.987.957*	*20*	32	4.647.008	4
12.04.2018	2.520	424.719.388	965	158.937.225	38	693	115.798.205	*118*	*14.932.535*	*13*	18	2.477.138	3
13.04.2017	1.694	281.044.143	639	105.351.787	38	191	28.405.025	*17*	*2.123.447*	*7*	9	1.212.810	5
14.04.2016	502	76.071.515	44	6.544.977	9	0	0	0	0				

Source: Own calculations after Archives of Projects Submission Session under the NRDP sub-measures 2014-2020, www.afir.info

After analysing the situation of the projects submitted, selected contracted, finalized but also cancelled during the period 11.04.2016-11.04.2019 (Table 2), it is found that for the above mentioned period only 39% of the total submitted projects were selected for financing, namely 985 projects, of which 877 projects were contracted. Of the total of the contracted projects, only 20% were finalized, a percentage that may seem small, most of the delays in their implementation being often the result of the lack of investment financing, but also delays caused by the evolution of online procurement procedures, especially where there are execution of works contracts. It is noticed that during the analysed period, 32 financing contracts were cancelled, and the largest number, namely 14 contracts, were cancelled in the last 12 months analysed.

One of the eligible actions requested for financing within the analysed Sub-measure is the request for funds for investments in agro-tourism boarding houses or leisure tourism activities. It is thus observed that from the selection reports related to sub-measure 6.4, the sub-measure analysed in the present paper, from all the selected projects, 32.84% have as activity either the establishment of agro-tourism boarding houses, or their modernization and leisure activities in tourism. Besides the projects of this type, the activities financed within the sub-measure are meant to transform the rural into a small urban, precisely to answer the needs that the citizens have (sewing, laundry, car services, dental medical offices, purchasing ambulances, veterinary offices, hairdressers, textile manufacture, clothing, etc.) in order to ensure the continuity of life in rural areas.

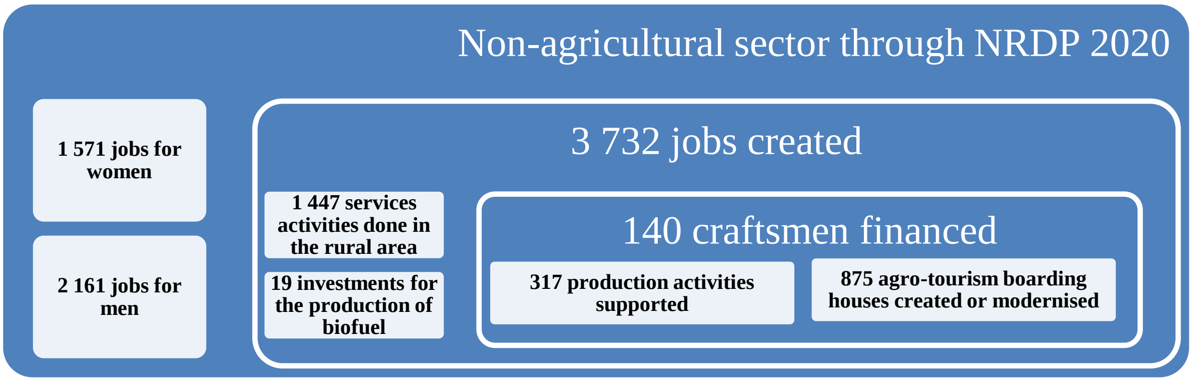

Figure 1. Non-agricultural sector trough NRDP 2020
Source: Own adaptation after AFIR 2018

The above Figure shows in a nutshell the main advantages of NRDP as regards the non-agricultural sector, mostly the creation of jobs and services in an area often lacking of these types of opportunities. The main advantages are those related to the need to keep the youth in and attract others to the rural areas. By creating all the infrastructure of the small urban centres into the rural areas and providing jobs we can reverse the depopulation process that affects most of the rural Romania.

Conclusions

Why does it matter to have a continuation of this type of financing? First of all, this type of financing serves what can be called a "political and legal" purpose as it helps to realise a more cohesive Europe, a social market economy as inscribed in the Treaties that generated a more cohesive Union, by eliminating the social and economic discrepancies.

Secondly, these activities create jobs in areas where the opportunities lack and help improve the local services infrastructures by adding new facilities for the residents of these rural areas. Thus we stop the depopulation process of the rural areas, we retain the youth and on the medium and long term we can hope to have a reverse trend – people coming to those areas from more crowded and expanding urban areas.

Having it maintain in the new CAP is a priority of many Central and Eastern European Members States, Romania included, as the above mentioned advantages and trends are what the EU needs in reducing the West and East discrepancies and rebalancing the continent from the socio-economic point of view, in a time when the global competitions is becoming more and more intense and affects us all.

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COW MILK ROUTE FROM ROMANIAN DAIRY FARMS TO MARKET

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Abstract

Milk is obtained in all countries of the European Union and occupies an important place in the economy of the Member States. In Romania the raising of dairy cows is a tradition among the inhabitants of rural and mountain areas. In this regard, the paper presents data on the production and marketing of cow milk for human consumption in Romania. The period covered is from 2010 to 2017. The following data were analyzed: the dairy cow herds, the total cow milk production in Romania and the place occupied with this production in the EU countries, the selling price of cow milk, the position occupied by Romania in the top of the main importers and exporters of cow milk worldwide. Following the analysis, it was noted that the dairy cow herds were steadily decreasing for the analyzed period, starting with 2010. The largest amount of cow and buffalo milk was produced in Macroregion One, on individual agricultural holdings, and the smallest quantity in individual agricultural holdings in the Macroregion Three. In 2017, Romania recorded the lowest price, among the EU countries, for 100 liters of raw cow milk, with an actual fat content. As far as exports were concerned, Romania was ranked 29th in the list of exporters of cow milk worldwide and ranked 19th among importers. The information found in this article was taken from specialized websites such as Eurostat, NIS, Faostat and ITC.

Keywords: *Cow milk, Dairy farms, Milk production, Romania.*

Introduction

Following the studies, it was concluded that approximately 85% of the milk produced worldwide comes from cows and the other 15% from goats, sheep, buffalos, camels, horses, and donkeys (Sawe, 2018).

The European Union is a very important player on the global dairy market, being the main exporter of many dairy products, especially cheese. Milk is produced in each member state of the European Union and represents 15% of the value of agricultural production, being the 2nd product in value.

Given the importance in the nutrition of people, taking into account the role it plays in the economy of the member states of the European Union, the market for milk and dairy products has been and will be one of the markets on which numerous regulations and measures are being acted upon, so that it becomes globally competitive.

The development of the world and European milk products market, as well as the modification of the consumers' options in the conditions of socio-economic expansion and the variation of the production due to the introduction of new technologies, will increasingly influence the sector of milk production and processing which is supported with the help of policy instruments (Giurcă *et al*., 2019).

Romania, even though it already holds the position of member state of the European Union, is facing important problems, which concern both the organization of farms in general and problems related to processing. Producers, processors and traders in this sector must comply with the European Union milk quality standards.

The Romanian milk market has come out over time with various problems, from the onset of mad cow disease, to the growth of cheap imports from countries outside the European Union (Florea *et al*., 2014).
The Romanian consumer milk market is about 1.5 billion liters. It offers three major product types: ultra-pasteurized milk (UHT); pasteurized milk, which together with the extra-pasteurized milk, totals about 10% of the quantity of consumer milk sold in Romania; unprocessed industrial milk, risky for the health of the consumer, but traditionally preferred and which still has a huge share (90%) of the milk sold in Romania.
The last product has an important share in the subsistence trade of the individual producers.
In this general context, the article will present the situation registered on the Romanian milk market, analyzing the official data on the production of cow's milk, the annual average price for fresh milk sold on the agri-food markets, the use of cow's milk and the situation of exports and imports of milk and cream between 2010-2017.

Material and Methods

The data used in this article was found at the National Institute of Statistics (NIS) and other internationally specialized sites. Various data has been consulted. The main tendencies in the production and commercialization of cow milk in Romania have been highlighted with the help of important indicators, such as: the number of cows in Romania and by development regions; total milk production achieved; average price for one liter of milk; annual milk and dairy products consumption per capita; imports and exports of milk. The main indicators analyzed in this paper were studied in their dynamics between 2010-2017. The results presented in the paper were shown in tables, interpreted and illustrated graphically.

Results and Discussion

The growth of milk cattle in Romania has become an increasingly profitable business for the producers who sell their milk at the farm gate with prices between 1-1,40 lei / l, depending on the quantity, quality and the distance to the processor factory that buys it.
From the processing plant to the shelf the price increases almost 5 times, the price difference includes: analysis, processing, packaging, payment of taxes plus the addition of supermarkets for the services of selling a liter of milk.
The price of milk is influenced by processors with 25-30%, the rest is influenced by what happens in the supermarkets.
The price of milk fell for various reasons: the decreased of raw materials and VAT, milk quotas were abolished and the offer increased by 35% due to the Russian embargo, as a consequence of the reorientation of European Union producers to the Romanian market.
Small and medium-sized producers will have to adapt on the go to the conditions of a liberalized market, where increasing imports generate a high level of competition.
Elimination of milk quotas means, for many of the Romanian farmers (small and medium-sized ones) that they face a competition they cannot face. There is the prospect of prolonging the situation in the long term due to the fact that, at European level, the offer of milk is greater than the demand. In this context, large processors have begun to give up contracts with local farmers, preferring to bring raw materials from outside.
Figure 1 shows the dynamics of dairy herds in 2010-2017. According to NIS, the highest value was registered in 2010, of 1,431,406 cows for milk, and the lowest in 2017 - 1,160,136.
While the number of cattle decreased, the number of sheep and goats increased. This was due to the high price of inputs and the low price of milk at the farm gate (Popescu, 2017).

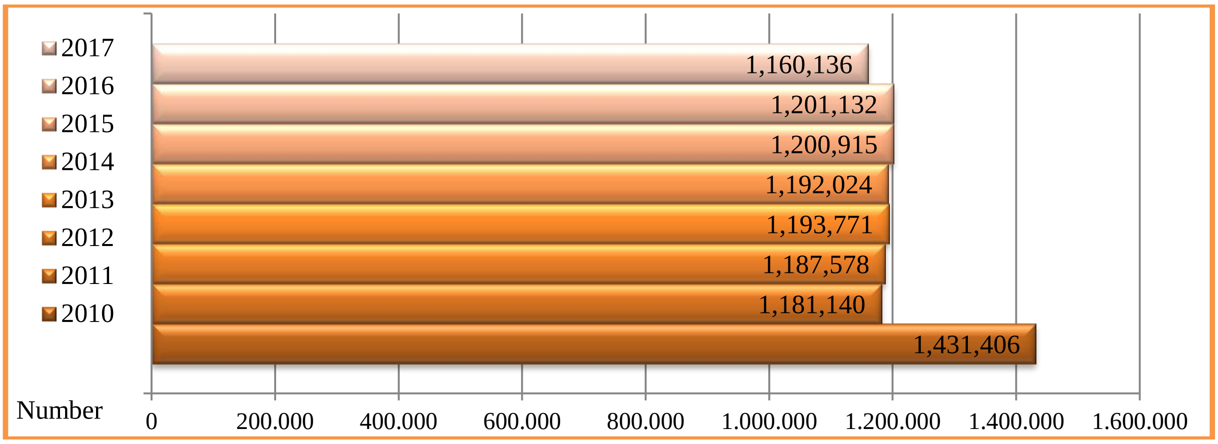

Fig 1 Herds of milk cows (number)

Source: NIS

Due to the number of heads registered in 2017, Romania ranked the 7th among the top dairy farmers in the European Union, where the first places are occupied by Germany, France and Poland (Eurostat)

At the level of macro-regions of development, for 2017 the situation is as follows: the highest number of dairy cows is registered in Macroregion One - 414,999 heads - and the smallest in Macroregion Three, 143,728 heads (NIS, Report Livestock and animal production in 2017).

It should be specified that Macroregion Three was in the last position because here is the Bucharest-Ilfov Region, where the share of land on which agricultural activities are carried out is very small.

Figure 2 provides data on the dynamics of milk production, between 2010-2017. It is observed that the production varies, the largest quantity of milk being obtained in 2014 - 44,015 thousand hl, and the smallest in 2017 - 40,385 thousand hl. With the production obtained in 2017 Romania was on the 13th place in the European Union (Faostat).

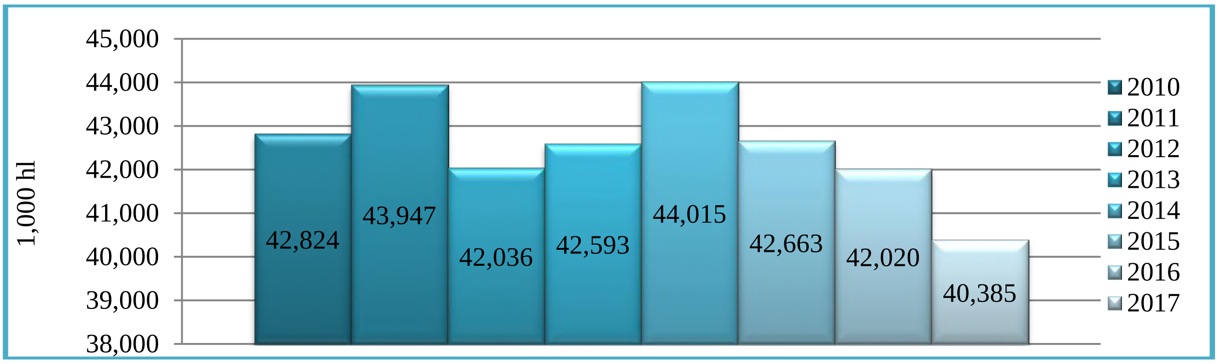

Fig. 2 Production of cow milk during 2010-2017 (1,000 hl)

Source: NIS

At the country level, the largest quantity of cow milk was produced in Macroregion One, 14,703 thousand hl, in the individual agricultural holdings, and the smallest in the individual agricultural holdings in the Macroregion Three, 4,968 thousand hl.

The production of cow milk decreased in 2017 by 3.4% compared to 2016. The development macro-regions registered decreases, except for Macroregion One (Center Region +2.1% and North-West +1%).

The counties that were the leaders in the production of cow milk in 2017 are those in the North of the country: Maramures - Macroregion One, Suceava and Botosani - Macroregion Two (NIS - Report Livestock and animal production in 2017).

Due to the lack of raw milk, processors were forced to buy raw milk from other countries (Popescu, 2017).
Table 1. shows the use of cow milk collected in 2017. As it can be seen, the largest quantity was destined to obtain cheese (13,680 thousand hl).

Table 1 Usage of cow milk - 2017

Specification		Quantity (mii hl)
Production of cow milk – total		40,385
Direct sucked milk		3,514
Milked milk		36,871
of which:	Milk for consumption	10,830
	Milk for butter and cream	1,482
	Milk for cheese	13,680
	Milk for yogurt	211
	Total other products	162
	Animal feed	482
	Delivered to the milk shop	9,990
	Differences and losses	34

Source: NIS, Report Livestock and animal production in 2017

According to the Eurostat Report on the European milk market, as of June 30, 2019, the Romanian milk sector ranked the 2nd, in milk-raw material deliveries to processors, registering an increase of 4.2% compared to June 30, 2018 (Demeter, 2019).

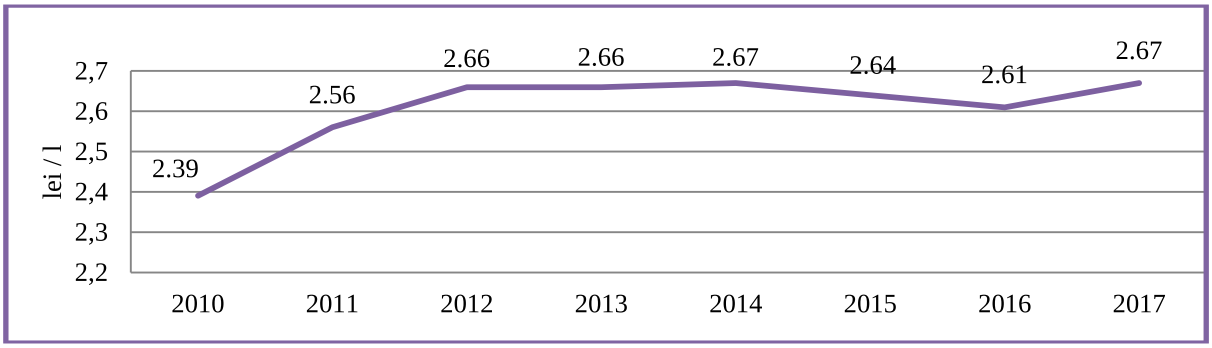

Fig 3 Average annual prices of fresh cow milk, sold in the agri-food markets
Source: NIS

While at the farm gate a liter of fresh cow milk was sold with values between 1-1.4 lei / l, in the agri-food markets it exceeded 2 lei during the analyzed period, as shown in Figure 3.
In 2017 the highest price was registered, of 2.67 lei / l, and in 2010, one liter of milk had the lowest price, of 2.39 lei / l.
The influence of milk prices on profitability is very important. To evaluate the profitability in dairy farms have to be considered both the input cost and the milk output as well as the milk market price (Popescu, 2014).
According to Eurostat, in 2017, the Austrians paid the most, for a liter of milk, 35.8 eurocents/ l and Romania with 28.8 eurocents / l was in the middle of the ranking.
Regarding the consumption of milk and milk products, Romania is not in a leading place in this category. In contrast to the large consuming countries, in 2017, in Romania there was an annual average consumption of milk and milk products, in milk equivalent of 3.5% fat, of 251.4 kg / per inhabitant (NIS). Table 2 presents the main exporters of milk and cream worldwide. Romania ranked the 29th, with a milk export value of 32,543 thousand USD.

Table 2. Exporters of milk and cream worldwide, thousand USD

Specification	Exporters	2014	2015	2016	**2017**	**2017/2014 %**
	World	9,738,031	7,490,713	7,622,325	9,429,135	96.83
1.	Germany	1,782,528	1,268,428	1,279,542	1,478,071	82.92
2.	Netherlands	654,060	523,777	658,368	954,021	145.86
3.	Belgium	822,224	618,904	552,459	792,471	96.38
4.	France	921,337	711,569	699,723	753,723	81.81
5.	New Zealand	207,706	217,389	293,251	492,016	236.88
…	…					
29.	Romania	26,466	20,286	25,053	32,543	122.96

Source: ITC, own calculation

Analyzing the data in the table, it is observed that the value exports increased by 22.96%, in 2017, compared to 2014.
The quantitative exports of milk and cream amounted 59,962 tons in 2017, with 59.91% more than in 2014, when 37,497 tons were exported (ITC).
The largest importers of milk and cream worldwide were Germany, Belgium and China (Table 3). Romania ranked the 19^{th}, with an import value of 110,027 thousand USD in 2017.

Table 3. Importers of milk and cream worldwide, thousand USD

Specification	Importers	2014	2015	2016	2017	2017/2014 %
	World	9,504,754	7,549,908	7,797,866	9,869,137	103.83
1.	Germany	1,411,358	1,099,978	1,090,729	1,705,594	120.85
2.	Belgium	898,567	617,044	714,788	1,015,885	113.06
3.	China	408,554	485,106	639,722	879,393	215.25
4.	Italy	1,277,736	891,843	763,201	814,153	63.72
5.	Netherlands	454,295	365,405	467,446	607,388	133.70
…	…					
19.	Romania	72,891	67,114	82,956	110,027	150.95

Source: ITC, own calculation

The value imports increased by 50.95% in 2017 compared to 2014.
The quantitative imports represented 208,145 tons in 2017, with 74.8% more than the quantity imported in 2014 (ITC).
The production value recorded fluctuations from year to year in the period 2010-2016. In 2016 was registered the lowest value - 1,977.751231 million US $, due to the decreasing of production. Production value has decreased by 19.53% in comparison with 2010, when the value was 2,457.855043 million US $ (Faostat).

Conclusions

The conclusions that can be drawn from this analysis are the following:
- The highest number of milk cows, 414,999 heads, is found in Macroregion One. A decrease of this indicator is remarked in the period 2010-2017;

- Cow milk production varied annually, the most significant production being recorded in 2014 (44,015 thousand hl) and the lowest in 2017 (40,385 thousand hl);
- The annual average consumption of cow milk and dairy products per capita in Romania, in 2017, was 251.4 kg/capita;
- Regarding Romania's quantitative exports and imports of cow milk, an increase during the analyzed period was noticed. Exports increased by 59.91% in 2017 compared to 2014, and quantitative imports by 50.95% in 2017 compared to 2014.

From the data concerning the imports and exports of Romanian cow milk for the period 2014-2017 it can be seen that the imports are ahead of the exports. This has a negative impact on the results obtained both at farm level and at macroeconomic level.

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LIVELIHOOD DIVERSIFICATION IN THE CONTEXT OF CLIMATE CHANGE: EVIDENCE FROM FARM HOUSEHOLDS IN SOUTH-EAST TUNISIA

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Abstract

Livelihood diversification reduces farm household vulnerability to the adverse effect of climate change. This study aims to analyze livelihood diversification of the farm households in southeast Tunisia. The research used a multistage random sampling technique to collect data from 50 farm households. Data were gathered through structured questionnaires and were described by means of descriptive statistics. The Simpson diversity index (SDI) was used to assess the level of livelihood diversification of households. Furthermore, with reference to the sustainable livelihood framework, the study showed the difference of capital endowments between livelihood groups. It also highlighted the determinants of livelihood diversification using a logistic regression model. The results showed that households with high levels of SDI (SDI > 0.5) were likely to have more sources of income. Furthermore, there was a significant difference ($p < 0.05$) in capital endowments between households that diversified their livelihoods and those that did not. Households with high SDI are more endowed with human, social and financial capital. Results of the logistic model highlighted that level of education, farm size, herd size, non-farm income and distance to the main plot are the key determinants of livelihood diversification.

Keywords:*Livelihood diversification, households, Simpson index, Sustainable livelihood Framework, Logistic regression.*

Introduction

Uncertainties and risks induced by climate change effects have been increased especially in the agricultural sector. Therefore, enhancing the adaptive capacity and the resilience of farm households are needed to deal with these threats. Diversification of livelihood strategies is a key solution to reduce the adverse impacts of climate change (Saha and Bahal, 2015). The concept of livelihood strategies is generally recognized as the combination of choices and activities performed by households in order to attain their livelihood goals (Khatun and Roy, 2012). It includes farm activities as well as non-farm activities. Previous studies showed that the impact of risk in agriculture and the pressure on households' farm income has stimulated the search of alternate revenue sources. Further, it has been reported that in low-income countries, farming activities hardly grant sufficient incomes that meet the needs of households (Fabusoro et al., 2010). Due to this fact, most farm households depend on a diversified portfolio of income sources to spread risks and to stabilize their revenues (Ellis, 1999).
To understand the factors affecting the livelihood strategies, several scholars have used the Sustainable Livelihood Approach (SLA), which was developed by the UK Department for International Development (DFID) (Chambers, 1987; Carney, 2003; Samsudin and Kamaruddin, 2013). The SLA takes into account a complex range of assets on which households depend for their livelihoods (Karl, 2002). This range includes tangible assets (money, land, agricultural equipment) and intangible assets (skills, social networks) (Xu et al., 2015). There are different ways to conceptualize these assets, but the most common is in the form of a pentagon representing five types of capitals: human, financial, physical, social and

natural capital (Carney, 2003). The SLA forms the conceptual and methodological basis for the "livelihoods" analysis that will be carried out in this paper.

Several international studies have been conducted to analyze the households' livelihood diversification (Fabusoro et al., 2010; Saha and Bahal, 2015; Ahmed et al., 2018; Gecho, 2017). For instance, Babatunde and Qaim, 2009 have assessed the households' livelihoods diversification in rural Nigeria. They employed the Herfindhal index to assess the livelihood diversification and the Tobit model to identify the determinants of diversification. They found that the male gender, the education, the productive assets, the pipe borne water and the access to credit are the major drivers of livelihood diversification. Likewise, another study conducted in West Bengal has analyzed the determinants of livelihood diversification. It showed that the educational level, the household experience, the training, the access to credit, the social status and the agro-climatic condition are the major factors affecting the livelihoods diversification (Khatun and Roy, 2012).

However, in Tunisia, there is a current lack of studies investigating the households' livelihoods diversification. This research tries to fill this gap through a case study of households from Medenine province, which belongs to South-East Tunisia. The purpose of this paper is to assess the level of diversification of these farm households and to analyze its main determinants.

Materials and Methods

The study was carried out in the Oum Zessar watershed, which is located in the northwest of Medenine province. It covers an area of 350 Km^2. It is spread over a territory of 11 imadas belonging to three districts: Beni khedache, North Medenine and Sidi Makhlouf. A multistage random sampling technique was employed. In the first stage, the three districts were selected to cover the entire watershed. In the second stage, out of the 11 imadas of the watershed, nine were selected randomly. In the third stage, households were selected randomly from each imada. Overall, 50 farm households were selected for this study. Data were gathered using a survey questionnaire with farm households.

To evaluate the level of households' livelihood diversification, this research used the Simpson Diversity Index (SDI), which was established by Edward H. Simpson in 1949 (Ahmed et *al.*, 2018). The SDI calculates both the number of livelihoods practiced by a household and the proportional contribution of each livelihood. We considered three types of livelihood strategies: crop-oriented, livestock oriented and off-farm-oriented. The SDI is calculated using the following equation:

$$\mathbf{SDI = 1 - \sum_{i=1}^{N} P_i^2} \qquad (1)$$

Where : N represents the number of income sources and P_i is the income proportion of the i-th income source. The value of the SDI is between 0 and 1. Households with less diversified livelihoods are associated with the littlest values of SDI. Those with a highly diversified income have a high SDI value. The SDI was calculated for each household and was used to group sample households into two groups according to their level of diversification. The level of livelihood diversification was interpreted using the SDI values: if SDI < 0.5, the household's livelihoods are lowly diversified; if SDI > 0.5 the household's livelihoods are highly diversified. Then, using the Sustainable Livelihood Approach (SLA), we compared the capital endowment between the two households' groups. Indicators used to quantify each capital (Table 1) were selected based on a literature review and on the availability of data in our database. Overall 17 indicators were chosen to apply the SLA framework. It was crucial to standardize each indicator because of the heterogeneity of units obtained from the raw data, the following formula was used:

$$\mathbf{Index\ I_h = (I_h - I_{min}) / (I_{max} - I_{min})} \qquad (2)$$

Where I_h is the original variable of household h, I_{min} and I_{max} are respectively the lowest and highest value of variable among all households.
In this paper, the decision to highly diversify livelihoods by households or not is considered a dichotomous variable, hence the use of a logistic regression model (McFadden, 1973).
For this dichotomous model, a logistic regression analysis using maximum likelihood was performed to test the effect of several explanatory factors on the probability of "livelihood diversification by households". If multicollinearity problem exists in the model, it is difficult to determine the effects of the explanatory variables on the dependent variable. Therefore, the multicollinearity problem between explanatory variables was tested before running the logistic model. Once we find two correlated variables, one of them should be eliminated to solve this problem (Ahmed et al., 2018). Finally, as shown in eqation (3), seven variables were maintained to run the logistic model. The obtained model is as below:

$$\textbf{LDI} = \beta_0 + \beta_1\ \textbf{Education} + \beta_2\ \textbf{Farm size} + \beta_3\ \textbf{Herd size} + \beta_4\ \textbf{Distance to the main plot} + \beta_5\ \textbf{Experience in agriculture} + \beta_6\ \textbf{Non-farm-income} + \beta_7\ \textbf{Distance to the market} + \mu_i \quad (3)$$

Where, LDI: Livelihood diversification index, β_0 = Intercept, μ_i = Error term

Results and Discussion

Study's results showed that all households are diversifying their livelihoods, while, the level of diversification differs among households. The average value of SDI for the whole sample was 0.41 with a standard deviation equal to 0.45. This indicates a great variability in the levels of diversification between households. This led us to classify the households into two groups using the binary logistic regression (0 for households with low level of diversification and 1 otherwise).

Table 1. Difference in capital endowment between the two groups of households

Livelihood Capital	Indicators	Group 1 (SDI<0.5)	Group 2 (SDI>0.5)
Human capital	Age	0.700	0.333
	Household size	0.448	0,614
	Dependency ratio	0.185	0.273
	Number of years of education of head of household	0.434	0.698
	Trainings	0.306	0.607
	Number of years of experience in agriculture	0.534	0.221
Physical capital	Value of agricultural equipment	0.308	0.002
	distance from the main plot	0.010	0.179
	Herd size	0.320	0.160
Natural capital	Farm size	0.308	0.154
	Irrigated area	0.149	0.002
	Number of olive tree	0.100	0.274
	Crop diversity index	0.412	0.189
Social capital	Membership in any organization	0.136	0.536
	Distance to the market	0.130	0.427
Financial Capital	Non-farm income	0.339	0.658
	Subsidies	0.089	0.000

Group 1 encompass 54% of the surveyed households that have a low level of diversification (SDI<0.5). Nevertheless, the other 46% have high SDI values. Table 1 and figure 1 compares the capital endowment between the two groups. There was a difference in the amount of livelihood capital between the two groups. Group 1 possess more abundant physical (0.212) and natural capital (0.242). Group 2 owns more abundant human (0.457), financial (0.328) and social capital (0.48). These differences were statistically significant (Figure 1).

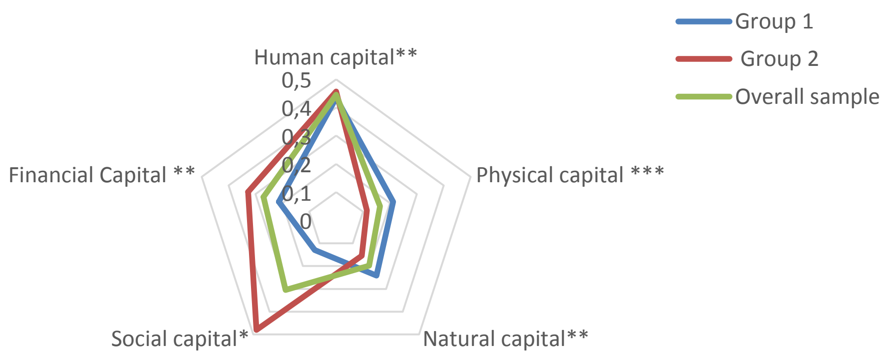

Figure 1. Difference in capital endowment between the two groups of households (*1% significance, ** 5 % significance, *** 10% significance)

Table 2 presents the results of the logit model estimation (Y) which explains the probability that a household will highly diversify its livelihoods. The model is statistically valid. Indeed, the validity of the estimated model is analyzed through the Wald $\chi^2(5) = 10.48$ statistic, which tests the equality to zero of all the model coefficients. On the other hand, he showed that the coefficients retained in the model were significantly different from zero at the 5 % threshold. Consequently, the hypothesis of nullity of the coefficients is rejected. The Wald χ^2 tested also the link between each of explanatory variables and the household's livelihoods diversification.

Using R^2 McFadden, good adequacy of the models was demonstrated. In fact, R2 has a satisfactory high value R2 =0.66, which prove the model performance.

Table 2. Predictability of the Logit model

Logit (LDI)	Coefficients	Pr > Chi²
Education	2.999	0.005*
Distance to the main plot	0.968	0.038**
Experience in agriculture	-0.521	0.205
Farm size	-2.364	0.014**
Herd size	1.126	0.089***
Distance to the market	0.756	0.182
Non-farm-income	0.928	0.092***
Wald χ^2	10.481	
R^2(McFadden)	0.662	
AIC	69.41	

*1% significance, ** 5 % significance, *** 10% significance

Then, we tested the independence of the explanatory variables with each other (Table 3). This led to a reduction in the number of variables and allowed us to avoid multi-collinearity. To test the existence of multi-collinearity, the explanatory variables were verified using a

variance inflation factor (VIF) which makes it possible to detect the multi-collinearity of the explanatory variables (Asfaw et al., 2017).
In our model, there is no multi-collinearity between the variables (Table 3). In fact, a variance inflation factor (VIF) and its inverse (1 / VIF) are used to verify the explanatory variables.
(1 / VIF) is greater than 0.1. Therefore, it can be concluded that the model variables explain significantly the household's livelihoods diversification.
Results analysis of the Logit econometric model (Y) presented in Table 2 shows that the significant variables were: level of education (1% threshold); land size and the distance to the main plot (5% threshold) and herd size and the non-farm income (10% threshold).

Table 3. Variance Inflation Factor (VIF)

Variables	VIF	1/VIF
Education	3,666	0,27275655
Distance to the main plot	1,281	0,78047636
Experience in agriculture	1,802	0,55486122
Land size	1,552	0,6441241
Herd size	1,190	0,84043628
Distance to the market	1,891	0,52881396
Non-farm-income	1,677	0,59629127

Logistic regression results indicate that the education variable is significant at the 1% level and positively correlated with the livelihood diversification (C = 2.999, Pr > χ^2 = 0.005). This suggests that a household head with a high level of education is more likely to diversify its livelihood. The high educational level of the household heads makes them able to look for employment activities and to undertake highly remunerative activities. This result is in line with previous researches, which found that the education is essential as it offers abilities and skills that enable households to secure well-paying jobs (Adebayo et al., 2012; Chaplin et al., 2002; Fabusoro et al., 2010). Thus, the vulnerability of their livelihoods may be reduced.
The findings from the logistic regression show that the farm size is significant at 5% threshold but with a negative sign (C =-2.364; Pr > χ^2= 0.014). This indicates that households with small farm size are more likely to diversify their livelihood strategies. The inverse relationship between the farm size and livelihood diversification have been revealed in several previous studies (Fabusoro et al., 2010; Adebayo et al., 2012; Sallawu et al., 2016). This affirmed that when agricultural activities offer sufficient revenue, households tend to decrease the diversification of their livelihood strategies. Indeed, households with large farm size are more likely to involve in agricultural activities as it requires more labor and enables them to ensure food security. Further, results in table 2 reported a positive coefficient of herd size, which was significant at 10% threshold. This indicates that the herd size was a determinant of household's livelihood diversification. Comparison of this finding with those of other studies confirms that the livestock endowment appears to be a crucial asset for improving diversification (Eneyew, 2012, Saha et al., 2015). A possible explanation for this might be that livestock help to generate more income and reduce liquidity constraints through selling animals. Moreover, the findings of this research suggest a significant (at 10% level) and a positive relationship of the non-farm income with livelihood diversification. The non-farm income is a source of investment for farm activities particularly in case of financial problem. It can further support the household's livelihood diversification and raise their standard of living. Finally, our model showed a positive and significant effect of the variable "distance to

the main plot". We can conclude that the proximity to the main plot is one of the factors that facilitate the management of the farm.

Conclusion

The study analyzed the livelihood diversification of farm households in the province of Medenine-Tunisia. The diversification seems to be a common practice. It was infrequent to find a household without non-farm income. However, the level of diversification differs between households which affect their capital asset. It was found that households who highly diversify their livelihoods have more abundant human, social and financial capital. Many determinants either negatively or positively affected the livelihood diversification. On the first hand, the education, the herd size, the non-farm-income and the distance to the main plot have positive and significant effect on livelihood diversification. On the other hand, the farm size and the experience in the agricultural sector have significant but negative effect on livelihood diversification.

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AN ANALYSIS OF THE CURRENT SITUATION OF SEED SECTOR IN TURKEY

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Abstract
Turkey has optimum climatic conditions, large lands, and geographical location advantage for the production of high-quality seed. In the country, as the public sector has been dominating the seed sector until the 1980s, the private sector has started to operate in the mid-1980s as a result of the legal and administrative regulations made at that time. Thus there has been a significant progress in the sector regarding human resources, capital accumulation and technology transfer with the cooperation of domestic and foreign companies. The Law on the Protection of Breeders' Rights for New Plant Varieties Law No.5042 and Seed Law No. 5553 were entered into effect respectively on 15.1.2004 and 08.11.2006. By Seed Law No. 5553, quality assurance in seed production has been provided; plant variety registration system in accordance with the EU and seed, seedling and sapling certification system have been established. The law has contributed to the development and strengthening of the sector through new regulations on the seed production and trade. In parallel with the developments in the sector, seed production and exports of Turkey have gradually increased over the years. The production amount increased from 145,227 tones in 2002 to 1 059 300 tones in 2018 and seed exports to 85 countries realized as 151,691 million US dollars and an amount of 102,786 thousand tones in 2018. Despite these developments, various problems still exist in the sector such as lots of small capital firms in the sector, lack of qualified technical staff, lack of capital to be allocated to R & D, problems regarding technology transfer, high production cost, the informal production and sale of seed, and the need for legislative amendment on some issues. In this study, the current situation in Turkey on seed sector is examined and basic problems and solutions to them in the sector are discussed.

Keywords: *Seed, Seed sector, Production, Trade*

Introduction
Although seed is the basic and most crucial input for crop production, it has become a product of industrial and economic value today. The use of high quality seeds is of great importance in increasing agricultural productivity and seed has a strategic importance for the agricultural sectors of the countries. With this in mind, developed countries have turned to policies that develop and strengthen the seed sector and have started research and development activities on this topic. As a result of these policies, the seed sector in these countries, which has a history of nearly 150 years, has developed greatly with their strong capital accumulation and advanced technological research opportunities and these countries have played an important role in the world seed trade today (Bağcı, 2011). The private sector-dominated seed sector has only a 30-year history in Turkey. In recent years the sector has improved considerably in terms of production and exports in the country. The production amount increased from 145,227 tones in 2002 to 1 059 300 tones in 2018 and seed exports to 85 countries realized as 151,691 million US dollars and an amount of 102,786 thousand tones in 2018.
Agricultural land in Turkey is around 38 million hectares, of which 23 million hectares consist of arable land and 14 million hectares of grassland and pasture land. The share of field crops in the arable areas is 39%, fruits 9%, vegetables 2% and fallow land 10%.

Furthermore, there are two Seed Gene Banks in the country. These are the National Seed Gene Bank of İzmir and the Seed Gene Bank of Turkey in Ankara. These institutions perform the collection, conservation and documentation of the plant genetic resources and make the other studies related to the seeds. As of 2015, 120,995 materials of 3,244 species have been preserved in the seed gene banks.
The main objective of this study is to examine the current situation of seed sector and discuss the basic problems and solutions to them.

Material and Methods

This study is based on the research of theoretical review and secondary data obtained from Turkish Statistical Institute (TÜİK), Ministry of Agriculture and Forestry (MoAF), Ministry of Trade (MoT), Sub-Union of Seed Industrialists and Producers (TSÜAB). The legal documents, articles, reports, websites and journals were employed to identfy the present situation of the seed sector and the basic problems and solutions to them in the sector.

Seed Sector in Turkey

Turkey has a big potential for seed production due to its favorable geographical condition and climate. The seed sector, which had a public dominated and monopolistic structure until the 1980s, has shown significant developments after these years as a result of substantial structural reforms and the transition to a free market economy (Elçi, 2000). With the liberalization of the economy and the abolition of restrictions on seed foreign trade, many domestic and foreign seed companies entered the sector either directly or through partnerships and seed sector has became a private sector dominated structure with the increase of the number and capacity of these companies in the sector.
Reviewing the sectoral structure of the seed industry, organizations operating in field crops and vegetable seed production and breeding can be grouped into four main headings. These are as follows:

1. Public Agricultural Research Institutions under the Ministry of Agriculture and Forestry and Universities
2. General Directorate of Agricultural Enterprises (GDAE) under MoAF
3. Unions and Cooperatives
4. Private Sector Seed Companies

Currently 26 public institutions, many union and cooperatives and 907 companies in various sizes which has either domestic or foreign capital exist in seed sector.

Legal Framework of Seed Sector in Turkey

Ministry of Agriculture and Forestry is responsible for both regulating and controlling the seed sector in Turkey and seed legislation and regulations of Turkey are largely in accordance with the ones of EU countries. The Seed Law No. 5553, which has come into effect since 1963, was amended by the parliament in 2006. By this law, quality assurance in seed production has been provided; plant variety registration system which is in accordance with the EU's and seed, seedling and sapling certification system have been established. Furthermore, some provisions has been added to the law for the establishment of professional organizations such as unions and sub-unions and upon its entry into force one union and 7 sub-union have been established in 2008.
The establishment of these unions and sub-unions has ensured the integrity of the sector. All seed industry and producers operating in Turkey must be a member of Sub-Union of Seed Industrialists and Producers. As of June 2019, the number of the members of TSÜAB is 907 (Sub-Union of Seed Industrialists and Producers, 2019).

Member companies of TSÜAB are engaged in activities such as plant breeding and variety development, protecting of varieties, cultivation, processing, packaging, marketing and distribution of original, basic and certified seeds, and export and import of seeds. Each stage of seed production is carried out in accordance with international rules in Turkey.
Additionally, Turkey's UPOV membership was approved by the national assembly and Turkey became an UPOV member in 2007.

Seed Production in Turkey

Seed production in Turkey is carried out by both public and private sectors. Wheat, potato, barley, corn, cotton and sunflower seeds are the leading products in certified seed production. Within seed supply system, Public Seed Agencies are currently engaged in a limited production and distribution activity focusing on self-pollinated plants such as wheat, barley and some forage crops. However, in recent years, private seed companies have also started to increase their market share significantly in these species (Table 1).

Table 1- Seed production inTurkey (tones)

Year	*Total Production (Public Sector +Private Sector)*	*Wheat*	*Barley*	*Soybean*	*Corn*	*Sunflower*	*Potato*	*Cotton*	*Vegetable*	*Forage Crope*
2003	Total Production	**100 101**	**11 194**	**343**	**21 399**	**5 628**	**25 395**	**7 662**	**992**	**2 600**
	Share of Private sector %	6	11	98	99	100	100	80	99	27
2006	Total Production	**210 788**	**28 195**	**4**	**16 107**	**7 670**	**75 138**	**18 856**	**2 283**	**3 897**
	Share of Private sector %	20	22	100	99	100	100	87	100	52
2009	Total Production	**227 852**	**36 144**	**1 170**	**28 921**	**9298**	**58 877**	**10 811**	**2 758**	**2 220**
	Share of Private sector %	45	49	99	100	100	100	100	100	59
2012	Total Production	**327 924**	**43 162**	**2 248**	**32 796**	**14 732**	**185 485**	**23 074**	**2 115**	**1 945**
	Share of Private sector %	58	73	97	100	100	100	99	94	56
2015	Total Production	**484 204**	**125 018**	**3 625**	**52 791**	**21 757**	**231 592**	**14 379**	**3 291**	**4 068**
	Share of Private sector %	64	83	99	99	100	100	100	100	74
2018	Total Production	**426 658**	**151 365**	**3 230**	**62 229**	**25 029**	**276 389**	**25 141**	**2 042**	**7 420**
	Share of Private sector %	60	85	100	100	100	100	100	100	79

Source: MoAF

Turkish seed sector has grown rapidly since the new Seed law came into force in 2006. Government policies supporting certified seed usage and domestic certicfied seed production has resulted in the rise of the production capacity of both the government and private sectors. Certified seed production has increased about three fold in last ten years, with a total production of 1 059 300 tones in 2018. Likewise, while the share of private sector in total production was 40% in 2003, this rate rose up to about 80,16 % in 2018. The private sector has produced all hybrid corn, hybrid sunflower, cotton, potato, soya, vegetable seed productions in Turkey since 2016 (Ministry of Agriculture and Forestry, 2019).

At present, an important part of certified seed production for wheat, barley, rice, and some forage crops and pulse seeds is made by using the varieties which have been bred in Turkey (Karahan, 2017).

Seed Trade

Turkish seed sector; both in terms of legislation, infrastructure and ecological opportunities, and with its cooperation and membership with international organizations, has an important advantage in domestic and international markets (Karahan, 2017).

The permission of the Ministry of Agriculture and Forestry is required for seed import and export in Turkey and the imported seed must comform with domestic requirements. The companies which are registered with the Turkish government as seed producers can get permission for seed import and import is allowed for certified seeds of registered varieties in Turkey.

Turkey seed market is mainly based on three categories, that is:cool climate grains, forage plants and industrial plants and vegetables. The largest share in the market belongs to hybrid vegetable seeds. Industrial plants consisting of hybrid seeds such as corn, sunflower, cotton and sugar beet are in the second place. The third group, particularly including certified wheat and barley seeds, ranks third in value but first in quantity (Ministry of Trade, 2016). Products which have the highest export rate in terms of volume in Turkey, are respectively, wheat, sunflower, corn, potato, barley, cotton and forage crops. Products which have the highest import rate in term of volume are potato, forage crops, knotgrass, corn and vegetable seeds (Ministry of Agriculture and Forestry, 2019).

Looking at the seed export data of Turkey, the export has been on the rise in recent years. While Turkey's seed export in 2012 was approximately 121 million dollars, this figure increased to approximately $ 152 million in 2018 with an increase of 25% in value basis.

On the other hand, the value of import has decreased from 197, 6 million dollars in 2012 to 178,8 million dollars with a decline of 9% in value basis in 2018. The ratio of exports to imports increased from 61 in 2012 to 84,8 % in 2018.

Tablo 2-Seed foreign trade figures

	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Export (Million dollars)	120.7	126.0	148.3	102.7	153.4	137	151.7
Import (Million dollars)	197.6	194.2	188.4	202.1	202.1	189	178.8
The ratio of exports to imports (%)	61	65	79	51	76	72,5	84,8

Source: MoAF

While Turkey exported seeds totally 76 different countries in 2016, this number increased to 85 countries in 2018. The number of export countries rising year by year. In 2018 Russia ranks first in export with the percentage of 12, 9%, then Ukraine and Iraq come second and third with the percentages of 12, 1% and 11,9% respectively in value basis. On the other hand, France, Italy and Netherland were the top three importers with the import share of 15.4%, 11.0% and 8.8 % respectively.

Seed Supports in Turkey

The government has given subsidies to the farmers for domestically produced certified seed usage in order to promote the use of certified seed since 2005. Likewise, seed producers producing certified seed in Turkey have been supported by the government since 2008. Support prices for domestic certified seed usage are shown in the Table 3.

Table 3-Subsidiy amount for farmers using domestic certified seed in 2018

Support for domesticall yseed usage (TL/da)	TL/Decare
Safflower canola, sesame	4
Rye, oat	6
Paddy Rice	8
Wheat	8,5
Barley	8,5
Trefoil	20
Clover	30
Chickpea, Bean, Lentil, Soybean	20
Potato	80

Source: MoAF

Seed Law No. 5553 and subsidies have contributed significantly to the development of the sector as well as the increase in certified seed usage and production.

Basic Problems and Solutions

Problems related to seed sector; legislation and legal problems; reseacrh and development (R & D) and technology problems; and high production costs, financing, inspection and marketing problems.

Legislation and Governance Issues:

Regulatory problems are due to the need for new regulations arising from changing conditions and trade patterns of the day, and problems related to existing legislation.

There are many sub-titles under this issue, such as market control, penalties, inspection of seed dealers to prevent unlicensed illegal seed sales, field controls, authority transfers from the public sector to the private sector, disintegration of authority on issues concerning seed sector, packaging and packaging, supports, production, local administrations, seed import and export. In addition, subsidies for certified seed production and use are insufficient and more support is needed by the sector. Likewise, R & D activities are not adequately supported.

Increasing state subsidies will contribute positively to the production, use and export of certified seeds.

Moreover, although the seed sector has extensive production and seed processing lines and factories, the sector cannot be considered as an Agri-Industrial Organization (SME) in Turkey and cannot benefit from the support of many SMEs. Granting these companies SME status will enable them to benefit from many domestic and international market supports (Turkish Seed Industry Association, 2016). The Seed Law No. 5553 provides for the establishment of specialised courts for issues related to seed. However, disputes regarding the seed remain in these courts for a long time and do not result in time.

In addition, more attention should be paid to auditing and the transfer of authority from the public sector to the private sector should be ensured for further development of the sector. (Bağcı and Yılmaz, 2016). Similarly, more emphasis should be paid to more effective cooperation between the public sector, civil society and the private sector in order to solve sector problems effectively in drafting and reviewing legislation.

High Productıon Costs:
The relatively high labor cost, especially high public land leases, expensive production inputs and high royalties paid to foreign companies and public institutions lead to high production costs. It is important that public and private sector representatives should carry out cost studies for determining production costs. Investment costs should also be taken into account when determining the cost per kg. of a product which have been bred (Ministry of Agriculture and Forestry, 2018).

Financing Problem:
Majority (83%) of the companies operating in the sector are composed of medium and small sized companies. Small firms has insufficient financial resources for infrastructure investment and R&D programs. This negatively affects the development of the sector and the volume of exports. In order to overcome this problem, it should be built up new funding and support programs.

R & D and Technology Problems:
Private sector R & D level is inadequate due to lack of qualified R & D personnel, the small size of the company, high laboratory and infrastructure investment costs and the inability of the majority of companies in the sector to afford R & D risks. The need for qualified personnel is one of the biggest problems for all companies of the sector.
One of the main reasons for importing seeds and varieties from abroad is that it is high costs and dependent on foreign agricultural technologies. It is expected that opening the technological and physical infrastructure of MoAF to the private sector, provided that R & D projects are implemented, and increasing R & D supports to the upper limits will contribute to solving the problems of the sector in this field (Ministry of Agriculture and Forestry, 2018). In this way, the competitiveness of the companies which are engaged in vegetable seed breeding and production will be increased in the domestic and foreign seed market.

Conclusions

Turkey has made significant progress in the seed sector in recent years and has completed its integration with the world by becoming a member of international organizations and establishing legal and technical infrastructure. Consequently, for the further development of the seed sector in the country, which is also advantageous in terms of climate and ecological conditions, the farmers should be more encouraged for the use of certified seed, R & D activities should be given more importance and supports for R & D activities should be increased, training of technical experts in the sector should be encouraged, variety breeding activities should be encouraged and policies aimed at improving competitiveness of the sector should be prioritised by taking into account developments both in Turkey and the world.
On the other hand, as a major producer country in terms of vegetables and field crops and with the development of the agricultural industry and greenhouse cultivation, Turkey has significant opportunities at international markets, particularly at Central Asia and Middle East countries, North Africa, Balkans and Eastern European countries
Therefore, a good market analysis in these regions, especially the harmonization of seed legislation and technical cooperation in the Seed Association of Economic Cooperation Countries (ECOSA) should be one of the country's future strategies (Bağcı, 2019).

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SALES MANAGEMENT IN THE DIGITAL ENVIRONMENT

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Abstract

The subject of this paper is the contemporary aspects of sales management in the digital environment. Sales management is a business discipline that focuses on the practical application of sales techniques and the management of a firm's sales operations. The growth of competition, the globalization of the market, the shorter product lifecycle, the increase in indirect competition in all spheres of business have all influenced the new approach to managing the sales process. The main goal of the paper is to analyze the significance and necessity of investment in information and communication technology and modern software solutions in sales management. Contemporary sales are becoming highly dependent on information and communication technologies, both for traditional commerce and especially for e-commerce. Global computer networks have brought tremendous benefits to commerce as well as new problems. Successful sale of agricultural products within a short period of their harvest is essential to the overall growth and survival of agribusiness. Agricultural producers are also trying to develop digital sales channels.

Keywords: *Sales management, Digital environment, Agribusiness*.

Introduction

The simplest way to think of the nature and role of selling, traditionally called salesmanship, is that its function is to make a sale (Jobber and Lancaster, 2015). In the traditional sense, selling encompasses a set of activities and tasks that organizations undertake to deliver goods and services. In economic terms, selling is the final stage of the process of production. Salespeople and sales teams are currently engaged in redefining their roles and adopting new forms of selling. These changes have been brought on by rapid developments in market conditions as well as by growing customer expectations. Sales management essentially boils down to planning, realizing and controlling the programs of personal contacts, designed with the goal of achieving a firm's sales and profit objectives (Jobber and Lancaster, 2015). Sales management is a business discipline which is focused on the practical application of sales techniques and the management of a firm's sales operations.

The trends of sales management give increasing importance to building customer relationships, team sales, sales profitability which requires strategic planning incorporated in selling and marketing programs, leadership due to the complexity of managing the sales personnel and, finally, they adopt a wider global market perspective. In addition, more stress is placed on the strategy of direct communication, which puts the emphasis on the dialogue with and feedback received from the customer that make product improvement possible. The growth of competition, the globalization of the market, the shorter product lifecycle, the increase in indirect competition in all spheres of business have all influenced the new approach to managing the sales process.

The digitalization as a social process refers to the transformation of the techno-economic environment and social-institutional operations via digital communication and applications. Digitalization is the use of digital technologies to change a business model and provide new revenue and value-producing opportunities (Gartner Group, 2019). It is the process of moving

to a digital business. Digitalization strongly influences both agricultural production and the ways in which agricultural products are sold.
The purpose of this paper is to provide an overview of the impact of digital transformation on sales management, in particular on agricultural product sales management and on agribusiness in general.

Material and Methods

The paper is based on an extended review of secondary data from relevant international institutions and the articles and literature of reference authors.
The technological, legal, economic and agribusiness aspects of digitalisation of sales management have been specifically considered.

Results and Discussion

The technological aspects of sales management in the digital environment

One of the most prominent technological trends is the use of IT resources as a service, not a product. This modern approach in IT is called *Cloud Computing*. The definitions of this term abound, but it seems that the definition provided by the *Computer Security Division of the U.S. Department of Commerce* (Mell and Grance, 2011) best captures the concept's essence. It reads: "*Cloud Computing* is a model for enabling convenient, on-demand network access to a shared pool of computing resources, e.g. networks, servers, storage, applications and services, that can be rapidly provisioned and released with minimal management effort or service provider interaction." Over the previous years, the concept of *Cloud Computing* has become more accepted in the marketplace. The *Cloud* model may hold special appeal for small firms that have limited IT personnel.
One of the pressing concerns in the development of the business software industry is how to create software tools for handling vast data volumes, since these have become commonplace in business systems. There is a need to provide storage for this data, but also to enable quick and efficient user access. This is why the concept known as Big Data will continue to gain ever greater significance in the future. This concept is described by *McKinsey Global Institute* using the 4V characteristics (Manyika *et al*., 2011): **V**olume (the enormous volume of data); **V**ariety; **V**elocity (the speed of gathering and turning data into knowledge); **V**alue (creating value based on the gathered data). IBM describes the *Big Data* concept using 3V (**V**olume, **V**elocity, **V**ariety) (Zikopoulos and Eaton, 2012).
The third technological trend that significantly influences changes in sales management is the concept of the IoT. The Internet of things (IoT) is a concept which expands the virtual world and the Internet to real-world physical things, thus enabling resource virtualization.

Economic aspects of sales management in the digital environment

The growth of the global economy and the global computer network enabled electronic commerce as a new segment of modern business. The market established on the World Wide Web provides new commerce possibilities, but requires a change in the approach to the very concept of commerce, which is termed e-Commerce. Communication-wise, e-Commerce comprises the delivery of information, products, services or paying for them via smart phones, computer networks or some other means. Business-wise, e-Commerce represents the application of technology to automate business transactions. Service-wise, e-Commerce is a tool that reduces costs, improves the quality of the goods and decreases delivery time.
E-Commerce provides new sales channels which make possible the interactive relationship between the seller, i.e. the supplier, and the buyer while increasing productivity and reducing costs. The model of networked global business has provided the following benefits to the firms that utilize it: cuts in business costs; customer satisfaction; a decrease in goods delivery

times and a reduction in the number of complaints; improved customer support; savings in distribution, etc. The electronic environment has given rise to software applications for managing the sales chain, which integrate the cycles of sales or orders and make them more efficient by increasing the speed of data exchange between the customer and the seller, which results in increased customer confidence and reduced purchase times. The companies that offer their products and services through a large number of channels require a new generation of e-Commerce applications. As the sales channels evolve, so does the process of selling. The companies face the decision of which new business practice to adopt to limit delays, support direct or self-service sales. E-Commerce enables competitive business and increases the survival rate in the marketplace. To develop e-Commerce, certain technological prerequisites have to be met. First and foremost, an information highway must be available, i.e. the infrastructure of sufficient capacity and an information transport service provider branched out in various communication networks.

Legal and regulatory aspects of sales management in the digital environment

In addition to technological prerequisites it is necessary to provide and improve the legal prerequisites which enable the unhindered development of electronic commerce, copyright and privacy protection and secure universal network access and suitable pricing policy for network access and information usage. All of this contributes to the existing dedication to activities at the national and global levels aimed at harmonizing laws and regulations on electronic commerce, electronic contracts, digital signature, remote payment, etc. The most important international regulations are: the UNCITRAL Model Law on Electronic Commerce; the EU Directive on certain legal aspects of electronic commerce in the Internal Market; the Directive of the European Parliament and of the Council on a Community framework for electronic signatures; the UNCITRAL Model Law on Electronic Signatures; the UNCITRAL Convention on the Use of Electronic Communication in International Contracts.

The EU Directive on the e-signature established a common framework for the development of the law on the e-signature in the EU, and therefore it promotes the legal recognition of the e-signature and its more widespread use. Only an advanced e-signature (supported by a qualified certificate and created with a secure private key) is considered a full legal equivalent to a handwritten signature, but all e-signatures are potentially admissible as evidence in the court of law. The objective of the Directive on e-commerce is to promote e-commerce in the EU. The Directive on e-commerce contains a framework for the member states' laws on e-commerce. In accordance with it, regulations have been recommended that deal with: the providers of certification services; e-contracts; the responsibility of intermediaries and the rules of conduct; dispute resolution and civil procedure. Each member state is responsible for regulating its e-sellers and may not restrict the activities of e-sellers established in another member state. E-sellers have the responsibility to: provide full information in advertisements (professionals must adhere to their professional advertising standards) and must confirm the receipt of the order without delay. The Internet service provider is not held legally responsible for the content of information if they act as a mere conduit, cache or host.

The Committee on Internal Market and Consumer Protection of the European Parliament passed the Digital Content Directive (DCD) that was provisionally agreed upon by the Council of Europe in 2019. The compromise ensures that the DCD conforms to the Directive on the sales of goods in classical commerce and provides a coherent and clear legal framework on digital content commerce. The Directive is intended to ensure a high level of protection and legal security to European consumers, especially when making purchases in other member states, as well as to provide support to the sellers, the small and medium-sized firms in particular, in offering their goods and services in the entirety of the EU market. The

Directive's objective is to harmonize to the greatest extent possible the rules of contracts on the provision of digital content in the whole of the EU, while allowing the member states to retain or introduce provisions in certain regulatory areas, e.g. a longer period of legal guarantee. Member states will also be able to regulate the general aspects of contract law, such as entering into, the validity, futility and termination of contract to the extent that they are not regulated by the Directive, or the right to compensation. Apart from the Digital Content Directive, the Proposal for the directive on the treaty on better implementation and modernization of consumer rights in the EU (the so-called omnibus directive, a part of the legal regulations on the digital commerce and services (e-commerce) in the strategy of the Single digital market) is in its preparatory stage.

Sales management in the digital environment from the aspect of agribusiness

E–agribusiness can be defined as any form of business transaction in which the parties interact electronically rather than by physical exchanges or direct physical contact. Doing agribusiness on-line through internet is generally referred to as electronic agribusiness. In short it is called as "E-agribusiness" (Dash and Mohapatra, 2017).

It is also referred to as application of e-commerce in agribusiness. Basically it is Information Technology (IT) based agribusiness. Therefore, E-agriculture is a promising area encompassing the agricultural value chain through the application of the Internet and related technologies.

Application of E-agriculture encompasses all agriculture and infrastructure projects in which ICT has the potential of enabling the empowerment of the community, such as: Providing Internet Demand Based Agriculture Information through ICTs, helping farmers to access information on commodity prices. Such practices are adopted for crop cultivation and in forging direct relationships with potential buyers in order to provide better value for their produce (Dash and Mohaptra, 2017).

How does the Netherlands, a country significantly smaller than Australia, manage to produce $158 billion in agricultural food exports compared to Australia's $50 billion? Why is agricultural food export earnings per hectare in the Netherlands $83,300 versus $109 (810 times more than Australia)? How it is that Australia has 185 times more land mass and 249 times more productive agriculture land, and yet the Netherlands exports three times more agricultural produce per year making them second in the world only after the USA?

To find the answers to these questions, one must look at the ways agribusiness in the Netherlands exploits the digital environment, especially the IoF and E-commerce. The Internet of Farms (IoF) is an umbrella term for all the farm devices connected to the Internet. From the simple connected switches to advanced soil sensors, almost all new sensors that hit the market have the Internet accessibility feature. The Netherlands has unlocked on-farm data by deploying a nationwide IoT communication networks that are helping to digitally transform the agricultural sector. The fourth industrial revolution enabled the Netherlands to become a globally recognized leader in the Ag 4.0 sector. Agriculture 4.0 is a term for the next big trends facing the industry, including a greater focus on precision agriculture, the internet of things (IoT) and the use of big data to drive greater business efficiencies in the face of rising populations and climate change.

The IoT enables physical objects to be sensed and remotely monitored to create a direct integration between the physical and digital worlds. It provided the agricultural workers with the information crucial for better decision-making or to change the way of conducting business. This benefits not only the farmer, but also the entire agricultural products supply chain. Using the IoT, the influence of farm connectivity can be extended all the way to the end consumer.

The project Internet of Food & Farm 2020 (IoF2020) examines the potential the IoT technologies hold for the European food and agriculture industries. It has set an ambitious goal: to make precise agriculture a reality and make that vital step in the more sustainable food supply chain. The IoT technologies increase the yields and product quality.

Big data has the potential to benefit the whole supply chain and will play a greater role than ever before in transforming the agriculture industry. The advanced connectivity of a global agriculture network provides a vast number of benefits up and down the supply chain: Farmers can use their data to apply the right products, at the right rates, and at the right time; distributors can use data to source inputs and position themselves for maximum advantage in the market; manufacturers can improve their means of production and better target their customer base (Proagrika, 2019).

The role of big data is one of change, likely the largest change seen in agricultural operations this century. Big data will make the whole chain more competitive and profitable.

For agribusiness firms upstream of the producer, it should be remembered that E-Commerce is not so much a product as it is a method for delivering agricultural inputs.

Conclusions

Agribusiness needs to adapt to the new "digital age" by supporting the development of information and communication technologies.

The attitude toward change of the individuals involved in agriculture management and the lack of skills and initiative to implement IT may frustrate the efforts to implement the concepts of expedited development and new business models focused on IT for agricultural companies. Understanding the way e-Commerce can affect the delivery of agricultural products would greatly benefit both the stakeholders and policy-makers.

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TRADE IN TERMS OF GREEN ECONOMICS

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Abstract

The issue of quality has always been present in trade, but mostly as a commercial requirement in the establishment and functioning of buying and selling relations. Historically observing, it can be concluded that the quality of the trade in market developed countries has evolved from elementary properties of assortment quality (durability, utility), over the quality of serving, to the eco-quality. Direction of quality evolution was determined by development of ecological awareness about the vulnerability of nature and environment. This is why the economies of certain countries are at different levels of ecological economics and eco- quality. In these circumstances, companies from different business segments, including trade, are identified as one of the key links in the environmental responsibility chain. Therefore, they are increasingly required to meet customer needs for products and services, while also taking into account the environmental impacts they have. Adequate identification of emerging trends that are reflected in, among other things, increasing demands for environmental responsibility, results in the creation of new ways to improve competitiveness. Thus, more and more companies are trying to integrate the concept of environmental responsibility into their business by introducing practices that have the epitome of "green". This paper will discuss the responsibilities of companies, especially trade, in the fight for environmental protection, the way companies incorporate environmental principles into their businesses, as well as the unfair practices that arise in pursuit of greater competitive advantage in a green economy.

Keywords: *Trade, Eco-quality, Eco-standard, Green economy.*

Introduction

The economic crisis of the 1970s and reports of the alarming consequences of uncontrolled economic growth on the environment have led to increasing demands for a change in approach to economic policy. The new economic policy should support the transition to an economy that respects the interests of future generations, as it requires the responsible use of scarce resources. Processes taking place globally make significant contribution to this goal. Initiatives from international environmentalists, the increasing influence of enviromentralist movements, and the media are increasingly influencing people's environmental awareness while creating new trends. In these circumstances, companies are designated as one of the key links in the environmental responsibility chain. Therefore, they are increasingly required to meet customer needs for products and services, while also taking into account the environmental impacts they have. Adequate identification of emerging trends that are reflected in, among other things, increasing demands for environmental responsibility, results in new ways to improve competitiveness. Thus, more and more companies are trying to integrate the concept of environmental responsibility into their operations by introducing "green" or "environmental" practices. This paper will discuss the corporate responsibility for environmental protection, how companies incorporate environmental principles into their operations in pursuit of greater competitive advantage in a green economy.

Green economy for sustainable development in trade

The rapid industrial development, in addition to positive changes such as significant production growth, accelerated economic development, higher employment and the living standard of people, has influenced the emergence of numerous forms of negative environmental consequences. Increased exploitation of resources has been necessary to enable mass production and meet the growing needs of the population. This has brought a number of environmental consequences such as pollution of water, soil, and air, destruction of biodiversity, climate change, and rising ocean levels. These phenomena have long been obscured, inter alia due to the lacking interest to consider the negative impacts of industrialization and high investment to prevent environmental consequences. Human negative environmental impact was only recognized globally in the early 1970s, at the first United Nations International Conference on the Environment in Stockholm. This conference is thought to have had a decisive influence on the growing interest of the international community in the growing environmental problems. The ensuing debates have focused on the role of man in the process, paying considerable attention to finding ways to stop these negative trends. In 1983, the United Nations adopted a Resolution establishing the World Commission on the Environment, and one of the historically significant accomplishments of this commission is the report "Our Common Future" (Brundtland report). Pointing to the dangers current economic development policies pose for future generations, the Commission has proposed adopting a sustainable development concept with responsibility at all levels, from global to local. The Commission's report was adopted at the Second United Nations Conference on Environment and Development in 1992, held in Rio de Janeiro. In addition, Agenda 21 was adopted, which defines the sustainable development goals and highlights the need to work towards sustainable development goals at the international, national, regional, and local levels. At subsequent conferences, countries confirmed their commitment. The first day of the Stockholm Conference in 1972, 5 June, was proclaimed World Environment Day. Agenda 2030 of 2015 extended the principles of sustainable development. Serbia has accepted the new agenda and in 2015 established an Interagency Group for the Implementation of the 2030 Agenda. The first Voluntary National Report on the Implementation of the 2030 Agenda for Sustainable Development was presented in 2019 (Agenda for Sustainable Development).

The concept of sustainable development integrates the economic, environmental, cultural and social component and is based on the principle of responsibility towards future generations when using resources. Consumption of scarce resources and environmental pollution have their limits (Ćuzović and Ivanović, 2017). Current generations must reconcile economic and overall development with these limits so as to ensure at least the same environmental quality for future generations. Sustainable development also means aligning development with the principles of social justice at the local, national, and global levels, as well as the transition from a market economy to an environmental economy. Thus, the concept of sustainable development emphasizes the deep link between economic development, the environment, and social well-being (Milenović, 2000). The issue of the environmental or green component of sustainable development came to the fore in 2012 at the UN Conference on Sustainable Development – Rio+20, dedicated to the green economy.

The Green Economy concept was first used by *Pearce et al.* (1989) in their report Blueprint for a Green Economy. (Merino-Saum *et al*., 2019). UNEP defines a green economy as one that contributes to human well-being and social equality while reducing environmental risks and environmental scarcities (UNEP, 2011). So, the new concept does not replace sustainable development, but further emphasizes that sustainable development goals are almost solely based on "correcting" the economy. This involves international coordination in promoting the economic "greening", the abolition of all policies, regulations, subsidies and benefits at the state level, which encourage environmentally-unfriendly business practices and the private

sector support to adopting green business practices. These efforts, as part of the UN Environment Program, address issues such as climate change, natural disasters, the use of scarce resources, and other phenomena that result from human negative environmental impacts. Governments also receive financial and advisory support to create public policies and adopt laws that preserve ecosystems. In this regard, programs involving international organizations, governments and non-governmental organizations have been launched, such as: Green Economy Initiatives (2008), Green Growth Knowledge Platform (2012), Partnerships for Action for Green Economy (2013) and others. In recent years, the concept of an inclusive green economy has evolved, which further emphasizes the need to strengthen solidarity, equality and promote human well-being and health. This requires that each country, within its capabilities, take some responsibility to meet these global challenges.
Numerous environmental measures are applied at different stages during the product life cycle. The rise of a green economy involves bringing together a large number of businesses involved in creating the final product and marketing it to the consumer. The most polluting economic sector is mostly the exploitation of natural resources that are to be used as raw materials for various products. During the exploitation, as well as extraction of metals and minerals from the ore, numerous processes are applied that greatly pollute the natural environment. In order to obtain 1 kg of pure copper, 50 to 200 kg of waste is produced, and 50 to 100 MJ of energy is spent (Kane, 2009, 9). As the value chain shifts, pollution is most often reduced, since manufacturers often pollute less than exploitation companies, while trade and services sectors are less harmful than manufacturers. Within the supply chain, economic and environmental benefits accrue. However, if consumers require non-polluting products whose production process is environmentally friendly, this is often beyond the direct control of the trader, since most of the pollution arises from exploitation and production of raw materials.
Consumers also play an important role in reducing pollution and preserving the environment. Much of the waste consists of inappropriately dismantled product packaging. Increasing consumer awareness of giving primacy to environment-friendly products will reduce the demand for products whose process of production and obtaining the raw materials significantly pollutes the natural environment. Also, awareness of the proper disposal of waste and recycling will reduce the need to exploit certain raw materials that can be obtained through recycling. The above example shows that all participants in the products value chain, from the exploitation of raw materials to consumers and recycling companies, play an important and indispensable role in reducing pollution.

Eco-labeling of products

Eco-labeling is a voluntary accepted certification by companies regarding the environment protection. In order to get the product an eco-label, the complete life cycle of the product is analyzed, from the raw materials used and how they were obtained, to the production process to the way of use and disposal. Customers appreciate the added value they can get when buying a product, so, in addition to price and convenience, retailers can provide customers with new quality by offering eco-labeled products (Ćuzović and Sokolov-Mladenović, 2015). The criteria for awarding the right to eco-label are defined on the basis of scientific research and knowledge, requiring the monitoring of the product life cycle and the application of complex formulas. Customers cannot carry out such calculations on their own, and are, in most cases, not competent to interpret them. The eco-label simply confirms to customers that the product meets the pre-established criteria, i.e. that it represents an environmentally friendly product. The first eco-label in the world was introduced in Germany in 1977, and then other countries introduced national eco-labels. National symbols are often used in combination with green, which increases their recognition. This also increases environmental awareness of customers, as a distinctive green logo can encourage customers to explore its purpose. According to a 2014 survey by the Hartman Group, 62% of respondents asked for product information that a manufacturer claims to be eco-friendly. In this way, customers simultaneously gain knowledge on the reasons for the eco-label, the criteria that the product must satisfy, and, in general, the importance of environmental protection. Thus, eco-labels educate customers, raise awareness of the importance of environmental protection, and, potentially, contribute to adopting environmental principles in all aspects of life and work. Due to the increasing number of labels suggesting a commitment to environmental responsibility, the International Organization for Standardization is developing standards concerning various aspects of eco-labeling of products and services. Eco-labeling standards belong to ISO 14020 – General principles and guidelines for the development and use of eco-labels and declarations. There are three standards within this group that reflect the ISO label classification (Georgeson *et al*., 2017):

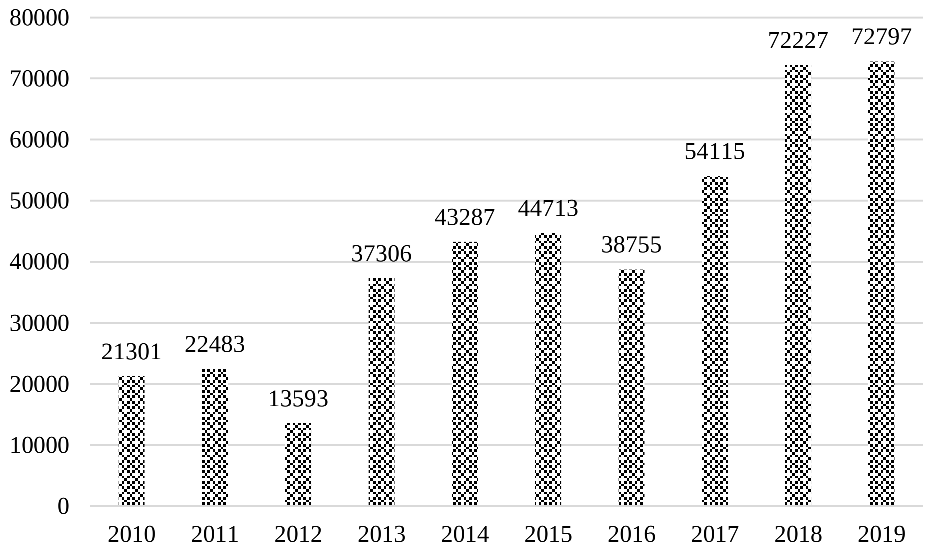
- Type 1 (ISO 14024) - eco-label awarded by a licensed independent organization if the eco-label applicant meets the defined criteria for the entire product life cycle. These may be national or regional eco-labels: "Environmental Choice" in Canada, "Blue Angel" in Germany, "European Flower" for EU members and other labels;
- Type 2 (ISO 14021) – claims that a product meets a certain environmental principle such as "produced from n% renewable material" or "reduced carbon dioxide emission". These are self-initiated claims, which independent organizations do not confirm, so ISO standards can contribute to their credibility;
- Type 3 (ISO 14025) – quantitative monitoring programs for the ecological performance of products throughout the life cycle based on parameters defined by a third, independent party, which verifies the data obtained. It applies to products belonging to a larger industrial group, and, due to the complex verification procedure, there are fewer labels of this type.

Serbia introduced the national eco-quality label in 2011 and is called "Friend of the Environment". This label, according to the ISO classification, belongs to the type I eco-label and is defined by the SRPS ISO: 14024. Accredited organizations verify the fulfillment of the criteria and award the label on the basis of the "Rules on closer conditions, criteria and procedure for obtaining the right to use the eco-label, elements, appearance and method of using the eco-label for products and services". Product groups and criteria for awarding the label by group are aligned with those applicable to the European Flower award.

Companies are increasingly recognizing the effects of eco-labeling, so labels such as "100% biodegradable", "environmental" or "eco friendly" can often be seen. Claiming environmental responsibility can increase sales revenue, business reputation and greater brand value. Among the effects of eco-labels is the advantage over competitors' products, since customers are increasingly comparing products and opt for a product that offers additional, "green" quality. These labels represent a significant marketing tool and have a psychological effect on customers, as they suggest that products and packaging are acceptable from an environmental point of view. Therefore, the increasing number of eco-labels raises concerns about the credibility of these claims. Today, the market has been characterised by the co-existence of a large number of ecolabels, claims and declarations. (Gruère, 2013). The question arises as to whether these labels suggest a genuine concern for the environment or the idea to increase the purchase of products. This is due to the lack of legal basis for regulating this field and the possibility of eco-labels being issued without verification by an independent institution.

Graph 2 shows data on eco-labeled products and services in the EU. In the observed period, the number of eco-labeled products and services has increased from 21,301 in 2010 to 72,797 in 2019. However, in 2012 and 2016, the number of eco-labeled products in the EU decreased. This decline is caused by the expiration of the licenses of a specific product group before the completion of the audit and renewal process. The countries with the highest number of eco-labeled products and services are Spain with 30,877, Italy with 8,630, Germany with 4,390, Belgium with 4,369 and France with 4,192.

Graph 2. EU Ecolabel Products and Services from 2010-2019



Source: European Commission https://ec.europa.eu/environment/ecolabel 20.09.2019.

Companies are increasingly recognizing the effects of eco-labeling, so labels such as "100% biodegradable", "environmental" or "eco friendly" can often be seen. Claiming environmental responsibility can increase sales revenue, business reputation and greater brand value. Among the effects of eco-labels is the advantage over competitors' products, since customers are increasingly comparing products and opt for a product that offers additional, "green" quality. These labels represent a significant marketing tool and have a psychological effect on customers, as they suggest that products and packaging are acceptable from an environmental point of view. Therefore, the increasing number of eco-labels raises concerns about the credibility of these claims. The question arises as to whether these labels suggest a genuine concern for the environment or the idea to increase the purchase of products. This is due to the lack of legal basis for regulating this field and the possibility of eco-labels being issued without verification by an independent institution. Eco-labeling can be misused by companies in order to attract customers. It is important to lay down rules to sanction such behavior, which could be a topic of further research.

Conclusions

Global efforts to reduce the impact of economic growth on the environment and secure the future of new generations have reflected, among other things, on corporate operations. Similar to macro-level activities, companies are required to design processes, operations, products and services as environmentally friendly. This means that, in a green economy, respect for environmental standards is a prerequisite for successful market positioning, especially for companies seeking to internationalize their businesses. This is why environmental thinking is increasingly becoming an integral part of the business decision-making process and the creation of product bundles. At the same time, growing customer interest in eco-labeled products has encouraged individual companies to use these labels as part of their offer, without a real commitment to environmental principles. Thus, false claims of compliance with environmental standards mislead customers and jeopardize the position of companies that make significant efforts to meet environmental requirements. International organizations have recognized this problem, and pay considerable attention to analyzing and making recommendations to avoid this unfair practice. However, states will play a key role in this process, enacting laws that protect customers' interests from unscrupulous retailers. Considering the growing interest of stakeholders in "greening" all business aspects, it is concluded that companies that are essentially committed to environmentally responsible business will have a great advantage in the future.

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THE OPERATIVE PROFIT MARGIN IN RETAIL FOOD

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Abstract

Both theoretical and practical importance have recently been attached to an analysis of the operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of the long-term performance of companies. In the integrated financial reporting it is presented through various indicators based on it. In view of this, we have made a comparative analysis of the operating profit margin and its impact on the performance of food trade companies in Serbia and comparable countries. Under the influence of different factors, the dynamics of the size of the operating profit margin of food trading companies in Serbia varies from comparable global food retailers in various countries. The EBITDA margin of the leading food trading companies in Serbia is lower than the in analyzed comparable food retail trade companies. It points to the conclusion that it is necessary to efficiently manage revenues, costs, profit, assets, and financial structure in order to improve the performance of food trading companies in Serbia in the future**.** The general conclusion is that it shows a growth tendency and is, nevertheless, lower in comparison to food trading companies from countries of a developed market economy. In order to increase the operating profit margin, as a measure of long-term performance, it is necessary to manage the financial structure of the food trading companies in Serbia as effectively as possible. (JEL codes: L81, M31, M41, O32)

Keywords: net profit, interest, tax, depreciation, amortization.

Introduction

Considerable theoretical and practical attention has been paid in recent years to analyzing the operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of the long-term performance of companies. On the basis of it, special indicators of long-term performance of companies have been developed. They are comparatively analyzed by individual companies (from the same and different sectors) and based on this –their long-term business success is recognized. Bearing this in mind, the subject of research in this paper is a comparative analysis of the operational profit margin of food retail enterprises in Serbia and comparable foreign retailers. The aim of this research is to thoroughly investigate the problems of the operating profit margin as one of the determinants of the long-term performance of food trading companies in Serbia and, on that basis, to propose the measures for its improvement in the future. This gap is to a certain extent filled with this paper, in what we find its scientific and professional contribution. The basic hypothesis of research in this paper is that the operating profit margin is a significant measure and determinant of the long-term performance of food trading companies. For these reasons it is necessary to investigate it more extensively on the example of food trade companies in Serbia, particularly the dynamics and factors of its size. In this paper, we will explore the dynamics of the size of EBITDA margin of well-known global food retailers, such as Wal-Mart, Tesco and Ahold Delhaize, in order to make comparisons of the EBITDA margin with Serbian trading companies. This provides the basis for proposing adequate measures to increase the size of the EBITDA margin, as a measure of long-term performance of food trading companies in Serbia. The EBITDA margin of the analyzed leading food trading companies in Serbia (Ahold Delhaize Serbia, Mercator-S and IDEA) is lower than that of analyzed comparable food retail (primarily food) trade companies from the developed market economies. Overall, more efficient management of the financial structure of capital, sales revenues, costs of goods sold (including operating costs, interest) and profit can significantly influence the increase in the EBITDA margin as a measure of the long-term performance of food trading companies in Serbia. This will definitely have a positive impact on the dynamics of the size and efficiency of investments, as a key factor in the performance of food trade companies in Serbia.

Material and methods

For the needs of the research of the treated problems in this paper, empirical data from the Agency for Business Registers of the Republic of Serbia were used. They are completely comparable to the same type of other global food retailers 'data and, in this sense, there are almost no restrictions on the obtained research results in this paper due to the fact that we used empirical data from their publicly disclosed financial statements in this study. With the defined aim and research hypothesis, the basic methodology in this paper is the comparative analysis and application of the relevant statistical analysis. Also, to a certain extent, the historical and normative methodology was applied in researching the treated problem in this paper. The operating profit margin or earnings before interest, taxes, depreciation and amortization (EBITDA margin) as a measure of performance has been used since the mid-1980s, especially since the 1990s in all companies, including wholesale and retail (Levy, 2014). There is extensive literature written on the subject of general problem of measuring the significance of gross operating margin in financial reporting for the needs of more efficient company management (Sui, 2017). However, a number of published papers dedicated to the specificities of gross operating margin analysis in commercial enterprises (food retailers) is significantly lower (Berman, 2013; Corona, 2014; Špička, 2016; Tan, 2016; Calva, 2017; Carstea et al, 2017; Ko et al., 2017; Hoe, 2017; Manini, 2017). This particularly applies to literature in Serbia (Lukic, 2017a, b; Lukic, 2018) – as far as we know, there is no complete work that has been published so far on the issue of the importance of measuring and analyzing gross operational margin in Serbia's trade companies (food retailers). As a measure of long-term performance of (food) retailers, the operating profit margin has its advantages and disadvantages. It is considered that during the usage of this criterion retailers are focused on the performance of fundamental business rather than on financial decision-making related to depreciation of fixed assets, interest and financial structure (lending instead of increasing equity by selling shares) (Levy, 2014). In view of this, it provides bankers, investors, creditors, fiscal authorities and others an insight into the long-term potential options for collecting their retailers' claims. More and more financial analysts are aware of certain problems of interpreting the EBITDA margin, and in order to overcome them, the model of economic additional value (the so-called EVA model) is recommended. Nevertheless, it should also be noted that the very model of economic value addition has its own weaknesses, which primarily relate to subjective assumptions regarding the calculation of capital costs. In conclusion, it is necessary to use both models (EBITDA margin, EVA model) concurrently when assessing the long-term performance of retailers. We are well aware of the fact that in recent years many global retailers, and what we consider quite right, also regularly report on the economic value added (for example, METRO group and others) in the context of integrated financial reporting. In this way, the problem of interpreting the EBITDA margin is partially mitigated. Due to the specifics of the nature itself, way of doing business and the applied financial management strategy, the dynamics of the size of the operating profit margin varies by individual types of trade (wholesale and retail), retail companies and countries in which they operate, retail chains (types of shops) and product categories. This is scientifically proven by the empirical analysis of EBITDA-size dynamics of retailers 'margin which has been carried out from different perspectives. Model of calculating the EBITDA margin, it is determined in the following way: EBITDA = Revenue - Expenses (excluding interest, taxes, depreciation and amortization), i.e. EBITDA = Net profit + Interest + Tax + Depreciation + Amortization. From this last formula it follows that: Net profit = EBITDA - (Interest + Tax + Depreciation + Amortization). For illustration purposes Table 1 shows the model for calculating the EBITDA margin in the global retailer Wal-Mart. Therefore, it is consistent with the model shown above.

Table 1. Model of calculating the EBITDA margin at Wal-Mart (USD $ million)

	January 31, 2018	January 31, 2017
Net income	9,862	13,643
Add: Net income attributable to non-controlling interest Less: Income from discontinued operations, net of income tax Add: Income tax expense	661 - 4,600	650 - 6,204
Earnings before tax (EBT)	15,123	20,497
Add: Interest expense, debt, capital lease and financing obligations	2,330	2,367
Earnings before interest and tax (EBIT)	17,453	22,864
Add: Depreciation and amortization	10,529	10,080
Earnings before interest, tax, depreciation and amortization(EBITDA)	27,982	32,944

Source: https: // www.stock-analysis-on.net, (May 10, 2018)

Results and discussion

The value multiplier is determined as follows:
Enterprise Value / EBITDA = (Market Value of Equity + Value of Debt-Cash) / EBITDA.
It shows how the market values the (retail) firm in accordance with the ability to generate operational profits (Enterprise value/EBITDA (EV/EBITDA). As an illustration, Table 2 shows a value multiplier on the example of a food value chain in the US.

Table 2. Food value chain value multiplier in US, January 5, 2018

	EV/EBITDA
Farm/Agriculture	13.07
Food processing	13.01
Food wholesale	10.43
Retail (grocery and food)	8.40
Restaurant/Dining	12.69

Note: Enterprise Value / EBITDA = (Market Value of Equity + Value of Debt-Cash) / EBITDA.

Source: Enterprise Value Multiples by Sector (US), January 5, 2018, http://pages.stern.nyu.edu/~adamodar/New_Home_Page/datafile/vebitda.html, (May 10, 2018)

The data in a given table show that the value multiplier is different for some members of the food value chain in the US. Thus, for example, it is significantly higher for farming/agriculture (13.07) than for retail (8.40). This is partly due to differences in the very nature of their business. In order to make in-depth analysis of the EBITDA margins in the food retail sector, Table 3 shows a value multiplier of the Wal-Mart retailer and its competitors for 2017 and 2018.

Table 3. Value multiplier,Wal-Mart (January 31, 2017 and 2018)

Wal-Mart Inc., EV / EBITDA calculation	January 31, 2018	January 31, 2017	January 31, 2016	January 31, 2015	January 31, 2014	January 31, 2013
Enterprise value (EV), (USD $ million)	305,207	260,427	260,724	306,165	300,184	297,926
Earnings before interest, tax, depreciation and amortization (EBITDA), (USD $ million)	27,982	32,944	33,640	36,433	35,861	36,489
Ratio EV / EBITDA	10.91	7.91	7.75	8.40	8.37	8.16
Benchmarking EV / EBITDA competition						
Amazon.com Inc.	-	43.74	31.40	33.08	33.45	41.58
Costco Wholesale Corp.	-	12.63	13.27	13.49	12.08	12.30
eBay Inc.	-	14.50	8.04	8.92	12.34	12.88
Home Depot Inc.	13.46	12.90	13.23	12.90	11.11	11.74
Lowe's Cos.Inc.	11.24	11.43	12.18	12.79	10.47	9.60
Netflix Inc.	-	18.03	11.80	10.59	8.22	9.62
Target Corp.	6.96	5.59	7.47	9.21	7.85	8.09
TJX Cos.Inc.	-	11.16	11.83	11.14	10.70	9.10
EV / EBITDA, Sector						
General retailers	-	15.92	12.72	12.34	10.93	10.95
EV / EBITDA, Industry						
Customer service	-	12.26	11.49	10.91	10.77	10.30

Source: https: // www.stock-analysis-on.net, (May 28, 2018)

The data in the given table show that the value multiplier differs between some food retailers. Thus, for example, on January 31, 2018, in Target Corp. it was 6.96 and in Wal-Mart 10.91, respectively. The Wal-Mart value multiplier is lower than the average of the sector and industry. These differences are certainly the result of the implementation of different financial management strategies (lending versus the increase in equity by selling shares). Earnings before interest, taxes, depreciation and amortization differ among individual food retail companies. Table 4 illustrates the dynamics of the EBITDA margin of the global retailer Wal-Mart for the period 2008 - 2017.

Table 4. Dynamics of EBITDA margin of Wal-Mart, 2008 – 2017

End of period	WMT
January 2008	NA
January 2009	7.3%
January 2010	7.6%
January 2011	7.9%
January 2012	7.7%
January 2013	7.7%
January 2014	7.5%
January 2015	7.5%
January 2016	7.0%
October 2016	6.8%
January 2017	6.8%
October 2017	6.6%

Source: https://finbox.io/WMT/explorer/ebitda_margin, (May 10, 2018)

Recently, the EBITDA margin has decreased in Wal-Mart compared to the previous period. Compared to some competitors it is larger and compared to others – smaller (for example, Target Corporation 9.9%) (Table 5). This is, partly, the result of the very nature of the industry operations of its own, sector, company size and business operations model (i.e. the applied financial strategy of the business).

Table 5. EBITDA margin of Wal-Mart and its competitors, 2017

Company	EBITDA margin
Spartan Nash Company (SPTN)	-0.3%
Smart & Final Stores, Inc. (SFS)	3.1%
Kroger Company (The) (KR)	4.5%
CompanhiaBrasileira de Distribuicao (CBD)	6.1%
Casey's General Stores, Inc. (CASY)	6.2%
Best Buy Co., Inc. (BBY)	6.2%
CVS Health Corporation (CVS)	6.6%
Wal-Mart Stores, Inc. (WMT)	6.6%
Target Corporation (TGT)	9.9%
Consumer Staples (SECTOR:STPL)	12.5%
Procter & Gamble Company (The) (PG)	25.6%
#ERROR! (CNCO)	N

Source: https://finbox.io/WMT/explorer/ebitda_margin, (May 11, 2018)

Table 6 shows the dynamics of EBITDA margin of Tesco for the period 2014 - 2018.

Table 6. EBITDA margin of Tesco, 2014 – 2018

Fiscal year March-February. All values are expressed in millions of pounds (GBP)	2018	2017	2016	2015	2014
Sales/Revenue	57,491	55,917	53,933	56,925	63,557
EBITDA	2,957	2,581	2,202	(1,733)	4,757
EBITDA growth	14.57%	17.21%	227.06%	-136.43%	-
EBITDA margin	5.14%	-	-	-	-
EBIT	1,663	1,284	-	-	3,225

Source: https://quotes.wsj.com/UK/XLON/TSCO/financials/annual/income-statement, (May 11, 2018)

The data in the given table show that the share of EBITDA margin in revenues is lower in Tesco (5.14%) than in Wal-Mart (6.6%). This is partly a consequence of a different model of doing financial operations. Table 7 shows the EBITDA margin of Ahold Delhaize, which operates in Serbia as Delhaize Serbia.

Table 7. EBITDA margin of Ahold Delhaize

	12/16A	12/17E	12/18E	12/19E
Revenue (€ million)	63,093	63,943	65,348	66,920
EBITDA (€ million)	4,142	4,267	4,507	4,836
EBIT (€ million)	2,420	2,386	2,638	2,923
EBIT growth (%)	7.9	(1.4)	10.6	10.8
EBITDA margin (%)	6.6	6.7	6.9	7.2
EBIT margin (%)	3.8	3.7	4.0	4.4
EV/EBITDA (x)	6.7	6.4	6.0	5.5
EV/EBIT (x)	11.4	11.4	10.3	9.2

Source:https://research-doc.credit-suisse.com/docView?language=ENG&format=PDF&sourceid=emgpm&document_id=1077229781&serialid=7%2F%2FS9ldDW4ewIdMX6A26zlMtYs6VxLxiTmpgD2zQdGM%3D, (May 22, 2018)

In Ahold Delhaize, the EBITDA margin is higher than at Tesco (5.14%) and is approximately the same as with Wal-Mart (6.6%). In the future, there is an estimated growing trend. The EBITDA margin is certainly different among observed countries in which Ahold Delhaize operates. Ahold Delhaize's operating profit margin, observed by individual countries in which it operates, is significantly higher in the US and the Netherlands than in Belgium and Central and Southeastern Europe (to which the Delhaize Serbia belongs). These differences are the result of different general business conditions and applied (financial) business strategies.Table 8 shows the EBITDA margin of the Russian company X5 Retail Group for the period 2012-2016.

Table 8. Dynamics of EBITDA margin of the company X5 Retail Group, 2012-2016

	EBITDA Margin (Rub bn)	EBITDA margin, %
2012	35,1	7.1%
2013	38,4	7.2%
2014	46,4	7.3%
2015	59,4	7.3%
2016	79,5	7.7%
2016/2015	33.8%	

Source: Q1 2017 Financial Results, X5 Retail Group, Moscow, Russian Federation 27 March 2017, https://www.x5.ru/en/Documents/X5-Q1-2017-Financial-results.pdf, (May 12, 2018

The data in the given table clearly show that the EBITDA margin of the company X5 Retail Group is higher than in Wal-Mart, Tesco and Ahold Delhaize. In other words, its profitability measured by cash flows from operations (using EBITDA margin) is slightly better than the observed retail companies.

In the analyzed period, the costs of goods sold by trade companies in Serbia have slightly increased dynamically until 2015 and from that year up to 2017 much faster, as can be seen from Figure 1. The annual growth rate of the costs of goods sold (3.3%) is thus lower than the annual growth rate of the EBITDA margin (4.99%).To sum up, the return on investments in Serbia's trade companies increased to some extent. In order to make anin-depth analysis of long-term trade performance in Serbia measured by the EBITDA margin, we will show the respective margin for three significant trade companies in Serbia for 2016 (Table 9). Based on the data presented in the given table, we can also conclude that the EBITDA margin of the leading (food) trading companies in Serbia is lower than the analyzed comparable food retail trade companies from the developed market economies.

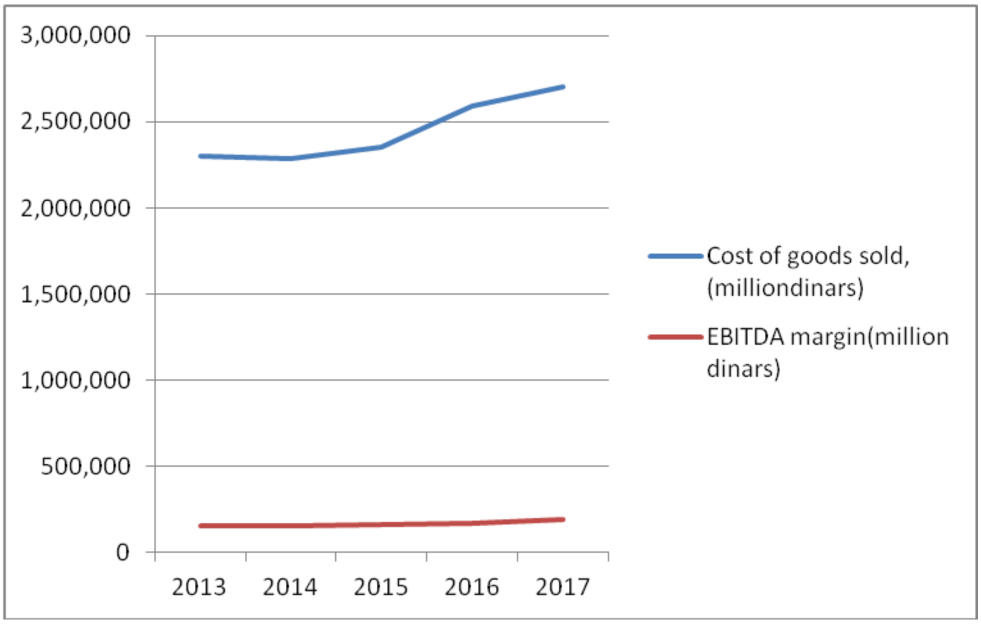

Figure 1. Cost dynamics of goods sold and EBITDA margin of trade companies in Serbia
Note: Figure illustrated by the author.
Source: Business Registers Agency.

The regression analysis show that the costs of goods sold, as a specific expression of investment, significantly affect the EBITDA margin (Pearson Correlation, 927, Sig. (1-tailed), 012 p <0.05). (The regression equation is: Y = -46285.142 + 0.087 X, where: Y = EBITDA margin, and X = costs of goods sold.) The method of financing working capital (especially inventories) is a significant determinant of the EBITDA margin of trading companies. In this context, we will examine the impact of interest costs on the costs of goods sold of the commercial enterprises in Serbia. The results of the correlation analysis show that there is a negative (statistically significant) strong correlation between the costs of sold goods and interest as a component of the EBITDA margin of trading enterprises in Serbia – this is quite logical – given their character.

Table 9. EBITDA margin of significant trade companies in Serbia, 2016

	EBITDA margin, (million dinars)	EBITDA margin, (% from sales)
Ahold Delhaize Serbia	3,719	4.3%
Mercator-S	3,081	2.9%
IDEA	117	3.99%

Note: Author's calculation.
Source: Business Registers Agency, Belgrade.

Conclusion

Due to the growing EBITDA margin trend and based on the analysis conducted in this paper, we can conclude that the long-term performance of trade companies in Serbia has recently improved. The average operating profit margin (EBITDA) of trading companies in Serbia expressed as percentage of revenue is slightly higher than 5% (Mean 5.1840). However, it is lower compared to the US, Canada, Europe, the Netherlands, Belgium, Central and Southeast Europe, Germany and Russia. More efficient management of the financial structure of capital (financial leverage = assets / capital) can influence the improvement of the return on sales measured by the relationship between the EBITDA margin and total revenues. Costs of goods sold, as a specific expression of the size of investments, significantly affect the EBITDA margin (Pearson Correlation, 927, Sig. (1-tailed), 012 $p < 0.05$). There is negative (but statistically significant) strong correlation between the costs of goods sold and interest as a component of the EBITDA margin, which is quite logical given their character. The EBITDA margin of the analyzed leading trading companies in Serbia (Ahold Delhaize Serbia, Mercator-S and IDEA) is lower than that of analyzed comparable retail (primarily food) trade companies from the developed market economies. Overall, more efficient management of the financial structure of capital, sales revenues, costs of goods sold (including operating costs, interest) and profit can significantly influence the increase in the EBITDA margin as a measure of the long-term performance of trading companies in Serbia. This will definitely have a positive impact on the dynamics of the size and efficiency of investments, as a key factor in the performance of trade companies in Serbia.

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THE NATIONAL RURAL INSURANCE SYSTEM IN MEXICO: PRIVATE PUBLIC PARTNERSHIP FOR RISK MANAGEMENT

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Abstract

In several countries there are initiatives to develop agricultural insurance schemes operated either directly by governments or by promoting this market with participation of private companies. Interest in the study of agricultural insurance arise from its impact on the sustainability of agricultural holdings by allowing farmers to conserve their means of production and maintain the capacity to reinvest in the next agricultural cycle in case of losses. In this paper an analysis of the National Rural Insurance System (SNARM), in Mexico is made. The participation of the government is provided by AGROASEMEX, a national insurance institution that functions as an agency for the development and research of the sector. Its functions include promoting the agricultural insurance market, providing reinsurance services to other stakeholders and granting subsidies to support cost of the insured's premium. It also provides support to the operation of the Insurance Funds. Also, the Ministry of Agriculture and Rural Development (SADER) has developed insurance schemes against natural disasters in recent years and provide additional support to producers with access to commercial insurance. There are six companies organized as corporations that have authorization to operate in agricultural and animal insurance. Finally, there is the participation of the producers who have set up 529 Insurance Funds. These are regional associations and they operate with non-profit and mutual principles. As a result, in Mexico there is a developed private-public partnership for risk management, adding to the government and private companies the participation of farmers providing self-insurance.

Keywords: *Risk management, agricultural insurance, insurance funds, catastrophic insurance.*

Introduction

Economic agents are subject to risks and uncertainty to at different degrees, according to the industry in which they operate. Agriculture production is characterized by highly volatile and unpredictable production results. Unlike most other entrepreneurs, agricultural producers cannot predict with certainty and reliability the amount of production they will obtain, due to the occurrence of risks such as weather, pests and diseases. The adverse events that occur during the establishment, development and harvesting of the crop can even cause the loss of the total production (Iturrioz, 2010).

The management of agricultural production risks is based on the optimal combination of technical and financial tools. Farmers and ranchers can absorb small but frequent losses through appropriate risk mitigation techniques in the production unit and with self-insurance tools. More severe but less frequent losses can be gradually transferred to cooperative / mutual insurance schemes, commercial insurers and reinsurers (Mahul & Stutley, 2010).

Agricultural insurance, among other risk management tools, can help improve agricultural productivity by helping farmers invest in more productive but potentially more risky agricultural practices. In a context, which is changing rapidly due to a more complex agri-food chain and climate change that may be increasing the frequency and severity of natural disasters and causing greater price volatility due to changes in the market structure, agricultural insurance is an important instrument to maintain long-term stability and growth of the agricultural sector, and facilitate access to credit, help reduce the negative impacts of natural disasters and encourage investment in improved production technologies (Porth & Seng Tan, 2015).

In the world, three agricultural insurance systems have been identified; the system controlled by the government, in which the supply of insurance predominates through a state entity; pure market systems, in which different insurance products are offered by insurance companies and public-private partnership systems with the participation of both the state and insurance companies (Iturroiz, 2010)

Mexico is a country that is highly exposed to a great variety of natural hazards. The southern Pacific coast of the country is located in the so-called Ring of Fire of tectonic activity. Tropical storms and hurricanes are a constant peril both the Pacific and the Atlantic coast. Droughts are increasingly affecting the entire country and even in typically humid regions, at the same time, a process of desertification is affecting vast northern regions. Under these conditions, natural hazards in Mexico have increased their economic impact in the last four decades (Saldana-Zorrilla, 2015).

The interest in agricultural insurance lies in its potential impact on the sustainability of agricultural holdings, because, in the case of a bad harvest, it allows farmers to keep their production tools and their ability to reinvest in the preparation of the next agricultural cycle (Sandmark , Debar, & Jaleran, 2014). This risk management instrument has been part of the public policies in Mexico since the 1940's. This paper identifies the current structure of the risk management system in Mexico from the perspective of the supply of agricultural insurance and the agents that comprise it.

Materials and Methods

Documentary review work was undertaken and databases of the main institutions involved in the National Rural Insurance System were compiled, such as AGROASEMEX, SA, the National Integrator of Insurance Funds (OINFA), as well as the regulatory entities of the system: the National Insurance and Bonding Commission (CNSF) and the Ministry of Finance and Public Credit (SHCP).

The information allowed identifying the agents that offered agricultural insurance directly to the producers as well as to those offering reinsurance, the area that has insurance coverage as well as the indicators related to the insurance of premiums and the payment of claims.

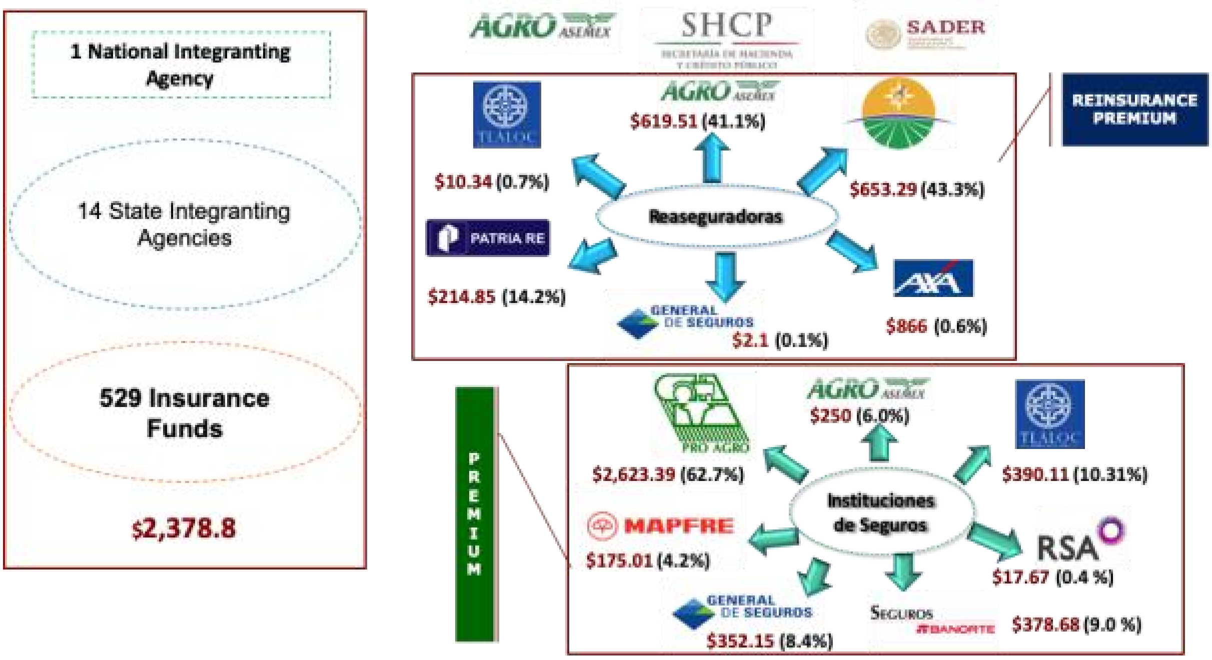
Results and Discussion

Structure of the National Insurance System for the Rural Environment

The transfer of the risk of loss, due to climatic or biological factors can be performed under three different modalities: (i) contract insurance with a private company, (ii) the organization of an insurance fund involving farmers directly each of whom pays the respective premium to a common fund, with the function of, compensating precisely those farmers who register claims, and (iii) limited coverage that can be accessed through the Agricultural, Livestock, Aquaculture and Fishing Catastrophic Insurance (SAC) component operated by SAGARPA and the governments of the states, which aims to be the address of the damages caused by natural disasters characterized for being atypical and unpredictable.

Thus, in the National System of Rural Insurance (SNAMR), the public, private and social sectors come together, giving rise to a public-private partnership. AGROASEMEX -is a national insurance institution-; is the part of the public sector that functions as an agency for development and research in the subject. Its functions include the promotion of the agricultural insurance market, the provision of reinsurance service to other actors and the payment of support as a subsidy to cover partially the cost of the premium of the insured as well as support for the operation of the Funds and the integrating organization. In the same public sector, in recent years the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food has developed insurance schemes against natural disasters and complementary support for producers with access to commercial insurance.

Figure 1. Structure of the provision of agricultural insurance in Mexico, (figures in millions of pesos)



Source. Author´s elaboration based on data from AGROAESEMEX and the National Insurance and Bonding Commission (2019)

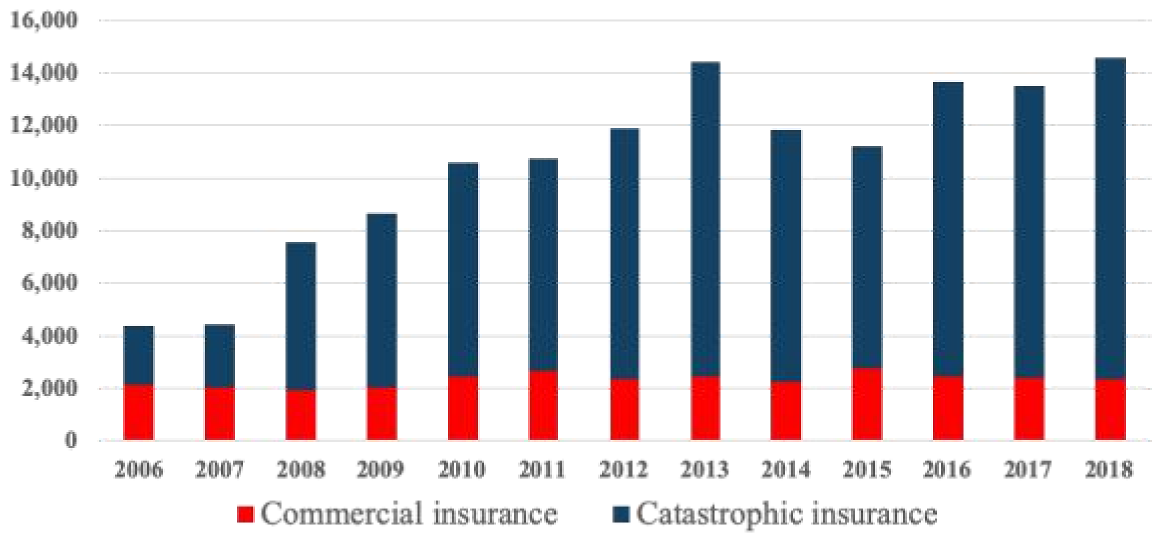
The private sector participates with 96 insurance institutions, constituted as corporations, of which 16 have authorization to operate the agricultural and livestock insurance. In the last 15 years, the maximum number of companies that made agricultural insurance was twelve, and at present only six companies operate agricultural insurance and five carried out reinsurance operations.

The organized producers constitute Insurance Funds, they are associations operating at the regional basis, working with non-profit and mutual principles. 529 funds have been set up (AGROASEMEX, 2018), and they are integrated at a higher level in 16 State Integrating Agencies and 1 National Integrating Agency supported by a specific legislation, the Law on Agricultural and Rural Insurance Funds.

Coverage of the insurance system

In Mexico, 21.9 million hectares of annual crops are sown, this is the first goal of where to provide protection by the agricultural insurance. There are two segments of the insurance market, commercial insurance mainly through which, a level of yield is protected allowing the recovery of the investment made in the crops until the time of occurrence of a loss through insurance policies or certificates. It is directly contracted by farmers.

Figure 2. Insured area by the National Insurance System for the Rural Sector (thousands of hectares)



Source: Author´s elaboration based on data from AGROASEMEX and SAGARPA

For historical reasons and due to the culture of risk management that is prevalent among farmers, the commercial insurance market has been closely related to the credit market. The natural insurance market is made up of those producers who plant relatively large areas, with irrigation, and who, by financing their investments with credit, have a greater propensity to protect them against the occurrence of adverse climatic and biological phenomena, thereby reducing the possibility of falling into insolvency and therefore being left out of the credit (Muñoz-Rodríguez, Santoyo-Cortés, Gómez-Pérez, & Altamirano-Cárdenas, 2018).

Until the year 2002, face the occurrence of a catastrophic year for agricultural activities in one or more states of the Republic, claims made to local government rose to such a degree that they compromised the state public finances, which invariably forced its leaders to request budget increases from the federation to attend the emergencies. Therefore, to avoid permanent pressure on public finances and have a foreseeable budgetary ceiling to address these contingencies, it was decided to create the second modality of access to insurance, catastrophic insurance in which relatively low sums are protected against risks of low occurrence but high impact. It is contracted by state governments with a federal and state subsidy.

Unlike commercial insurance, which has not grown in the last ten years, catastrophic insurance has recorded significant growth to represent an insured area that is four time larger than the covered by commercial insurance.

Catastrophic insurance is a massive type of insurance, acquired and paid for directly by state governments and the federal government to protect low-income producers when a natural disaster occurs that affects agricultural, livestock, fishing or aquaculture and it has limited coverage. The insured producers are not identified at the time of contracting, and it is the states that receives compensation in the case of catastrophic losses, then compensates the producers in the disaster areas. 11.5 million hectares are protected with catastrophic insurance schemes.

Result of the agricultural and livestock insurance

The indicator of technical and economic results for private equity companies and the state company show good performance in its operation. In the 25 years between 1991 and 2018, only in 3 years, was the amount paid for compensation higher than the amount of premiums collected. Especially in recent years, with the operation of catastrophic insurance this indicator has improved compared to the model focused on the commercial insurance offer.

Table 1. Amount of direct premiums, claims paid and branch loss index (data in thousands of pesos) *

Year	Premium (A)	Indemnity (B)	Loss Ratio (B/A)	Year	Premium (A)	Indemnity (B)	Loss Ratio (B/A)
1991	171,960	126,575	0.74	2005	740,892	679,991	0.92
1992	154,737	188,835	1.22	2006	798,147	407,809	0.51
1993	148,091	126,707	0.86	2007	902,667	430,117	0.48
1994	167,702	153,538	0.92	2008	1,584,593	1,072,251	0.68
1995	175,242	167,641	0.96	2009	1,730,331	1,432,811	0.83
1996	334,113	249,244	0.75	2010	1,921,968	943,960	0.49
1997	540,052	493,002	0.91	2011	2,437,247	3,080,374	1.26
1998	728,776	586,437	0.80	2012	3,424,180	1,251,890	0.37
1999	905,223	689,248	0.76	2013	4,272,057	1,742,790	0.41
2000	924,547	664,458	0.72	2014	4,222,997	1,816,847	0.43
2001	714,101	724,289	1.01	2015	3,853,562	2,388,561	0.62
2002	796,499	641,618	0.81	2016	4,080,334	1,857,847	0.46
2003	929,549	602,257	0.65	2017	4,069,011	2,383,305	0.59
2004	1,409,395	908,011	0.64	2018	4,187,005	2,179,397	0.52

* Does not include the information of Insurance Funds

Source: Prepared by the author with data from the National Insurance and Bonding Commission (CNSF).

At current values, premiums amounting to 46,325 million pesos have been issued, paying claims for 27,790 million, giving a loss ratio of 0.6. Despite these acceptable levels of loss rate, the fact is that almost 80% of the area insured at national level is under the catastrophic insurance modality and that really it is an insurance so that governments do not suffer an embezzlement in their budgets, more than insurance for producers, the coverage achieved by the system, especially in commercial insurance contracted directly by producers, is still low despite the high presence of risks that may be subject to protection and the relatively acceptable results of operation.

Conclusions

Mexico has a reliable system to provide of agricultural insurance; however, the coverage of this service is still low. From the perspective of the structure of the offer side, the participation of producers organized in Agricultural Insurance Funds with a wide coverage in the states of the country stands out, while by the private sector there are only six insurers out of 99 participate, which are registered in the country and only one of them concentrates more than 56% of the premiums in the market. The offer of reinsurance is even more limited, since only five companies provide the service and approximately 90% of the premiums from direct insurers are concentrated in them.

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7. FORESTRY AND AGRO-FORESTRY

FLAMMABILITY OF CERTAIN CONIFERS AND OAK SPECIES OF NORTH WEST ALGERIA

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Abstract

Fuel samples (*Quercus suber, Quercus ilex, Quercus, Pinus halepensis, faginea, Juniperus oxycedrus and Tetraclinis articulata*) were collected in a cork oak (*Quercus suber L)* forest in the southern part of the mountains of Tlemcen (Western Algeria). A series of flammability tests were carried out using a Mass Loss Calorimeter device (FTT ®). The results showed that the conifers species (*T. articulata, J. oxycedrus and P. halepensis*) were highly flammable because of their high combustibility and sustainability. A significant correlation was located between FMC and PHRR (combustibility, $r = -0.52$, $p < 0.05$) and between FMC and AEHC (sustainability, $r = -0.31$, $p < 0.05$).Analysis offreshfuels revealed *Juniperus oxycedrus*to be the most flammable species (FMC = 60,71 %, TTI = 46,85 s, PHRR = 190,72kW/m2, AEHC = 10,2 MJ/kg and RMF= 2,52). By contrast, Oak species presented low values of the four flammability parameters (high ignitability but low combustibility, sustainability and consumability). The fuel moisture content of Quercus spp. was significantly lower than that of other more flammable species. The findings suggest that the *P.halepensis* presents a very significant risk to the cork oak forests in Algeria.

Key words: *Cork oak forests, conifers, oak species, mass loss calorimeter, flammability, Tlemcen.*

Introduction

The Mediterranean basin is currently under severe water stress due to limited water resources and high demand (Milano et al., 2012). Unfortunately, this region of the world is not immune to the logic of fire, since more than 55 000 fires cover an average of 500 000 to 700 000 ha of Mediterranean forest each year, causing enormous ecological and economic damage, as well as loss of life. (Velez, 2000).The fire, especially at the peak of the summer period, poses a permanent threat to the forests of the Mediterranean region - especially in its western part - and is the main cause of destruction of forests and shrubby sub-forest stands (maquis, scrubland, scrub) (Ramade 1997).

According to M'hirit (1999), Mediterranean forests are mainly composed of areas of open forest or shrubs, maquis and grassy pastures dotted with woody vegetation such as the Oleaster thermophilic scrub (*Olea europaea* var oleaster) and lentiscus (*Pistacia lentiscus*); thermophilous coniferous forests of Aleppo Pine (*Pinus halepensis*), Maritime Pine (Pinus pinaster), Berber's Thuya(*Tetraclinis articulata*) and Juniper and Phoenician Juniper (*Juniperus phoenicea*); evergreen oak sclerophyllous forests: Holm oak (*Quercus ilex, Quercus rotundifolia*), Cork oak (*Quercus suber*), Kermes oak (*Quercus coccifera*); the deciduous forests of Zeen Oak (*Quercus faginea, Quercus canariensis*), Afares Oak (*Quercus afares*).

In Tlemcen mounts, conifers (*Pinus halepensis, Tetraclinis articulata* and *Juniperus oxycedrus*) occupy about 115 700 ha, or 56% of the forest area of the department. Oak species (*Quercus ilex, Quecus suber, Quercus faginea*) extends over 92 000 ha. It is the cork oak ecosystem that dominates in this region. Cork is the only product harvested from these stands. The major problem of these forests is the invasion of conifers with high flammability potential.

This mechanism causes an accumulation of an amalgam of dry and flammable forest fuel because of a strong presence of a sub-wood rich in essential oils and resins. In such climatic conditions and considering the properties of forest fuels, a mere even a small, increased heat can easily be at the origin of a fire and its spread (Kazakis and Ghosn, 2008). Between 1979 and 2009, the Algerian forest lost 1 162484 ha with a total number of reported fires of 41 644 (Bekdouche, 2010). During the year 2012, no less than 17 512 ha of cork oak forests disappeared (Abbas, 2013). In the western region of Algeria and specifically in the Tlemcen region and from 1970 to 2012, the average is 21 households /year. The area moderately burned between 1990 and 2015 exceeds 55 ha/year (DGF,2014). The concept of flammability still generates controversy (Schwilk 2015). Accordingly, flammability can be defined as the capacity of plant biomass to burn, i.e. to ignite and sustain a flame (Pausas et al. 2017). In the same vein, Anderson (1970) and Martin et al. (1994) approach it as a complex process with four compartments namely: ignitability or ease of ignition; the durability or ability of a material to maintain combustion and produce energy; combustibility or the speed with which combustion occurs; and the consumability or proportion of biomass consumed during combustion. Actually, several studies have been devoted to this parameter (Vallette, 1990; Hachmi et al.2011; Madrigal et al.2011). Analysis of the variations of the flammability of the fuel forest has a great importance in the hierarchy of forest between species and therefore in the improvement of the accuracy of the forest fires risk indices. Besides, they allow forest managers to better clarify forest formation and structures to guide forestry (Vallette, 1990; Marino et al.2011). As far as flammability assessment in the laboratory is concerned, a limited scale of experiment has been noticed (particle level or parts of plants, whole plants and stand scale, sensu White and Zipperer 2010) because the way in which plants are exposed to heat may not be comparable to wildfire conditions (Fernandes and Cruz 2012), and outdoor experimental fires are often limited.

Nevertheless, the heat release rate estimated at laboratory scale is a good index to characterise fire hazard and it can be used to classify fuels (Babrauskas and Peacock 1992). The characterization of flammability of companion species might help managers to prioritise treatments to lower the cover of high flammable species in order to reduce mainly the vulnerability of cork oak forests to recurrent forest fires (Catry et al. 2012). Therefore, the aim of the present study is to significantly evaluate the flammability parameters, at laboratory scale, by means of mass loss calorimeters in some frequent species of conifers and oaks that mingle strongly in the oak forests in Algeria.

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Materials and methods

Study site and Sampling

In the north of Algeria, the maquis is composed mainly of cork oak forests mixed with other hardwood and softwood species. For the purposes of the study, fuel samples were collected in a cork oak (*Quercus suber*L.) forest in the southern part of the mountains of Tlemcen (West of Algeria) (Fig. 1 left). The area is characterized by a sub-humid climate with 27 °C of average annual temperature and 500 mm of mean annual precipitation. The altitude varies between 1000 and 1282 m and the slope between 1.1 and 9.1%. In addition to *Quercus suber* as the main tree species, the following companion species (were considered: *Pinus halepensis* Mill., *Quercus ilex* L., *Quercus faginea* L., *Juniperus oxycedrus* L. and*Tetraclinis articulata* Vahl.). These species are frequent in cork oak forests and in the Mediterranean basin.

Fuel sampling was carried out during the fire season (September 2017) with the aim of assessing the effect of phenology on vegetation flammability and selecting low fuel moisture contents corresponding to high levels of fire hazard. Samples (approximately 500 g) of live

fine fuel (twigs Ø < 0.6 cm with foliage, according to Madrigal et al., (2009) were collected in five independent plots along one transect (34° 51′ 12.86″ N, 1° 21′ 07.99″ W) throughout the study area. In each plot, five trees and shrubs of each species were chosen at random. The samples were obtained with the aid of prune clippers and a telescopic saw.
Live fine fuel (approximately 500 gr) samples (twigs Ø <0.6 cm with foliage) were randomly collected from the upper, middle and lower part of the crown from different trees and shrubs, using prune clippers and a telescopic saw. It is wiser to record that all samples were placed into hermetic plastics bags and transported to laboratory in portable refrigerators within a few hours of the same day of collection. Once in the laboratory, one 100 g subsamples of each fine fuel sample were used to determine the fuel moisture content (FMC) by oven-drying the material in an oven at 100 ± 2°C during 24 h (until a constant weight was reached).

Experimental procedure
Indeed, 07 replicates were tested for each series in order to obtain at least 03 replicates which comply with repeatability criteria (Madrigal et al. 2009, 2013). Paired-samples were used in the experiment according to the methodology proposed by Madrigal et al (2013): one for the FMC determination using a moisture analyzer Computrac MAX ® 2000XL (Arizon Instrument LLC), the other for flammability tests (Mass Loss Calorimeter, FTT®) (Fig 1. right).In order to compare and classify the six species of the maquis and to thorough representation of the four flammability components sensu White and Zipperer (2010), the following parameters were measured: Time-to-ignition (TTI, s) for ignitability; Effective Heat of Combustion (AEHC, MJ/kg) for sustainability; Peak Heat Release Rate (PHRR, kW/m2) for combustibility; and Residual Mass Fraction (RMF, %) for consumability.

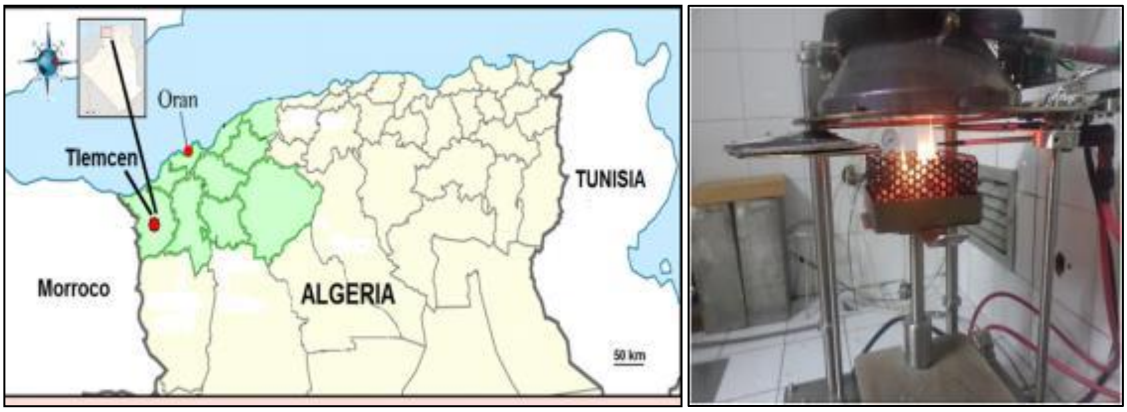

Figure 1. Left: Location of the study area Right: Flammability test in the Mass Loss Calorimete

Data analysis
Fuel moisture of fresh fuels (FMC) and flammability parameters (TTI, AEHC, PHRR, RMF) were compared by species using one-way ANOVA (n=30). Dunnest test was used to classify the forest fuel samples. SPSS.21® Software was employed to develop ANOVAs and to generate flammability classifications. The effect of FMC on flammability parameters was evaluated using a Pearson non-parametric correlation matrix.

Results and discussion

The results in the table show a marked variation between the averages recorded for the five parameters considered, a coefficient of variation varying between 17.66% (FMC) and 69.82% (ITT). Indeed, one may say that the ignitability under a heat source of 50 kW /m^2 varies from one species to another ($F = 49.36$, $p <0.000$). *Q. faginea* records the shortest time for ignition of the flame (12.42s) that is, this species has a high flammability potential. The minimum and maximum reached respectively vary from 6s to 19s respectively. On the other hand, Berber's thuya induces the slowest time (100.85s), with a minimum of 79s and a maximum of 122s. It is therefore the least flammable species.

Table 1. Measurements of the flammability parameters of the three species of oaks and conifers

Paramètres / Especes	FMC (%)	ITT (s)	PHRR (kW/m2)	AEHC (MJ/kg)	RMF (%)
J.oxycedrus	60,714(3,35)	46,85(18,56)	190,72(14,17)	10,22(2,52)	2,49(0,34)
P.halepensis	98,00(10,58)	89,57(21,29)	137,20(28,32)	7,42(2,92)	2,82(0,18)
T.articulata	71,42(71,42)	108,14(9,35)	128,67(89,53)	11,47(1,77)	3,55(0,30)
Q. suber	76,57(82,57)	28,14(12,79)	89,88(35,61)	7,52(2,50)	2,98(0,69)
Q. ilex	68,85(2,67)	31,85(11,92)	109,07(63,96)	8,60(1,74)	3,48(0,60)
Q.faginea	82,57(5.7)	12,42(4,57)	80,94(10,35)	4,45(1,61)	5,45(1,36)
Total	76,35(6,26)	52,83(37,33)	122,75(58,87)	8,28(3,08)	3,46(1,17)

As far as the Peak Heat Release Rate (PHRR, kW/m^2) is concerned, the variations are also marked between species ($F = 16.61$, $p < 0.000$). *Q. faginea* emits low heat among the six species, an average of 80.94 kW/m^2 with a minimum of 75.78kW/m^2 and 95.86kW/m^2. However, *J.oxycedrus* has the highest heat intensity, averaging 190.72kW/m^2, with a minimum of 160.71kW/m2 and a maximum of 199.60kW/m^2. With regard to durability measurements, Average Effective Heat of Combustion (AEHC, MJ / kg) records a very significant difference (($F = 8.50$, $p < 0.000$) Q. *faginea* is the weakest (4, 45 MJ / kg), with a minimum of 3.20 MJ/kg and a maximum of 7.20 MJ/kg, while *T. articiculata* records the highest values, an average of 11.47 MJ /kg, with a minimum of 9.60 MJ/kg, and a maximum of 14.30 MJ/kg.

Lastly, for the consumability measures, the differences are also detected (= 41.83, $p < 0.000$). *J.oxycedrus* induces the highest proportion of the residual mass among the six species, an average of 2.49%. In contrast *Q. faginea* holds the highest values (5.45%)

Effect of FMC on Flammability Parameters

The FMC was different among species collected during the same days (Fig.2). *P. halepensis* showed a FMC significantly higher than other tree species like *Quercus* (*Q.faginea, Q. ilex and Q. suber*) and *T. articulata*. Understory *J. oxycedrus.* presented similar FMC than *Quercus* spp.and *T. articulata* species.

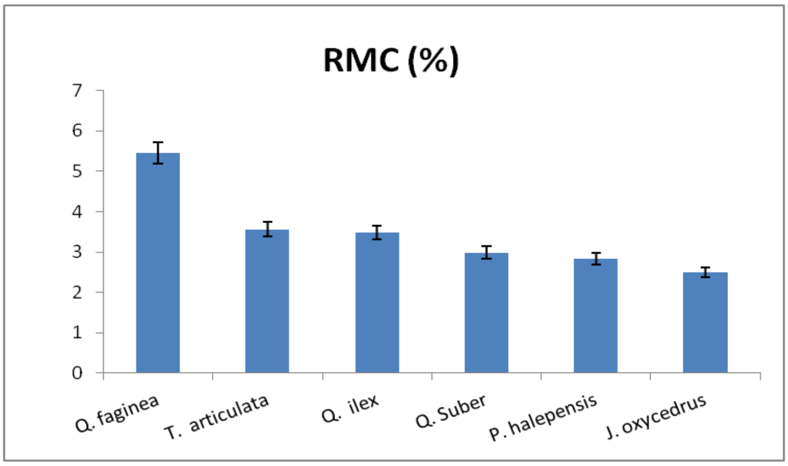

Figure 2. Fuel Moisture content (%) of selected species. Vertical bars show standard errors.

The effect of FMC on flammability parameters was evaluated using a correlation matrix. Surprisingly FMC did not show a significant correlation with TTI (ignitability) and RMF (consumability). A negative correlation was observed with PHRR (combustibility) ($p<0.01$) and with AEHC (sustainability) ($p<0.01$) (Table 2 and Fig.3).

Table 2. Correlation matrix (Pearson non-parametric correlation) for analyzed variables (*90% significance, **95% significance). Data (N = 42) correspond to 6 studied species (*Pinus halepensis*, *Juniperus oxycedrus*, *Tetraclinis articulata*, *Quercusilex.*, *Quercus faginea*, and *Quercus suber)*

	FMC	ITT	PHRR	AEHC	RMF
FMC	1,00	-0,15	-0,52**	-0,31**	0,13
ITT		1,000	0,39*	0,48**	-0,35*
PHRR			1,00	0,56**	-0,39**
AEHC				1,00	-0,34*
RMF					1,00

FMC fuel moisture content, TTI time to ignition, PHRR peak heat release rate, AEHC average effective heat of combustion, RMF residual mass fraction

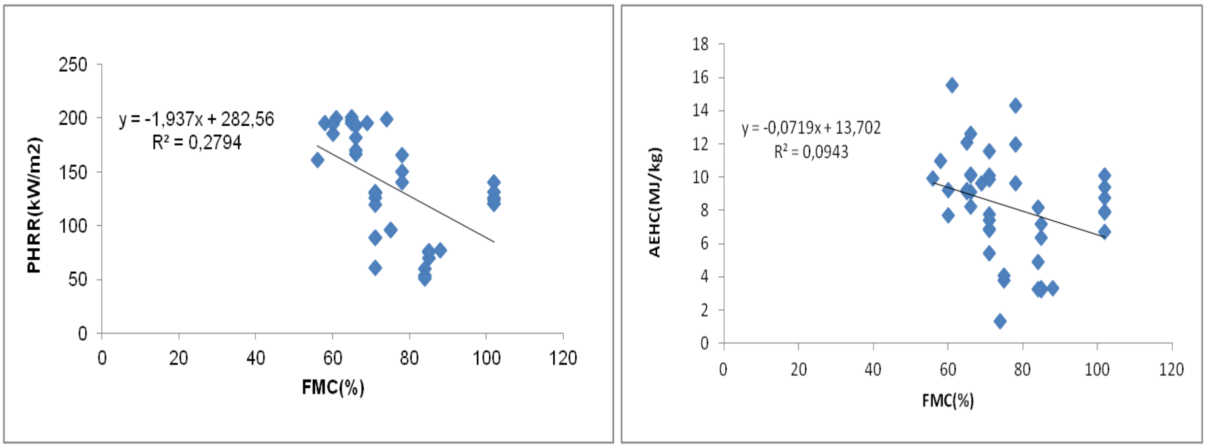

Figure 3. Correlation between Fuel Moisture Content (FMC, %) with (a) Peak Heat Release Rate (PHRR) and (b) Average Effective Heat of Combustion.

1.1.*Flammability of Cork Oak Forest Species*

Figure 4 reflects the comparison among analysed flammability variables for selected species. *Q. faginea* presented higher ignitability (lower time-to-ignition, fig.4a), lower combustibility (PHRR, fig.4b) and sustainability (AEHC, fig.4c) and higher consumability (RMF, fig.4d) than most of studied species. Therefore, *A. articulata* could be considered the lowest flammable of the series (fig.4 b).

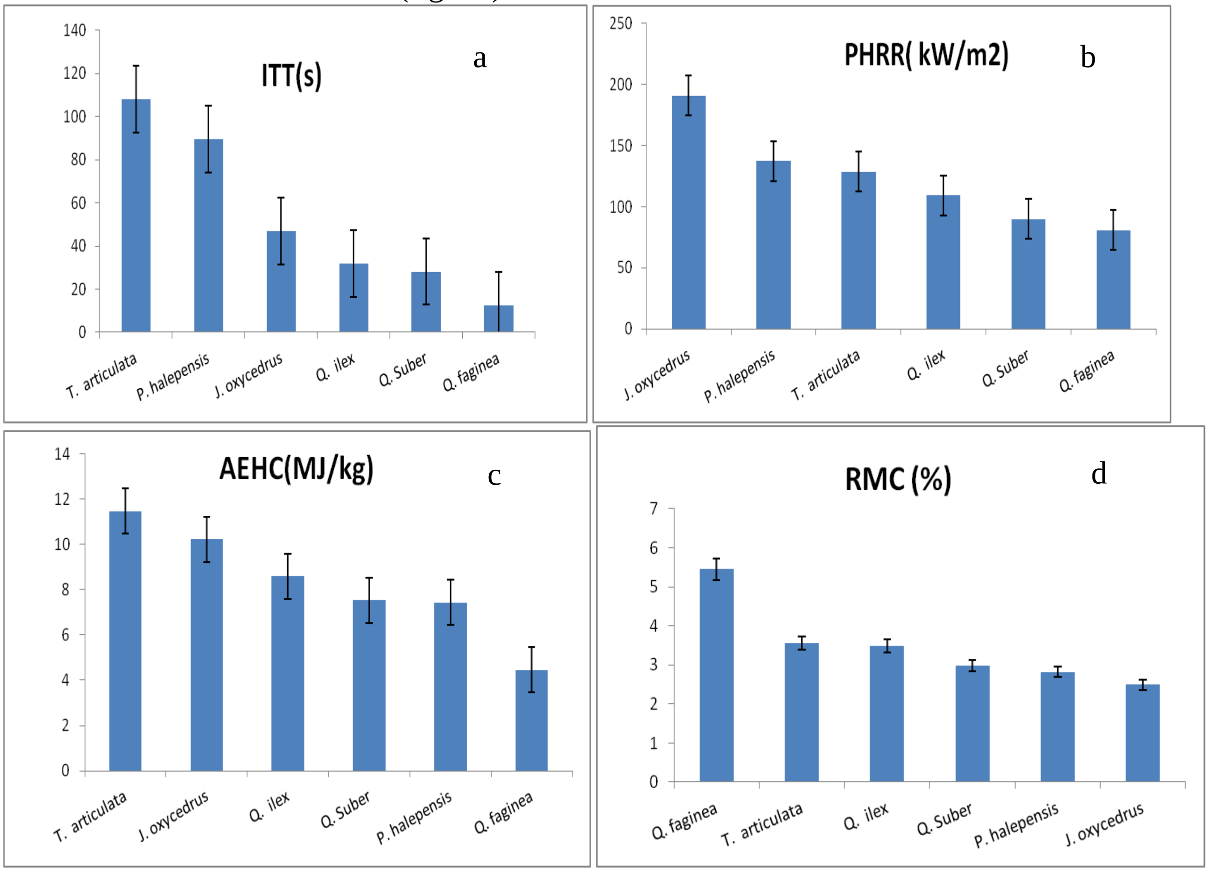

Figure 4. One-way ANOVA for selected flammability parameters (a) Time-to-ignition, TTI (b) Peak Heat Release Rate, PHRR (c) Average Effective Heat of Combustion, AEHC (d) Residual Mass Fraction (%). Vertical bars show average and standard errors. Different letters show significant differences ($p<0.001$).The flammability variables determined in the fresh samples were compared. The Dunnett test makes it possible to classify the species in homogeneous groups in relation to their respective parameters (Table 5).

Table 5. Comparison of averages of flammability parameters by one -way anova and Dunnett test

Legend: With: F: Observed function, ***: Highly significant differences at the 5% level; G.: Group.

Paramètres	F	Résultats du Test de Dunnett
Ignition of the flamme ITT(s)	49,36***	***G.A*** : T.*articulta, P. halepensis;;* ***G.B*** : *J.oxycedrus, Q. ilex, Q.faginea et Q.suber*
Peak Heat Release Rate PHRR (kW/m^2)	20,07***	***G.A*** :*T.articulta, J.oxycedrus, P.halepensis* **GB**: *Q.faginea* et *Q.suber, Q. ilex*

Average Effective Heat of Combustion, AEHC (MJ/kg)	8,39***	***G.A*** :*T. articulata,J. oxycedrus, Q. ilex* ***G.B*** : *P.halepensis, Q.faginea* et *Q.suber*
Residual Mass Fraction RMF (%)	15,77***	***G.A*** : *Q.faginea* ***G.B*** : *T.articulta, P. halepensi, Q. ilex , Q. suber*

Typically, a forest stand consists of various species possessing anatomical and physiological structures, very adapted to the conditions of the environment and to local ecological conditions (climate, soil and altitude). In the face of forest fire, none of these species is non-flammable, the ubiquitous flammability is hierarchized rather between a precipitated or delayed flammability in which the researchers evoke implicitly strongly, moderately or weakly flammable species. The water content of these trees (FMC or Fuel moisture content) is the main catalyst for flammability (Valette, 1990, Dimitrakopoulos & Mitsopoulos, 2006, Madrigal et al., 2009). The fire season in north Algeria extends from May to October. Such a period is characterized by the lack of rain and average daily temperature is higher than 30°C. Hence, a condition which generates water stress in the vegetation.

These two parameters, which jointly regulate soil water balance and evapotranspiration, are intercepted differently by forest trees. This implies that coniferous and broadleaved trees implement differentiated physiological regulations to avoid drought (Dimitrakopoulos and Mitsopoulos, 2006). Contrary to most previous studies, FMC did not have a significant effect on TTI (ignitability) or RMF (consumability) ($p > 0.5$). The moisture content of the fuel (FMC> 50%) generates high variability in the TTI (between 60,71% and 98%) and RMF (between 2,49 and 5.45%) values and, therefore, it was not possible to identify differences between species. Indeed, the effect of FMC of live fuels on forest fire behaviour at high radiant heat flux is controversial (Madrigal et al. 2013). Some authors consider that under such circumstances, the effect of the feedback from the fire and accumulation of VOCs may be stronger than the FMC (Viegas and Simeoni, 2011).

M 'Hamed et al. (2011) confirms this is probably due to the presence of high content of extractives and volatilized aromatic essential oils in the north-African foliage fuels specially in cork oak forests. Emission of VOCs is favored by evaporation of water due to the transportation of such compounds by water molecules during the evaporation process (Chetehouna et al., 2009). However, the results of the tests showed a considerable correlation between FMC and PHRR (combustibility, $r = -0.52$, $p < 0.05$) and between FMC and AEHC (sustainability, $r = -0.31$, $p < 0.05$) (Fig. 3). Thus, as the water content (FMC) decreased, the speed of combustion (PHRR) and heat release (AEHC) increased. Indeed, these findings show the importance of these variables in describing the combustion process (Babrauskas and Peacock 1992) and the possible involvement of VOCs in accelerating combustion (Viegas and Simeoni 2011, Chetehouna et al. 2014).

Oak species presented low values of the four flammability parameters (high ignitability but lowcombustibility, sustainability and consumability). The fuel moisture content of Quercus spp. is lower than that of other more flammable species (e.g. *P. halepensis*). Despite being highly flammable (due to its high surface area-to-volume ratio -Valette 2007- and low FMC in leaves), *Quercus* spp. do not store VOCs (Peñuelas and Llusia 2003) and have been described as less flammable than conifers (Pausas et al. 2015). This group of species can be considered as intermediate between "non-flammable" and "hot-flammable" under the new evolutionary concept defined by Pausas et al. (2017). Conifers species forms the most flammable group: *J. oxycedrus, T. articulata,* , *P. halepensis,* are phenologically and physiologically very similar and can be considered "hot flammable" under the new

evolutionary concept defined by Pausas et al. (2017). They are characterised by a very poorly lignified leaf, generally growing in southern areas on limestone and rockyground.
Juniperus oxycedrus is recognised as a highly flammable species in the Mediterranean region (Madrigal et al. 2011) because of its very high surfacearea-to-volume ratio and low FMC during the summer (Valette 1990; Elvira and Hernando 1989). In addition, *T. articulata and J. oxycedrus* both belong to the Cupressaceae fam.,in which it is characterised by the accumulation of VOCs (Della Rocca et al. 2015). *P. halepensis* is more flammable than the other species. The resin and essential oil (terpenoids) contents of*P. halepensis* tend to be very high, making these species extremely flammable (Dimitrakopoulos et al. 2013). As an indication, Aleppo pine forests cover more than 800 000 ha in Algeria and alone account for 1/3 of all burned areas in the Mediterranean region (Leone, 1999).
A proposed classification of some companion species of cork oak forest could potentially be used as predictors of the actual risk of fire in these stands. A recent proposal (Molina et al. 2017) shows that flammability rankings can improve fire risk indexes. In the same line of tought, Fares et al. (2017) stated that the use of flammability classifications, including the natural dynamics of live fuels might enhance fire risk indexes in the Mediterranean region. The new flammability evolutionary concept proposed by Pausas et al. (2017) opens the possibility to study potential links between biology, physiology and flammability (Schwilk 2015) to improve flammability classifications.

Conclusion

The results indicate that the problematic of live forest fuel in the region of Tlemcen is the same in the others Mediterranean's country, especially in the North Africa. The abandonment and mismanagement of the maquis led to uncontrolled spatial distribution of *T. articulata* and *P. halepensis*. The findings suggest that mixed maquis with *P. halepensis and/or T. articulata* present a major risk to cork production in scenarios with increased fire occurrence. This recommends that the *P.halepensis* presents a very important risk to the cork oak forests where the phenomenon of dominance of softwoods is very marked. Depth knowledge on forest fuel in the Algerian cork oak forests requires sampling consisting covering large areas. The results dictate the need for future research to put important points such as the interaction between FMC in the fresh and dried fuel, volatile compound and radiant heat flux. This would facilitate the fire risk mapping, mainly for the already managed forests with known ecological association units.

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ANALYSIS OF ROAD NETWORK DENSITY AND MEAN SKIDDING DISTANCE IN FOREST MANAGEMENT UNIT "MEŠTREVAC" USING MODERN TECHNIQUES AND METHODS

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Abstract

The paper shows the results of road network data analysis for associated compartments and forest categories in Forest Management Unit "Meštrevac" (the municipality of Foča, Entity of Republika Srpska, Bosnia and Herzegovina), performed in the GIS software package ArcMap 10.5. The real mean skidding distance is obtained as a product of the mean geometric skidding distance and correction factor. The correction factor is calculated as the average value of the correction factor by terrain slope and correction factor by relief areas. According to the current state, the entire road network is made up of roads with a macadam type of pavement. The total length of mentioned roads is 124,95 km. Due to the spatial position of certain sections that unilaterally open the forest area, the total length of the roads is taken into account with 79.31% of its length (99,10 km) and so openness of FMU "Meštrevac" has a value of 8.63 m/ha. From a total of 157 analyzed compartments, 41 of them are not opened with forest road network at all. The mean geometric skidding distance has value of 330.96 m. With the calculated correction factor of 1.48 in mind, the real mean skidding distance has value of 489.82 m. Based on characteristic cases of the spatial position of some sections in the road network, the criteria used to determine road network density are subsequently supplemented by the corresponding criteria.

Key words: *road density, openness, mean skidding distance, correction factor, GIS.*

Introduction

A well-built network of forest roads is the starting point for planning and implementing measures of care, cultivation and use of forest resources. Building a primary network of forest roads in the forests, regardless of the ownership structure, has a wider social significance, whether if the subject of the opening are private forests, spatially separated forest complexes of state forests or state forests in general (Đuka *et. al.*, 2017). According to Petković and Potočnik (2018), forest roads should be built in areas that are suitable to their construction, and suitability is determined by grading the terrain, habitat conditions and the existing network of forest roads, i.e. current openness. However, road construction is often limited due to the current needs, the most pressing technological and technical problems and financial possibility of a legal entity that manages the forest area.

The first step through which we can point out the basic drawbacks and lay the foundation for the future upgrading of the existing forest road infrastructure is to determine the road network density. Although Pentek (2011) states that road network density is a quantitative parameter that, besides the numerical data on the amount of road infrastructure on a certain surface, does not say anything about the quality of its spatial distribution. Using modern technologies (GPS devices and GIS software) and applying the criteria for determining road network density developed by Poršinsky *et al.* (2017), the entire job of recording and inventoring of roads is extremely easy and simplified. The efficiency of using GPS devices for recording the existing forest road network has been demonstrated by Potočnik *et. al.* (2009), stating that the use of GPS devices for the same time period allows even 6 shots more than classic way of recording. Within the GIS software platform, we can display the collected data in raster and vector form, then systematize them into the appropriate bases and perform multi-criteria analysis. As the positive correlation between openness and mean skidding distance is evident, according to Petković *et. al.* (2015) determining the mean skidding distance is imposed as one of the most important tasks especially in the process of forest openness planing. This is supported by the fact that the size of the mean skidding distance directly affects the unit labor costs in the 1st stage of timber assortments transport. According to Simić (2007: Kulušić, 1979), in total forestry production costs, the timber assortments transport costs account for as much as 60%. The modern approach to determining the mean skidding distance involves assisting the GIS platform and applying different methods within them. According to Pentek *et. al*. (2005); Danilović *et. al.* (2012, 2013); Danilović & Stojnić (2014); Petković *et. al.* (2015, 2017), the mean skidding distance is the product of the geometric mean skidding distance, which is determined using the raster method, grid point method, the buffer strips method (Enache *et. al.,* 2015) and the correction factor, determined on the basis of terrain relief characteristics and analytical methods (Petković *et. al*., 2015, 2017, Lotfalian *et. al*., 2011). The aim of this paper is to determine road network density and the mean skidding distance in the Forest Management Unit "Meštrevac" with the application of modern methods and techniques, with an overview of the advantages and disadvantages of the used methodology.

Material and Methods

The research is carried out at the FMU "Meštrevac", located in the southeastern part of the Republic of Srpska/Bosnia and Herzegovina, on the territory of the Municipality of Foča (Figure 1). This Forest Management Unit belongs to the Forest Management Area "Gornjedrinsko", which is managed by the Forest Enterprise "Maglić" Foča. It ranges from 18°54'22" E to 19°05'20" E and from 43°15'01" N to 43°27'22" N. The value of FMU "Meštrevac" area listed in Forest Management Plan is smaller by 155.50 hectares or 1.35% compared to GIS determined area (Table 1.). The terrain relief is typically mountainous (highest point - Ljubišnja 2238 m), with narrow canals and river canyons (Tara and Ćehotina). According to the Forest Management Plan, the total area of FMU "Meštrevac" is 11300.36 ha (IRPC, 2010). Cuting volume for ten-year period is 416,242 m^3 of wood, where 340,535 m^3 is from coniferous and 75,707 m^3 from deciduous trees (IRPC, 2010).

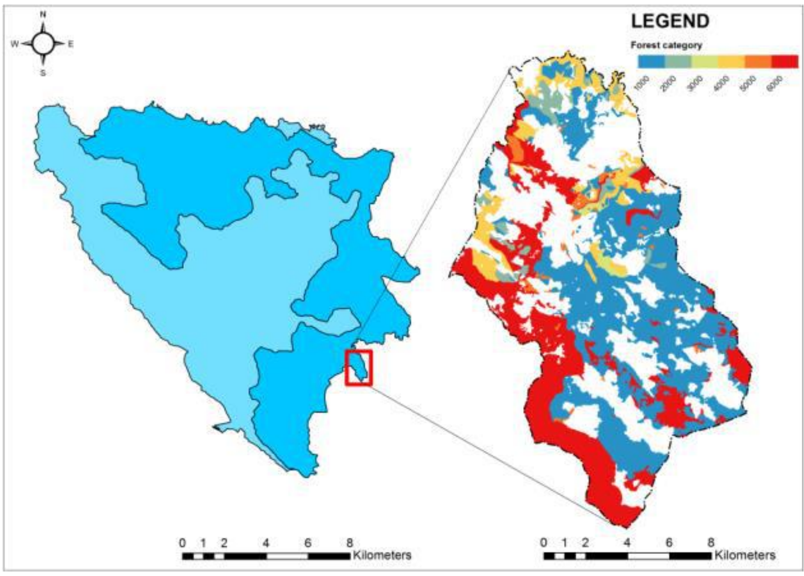


Figure 1. Geographical location and spatial distribution of forest categories in FMU "Meštrevac"

Table 1. Overview of Forest Management Unit "Meštrevac" area according to the forest categories

Forest category	**Area**		**Relative share**
	FMP	**GIS**	
	ha		**%**
1000 – High forests with natural regeneration	5980.74	5681.12	49.59
2000 – High degraded forests	334.71	501.38	4.38
3000 – Forest plantations	112.24	108.13	0.94
4000 – Coppice forests	1125.01	1084.38	9.47
5000 – Surfaces suitable for afforestation and forest management	149.65	324.57	2.83
6000 – Surfaces unsuitable for afforestation and forest management	3710.25	3755.83	32.79
TOTAL	**11300.36**	**11455.41**	**100**

Larger part of the primary forest road network is recorded by forestry engineers from FE "Maglić" using the GARMIN Oregon 600 GPS device, while the rest is recorded using the Garmin Etrex 10 GPS device by first author. The recording is performed by driving on a one way direction.
Transformation of recorded data from gpx format into shapefile format, suitable for processing in ArcMap 10.5 software package, is done using the DNR GPS software.
Correction of the spatial position of the recorded roads is made using a orthophoto image with resolution of 1 m.
Vector data (the boundary of the Forest Management Unit, compartments and forest categories) is obtained by digitizing forestry thematic maps of scale 1:25 000 from Public Forest Enterprise "Šume Republike Srpske".
The road network density is calculated using the expression (1):

$$R_d = \frac{L_r}{A_t} \quad (1)$$

Where is:
R_d - primary road network density (m/ha)
L_r – primary road network lenght (m)
A_t - total area (ha)

Calculation of road network density is done using criteria developed by Poršinsky *et. al*. (2017):

General criteria:
a) the road has an upper structure,
b) the road to a greater extent meets the essential minimum technical characteristics required for the transport of wood by truck,
v) there is no traffic signal on the road with a regulated axle load limit of less than 10 tonnes and a total load less than 26 tonnes,
g) wood can be skidded to the road, while forest vehicles will not move on non-forest soil (agricultural land, urbanized land, etc.).

Special (spatial) criteria:
1. forest, public or "unclassified road", or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, which, through its entire length, passes through the forest and/or forest land and which opens forest from two sides, is taken into account with all its length (100% of length),

2. forest, public or "unclassified road", or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, which through its entire length passes through the forest and/or forest land; and which opens the forest unilaterally, due to various restrictions, is taken into account with half of its length (50% of its length).
3. forest, public or "unclassified road", or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, passing along the the Forest Management Unit boundary (hereinafter: the border), or up to a maximum of 250 m from the boundary from its external or up to 125 m distance from the border on its inside, whose route generally follows the direction of the border, is taken into account with half of its length (50% of its length).
4. forest, public or "unclassified road", or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, which extends to the Forest Management Unit boundary (hereinafter: the border) at right angle approximately (90°±20°) and ends at the boundary, is taken into account with a length of 250 m. If the length of the mentioned section of the primary road network is less than 500 m, it shall be taken into account with half of its length (50% of length). If the same section does not end at the border but enters in road opened area, on it, within the mentioned border, other general and specific criteria for determining road network density are applied.
5. forest, public or "unclassified road", or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, which comes close to the Forest Management Unit boundary (hereinafter: the border) but which is the subject of forest opening by primary road network at approximately the right angle (90°±20°), but ends on the outside of the boundary, is taken into account with a length of 250 m, reduced by half the distance of its ending from the border.

Determination of the geometric mean skidding distance is carried out using the grid point method - G100 (Enache *et al*., 2015), with *Arctoolbox Fishnet* tool. Using this method, 8359 points are placed within the area of FMU "Meštrevac". The shortest distance from each point to the road network is calculated. Average value of these distances for/inside every single opened compartment is taken as its mean geometric skidding distance (2) (Đuka *et. al.*, 2017):

$$\bar{\mathrm{S}}_{\mathrm{g}} = \frac{\sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{S}_{\mathrm{g_i}}}{n} \quad (2)$$

Where is:
$\bar{\mathrm{S}}_{\mathrm{g}}$- mean geometric skidding distance (m),
$\sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{S}_{\mathrm{g_i}}$- the distance of all points within the compartment (m)
n – the number of uniformly distributed points over the compartment surface.

Using the *Arctoolbox Slope* tool, an slope analysis is performed with 25 m resolution Digital Elevation Model (EU-DEM v1.1) from the Copernicus program[17]. The resulting slope raster is classified into 5 categories with intervals: 0-15%, 16-30%, 31-45%, 46-60% and 61-140% (Figure 4). Summating the products of all 5 slope categories area relative share in investigated area and average correction factor for every single terrain slope category, a correction factor is obtained (Petković *et. al*., 2017, according to Sokolović & Bajrić 2013).

With additional analysis of the Digital Elevation Model we determined relative participation of relief categories in the FMU "Meštrevac" investigated area, where, acording to Petković *et*

[17] **Copernicus** is the European Union's Earth observation programme coordinated and managed by the European Commission in partnership with the European Space Agency (ESA), the EU Member States and EU Agencies.

al. (2015: Bertović 1999), areas located up to 200 m above sea level are characterized as lowlands, from 200 to 500 m as hilly area, from 500 to 1000 m as low mountains, from 1000 to 1500 m as medium high mountains and from 1500 to 2000 m as high mountains. Pentek *et. al.* (2005: Segebaden 1964, 1969 and Abegg 1978) states that the correction factor ranges from 1.5 to 1.65, in average 1.58 for mountain areas in Sweden and Switzerland.

The overall geometric mean transport distance correction factor is determined as the average value of the terrain slope correction factor and correction factor for the relief areas (Petković *et al.,* 2015).

The area of all open compartments in FMU "Meštrevac" including buffer whose border reaches up to 250 m from the boundary of open compartments is taken as investigated area.

The real mean skidding distance is calculated as the product of the mean geometric skidding distance and the overall correction factor (3).

$$\overline{\mathrm{S}}_d = \mathrm{S_g} * f_k \quad (3)$$

Where are:
$\overline{\mathrm{S}}_d$ – real mean skidding distance (m),
$\mathrm{S_g}$- mean geometric skidding distance (m),
f_k – correction factor.

Results and Discussion

According to the current state, the entire road network is made up of roads with a macadam type of pavement. The total length of the roads that open FMU "Meštrevac" (Figure 3) is 124.95 km. Due to the spatial position of certain sections that unilaterally open the forest area, the total length of the roads is taken into account with 79.31% of its length (99.10 km) and so openness of FMU "Meštrevac" has a value of 8.63 m/ha. In compartments with an openness greater than 10 m/ha (Figure 5), high forests with natural regeneration occupy 54.52% of the area. Moreover, the largest part of the forest road network has been built within the area of high forests with natural regeneration (Table 2). This result unequivocally indicates that the construction of roads in FMU "Meštrevac" is in direct dependence on planned revenues from exploitation of forest areas that are the subject of the opening. Although the openness of forest plantations and surfaces suitable for afforestation and management is at a satisfactory level (Table 2), the current state is undoubtedly a consequence of the relatively small share of the mentioned categories area in FMU "Meštrevac" (Table 1) and proximity to existing roads that open high forests with natural regeneration. In accordance with the silvicultural and economic potential, perspective for opening coppice forests and areas unsuitable for management in the past did not exist, so their openness today is at an extremely low level. Since modern approach to forest management implies respecting the principles of polyfunctionality, this area will be the subject of opening in the future, as evidenced by the construction of the road along the Tara River canyon for the purpose of tourism development.
Most "well-opened" compartments (30) are opened in the range of 10.01–15.00 m/ha (Figure 2). For mountain areas in Croatia, as is the area of FMU "Meštrevac", the minimum recommended openness is 15 m/ha (Pentek *et al.,* 2011) or 20 m/ha (Hodić & Jurišić 2011). In Austria, whose productive forests are mainly located in the mountainous area, openness is at a high level with 45 m/ha (Findeis, 2016). Compared to the above, the openness of FMU "Meštrevac" cannot be considered satisfactory.

Table 2. Average density and length of forest road network by forest category in FMU "Meštrevac'

Forest category	Primary road network	Road network density
	m	m/ha
1000	68298.27	11.81
2000	2062.83	4.48
3000	2079.71	20.71
4000	3439.59	3.23
5000	5329.46	20.34
6000	26201.51	6.93

By analyzing the DEM it is found that the investigated area in FMU "Meštrevac" consists of 0.28% of hilly and 99.72% of mountainous areas (low mountains: 22.01%, medium high mountains: 58.32% and high mountains: 19.38%). Since the participation of hilly areas is negligible, the area of FMU "Meštrevac" can be fully defined as mountainous and as such it has a correction coefficient of 1.58. Taking into account that the total correction factor is equal to the average value of the correction factor by terrain slope (Table 3) and correction factor by relief areas, the total correction factor has a value of 1.48.

Table 3. Calculated corection factor acording to terrain slope in FMU "Meštrevac"

Slope category	Corection factor	Relative share in total area (%)	Total
0 - 15	1.1035	25.11	0.28
15.01 - 30	1.2305	33.80	0.41
30.01 - 45	1.3625	19.00	0.26
45.01 - 60	1.5815	11.41	0.18
60.01 - 140	2.3265	10.68	0.25
Total	/	**100**	**1.38**

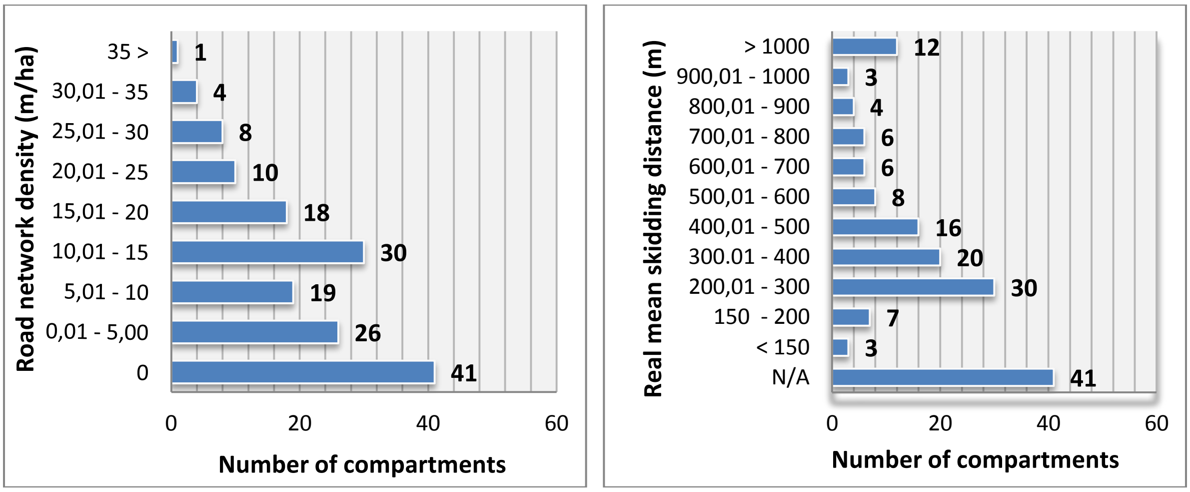
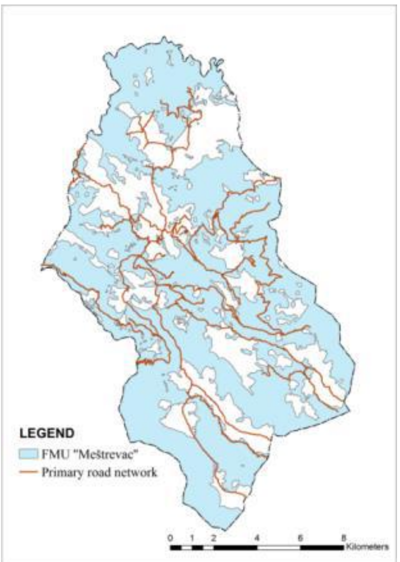
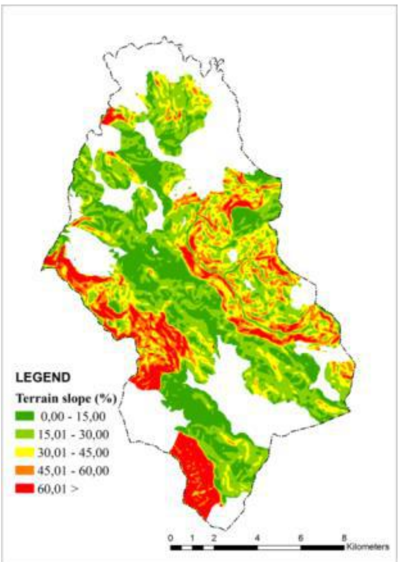


Figure 2. Numerous distribution of compartments according to the classes of openness (left) and real mean skidding distance (right) in FMU "Meštrevac"

The mean geometric skidding distance in FMU "Meštrevac" is calculated with value of 330.96 m. According to the calculated correction factor for FMU "Meštrevac", the real mean skidding distance will be 489.82 m. The mean skidding distance obtained by this methodology is often approximate, but satisfactory to the level of strategic planning. Figure 2 shows the numerical distribution of open compartments according to the classes of the real mean skidding distance, where we can see that the largest number of compartments is located whitin range of 200.01-300.00 m. The spatial distribution of these compartments is shown in Figure 6.





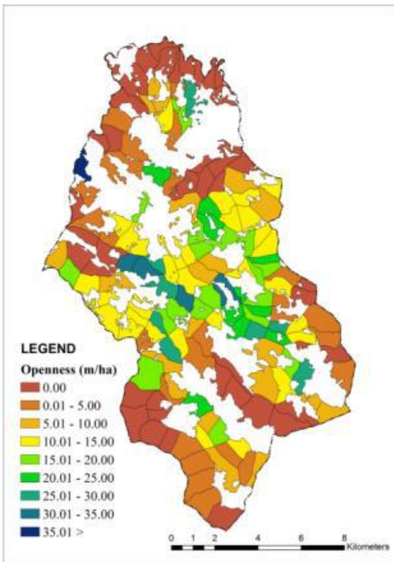
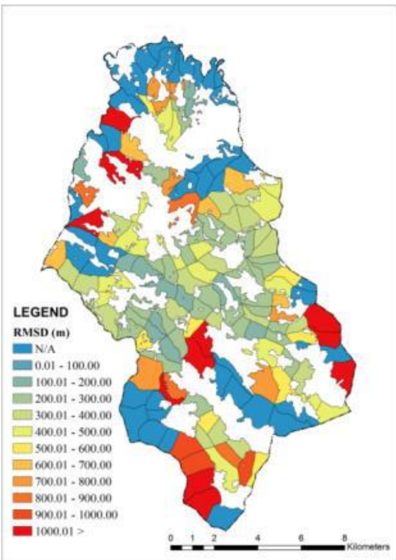
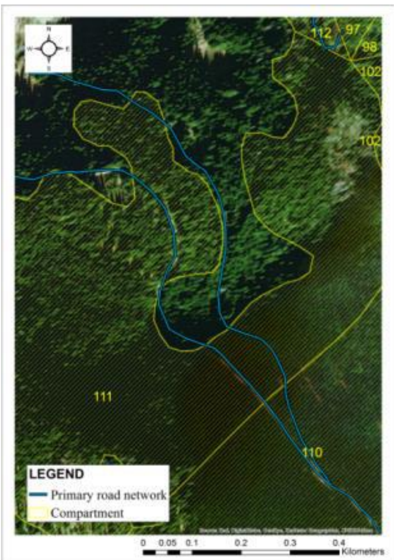


Figure 3. Primary forest road network in FMU "Meštrevac" (left),
Figure 4. Terrain slope in FMU "Meštrevac" (middle)
Figure 5. Road network density by compartments in FMU "Meštrevac" (right),





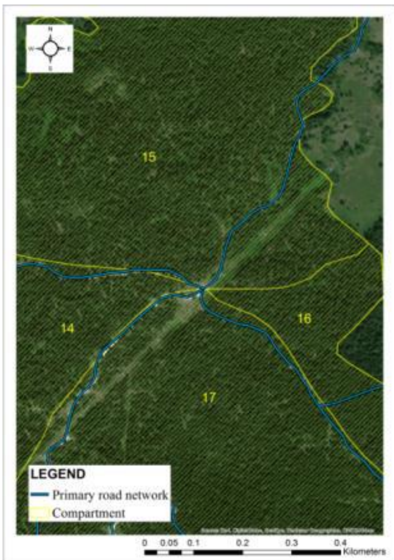


Figure 6. Real mean skidding distance by compartments in FMU "Meštrevac" (left)
Figure 7. Characteristic case of road spatial position in FMU "Meštrevac" – example 1 (middle)
Figure 8. Characteristic case of road spatial position in FMU "Meštrevac" – example 2 (right)

Conclusions

Numerical values of openness obtained according to the criteria developed by Poršinsky *et al.* (2017) at the same time represent an assessment of the spatial distribution of the primary road network, since the spatial position of the roads defines the numerical value that is taken into account. However, as mentioned by the authors, possible cases in operational/practical forestry that can be encountered in determining of primary forest road network density, are almost countless. In order to complement the mentioned criteria, it is necessary to analyze as many areas as possible with their typical field conditions. Its the only way that undefined cases can be spotted, processed and channeled into a new criterion.

According to characteristic cases from FMU "Meštrevac" section of special criteria should be supplemented with:

1. The forest, public or "unclassified road", or its particular section which, due to its characteristics, is taken into the account for openness calculating under paragraphs (1), (2)

and (3), which is located nearby, according to the above criteria relevant, other forest, public or "unclassified road" so that its route closely follows its direction is taken into calculation with (Figure 7):

a) 50% of the length: 225 ≤ 250 m from another forest, public or unclassified road,
b) 45% of the length: 200 ≤ 225 m from other forest, public or non-classified roads,
c) 40% of the length: 175 ≤ 200 m from other forest, public or unclassified roads,
d) 35% of the length: 150 ≤ 175 m from other forest, public or non-classified roads,
e) 30% of the length: 125 ≤ 150 m from another forest, public or unclassified road,
f) 25% of the length: 100 ≤ 125 m from other forest, public or non-classified roads,
g) 20% of the length: 75 ≤ 100 m from other forest, public or non-classified roads,
h) 15% of the length: 50 ≤ 75 m from other forest, public or unclassified roads,
i) 10% of the length: 25 ≤ 50 m from other forest, public or unclassified roads,
j) 5% of the length: <25 m from another forest, public or unclassified road.

2. Forest, public or non-classified road, or its particular section, which can be used for sustainable forest management, mainly for the loading of timber assortments, which is connected to another forest, public or non-classified road at approximately right angle (90°±20°), is taken into account with a whole length but reduced by 250 m, while the other criteria for determination of forest road netwerk density are applied to the reduced length (Figure 8).

The determination of the mean skidding distance in this paper carries certain characteristics. Unlike Petković *et al.* (2015, 2017), which analyzed terrain slope on the entire area of FMU, in the case of FMU "Meštrevac" terrain slope is analyzed only on areas where by using Poršinsky *et al.* (2017) general and special criteria a certain level of openness is determined. In this way, only those areas where theoretically timber assortments could be transported with transportation means in first stage of timber assortments transportation are taken into account, assuming that transportation of timber assortments cannot be done in unopened compartments. The value of the mean skidding distance obtained in this way is not burdened by analyzes of those areas where transportation of timber assortments will not be carried out, i.e. in those areas that often cannot be opened by the primary and secondary forest road networks, and the results thus obtained can be considered more precise.

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BIOINDICATION OF VITALITY ON THE BASIS OF DIAMETER INCREMENT OF EUROPEAN SILVER FIR (*Abies alba* Mill.) ON THE MOUNTAIN BORJA

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Abstract

In this paper, the diameter increment of European silver fir is analysed on the basis of bioindication of the vitality of trees and forest stands. The research is accomplished by examining characteristics of the annual diametre increment on Mt Borja for the period of 50 years. The required data are collected on five trial plots in various stands, with four of them placed in mixed stands of fir and beech, and one being an exclusive fir stand. A total of 75 trees of the first, second, and third biological sites respectively are cored. In order to minimise the influence of age, to better detect the reaction of trees to exogenous factors on the radial growth and the evaluation of vitality of trees and stands, indexes of the tree rings width are calculated. The average values of the tree ring width are obtained through levelling in the ANTEVS programme (using the so-called smoothing cubic spline). Summarising the explored stands, it can be concluded that their vitality is solid and that possible devitalisation in the future can be prevented by the application of appropriate silviculture measures.

Keywords: *European silver fir, diameter increment, vitality, bioindication, Borja*

Introduction

In all European countries, modern-day forestry has been confronted with the challenge of securing permanent efficiency of forest ecosystems with a view to fulfilling their basic economic and ecological functions. This primarily means that it is necessary to maintain the stability of forests and their key features, such as biological diversity, renewability, production of biomass etc. Thus, defining the optimal structure of forest stands and an objective assessment of their current condition represent an important prerequisite for viable forest management in terms of economy and ecology (Vučković *et al*., 2004).

With regard to Bosnia and Herzegovina, the fir is usually found in mixed beech and fir, that is, beech, fir, and spruce forests, extremely rarely in exclusive fir forests, covering more than 50% of total high forests (Ballian and Halilović, 2016). The fir is the most common conifer in Bosnia and Herzegovina. The surface area of conifer forests and mixed conifer/broadleave forests, with regard to fir, beech, and spruce, is 599, 000 ha or 28% of productive forest area (Čabaravdić *et al*., 2016).

The examination of trees affected by drying points to the fact that their increment may show a long-term tendency of deviating from the 'normal' trend and that the process of losing vitality may begin much before there are visual symptoms evident (Vučković and Stamenković, 2000; Vučković *et al*., 2008).

Increment can be considered a reliable bioindicator of the tree vitality and the ecological conditions in which the tree grew and a parametre for forecast, that is, prediction of devitalisation and dying-off of trees in future. Consequently, the increment of dendromass can be used as a reliable indicator of general functionality of forest ecosystems. Numerous research have shown that changes in increment and its deviations from typical trends represent a bioindicator of tree and stand vitality. The changes in increment may point to the beginning of destructive processes even before there are visual symptoms of tree devitalisation, where the drying featuring the latter symptoms is only the final phase of a devitalisation process, which may pass unnoticed for decades. The monitoring of diameter increment of trees and stands and the forecast of their trend may enable a timely discovery of devitalisation, as well as minimising a possible damage in the period prior to visual symptoms of drying (Fritts, 1976; Prpić *et al*., 1994; Vučković *et al*., 1998, Bigler and Bugman, 2003, 2004; Vučković *et al*., 2004, 2005; Dukić and Maunaga, 2007, 2009; Stajić, 2010, Stajić *et al*., 2017 itd.).

Given the aforementioned, the examination of fir diameter increment on Mt Borja should contribute the applicability of increment analysis with a view to timely detecting of negative processes and decrease in tree and stand vitality, that is, detecting these processes prior to the occurrence of visual symptoms of drying to the extent that unanimously points to the decay of stands. Apart from scientific contribution, the results gained can be applied in forest management of pure stands of fir and mixed stands of fir and beech on Mt Borja.

Material and Methods

The researched area is Mt Borja, located in the northern part of Central Bosnia, between the rivers of Velika and Mala Usora, with its highest peak, Velika Runjavica (1,077m) dominating its north-west part. In most cases, the mountain is covered by mixed stands of fir and beech, with occasional occurrence of pure stands of fir (uneven-aged stands). Within the project of comprehensive research of increment of European silver fir, there have been 15 trial plots (50m x 50m) of pure stands of fir and mixed stands of fir and beech. Out of these, there are 5 trial plots (TP) selected (Trial plots: 1, 3, 5, 10 and 13) characterised by various conditions in terms of their habitat and stands. With regard to the aforementioned, the data necessary for bioindication vitality, based on the characteristics of diameter increment. The trial plots 1, 3, and 10 are set up in the *Rusco hypoglossi-Abietetum* association Brujić 2004, and the plots 5 and 13 in the *Galio rotundifolii-Abietetum* association Wraber 1959. On these plots, all trees with the diameter at breast height (dbh) larger than 5 cm have been subjected to the measurement of basic elements of growth: dbh, tree height (h), and the crown projection area, as well as biological position and tree vitality. Based on the extent to which their canopies are developed and the extent of light availability/ accessability, the trees are distributed into three biological classes (position), according to Bunuševac (1951): dominant trees (First biological position, Kraft 1 and 2), codominant trees (Second biological position, Kraft 3 and 4a), and overtopped trees (Third biological position, Kraft 4b and 5). With regard to vitality, all fir trees are classified in three categories (Innes, 1990): good vitality (1), medium vitality (2), and poor vitality (3).

As far as the researched stands in the aforementioned plots are concerned, the number of trees per ha ranges from 392 to 732 (560 on average), that is, the number of fir trees ranges from 200 to 604 (440 on average). With regard to the fir, the quadratic mean diameter is within the 22.8 – 39.3 cm range. When the stand basal area is concerned, it ranges from 24.9 to 35.1 m^2 ha^{-1}, with the volume of dendromass in the stands featuring the interval from 273.7 to 710.1 m^3 ha^{-1}.

On each trial plot, 5 trees from the first, 5 trees from the second, and 5 trees from the third biological position are drilled using Pressler increment borer for extracting long wood cores. The samples (wood core) are sent to a laboratory, placed and glued to guttered wooden supports, and then sanded down using sandpaper. The samples processed are scanned with a high-performance scanner (Epson Perfection V30 Photo). The measurement of ring width is performed by means of the CooRecorder 7.6 and CDendro 7.6 software respectively. With a view to removing, that is, minimising the influence of age on the increment trend, and in order to better detect the reaction of trees to exogenous factors on the radial growth and the evaluation of vitality of trees and stands, the following indexes of the tree rings width are calculated:

$I_r = \frac{i_r}{\hat{i}_r}$, I_r - ring width index, i_r - actual ring width , and $\hat{i}_r$ - estimated ring width.

The average values of the tree ring width are obtained through levelling in the ANTEVS programme (Rayburn and Vollmer, 2013; Vollmer, 2016), using the so-called smoothing cubic spline, thus providing estimated ring width values. Spline is a special function defined section by section over a polynome, so that it levels the connection between n spots defined in the Cartesian co-ordinate system (Pretzsch, 2009).

Results and Discussion

Table 1 shows the elements of growth of the trees encompassed by the analysis of diameter increment, with regard to their respective biological positions. According to this parametre, the arithmetic means of the diametres ranges from 11.7 cm to 43.1 cm, and of the heights from 9.7 to 28.5 m.

Table 1. Characteristics of the analyzed trees

Biological positions	Number of trees	dbh (cm)		h (m)		Crown projection area (m^2)	Vitality
		Means	Std. Dev.	Means	Std. Dev.		
I	25	43.1	8.7	28.5	3.5	13.5	1.2
II	25	22.6	6.2	18.6	3.5	9.2	2.3
III	25	11.7	3.0	9.7	2.3	3.8	2.7
All	75	25.8	14.6	18.9	8.4	13.6	2.1

The series of tree increment rings, with reference to the past 50 years (1965 - 2014) are divided by the levelled values (using the so-called smoothing cubic spline) by biological positions, resulting in the ring width indexes that enable the identification of the trees with hidden symptoms of devitalisation, that is, the trees that may feature vitality loss and be affected by drying processes. The trees that, over the period monitored, have displayed a significant decrease of the index, where this value has constantly been under 1 for the minimum of past 15 years, are marked as the trees with hidden symptoms of devitalisation.

Figure 1 shows the relation of the ring width index of a vital tree (tree number 150) and that of a tree with hidden symptoms of devitalisation (tree number 149) on trial plot 1. The trees are of nearly matching dimensions, with the former measuring 49.5 cm in diameter and 30.2 m in height, whereas the measures of the latter are 45.5 cm and 29.2 m respectively. According to Vučković et al. (2005), trees with a small ring width do not have enough capacity for transporting water and nutrients, which, in case of dominant trees, due to the extent to which their crown are developed and their great 'transporting distance', often leads to the disbalance between the intake and consumption of water, that is, to devitalisation and dying. Figure 2 shows the ring width indexes of the trees of the second biological position with hidden symptoms devitalisation.

There are analyses conducted of the differences between the vital trees and the ones with hidden symptoms of devitalisation in terms of dbh, height, crown projection area, and vitality by means of the t-test and F-test. The results show that, in relation to biological positions, there are no statistically significant differences between the two groups with respect to the parametres observed.

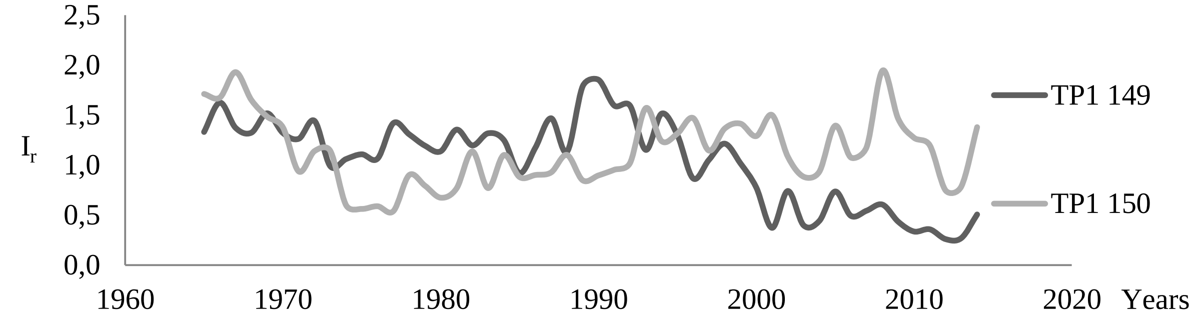

Figure 1. Vital trees (tree number 150) and tree with hidden symptoms of devitalisation (tree number 149)

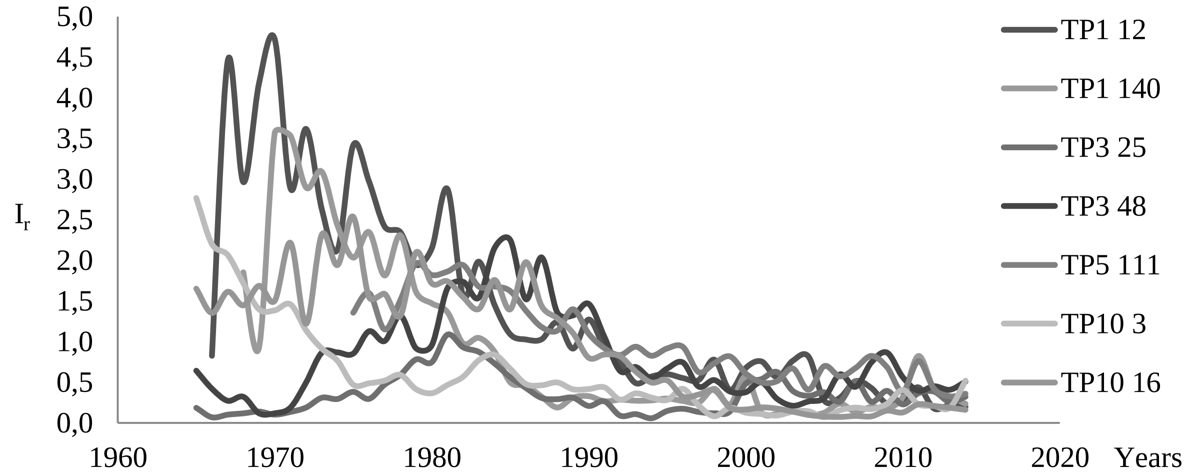

Figure 2. Ring width indexes of trees of second biological positions with hidden symptoms of devitalisation

Table 2 shows the respective share of the vital trees and the trees with hidden symptoms of devitalisation by trial plots and by biological classes. When dominant trees are concerned, the largest share of trees with hidden symptoms of devitalisation is found on trial plot 5, whereas the least share refers to trial plots of 3 and 13, where there are no such trees. In total, with regard to the researched stands, the share of dominant trees with hidden symptoms of devitalisation is 28%. Dukić and Maunaga (2009) applied the same method while researching on mature stands of sessile oak, and their results showed the share of dominant trees with hidden symptoms of devitalisation within the 31-35% range.

As far as codominant trees are considered, the largest share of trees with hidden symptoms of devitalisation is found on trial plots of 1, 3, and 10. Finally, with overtopped trees, the largest share of the trees with hidden sympotms of devitalisation is found on trial plots of 3 and 13.

Table 2. Share of vital trees and trees with hidden symptoms of devitalisation

Trial plots	First biological position		Second biological position		Third biological position	
	vital	devitalised	vital	devitalised	vital	devitalised
	(%)					
1	80	20	60	40	100	0
3	100	0	60	40	60	40
5	20	80	80	20	80	20
10	60	40	60	40	80	20
13	100	0	100	0	60	40
All	72	28	72	28	76	24

On the five trial plots researched, the number of fir trees per ha determined is 440 (206, 104, and 130 trees of the first, second, and third biological position respectively), that is, 560 with beech trees. Taking the aforementioned numbers and established shares as the starting point, there are 58, 29, and 31 trees of the first, second, and third biological position respectively with hidden symptoms of devitalisation. In total, the share of trees with hidden symptoms of devitalisation is 21%. In addition, we should take into consideration the fact that trends of diameter increment, which point to vitality loss in some trees, may, in fact, be the consequence of a somewhat less favourable position of the tree in question within its respective stand, due to a greater amount of shade provided by neighbouring trees, that is, due to increased competition between the trees in a stand, which becomes more pronounced as the trees progress in growth.

Conclusions

This research utilises a ring width index analysis for bioindication of tree/stand vitality. Ring width indexes are calculated in order to minimise the influence of age on the increment trend, that is, to better view the reaction of trees to exogenous factors. With regard to biondication of vitality, with reference to ring width index specifically, we can conclude that trial plot 13 features the best results, while trial plots 5 and 10 feature potentially the worst ones.

Summarising the explored stands, it can be concluded that their vitality is solid and that possible devitalisation in the future can be prevented by the application of appropriate silviculture measures.

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MONITORING ON APPEARANCE AND SPREAD OF HARMFUL INVASIVE PATHOGENS AND PESTS IN BELASITSA MOUNTAIN

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Abstract

Belasitsa Mountain is located on the territory of Southwest Bulgaria, Northern Greece and Northern Macedonia. The Bulgarian part of the mountain includes most of its northern slopes and ridge territories, characterized by variety of vegetation zones and plant communities. At lower altitudes, *Quercus* spp., are the most distributed, but gradually replaced by the sweet chestnut forests (*Castanea sativa*). Chestnuts are distributed between 400-1300 m a.s.l., forming the largest natural locality in the country. Along the river valleys in the mountain, natural population of *Platanus orientalis* occurs. Since 2018, a system for monitoring of two pathogens (*Cryphonectria parasitica* on *Castanea sativa* and *Ceratocystis fimbriata* on *Platanus orientalis*) and three invasive pests (*Corythucha arcuata* on different oak species and chestnut, *C. ciliata* on *P. orientalis* and *Dryocosmus kuriphilus* on chestnut forests) has been implemented. Until now, a high density of *C. ciliata* was established on *P. orientalis* causing significant damages on host trees. *C. arcuata* has spread in Bulgaria since 2013. Over the next few years, the species penetrated many regions of the country by affecting predominantly oak forests, but in 2017, it was registered for first time on the chestnut trees. In Bulgaria, *D. kuriphilus* has not been found yet, but as the pest was established in Greece in 2014, natural spreading of the species to Belasitsa Mt. could be expected. The possible penetration of this pest could increase the rate of damaged stands that had already been severely attacked by the fungal pathogen *C. parasitica.*

Key words: *invasive pests, monitoring, Belasitsa Mt., Bulgaria*

Introduction

Belasitsa Mountain is located on the territory of three countries: Bulgaria, Northern Greece and Northern Macedonia. The mountain is about 60 km long and 7 to 9 km wide. Its main massif is oriented in east-western direction. The climate is characterized by strong Mediterranean influence: precipitation is at its highest in the autumn and winter, and at its lowest in the summer. Bulgarian part of the mountain includes most of its northern slopes and ridge territories. It is characterized by variety of vegetation zones and plant communities. *Castanea sativa* Mill. population located on the northern slopes of Bulgarian part of Belasitsa Mt., at attitudes from 500-600 up to 900-1000 m a.s.l. This population is the largest and most valuable one at the Balkan Peninsula. It is also among the few well preserved autochthonous populations of this species in Europe. Most investigations on natural *C. sativa* forests in Europe and at the Balkan Peninsula in particular, reveal progressive degradation of this species, even in nature protected territories. The area occupied by the *C. sativa* forests on the northern slopes of Bulgarian part of Belasitsa Mountain is around 1400 ha. Natural stands cover an area of about 650 ha. More than 90% of them are older than 100 years of age, and around 75% are older than 140. Most natural stands are in poor health condition: with dieback in the crowns.

Pests and fungal pathogens are a part of the natural biocenoses complex inhabiting different types of ecosystems. Their development in plants causes destructive processes that have negative influence on the vitality and productivity of the trees. During the last years,

introduced pests and pathogens have turned into growing threat for the natural plant species disturbing the biodiversity and ecological dynamics in forest ecosystems.
The aim of study was to determine penetration of invasive pathogens and insect pests threatening tree vegetation in Belasitsa Mountain.

Materials and methods

In 2018, a network of 14 sample plots (SPs) was established in Bulgarian part of Belasitsa Mt. in outskirts of six settlements (Table 1). Three SPs were selected in *Platanus orientalis* L. stands for monitoring of *Corythucha ciliata* (Say, 1832) (Hemiptera: Tingidae) and *Ceratocystis fimbriata* Ellis & Halsted *f.sp. platani* (J.M. Walter) Engelb. & T.C. Harr (Microascales: Ceratocystidaceae) and the remaining ones – predominately in *Castanea sativa* and *Quercus petraea* Liebl. stands for monitoring of *Cryphonectria parasitica* (Murrill) Barr. (Diaporthales: Valsaceae), *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) and *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae).

Table 1. Main characteristics of sample plots in Belasitsa Mt.

№	Locality	Geographical coordinates		Altitude (m)	Stand age	Stand composition*
		Latitude (N)	Longitude (E)			
1	Petrich town	41.382000	23.204890	313	70	P.o.; C.s.; Q.p.
2	Petrich town	41.367282	23.199985	739	65	C.s.; F.s.
3	Petrich town	41.365502	23.196742	832	50	C.s.; F.s.
4	Belasitsa vill.	41.356295	23.104209	664	75	C.s.; Q.p.
5	Kolarovo vill.	41.361280	23.108780	459	80	P.o.; C.s.; Q.p.
6	Kolarovo vill.	41.360740	23.110676	505	65	C.s.; Q.p.
7	Kolarovo vill.	41.359445	23.095323	650	70	C.s.; Q.p.
8	Samuilovo vill.	41.371220	23.08514	316	65	P.o.; C.s.
9	Samuilovo vill.	41.364934	23.090233	481	60	C.s.; Q.p.
10	Samuilovo vill.	41.364357	23.083757	523	65	C.s.; Q.p.
11	Yavornitsa vill.	41.359567	23.045905	589	70	C.s.; Q.p.
12	Yavornitsa vill.	41.358411	23.052411	614	70	C.s.; Q.p.; F.s.
13	Kluch vill.	41.357716	23.019078	559	60	C.s.; Q.p.
14	Kluch vill.	41.356752	23.059601	663	65	C.s.; F.s.

* P.o. – *Platanus orientalis*; C.s. – *Castanea sativa*; Q.p. – *Quercus petraea*; F.s. – *Fagus sylvatica*

Defoliations caused by biotic and abiotic stresses were assessed according to the European methodology of the UNECE International ICP Forests Program (Eichhorn et al., 2010).
The studies were carried out by periodical observations and collection of biological material (leaf, twigs, bark and branches) for laboratory testing at the Forest Research Institute, Sofia. Isolation of the fungi into pure cultures on PDA (Difco) nutrient media was done. The colonized fungi were further identified by combination of colony macroscopic (colour, morphology and growth rate) and microscopic characteristics (spore shape, size, colour and morphology).
During the growing seasons, samples of leaves and shoots were collected regarding the presence/absence of studied species and the intensity of the attack (expressed as percentage of injured leaves). The attack intensity was assessed by the degree of foliage fading, as a deviation from the normal colour of the host tree leaves. Larvae of insect pests were placed in entomological boxes and photo eclectors to obtain adult emergence. Adult insects, nymphs and eggs were also collected for species identification.

Results and discussion

In this study, only *Cryphonectria parasitica* was established on *Castanea sativa*, and *Corythucha ciliata* – on *Platanus orientalis* L. The remaining invasive insect pests, *Corythucha arcuata* and *Dryocosmus kuriphilus*, as well as the pathogen *Ceratocystis fimbriata f.sp. platani* were not detected in Belasitsa Mt. Distinct decline in crown condition was observed in sampled chestnut trees.

The assessing of the distribution of *C. parasitica* carried out in this study shows that from the sites of primary infection, the disease has been spread across the whole mountain, between 400 and 800 m a.s.l., affecting 85% of the surveyed trees. Only 15% of chestnut trees were classified as healthy (defoliation of up to 25%). In the areas, located at higher altitude (between 700 and 900 m a.s.l.), the rate of infected by *C. parasitica* trees were only 10%. The effect of trees decline varied from partial crown dieback to completely death trees (18%). In individual SPs, chestnut blight incidence ranged from 18% to 100% of the trees in the SPs, and mortality caused by the fungus was between 2% and 80%. The results obtained indicated that chestnut blight had spread into most chestnut stands of Belasitsa Mt., the most affected stands between 400-800 m. a.s.l.

The pathogen *Cryphonectria parasitica* was found to be widely distributed in all studied SPs in in Belasitsa Mt. It causes chestnut blight disease and is considered to be the most destructive pathogen on both American (*Castanea dentata* (Marsh.) Borkh.) and European chestnut (*C. sativa*) (Anagnostakis, 1987). The first documented observation of *C. parasitica* in Bulgaria was on chestnut trees in pure and mixed stands on the northern slopes of Belasitsa Mt. and on the Eastern Rhodopes mountain in 1993 (Petkov, Rossnev, 2000). The pathogen is detected in both largest localities of chestnut in Bulgaria – in Belasitsa Mt. and West Balkan Range, and causes serious damages on stems and branches (Georgieva et al., 2013). *C. parasitica* attacks bark tissues producing cankers that can develop as sunken regions due to tissue collapse, damage to vascular tissues produces wilts and diebacks. A high infection rate and crown defoliation of the chestnut trees manifest that chestnut blight disease was a major stress factor and likely an important driver of chestnut decline. Epidemic of disease reduces the abundance of a dominant native species, resulting in a change in landscape structure. The infected dead branches with wilted, yellow or brown leaves which remain hanging on the branches, producing a so-called flag. The stems developing numerous epicormic shoots below the necrotic areas. A parasitic fungus causing necrotic lesions (cankers) on the bark of chestnut stems and branches. The pathogen attacks all the aerial parts of the tree: the main stem, branches and young suckers; infecting the tree via wounds in the bark and forming sunken canker due to necrosis and collapse of bark tissue (Hebard et al., 1984). The buff-brown mycelia of the fungus invade and destroys the inner bark (i.e. phloem, vascular cambium and xylem) resulting in the death of host tissue distal to the point of infection. Thus, the disease leads to the loss of an important part of the chestnut production and the progressive death of the tree.

The sycamore lace bug, *Corythucha ciliata* was found at high density in the three studied SPs (Petrich, Kolarovo and Samuilovo) in Belasitsa Mt. Additional surveys showed that the species was penetrated in *Platanus orientalis* stands along the rivers and streams in the mountain. It is naturally distributed in USA and south parts of Canada. In Europe the species was found for first time in Italy in 1964 and later in other countries (France, Switzerland, Croatia, Greece, etc.). In Bulgaria the pest was found for first time in 1989 in Sofia (Josifov, 1990). Currently, it is distributed all over the country. The species is trophycally related to plane trees (sycamore) (*Platanus* spp.). In urban environment in Bulgaria it frequently feeds on *Platanus x acerifolia* (a hybrid between *Platanus occidentalis* and *P. orientalis*). It has penetrated into all natural localities of *P. orientalis* in the country. In Europe, *C. ciliata* usually develops two generations per year, but in some south regions with Mediterranean

climate it has three. The female lay eggs around the nerves on the lower leaf's surface. The larvae and adults feed in groups on the lower surface and the colonies could reach scores and hundreds of specimens. The generation develops within 45 days. The second generation appears at the end of August/beginning of September. It winters as imago in bark crevices of thick branches and stems. At the places where larvae and imago were feeding, small white spots appear. Later the spots grow, and this leads to leaf depigmentation and yellowing. In case of massive attack, the leaves dry and fall prematurely at the end of summer. In combination with other stress factors the massive development of *C. ciliata* during some consecutive years could lead to ruin of the attacked trees. It should be noted that the damages caused by the pest are most significant in urban conditions (Georgiev et al., 2017).
The oak lace bug, *Corythucha arcuata*, is naturally distributed in North America. In Europe the species was found for first time in Italy in 2000 (Bernardinelli, Zandigiacomo, 2000). In 2002 was reported for Switzerland (Forster et al., 2005), and in 2003 for Turkey (Mutun, 2003). In Bulgaria it was found a new species for the Balkan Peninsula in 2012 in the region of Plovdiv and Simeonovgrad (Dobreva et al., 2013). Later, invasion of the oak lace bug (*C. arcuata*) was reported in it in Hungary (Csóka et al., 2013), Croatia (Hrašovec et al., 2013), Romania (Tomescu et al., 2018) and other countries. In its natural area, *C. arcuata* feeds mainly on *Quercus* spp., and rarely representatives of other genera (*Castanea*, *Acer*, *Pyrus*, *Malus*, *Rosa*). In Europe, it was found on *Quercus petraea*, *Q. robur, Q. pubescens, Q. cerris, Q. rubra*, *Rubus idaeus*, *R. ulmifolius, Castanea sativa* and *Rosa canina* (Dobreva et al., 2013). *C. arcuata* biology and ecology is similar to that of Sycamore lace bug (*C. ciliata*). The feeding of larvae and adult colonies causes depigmentation, aging and premature leaf falling. Recently, the species was found on 8 oak species in high number nearly all over Bulgaria, on *Castanea sativ*a in Plovdiv region and on 5 occasional tree and shrub hosts (Simov et al., 2018). In case of penetration in Belasitsa Mt., *C. arcuata* could turn into a danger for chestnut stands due to progressive degradation and the poor health condition of the species.
The quarantine pest *Dryocosmus kuriphilus* causes severe damage to species of *Castanea* genus. It is widely spread in China, Korea and Japan, and in 2002, it first appeared in Italy. After the first penetration of the pest in Europe, it has spread rapidly in several other European countries, probably due to the trade in chestnut plant material. Despite the measures taken to prevent further spread of this pest in other countries, it was first obtained in Greece in 2014 (Michaelakis et al., 2016). However, studies show that the pest is still very limited in Greece. In Bulgaria, the species is not found at present, but its possible penetration at Belasitsa Mt. will increase the damage caused by *Cryphonectria parasitica*, which has caused significant losses in chestnut forests over the last 20 years.
The pathogen *Ceratocystis fimbriata* is an introduced fungal pathogen from North America to South Europe, distributed on species from genus *Platanus*, causing destructive tracheomicotic disease. Since 2004, the disease has been detected in southwest parts of Greece, where it caused dead of natural plantations and urban trees (Tsopelas, Angelopoulos, 2004). *Platanus* species are the only hosts for pathogen, with highest sensibility being *P. acerifolia*, often used for planting in urban environments in the countries with moderate climate. In transversal cut of the stem blue-black coloration growing radially to the periphery, with fusiform patterns could be observed. The dead cambial tissue provokes appearance of dark longitudinal spots on the bark surface. These spots are very important characteristics for diagnostics even if the infection has penetrated through the roots. Infection with the pathogen *Ceratocystis platani* f.sp. *platani* develops in the places of injuries on the bark or root, stem or branches, timber during pruning or other mechanical damages. After fungus spores appear on the plant tissue fast distribution starts in the xylem tissue along the stem and radially to its core. The fungus

could overwinter at temperatures up to -17°C, but does not develop under 10°C and over 45°C.

Conclusion

Finally, it should be noted that monitoring of invasive fungal pathogens and insect pests threatening forest vegetation in Belasitsa Mt. should continue to take adequate measures in case of penetration of highly aggressive species. This is mainly true for species whose health status is severely degraded, such as chestnut (*Castanea sativa*), as well as another species of high conservation significance, the eastern plane (*Platanus orientalis*).

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HEALTH STATUS OF PINE STANDS AS A PART OF THE EXTENSIVE TREE HEALTH MONITORING NETWORK IN BULGARIA

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Abstract

Since 1986, Bulgaria has been a part of the International Cooperative Program 'Forests' launched in 1985 under the auspices of the United Nations for European Economic Commission (UN/ECE). During the period 1986-1989, a large-scale network of 256 observation plots were set in ten regions throughout the country in order to monitor the health condition and dynamics of changes occurring in forest ecosystems. During the period 1986-2009, experts from Forest Research Institute, Bulgarian Academy of Sciences, annually conducted assessments in 130 permanent observation plots in five regions of the monitoring network. Among all plots, 54 were established in Austrian pine (*Pinus nigra* Arn.) and Scots pine (*P. sylvestris* L.) stands. In 2009, the number of plots was reduced to 80, as twenty-seven of them (predominantly plantations) were set in pine stands with 696 *P. sylvestris* and 384 *P. nigra* trees. The health status of trees in natural pine stands remained unchanged for the entire studied period. In recent years, worsening of *P. sylvestris* plantations has been observed at lower altitudes (below 700 m a.s.l.). Insect pests mainly bark beetles, and fungal pathogens, were the most frequent causers of damages. Snow- and wind-throws damages often occurred provoking the bark beetle attacks.

Key words: *pine stands, monitoring, health status, Bulgaria.*

Introduction

The forests of Bulgaria cover 4.1 million ha that is 37.7% of the territory. Broadleaved forests account for 68% of the forest area, and conifers account for 32% of the area. In 1960s, large-scale afforestation was carried out to converse the abandoned land with tree plantations to control the deterioration and soil erosion. The most commonly planted coniferous species comprised native Austrian pine (*Pinus nigra* Arn.) and Scots pine (*P. sylvestris* L.). The share of forest plantations amounts to 20.9% from the total forest area in the country. The International Cooperative Program 'Forests' launched in 1985 in Freiburg under the auspices of the United Nations for European Economic Commission (UNECE) with the adopted methodology for forest assessment and monitoring. Since 1986, Bulgaria is actively involved in the implementation of the ICP Forests. During the period 1986-1989, a network of 256 permanent observation plots (POPs) were set up in ten regions throughout the country to monitor the health status and dynamics of changes occurring in forest ecosystems. Observation plots were set in a systematic transnational grid of 16 × 16 km in 10 regions throughout the country to gain insight into the geographic and temporal variations in forest condition. Scientific experts of the Forest Research Institute - BAS have conducted observations in five of them (2nd - northern slopes of Middle Stara Planina Mt., 3rd - Eastern Stara Planina Mt., 4A- southern slopes of Middle Stara Planina and Sredna Gora Mts., 5th - Osogovo, Kraishte, western slopes of Rila and Vitosha Mts. and 6th - the mountains of Southwestern Bulgaria). During the period 1986-2009, annual observation was conducted in 130 permanent observation plots. Among them, 54 were established in *P. nigra* and *P. sylvestris* stands. In 2009, the number of observation plots was reduced to 80.

The annual reports present the scientific findings for Bulgaria, covering major challenges regarding the future preservation and management of forest ecosystems under environmental change. The results have shown that environmental factors and damages caused by abiotic and biotic agents fluctuated in years and need to be considered for assessment of tree health condition (Mirchev et al., 2006; Velizarova, 2010, Velizarova et al., 2013). The fluctuations add another degree of uncertainty to the survival of observed tree species. Special attention has been paid to the worsening of the health status of pine plantations in Southwestern Bulgaria for the period of 1986-2005 (Rossnev et al., 2008) and for 2000-2012 (Mirchev et al., 2014). This current study presents data on the health status of pine plantations in the five observed regions, covering the period 2009-2018.

Material and methods

Duringthe period 2009-2018, twenty-seven plots (predominantly plantations) were monitored in pine stands with 696 *P. sylvestris* and 384 *P. nigra* trees. All *P. sylvestris* plots were located in Southern Bulgaria andfive of them werelocatedin natural stands at 1302-1520 m a.s.l. These stands aged from 70 to120 years (table 1). Thirteen pots were set in tree plantations that aged from 50 to78 years and these stands were located at an altitude from 320 to 1298 m. All ten observation plots are set in *P. nigra* plantations in both Northern and Southern Bulgaria at 80 m a.s.l. (Varna) to 951 m a.s.l. (Klisura, Sofia distr.) (table 2). Most observation plots were at flat terrains and predominantly exposed in south direction.

An observation plot in Petrich (6^{th} region) represented mixed forest of 23 *P. nigra* and 17 *P. sylvestris* trees. The assessment of the general health status of 1084 trees was carried out according to the Manual of assessment of crown conditions of ICP 'Forests' (Eichhorn, 2010). Two physiological indicators were included in the complex assessment of the trees: defoliation and discoloration, characterizing the health of the trees, affected by several anthropogenic and natural factors. The surveys were conducted in combination with detailed assessments of biotic and abiotic damage causes. Defoliation was estimated in 5% steps, ranging from 0% (no defoliation) to 100% (dead tree), grouped into five classes: '0' (0-10% - no defoliation), '1' (>10-25% - slight), '2' (>25-60% - middle, '3' (>60-<100% - severe) and '4' (100% dead trees).

The general health status was quantified as a weighted arithmetic mean of the complex assessment on health status obtained by the formula:

$$Weighted\ Arithmetic\ Mean = \frac{\sum 0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4}{\sum n_0 + n_1 + n_2 + n_3 + n_4}$$

where:

0, 1, 2, 3, 4 - complex assessment;

n_0, n_1, n_2, n_3, n_4 - number of tree in the respective degree.

Results and discussion

For the period 2009-2018, a serious deterioration of Scots pine (*P. sylvestris*) health status was noticed. Mean defoliation increased from 1.4 (slight damaged) in 2010 to 2.0 (middle damaged) in 2018 in both natural stands and plantations (fig. 1). In all natural stands, withering was found at the end of observation period with a more significant degree at Leeve, Rila Mt. (table 1) after snow damages in 2015 that changed stand structure. Disturbances, caused by biotic agents, were the most frequent in Scots pine plantations. In 69.2% of them, symptoms of drying were observed for the entire studied period. In 2016, in Zavala (Kraishte Mt.), the share of completely dead trees reached up to 90% due to root rot fungus

(*Heterobasidion annosum* (Fr.) Bref) and bark beetle (*Ips acuminatus* Gyllenhal, 1827). The assessment of health status in observation plots in Vitosha Mt. (Selimitsa and Dren) and in Osogovo Mt. (Lisets) (table 1) obtained similar results. A long-term increase of mean defoliation and a significant decrease in the tree vitality was observed related to isolated water shortages and outbreaks of bark beetles. The value of mean defoliation rose by 76.9% at the end of the observation period, which was indication for deteriorated health status of all Scots pine plantations. General deterioration was observed in Austrian pine (*P. nigra*) that was more evident in the period 2016-2018 (fig. 1). The value of mean defoliation increased from 1.05 (slight damaged) to 2.1 (middle damaged). The percentage of healthy trees decreased but so far in lower rate than *P. sylvestris*. The number of dead or missing trees has slightly decreased and reached 40% for the studied period. The mortality of trees is mainly due to decline processes related to pathogens *Diplodia sapinea* and *Dothistroma* spp., caused damage to all trees in the plots. The data for the whole study period show that in the studied regions of Bulgaria the health status of *P. sylvestris* and *P. nigra* were similar or very close in values. The fluctuations observed during the years are synchronous for both species. After 2015, there was a steady trend of deterioration in the health status of both tree species (fig. 1).
With regards to the determined biotic factors, the population of pine bark beetle (*Ips acuminatus*) dramatically increased and caused serious damage to the Scots pine stands in the monitored sample plots. Limited damages were observed by *Tomicus piniperda* (L). and *Thaumetopoea pityocampa* (Den. Et. Schiff.) which caused physiologically weakening of trees in pine plantations. The presence of the fungus *H. annosum* was observed in both the *P. sylvestris* and *P. nigra* stands. The pathogens *Dothistroma pini* Hulbary, *D. sapinea* and *Cyclaneusma minus* (Butin) DiCosmo, Peredo and Minter were also found in these plantations. These pathogens cause damage to needles and shoots, and therefore pose a threat to the monitored tree stands. Numerous factors determine the health condition of forests. These include climatic factors, disease and insect damage as well as other biotic and abiotic factors. Long-term monitoring provides baseline data on the distribution, occurrence and harmfulness of biotic agents or damage factors in Europe. These data are contributing to other aspects relevant for forest policy like sustainable forest management (Eichhorn et al., 2016).
An increased incidence of trees with severe defoliation (class '3' and '4') was found out both in *P. sylvestris* and *P. nigra* plots almost throughout the whole territory of the country, affected by a bark beetle calamity, mainly at lower altitude. The ecological problems were particularly manifested in plantations. Some of pine plantations were planted for the restoration of degraded lands, in atypical habitats at lower altitudes where early pine mortality was registered (Popov et al., 2015, 2016). A process of incremented damages by biotic and abiotic factors contributes significantly to the rapid destruction of pine plantations in the country. Among the abiotic factors responsible for the deterioration of pine plantation, damages caused by wind, wet snow and ice have the most negative impact. Damages from natural anomalies are regularly observed in Bulgarian forests on larger or more limited areas. However, their frequency and strength are increasing, creating prerequisites for appearance of xylophagous pest calamities and disease development. Among xylophages, the most dangerous and economically harmful for pine trees is *Ips acuminatus*, and among diseases - root rot caused by the fungus *Heterobasidion annosum* (Mirchev et al., 2016).

Table 1. Main characteristics of studied areas of *P.sylvestris* and changes in the health condition of the trees during the period 2009-2018

Sample plots	Coordinates N	Coordinates E	Age of trees by 2018	Altitude, m	Gradient	Exposure (°)	Mean assessment 2009	Mean assessment 2018	Withering during the period 2009-2018,%
natural stands									
Leeve	+420620	+234237	87	1590	S	30	1.75	2.28	25.0
Sandanski	+414057	+232433	120	1490	N	30	0.55	0.75	0.0
Belitsa	+420154	+233458	104	1372	SW	15	0.95	2.18	0.0
Dobrinishte	+414550	+233316	70	1387	NE	20	0.80	1.75	0.0
Nadaritsa	+421515	+232621	110	1302	N	6	2.03	2.13	7.5
Plantations									
Lisets	+421605	+223410	66	971	S	15	2.40	2.25	52.5
Zavala	+424941	+224735	51	1092	NE	20	2.43	3.25	70.0
Mali Varbovnik	+421518	+225956	53	726	N	12	1.70	2.58	15.0
Radomir	+423409	+225733	57	778	N	8	2.10	2.53	25.0
Ovchartsi	+421619	+231318	52	809	N	12	1.49	1.48	0.0
Dren	+422344	+230821	78	838	NW	21	1.75	2.20	37.5
Selimitsa	+423440	+231143	78	1298	NW	19	2.08	2.38	47.5
Klisura	+424245	+242328	71	1080	SW	20	1.15	1.53	7.5
Kazanlak	+423709	+251900	64	355	SW	5	1.48	1.38	0.0
Sevlievo	+425409	+245737	64	575	SE	12	1.95	1.50	30.0
Petrich	+412230	+230459	50	320	N	5	1.10	2.40	0.0
Harsovo	+420114	+231203	57	802	N	25	0.83	2.25	12.5
G. Delchev	+413404	+233937	55	1180	S	14	0.85	2.23	5.0

Table 2. Main characteristics of studied areas of *P. nigra* and changes in the health condition of the trees during the period 2009-2018

Sample plots	Coordinates		Age of trees by 2018	Altitude, m	Gradient	Exposure (°)	Mean assessment 2009	Mean assessment 2018	Withering during the period 2009-2018
South Bulgaria									
Garmen	+413714	+234856	67	647	SW	10	1.28	2.83	0.0
Tsaparevo	+413700	+230637	44	785		0	1.15	2.55	0.0
Aldomirovtsi	+425136	+225921	54	672	S	4	2.18	2.03	20.0
Klisura-Sofia	+424335	+230427	63	951	SE	16	1.88	1.83	0.0
Gorna Banja	+424335	+230427	63	835	S	6	1.80	1.75	12.5
Osogovo	+421548	+224040	59	836	NW	11	1.43	2.10	0.0
Petrich	+412230	+230459	50	320	N	5	1.33	2.26	8.7
North Bulgaria									
Shumen	+430929	+270619	54	305	S	5	1.15	1.60	0.0
Varna	+431133	+275303	59	80	E	4	1.95	2.80	0.0
Gostilitsa	+430226	+252230	67	475		0	1.83	1.53	17.5

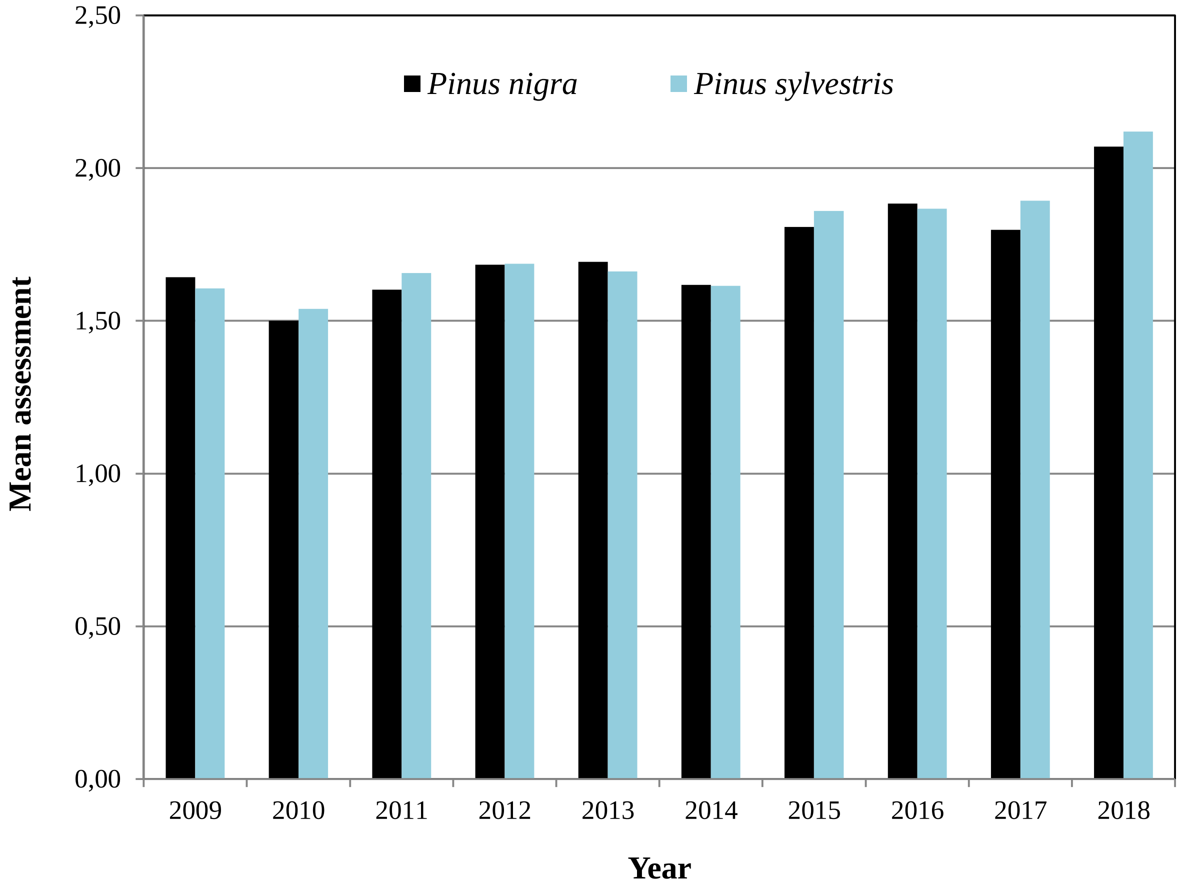

Figure 1. A weighted arithmetic mean of complex assessment on health status of studied *P. sylvestris* and *P. nigra* during the period 2009-2019

Conclusions

In conclusion, it should be noted that the health status of pine trees in natural stands remained unchanged for ten-year period. In the plantations, worsening of trees has been observed at lower altitudes (below 700 m a.s.l.). Insect pests mainly bark beetles, and fungal pathogens, were the most frequent causers of damages. Snow- and wind-throws damages often occurred provoking the bark beetle attacks. Among the abiotic factors responsible for the deterioration of pine plantation, damages caused by wind, wet snow and ice have the most negative impact.

The long-term monitoring provides baseline data on the distribution, occurrence and harmfulness of biotic agents or damage factors. The assessment of harmful impact and spread of the most important insect pests and fungal pathogens causing damages in Bulgarian forests is essential for making decisions about management of pine plantations. The identification of the pests and pathogens and the determination of their specific ecological features will allow the development of integrated measures for limiting their negative impact.

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INVESTIGATING FACTORS AFFECTING CONSUMPTION OF CONSTRUCTION TIMBER FOR TEN SELECTED EUROPEAN COUNTRIES. A FOOTPRINT APPROACH

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Abstract

Europe is a world leading producer and consumer of wood products, which generates a substantial forest "footprint" both inside and outside European borders. However, there has been limited research on what factors shape consumption, and in particular, the relative roles of local forest abundance, forest production and economic wealth. This paper helps to address this issue, by assessing the relationship between few key variables–consumption of construction timber, forest area, GDP and finally forest products footprint. Ten European countries were selected, ranging from countries with very high forest cover and low population density to those with very low forest area per capita. Pearson correlation was employed. It was found that, on average, the wood products consumption is more related to people in more forest-endowed countries than those in countries with less forest per capita. However, this relationship shows regional variation. In other words, higher rates of forest areas appeared to support higher rates of consumption rather than lower imports or "external footprints". The results for per capita GDP revealed a significant correlation with per capita consumption, primarily within the examined emerging economies. This decoupling of wood consumption from GDP in wealthier countries is consistent with past studies. Finally, significant correlation was found between footprint and sawnwood consumption almost across all the case studies, while in only three of them wood panels consumption was related to footprint. All of these observed patterns, taken together, highlight the need for additional research to understand the causal factors and net environmental and social impacts.

Keywords: *Consumption, forest products, forest footprint, GDP, correlation.*

Introduction

The focus of this article is on the EU forest sector. Europe is a world leading producer and consumer of wood products and this leaves a substantial 'footprint' both inside and outside its boundaries. The ecological footprint measures human appropriation of ecosystem products and services in terms of the amount of bioproductive land and sea area needed to supply these services (Wackernagel and Rees, 1996). The ecological footprint and biocapacity accounts for six land use types: cropland, grazing land, fishing ground, forest land, built-up land, and carbon uptake land (to accommodate the carbon footprint) (Ewing et al., 2010). To date international concern has focused primarily on the EU's external forest footprint, and the extent to which it can be linked to illegal logging (e.g. Lawson and MacFaul, 2010) or consumption of products, such as palm oil and soy, that are driving deforestation elsewhere (e.g. Cuypers et al., 2013). However, there has been relatively little attention to the factor's driving the total size of Europe's wood product consumption.

Considering only the volume of wood consumed, European consumption has remained well within its capacity to produce that wood (e.g. Global Footprint Network 2012; UNECE, 2013). Nevertheless, there is considerable variation in the environmental and social impacts of wood production across the many countries from which Europe sources its wood. Furthermore, regardless of where the wood is produced, there are inherent tradeoffs between

production and other social and ecological values associated with forests. For all of these reasons, it is important to better understand the key factors driving total wood consumption. There has been very little empirical research to date examining the variables driving per capita consumption of wood products, with the exception of (Haripriya and Parikh, 1998) and (Humerkoski et al., 2015). In addition is reported by O’Brien et al., (2018) that the existing subsidy schemes for bioenergy in the EU are expected to increase dependency on wood imports (Schulze et al., 2012; UNECE et al., 2012), creating -extra- future global land demand, on behalf of EU. Nevertheless, most predictions of future wood consumption assume a close relationship between per capita consumption rates and per capita GDP, with a leveling off past a certain threshold of economic development (e.g. Jonsson, 2012). Yet countries that have passed that threshold still vary considerably in the quantity of wood the average resident consumes. This raises questions about what other variables might explain this variation. Past research has highlighted important regional differences in wood consumption (Buongiorno, 2009), and the relative importance and persistence of local building traditions in determining the choice of construction material (Mahapatra and Gustavsson, 2008). In partial explanation of such differences, Humerkoski et al.’s (2015) study of European sawnwood consumption suggests a correlation between a country’s forest growing stock per capita and consumption per capita. This would suggest, not surprisingly, that there is a historical tradition of using wood for construction in countries where there is a relative abundance of wood, and that these traditions have persisted despite the globalization of wood trade. However, Humerkoski et al., also find that the precise relationship between forest abundance and per capita consumption varies by region (ibid), and hence other variables must also be significant.
There is growing international interest in the concept of ecological footprints, or related measures that assess and compare consumption levels and their impacts on the earth’s productive capacity (e.g. Steen-Olsen et al., 2012; Wiedmann et al., 2013; and Steffen et al., 2015). However, there is limited research attempting to explain differences in consumption levels, particularly among countries with similar levels of economic development.
This article tries to approach this issue, by assessing the relationship between three key variables which could be related to footprints – i.e. per capita consumption, forest area and GDP. Finally, it investigates which subcategory of construction timber sawnwood seems to play more important role in footprint creation for each case study.

Material and methods

The analysis was focused on consumption of construction timber as primary timber (sawnwood and wood based panels) in ten case study countries based on the reasoning that levels of forest endowment are more likely to affect choices in building materials for which many substitutes are available, than use of products like paper for which there are fewer substitutes. Paper production is also subject to intense global price competition, favoring large-scale, high technology production and locations with high fiber productivity.
Figures for the consumption of construction timber per capita and the GDP per capita were collected from FAOSTAT and the EUROSTAT database (EUROSTAT, 2014) for the period 1995-2013. For construction timber we combined the FAO data on sawnwood and wood-based panels. The former consisted of the sawnwood+ (total) category, which includes coniferous and non-coniferous production in cubic metres. The latter consisted of the wood panels + (total) category, which includes veneer sheets, plywood, particle board and fibreboard as defined by FAO (FAO, 2012). Population data were drawn from (FAOSTAT, 2014) in order to convert the consumption figures into units per capita.
The analysis was centered around the functions of

$$\frac{Consumption}{capita} = f\left(\frac{production}{capita}, \frac{GDP}{capita}\right) \qquad (1)$$

for construction timber, with consumption/capita being the dependent variable and production/capita and GDP/capita being the 'independent' variables. The use of GDP of is very common to the forest sector models which are defined by Solberg (1986). EFSOS (European Forest Sector Outlook Study) model (Kangas and Baudin, 2003) and EFSOS II (UNECE/ FAO, 2011) use GDP as the best reflecting variable for economic growth in order to make projections about forest products demand, supply and trade in Europe. The second updated part of the analysis is aiming to determine if there is a statistically significant relationship between the forest products footprint and the consumption of construction timber (function 2).

$$\frac{\text{Forest products footprint}}{\text{capita}} = f\left(\frac{\text{sawnwood consumption}}{\text{capita}}, \frac{\text{woodpanels consumption}}{\text{capita}}\right) \qquad (2)$$

The data regarding forest products footprint for each country were received from the Global Footprint Network open database (Global Footprint Network, 2018) for the period 1990-2016. Correlation analysis was employed using the Pearson correlation coefficient. The statistical analysis was performed with SPSS (version 22.0) statistical package (Norusis, 1997).

Results and discussion

Ten European case study countries (involved in INTEGRAL project) were chosen for the analysis, representing a wide range of per capita production rates and/or forest areas (related to potential production rates) (Table 1). Sweden, Lithuania and Portugal represent countries with high levels of per capita industrial roundwood production followed by France, Germany, Ireland and Slovakia with medium levels of per capita production. The case study countries with low levels of industrial roundwood productivity are the Netherlands, Bulgaria and Italy. Bulgaria is unusual among the case studies, in that its forest area/capita is relatively high but its levels of industrial forest production are relatively low.

Table 1: Case study countries industrial production and forest area per capita

	N Europe	CE Europe	S Europe
High production/capita	Sweden	Lithuania	Portugal
Industrial production (cum)/capita (Average 2000-2013)	7.05	1.45	0.91
Forest area/capita (2012)	2.97	0.72	0.33
Medium production/capita	Germany	Slovakia	France
Industrial production (cum)/capita (Average 2000-2013)	0.56	1.37	0.47
Forest area/capita (2012)	0.21	0.36	0.25
	Ireland		
	0.59		
	0.17		
Low production/capita	Netherlands	Bulgaria	Italy
Industrial production (cum)/capita (Average 2000-2013)	0.05	0.39	0.05
Forest area/capita (2012)	0.02	0.55	0.15

Source: FAOSTAT 2014

The general trend of correlation between consumption of forest products and economic growth (and population sometimes), measured by GDP, is intuitive given the wide variety of usage ranging from construction material to furniture or paper. One known major exception of this trend is fuelwood for which a decline in consumption is observed as GDP per capita rises as people shift to more convenient fuel sources once these become affordable for them (Buongiorno et al., 2003; Whiteman et al., 1999). In doing so, was realized the converse role of production in the function as long as increased consumption can increase demand for timber and drive increases in domestic production. At the same time, we the possibility of the converse relationship – that is whether higher levels of domestic production (due to changes in price, ease of availability or other factors) might spur consumption, seems really interesting. The existing observations about higher wood consumption in leading wood producing countries, coupled with evidence that in most cases GDP alone does not explain consumption, suggests the possibility of this latter effect. If domestic production spurs domestic consumption, in addition to consumption spurring domestic production, this needs to be taking into account in assessing strategies to reduce ecological footprints.
Given the dearth of existing studies on exactly which variables distinguish one regional wood culture from another, it is likewise not possible to classify which countries belong to which regional grouping. However, in the EU at least, even simple divisions between north, east, west and south yield much stronger regional correlations between wood abundance and consumption than for the EU as a whole. For example, a strong positive correlation was found, between per capita forest area and per capita consumption of construction wood (sawnwood, wood based panels) among the eastern emerging economies of the EU, consisting of Hungary, Poland, the Czech Republic, Romania, Slovakia, Croatia, Bulgaria, Slovenia, Lithuania, Latvia and Estonia (p=0.001) (Table 2). Likewise, there is a significant correlation in the Scandinavian countries of Denmark, Sweden and Finland (p= 0.01) and an equally significant correlation among other Northwestern countries. In contrast, there is no apparent correlation in the more Southern EU countries of Italy, Cyprus, France, Portugal, Greece and Spain. This latter result may be explained, for example, by the risk of fire or other climatic variables shaping wood use.[18]

Table 2. The correlation between forest area and consumption for selected groups of EU countries.

		Pearson correlation coefficient	**Significance level**
EU countries		Forest area/cap	
E	total cons/cap	0.864	P<0.05
N	total cons/cap	1	P<0.05
NW	total cons/cap	0.773	P<0.05
S	total cons/cap	-0.348	**P=0.449**

East group includes: Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Romania and Slovenia
North group includes: Denmark, Finland and Sweden, Northwestern group includes: Austria, Belgium, Germany, Ireland, Luxemburg, Netherlands and UK
South group includes: Cyprus, France, Greece, Italy, Portugal and Spain

[18]The source of the data for these calculations was (FAOSTAT, 2014) and the p values are based on data from the year 2012.

Whatever the precise combination of variables that determine regional differences, the above evidence is sufficient to suggest a significant relationship between wood abundance and wood consumption that merits further exploration. Furthermore, it is also important to interrogate how much of this relationship is based on durable traditions of wood use and/or whether fluctuations in wood availability within a given country are accompanied by changes in consumption. The following analysis addresses the latter by comparing the production of construction wood (sawnwood, wood based panels) per capita with forest apparent consumption[19] per capita over a period of 18 years within ten case study EU countries. In addition, we include GDP per capita as another important variable, independent of wood culture. The results of this analysis which described in methodology for the case study countries are presented below [tables 3,4].
Correlation analysis based on Pearson’s r indicated that regarding sawnwood there is a positive relationship between consumption/capita and production/capita for all the countries. The Pearson correlation coefficient for this variable ranged from 0.53 for Germany to 0.94 for Slovakia (Table 3). On the other hand, the Pearson correlation coefficient ranged from 0.44 for Sweden to 0.84 for Lithuania regarding the GDP/capita. Positive relationships between GDP per capita and sawnwood/capita were found for all three Eastern emerging economies, i.e. Lithuania, Slovakia and Bulgaria, but were not found consistently for developed economies. This is consistent with other research, which indicates that wood consumption tends to increase with GDP and then levels off among fully industrialized countries (e.g. Jonsson, 2012). For France, Germany and Ireland there wasn’t any correlation between sawnwood consumption/capita and GDP/capita. Thus, production/capita is the variable that correlates most strongly with consumption/capita and GDP/capita has either a positive or a negative (Netherlands and Portugal) correlation, probably determined in part by level of economic development.
As regards to wood panels there is a positive relationship (variables tend to increase together) between consumption/capita and production/capita for the eight of 10 case studies and in one (Sweden) there is a negative correlation. In the Netherlands there is no significant correlation. The Pearson correlation coefficient for this variable ranged from 0.59 for Ireland and Portugal to 0.91 for Slovakia (Table 3). The Pearson correlation coefficient ranged from 0.56 for Portugal to 0.99 for Lithuania regarding the GDP/capita. For the Netherlands, Germany and Ireland there wasn’t any significant correlation between consumption/capita and GDP/capita. Thus, wood panels production/capita and GDP/capita correlate quite strongly with consumption/capita for most of the countries. It’s important to underline here that in most of the cases above, the findings are designated as significant ($p<0.05$).

Table 3. Case studies countries correlation results. Linear dependence of production/capita, GDP/capita and forest area/capita on Consumption/capita for the construction timber

		Pearson correlation coefficient			
	Consumption/cap	**Production/cap**		**GDP/cap**	**Significance level**
		sawnwood	**Wood panels**		
Bulgaria	sawnwood	0.86		0.83	P values <0.05

[19] The sum of production plus imports minus exports. The difference between 'apparent' consumption and 'real' consumption is that the latter definition also recognizes changes in stock levels and imports or exports of highly processed products.

	Wood panels		0.85	0.75	
France	sawnwood	0.86		n.s.	P values <0.05
	Wood panels		0.70	0.88	
Germany	sawnwood	0.53		n.s.	P values <0.05
	Wood panels		0.71	n.s.	
Ireland	sawnwood	0.86		n.s.	P value <0.05
	Wood panels		0.59	n.s.	
Italy	sawnwood	0.76		0.53	P values <0.05
	Wood panels		0.86	0.92	
Lithuania	sawnwood	0.72		0.84	P values <0.05
	Wood panels		0.96	0.99	
Netherlands	sawnwood	0.89		-0.70	P values <0.05
	Wood panels		n.s.	n.s.	
Portugal	sawnwood	0.88		-0.50	P values <0.05
	Wood panels		0.59	0.56	
Slovakia	sawnwood	0.94		0.80	P values <0.05
	Wood panels		0.91	0.82	
Sweden	sawnwood	0.86		0.44	P values <0.05
	Wood panels		-0.51	0.84	

n.s: not significant

Table 4. Case studies countries correlation results. Linear dependence of forest products footprint /capita, with construction timber consumption/capita.

	Pearson correlation coefficient		
Footprint/cap	**Consumption/cap**		**Significance level**
	sawnwood	**Wood panels**	
France	0.803	n.s.	P value <0.05
Germany	0.337	n.s	P value =0.05
Italy	0.770	0.645	P values <0.05
Lithuania	0.888	0.958	P values <0.05
Netherlands	0.735	0.820	P values <0.05
Portugal	0.472	n.s.	P value <0.05
Slovakia	0.472	n.s	P value <0.05
Sweden	0.419	n.s	P values <0.05

Source: Global Footprint Network 2018, no data available regarding Bulgaria and Ireland.
n.s: not significant

Finally, the last part of our analysis indicated that there is significant correlation between forest products footprint and construction timber consumption mostly regarding the sawnwood consumption. (table 4). As it was expected all of them are showing quite strong relationship among forest products footprint and sawnwood consumption. This relationship varies in significance starting from Germany with r=0.337 reaching to r=0.889 regarding Lithuania. On the other hand, among the seven countries that were examined only three of

them (Italy, Lithuania and Netherlands) wood panels consumption seems to be related with the footprint. In all the above cases the significance level of the correlations was below 0.05.
The results indicate that production per capita has the strongest relationship with consumption per capita for sawnwood and panels in the examined countries during the studied period. It is indeed not surprising that production would rise with rising demand for wood products. However, the fact that this is somewhat decoupled from changes in per capita GDP in many countries suggests that production could also be driving demand. In other words, it suggests that an increase in domestic production can contribute to increased consumption, and hence an increase in forest footprint. This would belie assumptions that increasing local production will reduce imports, or external forest footprints. Rather it suggests that factors driving internal and external footprints must each be subject to their own systematic examination.
Income plays a smaller role in consumption but not a minor one, particularly in the emerging economies. In most of the countries (Netherlands for both categories and Portugal for sawnwood are excluded) there was a positive correlation between consumption and GDP per capita. However, in a few countries the correlation was negative, while in others GDP seems to not play a significant role in consumption either way, for example in sawnwood consumption in France or for sawnwood consumption and panels in Germany and Ireland.
Finally, most of the examined countries are showing strong significant relationship between the forest products footprint and the sawnwood production, while only in three of them an important relationship between panels consumption and forest footprint is observed. The latter observation, could reveal connections of the apparent consumption of construction timber with the national footprint creation meaning that except sawnwood important quantities of production or imports of wood panels could have contribution to the footprint creation. If we consider the connection between wood panels and furniture industry, seems that furniture industry development plays its role in footprint creation inside and outside of the country (i.e. Italy is one of the biggest producers in the word). This suggestion of course does not cover the subject in total and surly demands further investigation. Even if furniture production motivates footprint creation, other parameters like the percentage of certified wood production that used for furniture production, the national forest products imports policy, the efficiency of production and the origin of imports must be considered.
Categorically, it is important to underline several caveats to the above observed patterns. Firstly, they apply only to the ten case study countries. More such analysis would be needed to assess whether such patterns hold across the rest of Europe. Secondly, this research does not, by itself, enable conclusions about whether more or less domestic production is sustainable from a footprint perspective. This would require, for one, a more holistic material flow analysis which takes into account product substitutions. For example, if increased domestic production leads to increased consumption of construction timber this could reflect an increase in the net ecological footprint, or it could reflect a switch from non-wood to wood building materials and a decreased footprint. Likewise, the study's focus on wood volumes does not address the sustainability of how the wood is produced. It is possible, for example, that increasing domestic production contributes to improved production efficiencies and/or improved local knowledge and engagement in sustainable forest management, both at home and abroad. Europe is relatively well endowed with forests, and it is arguably the environmental and social "quality" of its forest footprint that matters as much as its quantity.

Conclusions

This paper analyzed construction timber (sawnwood and wood-based panels) consumption, production and GDP per capita in ten EU case study countries, ranging from heavily forested, high producing countries to low forest, low producing countries. The volume of domestic production per capita was strongly correlated with consumption per capita in most of the case

studies. The role of GDP per capita was smaller overall, and less consistent within the Western European economies, but does appear to drive demand for construction timber in the eastern European countries examined. From a footprints perspective, this illustrates that increasing domestic production may lead to an increase in a country's total forest footprint. This suggests, in turn, that increasing local production may not reduce a country's imports, or external forest footprint, unless it is accompanied by other changes or policy measures that address total consumption and import activity. Moreover, was confirmed that between the two subcategories of construction timber sawnwood consumption seems to play more important role in footprint creation than wood panels.
The findings in this study are limited to the EU countries analyzed. This, plus the highly dynamic nature of global wood markets and associated socioeconomic and technological contexts, means that these findings should not be used in isolation to investigate the factors that forest products footprint creation extensively. Furthermore, the examination of a single product category – i.e. construction timber – does not allow us draw conclusions about effects on net ecological footprints; rather doing so would require attention to other factors such as net material flows and production substitutions. Nevertheless, what this research does clearly illustrate, is the importance of understanding what shapes the nature and size of ecological footprints. Such understanding is crucial if we are to design effective policies to improve and reduce them.

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CHARACTERIZATION OF *POPULUS* HYBRIDS BY THEIR DIFFERENT RESPONSES TO ABSCISIC ACID DURING *IN VITRO* CULTURE

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Abstract

The aim of the study was to determine the effect of phytohormone abscisic acid (ABA), known for its accumulation in plants during stress (e.g., drought), on four selected *Populus* genotypes. These represented the crossings of Eurasian aspen with either American aspen (*P. tremula* × *P. tremuloides*; genotypes L191, Wa13, and 174/10) or white poplar (*P. tremula* × *P. alba*; genotype 91/78). Experiments were conducted on clonal *in vitro* cultures through two stages. First, explants – apical shoot segments – were cultured on different media, either hormone-free or enriched with 3 μmol l^{-1} ABA, in darkness (thus, genotype responses to the absence of light were also tested). Afterwards, the apical segments of control and ABA-treated shoots were excised again and transferred onto fresh hormone-free medium under standard light conditions. The results showed that genotype L191 was most negatively affected by darkness and further suppressed by ABA. In comparison to L191, Wa13 was less affected by darkness, but its root formation and development remained suppressed by ABA even during the following subculture on hormone-free medium. Genotype 174/10 developed relatively well in darkness and, among the tested genotypes, was least affected by ABA. Meanwhile, genotype 91/78 showed the most complex response to ABA: it had weaker shoot growth and root development under direct ABA treatment. However, on the fresh hormone-free medium, the ABA-affected 91/78 explants had longer shoots and dominant roots than their counterparts from the control variant. This suggests that, in crossings with aspen, white poplar genes can improve a proper response to stress hormone ABA.

Keywords: *Abscisic acid, Aspen, White poplar, Hybrid tree, Rooting*

Introduction

Because the global resources of fossil fuels are declining steadily, the world-wide interest in fast-growing tree genera, such as willows (*Salix*) and poplars (*Populus*), is increasing and the representatives of these genera are widely used for the establishment of forest plantations aimed at fast biomass production (Rosso *et al*., 2013). In Northern and Central Europe, Eurasian aspen (*Populus tremula*), a native tree species of this region, is often involved in *Populus* breeding and selection programmes (Müller *et al*., 2012). *P. tremula* hybridization with, e.g., American aspen (*Populus tremuloides*) and white poplar (*Populus alba*) can result in genotypes with high growth rate and biomass productivity (Niemczyk *et al*., 2019). Hybridization between *P. alba* and *P. tremula* occurs also naturally, resulting in the hybrid poplar species *Populus* × *canescens* (Santos-del-Blanco *et al*., 2013). Poplar species, including *P. tremula*, are characterized by high intraspecific genetic variation (Zhang *et al*., 2015); therefore, even genotypes derived from a same interspecific crossing combination can be considerably different from each other and the use of different species further increases variability among hybrid *Populus* genotypes. In the context of this variability, it is important to select *Populus* genotypes with potentially best phenotypic qualities.

One of the most important characteristics of promising tree genotypes would be their ability to maintain growth after stress. The current knowledge about the regulation of plant stress

responses, as summarized, e.g., by Brunner *et al*. (2015), indicates that plants, once under stress conditions, accumulate higher levels of the hormone abscisic acid (ABA). This usually leads to the above-ground growth inhibition, helping to save energy and nutrient resources. Although ABA, as an inducer of stomatal closure (Liu *et al*., 2001), is especially important under drought stress, this hormone enables plants to survive under various sorts of unfavourable conditions, including cold (Bertrand *et al*., 1997), heat (Talanova *et al*. 2006), and increased concentration of salt or toxic materials (Hsu and Kao, 2005). Since one of the primary steps in *Populus* selection would be the identification of genotypes with a potential to continue growing after encountering unusual or unfavourable environmental conditions, the ability of a particular genotype to restore its growth parameters after the treatment with ABA could be suggested as one of the early test criteria.
Accordingly, the present *in vitro* study tested four hybrid *Populus* genotypes, derived from the crossing combinations *P. tremula* × *P. tremuloides* and *P. tremula* × *P. alba*, for their responses to ABA. The initial experiments involved culturing of *Populus* explants on the hormone-free or the ABA-supplemented medium variants in darkness because of the recorded susceptibility of ABA molecules to light-induced destruction (Wenjian *et al*., 2013). The culture of explants on the hormone-free medium in light was involved as a separate experimental treatment at this stage in order to see how darkness itself affects the development of the studied genotypes. Further, the second stage of the study was designed to identify the differences among the *Populus* genotypes in respect of their ability to recover after ABA treatment and, for this aim, the explants from both the control and the ABA-treated variants were transferred onto a fresh hormone-free medium.

Material and Methods

The data on the four *Populus* genotypes involved in the study are given in Table 1. The experiments were conducted on the clonal shoot cultures of these genotypes, maintained *in vitro*. In both the pre-experimental and the experimental treatments, *Populus* shoots were cultured on the solid Murashige and Skoog (MS) nutrient medium containing 20 g l^{-1} sucrose and 4 g l^{-1} Gelrite (commercial gelling agent based on gellan gum), with the pH value set at 5.8. The medium, used for the multiplication of shoots before the start of experiments, was supplemented additionally with 0.6 mg l^{-1} 6-benzylaminopurine and 0.1 mg l^{-1} 1-naphthylacetic acid. The medium and its additional components, including the hormone ABA used for the experiments, were purchased from Duchefa Biochemie, The Netherlands.

Table 1. *Populus* genotypes used in the experiments of the study

Genotype	Crossing combination	Place of origin
91/78	*Populus tremula* × *P. alba*	Forest Research Institute, Poland
L191	*Populus tremula* × *P. tremuloides*	Thünen Institute, Germany
Wa 13	*Populus tremula* × *P. tremuloides*	Thünen Institute, Germany
174/10	*Populus tremula* × *P. tremuloides*	Thünen Institute, Germany

The MS nutrient medium used for the first stage of experiments was either hormone-free (for control) or supplemented with 3 μmol l^{-1} ABA. 10-mm-long apical shoot segments cut from the *in vitro*-grown *Populus* shoot cultures were used as explants. These were placed in Petri dishes, 13 mm of height and 55 mm of diameter, and cultured for three weeks in darkness. Each Petri dish contained 12 ml of nutrient medium and five explants. The additional, third, experimental variant at this stage contained explants cultured on the hormone-free medium but under 16-hour white-light photoperiod. After the period of three weeks, both the control and the ABA-treated shoots, cultured in darkness, were used to prepare new 10-mm-long apical explants. These were transferred onto the fresh hormone-free medium and cultured for subsequent six weeks in glass jars under 16-hour white-light photoperiod. Each jar, 75 mm of height and 70 mm of diameter, contained 30 ml of nutrient medium and four explants.

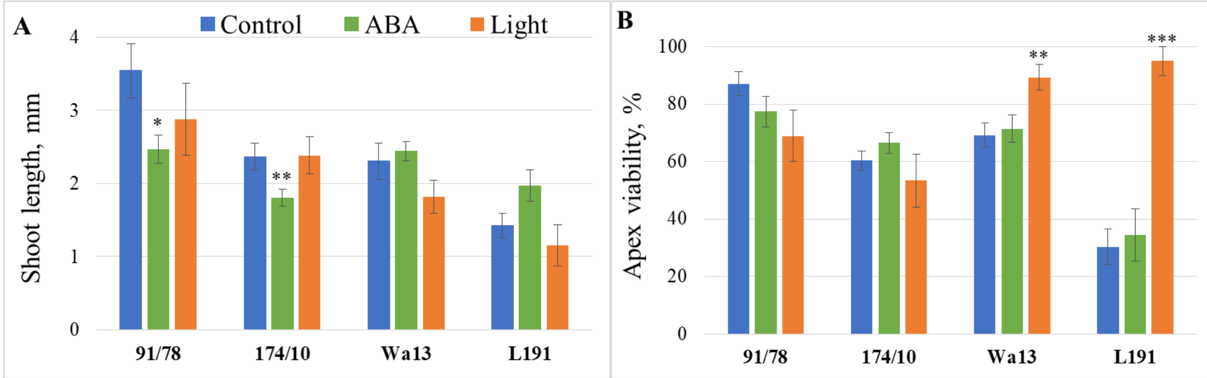
Each experimental variant (genotype × treatment) consisted of three replicates, 20-25 explants per replicate, and these were organized in a completely randomized design. The results presented in this study include the following parameters: the rate of explants with green shoot apex (apex viability, %), the increase of shoot length starting from the original 10 mm (shoot length, mm), and the rate of explants with visible roots (rooting, %). Because of the low rooting rate under direct ABA treatment, the data on root length are given only for the second experimental stage. These data include the length of the largest root of an individual explant and the total root length per explant. For the comparison of the obtained results, a two-tailed Welch's *t*-test (Welch, 1947) intended to compare samples with possibly unequal variances was performed to calculate the probability that the means of the different variants are equal.

Results and Discussion

The results of the first experimental stage are shown in Figure 1. On the hormone-free medium, the differences among the four genotypes in shoot elongation both in darkness and in light (Figure 1A) were closely related to the ability of shoot apex to remain green in darkness (Figure 1B). The genotype 91/78, which, differently from the other three genotypes, was obtained after crossing of *P. tremula* with *P. alba* and not with *P. tremuloides*, had the highest average shoot length increase both in darkness and in light as well as the highest shoot apex viability rate in darkness (87.1±4.3%). 91/78 contrasted most strongly with the genotype L191 which had the lowest average shoot length increase both in darkness and in light as well as the lowest shoot apex viability rate in darkness. The particularly low shoot apex viability rate of L191 in darkness (30.4±6.2%) contrasted strongly with the same genotype's high rate of shoot apex viability in light (95.0±5.0%). Another genotype which had a significantly lower shoot apex viability rate in darkness than in light was Wa13 but the difference here was much less dramatic than in L191. In the other two genotypes, 174/10 and 91/78, the shoot apex viability rate in light was not higher than in darkness (Figure 1B).

Regarding the light-dependent patterns of adventitious rooting, the genotypes 174/10 and Wa13 (both *P. tremula* × *P. tremuloides*) had much higher rooting rates in darkness than in light (Figure 1C). The negative effect of light on the rooting of *P. tremula* × *P. tremuloides* clones is also reported by Stenvall *et al.* (2005) who performed experiments on root cuttings. This negative response of *P. tremula* × *P. tremuloides* to light was in strong contrast to the observation made on the genotype 91/78 (*P. tremula* × *P. alba*) which had a 2.4-fold higher rooting rate in light than in darkness (Figure 1C).

As it is shown in Figure 1A, the hormone ABA decreased shoot growth significantly, although not dramatically, in the genotypes 91/78 and 174/10 (to 69.7% and 76.0% of the control variant, respectively). ABA did not affect the viability of shoot apex itself in any of the tested genotypes (Figure 1B). The most significant effect of ABA during the first experimental stage was found in respect of rooting whose rate was decreased by this hormone in all the studied genotypes (Figure 1C). In the genotype 174/10, the negative effect of ABA on rooting was relatively slight but, in the other genotypes, it ranged from an 8-fold decrease (in 91/78) to even a 16.7-fold decrease (in Wa13).



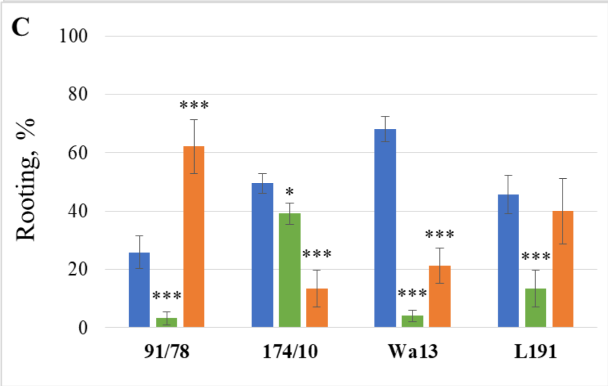


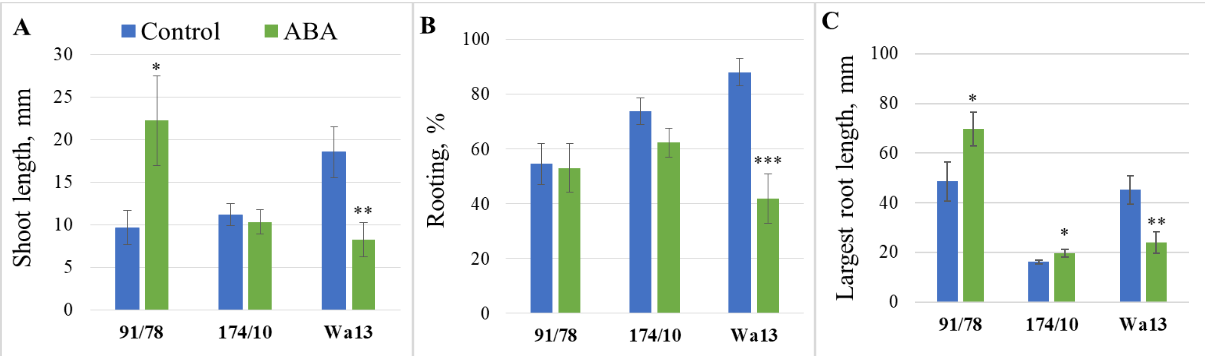
Figure 1. Characterization of the shoot explants from four *Populus* genotypes after three weeks of *in vitro* culture: A – shoot length; B – rate of explants with green shoot apex; C – rate of explants with adventitious roots. Different culture conditions are denoted: "Control" – hormone-free medium, darkness; "ABA" – medium with 3 µmol l^{-1} of abscisic acid, darkness; "Light" – hormone-free medium, 16-hour photoperiod. Significant differences between the control and the other two treatments are indicated: *P < 0.05, **P<0.01, *** P <0.001.

After the first experimental stage, the viable shoots were used for the preparation of new explants that were transferred onto the fresh hormone-free medium in jars for subsequent observation. This second experimental stage involved only three of the four initial genotypes, since the particularly low shoot apex viability of the genotype L191 explants cultured in darkness did not allow to continue with this genotype into the second stage. The results obtained with the three remaining genotypes during the second experimental stage are given in Figure 2. The major contrast in the development after the ABA treatment was observed between the genotypes Wa13 (*Populus tremula* × *P. tremuloides*) and 91/78 (*Populus tremula* × *P. alba*). The Wa13 explants taken from the ABA-supplemented medium were 1.9 times shorter than their counterparts from the hormone-free medium (Figure 2A) and had a 2.1 times lower rooting rate (Figure 2B). The root length in Wa13 was also significantly decreased by the previous ABA treatment (Figure 2C-D). Meanwhile, the second genotype of *P. tremula* × *P. tremuloides* – 174/10 – remained almost unaffected by the previous ABA treatment, except a slight (1.2-fold) increase in the average largest root length (Figure 2C).

In contrast to Wa13, the genotype 91/78 (*P. tremula* × *P. alba*) was affected positively by the previous ABA treatment. Here, the average shoot length after the ABA treatment in the previous subculture was 2.3 times higher than in the control variant (Figure 2A). The average largest root length was also affected positively by the previous ABA treatment, with a 1.4-fold increase in comparison to the control variant (Figure 2C).

The obtained results indicate that the genotype 91/78, derived from a crossing of aspen with white poplar (*P. alba*), had the most desirable response to the exogenously applied stress hormone ABA among the studied *Populus* genotypes. There are reports indicating a superior resistance, for example, to toxic stresses of both *P. alba* (Castiglione *et al.*, 2009) and its hybrids with *P. tremula* (Bittsánszky *et al.*, 2009). Interestingly, in the report by Bojarczuk

(2000), it was found that microcuttings of hybrid poplar *P. tremula* × *P. alba* had better developed root system in the substrate with aluminium (a toxic factor) in the case they were grown previously on the *in vitro* medium with the same toxic factor. As ABA is known as one of the most important signals that enables poplars to cope with the environmental toxicity (Shi *et al.*, 2019) and other abiotic stresses (Arend *et al.*, 2009), the ability of the genotype 91/78 to increase its growth during the subsequent culture stage after ABA treatment makes this genotype particularly interesting for further studies aimed at the selection of promising *Populus* genotypes.



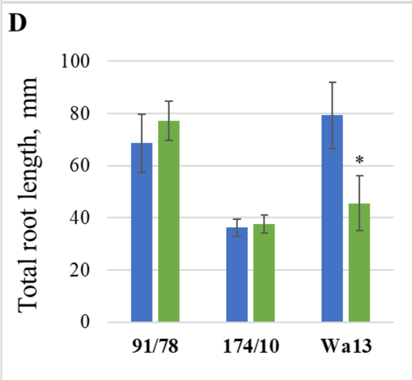


Figure 2. Characterization of the morphogenesis on the shoot explants from three *Populus* genotypes after six weeks of *in vitro* culture under 16-hour photoperiod: A – shoot length; B – rate of explants with adventitious roots; C – largest root length; D – total root length. During the previous subculture, the donor shoots used for explant preparation were cultured in darkness either on the hormone-free medium ("Control") or on the medium with 3 µmol l^{-1} of abscisic acid ("ABA"). Significant differences between the control and the ABA treatments are indicated as follows: *P < 0.05, **P<0.01, *** P <0.001.

Conclusions

Among the studied *Populus* genotypes, L191 (*P. tremula* × *P. tremuloides*) was most negatively affected by darkness and further suppressed by ABA. In comparison to L191, Wa13 (*P. tremula* × *P. tremuloides*) was less affected by darkness, but its root formation and development remained suppressed by ABA even during the following subculture on hormone-free medium. Genotype 174/10 (*P. tremula* × *P. tremuloides*) developed relatively well in darkness and, among the tested genotypes, was least affected by ABA. Meanwhile, genotype 91/78 (*P. tremula* × *P. alba*) showed the most complex response to ABA: it had weaker shoot growth and root development under direct ABA treatment but, on the fresh hormone-free medium, the ABA-affected 91/78 explants had longer shoots and dominant roots than their counterparts from the control variant. This suggests that, in crossings with aspen, white poplar genes can improve a proper response to the stress hormone ABA.

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BENEFITS BROUGHT BY THE ABUNDANCE AND IMPORTANCE OF FOREST FRUITS FROM SATU MARE COUNTY, ROMANIA

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Abstract

Non-timber forest products (NTFPs), also known as non-wood forest products (NWFPs), minor forest produce, special, minor, alternative and secondary forest products, are useful substances, materials and commodities obtained from forests which do not require harvesting (logging) trees. NTFPs is an important source of food and income. Several million households world-wide depend heavily on NWFPs for subsistence or income. Some 80 percent of the population of the developing world use NWFPs for health and nutritional needs. NWFPs have also attracted considerable global interest in recent years due to the increasing recognition of their contribution to environmental objectives, including the conservation of biological diversity. It is an important "branch" of forest management which not include the harvesting. Due to the fact that Romania has a wide variety of relief forms, numerous NWFPs can be found on her territory, the most important ones being forest fruits. Across the country, the distribution of NWFPs is not so uniform. These are rich in vitamins and have antioxidant properties, being used in alimentation, medicine and the cosmetic industry. The present article exposes the most representative forest fruits from Satu Mare County. Their classification is also realized based on an analytical hierarchy that takes into account 19 well-established criteria.

Based on an analytical hierarchy, their classification it was been realized by 19 well-established criteria. The abundance was dominated by fruits like sweet chestnuts, raspberries and wild pears.

Key words: *analytical hierarchy process, forest fruits, harvesting period, perishability, NTFPs*.

Introduction

During the past decades, the role of the forest has changed in Europe. NTFPs obtained from plant resources, including seeds, flowers, fruits, leaves, roots, bark, latex, resins and other non-wood plant parts, have gained much attention in conservation circles (Ticktin, 2004). Besides wood, its principal source, non-timber resources have become of interest. According to the Food and Agriculture Organization (FAO), the term which characterises these resources is "non-timber forest products" (NWFPs). The FAO organization adopted the working definition that: "Non-wood forest products consist of goods of biological origin other than wood, derived from forests, other wooded land and trees outside forests". NWFPs exclude timber and that the product, benefit or service should come from the forest (Wong *et al.*, 2001). Non-timber forest products (NTFPs) have been harvested by human populations for subsistence use and trade over thousands of years. FAO estimates that about 80% of populations from developing countries use NWFPs in food and medical industries (http://www.fao.org/).

The scope of this present paper is to identify the most important forest fruits from Satu Mare County. From a geographical point of view, Satu Mare County is situated in North-Western Romania. It was established in 1968 by the territorial reorganization of the former Maramures Region (from Satu Mare, Carei and Negresti-Oaş districts). Most of the territory of today's county was part of Satu Mare County (interwar) and of Satu Mare County (antebelic) respectively (https://ro.wikipedia.org/). The county seat and cultural, educational and economic center of the county. Its estimated surface reaches 4.418 km² (1,9 % from national territory). Land use includes : 72 % agricultural territories, 18 % forests, 3 % rivers, 7 % another surfaces (https://ro.wikipedia.org/).

Fig.1 Location of Satu Mare County, source: https://www.wikipedia.org/

The following forest fruits can be found in Satu Mare County: rosehips (*Rosa canina* L.*)*, raspberries (*Rubus idaeus* L.), black shock (*Sambucus nigra* L.), blackthorns (*Prunus spinosa* L.*)*, hawthorns *(Crataegus monogyna* L.*)*, horns (*Cornus mas* L.), sweet chestnuts (*Castanea sativa* Mill.) and wild pears (*Pyrus pyraster* L.) (Vasile *et al.*, 2016).

Material and methods

Thomas Saaty has created during the last several decades, an analysis decision model based on multiple criteria. The Analytical Hierarchy Process (AHP) was used in taking decisions in numerous domains (Saaty, 2008). Thus, the same process was used in determining the most important forest fruits from Satu Mare County. Expert Choice Desktop (v. 11.5.1683) was used an analysis software. The following criteria were taken into consideration for the analysis of the main forest fruits from Satu Mare County: harvesting period, harvested quantity by one worker in 8 hours, harvesting cost, harvesting knowledge, tools needed for harvesting, complexity of harvesting process, the development of the harvesting process, recognition knowledge, distribution range, biotic threats, abiotic threats, perishability, market potential, market demand, "celebrity" of the product on the market, the price of raw product, the price of the derived product, portfolio of derived products and transport from the harvesting point to the storage centre. These criteria have also been used in other similar studies from different counties such as: Maramureș (Enescu *et al.*, 2017), Gorj (Vechiu *et al.*, 2018), Prahova (Enescu *et al.*, 2018), Timiș (Enescu *et al.*, 2018) and Bihor (Timiș-Gânsac *et al.*, 2018).

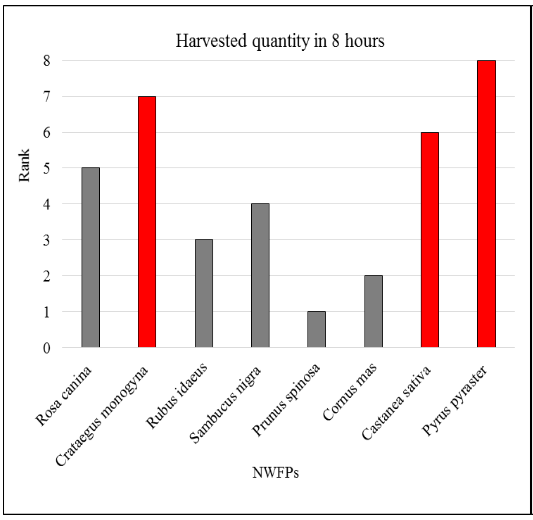
Results and discussion

In the (Table 1.) were presented the 19 criteria taken into consideration for analysing the most important forest fruits from Satu Mare County. The criteria have been approved by specialists.

Table 1. AHP alternative ranking

Criterion		Berries							
		Rosa canina	*Crataegus monogyna*	*Rubus idaeus*	*Sambucus nigra*	*Prunus spinosa*	*Cornus mas*	*Castanea sativa*	*Pyrus piraster*
		1	2	3	4	5	6	7	8
1	Harvesting period	8	5	1	3	7	2	6	4
2	Harvested quantity / worker / 8 hours	5	7	3	4	1	2	6	8
3	Harvesting cost	7	5	1	3	4	2	6	8
4	Knowledge for harvesting	3	5	1	4	6	2	8	7
5	Tools needed for harvesting	5	6	1	4	3	2	8	7
6	Complexity of harvesting process	5	6	1	2	4	3	8	7
7	Development of harvesting process	3	6	1	2	4	5	8	7
8	Knowledge for recognition	2	5	1	6	7	8	4	3
9	Distribution range	8	7	5	4	3	2	1	6
10	Biotic threats	2	1	7	4	3	5	8	6
11	Abiotic threats	2	3	7	5	1	4	8	6
12	Perishability	1	4	8	6	3	5	2	7
13	Market potential	6	5	7	4	1	3	8	2
14	Market demand	7	5	8	3	1	2	6	4
15	"Celebrity" of the product on market	6	2	8	5	1	3	7	4
16	The price of raw product	5	3	7	2	1	6	8	4
17	The price of the derived products	4	2	7	3	1	6	8	5
18	Portfolio of derived products	7	2	8	4	1	6	5	3
19	Transport (harvesting - storage center)	3	1	8	2	4	5	7	6

The harvested quantity of wild pears, hawthorns and sweet chestnuts within 8 hours is larger in comparison with rosehips, black shocks and raspberries (Fig.2). Furthermore, the harvesting cost was bigger in the case of wild pears, rosehips and sweet chestnuts (Fig.3). On the other hand, raspberries, wild pears and black shocks are the most perishable forest fruits (Fig.4).



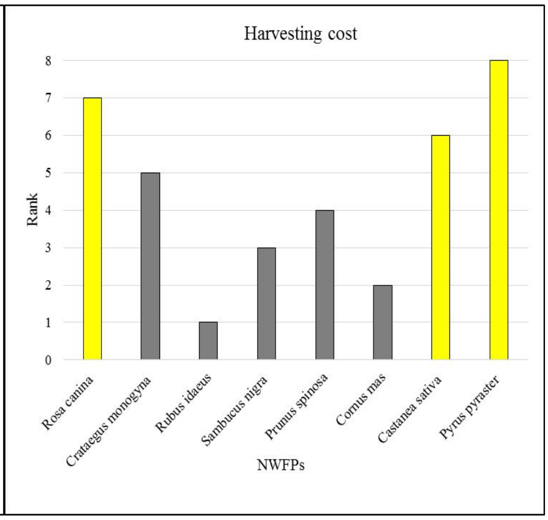


Fig.2 Rank of the harvested quantity in 8 hours Fig.3 Rank of harvesting cost

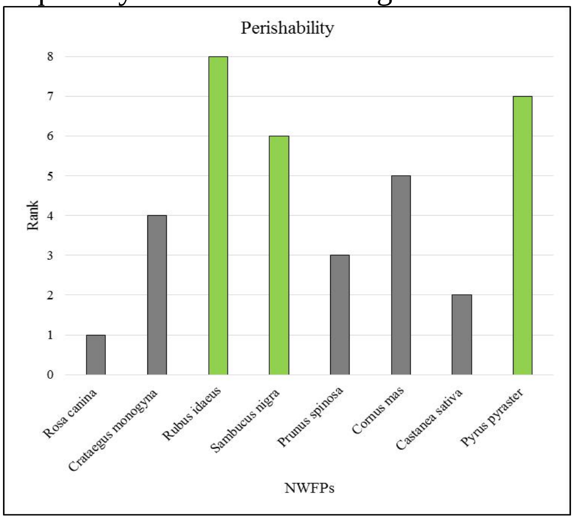


Fig.4 Rank of perishability

The next figure presents the presence of each species through the Expert Choice Desktop software (v. 11.5.1683).

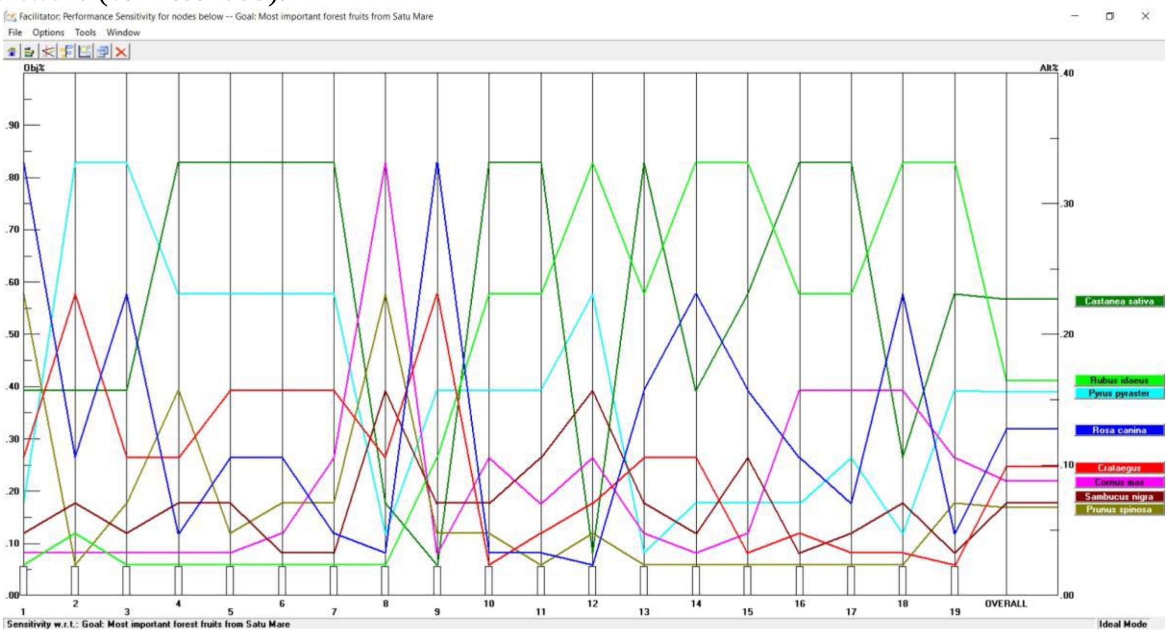


Fig. 5 Ranking of the eight forest fruits

Based on this software program, the most popular and requested forest fruits are sweet chestnuts, raspberries and wild pears (Fig.5). The most important species will be short described in the rows below following their importance in different domains.
Castanea sativa Mill. *(C. vesca* Gaertn.). The food industry uses aproximatevely 7000 t/year of chestnuts in the production of chestnut purée, marron-glacé, etc. The shell as a waste product, which represents ~10% of the weight of whole chestnuts is used as fuel (Vázquez, G. et al., 2008). Wood is of superior quality, comparable to that of oaks. Duramen is very well represented, brown-dark, relatively heavy and hard. It can be used in the furniture industry as well as for parquet, friezes, wainscotings. It is also used for construction (Şofletea and Curtu, 2007). At the same time, chestnut is highly appreciated for its edible, highly nutritious fruit. Contains starch, glucose, sucrose, dextrin, vitamin. By processing, they produce spirits, sugar, oil etc (Şofletea and Curtu, 2007).
Rubus idaeus L. *Rubus idaeus* is part of *Rubus* Genus, subfamily *ROSOIDEAE* FOCKE. By combining gas chromatographymass spectrometry it will be obtain the volatile components of fresh wild raspberries. They were found 75 components, which coresponding to about 64 ppm raspberry oil obtained in the press juice (Honkanen *et al*., 1980).
Fruits are apocarpic, multiple and constitute a globular polidrup, mature red, succulent, sweet. Raspberry is an indicator of rich soil, well supplied with water and nitrogen. He is fructifing abundantly, especially in the light (Şofletea and Curtu, 2007). Fruits are used in the food industry: juices, jams, syrups, etc.
Pyrus pyraster (L.) Burgsd. belong to the plant family Rosaceae. *Pyrus pyraster* does not occur naturally only in the north European countries. Wild pear are autochthonous in several European countries intensive conservation measures are still lacking with the exception of Germany (Turok *et al.*, 1998). The wood is beautifully colored, red-brown, very dense, homogeneous, hard, resistant. It polishes beautifully and is appreciated for sculpture, handicrafts, musical instruments. Fruits are consumed by wild animals. The fruits are obovoidal or globular, stony, dark green to brown, with persistent calyx, astringent (Şofletea and Curtu, 2007).

Conclusions

Satu Mare County has a wide range of forest fruits on its territory. With the help of the analytical hierarchy based on the criteria taken into account, the most representative forest fruits from this region are: rosehips (*Rosa canina* L.*)*, raspberries (*Rubus idaeus* L.), black shock (*Sambucus nigra* L.), blackthorns (*Prunus spinosa* L.*)*, hawthorns *(Crataegus monogyna* L.*)*, horns (*Cornus mas* L.), sweet chestnuts (*Castanea sativa* Mill.), wild pears (*Pyrus pyraster* L.). and others. Based on Expert Choice Desktop software, the third species are the most representative: sweet chestnuts (*Castanea sativa* Mill.), raspberries (*Rubus idaeus* L.) and wild pears (*Pyrus pyraster* L). Forest fruits dispose of necessary conditions for growth and development because of the fact that the region presents different relief forms which comply with the ecological requirements of the species.
Furthermore, it was observed that most species originate from hills and plains, but rarely they are rising also in the montain zone, until altitude reach 700-800 metres, where the climate is warmful and the humidity is lower than 50%. As such, specialists should take into account the areas populated with all these species and to try different methods for conserving and extending them.

Acknowledgements

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APPLICATION OF MULTISPECTRAL SENSOR AND SMALL UNMANNED AERIAL SYSTEMS FOR EARLY DETECTION OF STRESS IN FOREST STANDS OF WESTERN SERBIA

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Abstract

The main goal in the sustainable forestry is to achieve healthy forest stands which will be passed on to the next generations. Healthy ecosystems and economies rely on high-quality forests. Massive forest decline has occurred in many countries in the last several decades caused by high-number of pest infestation induced by draught and climate change. After the trees have been already physiologically stressed, pests come as the secondary infestation. Many forest pests are capable of devastating the entire forests. In the forest health protection, the key of success is to prevent major pests to breed in high number, as well as early detection of symptoms invisible to the naked eye. The object of study chosen for the application of the multispectral sensor mounted on unmanned aerial system (UAS), were coniferous forest stands, located at Mt. Kopaonik, W Serbia. Utilization of the multispectral imagery have provided us with sufficient information on the forest health and vigor. This was the first application of 5-sensor high-resolution multispectral imagery for the identification of stress of forests in Serbia. Through applications of algorithms and vegetation indices, it was possible to identify not only the dead trees in the remote and hard-to-reach areas, but also the trees which were still physiologically active, but possibly threatened by pests, although without any visible signs of health problems. The results of the study proved that the use of the multispectral sensor and small UAV has in early detection of stress for purpose of improving the health of forest ecosystems were justified.

Keywords: *Remote sensing, Forest protection, Early detection, Forest pests, Multispectral camera.*

Introduction

Due to the climate changes, extreme climate conditions, and extended periods of drought, many forest stands have been diagnosed with a decline of health and productivity. As a modern phenomenon, desiccation of mainly autochthonous spruce stands is present in most countries of Central and Southeastern Europe (Tabaković-Tošić, Milosavljević, 2018). Classified as a national park, Mt. Kopaonik, with over 1600 plant species and its valuable coniferous forest was of special interest. It is situated in western Serbia, with the highest peak of 2017 meters above sea level, and the total area of the national park of 121.06 km^2. Mt. Kopaonik has a subarctic climate, with snow season lasting from November to May. In spite of abundant amounts of snow, the latest observations showed many issues with mortality of large areas of native woodland. The physiological stress caused by drought made individual conifer trees and entire areas suitable for pest infestation which furthermore caused devastation of wide areas. Many of those areas on Mt. Kopaonik suffered from both drought and secondary pest infestations.

During 2018. salvage logging was done in the areas identified with total loss of vitality of groups of trees, and in areas infested with two species of bark beetle - *Pityogenes chalcographus* L. and *Ips typographus* L. In total 11,330 m3 of wood was removed.

Another important effect bark-beetle infestation is the production of fuel for wildfires. The changes in foliage chemistry and reduction of moisture increase flammability of the tree and reduces heat requirements for ignition (Jolly *et al.*, 2012).
Use of modern technology would allow precise identification of areas not only already infested, but also the areas prone to the infestation. This information would increase the effectiveness of prevention measures. Two study areas were chosen for this research.
The purpose of the study was testing the combination of small Unmanned Aerial Systems (sUAS) and ground-truthing for early detection and identification of apparently healthy, and physiologically active trees, that may be prone to the secondary infestation. This was the first application of sUAS in combination with 5-band multispectral imagery in forestry in Serbia.

Materials and Methods

Remote sensing approach chosen for study areas consisted of multispectral and RGB sensors mounted on small Unmanned Aerial Systems. The multispectral sensor was MicaSense RedEdge M. This multispectral sensor consists of 5 individual sensors, each capable of taking images in a single discreet narrow band. Medium wavelengths for sensors are: Blue (475nm), Green (560 nm), Red (668), Red Edge (717nm), Near Infrared (840nm). The resolution of each sensor is 1.2 Mpix. The multispectral sensor is also equipped with its own GPS and magnetometer module, and Downward Light Sensor for correction of sensor exposure during the flight.Before and after each flight, the calibration images were taken by a multispectral sensor. Images of calibration panel with predefined spectral values are used during the processing of the images so that sensitivity to different light conditions of reflectance values in the resulting orthomosaics are minimal. All images used from the multispectral sensor were in 16-bit TIFF format. RGB sensor has a standard Bayer pattern sensor for taking images in the visible part of the electromagnetic spectrum. It has a resolution of 20 Mpix, and the sensor was mounted on 3-axis gimbal. Small UAS used as a carrier platform for both sensors was quadcopter DJI Phantom 4 Pro. Both sensors were mounted simultaneously while sUAS was airborne. Flight altitude of first flights was 100m above ground level (AGL), above the point of take-off, while the second area was imaged from 80m AGL. The altitude remained constant throughout the flight – aircraft did not follow terrain change. The first study area was mapped in automatic flight mode via 3rd party professional UAS surveying app Datufly, while the second flight was flown manually, due to the combination of environmental factors – flight altitude, the height of trees, high wind velocity, and area of coverage.
Imaging was done in the middle of the April, and there were patches of snow still present on the ground but canopies were 100% clear and visible. Processing of the images was done in professional photogrammetry software Agisoft Metashape

Rationale

Multispectral imaging has been in use for over 60 years in agriculture and precision agriculture. Reflectance values have been related to vegetation characteristics such as plant biomass or fraction of intercepted photosynthetically active radiation (Barnes, Clarke, Richards, 2000). With recent improvement and development of technologies, the sensors became more compact, and in combination with small UAS, it provided the opportunity to transfer knowledge and technology into other industries. In forest inventory mapping high-resolution multispectral imagery became an essential, and an often critical tool for effective forest management (Moskal *et al*., 2002). Many technologies and innovation transcended from agriculture to forestry. The same vegetation indices, developed for estimation and quantitative evaluation of crop health, are also used in forestry.

Since bark beetles can affect large spots in hard to reach areas, remote sensing is the most promising way to cover large areas, identify the potential infestation, measure it and provide information for future decision making (Stoyanova *et al.*, 2018).
Vegetation indices (VI) are a mathematical algorithm based on digital brightness (spectral) values. Those values are added, subtracted, divided and multiplied to yield a single value that indicates the amount of vigor of vegetation in pixel. High values of VI corresponds with the areas covered by a substantial amount of healthy vegetation (Cambell, Wynne, 2011).
High-resolution multispectral imaging is the latest tool used in forestry to prevent wide area pest infestation and devastation. Five narrow band individual sensors allowed the application of multiple vegetation indices:

- NDVI – Normalized Difference Vegetation Index
- NDRE – Normalized Difference Red Edge

NDVI is the most widely used VI. It represents the band ratio of two spectral behaviors with inverse relationship – absorption of Red light by chlorophyll, and strong reflectance of Near-Infrared light by mesophyll tissue. Physiologically active and grooving plants have high values of NDVI, whereas bare lands, water, snow, man-made objects, and dry vegetation have low index values (Cambell, Wynne, 2011).
NDVI values are calculated through formula:

$$\frac{R_{NIR} - R}{R_{NIR} + R}$$

R_{NIR} – Reflectance values in Near Infrared band
R_{RED} – Reflectance values in Red band
NDRE is the modification of the NDVI which uses Red Edge portion of the electromagnetic spectrum instead of RED light. Due to the longer wavelength, it is capable of penetrating deeper in the canopy, thus providing more relevant information on the health status of a plant, and it is more sensitive to the chlorophyll status than indices relying on RED. Using the information from Red Edge part of the spectrum has the potential to significantly improve forest stress monitoring (Eitel *et al.*, 2011) and NDRE is potentially the best index for forest stand assessment since it is less sensitive to the changes in different study area parameters (Modzelewska *et al.*, 2017)
NDRE values are calculated through formula:

$$\frac{R_{NIR} - R_{EDGE}}{R_{NIR} + R_{EDGE}}$$

R_{NIR} – Reflectance values in Near Infrared band
$R_{RED\ EDGE}$ – Reflectance values in Red Edge band
Both indices have a range of values from -1 to +1. Vegetation index values are represented through color-coded thematic maps for easy visualization. To increase visibility HEAT palette was used with high levels of the index in red color, while low levels were in blue.

Study areas

For this research two study areas were chosen. Before the application of sUAS, both areas were surveyed by personnel of the Institute of forestry, and health status of each area was diagnosed by routing and random sampling methods.
The first study area (management unit Samokovska Reka, forest compartment 114/d) had a total area of approximately 35 ha. It consists of tall spruce trees of various ages. Maximum terrain altitude was 1530 m a.s.l., while the minimum was 1440 meters. The forest was predominantly coniferous, with no, or very little signs of desiccation. Majority of forests were situated in the lower parts of the study area. There was no predefined area of interest, the entire area was imaged with both sensors and multiple vegetation indices we applied.

The second area (management unit Samokovska Reka, forest compartment 95/b) had a total area of approximately 2.3 ha, and altitudes form 1540 – 1570m a.s.l. This area was heavily affected with infestation with a lot of dry and fallen trees, surrounded by apparently healthy vegetation.
Imaging of both study areas took approximately 30 minutes in total.

Results and Discussion

The products of the imaging and processing were dense point cloud, digital elevation model (DEM), and ortho-mosaics. We have produced both RGB (Figure 1) and vegetation indices (Figure 2 and Figure 4) orthomosaics. High resolution of the images provided orthomosaics with a resolution of approx. 3.4 cm/pixel for RGB and 8 cm/pixel for multispectral. This allowed the application of indices not only on tree level but also on parts of the trees.
Mapping of both study areas showed several areas with daed trees which are easily visible even in the RGB orthomosaic. However, the purpose of this research was not the identification of already dead trees, but the identification of the areas that will present the future potential of infestation. Also, the areas of dry trees provided a good starting point for ground-truthing. Especially interesting were areas in the vicinity of dry trees with individuals and groups of trees with medium to low VI values.

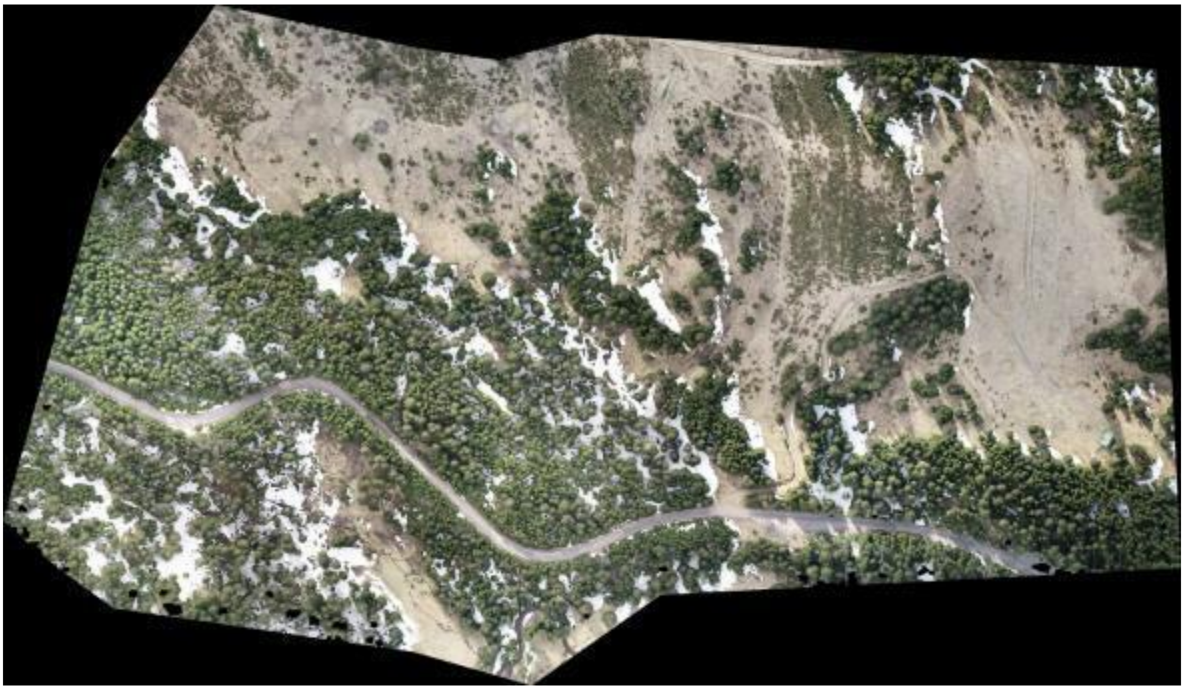

Figure 1. Study area 1 – RGB orthomosaic

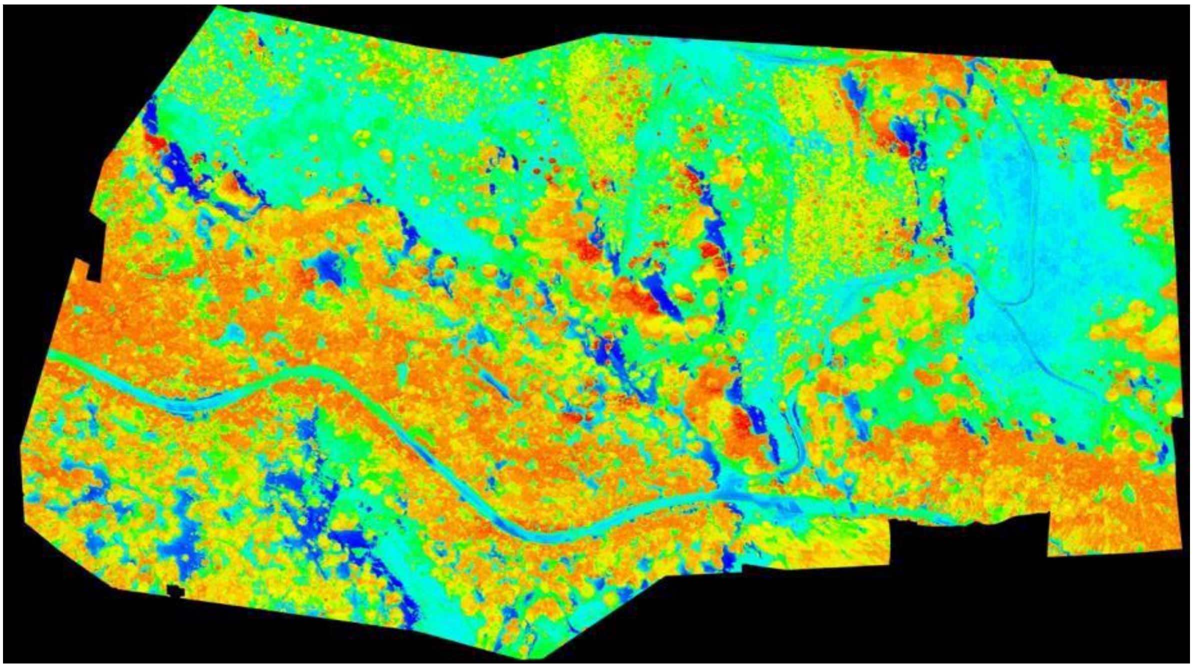

Figure 2. Sturdy area 1 – NDVI index

Application of the NDVI vegetation indices showed several areas with lower index values that in the RGB orthomosaic looked healthy and physiologically active (Figure 3). Aerial imaging also showed exact locations of dead and fallen trees, which have remained in the forest. Terrestrial (*in-situ*) observations confirmed the poor state of the stand with a high number of dying trees, with the presence of fungi, and old entering and exiting holes of bark beetle. To the poor health of the forest has also contributed poor extraction of the dead and fallen trees which could serve for development and rapid multiplication of highly aggressive xylophagous insect pests (Dimitrov *et al.*, 2019).

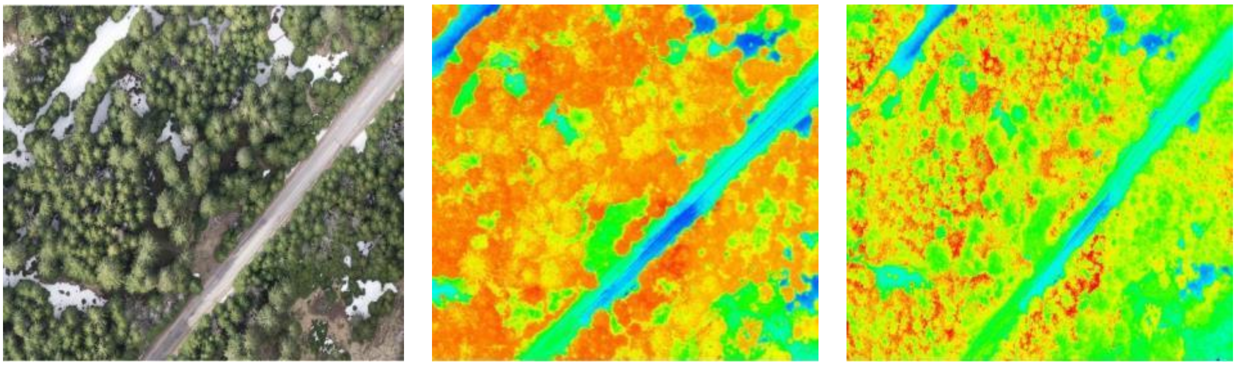

Figure 3. Study area 1 – comparison of same area in RGB, NDVI, and NDRE maps

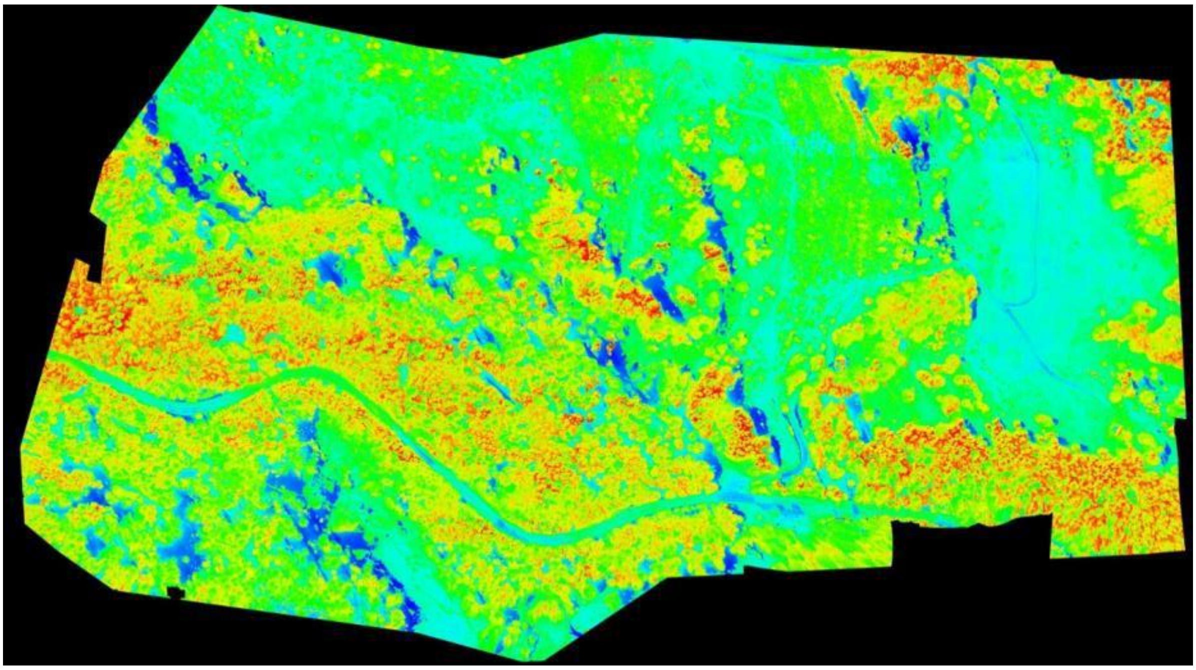

Figure 4. Study area 1 – NDRE index

Application of NDRE vegetation index confirmed NDVI map, but it also showed more dramatic status with more pronounced low levels of the physiological activity of vegetation as shown in Figure 5. Since this was the first imaging early in the spring, another imaging in the summer should add more data to this first map. It should also provide more information on the physiological activity of the trees. NDRE showed many areas and trees with low index values. Regular imaging and application of NDRE index have the potential for providing relevant information on the health status of the forest throughout the year. Especially since indices relying on NIR and RED tends to saturate at medium to fully developed canopies (Xie Q *et al*., 2018).

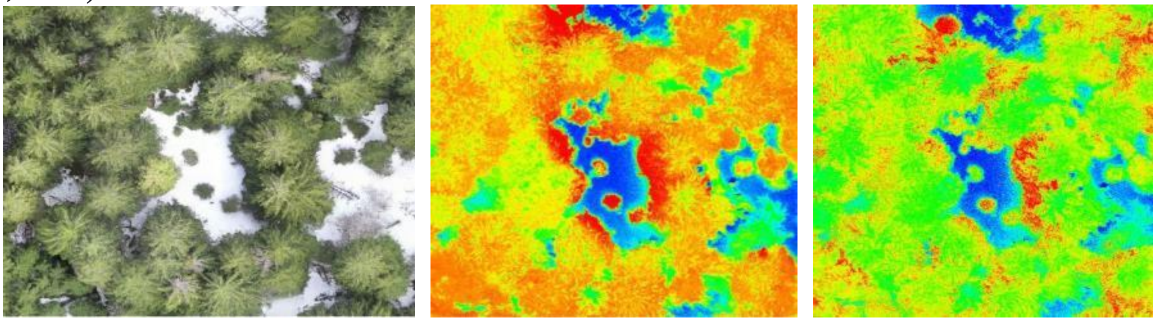

Figure 5. Part of study area 2 – from left to right - RGB, NDVI, NDRE

Conclusion

Use of small unmanned aerial vehicles equipped with RGB and multispectral sensors are a fast and easy tool for acquiring the high amount of precise information which can be used for assessment of health status of vegetation, identification, and geolocation of the problematic areas. This kind of imaging can be used as a foundation for further land cover classification for field-work and forest care planning. This kind of high-resolution imaging provided high levels of details, in 2D and 3D color-coded multispectral vegetation indices. All processing and assessment were performed on a pixel level. Future exploration of this technology would require a comparison of these results with object-based classification, alongside further and continuous ground-truthing to provide the most reliable information.

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PARASITOID COMPLEX OF *BRUCHIDIUS TERRENUS* IN BIOCONTROL OF FABACEAE HOST PLANTS AND ITS SPERMATOPHAOGOUS ENTOMOFAUNA

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Abstract

Bruchidius terrenus (Sharp) Coleoptera: Bruchinae, is suppressor of generically potencial of introduced leguminous *Albizia julibrissin*, Durazz in significant number as a seed beetle. So far this weevil has been proven as a monophagous species in Serbia. The name of the species in the Serbian language, according usually for taxonomic affiliation and the host plant, is mentioned for the first time in our research as a Persian silk tree, or pink silk tree seed beetle. Adults were first found in 2013, in Ruma town in Autonomous Province of Vojvodina (Serbia) with the observation of insects wintering in adult stage, waiting for the silk tree flourishes from June to August. The pods are ripe from September to November. In 2014, the young adults start emergence of ripe pods (this is the latest stage of ripening) in late summer. These Bruchine can be considered as successfully adapted, and with regard to the host, i.e. with high potentional bioagent agenst host plant. The data on the percentage of infestation for the season 2013/14, varied from the dissection of pods and the maximum was 88 %. In the same period, parasitoids appear for the first time.

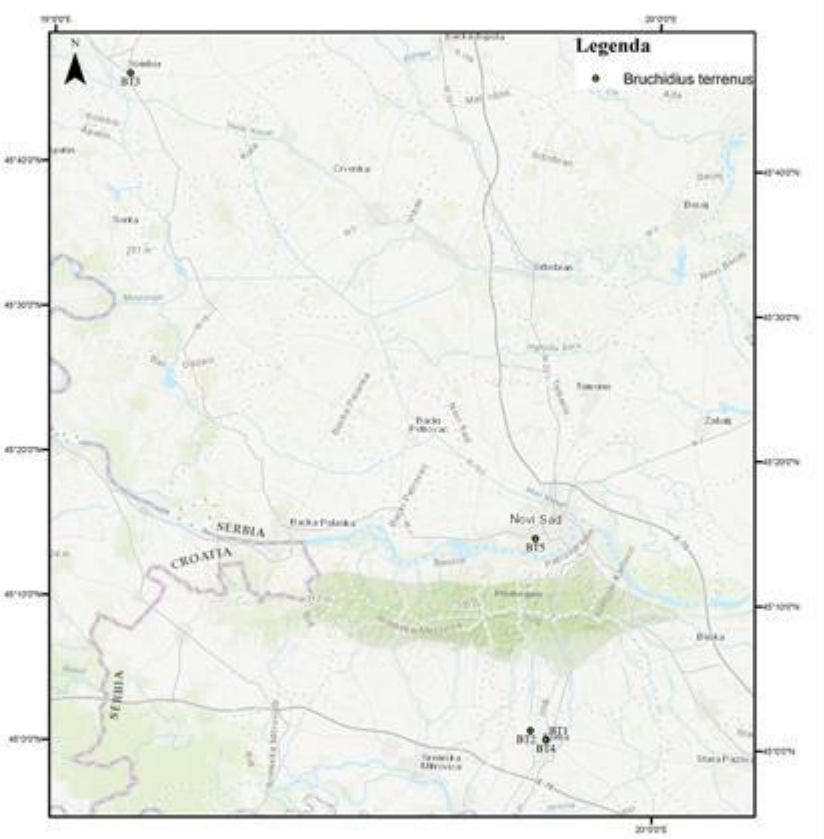
Key words: *parasitoids; Albizia; Bruchidius; Chalcidoidea; Serbia.*

Introduction

The genera Bruchidius (Coleoptera: Chrysomelidae: Bruchinae), which is described by Schilsky in 1905, is distributed in the old world and includes about 300 seedbeeetle species (Hoebeke at al, 2009). Bruchine are economically important as pests of agricultural storage products; for example (Ebadollahi et al., 2013; Loni & Panahi, 2015). Larvae of Bruchidius species for host plants have leguminous plants, family (Fabaceae). *Albizia julibrissin* (Fabales: Fabaceae) was introduced in Serbia, and planted as ornamental trees (Vukićević, 1996, Jovanović, 2000). During the research since 2012 in Serbia, the species *Bruchidius terrenus* (Sharp), which are parasitized by *A. julibrissin* seeds, and their parasitoids (Gagić-Serdar et al., 2012; Gagić-Serdar et al., 2013) had been recorded for the first time.

Materials and methods

The collected Populations (Figure 1) were cultivated under controlled conditions in order to obtain data on the length of each development stage. In addition to the development stages of seedbeetles, development stages of their parasitoids have also been recorded, which are further grown in plastic boxes with mesh and petri dish for the purpose of determination. The discovery of randomly selected pods revealed seed infestation according to Tuda's methodology and associates (2001). Parasitoids are determined by using interactive act keys like De Graham (1969); Trâpicéyn (1989); Medvedev (1978) & Bouček(1991) by prof.dr Ljubodrag Mihajlović.



Picture1. Collecting sites, host plant with live Bruchinae material

Table 2. Locations of collection of pods from which the obtained insects *Bruchidius terrenus* appeared

Seed pests BRUCHINAE	Host plant	List of sites, Date of collection of materials for each season
Bruchidius terrenus (Sharp)/ Persian silk tree	*Albizia jullibrisin* (Durazz., 1772. non sensu Baker, 1876)	1.Рума, Детелине –6th August , 2012
		2.Ruma, Кудош – 10th. August , 2013
		3.Sombor –04th October 2014
		4.Ruma, Deteline, - 05th October 2014.
		5.Novi Sad, Somborski bulevar- 11th November2014

Source: Author s' elaboration based on the obtained results.

Results and discussion

Since start of research many number of species of parasitic wasps had been determined and registered as autohtonous fauna; thez were determined as the natural enemies of granivorous *Bruchidius terrenus* (Sharp). The parasitod complex consist primary of the *Dinarmus acutus* (Hymenoptera: Chalcidoidea: Pteromalidae) and an entirely new species of Eurytomidae (Hymenoptera: Chalcidoidea). The adaptation process is still ongoing; parasitoids are indigenous species, trying even to be synchronous with development stages of Bruhinae. *D. acutus*, which is fed inside the pods, is fed in the larvae within the originally infested healthy generative material of the silk tree, and is considered to be solitary ectoparasitoid. Nevertheless, they were found by numerous species of Eulophidae (Hymenoptera: Chalcidoidea) as the base, as parasitoids of the second or third order-hyperparasitoids: Eulophid generas *Aprostocetus* and *Tetrastichus*. Nothing less important for the abundance of germination of seeds is also mentioned parasitoids that have about 15-20% of the total number of infested seeds. Information on the, biology, identity, phenology, distribution and impact of silk tree key parasitoid species in Serbia still need to be investigated in the future.

Figure 1. Beetle *Bruchidius terrenus* after ecclosion, 2017, site Ruma (Original)

Figure 2. Darker appearance of the adult elitrae cover of *Bruchidius terrenus*, site Novi Sad, 2014. (Original)

Figure 3 and Figure 4, parasitic, wasps fam. Eurytomidae (Hymenoptera: Chalcidoidea; Eurytomidae), by Albicija weevil (Original)

Figure 5 and Figure 6. Parasitic wasp family (Hymenoptera: Chalcidoidea: Eupelmidae), *Eupelmus vesicularis*, Albicija’s Weevil (Original)

Figure 7 and Figure 8.
Parasitic wasps family Pteromalide, *Dinarmus acutus* (Hymenoptera: Chalcidoidea: Pteromalidae) Albicija’s weevil(Original)

Figure 9.
Detail of the wing of the Dinarmus acutus parasitoid of
Bruchidius terrenus Albicija's
Weevil (Original.)

Figure 10 Adults of *Bruchidius terrenus* Albicija's
weevil, flew out of the legume pods (seeds) of *Albizia jullibrisin*
collected in August 2014; Ruma Deteline site, parasitoids, and details in
appearance compared of adults and green pods with damage and
emerging wholes (Original)

Conclusions

Parasitoids that are shown and photographed in this paper, the first time were recorded and found as *Albizia jullibrisin* weevil parasitoids and they are indigenous species that have adapted to non-native host (host plant as nonnative seed beetle). That's opens many question about their bionomy and ecology which is in a adaptive, denizen state.

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EFFECT OF DROUGHT ON GROWTH AND MULTIPLICATION OF WHITE POPLAR GENOTYPES IN TISSUE CULTURE

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Abstract

The aim of this study was to examine the response of white poplar genotypes on drought stress based on six morphometric, survival and biomass parameters. The microshoots of five genotypes (Villafranca, L-12, L-80, LBM and LCM) were cultivated on media with two different concentrations of polyethylene glycol (PEG) 6000: 20 g/L and 50 g/L, as well as on control treatment without PEG. The media were based on ACM (Aspen Culture Medium) with 9 gL^{-1} agar, 20 gL^{-1}sucrose, 1µM kinetin, 1µM benzylaminopurine (BAP) and 100 mg L^{-1} myoinositol. Results of two-way ANOVA and LSD tests suggested that the effect of media was significant for all examined traits, with clear increase in inhibitory effect by the increase in concentration of PEG. The effect of genotype was significant for number of new shoots and fresh and dry shoot mass. The effect of interaction genotype × medium was significant only for number of new shoots. The difference between genotypes was the highest on medium with the highest PEG concentration (50 g/L). This medium was suggested for further drought studies in white poplar *in vitro* including drought tolerance evaluation. The best performance was achieved with genotype L-80, and the worst with Villafranca. The study suggests significant potential of tissue culture in the research of drought tolerance in controlled conditions.

Keywords: *Populus alba, micropropagation, drought tolerance.*

Introduction

Drought is one of the principal abiotic factors that limit agricultural production. It is estimated that it globally decreases yield by 17% (Ashraf and Foolad, 2007). In wood production, the losses caused by drought are increasing. Evaluation of tolerance to abiotic factors in plants is complex process due to interactions between stress factors and physiological and biochemical processes that influence plants' growth and development (Razmjoo et al., 2008). Many researchers suggest that the basic approach to the mitigation of climate changes should be breeding and cultivation of tolerant cultivars, particularly of forest tree species tolerant to drought. In this process, the knowledge of basic mechanisms of plants' tolerance, especially of forest tree species to drought, is of crucial importance. Rubio et al., (2002) emphasize that effects typical to drought could be found as a result of influence of other abiotic factors, such as stresses caused by salt, high and low temperature. Research on tolerance of abiotic factors *in vitro* has many advantages, considering relatively fast procedures methodology, high number of repetitions in controlled conditions, etc. (Cui et al., 2010; Kovačević et al., 2013a; Kovačević et al., 2013b, Vuksanović, et al., 2019).

In spite of its high adaptability, white poplar is considered endangered species in Serbia, which gives special importance to its use in preservation and improvement of biodiversity in general (Kovačević et al., 2010a). Beside the establishment of plantations dedicated to wood production (Rédei et al., 2013), this species is used in horticulture and landscape architecture as well, especially genotypes with pyramidal crown form (Kovačević et al., 2010b). White

poplar has important role in biotechnological research, where it is considered as model tree species (Confalonieri et al., 2000). Generally, poplars are good candidates for such research considering the fact that the genome of *Populus trichocarpa* has been completely sequenced (Tuskan et al., 2006).
The main aim of this study was to evaluate *in vitro* tolerance of white poplar genotypes to PEG-6000 induced drought conditions, in order to find tolerant genotype which could be used in horticulture, landscape architecture and wood production.

Material and methods

Five genotypes of white poplar (*Populus alba* L.) were used in this study: experimental Serbian genotypes: L-80, L-12, LCM and LBM and well-known Italian genotype *cl.* Villafranca, regarded as control genotype. Examined group of genotypes genotypes are characterized by vigorous growth in tissue culture and genetic diversity (Guzina and Tomović, 1989; Kovačević et al., 2013b). Shoots 10-15 mm high were cultivated 35 days on modified Aspen Culture Medium (Ahuja, 1984) for multiplication with added 9 g/L agar, 20 g/L sucrose, 1μM kinetin, 1μM BAP and 100 mg myo-inositol. Effect of two concentrations of polyethylene glycol (PEG) 6000: 20 g/L and 50 g/L added in multiplication medium were examined, while medium with no PEG was used as Control. Sterilization was performed by autoclaving. Cultures were cultivated in jars 120 mL in volume, with 25 mL on medium in each, on temperature t = 26 ± 2°C, white light provided by LED lamps with 3500 lx/m^2 in 16h/8h day/night regime. After 35 days of *in vitro* cultivation following parameters were measured: length of the longest shoot, number of new shoots, survival rate, multiplication rate, fresh and dry shoot mass.

Statistical analysis

The significance of the influence of examined sources of variation and differences between treatments were tested by two-way ANOVA and Fisher's LSD test at the level of α=0.05. All statistical procedures were performed by Statistica for Windows version 13 (TIBCO Software Inc., 2017).

Results and discussion

After testing of applied PEG concentrations, results of analysis of variance showed significant effect of media on all examined parameters. Effect of genotypes was statistically significant for number of new shoots, fresh and dry mass, while effect of interaction genotype × medium was significant only for number of new shoots (Table 1.).
Drought caused by polyethylene glycol had significant inhibitory effect on all examined parameters. According to the totals for genotypes, best general performance achieved genotype L-80, especially according to the results for fresh and dry shoot mass. (Table 2.).The poorest results had genotypes LCM and Villafranca. Generally, plants on medium with 50 g/L PEG achieved 20% lower shoot height, 37% less new shoots, 30% lower multiplication rate and 33% less fresh shoot mass comparing to the Control.
Results of LSD-test confirmed significant effect of tested PEG concentrations on examined morphological and biomass parameters (Table 3.). The shoot height on the highest examined concentration of PEG (50 g/L) varied from 10.6 mm (L-12) to 14.13 mm (LCM). The highest survival rate on 50 g/L PEG achieved L-80, and the lowest L-12. The lowest multiplication rate achieved Villafranca. The highest fresh and dry shoot mass per jar on medium with 50 g/L PEG achieved L-80. No significant differences between genotypes on this medium were found for number of new shoots.

Table 1. The results of two-way analysis of variance for examined white poplar genotypes and media [a)]

Character	F-test		
	Genotype (A)	*Medium (B)*	*Interaction A × B*
Shoot height (mm)	1.10	26.24 ** b)	2.15
Shoot number	3.54 *	94.78 **	5.47 **
Percentage of survival	0.76	4.13 *	0.53
Percentage of multiplication	0.97	40.55 **	2.03
Shoot fresh mass (g)	9.79 **	5.26 *	1.08
Shoot dry mass (g)	15.05 **	8.44 **	1.38

Legend:[a)] *Degrees of freedom for genotype was DF_A = 4, degrees of freedom for medium DF_B = 2, degrees of freedom for interaction genotype × medium $DF_{A\times B}$ = 8, degrees of freedom for error DF_{ERR} = 30 and degrees of freedom for total DF_T = 44.*
[b)] *Labels for F-test: * - significant at the level α=0,05; ** - significant at the level α=0,01*

Table 2. LSD test for examined parameters on tested genotypes and media.

	Shoot height (mm)	Shoot number	Survival rate (%)	Multiplication rate (%)	Shoot fresh mass (g)	Shoot dry mass (g)
			Genotype			
L-12	15.02 a	1.18 a	98.94 a	98.38 a	0.30 a	0.04 b
L-80	14.71 a	1.19 a	100.00 a	95.99 a	0.31 a	0.05 a
LBM	14.23 a	1.13 a	99.73 a	94.98 a	0.21 b	0.03 b
LCM	15.54 a	1.00 b	98.38 a	91.77 a	0.15 b	0.02 c
Villafranca	16.00 a	1.14 a	99.73 a	93.30 a	0.15 b	0.02 c
			c (PEG-6000) (g/L)			
0	17.92 a	1.42 a	100.00 a	100.00 a	0.27 a	0.04 a
20	14.63 b	1.01 b	99.90 a	99.31 a	0.22 ab	0.03 a
50	12.75 c	0.89 c	97.16 b	69.83 b	0.18 b	0.03 b

a) The differences among values of particular characteristic marked with the same letter are not significant at the level of α0.05.

Results of analysis of variance and LSD-test for examined parameters suggest decrease of shoot height with the increment of PEG-6000 concentration in medium in all examined genotypes, which is in concordance with recent studies (Lei et al., 2006; Yin et al., 2005; Yang and Miao, 2010). Achieved results also present difference between examined genotypes in their response to the drought caused by PEG, with the strongest inhibitory effect of 50 g/L PEG concentration. Also, the most evident differences between examined genotypes have been found on 50 g/L PEG-a, which purposed this medium for further drought tolerance research.

The smallest decrement of shoot height on medium with 50 g/L PEG was found for genotype L-80. Also, this genotype achieved 100% survival on the driest examined medium. The strongest inhibitory effect on biomass parameters of this medium was found in genotype Villafranca, and the weakest for L-80. Studies on drought tolerance *in vitro* was performed also by Abdulmalik et al., (2018). They examined drought tolerance of peanut cultivars on modified MS media with different PEG-6000 concentrations (0, 20, 40 and 60 g/L). They

found decrement of examined morphometric parameters (shoot height, root length, number of shoot and roots per plant) with the increment of PEG-6000 concentration.
After study of drought tolerance on media with 20, 40 and 60 g/L PEG after one month long cultivation *in vitro*, Manoj and Uday, (2007) observed that root length, shoot height, as well as shoot and root mass decreased with increment of PEG concentration in medium, with stronger effect of drought on root length then on shoot height.

Table 3. LSD test for examined parameters at the level of interaction genotype × medium.

Genotype	c (PEG) (g/L)	Shoot height (mm)	Shoot number	Percentage of survival	Percentage of multiplication	Shoot fresh mass (g)	Shoot dry mass (g)
L-12	**0**	20.00 [*)a]	1.51 [a]	100.00 [a]	100.00 [a]	0.37 [a]	0.04 [ab]
L-80	**0**	15.80 [bcde]	1.56 [a]	100.00 [a]	100.00 [a]	0.33 [a]	0.05 [a]
LBM	**0**	18.67 [ab]	1.49 [a]	100.00 [a]	100.00 [a]	0.33 [a]	0.05 [a]
LCM	**0**	17.53 [abc]	1.03 [b]	100.00 [a]	100.00 [a]	0.14 [bc]	0.02 [cde]
Villafranca	**0**	17.60 [abc]	1.46 [a]	100.00 [a]	100.00 [a]	0.17 [bc]	0.02 [cde]
L-12	**20**	14.47 [cde]	1.06 [b]	100.00 [a]	100.00 [a]	0.31 [a]	0.04 [abc]
L-80	**20**	14.40 [cdef]	0.99 [bc]	100.00 [a]	100.00 [a]	0.34 [a]	0.05 [a]
LBM	**20**	12.87 [efg]	0.93 [bc]	100.00 [a]	100.00 [a]	0.16 [bc]	0.03 [cd]
LCM	**20**	14.95 [cde]	0.99 [bc]	97.63 [ab]	83.64 [b]	0.14 [bc]	0.02 [de]
Villafranca	**20**	16.47 [bcd]	1.06 [b]	100.00 [a]	100.00 [a]	0.17 [bc]	0.02 [de]
L-12	**50**	10.60 [g]	0.86 [bc]	90.75 [b]	86.05 [b]	0.24 [ab]	0.03 [bcd]
L-80	**50**	13.93 [def]	0.94 [bc]	100.00 [a]	67.70 [bc]	0.25 [ab]	0.04 [ab]
LBM	**50**	11.17 [fg]	0.86 [bc]	97.63 [ab]	60.64 [bc]	0.13 [bc]	0.02 [de]
LCM	**50**	14.13 [def]	0.98 [bc]	94.88 [ab]	80.57 [b]	0.16 [bc]	0.02 [de]
Villafranca	**50**	13.93 [def]	0.79 [c]	97.63 [ab]	50.00 [c]	0.10 [c]	0.01 [e]

**) The differences among values of particular characteristic marked with the same letter are not significant at the level of α=0.05*

Conclusions

According to the results of our study, genotype L-80 achieved good shoot growth, high survival rate, high fresh and dry mass accumulation, and in all these parameters it exceeded Villafranca, that was considered as standard genotype in this study. That is why L-80 could be suggested as the most tolerant genotype within examined group of genotypes for cultivation on habitats burdened with drought. Results of *in vitro* tests in this work suggest that the examined parameters could be indicative for the selection of white poplar genotypes for drought tolerance. Thus, further research of drought tolerance should be directed to examination of larger group of genotypes and species by implementation of same or similar methodology.

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CURRENT STATUS AND POTENTIAL OF SOME IMPORTANT SPECIES OF MANGROVE FOREST IN KIEN GIANG AND CA MAU PROVINCES, VIET NAM

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Abstract

Identifying the component and distribution of some important species of mangrove forest in Kien Giang and Ca Mau will be useful for the strategy of mangrove forest protection development. After a preliminary survey of the entire forest, random measurements of 3 plots were used to calculate the coefficient of variation as a basis for calculating the sample size needed to conduct forest surveys. The area is required for the study is 1,666 ha, with a 95% confidence level and 10% error, each plot needs 100m^2. The results of survey show that natural forests and coastal forests (apart from shrimp farms) are the main protective forest belt of the coastal line, so there are only 17 species belonging to 11 families of plants including 14 species woody plants, three shrub species, in which the *Rhizophora* (*Rhizophora apiculata*-Duoc) and *Avicennia* (*Avicennia* sp. -Mam) families are dominate. Regions can be grouped into four sub-regions depending on number of species as well as component of each species. At the similarity level of 40% of the plots, it can be divided into 5 groups. The analysis results show as follows: Group 1 has dominant species like Euphorbiaceae (*Excoecaria agallocha*-Gia), Group 2 dominant species is White *Avicennia* (*Avicennia alba* -Mam trang), Group 3 has 4 dominant species: White *Avocado*, *Avicennia*, *Sonneratia* (*Sonneratia* sp.), *Rhizophora*. Group 5 has 2 dominant species as Black *Avicennia* (*Avicennia officinalis*- Mam den) and *Rhizophora* (*Rhizophora apiculata*-Duoc). In conclusions, Mangroves and Black *Avicennia*, it should be considered for developing and protecting mangrove forest.

Keywords: *Avicennia (Mam), Some imforttant species, Mangrove forest, Myrsinaceae (Su), Rhizophoraceae (Vet, Duoc),*

Introduction

Western mangroves in the Mekong Delta, mostly located in Kien Giang province and part of Ca Mau province borders An Minh district of Kien Giang; from An Minh to Ca Mau due to erosion of the sea, the area of natural forest in this area is severely damaged, so the area of natural mangrove forest is mostly in Kien Giang province. Vietnam has 3,260 km from Mong Cai, Quang Ninh province to Dat Mui, Ca Mau province. With a long coastline, many regions and regions have different natural conditions, so the distribution of mangrove species is also different. Particularly, mangrove forests in the west of Kien Giang and Ca Mau have more than 308 km from the Cambodian border to the boundary of Ca Mau Cape. Mangroves are typical ecosystem in the estuary of tropical and subtropical coastal areas, with rich and diverse biological resources, which are the boundary between land and sea, so it plays a particularly important role in the protection of sea dykes, fixed sediment encroachment on the sea, limiting the harms of storms, protecting accretion, preventing coastal erosion, limiting saltwater intrusion, protecting ecological environment.

The Western Sea region of Kien Giang province including An Minh, An Bien and Rach Gia city, Hon Dat, Kien Luong and Ha Tien town have more than 208 km of coastline, of which about 170 km of mangrove forest distribution with a total area of forests and coastal protective forest land of 8,365 ha (Kien Giang Department of Agriculture and Rural Development, 2012). In recent years, due to various reasons, the area of mangrove forests has been seriously destroyed, affecting production and people's lives in coastal areas. According to data from the Ministry of Agriculture and Rural Development, the area of mangrove forests in the whole country in 1943 was 408,500 hectares, until 1982 only 252,000 hectares (General Department of Forestry, 2010).

The surface water environment of the coastal area is affected by many factors such as extensive and intensive shrimp farming areas, residential areas and aquatic processing facilities along the region. The rule of delta formation is due to the accumulation of accreted sediments, the mudflats gradually rise and rise, at low tide the ground is exposed. These are conditions for suitable species to spread seeds to grow, then stabilize and develop into a forest related to a group of Mam species (*Avicennia* sp). When the population of Avicennia forest has formed and the mudflats are stable, other species such as Su and Vet ... will follow to continue strengthening soil, until the mud soil has stabilized. For those reasons, the topic: "Identification of the component and distribution of some important species of mangrove forest in Kien Giang and Ca Mau" was carried out to determine the composition and distribution of some important species of mangrove forests in Kien Giang and Ca Mau provinces, from that strategy for protecting mangrove forest will be done in future.

Material and Methods

Descriptives of Research place

Kien Giang and Ca Mau are two provinces in the Mekong delta, located from 101^0 30' to 105^0 32' Eastern Longitude and from 9^0 23' to 10^0 32' Northern Latitude. This area is shown in Figure 1.

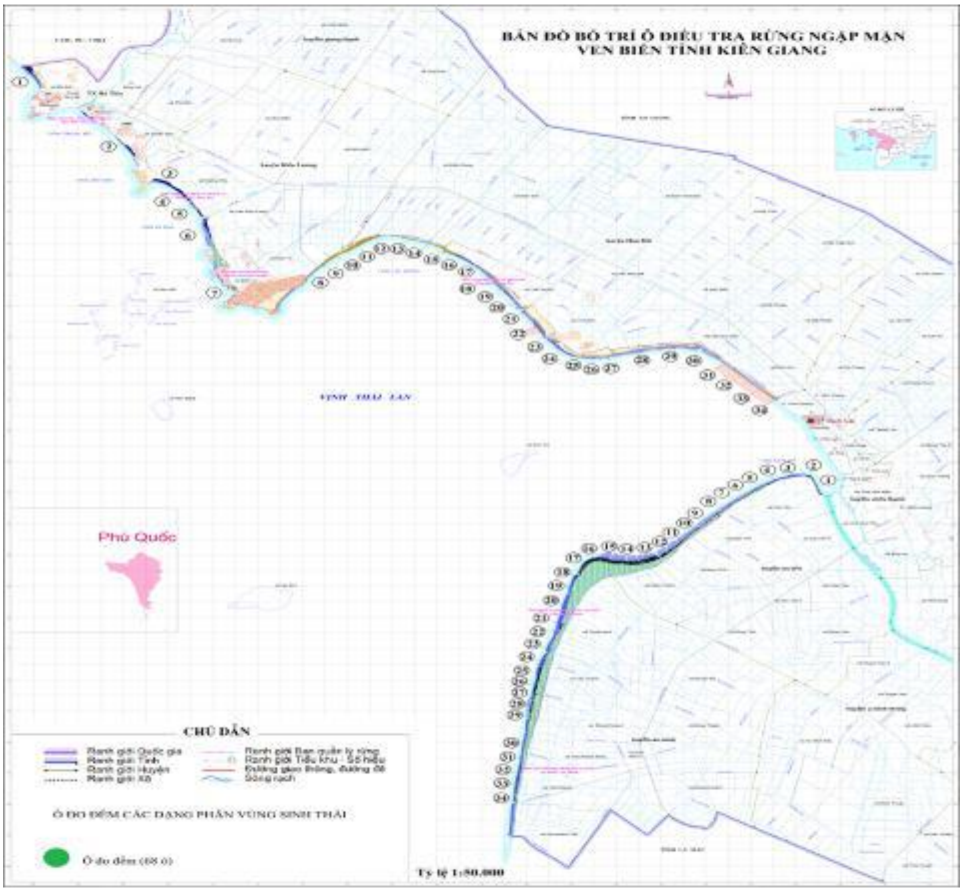

Figure 1. Map of plot arrangement measuring mangrove forest in the study area.

Survey methods

- After a preliminary survey of the entire forest, random measurements of 3 plots were used to calculate the coefficient of variation.
- Depending on the purpose of the investigation, the accuracy is controlled differently. In the given agro-forestry research, the reliability is usually equal to 95% and 10% of the given error. The number of sample plots required for investigation ensures reliability is calculated according to the formula (Nguyen Ngoc Binh, 2006):

$$n = \frac{4N(S\%)^2}{N(\Delta\%)^2 + 4a(S\%)^2}$$

where: n: Number of plots N: Total capacity (N=f/a)

a: Area of sample plot F: Area of survey region

Δ%: Preset Error (10%)

S%: Coefficient of Variation; S% = S/ $\overline{x}$*100

where: S: Sample Standard Error

$\overline{x}$: Sample average

Applying the above method to calculate the number of sample plots to be investigated, it is necessary to first investigate 3 sample plots to calculate the coefficient of variation on trunk diameter at 1.3 m (D1,3) of sample plots. Area of each plot is 10m x 10m = 100m^2 due to typical determination, so the distance determined based on the forest distribution surveyed and the previous coordinates determined, determine the relevant values to calculate, the results are as follows:

Average = 6,73787 Stdev = 4,787777

S = 0,2108 S% = 21,08%

$$N = \frac{F}{a} = \frac{\textit{Area of survey region}}{\textit{Sample plot Area}} = \frac{1.666ha}{100m2} = \frac{16.660.000m2}{100m2} = 166.600$$

$$N = \frac{4N(S\%)^2}{N(\Delta)(\Delta\%)^2 + 4a(S\%)^2} = \frac{4*166600*444.37}{166600*100+4*100*444.37} = 8,8$$

Based on the West Sea mangrove current status map of Kien Giang and Ca Mau provinces, survey lines were arranged from the mainland to the sea so that the plot was located at the center of the forest belt. The points and survey plots were defined in advance coordinates on the digital map as a basis for determining the field location. The total number of plots was 68 plots on 2 routes, each of which has 34 plots shown in Figure 1.

- Characters for measuring: Determining tree species, trunk diameter at breast height (D1,3), peak height (Hvn), average diameter of tree canopy (Dt) perpendicular 2 toward the East-West and South-North, deduce the canopy section (Gt) by the formula:

$$Gt = (\frac{D}{2})^2 \times \pi$$

Growth level: Quality classification of standing trees (He) is the growth level of trees according to a scale of 5 (1 point is dead tree; 2 top tops; 3 normal growth plants; 4 good growth trees of copper 5 plants with strong growth, outstanding height, straight body, high branches).

Data analysis methods

Microsoft Excel 2013 was used to analyse data and graph. PRIMER 6 software was used to analyse species similarities: Variables where standardized by Square root method, then set the same matrix according to Bray-Curtis method and draw branch diagrams by group average (Group average) to consider at similar levels. SPSS version 10.0 software was used to process data, using the ANOVA method and Duncan test at the 5% significance level to compare the differences among the study areas.

Results and Discussion

Components of plant species

The results of the mangrove forest survey show that natural forests and coastal forests (apart from shrimp farms) are the main protective forest belt of the coastal line, so there are only 17 species belonging to 11 families of plants including 14 species of woody plants, three species of shrubs, in which the Rhizophora (Duoc) and Avicennia (Mam) families are dominate. Details of plants and species of mangrove forest are shown in Table 1.

Table 1. Plant species component of mangrove forest in Kien Giang province.

No.	Families	Scientific name	Local name
1	Myrsinaceae	*Aegiceras corniculatum*	Su
2	Avicenniaceae	*Avicennia alba*	Mam trang
3	Avicenniaceae	*Avicennia marina*	Mam bien
4	Avicenniaceae	*Avicennia officinalis*	Mam den
5	Rhizophoraceae	*Bruguiera cylindrical*	Vet tru
6	Rhizophoraceae	*Bruguiera gymnorhiza*	Vet du
7	Euphorbiaceae	*Excoecaria agallocha*	Gia
8	Combretaceae	*Lumnitzera littorea*	Coc do
9	Combretaceae	*Lumnitzera racemosa*	Coc trang
10	Areecaceae	*Nypa fruticans*	Dua nuoc
11	Rhizophoraceae	*Rhizophora apiculata*	Duoc
12	Rhizophoraceae	*Rhizophora mucronata*	Dung
13	Sonneratiaceae	*Sonneratia caseolaris*	Ban chua
14	Sonneratiaceae	*Sonneratia ovate*	Ban oi
15	Annonaceae	*Annona reticulate*	Binh bat
16	Verbenaceae	*Clerodendro inerme*	Chum gong (Ngoc nu bien)
17	Malvaceae	*Hibiscus tiliaceus*	Tra

Research area depending on ecological conditions can be divided in four sub-regions that are shown in Figure 2.

Growth characteristics of mangrove species in sub-region 1

In sub-region 1, there are 7 species with density of 6,100 trees/ha. Species with number of dominant trees are Black Avicennia, accounting for 45%; The dominant species in the high tier is Ban oi, which accounts for a low proportion in the community of 11.75%; *Rhizophora* is the species with the second high ratio with the density of 32.5%; *Avicennia* (Mam trang) has a low ratio of 4.9%; Vet du, though accounting for a low rate of 3%; Gia is only 1.9%.

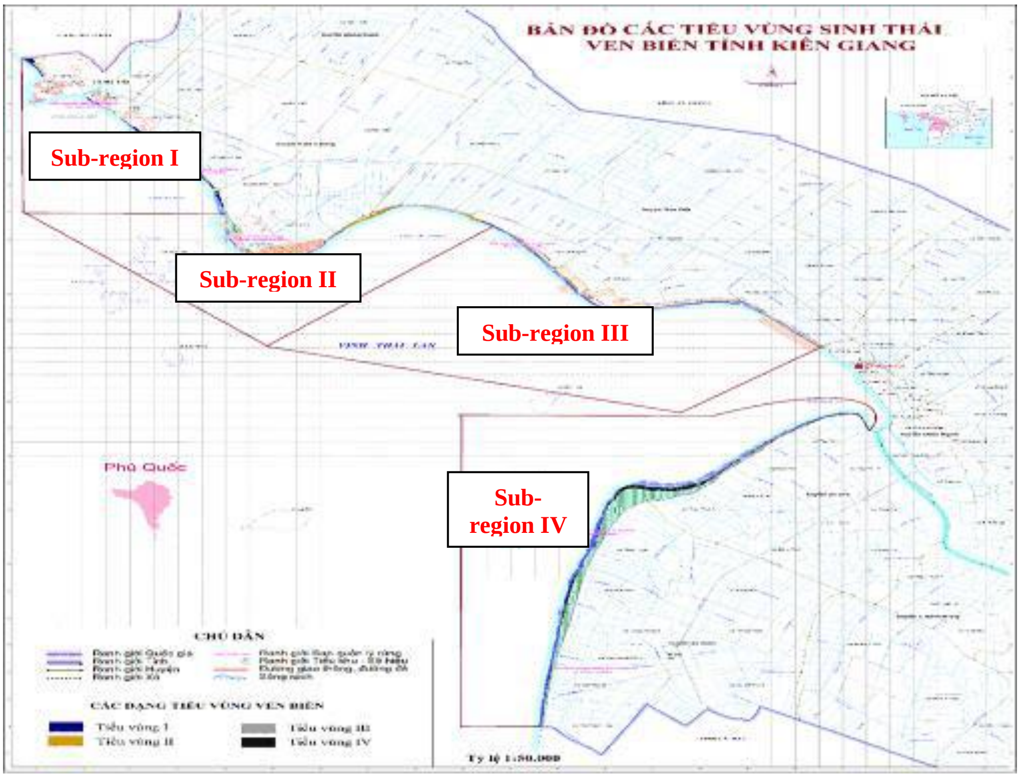

Figure 2. Four sub-regions represent for different ecological conditions in study area.

Growth characteristics of mangrove species in sub-region 2

Sub-region 2 has 12 species, expressing biodiversity here. It is higher than other sub-regions; for example, species composition is high, an endemic species is known as Coc do species. This species is being conservation of genetic resources. Besides, the density of forest trees here is quite high, 4,100 trees/ha; regarding density structure, the density of black Avicennia is 1,190 trees/ha, accounting for 29%; the second species has high density is White Avicennia 20%, the third highest density is Gia about 18%. Species have the fourth highest density is Rhizophora with 13%, other species account for less than 1% to 6% including Coc do, Coc trang, Su, Vet du, Ban chua, Tra, Binh bat, Ngoc Nu.

Growth characteristics of mangrove species in sub-region 3

In the ecological sub-region 3, there are 8 species: the dominant species with the highest density are Mam trang with 32%, the species with average appearance rate of 13-21% having 3 species of Rhizophora, Mam bien, Nypa palm; and species with low percentage smaller than 7% such as Gia, Vet du, Ban chua and Dung.

Growth characteristics of mangrove species in sub-region 4

In sub-region 4, there are 10 species and an average density with 2,450 trees/ha. This is the form of forest with many mixed species with 10 species surveyed: species with dominant density are Black Avicennia with 49% in all sub-regions, other species with high density such as White Avicennia accounts for 27%, Rhizophora accounts for 15%, other species such as Gia account for nearly 6%, the rest are accounted for below 1% like Ban oi, Ban chua (Sonneratia), Vet du, Coc trang, Avicennia, Vet tru.

The relationship among communities

There is not significant at similarity relationship of 20%. At the similaity level of 40% of the plots, it can be divided into 5 groups. In each community group or main forest structure type, each forest type has different dominant species.

- Group 1 has dominant species like Gia, and there are 3 types of structure with total of 3 cells, accounting for 4.41%.
- Group 2 dominant species is White Avicennia, and there are 5 types of structure with total number of cells is 14 accounting for 20.58%.
- Group 3 has 4 dominant species: White Avocado, Avicennia, Sonneratia, Rhizophora. Of which, White Avicennia has 7 types of structure with a total of 7 cells occupy 10.29%. The Avicennia species has 4 types of structures with a total of 4 plots make up 5.88%. There are 2 species of Sonneratia and Rhizophora, each species has 1 type of vicwstructure and only 1 cell, accounting for 1.47%.
- Group 4 has 2 dominant species: Rhizophora and White Avicennia. In particular, Rhizophora is the main dominant species, and there are 9 types of structures with 9 cells account for 13.23%. The rest are White Avicennia species with 1 structure type and only 1 cell accounting for 1.47%.
- Group 5 has 2 dominant species as Black Avicennia and Rhizophora. In particular, Black Avicennia is the main dominant species accounting for 22 cells/28 cells. There are 11 types of structures, total of 22 cells accounting for 32.35%. The dominant species is Rhizophora with 6 types of structure, a total of 6 plots account for 8.82% of the measured plots.

Conclusions

The composition and distribution of some species in the West Sea mangrove forest have 17 species of 11 plant families including 14 species of woody plants, 3 species of shrub species. About the relationship between species in the study area at the same level of 40% of plants divided into 5 main groups with 47 community types, 6 dominant species, including Mam đen [*Avicennia officinalis*], Mam trang [*Avicennia alba*], Duoc [*Rhizophora apiculata*], Gia [*Excoecaria agallocha*], Ban oi [*Someratia caseolaris*]. Vet du [*Bruguiera yipamoriza*]. The distribution of species showed that Mam den [*Avicennia officinalis*] has the highest rate, the second species is Duoc [*Rhizophora apiculata*], and the third species is Mam trang [*Avicennia alba*]. It should be proposed in the afforestation on new land warped in the study area.

Sub-region 1, the composition of mudflats has a ratio of clay composition of 12.53%, flesh soil composition of 19.45%, sand of 68.02%. Due to the site conditions, this sub-region has very high sand content of 68%. No new forest planting should be conducted, but natural regeneration should only be conducted so that seedlings can adapt themselves to places where there are favourable conditions.

Sub-region 2 has natural conditions with the ratio of 4.19% organic, 54.71% clay, 36.10% meat, 9.19% sand, total salt in soil 6.33 ‰, soil pH 8,11, and EC = 9,89mS/cm. This sub-region has a favorable condition of natural fertility, with a sufficient amount of sand, a high content of flesh soil, and a high percentage of organic ingredients, thus it only increaseas the composition of plant humus into the potting medium with a rate of 5-10% enough.

Sub-region 3 has ratio of 7.29% soil organic matter, 52.29% clay, 45.89% flesh soil, 1.82% sand, total salt dissolved in 6.44‰ soil, pH 6.89, and EC = 10,06 mS/cm. As this sub-region has high clay and flesh soil content, low sand ratio, high organic composition, it is suitable for plantation and afforestation.

Sub-region 4, there are ratio of soil organic matter 2.34%, clay 55.59%, flesh soil 43.84%, sand 0.57%, total soluble salt 8.83 tan, soil pH 7.54, and EC = 13.8 mS/cm. This is a place with a fairly large mudflat, high clay and flesh sol content, low organic matter content in the soil, so it only pays attention to increase the ratio of organic matter in the potting mix from 5 - 10% to increase porosity and humus content for seedlings.

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CHARACTERISTICS OF ALKALIZED SOILS IN THE BAČKA REGION (SERBIA) AND THE POSSIBILITY OF THEIR AFFORESTATION

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Abstract

Alkalized soils occupy 80,333 ha or 3.75% of the total area of Vojvodina and represent the areas fragmentally covered with scarce halophytic shrubby vegetation. Besides alkalization processes endanger about 34,000 ha, or 1.60% of primary agricultural land, which represents in total 5.35% areas endangered with alkalization in Vojvodina. Bearing in mind the surfaces that are endangered by alkalization, in the Bačka region, the study of alkalized soils was carried out on relief forms in the form of depressions representing river basins from the geological past. In these relief areas of Bačka, where alkalisation is present, alkalized soil is formed at the soil type designated as solonetz, class solonci. The paper presents the properties of solonetz soils, ie their physical and chemical properties. The study of these soils is significant for the purpose of finding a suitable method for the melioration of such areas. On the basis of the soil studies, the tree species that can be planted on these habitats will be determined. The soil quality of these areas will be improved through forest establishment.

Keywords: *Alkalized soils, Afforestation, Forest melioration*

Introduction

Alkalised soils occupy mostly lower relief positions and appear in spots, on sedimentary substrates, loess, loam and clay. Their formation is accompanied by a series of complex processes, and the basic process is sodium embedding in the adsorptive complex and its relatively high presence. In the area of Vojvodina, these lands are mostly widespread in Banat, somewhat less in Bačka and the least in Srem. According to Ivanišević et al. (2011) alkalisation, as a degradation process, endangers also the peripheral parts of agricultural soils. According to Miljković, (2005) solonetz is most oftenly located in the complex with solonchak and other saline soils of automorphic and hydromorphic origin, which are affected by processes of alkalization, salinization and desalination. The usage of the production potential of these soils requires different meliorative operations, and the solonetzes, due to their unfavorable chemical and physical properties, are used as natural, rather scarce pastures. According to the benefits for plant production, these lands belong to the seventh grade (Hadžić, et al. 2002). Given the unfavorable conditions for the use of these soils, Ivanišević et al. (2013) propose the establishing of tree plantations to prevent the process of further salinisation of these soils, so tree plantations would have a semimelioration function. According to Roncevic (2014), as stated by Kadovic, (1983); Galić, (2006) and Ivanišević et al. (2013), it is important to choose woody species that are tolerant to these unfavorable soil conditions and which can contribute to mitigation of further degradation processes. The aim of this research was to show the characteristics of alkalized soils and the possibility of their afforestation with certain types of trees.

Material and methods

In this paper, alkalized soils in the region of Bačka (Serbia) were studied. Soil surveys were carried out in the fields of the towns Futog-Begeč and Kovilj. Pedological profiles were opened and their external and internal morphology were described. Soil samples were taken in deteriorated state, and physical and chemical analyzes of samples were made in the

laboratory. The following analyzes were performed:
Determination of granulometric composition of soil according to international B-pipette method by preparation in sodium pyrophosphate (Bošnjak et al. 1997)
Determination of mechanical elements was made by Atteberg, and the texture classes were determined according to the American classification (Belić et al. 2014)
Determination of humus content in soil by the Tjurina method by Simakov modification, 1957
Determination of the content of CaCO3 in the soil, volumetric with the Scheibler calcimeter,
Determination of chemical reaction of soil, pH in water electrometric with glass electrode,
Determination of the content of total water soluble salts in soil by the method of measuring electrical conductivity in the saturated soil trap.

Picture 1 Location of pedological profiles

Results and discussion

Analyzing the granulometric composition of the studied soils (Table 1), it can be seen a very small fraction of coarse sand, on average only 0.8 to 1.8%, and an increase in the fine sand fraction ranging from 36.0 to 56.3% , as well as the share of silt whose values are from 26.6 to 37.3%, while average share of colloidal clay was slightly lower, or from 15.2 to 26.6%. According to the total content of sand and clay, we can conclude that the average content of total sand is in the range of 36.9 to 58.1%, while the average content of total clay is from 41.8 to 63.1. According to Ćirić, (2012) this soil has a heavier mechanical composition with more than 30% of clay. Characteristics of alkalized soils have been investigated in detail in the works of Belić et al. (2004) and Belić (2005).

Table 1 Granulometric composition

No. profil	Horizon	Depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Total sand (%)	Total clay (%)	Texture class
P1	AE	0-20	3.9	44.1	34.4	17.4	48.1	51.8	Loam
	$Bt,_{na}$	20-50	0.2	42.9	30.3	26.4	43.1	56.8	Claly loam
	C_{ca}	50-150	1.0	58.5	27.9	12.4	59.6	40.4	Sandy loam
	Gr	150>	1.9	79.7	13.8	4.44	81.6	18.3	Loamy sand
	Average		**1.8**	**56.3**	**26.6**	**15.2**	**58.1**	**41.8**	
P2*	AE	0-15	3.8	47.2	32.3	16.6	51.1	48.9	Loam
	$Bt,_{na}$	15-60	0.4	28.4	28.3	42.9	28.8	71.2	Clay
	C_{ca}	60-130	1.1	21.5	45.5	31.8	22.6	77.4	Claly loam
	R	>130	1.1	48.9	35.0	15.1	50.0	50.0	Loam
	Average		**1.6**	**36.5**	**35.3**	**26.6**	**38.2**	**61.8**	
P3	A_{oh}	0-35	1.5	45.8	34.7	18.0	47.3	52.7	Loam
	$Bt,_{na}$	35-65	0.1	26.1	28.7	45.1	26.2	73.8	Clay

C_{ca}	65-150	1.7	19.6	53.2	25.6	21.2	78.8	Clay
R	>150	0.2	52.6	32.6	14.5	52.8	47.2	Loam
	Average	**0.8**	**36.0**	**37.3**	**25.8**	**36.9**	**63.1**	

P2 *Rončević et al. (2014)

It is particularly evident that in iluvial $Bt,_{na}$ the horizon of these soils the content of total clay was increased. This horizon with its morphological, physical and chemical characteristics is the basic diagnostic horizon of this type of soil. The textural classes of this iluvial horizon are: clay loam and clay, while the remaining horizons of these lands are: loamy sand, sandy clay and clay.

Table 2 Chemical properties

No profil	Horizon	Depth (cm)	pH	Humus (%)	$CaCO_3$ (%)	Total salt (%)
P1	AE	0-20	7.93	2.69	16.11	0.09
	$Bt,_{na}$	20-50	9.39	2.31	16.47	0.25
	C_{ca}	50-150	9.45	0.69	15.38	0.09
	Gr	>150	8.85	0.50	14.36	0.09
		Average	**8.90**	**1.54**	**15.58**	**0.13**
P2*	AE	0-15	7.42	0.90	15.91	0.08
	$Bt,_{na}$	15-60	9.76	0.47	17.66	0.30
	C_{ca}	60-130	9.73	0.13	18.24	0.16
	R	>130	9.31	0.03	15.98	0.02
		Average	**9.05**	**0.38**	**16.95**	**0.14**
P3	A_{oh}	0-35	6.92	4.39	16.17	0.01
	$Bt,_{na}$	35-65	7.38	0.16	17.19	0.12
	C_{ca}	65-150	9.32	0.03	18.38	0.14
	R	>150	9.32	0.05	15.79	0.05
		Average	**8.24**	**4.63**	**16.88**	**0.08**

P2 *Rončević et al. (2014)

Analyzing the chemical properties of this soil (Table 2), it is estimated that the average pH value for these soils was from 8.24 to 9.05, and the tested soils are medium to very alkaline. Observing the humus content, it is evident that the highest humus content is in surface horizons, and the humus content decreases with the depth of the profile. The average values of the humus content are in the range from 0.38% to 4.63% and classify this land in very poorly humid to quite humid. The carbonate content grows slightly with depth of soil, except in the lowest Gr or R horizons. The average values of carbonates are 15.58% and 16.95% respectively, and according to the classification, these lands are classified into highly carbonate soils. The total salt content varies in these soils and is highest in the eluvial $Bt,_{na}$ the horizons with values of 0.25%, 0.30% and 0.12%. It is also noticeable that the increased salt content in the horizons below $Bt,_{na}$ the horizon, or in C_{ca} horizons in the P2 and P3 profiles, with values of 0.16% and 0.14% indicating the occurrence of increased sedimentation in these horizons for the tested soil. Since soils in degradation have heavier mechanical composition and the alkalization process (Pekeč, 2016), it can be concluded that the ecological and production value of these soils depends largely on the content of the total water-soluble salts and the mechanical composition of the soil.

The analyzed alkalized soils are solonetz type, morphological structure AE-$Bt,_{na}$-C_{ca}-Gr (R) or A_{oh}-$Bt,_{na}$-C_{ca}-R. Texture migration, due to eluvial-illuvial processes from surface horizons in the illuvial horizon, is present in these soils. The horizon of the determination of these soils is iluvial Bt, na a horizon at a depth of 20-50cm, 15-60cm and 35-65cm, which is characterized by a high proportion of total clay from 56.8 to 73.8%. It is very compact and also

distinguished with high content of total salts (0.12-0.30%) and high pH values. The power of this horizon is 30-45cm with stubble structure and unfavorable water-air features.
High content of sodium and heavy mechanical composition in Bt_{na} horizon are the cause of the lack of favorable water, air and heat regime of this horizon (Hadžić et al., 1991)
Given these poor physical and chemical characteristics, there are no woody species on these soils and mainly pastures are present. Large areas with this kind of soils are under halophytic vegetation, without woody species, with sparsely arranged bushy formations of *Cornus mas*, *Prunus spinosa* and *Pyrus pyraster*. Studying the reception of various species of trees at the solonetz, Rončević et al. (2014) highlighted the particularly good reception of narrow-leaved ash (*Fraxinus angustifolia*), domestic white poplar (*Populus alba*), Russian olive (*Eleagnus angustifolia*), white mulbery (*Morus alba*) and Turkey oak (*Quercus cerris*).
It is important to emphasize that such soils could be afforested only with species with high increase. According to Pekeč et al. (2010), bearing in mind the characteristics of these soils, wood species for their afforestation should be tree species that can endure such unfavourable habitat conditions, primarily the following types of trees: wild pear (*Pirus piraster*), Russian olive (*Eleagnus angustifolia*), various species of the genus *Prunus sp*., white poplar (*Populus alba*), pedunculate oak (*Quercus robur*), Turkey oak (*Quercus cerris*) and narrow-leaved ash (*Fraxinus angustifolia*). It can certainly be said that such soils can be afforested only by a narrow spectrum of forest trees which are resistant to such unfavorable edaphic conditions.

Conclusion

In the Bačka region, individual parts are exposed to degradation processes of alkalization, which occupy generally lower relief positions. The use of the production potential of these soils requires different meliorative operations, and the solonetzes are used as natural pastures due to unfavorable chemical and physical properties. The analyzed alkalized soils are of solonci class, solonetz type, morphological material AE-$Bt,_{na}$-C_{ca}-Gr or Aoh-$Bt,_{na}$-C_{ca}-R. Texture migration due to eluvial-illuvial processes is present in these soils from surface horizons to the horizon of illuviation. The horizon for the determination of these soils is illuvial Bt, na the horizon which is at the depth of 15-65 cm in the studied soil, with power of 30-45 cm. It is characterized by a high proportion of total clay, increased alkalinity and increased content of total salts, which is a limiting factor for plant production. The soils with such unfavorable properties can be afforested with a narrow spectrum of forest trees which are resistant to these unfavorable edaphic conditions. The afforestation would partially improve the properties of these soils through forest meliorations.

Aknowledgement

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THE EFFECTS OF CLIMATE CHARACTERISTICS ON THE DIAMETER INCREMENT OF DOUGLAS-FIR IN THE CITY OF BELGRADE (SERBIA)

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Abstract

The paper presents the results of a study dealing with the effects of climate factors on the size of the early and late wood as well as the total diameter increment of Douglas-fir. For the purpose of this analysis, 30 increment bores were taken at breast height. The research was conducted in the area of Belgrade (Serbia) on the site of European Turkey oak and Hungarian oak (*Quercetum farnetto-cerris aculeatetosum*). The obtained data were related to the mean-monthly air temperatures (in the period from April to October) and the monthly rainfall totals (in the period from April to September). The research included another very important parameter - the age of trees. The amount of rainfall in April, as well as the air temperatures in May, August and September, had a negative effect on the total diameter increment. The share of latewood was negatively affected by the amount of rainfall in April and September, as well as by the air temperatures in June, August and September. The share of earlywood was negatively affected by the amount of rainfall in April and May, as well as the temperatures in May. The analyzed parameters explained 54.2% of the current diameter increment, 33.9% of the share of latewood and 51.1% of the share of earlywood. In order to meet future requirements of forest management, growth models sensitive to climate characteristics and adaptability of Douglas-fir to changing environmental conditions have been developed.

Keywords: *adaptability, climate change, Douglas-fir, earlywood, latewood.*

Introduction

Apart from extending the growing season and increasing the storage of CO_2 and nitrogen, climate change contributes to the rapid growth of forest trees. Forest growth in changing climate conditions cannot be accurately predicted without studying the impact of climate factors on trees. A great number of studies indicate that the accumulation of gases causes the greenhouse effect (IPCC 2001). In the past 200 years, new species have been introduced from different parts of the world into European forests. Since they occupy a significant area today, the need for forest management practices in a changing environment has arisen. Dendroclimatological research can be used to identify the major climate condition factors that affect the radial growth of trees (Lopatin et al, 2008; Wager and Baker, 2003.

Douglas-fir was introduced to Europe as a decorative tree for arboretums and parks in 1827. Since the end of the 19th century, it has been planted in the forests of many European countries, especially after World War II (Vrcelj-Kitic, 1982). Today, Douglas-fir is the most commonly used allochthonous tree species in European forests and its forests cover more than 800,000 ha. Table 1 shows the general characteristics of natural habitats and the sites of the study stand in the area of Belgrade.

Table 1. Climate data for five subdivisions of the range of Douglas-fir

Climate data	Pacific Northwest		Rocky mountains			Belgrade (Serbia)*
	Coastal	Mountainous	Northern	Central	Southern	
Temperature (^{0}C)						
July	20-27	22-30	14-20	14-21	7-11	19.8 to 27.0
January	-2.5 to 2.5	-9.0 to -2.5	-7.0 to -2.5	-9.0 to -6.0	0 to 2.0	-2.0 to 7.6
Precipitation (mm)						
Annual	760-3000	600-3000	560-1020	360-610	410-760	367 to 1095

Source: Valeriu – Norocel, 2019, *Ratknic, 2019.

The aim of this study was to determine the impact of climate characteristics on the diameter increment and the share of earlywood and latewood.

Material and methods

The research was carried out in an artificially-established Douglas-fir stand in Suplja Stena area near Belgrade (Serbia). The studied stand was planted in 1961 on the site of European Turkey oak and Hungarian oak (*Quercetum farnetto-cerris aculeatetosum*). The data were collected during 2018. It is at an altitude of 276 meters with a slope of 50º and a western aspect. The stand is complete and well-preserved. The bedrock underlying the stand is made of serpentine. The total number of trees is 208 per ha. Most trees range from 37.5 cm to 42.8 cm in diameter. The mean height of the stand is 43.35 cm, and the mean diameter is 43.1 cm. It has a tree distribution line typical of even-aged stands. The volume distribution line is also typical – characteristic of even-aged stands. The total basal area is 30.7 m^2ha-1, while the total wood volume amounts to 275.97 m^3ha-1. The distribution of wood volume by diameter classes is the result of the distribution of the tree number. The maximum is in the diameter class of 52.5 cm. The general data on the artificially-established stand are shown in Table 2.

Results and discussion

The dependence of the current diameter increment and width of earlywood and latewood on climate characteristics (precipitation by months and monthly air temperature averages during the growing season – from April to September) was investigated. Independent variables were precipitation sum in April (AP_P), precipitation sum in May (MA_P), precipitation in June (JU_P), precipitation in July (JL_P), precipitation sum in August (AV_P), precipitation sum in September (SE_P), mean air temperature in April (AP_T), mean air temperature in May (MA_T), mean air temperature in June (JU_T), mean air temperature in July (JL_T), mean air temperature in August (AV_T), mean air temperature in September (SE_T). A linear regression model of the impact of the age and the analyzed climate factors on the current diameter increment (Zi), share of latewood (Ka) and share of earlywood (Ra) was constructed. The model parameters are given in Table 3.

The amount of rainfall in April, as well as the air temperatures in May, August and September, had a negative effect on the total diameter increment. The share of latewood was negatively affected by the amount of rainfall in April and September, as well as by the air temperatures in June, August and September. The share of earlywood was negatively affected by the amount of rainfall in April and May, as well as the temperatures in May.

Table 2. General data on the artificially-established Douglas-fir stand

Diameter class (cm)	N		H_{mean}	G		V (m^3)	
	1 ha	%	(m)	(m^2/ha)	%	1 ha	%
27.5	16	0.9	21.45	0.95	3.1	6.93	2.5
37.5	48	6.2	25.16	6.18	20.3	57.22	20.7
42.5	48	5.7	26.02	5.67	18.6	50.70	18.4
47.5	50	14.2	25.98	14.17	46.5	124.85	45.25
52.5	16	3.5	29.95	3.46	13.4	36.19	13.12
∑	**208**	100.0		**30.45**	**100.00**	**275.89**	100.0

Source: Original

Principal Component Analysis (PCA) was applied to determine the variability of data between and within the analyzed climate characteristics in order to select the best variables for discrimination. The results of these analyses are presented numerically and graphically.

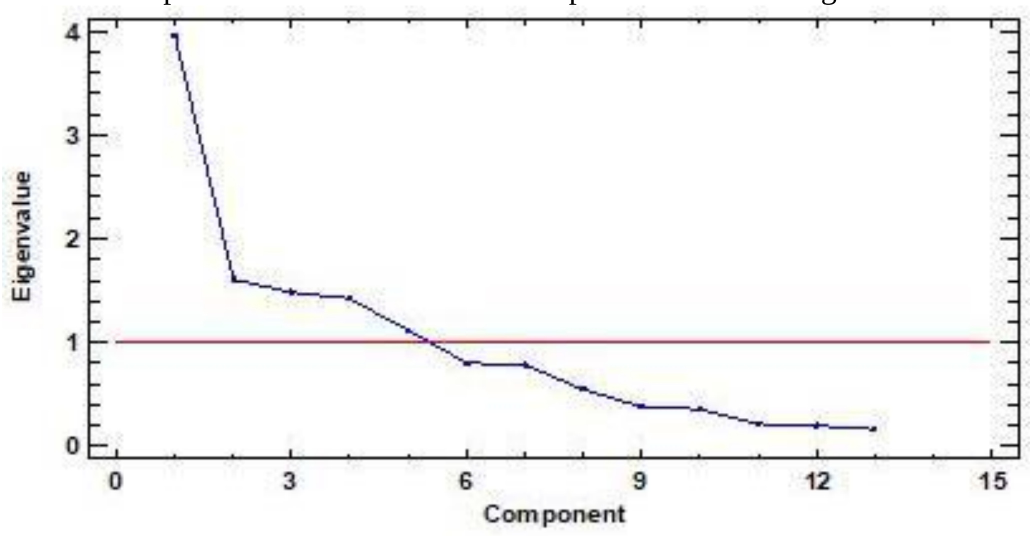
Table 3. The influence of age and analyzed climate factors on the current diameter increment (Zi), share of latewood (Ka) and share of earlywood (Ra)

Independent variable	Dependent variable					
	Zi		Ka		Ra	
	Parameters	Error	Parameters	Error	Parameters	Error
CONSTANT	317.45400	40.76610	34.43580	12.90860	283.01800	35.72160
Year	-0.16005	0.02270	-0.01627	0.00719	-0.14378	0.01989
AP_P	-0.00371	0.00761	-0.00017	0.00241	-0.00355	0.00667
MA_P	0.00109	0.00463	0.00142	0.00147	-0.00032	0.00406
JU_p	0.00235	0.00446	0.00170	0.00141	0.00065	0.00391
JL_P	0.00937	0.00405	0.00244	0.00128	0.00693	0.00355
AV_P	0.00557	0.00618	0.00114	0.00196	0.00443	0.00542
SE_P	0.00210	0.00631	-0.00258	0.00200	0.00468	0.00553
AP_T	0.12271	0.13334	-0.05046	0.04222	0.17317	0.11684
MA_T	-0.01034	0.14862	0.11082	0.04706	-0.12116	0.13023
JU_T	0.03731	0.17976	-0.06513	0.05692	0.10244	0.15751
JL_T	0.09297	0.19453	0.02702	0.06160	0.06594	0.17046
AV_T	-0.01046	0.16034	-0.05173	0.05077	0.04127	0.14050
SE_T	-0.00997	0.13181	-0.03983	0.04174	0.02986	0.11550
R	0.7363		0.5826		0.7153	
R^2	54.2167		33.9420		51.1657	
Standard error	2.0777		0.6579		1.8206	
F-test	12.0200		5.2200		10.6400	
Durbin-Watson statistic	0.8950		1.4718		0.9341	

Source: Original

Five components were isolated in the principal component analysis (PCA) (Graph 1). The results of this analysis are shown in Table 4. According to the eigenvalues and percentage values obtained, the first five components (coordinates) are sufficient to explain 73.716% of the total variability of data. The value each variable (climate factor) contributes to the overall variability of data (according to the first, second and third axes) is shown in Table 5. The scatter plot (Graph 2) shows the geometric distance between the studied climate parameters and the variability between them.

Graph 1. Number of identified components and their eigenvalue



Source: Original

According to the first breakpoint shown in Graph 2, two components were further analized in PCA.

Table 4. Eigenvalues and percentage values with the participation of each coordinate in describing the total variability of data

Component number	Eigenvalue	Variance percentage	Cummulative percentage
1	3.95977	30.460	30.460
2	1.60379	12.337	42.797
3	1.47997	11.384	54.181
4	1.42009	10.924	65.105
5	1.11951	8.612	73.716
6	0.80045	6.157	79.874
7	0.78265	6.020	85.894
8	0.54652	4.204	90.098
9	0.37562	2.889	92.988
10	0.34896	2.684	95.672
11	0.21039	1.618	97.290
12	0.19595	1.507	98.798
13	0.15632	1.202	100.000

Source: Original

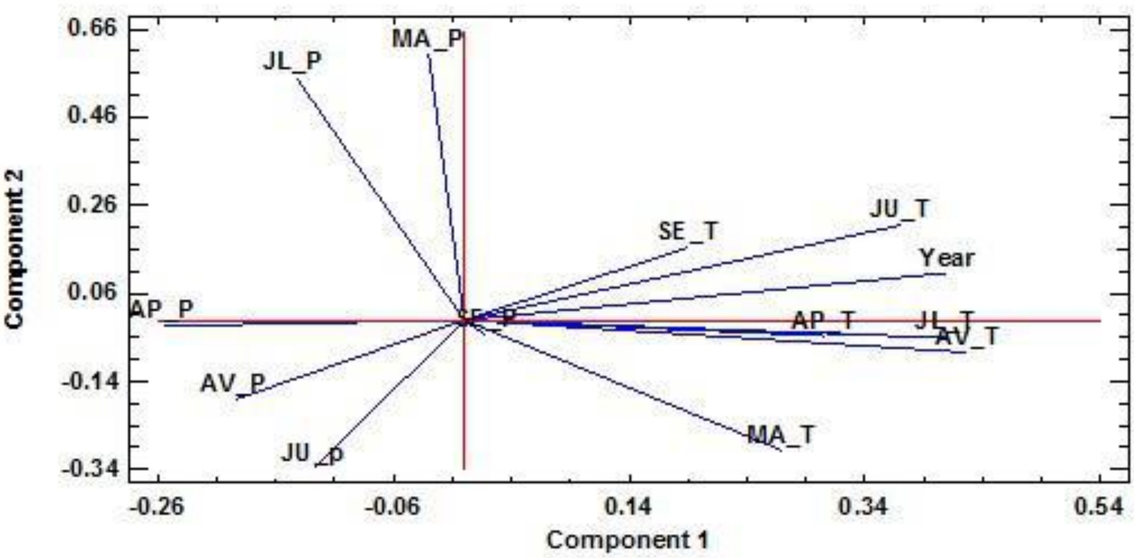
Based on the transient diagram shown in Graf 2, it can be seen that of the 13 tested factors, two components best explain the variability in the sample. The correlation between the mean air temperature in August (AV_T), year, mean air temperature in July, mean air temperature in June (JU_T), and mean air temperature in April (AP_T) determines the basic characteristics of component 1. The connection between the precipitation sum in May (MA_P), precipitation in July (JL_P), and precipitation in June (JU_P) defines component 2.

Table 5. Table of Component Weight

Factor	*Component*				
	1	2	3	4	5
Year	0.40959	0.10608	0.08611	0.17479	0.31939
AP_P	-0.25601	-0.01402	0.25724	0.18465	0.42858
MA_P	-0.03020	0.60315	0.19185	0.30646	0.00867
JU_p	-0.12817	-0.33915	-0.14322	0.58305	-0.08587
JL_P	-0.14324	0.55253	-0.11676	-0.12468	0.08287
AV_P	-0.19511	-0.18149	-0.10309	-0.01512	0.73212
SE_P	0.01834	-0.03358	0.71682	0.11160	-0.02230
AP_T	0.30599	-0.03532	0.07652	0.26808	-0.16052
MA_T	0.26978	-0.29746	-0.00111	-0.38051	0.10599
JU_T	0.37112	0.21563	0.00940	-0.33172	0.17897
JL_T	0.40901	-0.03974	0.05165	0.22992	0.27241
AV_T	0.42728	-0.07493	0.06549	0.06924	-0.11633
SE_T	0.19091	0.16424	-0.56401	0.29818	0.08211

Source: Original

Graf 2. Two-dimensional data distribution (scattering points)



Source: Original

Conclusions

A clear long-term trend of changing climate has been identified. It can be concluded that the analyzed parameters explain 54.2% of the current diameter increment, 33.9% of the share of latewood and 51.1% of the share of earlywood. For forest management purposes, new growth models that are sensitive to climate characteristics and their changes in forest ecosystems need to be developed.

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THE CHOICE OF OPTIMAL TECHNOLOGY OF PROCESSING OF WOOD GREENERY IN THE CONDITIONS OF INTEGRATED UTILIZATION OF FOREST DENDROMASS

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Abstract

In our forests, around 2.94 mil. m^3 of dendromass is cut down each year. Of these, about 4-6% or 117,000 to 176,000 m^3 makes wood greenery - twigs with needles or leaves, with 10 mm diameter at thick end, measured with bark. Based on the experience of others, first of all from Russia, the Baltic and Scandinavian countries, it is known that wood greenery represents valued raw material in different sectors of economy: agriculture, pharmaceutical and cosmetics industry, and others. The entire annual attack of wood greenery remains unused, with us. There are two reasons for this: a) because there are still no elaborated and proven practices of rational collection and concentration of raw materials to processing plants; and b) because we are not familiar enough with the methods of wood greenery processing. A detailed analysis of the technology of wood assortment production in our forestry practice has been carried out. Based on these findings, a model of integrated forest utilization was conceived, including wood greenery. Then, the technology of wood greenery processing was analyzed in the following ways: mechanical drying and fractionation processes, chemical extraction processes, thermal processes by classical heating and energy use of the microwave electromagnetic field and their combinations: mechanical-chemical methods and thermo-chemical methods. Finally, such technology of wood greenery processing has been selected to optimally matches with the integrated use of forest resources, in terms of the following criteria: minimal negative environmental impact, maximum safety at work, low energy consumption, primarily from renewable sources, maximum quality of final products.

Keywords: *wood greenery, processing technology, forest dendromass*

Introduction

Wood greenery (WG) consists of branches with needles or leaves with a diameter of up to 10 mm measured over bark from the butt end (Fig. 1); it is also referred as technical greenery. WG is made from brushwood of coniferous and hardwood trees. WG is a raw material for the production of several products, with a variety of usable values such as: vitamin-mineral flour, chlorophyll - carotenoic paste, essential oils, fitoinsecticides, phytopharmaceuticals, plant growth regulators, primary forms of medicinal preparations in human medicine etc. (Vučić *et al*., 2019).

In our forests, around 2.94 mil. m^3 of dendromass is cut down each year. Of these, about 4-6% or 117,000 to 176,000 m^3 makes WG (Ljubojević *et al*., 2007). The entire annual attack of WG remains unused, with us. There are two reasons for this: a) because there are still no elaborated and proven practices of rational collection and concentration of raw materials to processing plants, and b) because we are not familiar enough with the methods of WG processing.

Figure 1: Wood greenery of fir (*Abies alba* Mill.); (photo: S. Ljubojević)

The aim of the paper is to analyze the technology of production of wood assortments in our forestry practice and, based on this knowledge, to propose the concept of integral forest exploitation, which includes WG. Then to analyze the technologies of WG processing and to choose the solution that most completely satisfies the given criteria.

Material and method

The subject of research is WG of domestic conifers: fir (Abies alba Mill.), spruce (Picea excelsa Link.), Scots pine (Pinus silvestris L.) and black pine (Pinus nigra Arnold). In comparison to the raw material of the broadleaf (deciduous) species, the coniferous raw materials are of no seasonality and can be used throughout the year. An analysis of the production technology of wood assortments in our forestry practice was carried out by monitoring three felling areas in different harvesting season. The basic characteristics of the observed objects are given in Table 1. Harvesting and processing technology was observed using method of continuous timing during one full working day per felling area. In this way, a chronological sequence of work elements was established, from the moment when the cutting begin until the moment when the timber assortments are dispatched to the timber yard. Along with this, not disturbing the regular production process, three organizational solutions were tested.

1. WG was separated from the branchwood and brushwood at a felling site using simple garden tree shearing scissors, then put in 70 liters bags, loaded on pack-horses and carried to the storage place along the truck road.
2. Brushwood was separated from the branchwood at the felling site using axes and strong bypass lopper and than stacked in small bundles. From here the raw material was handed over to the iron baskets arranged along the skid road; in regular production these baskets are used for the transportation of stacked wood. Baskets were mounted on the front and rear of the skidder and forwarded to the storage place, where the finally separation of WG was carried out.
3. The third organizational solution was applied in a strip clear cut in pine plantation on a steep terrain, where it is not justified to build permanent roads (skidding trucks and forest

roads). The trees were cut and hauled as a whole or split by half, using pair of winches: „Ackja“ winch for skidding from stupms to the ropeway and „Gantner HSW 20“ winch for extraction down the mainline.
WG was separated from the branchwood and brushwood by hand tools at the and of the ropeway.

Table 1: The basic characteristics of the observed objects

Basic characteristics	Observed objects		
Location	Industrial plantations „INCEL“	Forest estate „Borja“ Teslić	Forest estate „Gostović“ Zavidovići
Type of ownership	Joint-stock company	State-owned enterprise	
Forest type	Pine plantations	High forest of pines and pine plantation	
Used means of work	*Working phase - harvesting*		
	Harvester Lokomo 990	Chainsaw	
	Working phase - wood bunching and extracting		
	Skidder LKT-81	Skidder LKT-80	Sledwinches Ackja and Gantner HSW 20
Harvesting system	Assortment method	Assortment method	Full-tree method
Appearance form of wood greenery (WG)	WG integrated with branchwood - at felling site	WG integrated with branchwood - at felling site	WG integrated with branchwood - at timber yard

To better understand the working conditions on preparation of WG, the average moisture content by the tree species and the felling season was determined, in the usual way and based on small samples ($ni \leq 30$). For the same reasons, the bulk density of WG was evaluated as well. With that regard, a wooden container of 1 x 0.5 x 0.5 m was made. It was filled with material to the top and weighed on the field scale, with a reading of 0.1 kg. The obtained values are converted to the standard unit kg/m3.
By inspecting the scientific and professional papers, technical documentation and patents, processing technologies of WG have been analyzed and a solution that satisfies the following criteria has been selected: a) a mass-usable product is produced without further fractionation, b) waste that is generated during production is usable, c) production takes place without the use of chemical agents, d) production is flexible with respect to raw material origin - it is possible in mobile and stationary plants, f) selected solution is proven in practice. Also, chosen solution does not adversely affect the environment, provides all the necessary safety at work and requires low energy consumption, primarily from renewable energy sources.

Results and Discussion

To make WG a kind of raw material, it is necessary to conduct several working operations: to collect branchwood from the felling site, to separate WG from the branchwood and to gather it in a form suitable for transportation, to transport it to the processing site. In doing so, we distinguish between internal transport, on a move from stumps to the storage place, and external transport, from the storage place to the processing plant. Collecting WG from standing trees is not reasonable for reasons that need not to be specifically explained. On the contrary, the only possible solution is the separation of the tree's greenery immediately after the tree felling in any sitations: regular felling, sanitation felling, random yield etc.

Manual preparation of wood greenery

In a typical case, manual preparation of WG involves four work elements: 1. collecting brunchwood left behind after cutting the trees, 2. separation of WG from brunchwood, 3. carrying of WG to the containers for transport, 4. stacking of WG at the containers for transport. The following average working time structure was determined for the implementation of the described work elements in the eighth hours working day (WD): Effective working time (EWT) - 6 h (75% of WD), Delay time (DT) - 2 h (25% of WD), where under the EWT we mean a period of useful work, and under the DT allowed breaks in work, such as: meal time, rest and personal time, interference time. The following average structure of EWT was determined: Preparatory time – 14.4 min (4% of WT), Time of transition (from tree to tree) - 24.8 min (8% of WT), Separation of WG from branchwood - 277.2 min (77% of WT), Disposal and stacking of WG at the container - 39.6 min (11% of WT). Average production rate per worker was determined as 161.4 kg of WG per WD, with variation interval: 134 - 189 kg of WG per WD. These results point to the conclusion that manual preparation of WG is not economically justified due to the low productivity.

Internal transport

Relatively high moisture content in fresh WG and its low bulk density (Tab. 2) make it difficult to find rational solutions with the transport means currently in use in our forestry, whether the raw material is carryng from the forests in bags on pack-horses, or in iron baskets mounted on the skidder. The only economically viable solution is to transport entire trees using wire-rope systems. However, this technological solution rarely meets our forestry practice, and as a such, it does not have a decisive significance in the eventual organization of mass production. Conversely, mechanical hauling of whole trees or parts of them is not acceptable due to the high pollution and damage of raw material (Ljubojević, 2008).

Table 2: Moisture content in fresh wood greenery and its bulk density

Harvesting season	Tree species			
	Scots pine	Black pine	Fir	Spruce
Moisture content (%)				
Winter	51	51	50	48
Summer	51	53	50	47
Bulk density (kg/m^3)				
Winter/summer	57,6	82	142	173,2

Concept of primary collection and concentrations of wood greenery to the processing site

An applicable techno-economic solution assumes the mechanized gathering and palletizing of forest residues in the form of biomass bundles and their delivery to the processing plants (Il. tab. 1, production lines 1 and 2; Ljubojević, 2016). In this way, it significantly shortens the time of primary collection and concentration of raw material to the processing site, ensures better raw material quality and at the same time increases the bulk density of the load. Two-stage separation of biomass is performed at the processing site. In the first step, branchwood and brushwood are separated by a light hydraulic crane. Branchwood is directed to the production of solid biofuels (woodchips, briquettes, pellets). Brushwood is mechanically separated into ligno-cellulose

1	Logging residues at the felling site	Making bundles with the slash bundler at the felling site	Collecting bundles over felling site and transfering to the storage place	Transporting bundles to the processing plant
				
2	Logging residues at the felling site	Collecting and transporting forest residues to the storage place	Making bundles with the slash bundler at the storage place	Transporting bundles to the processing plant
				

Illustrated table 1: Production lines for bundling of logging residues in regular felling skeleton and wood greenery. Separation of WG from the wooden skeleton is the most important operation in the phase of WG preparation. By switching from manual to mechanized production, it significantly increases productivity and reduces production costs (Hakkila, 1989; Levin et al., 1984; Nikitov, 1985). Separator "ODZ-12A" is an automatic machine constructed and introduced into regular production in the former USSR (Tomčuk and Tomčuk, 1966). The separator consists of five basic parts and mechanisms mounted on a rigid chassis: 1. belt conveyor (internal), 2. roller inserter, 3. roller ejector, 4. separation drum with blades, 5. bunker for receiving WG. Automatic feeding of separation drum with WG and discharge of bare branches is carried out by belt conveyor. Top layer of belt consists of metal rolls with welded ribs - a thin cylinders in the form of non-head nails. Its working position and strength is regulated by tensile rowels. Drum with blades is the main part of separator. It devides green parts from wooden skeleton by means of knives fixed with clamps. Rubber shock absorbers prevent collisions during rotation. External belt conveyor inserted WG in a shape of brushwood into the separator, piece per piece with a thicker end forward. Main technical characteristics of the separator are: capacity - 1.5 t WG/h, separation purity - 98%, power - 3.8 kW, drum rotation speed 500 - 650 /min, working volume of bunker - 0.5 m^3.

Choice of optimal wood greenery processing technology

WG serves as a raw material for obtaining a large number of products with a wide usage value, for which various technological processes have been developed (Tab. 3). Fresh unprocessed branches with needles are used as a protective cover for young agricultural crops, soil protection from moisture loss, as an addition to fodder and as a raw material for compost production (Duryea and Edwards, 1997; Daugavietis et al., 2015). When domestic animals and poultry are fed, a previous smoothing with water vapor is practiced.

If the simplified review of processing technologies is filtered through the set of criteria, the production of vitamin-mineral flour (VMF) comes out as an optimal solution. VMF can be produced in mobile facilities and in stationary plants, both of which operate according to the same principles (Hakkila, 1989; Levin et al., 1984; Nikitov, 1985; Tomčuk and Tomčuk, 1966). Only exception is WG of fir which, due to the high content of essential oil, requires additional technological intervention, combining hydrodestillation with short-term processing of the raw material by high pressure steam (so-called steam cracking) (Levdanskij et al., 1992).

Table 3: Simplified review of wood greenery processing technologies

Products obtained from wood greenery	Essence of the processing technology	Source
Vitamin-mineral flour	Thermal and mechanical treatment by combining drying, sorting and grinding processes	Hakkila (1989), Levin (1984) Nikitov (1985), Tomčuk (1966)
Essential oils	Hydrothermal treatment by aqueous distillation	Daugavietis (2014), Tomčuk (1966)
BAC* of law molecular weight	Chemical treatment by extraction with aqueous sodium hydroxide solution**	Karmanova (2005), Terenteva (2016) Šanina (2004)
BAC* of high molecular weight	Thermochemical treatments	Karmanova (2005), Terenteva (2016)
Chlorophyll-carotenoic paste	Thermohemical treatment by gas extraction process in combination with distillation of essential oils	Levin (1984), Tomčuk (1966)
Primary forms of medicinal preparations in human medicine	An infusion in water or in diluted ethanol; coniferous therapeutic extract	Levin (1984), Paršikova (2015)

*Biologically Active Compounds
**This process yields more useful products. Lipids are used in perfumes, medicine and other industries. Concentrate of neutral ingredients (provitamin concentrate), separated by emulsion extraction, can be used as a bioactive additive in the perfumery and cosmetic industry and as an additive in domestic poultry and cage breeding fur animals.

Production of VMB is carried out according to the following procedure (Tomčuk and Tomčuk, 1966; illustration No. 17): WG from the collecting bunker drops onto the screw conveyor which brings it to the crusher. Rough chips comes out of the crusher as a mixture of needles and particles of wood and bark. It is carried through conveying duct by compressed air into the bunker. At the bottom of the bunker there is a dosing unit by which rough chips is evenly distributed to the first drying column. A mixture of greenery and particles of wood and bark floats in the column space. Stream of hot air is programmed so that only the hardest fraction (particles of wood) can fall down. This fraction is collected into the container below the first drying column, from where it is periodically inserted into the furnace. From the first column, the dried material goes to the second and the third column for further drying. A part of the material that is not sufficiently dried and, therefore is heavier, is separated and via feedback sent for redrying. Completely dried material goes through the cyclone and the dosing unit to the mill in which it turns into flour. It passes through a sieve of 1.5-2 mm diameter and through conveying duct goes to the cyclone with WMF bunker and with line for filling and sewing bags with VMF. Bare branches falls into the press for briquettes which are intended for market or used on-site for own needs. Compared with mentioned plant, Hakkila (1989) describes a slightly smaller, more compact and more mobile chip sorter "SIKO-2". Recently, processing of WG into VMF using the energy of the microwave electromagnetic field, have been described by Posmetjev and Latiševa (2018). The essence of this process is to convert the energy of the microwave electromagnetic field into the heat. According to the authors, with applying their solution one can achieve separation purity of 90-95 % by weight without prior mechanical processing (as made by „ODZ 12A" separator). Unfortunately, this solution has not yet lived in practice, inspite the fact that it has been officially patented in 2007 (Posmetjev *et al*.).

Conclusions

So as to become a raw material, it is necessary to conduct several operations over WG: to gather branchwood from the felling site, to separate WG from branchwood, to gether it in a form suitable for transport and to deliver it to the processing site. Manual preparation of WG is not economically justified due to the low productivity. Relatively high moisture content in fresh WG and its low bulk density make it difficult to find rational solutions with the transport means currently in use in our forestry, whether the raw material is carryng from the forests in bags on pack-horses, or in iron baskets mounted on the skidder. The only economically viable solution is to transport entire trees using wire-rope systems. However, this technological solution rarely meets our forestry practice, and as a such, it does not have a decisive significance in the eventual organization of mass production.

An applicable techno-economic solution assumes the mechanized gathering and palletizing of forest residues in the form of biomass bundles and their delivery to the processing plants. In this way, it significantly shortens the time of primary collection and concentration of the raw material to the processing site, ensures better raw material quality and at the same time increases the bulk density of the load.

Separation of greenery from the wooden skeleton is the most important operation in the phase of preparation. By switching from manual to mechanized production, it significantly increases productivity and reduces production costs.

Of the many products that can be obtained from WG, VMF is especially distinguished as a mass-consumable product in the feeding of domestic animals. Its production is flexible in relation to the source of raw materials - it is possible in mobile and stationary plants, and waste generated during production is usable. Production takes place without use of chemicals and any negative impacts on environment.

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ECONOMIC ASSESSMENT OF THE WATER ECOSYSTEM SERVICE PROVIDED BY FOREST AREAS WITH WATER PROTECTION FUNCTIONS IN NORTHWESTRN BULGARIA

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Abstract
The EU Biodiversity Strategy for 2020 (2011) adopted by the European Commission recommends that Member States map and assess the state of ecosystems and their services in their national territory, assess the economic value of such services, and promote the integration of these values into supervisory and reporting systems at EU and national level. The Bulgarian legislation (Forestry Act, 2011) defines 9 paid services provided by forest areas, as well as the possibility that certain forest areas be designated in regional development plans as areas providing paid ecosystem services. The money collected from these payments are going to be divided among the owners of the forest areas – territorial units of forest and hunting holdings, municipalities, etc. The question should be answered: what a fair income the forest owner should receive from the water protection function as a part of the gross added value created in the country's water sector. The purpose of the latest study is to make an economic assessment of the water ecosystem service provided by forest areas with water protection functions of two municipalities in the North-West region in Bulgaria. The results show that about 2.5% of the cost of water that is used and paid for by water users and water-producers must be given to forest owners. When the water is used for irrigation, the cost of the ecosystem service is 0,007 BGN for 1 m^3 water, for electricity production - 0,003 BGN for 1 m^3 water and for drinking needs - between 0,018-0,026 BGN for 1 m^3 water.

Keywords: *Ecosystem services, Economic assessment, Forest areas, Bulgaria*

Introduction
The importance of ecosystems, their services and sustainable use for long-term human well-being is being increasingly recognised (MA 2005, TEEB 2010). The EU Biodiversity Strategy for 2020, adopted by the European Commission recommends that Member States map and assess the state of ecosystems and their services in their national territory, assess the economic value of such services, and promote the integration of these values into supervisory and reporting systems at EU and national level. (European Commission 2011)
The importance (value) of ecosystems and their services can be expressed in different ways but basically, there are three value domains: biophysical, socio-cultural and economic (Groot et al. 2010, Martín-López et al. 2014).
The Bulgarian legislation (Forestry Act) defines 9 paid services provided by forest areas, as well as the possibility that certain forest areas be designated in regional development plans as areas providing paid ecosystem services. The money collected from these payments are going to be divided among the owners of the forest areas – territorial units of forest and hunting holdings, municipalities, etc. (Forestry Act, 2011)
In Bulgaria, 85% of the water resources are derived from mountain catchments where forests are the predominant type of vegetation, and 60% of irrigable areas are irrigated by water coming from forest catchments. Forest areas provide water resources that account for 60% of the demand for drinking water. (Yovkov, Shuleva, 2011)
The forest areas with water protection functions in Bulgaria cover an area of 248,943 ha, which equals 6.1% of the forest fund. 72.64 % of these territories are state land, 11.41% -

municipal land, 9.89% - private land, and 6.06% - other land. Forests with water protection functions accumulate between 1-1.5 billion m3 of water annually. These forests play the role of a multi-annual equalisation tower which provides an all-year-round uniform flow of presumably clean water, which is brought to water consumers and water users through aquaculture facilities.
In order to obtain this product, the forest owners are obliged to manage them in a special regime. In the water protection regime, the goal is not to get wood from the forest, but to manage it in order to preserve or even increase the water protection systems and water regulating natural properties of forest ecosystems. Every forest owner would only manage his forest as a water protection if he could get income from that property. Such type of management is possible, if the water protection properties of the forest ecosystems become a function of production for the forest owners. The institutions should protect investment in water protection forests so that each forest owner is able to calculate his future incomes through water use and water consumption chain. The question should be answered: what a fair income the forest owner should receive from the water protection function as a part of the gross added value created in the country's water sector. (Yovkov, Shuleva, 2011)
The purpose of the latest study is to make an economic assessment of the ecosystem service „water", provided by forest areas with water protection functions of two municipalities (Varshets, Berkovitsa) in the North-West region in Bulgaria.

Material and Methods

To determine the economic assessment of the water ecosystem service provided by forest areas with water protection functions, the following methodology is used:
1. Determining the reserve of standing wood (V) of the tree species, included in the structure of the plantation, m^3;
2. Determining the current annual growth by volume of the available stand of trees ($Z_v^{тек}$), m^3;
3. Assortment of the timber from current growth;
4. Determining the cash value of the current growth ($Wz_v^{тек}$), BGN;
5. Determining the present value of the permanent costs (FVv), BGN;
6. Determining the net financial contribution of the plantation (NFC) BGN, based on the following formula:

NFC = $Wz_v^{тек}$ – FVv, BGN/hectare,

where: $Wz_v^{тек}$ is the cash value of the current growth for a 10-year period, BGN;

FVv – present value of the permanent costs of production, BGN

7. Determining the rate of return (profit) (RR), % based on the following formula:

$$RR = \frac{NFC}{Wv - Wz_v^{тек}}.100\,,\ (\text{в } \%)$$

RR – rate of return (profit) on investment;
NFC - net financial contribution (NFC) per 1 hectare, for a certain reserve of the stand of trees, BGN/hectare

$Wz_v^{тек}$ - the cash value of the current wood growth for a 10-year period, BGN/hectare;

W_v – the cash value of the reserve of the stand of planted trees, BGN/hectare.
8. Determining the cost of the ecosystem service „water" (CES), BGN for the respective municipality, based on the following formula:

$$\boldsymbol{CES} = RR * P_W^D Q_W^D + RR * P_W^I * Q_W^I + RR * P_W^E * Q_W^E ,$$

where RR is the rate of return (profit) on investment, %;

P_W^D – average market price of drinking water, BGN/m^3;

Q_W^D – quantity of drinking water used per 1 year, m^3;

P_W^I – average market price of irrigation water, BGN/m^3;

Q_W^I – quantity of irrigation used per 1 year, m^3;

P_W^E – average market price of electricity, BGN/KW;

Q_W^E – quantity of water used for the production of electricity per 1 year, m^3;

Results and Discussion

Economic assessment of the water ecosystem service provided by forest areas with water protection functions in the scope of the Varshets Municipality

The forested area of the forest territories in the scope of the Varshets Municipality, which provide the ecosystem service "water" is 1084,4 hectares, which accounts for 9,45 % of the total area of the forest territories in the municipality. The average age of the plants is 104 years, average reserve - 247 m^3/hectare, and the average growth is 2,96 m^3/hectare. The beech is the most common tree species - 987,3 hectares or 91 % of the total forested area (Table 1)

Table 1. Allocation of the forest areas by wood types and estimated productivityof the forest territories in the scope of the Varshets Municipality, providing the ecosystem service "water"

Wood species	Forest area (hectares)						Average
	I P	II P	III P	IV P	V P	Total	Productivity
Beech	51,5	325,9	491,6	104,1	14,2	987,3	2,7
Other	40,7	12,6	33,2	9,6	1,0	97,1	2,7
TOTAL (hectares)	92,2	338,5	524,8	113,7	15,2	1084,4	2,7

*Source: Forest management project of TEF "Petrohan"

The average productivity of all forests, providing the ecosystem service "water" on the territory of the Varshets Municipality is 2,7. The plantations with Average Productivity II account for 50% of the forested area or 524,8 hectares. The prevailing plantations of the VIII class of age (over 140 years) - 499 hectares or 46 %.

The average market price of the timber sold by TEF "Petrohan" in 2018 year are: for deciduous tree species - Large Construction Timber /LCT/ - 111,84 BGN/m3, Medium Construction Timber /MCT/ - 75,47 BGN/m^3, Small Construction Timber /SCT/ - 74,07 BGN/ m^3 and Wood - 65,07 BGN/m^3; for coniferous tree species - LCT - 86,29 BGN/m^3, MCT - 60,00 BGN/m^3, SCT - 47,00 BGN/ m^3 and Wood - 50,00 BGN/m^3.

The results of the separate elements of the methodology for the calculation of the Net Financial Contribution (NFC) are presented in Table 2.

Table 2. Evaluation of the Net Financial contribution of 1 hectare of beech – estimated productivity III on the territory of the Varshets municipality

Age of the stand of trees u	Reserve in standing wood V	Current periodic growth Zv^{cur}	Cash value of 1 m^3 defoliated wood: of standing wood Wv^{av}	Cash value of 1 m^3 defoliated wood: of current growth Wz_v^{av}	Cash value: of reserve in standing wood $Wv=V*Wv^{av}$	Cash value: of current period. growth $Wz^{cur}=Zv^{cur}*Wz_v^{av}$	Administrative: yr. costs v	Administrative: Future value FVv	Net financial contribution: in 10 years NFC	Net financial contribution: in one year $NFC_{yr}=NFC/10$	RR
yr.	m^3	m^3	BGN/m^3	BGN/m^3	BGN	BGN	BGN	BGN	BGN	BGN/yr.	%
80	148,0		64,96	77,02	9613,84		10	130,36	-130,36	**-13,04**	**-0,14**
90	186,5	38,50	65,65	81,12	12243,93	3123,19	10	130,36	2992,83	**299,28**	**3,28**
100	225,5	39,00	66,80	82,21	15062,84	3206,30	10	130,36	3075,95	**307,59**	**2,59**
110	263,0	37,50	68,29	83,30	17961,40	3123,90	10	130,36	2993,55	**299,35**	**2,02**
120	297,5	34,50	70,46	84,50	20960,60	2915,22	10	130,36	2784,86	**278,49**	**1,54**
130	329,0	31,50	72,64	84,86	23897,90	2673,18	10	130,36	2542,82	**254,28**	**1,20**
140	357,0	28,00	75,98	85,23	27125,07	2386,34	10	130,36	2255,99	**225,60**	**0,91**

*Source: Author's calculations.

The economic evaluation of the forest territories, ensuring the ecosystem service „water" on the territory of the Varshets Municipality shows that the largest financial contribution per 1 hectare of beech, which accounts for 91% of the area is 307,59 BGN/year and it is obtained with reserve of the stand of trees – 226 m3/hectare. The rate of return (profit) is 2,59%.

Table 3. Water users and water consumers from the Varshets Municipality

Water Supply and Sewerage operator	Cost of Water BGN/m^3	Cost of Energy BGN/kWh	Cost of Ecosystem Services at RR=2,59% BGN/m^3
"ViK"OOD- Montana	1,01		0,026
"Napoitelni Sistemi"AD, Sofia	0,26		0,007
"ENERGO-PRO Sales" AD		0,13476	0,003

*Source: Accounts of Companies

On the territory of the Varshets Municipality the water users and water consumers will have to pay a price for the ecosystem service „water" between 0,003 and 0,026 BGN for 1 m^3 water consumed (Table 3)
The total cost of the ecosystem service „water" for the Varshets Municipality was calculated based on the following formula:

CES = $0{,}0259*1{,}01*Q_W^D + 0{,}0259*0{,}26*Q_W^I + 0{,}0259*0{,}13476*Q_W^E$,

where P_W^D is average market price of drinking water, BGN/m^3; Q_W^D – quantity of drinking water used per 1 year, m^3; Q_W^I – quantity of irrigation used per 1 year, m^3; Q_W^E – quantity of water used for the production of electricity per 1 year, m^3;

Economic assessment of the water ecosystem service provided by forest areas with water protection functions in the scope of the Berkovitsa Municipality
The forested area of the forest territories in the scope of the Berkovitsa Municipality, which provide the ecosystem service „water" is 6902,2 hectares, which accounts for 32,77 % of the total area of the forest territories in the municipality. The average age of the plants is 112 years, average reserve - 285 m^3/hectare, and the average growth is 2,96 m^3/hectare. The beech is the most common tree species - 5864,4 hectares or 85 % of the total forested area (Table 4)

Table 4. Allocation of the forest areas by wood types and estimated productivity of the forest territories in the scope of the Berkovitsa Municipality, providing the ecosystem service "water"

Wood species	Forest area (hectares)						Average Productivity
	I P	II P	III P	IV P	V P	Total	
White pine	6,9	64,6	93,7	83,2	0	248,4	3,0
Spruce	202,6	51,9	86,5	21,7	7,6	370,3	1,9
Beech	468,4	2927,7	1903,4	391,5	173,4	5864,4	2,5
Other	67,1	73,7	123,9	121,2	33,2	419,1	3,0
TOTAL (hectares)	745,0	3117,9	2207,5	617,6	214,2	6902,2	2,5

*Source: Forest management project of SOF "Berkovitsa"

The average productivity of all forests, providing the ecosystem service „water" on the territory of the Berkovitsa Municipality is 2,5. The plantations of Productivity II and III account for 77% of the forested area. The prevailing plantations of the VIII class of age (over 140 years) – 2630,6 hectares or 38,11 %.

The average market price of the timber sold by SOF "Berkovitsa" in 2018 year are: for deciduous tree species - LCT - 111,84 BGN/m^3, MCT - 75,47 BGN/m^3, SCT - 74,07 BGN/ m^3 and Wood - 65,07 BGN/m^3; for coniferous tree species - LCT - 86,29 BGN/m^3, MCT - 60,00 BGN/m^3, SCT - 47,00 BGN/ m^3 and Wood - 50,00 BGN/m^3.

The results of the separate elements of the methodology for the calculation of the Net Financial Contribution (NFC) are presented in tables 5,6,7.

Table 5. Evaluation of the Net Financial contribution of 1 hectare of beech – estimated productivity II on the territory of the Berkovitsa municipality

Age of the stand of trees	Reserve in standing wood	Current periodic growth	Vash value of 1 m^3 defoliated wood		Cash value		Administrative costs		Net financial contribution		RR
			of standing wood	of current growth	of reserve in standing wood	of current period. growth	yr. costs	Future value	in 10 years	in one year	
u	V	Zv^{cur}	Wv^{av}	Wz_v^{av}	$Wv=V*Wv^{av}$	$Wz^{cur}=Zv^{cur}*Wz_v^{av}$	v	FVv	NFC	$NFC_{yr}=NFC/10$	
yr.	m^3	m^3	BGN/m^3	BGN/m^3	BGN	BGN	BGN	BGN	BGN	BGN/yr.	%
60	108,00		64,70	77,15	6988,06	0,00	24	315,72	**-315,72**	**-31,57**	**-0,45**
70	152,50	44,50	66,13	85,54	10084,42	3806,54	24	315,72	**3490,82**	**349,08**	**5,56**
80	197,00	44,50	67,55	91,10	13307,43	4053,94	24	315,72	**3738,22**	**373,82**	**4,04**
90	244,00	47,00	68,86	94,37	16801,51	4435,54	24	315,72	**4119,82**	**411,98**	**3,33**
100	291,00	47,00	70,17	95,10	20418,57	4469,72	24	315,72	**4154,00**	**415,40**	**2,60**
110	334,00	43,00	73,66	96,30	24601,45	4140,71	24	315,72	**3824,99**	**382,50**	**1,87**
120	373,00	39,00	77,15	97,39	28775,91	3798,08	24	315,72	**3482,36**	**348,24**	**1,39**
130	408,00	35,00	62,24	97,57	25391,88	3414,90	24	315,72	**3099,18**	**309,92**	**1,41**

*Source: Author's calculations.

Table 6. Evaluation of the Net Financial contribution of 1 hectare of white pine – estimated productivity III on the territory of the Berkovitsa municipality

Age of the stand of trees	Reserve in standing wood	Current periodic growth	Vash value of 1 m^3 defoliated wood		Cash value		Administrati		Net financial		RR
			of standing wood	of current growth	of reserve in standing wood	of current period. growth	yr. costs	Future value	in 10 years	in one year	
u	V	Zv^{cur}	Wv^{av}	Wz_v^{av}	$Wv=V*Wv^{av}$	$Wz^{cur}=Zv^{cur}*Wz_v^{av}$	v	FVv	NFC	$NFC_{yr}=NFC/10$	
yr.	m^3	m^3	BGN/m^3	BGN/m^3	BGN	BGN	BGN	BGN	BGN	BGN/yr.	%
40	75,0		31,29	43,41	2346,75	0,00	10	130,36	**-130,36**	**-13,04**	**-0,56**
50	102,5	27,50	35,36	47,59	3623,89	1308,78	10	130,36	**1178,42**	**117,84**	**5,09**
60	130,0	27,50	39,42	52,30	5124,60	1438,20	10	130,36	**1307,85**	**130,78**	**3,55**
70	158,0	28,00	41,42	54,14	6543,57	1515,88	10	130,36	**1385,53**	**138,55**	**2,76**
80	186,0	28,00	43,41	55,98	8074,26	1567,41	10	130,36	**1437,05**	**143,71**	**2,21**
90	214,5	28,50	45,50	57,29	9759,95	1632,86	10	130,36	**1502,51**	**150,25**	**1,85**
100	243,0	28,50	47,59	58,35	11564,83	1662,83	10	130,36	**1532,48**	**153,25**	**1,55**
110	269,0	26,00	49,95	58,87	13435,23	1530,64	10	130,36	**1400,29**	**140,03**	**1,18**
120	292,5	23,50	52,30	58,80	15297,25	1381,72	10	130,36	**1251,37**	**125,14**	**0,90**

*Source: Author's calculations.

Table 7. Evaluation of the Net Financial contribution of 1 hectare of spruce – estimated productivity II on the territory of the Berkovitsa municipality

Age of the stand of trees u	Reserve in standing wood V	Current periodic growth Zv^{cur}	Vash value of 1 m^3 defoliated wood: of standing wood Wv^{av}	of current growth Wz_v^{av}	Cash value: of reserve in standing wood $Wv=V*Wv^{av}$	of current period. growth $Wz^{cur}=Zv^{cur}*Wz_v^{av}$	Administrative: yr. costs v	Future value FVv	Net financial contribution: in 10 years $NFC=Wz^{cur}-FVv$	in one year $NFC_{yr}=NFC/10$	RR
yr.	m^3	m^3	BGN/m^3	BGN/m^3	BGN	BGN	BGN	BGN	BGN	BGN/yr.	%
50	129,9		36,47	49,54	4737,45	0,00	10	130,36	**-130,36**	**-13,04**	**-0,28**
60	175,5	45,55	42,07	53,96	7381,18	2457,79	10	130,36	**2327,43**	**232,74**	**4,73**
70	221,0	45,55	47,70	60,10	10541,70	2737,77	10	130,36	**2607,41**	**260,74**	**3,34**
80	272,4	51,35	47,96	61,68	13061,91	3167,38	10	130,36	**3037,02**	**303,70**	**3,07**
90	323,7	51,35	48,27	63,89	15625,94	3280,51	10	130,36	**3150,16**	**315,02**	**2,55**
100	376,0	52,30	48,22	64,67	18131,81	3382,45	10	130,36	**3252,10**	**325,21**	**2,20**
110	426,0	50,00	49,54	65,73	21102,25	3286,28	10	130,36	**3155,93**	**315,59**	**1,77**
120	474,0	48,00	51,33	65,99	24331,32	3167,45	10	130,36	**3037,09**	**303,71**	**1,44**
140	517,0	43,00	53,96	66,25	27896,29	2848,81	10	130,36	**2718,46**	**271,85**	**1,09**

*Source: Author's calculations.

The economic evaluation of the forest territories, ensuring the ecosystem benefit „water" on the territory of the Berkovitsa Municipality shows that the largest financial contribution per 1 hectare of beech is 415,40 BGN/year and it is obtained with reserve of the stand of trees - 291 m^3/hectare, for the white pine - 153,25 BGN/year at 243 m^3/hectare and for the spruce - 325,21 BGN/year at 376 m^3/hectare. The average rate of return (profit) is 2,54%.

Table 8. Water users and water consumers from the Berkovitsa Municipality

Water Supply and Sewerage operator	Cost of Water BGN/m^3	Cost of Energy BGN/kWh	Cost of Ecosystem Services at RR=2,54% BGN/m^3
"ViK - Berkovitsa" EOOD	0,71		0,018
"Napoitelni Sistemi"AD, Sofia	0,26		0,007
"ENERGO-PRO Sales" AD		0,13476	0,003

*Source: Accounts of Companies

On the territory of the Berkovitsa Municipality the water users and water consumers will have to pay a price for the ecosystem service „water" between 0,003 and 0,018 BGN for 1 m^3 water consumed (Table 8).

The total cost of the material ecosystem service „ensuring the quantity and quality of water" for the Berkovitsa Municipality was calculated based on the following formula:

$$CES = 0{,}0254*0{,}71*Q_W^D + 0{,}0259*0{,}26*Q_W^I + 0{,}0259*0{,}13476*Q_W^E$$

Conclusions

In general, forests and forest areas with water protection functions have real owns and management organizations. The water management function of forests is between 25% and 100% in the multipurpose management target models. The realization to these target models in market economy conditions should be linked to the contribution of each individual investment, incl. and water-related functions. In order to trigger a new price mechanism for the implementation of water management function it is necessary to consider forests not just as a fund (accumulation) but to obtain public recognition of capital. Investing in forests with varying degrees of plumbing in pluralistic ownership has the character of acquiring assets with different yields. The state's constitutional law on water resources does not prevent it from entering into a public-private partnership with the various forest owners. It is also not an obstacle to the economic forestry organizations that it has set up to allocate in the form of a rent income a part of the gross added value of the water business organizations. Moreover, the recognition of market character relation between water-borne forests and water consumption can reduce the pressure on the use of wood. This pressure is now becoming more and more significant and damaging to the water functions of forests. Impacts, water scarcity, impaired drainage modules of forest ecosystems in the period since 1997 are those empirical facts that point to institutional changes in the direction of acquisition of a productive nature of the water protection function of forests and economic assessment of the ecosystem service "water".

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PLANNING THE SIZE OF SAMPLE PLOTS IN THE RESEARCH MACEDONIAN PINE STANDS IN THE STRICT NATURE RESERVATE "MALI BELEG" IN SOUTH SERBIA

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Abstract

Determining the size of the sample plots is a demanding and complex issue and it depends largely on the character of the planned research. The approach of defining the stands as a set of trees was respected in this paper. Methodological procedure on the dendrometric-statistical basis was fully applied. Planning the size of the sample plot started from choosing an optimal number of trees in it using the classical statistical formula to determine the sample size. In even-aged, pure stands of Macedonian pine, optimal number of trees was analyzed on the basis of the predefined level of accuracy and the variation of their properties. In the pre-investigation process the coefficient of volume variation (CV%) was 78.59% based on 145 measured trees at a elementary test surface of 0.20 ha. The density of the stand is determined on the basis of 30 test surface. The optimal number of 371 trees was determined according to the usual level of the desired accuracy of volume determination ($\pm$8%). The size of sample plot of 0.58 ha was established using the principles of proportionality. Derived values can vary within the determined accuracy of the estimated density stand of $\pm$9.10%. The value of the sample size had an orientational character. When determining the surface it was necessary to respect all the factors influencing the definition of the optimal real size of the sample plot.

Keywords: *Planning, sample plot, stand, Macedonian pine, south Serbia*

Introduction

Macedonian pine (*Pinus peuce* Griseb.) belongs to a group of relic, endemic, rare and endangered species of trees (Medarevic, 2006). In Serbia it is represented on a small area within its wider distribution along the mountain range Hajla-Stedim-Zljeb-Beleg-Pogled (Fukarek, 1949).

When researching forest ecosystems choosing adequate sample values is the starting point for successful achievement of the set goal. The problem of sample planning in our conditions is still underdeveloped areas even it has vital role in all aspects of forest management. Generally, forest inventory has an important role in forest management and it is providing necessary high-quality data from forest resources (Georgakis and Stamatellos, 2019). Therewith, sampling is a fundamental postulate of forest inventory (Kershaw Jr, Ducey, Beers and Husch, 2016). While planning a sample size, the basic principle is to achieve the highest precision (accuracy) of estimation at minimal cost (Koprivica, 2004). Sampling design balance between precision and cost constraints (Yang et al., 2017). According to all the difficulties of making decisions on small samples and uneconomical nature of large-sample research, the adequately established value of its size is one of the most significant segments. The procedures for determining the sample plot size are different in case of investigating the characteristics of stands. Sampling may take one of two forms: subjective sampling (experientially) or probability sampling (objectively) (Gyde Lund and Thomas, 1989). Sometimes using statistical sampling is justified in combination with subjective ones (Cunia, 1982). The theory of probability sampling refers to the basic rules of determining them. The basic values that make up a sample depend on its design and refer to the layout, size and number of repetitions. Specific sample size planning solutions have been applied in some studies (Bankovic, 1981; Koprivica, 2004; Pantic, 2002). The paper examined the adequate size of the field (sample plot) in the Macedonian pine stands within the study site. As the subjective methods of establishing sample size are foreseen for short-term research (Gyde Lund and Thomas, 1989), the statistical sampling (probability theory) method has been applied in the paper with reference to the long-term plan for researching Macedonian pine stands.

Materials and methods

The study was performed in south Serbia, Strict Nature Reservate (SNR) of Macedonian pine forest "Mali Beleg" (Figure 1).

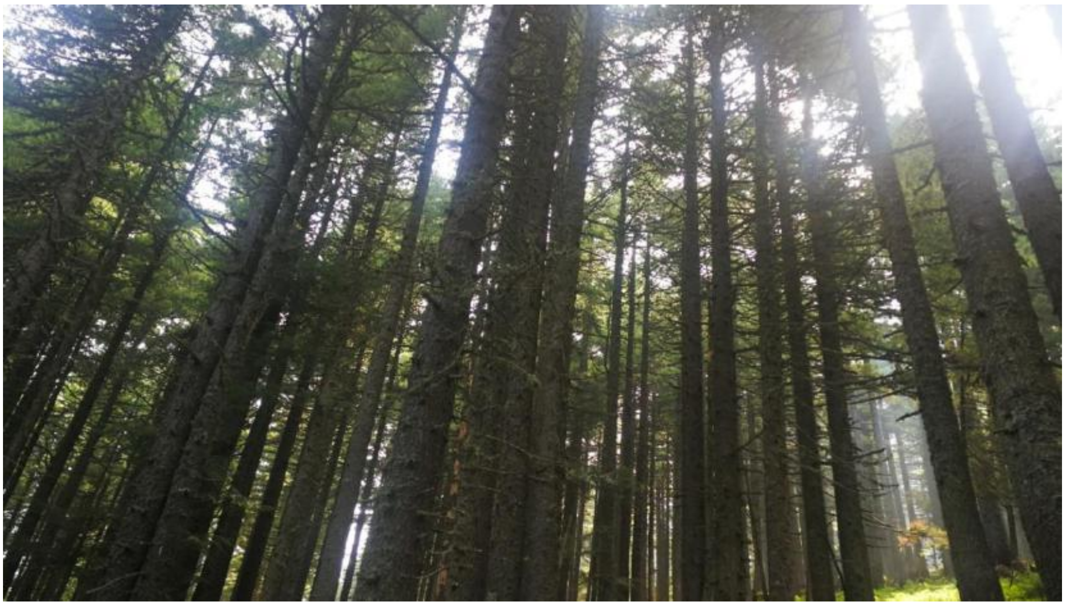

Fig.1. Natural pure stand of Macedonian pine in the SNR "Mali Beleg" (Photo: Popovic Aleksandar, 2019)

The combined statistical-dendrometric method of determining the optimal size of the sample plot was applied. In order to determine the optimal number of trees on the surface, classical formulas (1) were used to determine the size of a simple random sample (Freese, 1962; Hadzivukovic, 1991; Koprivica, 2015; Bankovic and Pantic, 2006).

$$n = \frac{t^2 \cdot CV_{\%}{}^2}{m_{\%}{}^2} \quad (1)$$

An approach was used to define a stand as a set of trees (Koprivica, 1999). The optimal set (number) spatially defined is determined on the estimated density of the stand.

The variation of the characteristics of trees is defined according to 145 trees within an elementary test surface of square shape (0.20 ha). This is based on fact that application of classical formulas requires knowledge of the variability of taxation elements in advance. The values of the optimal number of trees were determined by the coefficient of variation of diameter ($d_{1.3}$), height (h), basal area (g) and volume (v) of individual trees at different levels of desired accuracy ($m_x \rightarrow 5\%$, 8% and 10%). Tested accuracy levels are common and regularly used in forestry researches. Their value depends on the importance and intensity of researches. Tree volume values were determined based on two-way table (Parisko, 1962).

In order to determine the density of the stand *($N \cdot ha^{-1}$)*, 30 test surfaces of a circular shape with a constant surface of 0.05 ha were measured (Figure 2). Surfaces were set up within a predefined target group of stands. There were included pure, even-aged, closed, dense and very dense canopy (0.7; 0.8-0.9; 1.0) stands. The circles were sampled on the principles of random sampling, and all that did not meet the requirements of canopy and mixability were not considered as adequate. The planned optimal sample plot area was determined based on proportionality principles concerning the previously determined optimal number of trees.

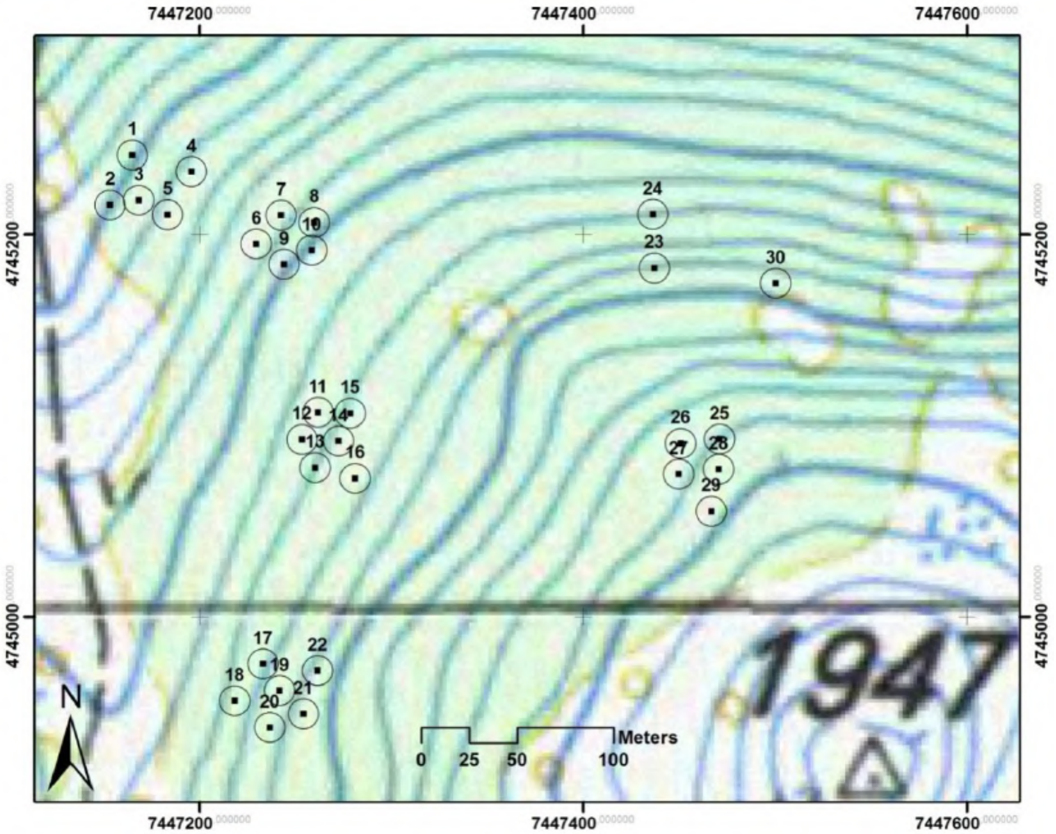

Fig. 2. Random sample test surfaces in pure stands in SNR "Mali Beleg"

According to the formulas for a simple random sample, the relative error of the arithmetic mean (2) of the characteristics of the trees and sampled surfaces was determined:

$$m_{\%} = \frac{CV_{\%}}{\sqrt{n}} \cdot t \quad (2)$$

wherein the:

$m_{\%}$ - relative error of arithmetic mean ($\pm$ in %)

$CV_{\%}$ - coefficient of variation of the studied sample properties (in %)

t - table value (n-1 and 95%)

Descriptive statistics methods in the appropriate software package were applied for the purpose of data processing.

Results and discussion

The elementary test surface was set in slope conditions at an average slope of 22°, north-western exposure (298°), and an altitude of 1.820 m. The stand is pure, dense canopy (0.8-0.9) with even-aged character. The stand has a rainforest character due to its strict protection regime and it was selected as a representative sample of the investigation stands. The average number of trees on the surface is 725 $N \cdot ha^{-1}$.The tree sample has the following statistical parameters (Table 1) of the variation of their characteristics.

Tabela 1. Statistical indicators characteristic of Macedonian pine trees (d, h, g, v)

Descriptive Statistics	**$d_{1.3}$ (cm)**	**h (m)**	**g (m^2)**	**v (m^3)**
Mean	30.47	15.69	0.0821	0.6597
Std. Error of Mean	0.9021	0.2924	0.0046	0.0430
Std. Deviation	10.86	3.52	0.0554	0.5185
Variance	118.00	12.40	0.0031	0.2688
Coef. of Variacion (%)	35.64	22.43	67.47	78.59
Minimum	10.55	4.20	0.0087	0.0356
Maximum	57.40	22.30	0.2586	2.2500
Sum	4,419.00	2,275.00	11.90	95.65

The mean value of the established diameter is 30.47 cm, and the height is 15.69 m. The maximum established diameter is 57.40 cm, and it's height is 22.30 m. The largest variation is shown by the volume values of individual trees, which is 78.59%. The value of the coefficient for the ratio of the variation of volume to diameter in the investigated stand is 2.20. The obtained value corresponds to the established regression relations in previous studies (Hlavcek, 1965 in Koprivica, 1999). The established basal area on the elementary test surface is 11.9 m^2 (59.50 $m^2 \cdot ha^{-1}$) and the volume is 95.65 m^3 (478.25 $m^3 \cdot ha^{-1}$).

Based on a sample of 30 circular test surfaces, the average density of the stands in the reservate was determined. The sample has the following statistical characteristics (Table 2) of the number of trees.

Table 2. Statistical indicators of trees number (*N*) characteristics from test surfaces *(TS_{30})*

Statistics Descriptive	*$N(TS_{30})$*
Mean	31.9
Std. Error of Mean	1.4234
Std. Deviation	7.79

Variance	60.77
Coef. of Variacion (%)	24.43
Minimum	21
Maximum	49
Sum	957

The average number of trees on area of 0.05 ha is 31.9, determined according to the sample with a coefficient of variation of 24.43%. On a total surface area of 1.5 ha, 957 trees were identified, where average is 638 $N \cdot ha^{-1}$. The number of trees on the surfaces ranges from 21 (min) to 49 (max).
The differences in the values of the average number of trees from the surfaces (87) are due to the different shapes and size of the samples on which they were determined.
In order to conduct the research with a known level of statistical significance, relative errors of arithmetic means of the studied taxation characteristics were determined on the basis of the starting sample (Table 3).

Table 3. The relative error of determining the arithmetic mean of the sampled trees

Standard Error of Mean (± %)	**Characteristics of trees**			
	$d_{1.3}$	**h**	**g**	**v**
P=95%; **n=144;**	5.80	3.28	10.98	12.79

The largest error in the arithmetic mean was found at value (±12.79%), as expected due to the largest variation in the volume of individual trees. The smallest value obtained is the relative error of height estimation (±3.28%), which is ± 0.51 m in absolute values. The relative error of mean diameter is ±5.8% (±1.76 cm).
The relative error of determining the average number of trees based on a sample of 30areas at a significance level of 95% is ±9.10%. It is ±58 trees on estimated amount of 638 $N \cdot ha^{-1}$. The estimated density stands is in the range of 580 to 696 trees per hectare. The values determined this way will form the basis of the calculation of optimal surface of the sample plot.
The established error values are within the limits that can be considered as acceptable according to character of the research. Therefore, the sample can serve as a reliable basis for determining the optimal number of trees on the sample plot (Table 4).

Table 4. Optimum number of trees calculated on different basis and levels of desired accuracy

Optimal number of trees on the sample plot ($N_{opt.}$)				
$m_{x\%}$	Basis of calculation			
	$d_{1.3}$	**h**	**g**	**v**
5	195	62	700	949
8	76	24	273	371
10	49	16	175	237

The smallest values were determined on basis of height (16, 24, 62), while the highest values were obtained on volume basis (949, 371, 273). Values were determined in the target at different levels of desired accuracy ($m_{x\%}$).

Considering that the primary precision is to determine the volume, the basis for calculating the optimal surface was the determined by value of this parameter. The area was determined on the ration of the determined number of trees per hectare and the optimal number of trees according to the coefficient of variation (volume) of individual trees (3) at different levels of desired accuracy ($m_{x\%}$).

$$P_{opt.} = N_{opt.(V)} / N \cdot ha^{-1} \quad (3)$$

$P_{opt.} = 1.49\ ha$ (m_x=5%)

$P_{opt.} = 0.58\ ha$ (m_x=8%)

$P_{opt.} = 0.37\ ha$ (m_x=10%)

Derived values can vary within the determined accuracy of the estimated density stands of $\pm$ 9.10%.

Conclusion

The paper establishes indicative values of the size of the sample plot in order to estimate the volume of pure and even-aged stands of the Macedonian pine at SNR "Mali Beleg". The determined surface values vary depending on the desired level of accuracy of the estimated results. At the level of 5% the area ranges from 1.36 ha to 1.63 ha. At the usual level of accuracy volume estimates in Serbia of 8% the area ranges from 0.53 ha to 0.64 ha. At the level of accuracy of 10% it ranges from 0.34 ha to 0.41 ha. In order to estimate the average diameter, it is necessary to measure from 49 to 193 diameters and to estimate the mean height it is necessary to measure from 16 to 62 heights.

The values obtained for estimating the mean height stand are relatively low what is a consequence of the low height variation as an expression of specific stand and habitat conditions. The values have orientational character and are related to the target group of stands at the studied site. In addition to the determined values of the sample size, it is necessary to determine the schedule and the necessary number of repetitions when forming the optimal plan of the experiment. The mentioned values depend on the purpose of future research and that was not the subject of this paper.

While deciding the final sample size, different factors must be included that affect the successful implementation of the research. This particularly refer to financial frameworks which limits every research. The obtained values are applicable only within the given frameworks and conditions of the researching stands of the Macedonian pine in the SNR "Mali Beleg".

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VARIABILITY OF CONCENTRATION OF PHOTOSYNTHETIC PIGMENTS IN LEAVES OF SYCAMORE MAPLE (*Acer pseudoplatanus* L.) ONE-YEAR-OLD SEEDLINGS OF DIFFERENT HALF-SIB LINES

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Abstract

The variability of concentration of photosynthetic pigments in leaves of Sycamore maple (*Acer pseudoplatanus* L.) one-year-old seedlings was studied at the level of 11 half-sib lines in the seedling nursery conditions. Three types of pigments have been examined: chlorophyll a, chlorophyll b and carotenoids. Their concentration at the level of the half-sib lines as well as the interdependence was determined. The seedlings were produced in the seedling nursery of the Institute of Forestry in Belgrade (Serbia) in equal environmental conditions and from the seed of known origin. The leaf sampling was carried out in the middle of the growing season. The highest average value of chlorophyll a (0.878 mg/g) was determined in the half-sib line 9, and the lowest in the half-sib line 7 (0.496 mg/g). The highest average value of chlorophyll b (0.952 mg/g) was determined in the half-sib line 5, and the lowest in the half-sib line 11 (0.568 mg/g). The highest average value of carotenoids (0.462 mg/g) was determined in the half-sib line 9, and the lowest in the half-sib line 8 (0.197 mg/g). The strongest interdependence was determined between carotenoids and chlorophyll b (R^2 =0.0053), and the weakest between carotenoids and chlorophyll a (R^2 =0.0005). The researches have shown that the concentration of the photosynthetic pigments in the Sycamore maple leaves changes depending on the origin, or in this case the mother tree. The conclusion arising from the obtained results is that during the selection of mother trees the attention has to be paid to concentration of the photosynthetic pigments in leaves and the intensity of the photosynthesis because that will significantly affect the elements of seedlings' development.

Key words: *Sycamore maple, half-sib line, photosynthetic pigments, leaves.*

Introduction

The Sycamore maple (*Acer pseudoplatanus* L.) is the species that has been present on European continent since glacial (Svenning, J.C., Skov, F., 2004, 2005). It is widespread in parks as a decorative species. Forest plantations of Sycamore maple are rare in Serbia (Isajev, V., Mančić, A., 2001). As in their studies stated Ivetić, V., Tucović, A. (2003), "...the analysis of the variability of trees within the species in nature (Jovanović, B., 1967) and cultivated stands (Bojović, S., 1989) has been conducted since 1985 and for that reason this species lagging behind other species in terms of scientific researches...". Forestry as a science pays greater attention to studying the Sycamore maple in recent years.

Importance of photosynthetic pigments is reflected in the absorption of light necessary for the process of photosynthesis in plants. Chlorophylls are the primary photosynthetic pigments. They reflect green part of the spectrum of visible light, while carotenoids reflect yellow, orange or red part of the spectrum. The ability to absorb certain wavelengths of light is more important for the process of photosynthesis than the ability of reflection of the part of the light (Popović et al., 2015). Due to different size and shape as well as different concentration of pigments, the leaves have different ability to absorb visible light and to transform it into energy of chemical bonds (Čivić et al., 2003). A foliar concentration of the main photosynthetic pigments chlorophyll **a** and **b** is considered to be a bio-indicator of the total primary production of biomass (Gitelson, A., Merzlyak, M. N., 1994).

Bearing in mind that beside transforming light energy into organic compounds the concentration of the photosynthetic pigments affects the dominant coloration of higher plants, and increased adaptability of trees and stands, and is important as a raw material for pharmaceutical industry, so the knowledge on variability within the species is of increasing importance (Mataruga et al. 2000). The determination of the concentration of the photosynthetic pigments in leaves is justified because the intensity of photosynthesis depends greatly on it and also significantly influences the growth and development of seedlings.
The aim of the research in this paper is to determine how the origin of the seed which the seedlings were produced from affects the concentration and variability of photosynthetic pigments in the leaves of the Sycamore maple (*Acer pseudoplatanus* L.) one-year-old seedlings.

Material and Method

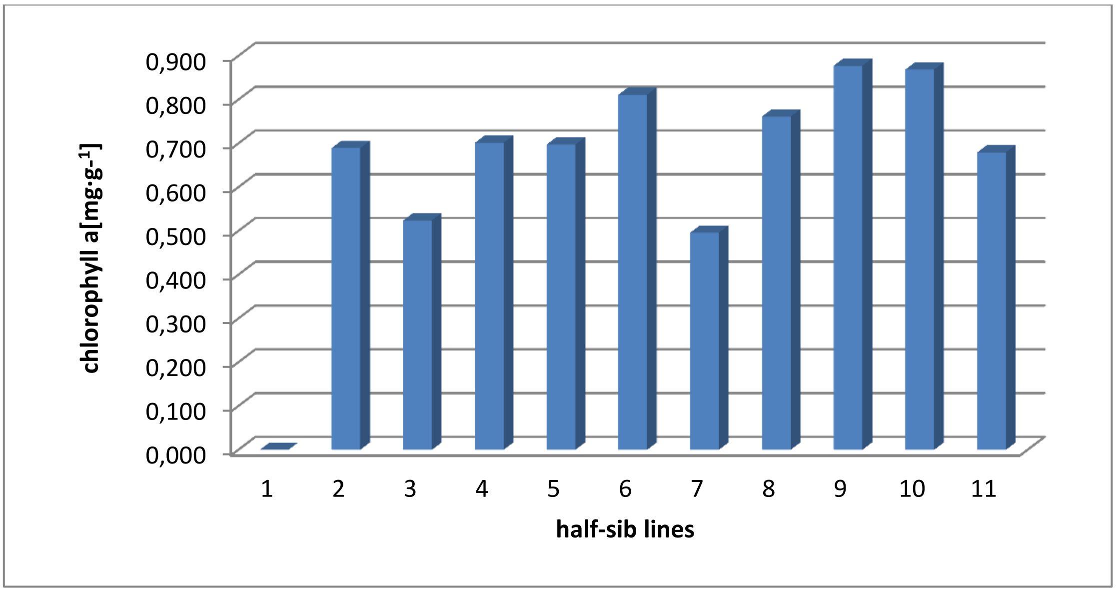
The seedlings were produced from the seed collected from micro-populations on the territory of Belgrade (Serbia). The seed was stratified by putting in cold, moist in sand for 5 months and in that way prepared for the spring sowing. The sowing was conducted in April 2016 in the seedling nursery of the Institute of Forestry in Belgrade (Serbia), in a hotbed size 1x10 meters. The space between rows in the hotbed is 15 cm, and the rows are parallel with the longer side of the hotbed.
Leaf sampling of Sycamore maple seedlings was conducted in the last week of July 2017. A sample of 1 g was homogenized using a mortar and pestle. For better homogenization of the sample 2 g of quartz sand was added in the mortar before mechanical grinding. The paste was for 3 minutes treated with 15 ml of 80% acetone. To this mixture was added 1 mg of $MgCO_3$ in order to prevent acidification of the solution. The resulting green solution was applied by a small glass rod on a glass filter and using a water spray vacuum pump it was filtered into the vacuum test tube. The resulting filtrate was the pigment extract which is transferred from the test tube to the regular 25 ml vessel and supplemented with 80% acetone to the line. To perform reading in a spectrophotometer the obtained extract has to be diluted. 1 ml of the obtained extract was taken by pipette and into that was added 9 ml of acetone and then it was transferred to the test tube. Thus prepared extract was poured into the cuvette and read on the spectrophotometer, the absorption was at the wavelengths 662, 644 and 440 nm. The formula of Holm and Wetsttein was applied to calculate the concentration of the pigment in the extract in mg / dm^3.
Preparing and reading on the spectrophotometer as well as calculating was performed using standard methods (Oljača and Srdić, 2005).

Results and Discussion

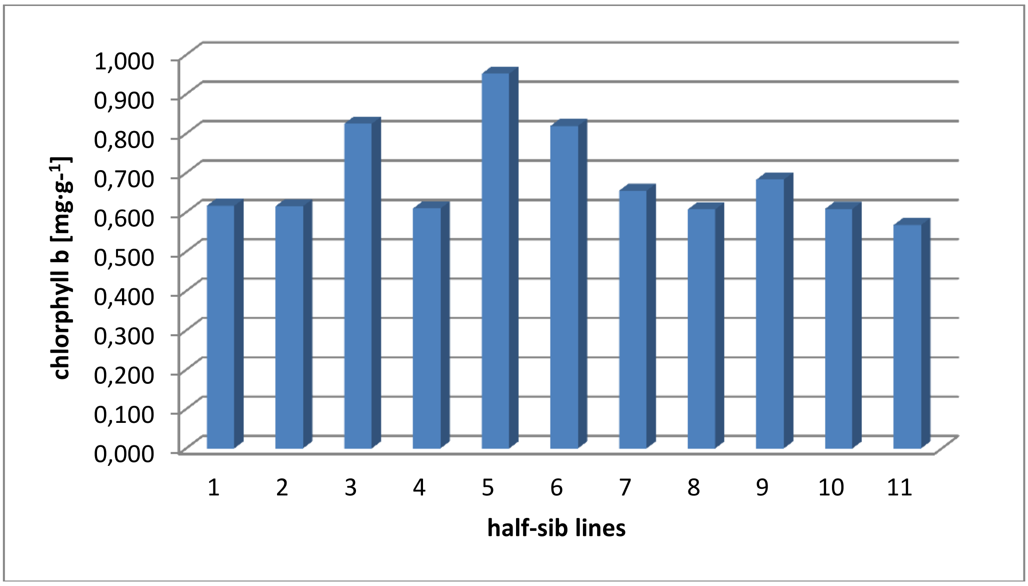
The review of the concentration of photosynthetic pigments in the leaves of the one-year-old Sycamore maple seedlings as per half-sib lines is shown in the Table 1 and Graphs. Based on the results can be concluded that the concentration of photosynthetic pigments changes depending on origin of the seed. The highest average value of chlorophyll a (0.878 mg/g) was determined in the half-sib line 9, and the lowest in the half-sib line 7 (0.496 mg/g). The highest average value of chlorophyll b (0.952 mg/g) was determined in the half-sib line 5, and the lowest in the half-sib line 11 (0.568 mg/g). The highest average value of carotenoids (0.462 mg/g) was determined in the half-sib line 9, and the lowest in the half-sib line 8 (0.197 mg/g).

Table 1. The concentration of photosynthetic pigments in the leaves of the Sycamore maple seedlings as per half-sib lines

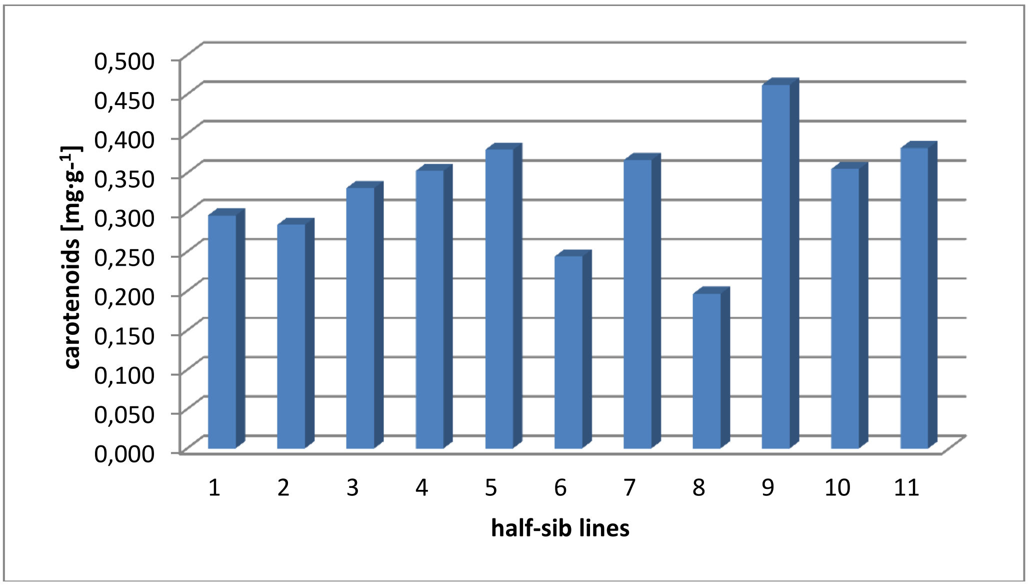
Half-sib line	Chlorophyll a mg/g	Chlorophyll b mg/g	Carotenoids mg/g
1	0.797	0.617	0.296
2	0.689	0.615	0.284
3	0.524	0.825	0.331
4	0.701	0.610	0.353
5	0.698	0.952	0.380
6	0.811	0.819	0.244
7	0.496	0.655	0.366
8	0.761	0.607	0.197
9	0.878	0.683	0.462
10	0.869	0.609	0.355
11	0.679	0.568	0.382



Graph 1. Concentration of chlorophyll a in leaves of Sycamore maple seedlings as per half-sib lines

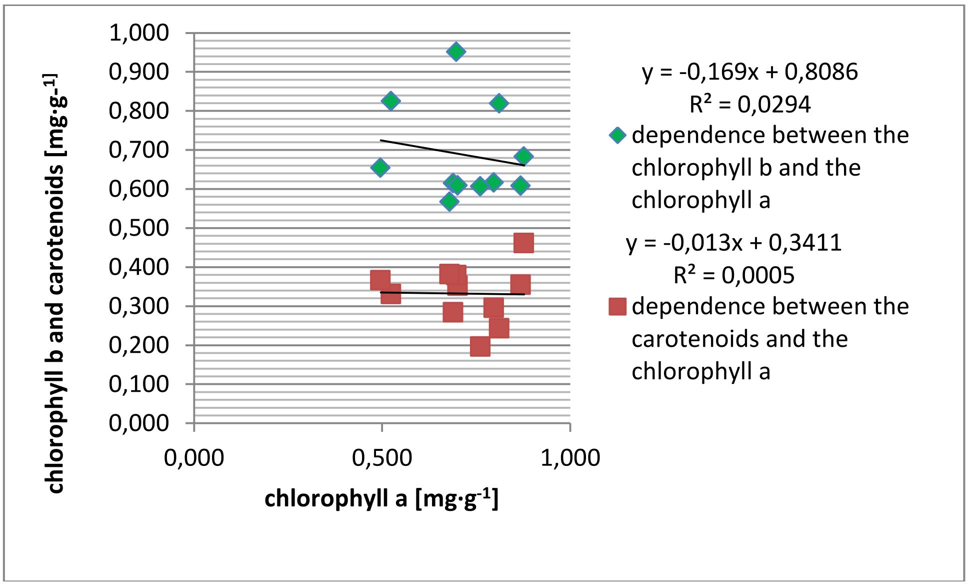


Graph 2. Concentration of chlorophyll b in leaves of Sycamore maple seedlings as per half-sib lines

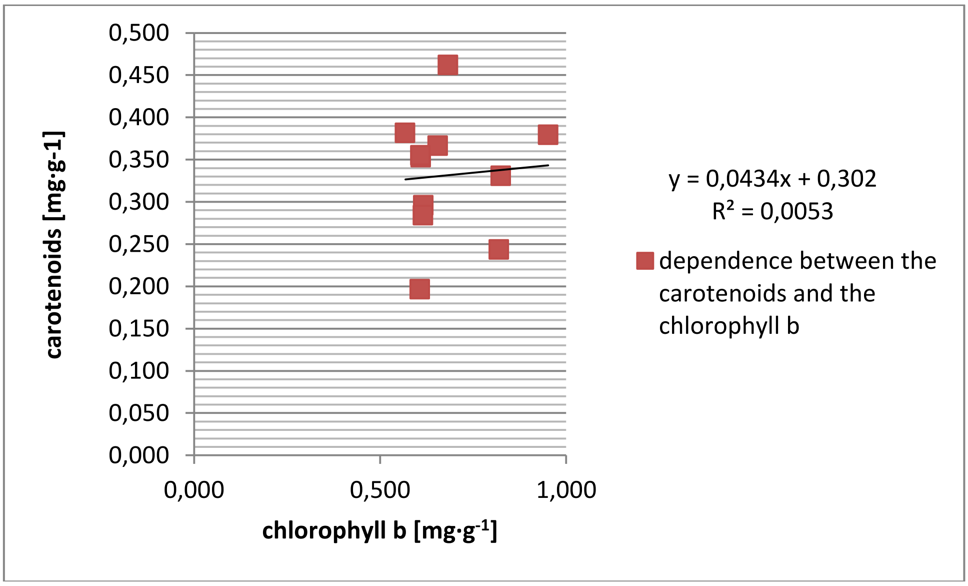


Graph 3. Concentration of carotenoids in leaves of Sycamore maple seedlings as per half-sib lines

The Graphs 4 and 5 show the interdependence of the studied photosynthetic pigments. The strongest interdependence was determined between carotenoids and chlorophyll b (R^2 =0.0053), and the weakest between carotenoids and chlorophyll a (R^2 =0.0005).



Graph 4. Dependence of the content of chlorophyll b and carotenoids on the content of chlorophyll a



Graph 5. Dependence of the content of carotenoids on the content of chlorophyll b

Given that the environmental conditions in the seedling nursery are equal, the determined differences in the concentration of pigments between seedlings of different half-sib lines are probably due to their genetic constitution. The existence of these differences can be used when selecting the best genotypes, mother trees, and breeding in general in order to maximize the use of the available gene pool of trees.

Conclusion

The obtained results have shown that the concentration of the photosynthetic pigments in the leaves of the Sycamore maple one-year-old seedlings changes depending on their origin. The significant amounts of pigments were found in the all analysed half-sib lines, but, as a rule, the woody plants have the surplus of pigments. The performed analyses confirm the paying attention to the physiological characteristics when selecting and using mother trees for the planned seed production. For a more comprehensive understanding of importance of knowing the concentration of pigments, it would be good to determine their concentration in different periods of growing season, as well as the interdependence of the concentration of pigments and the elements of seedlings' growth.

Acknowledgements

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ANALYSIS OF YIELD QUALITY OF BALD CYPRESS (*Taxodium distichum* L. Rich.) AT THE LEVEL OF SEED STAND RS-2-2-tdi-00-240

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Abstract

Yield quality analyses of Bald cypress at the level of the seed stand RS-2-2-tdi-00-240 have been presented in the paper. Cones were collected from 20 mother trees evenly distributed throughout the seed stand. Further processing and yield quality analysis were evaluated in the laboratory of the Institute of Forestry in Belgrade (Serbia). . The following yield quality parameters were analyzed: coefficient of extraction, absolute seed weight, and technical germination in the field trial. The obtained results showed that average value of the coefficient of extraction per mother tree ranges from 13.14% to 17.38%. The absolute seed weight ranges from 96.5 g to 134.7 g and the technical germination ranges from 57.2% to 73.4%. The analyzed yield quality indicators are very important for planning nursery production because knowledge on these indicators can considerably influence the optimization of production costs. The analyzed yield quality indicators can also be used to assess the genetic quality of seed stand and to make recommendation for the transfer of seeds. The obtained results serves for improvement of mass production of high quality reproductive material of Bald cypress in Serbia and are good starting point for the future breeding program in forestry. .

Key words: Bald cypress, seed stand, yield, germination.

Introduction

Bald cypress naturally grows on flooded and periodically flooded soils in the southeast and Gulf areas of the United States, from Louisiana to Florida, Mexico and southeastern China. The Taxodium genus has been widespread in Europe and North America in the past. During the Holocene era in the Balkans was occurred numerous forest formations rich in species and valuable genetic resources (Alexandrov and Velkov, 2000). Some of these formations, originating from the Paleozoic era, were located in the Dinaric Alps, the Rhodope massif and a part of the Traki-Macedonian Mountains. The presence of the Taxodium genus (Palamerov, 1997) was recorded during the middle and late Oligocene era (Palamerov, 1997). Paleo-palynological examinations of pollen and spore samples from the wells in the Kolubara-Tamnava lignite basin showed that this area was within the wetland region, and the main representatives of forest vegetation were swamp conifers of the Taxodium genus and swamp broadleaves of the Nyssa genus (Dražić and Batos, 2002). The same research was conducted in the Rhine hard coal mine in Germany where the presence of identified species was determined with the domination of the species of the Taxodium genus. According to environmental and botanical characteristics the four species stand out on the stands in the natural area of distribution: *Taxodium distichum* L. Rich., *Taxodium ascendens* Brog., *Taxodium mucronatum* Ten. and *Taxodium heterophyllum* Brog. The first two species grow in USA, the third in Mexico, and the fourth in China.

It was introduced to Europe in 1640 on extremely humid soils and in areas with high underground or residual water. It is mostly grown as an ornamental species, but it can also be used for raising forest plantations (Vidaković, 1982). In Serbia mainly grows *Taxodium distichum* L. Rich. in the urban green areas (Belgrade, Novi Sad, Vršac etc.), although *Taxodium ascendens* Brog is also described in the park of Koviljaca spa (Tucović and Ocokoljić, 2005).

In the 1950s, Bald cypress as an allochthonous conifer species on the territory of the former Yugoslavia has come into focus of the professional public (Petrović, 1951; Špiranec, 1959). At the beginning of the 1970s, authors Tucović and Stilinović concluded that in our environmental conditions Bald cypress can be considered as a species of rapid growth, one of the rare coniferous species that can be suitable for introduction of conifers in lowland environments, and especially, flooded habitats where it can achieve high productivity. In Serbia, Bald cypress that grows in swamp plays an important role in horticulture where it is grown in groups or as soliter tree on wet terrains where other conifers fail.
The interest of the scientific community in Serbia for Bald cypress has been intensified recently, so the specificities and genetic variability of adult Bald cypress trees, as well as the characteristics of seeds and seedlings at the earliest stages of development have been recorded (Vukićević, 1987; Ninić-Todorović and Ocokoljić, 2001; Šijačić-Nikolić et al., 2011; Popović et al., 2012; Popović et al., 2012a; Popović et al., 2012b; Popović, V. et al., 2012c; Popović, V. et al., 2012d; Popović, V. et al., 2013; Popović, V., 2014; Popović, V. et al., 2014; Popović, V. et al., 2014a; Popović, V. et al., 2014b; Popović, V. et al., 2015;).
The aim of the study in this paper is to analyze the quality of Bald cypress (*Taxodium distichum* L. Rich.) yield at the level of the seed stand RS-2-2-tdi-00-240. The obtained results can serve for acquiring preliminary knowledge on genetic variability of the studied seed stand and for the improvement of the production of high quality seed and seedlings of Bald cypress in Serbia.

Material and Method

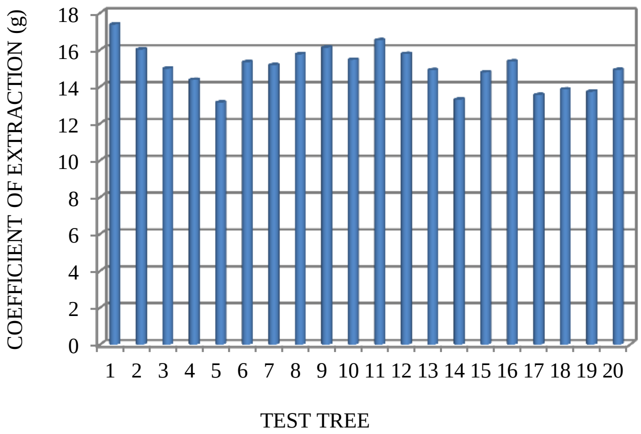
Cone sampling was performed in the seed stand RS-2-2- tdi-00-240 at the level of 20 test trees, 300 cones per tree were collected. The cones were manually harvested using hydraulic lifting platform and grouped into special bags for easier handling and proper monitoring of further studying. Afterwards they were transported to further processing at the Laboratory for Genetics, Seed Production, Physiology and Plant Breeding at the Institute of Forestry in Belgrade (Serbia). Fifty cones per each of 20 test trees were selected using a method of random sampling for the yield quality and cone processing analysis.The cones were then transferred to a dryer where they were heated at 40° C for 48 hours. After the cones opened the seed was separated by test trees. The absolute seed weight and the coefficient of extraction for each test tree were then measured. The weight of seeds and cones was measured on an electronic scale with an accuracy of 0.01 g.
In April next year the seed was sown in treset ???containers of type Bosnaplast 12 filled with the Tref TPS fine brown substrate in order to determine the field germination. The containers were disinfected before sowing with Benomyl solution. Four hundred seeds were sown per each test tree. The seeds were immersed in a citric acid solution of 100 ppm concentration before sowing and left to soak or to dry or in petri dish…please specify ??? for 48 hours. The temperature during germination was 18-20° C, and the germination rate was monitored for 28 days.

Results and Discussion

The values of the coefficient of extraction of 20 Bald cypress test trees respectively are shown in Table 1 and Graph 1. The coefficient of extraction is important in the process of planning the appropriate seed sampling rate. Coefficient of extraction stands for a quotient of the total seed weight and the total cone weight per one test tree. Correctly determined coefficient of extraction facilitates and ensures seed and seedling production. In addition to the production significance, it can also be used in determining the variability of the yield quantity between the test trees. The obtained results of the analysis shows variability of the coefficient of extraction between 20 Bald cypress test trees. The highest value of the coefficient of extraction was determined in the test tree number 1 (17.38%), and the smallest in test tree number 5 (13.14%).

Table 1: Coefficient of extraction

Test tree	Cone weight (kg)	Seed weight (g)	Coefficient of extraction (%)
1	3742	651	17.38
2	3540	567	16.02
3	4112	616	14.98
4	4322	621	14.37
5	3911	514	13.14
6	3424	525	15.33
7	3654	552	15.17
8	3821	602	15.76
9	4101	661	16.12
10	4082	631	15.46
11	3566	589	16.52
12	3621	572	15.78
13	3765	561	14.90
14	3828	509	13.30
15	4255	629	14.78
16	3910	601	15.37
17	4221	572	13.55
18	3992	553	13.85
19	4312	592	13.73
20	3898	581	14.91

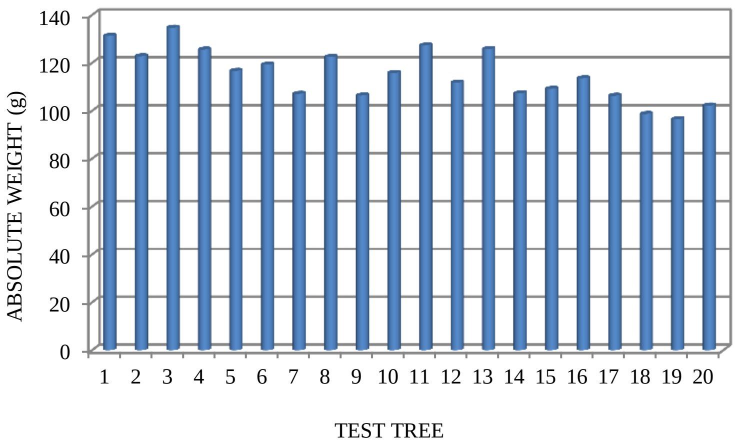


Graph 1: Variability of the coefficient of extraction

In the Table 2 and Graph 2 the values of the absolute seed weight at the level of 20 Bald cypress test trees were shown.

Table 2: Absolute seed weight

Test tree	Absolute seed weight (g)
1	131.3
2	122.8
3	134.7
4	125.6
5	116.7
6	119.3
7	107.1
8	122.5
9	106.4
10	115.8
11	127.3
12	111.8
13	125.8
14	107.3
15	109.2
16	113.6
17	106.3
18	98.7
19	96.5
20	102.2



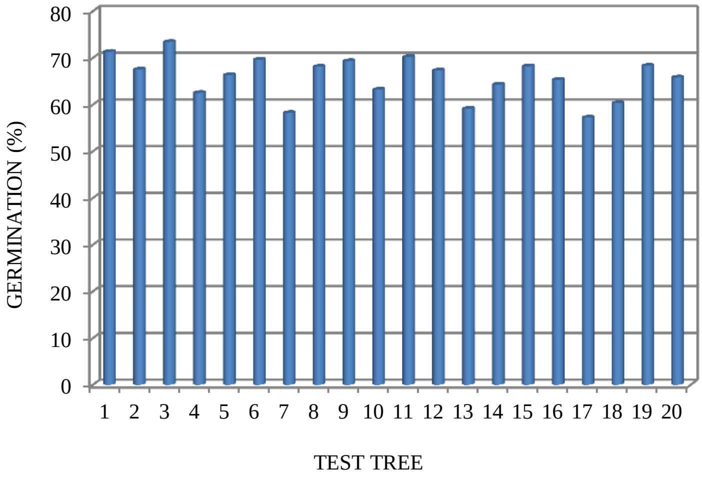
Graph 2: Variability of the absolute seed weight

The absolute seed weight is the weight of 1000 air-dried seeds expressed in grams. For the planning of nursery production, the knowledge of the absolute seed weight is of particular importance. In addition, it can be used to determine the variability of the yield quantity between the test trees, as well as the variability within population. The analysis of the obtained values of the absolute seed weight at the level of 20 Bald cypress test trees shows that the highest value was determined in the test tree number 3 (134.7 g), and the smallest in test tree number 19 (96.5 g).

Table 3 and Graph 3 show the values of technical germination in percentages at the level of 20 Bald cypress test trees. Knowing the percentage of seed germination is necessary when determining the sowing norm, and can also be used to determine the variability of the yield quality between the test trees. According to studies of many authors (Monk, C. D., Brown, T. W., 1965; USDA Forest Service, 1965, 1974; Faulkner, S. P., 1982; Gunderson, L. H., 1984; Krauss, K. W. et al., 1998) the natural germination of Bald cypress ranges from 2 to 15%. Stilinović, S. (1985) stated that in the conditions of Serbia the Bald cypress full-seed is up to 50%.

Table 3: Technical seed germination

Test tree	Technical germination (%)
1	71.3
2	67.5
3	73.4
4	62.5
5	66.3
6	69.6
7	58.2
8	68.1
9	69.3
10	63.2
11	70.2
12	67.3
13	59.1
14	64.3
15	68.2
16	65.3
17	57.2
18	60.3
19	68.3
20	65.8



Graph 3: Variability of technical germination

The analysis of germination at the level of 20 Bald cypress test trees showed that the germination ranges from 57.2% in the test tree number 17 to 73.4% in the test tree number 3. If these values compare with the literature data, it can be concluded that the studied test trees have germination above average. Such a high germination rate is probably the result of pre-sowing seed treatment. The seed was immersed in the citric acid solution of 100 ppm concentration for 48 hours before sowing (Popović, V. et al., 2012c). Based on the obtained results this pre-sowing seed treatment can be recommended in regular production of Bald cypress seedlings.

Conclusions

The research carried out in the seed stand showed that Bald cypress has been well adapted to the conditions of the habitat in this locality, and that the yield quality is mostly conditioned by climate factors. The obtained results indicate the high gene-environmental potential of Bald cypress at the level of the seed stand as well as the pronounced variability of the analyzed characteristics at the level of the test trees.

The conducted analyzes point to the satisfactory seed yield quality at the level of the seed stand, which represents a good starting point for the improvement of mass production of seed of this species in Serbia.

The applied pre-sowing seed treatment method, described in this paper, contributes to the increase of seed germination, and the development of the technology of production of planting material can in many ways contribute to the mass production of Bald cypress in Serbia.

Acknowledgements

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VASCULAR PLANT DIVERSITY AS A VALUABLE TOOL FOR THE ECOLOGICAL AND CULTURAL PROJECTION OF THE NATIONAL PARKS IN GREECE

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Abstract

National Parks are natural areas that usually possess high ecological and cultural value. Their intact landscapes, the representativeness of their natural ecosystems and the high diversity of their biota give them outstanding ecological, aesthetic, cultural, educational and scientific values. They have high conservation value and attract numerous visitors aiming to experience nature. A recent project co-funded by the European Union and the Greek National Funds is AdVENt*: Augmented Visitor Experience in National Parks*, started in 2018 and has a 3-year duration. The AdVENt project aims to develop innovative applications to enhance visitors touring experience in protected areas of particular environmental interest and natural beauty, as well as the production of rich and technologically advanced multimedia content for their promotion, to highlight their natural wealth as a remarkable and attractive touristic product. More specifically, the project focuses on the mountain complex of the Region of Central Greece, which includes the National Parks of Oiti and Parnassos. Both areas host a remarkably diverse flora and fauna, while Mt Parnassos is considered as a hotspot of plant species diversity and endemism. Important archaeological sites like Delphi, major ski centers and popular hiking trails are combined with remarkable natural fir forests and other mountain habitat types and a rich endemic flora. It is noteworthy, that AdVENt project will create: a) a vascular plant database of the National Parks of Oiti and Parnassos, available to the scientific community and the general public, which is expected to promote research in the relevant fields of botany, ecology etc., b) an integrated Augmented Reality (AR) mobile application for enhancing the visitors touring experience and c) the development of cutting-edge technology for the visual identification of vascular plant species.

Keywords: *Vascular plants, Application, Augmented Reality, National Parks, Natura 2000.*

Introduction

Mediterranean Basin is characterized by high geomorphological and climatic heterogeneity and has been acknowledged as one of the 18 biodiversity hotspots worldwide (Blondel & Aronson, 1999). Mediterranean mountains host an especially high plant diversity and endemism, rendering them an ideal stage for biodiversity and biogeographical studies. The flora of the Mediterranean Basin includes approximately 25,000 species or nearly 30,000 species and subspecies (Médail, 2008), a fact that renders it the richest among regions hosting Mediterranean ecosystems.

Greece, located at the crossroad of three continents, namely Europe, Asia and Africa, is an important component of the Mediterranean Basin hotspot (Sainz Ollero, Moreno Saiz 2002; Martín-Brano et al. 2010). The largest part of Greece is characterized by a typical Mediterranean climate with mild, humid winters and hot, dry summers although differences can be found regionally or locally due to the diversity of its topography (Hobbs et al., 1995). It is noteworthy, Greece hosts a large variety of Mediterranean habitats included in the reference list of the Natura 2000 initiative with an outstanding biodiversity, which is well protected within the boundaries of Greece's National Parks. National Parks are natural areas that usually possess high ecological, aesthetic, cultural, educational and scientific values. They host a high conservation value and attract numerous visitors aiming to experience nature. The vascular flora of Greece comprises 5758 species and 1970 subspecies (native and naturalized), representing 6620 taxa, belonging to 1073 genera and 185 families (Dimopoulos et al. 2013, 2016). The endemic vascular flora of Greece includes 1459 taxa (22% of the total number of taxa in Greece), corresponding to 1,274 endemic species (22.1% of the total number of Greek species) and 450 endemic subspecies (22.8% of the total number of Greek subspecies) (Dimopoulos et al. 2013, 2016). In order to achieve an increased direct and vicarious experience of nature for visitors in Oiti and Parnassos National Parks, a recent project co-funded by the European Union and the Greek National Funds AdVENt (*Augmented Visitor Experience in National Parks*) started in 2018 and has a 3-year duration. The AdVENt partnership includes: a) the inDigital SA, Greece (Coordinator), b) Institute of Mediterranean and Forest Ecosystems, Hellenic Agricultural Organization ″DEMETER″, Greece, c) Research and Innovation Information Technologies ″ATHENA″, Greece and d) Organization for Sterea Ellas Greece.

Project area

The project focuses on the mountain complex of central Sterea Ellas, which includes the National Parks of Oiti (Fig. 1) and Parnassos (Fig. 2). These National Parks**,** include remarkable natural and cultural richness, such as hiking trails, dense fir forests, endemic and rare plant species, landscapes of special natural beauty, historical and archaeological sites, as well as popular tourist destinations (Delphi and Parnassos ski center).

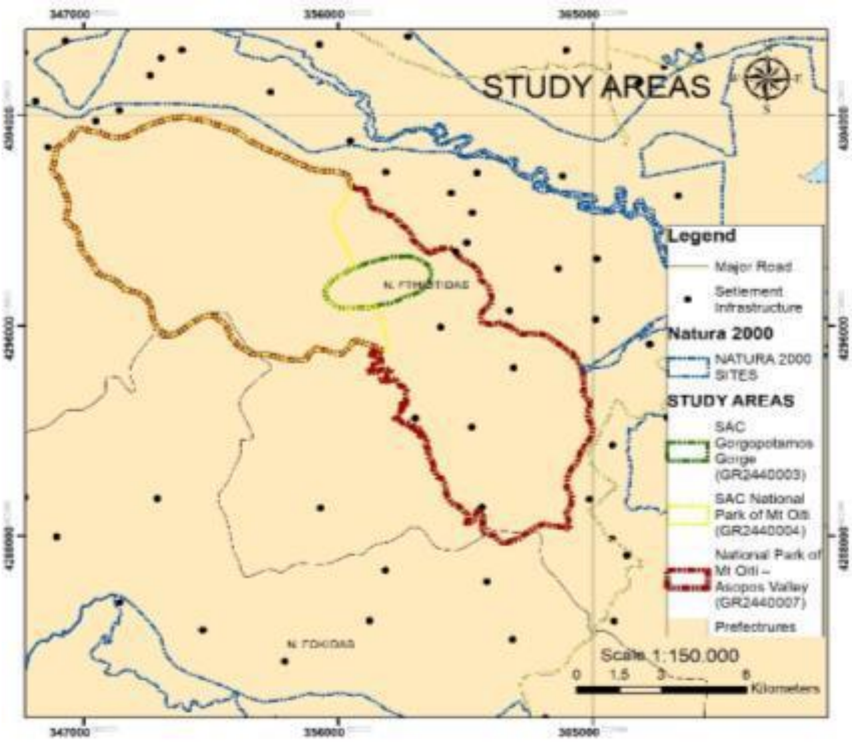

Figure 1. Oiti National Park (QGIS 3.6).

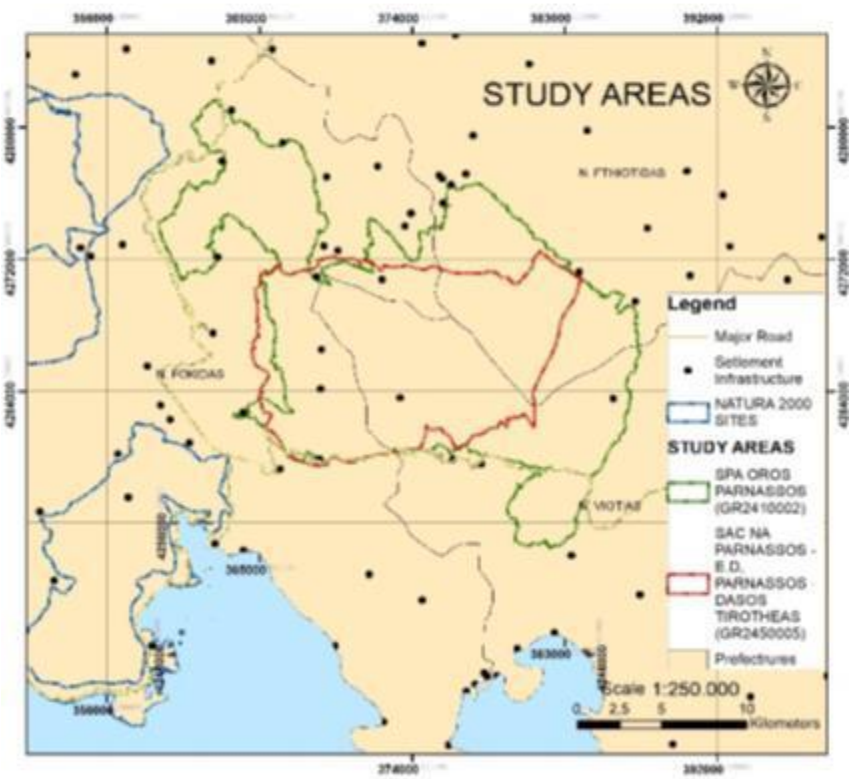

Figure 2. Parnassos National Park (QGIS 3.6).

Project objectives

National Parks of Oiti and Parnassos host a remarkably diverse flora, fauna and landscapes, while Mt Parnassos is considered as a hotspot of plant species diversity and endemism.

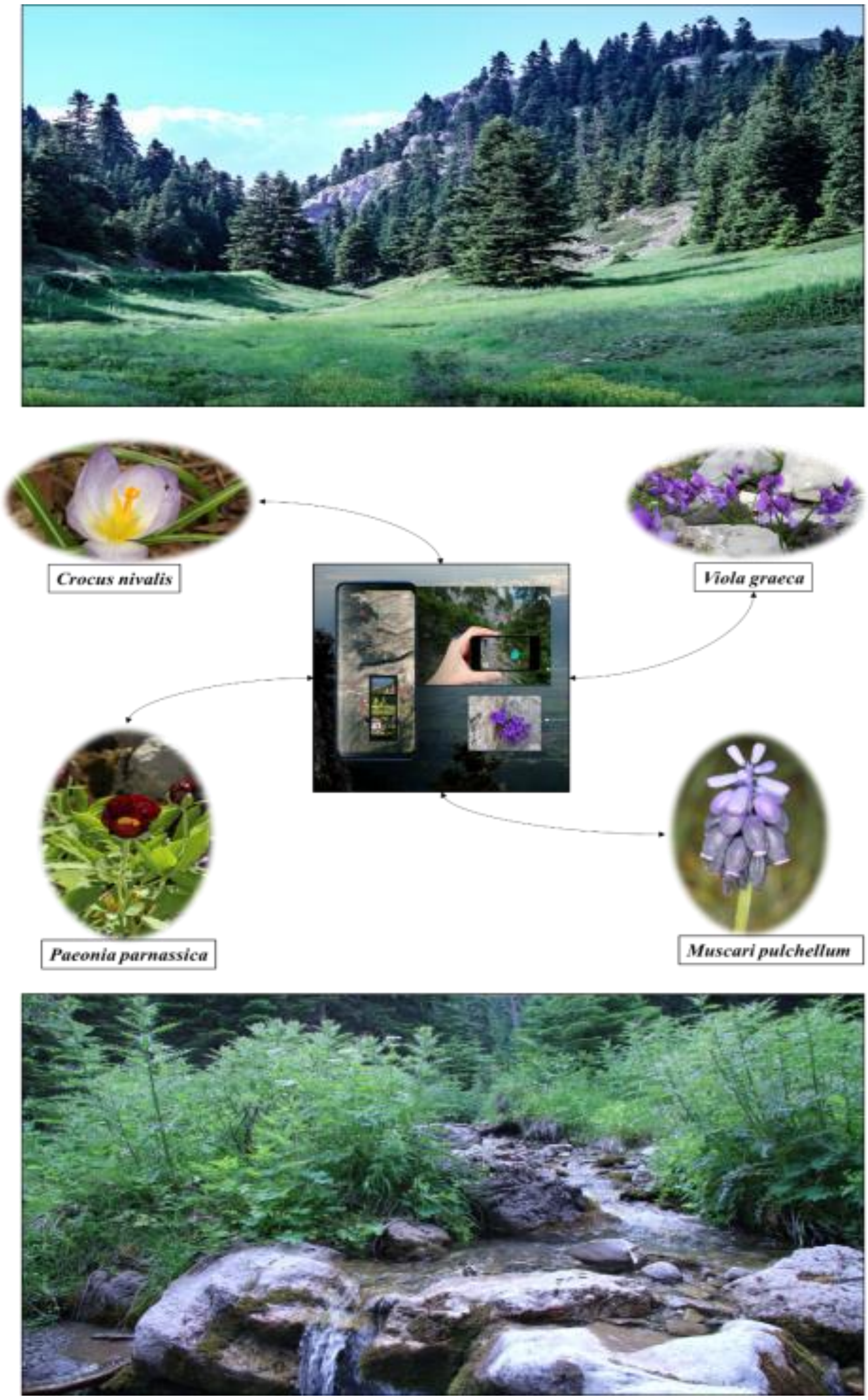

Figure 3. Visual identification of vascular plant species.

Mt Oiti, also known as the mountain of flowers and of legendary hero Hercules, is a mountain of unique beauty, with vast fir forests, rare as well as impressive plant species, rich fauna and abundant waters, which most of the times flow abruptly, through steep and beautiful gorges. It is the fifth highest mountain in Central Greece and its highest peak is Pyrgos (2.152 m) (Management Body of Oiti National Park, Sperchios Valley and Maliakos Gulf, 2019).
Also, Mt. Parnassos consists of spectacular cliffs and rocky areas. It mainly consists of hard limestones (76.6%), which forms particularly impressive karsts; some formations, such as the

Sinkhole of Lilaia and the Corycian Cave, are especially famous. Last but not least, Parnassos is a place of enormous cultural - historical heritage, i.e. the Sanctuary of Apollo and the Oracle of Delphi. In addition, the great cultural - historical value of the area is enhanced by the ancient and modern monuments, as well as the rich history of the region (Management Body of Parnassos National Park, 2019).
The main objectives of AdVENt project are the development of: a) a vascular plant database of Oiti and Parnassos National Parks, available to the scientific community and the general public, which is expected to promote research in the relevant fields of botany, ecology etc., b) an integrated Augmented Reality (AR) mobile application for enhancing the visitors touring experience and c) a cutting-edge technology for the visual identification of vascular plant species (Fig. 3). In addition, AdVENt aims to promote, enrich, and therefore enhance the visibility and competitiveness of the local tourism product.

Expected results

The first system of plant species identification in Greece is expected to be developed during AdVENt project. A cognitive database for the plant species of Oiti and Parnassos National Parks will also be created. This basis is going to be available not only to the scientific community, but also to the public and will contribute to knowledge enhancement of the relevant fields (botany, ecology etc.). The project will upscale and promote the applied research on the 3D digital illustration of natural areas at multiple spatial scales. Also, the sharing of the know-how between the enterprises and the research institutes will undoubtedly increase their competitiveness.
This project will unfold and provide innovative technological implementations suitable to underline the environmental wealth, natural beauty and cultural background of these specific mountainous areas. Creating thus, an attractive, modern touristic product with direct positive results and developmental possibilities for the local and also regional economy and society. In a way, the project will "equip" the local touristic product with a tool, which enriches the interaction of visitors with the natural environment, enhancing their overall experience. The idea of interacting and understanding the local culture of a place is very common among eco tourists who support the local economy preferring and advertising local produce and services.

Acknowledgments

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RESTORATION, MANAGEMENT AND VALORISATION OF PRIORITY HABITATS OF MEDITERRANEAN COASTAL AREAS

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Abstract
The Mediterranean Basin is one of the most bio-diverse regions and the third most important plant diversity hotspot worldwide. However, the Mediterranean region is also known as one of the planet most threatened territories, being the 4th most significantly altered biodiversity hotspot of the planet, and the 2nd in habitat area loss. Therefore, one of the recent projects co-funded by the EC is LIFE17 NAT/GR/000511: "*Restoration, management and valorisation of PRIority habitats of MEDiterranean coastal areas*" (project areas: Anatoliki Makedonia – Thraki, Greece and Lazio, Italy). The primary aim of the project is to improve the conservation status of the habitats included in the Habitat Directive 92/43/EC: 1. Annex II (a) 3170* Mediterranean temporary ponds; (b) 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*; (c) 5230* Arborescent matorral with *Laurus nobilis;* 2. Annex I (d) 91M0 Pannonian-Balkanic turkey oak-sessile oak forests, as well as of the species included in the Annex I of the Habitat Directive 92/43/EC: (e) *Eurotestudo hermanni*, (f) *Emys orbicularis*, (g) *Callimorpha (Euplagia) quadripunctaria**, through interventions taking place within SCI GR1150010 and SCI IT6030022. It is noteworthy that the accurate and innovative interventions, that will be implemented in this project, can represent a good opportunity for conservation and restoration of biodiversity and ecosystem services, providing solutions for mitigating the climate change effects. Furthermore, the innovative interventions will be implemented and valorised during the project's duration so that replication and transportation to other similar environmental contexts will be feasible, thus promoting and enabling the long-term conservation of these habitats and species.

Keywords: *Restoration, Management, Valorization, Natura 2000, Mediterranean.*

Introduction
The Mediterranean Basin is one of the most bio-diverse regions and the third most significant plant diversity hotspot worldwide. It hosts 25,000 plant species, of which more than half are found nowhere else (Myers et al., 2000). However, this region is unfortunately known as one of the planet's most threatened territories, considered as the 4th most significantly altered biodiversity hotspots of our planet (Mittermeier et al., 2004) and the 2nd in habitat area loss. While, at the same time it has been identified has been identified as a climate change hotspot (Diffenbaugh and Giorgi, 2012). The observed seasonality in the distribution of temperature and precipitation is a characteristic of the Mediterranean climate. On the whole, there is a remarkable water stress in the species found in Mediterranean ecosystems due to hot and dry summers, while winters tend to be cool and wet.

We must take into consideration that thousands of years of human settlement and habitat modification have taken their toll. More specifically, rapid and abrupt land use changes due to agricultural land expansion or poor forest management technics including overexploitation, along with intensive grazing, frequent wildfires and infrastructure development, particularly in the coastal areas have contributed to the natural habitats' loss and degradation. Therefore, it is apparent, that habitat loss and degradation are mainly caused by human activities and pose as the main threat for the Mediterranean basin species. Extreme climatic events such as fires or severe droughts are expected to increase the threat further (Cuttelod et al., 2008). Hence, one of the recent projects co-funded by the EC is LIFE17 NAT/GR/000511: "*Restoration, management and valorisation of PRIority habitats of MEDiterranean coastal areas*", project areas: Anatoliki Makedonia – Thraki, Greece and Lazio, Italy) which will be contribute to improve the conservation status of the habitats. The project started in 2018 and has a 5-year duration.

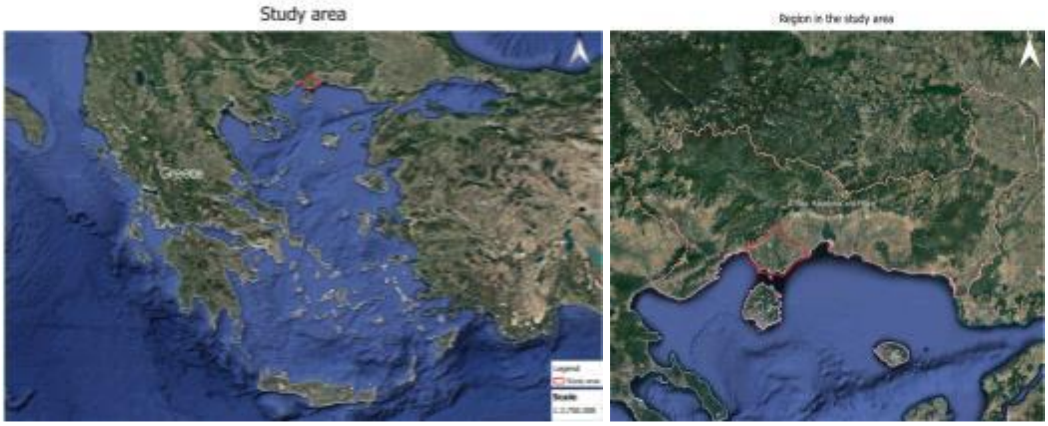
The LIFE PRIMED partnership includes: a) the Hellenic Society for the Protection of Nature (HSPN) (Coordinator), b) the Institute of Mediterranean and Forest Ecosystems, Hellenic Agricultural Organization "DEMETER", Greece, c) the Management Body of Nestos Delta - Vistonida - Ismarida, Greece, d) (i) the Department of Environmental Biology and (ii) the Department of Civil, Constructional and Environmental Engineering - Sapienza University of Rome, Italy, and e) the AGENZIA REGIONALE per lo SVILUPPO e l'INNOVAZIONE in AGRICOLTURA, Italy.

Project area

The LIFE PRIMED project is being carried out in the Nestos Delta, Kavala Greece – SCI GR1150010 [surface area (ha): 22,484.630] (Map 1) and in the Bosco di Palo Laziale [surface area (ha): 129.000] (Map 2).

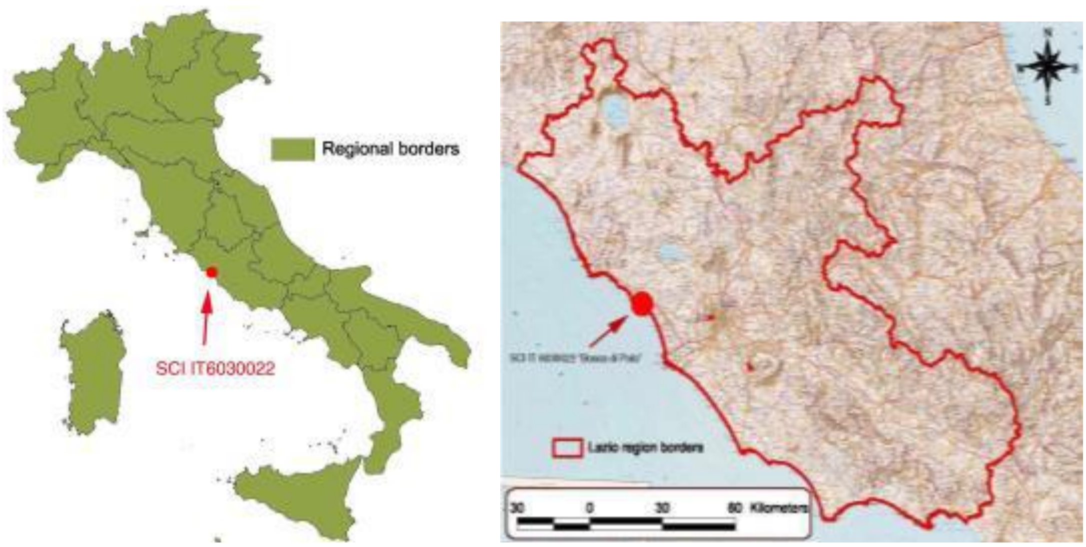
More specifically, the Nestos River arises in Bulgaria and flows into the Thracian Sea, forming the natural boundary between the Regions of Macedonia and Thrace, in northeastern Greece. The Nestos Delta, created by the alluvial deposits of the river that have extended the land into the sea, covers an area of 55,000 ha and is fan-shaped. Its top is at bridge near the village of Toxotes, and its base is the coastal zone opposite of Thassos island. The eastern side of the delta reaches the lagoons of Avdira. On its western side there are nine more lagoons (Vassova, Erateino, Agiasma, Kokkala, Haidefto, Keramoti, Gefyraki, Palaias Koitis of Nestos, and Monastiraki). The lagoons are surrounded by extensive salt marshes and are the most productive fish farms of Greece. The nearest cities are Xanthi 16 km to the east, and Kavala, 25 km to the west. Because of its size and the variety of its habitats, the Nestos Delta is considered among the most important wetlands of Greece and Europe. A significant feature of SCI GR1150010 is the riparian forest known as "Koca Orman" (Great Forest), one of the largest of its type in Mediterranean area. It is noteworthy that the main type of land use in Nestos is agriculture (20%), aquaculture/fisheries (30%), forestry (10%), tourism / recreation (5%) and water management (including watershed management) (50%).

According to the ranking of our country's bio-climate (Mavromatis, 1980), the area belongs to the semi-dry bioclimatic storey with a cold winter and intense mean-Mediterranean character of the bioclimate.



Map 1. Study area in Greece.

Also, the Palo Laziale wood is located along the coastline of the Lazio Region, in the province of Rome, about 40 km NW of Italy's capital, within the territory of the Municipality of Ladispoli. It is set within an entirely fenced-off private property within the SCI IT6030022 “Bosco di Palo Laziale”. It is a flat area of about 50 hectares, with an altitude of between 3 and 10 meters above sea level and about 100 meters far away from the coastline.
According to the bioclimatic features, the area is located within the Mediterranean region, as defined by the compensated summer ombrothermic index (Rivas Martinez, 2008). During the summer, the high temperatures and the low precipitation give rise of a dry period and negative water balance of the soil due to the high evapotranspiration.
The area of greatest naturalistic interest within the SCI is the Wood of Palo in which the actions of the Project are concentrated. It lies in the core of the SCI covering about 50 hectares. It is mainly composed by a planitial oak wood (Habitat 91M0), Mediterranean temporary ponds (Habitat 3170*), and high sclerophyllous maquis as well as a small portion of ″Arborescent Matorral with *Laurus nobilis*″ (Habitat 5230*). A strip of meadow extends for about 18 hectares between the wood and the beach. The remaining part is predominantly affected by strong anthropic use.



Map 2. Study area in Bosco di Palo.

Project objectives

The primary aim of this Project is to improve the conservation status of the habitats included in the Annex II of the Habitat Directive 92/43/EC: (a) 3170* Mediterranean temporary ponds; (b) 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*; (c) 5230* Arborescent matorral with *Laurus nobilis;* (d) 91M0 Pannonian-Balkanic turkey oak-sessile oak forests, as well as of the species included in the Annex I of the Habitat Directive 92/43/EC: (e) *Eurotestudo hermanni*, (f) *Emys orbicularis*, (g) *Callimorpha (Euplagia) quadripunctaria**, and in the Annex II and III of the Birds Directive 2009/147/EC: (h) *Phasianus colchicus colchicus,* through interventions taking place within the SCI GR1150010 Delta Nestou Kai Limnothalasses Keramotis (Delta of River Nestos) and the SCI IT6030022 (Bosco di Palo Laziale).

The innovative solutions applied and tested during the Project will be actively replicated and transferred to other similar environmental contexts in order to promote and enable the long-term conservation of these habitats and species in the whole Mediterranean coastal area.

The specific objectives of the Project are the following:

- ✓ To quantify the exact causes that have led to the current unfavourable conservation status and to define and implement appropriate and innovative actions capable to restore the affected ecosystems.
- ✓ To develop mechanisms that will ensure the continuation of ecosystem conservation and the management actions even after the end of the Project.
- ✓ To present the outcomes of the Project to the scientific community and to relevant institutions in order to scale-up the restoration, management and valorisation approach in other similar coastal ecosystems of Italy, Greece and Europe.
- ✓ To support the local and regional economy by engaging residents in the Project activities and in the further management and touristic use of the sites.
- ✓ To promote knowledge of the areas, its habitats and species, through outreach and environmental education initiatives.

Expected results

The expected results of the project are described below:

- ✓ Increasing the current surface of the 3170* in the SCI of Palo Laziale up to 0.8 ha and in Nestos Delta by 0.02 ha;
- ✓ Recovering 40 ha of 91M0 of the SCI of Palo Laziale, currently highly degraded, restored;
- ✓ Improving the chemical-physical conditions of the soil in 91M0 (Italy) and 91E0* (Greece), through the provision of water during dry season and the consequent washing out of the substrate sodicity, which is one of the stress factor causing the wood decline;
- ✓ Increasing the population size of *T. hermanni, E. orbicularis, C. quadripunctaria**, *P. colchicus* by improving the conservation status of the SCIs' habitats;
- ✓ Disseminating good-practices on the restoration and management of lowland ecosystems, and mainstreaming the methodological approach of the Sustainable Forest Management Strategic Plan (SFMSP);
- ✓ Involving at least 400 people during the International Workshops that will be organised to scaleup the Project outcomes among the scientific community and relevant institutions across Europe (e.g. managing authorities of Natura 2000 sites, NGOs);
- ✓ Training at least 200 people during tailor-made ToT trainings on conservation engineering (e.g. training on the job to engineering students), restoration ecology, PAs management, EU funding Programmes (e.g. LIFE programme, Interreg, IPA, etc.),

sustainable tourism, sustainable agriculture, with the aim to create new jobs and/or new curricula in the topics targeted by the Project;

- ✓ Engaging at least 2,500 among tourists (local and foreign; "traditional" and cruisers) and residents in the touristic-recreational initiatives facilitated by the Project (e.g. local food festivals and/or markets, guided naturalistic tours, Science and Junior Cafe, etc.);
- ✓ Engaging at least 2,500 people among the local communities in the environmental awareness activities focused on the LIFE programme, Natura 2000 Network and its value for generating incomes, jobs, ecosystem services benefits and quality of life improvements (e.g. guided tours, schools, volunteer campaigns such as Bioblitz, Citizen Science, etc.).

Demonstration character of the Project

A multidisciplinary approach for the conservation of priority habitats and species will be applied to Mediterranean ecosystems. This approach for evaluation and applied conservation actions in such ecosystems will be widely communicated as a good practice. Foresters, biologists, geologists, botanists, zoologists and environmental engineers will be called to apply the latest methods in ecosystem restoration and to adapt them to each concrete case in an innovative and replicable way. The project Actions will utilize scientific and technical knowledge on the restoration of temporary ponds and floodplain woodlands to improve their resilience capacity and to apply adapted management practices. At the same time and in a synergic approach, hydrological works will be implemented, taking into account the restoration and conservation requirements and limiting the environmental factors that produce plant stress conditions. Restoration of adequate hydrologic regime and functions of the habitats will also contribute towards mitigation of climate change effects in the Mediterranean area.

The project will apply and monitor for the first time a process for establishing new temporary ponds, including species reintroduction and management regime, in the ecological conditions and for the temporary pond species assemblages of the specific association of ponds and woodland. The response of these communities to the proposed management measures will be determined through monitoring and management techniques will be further adapted. The results produced will be available to managers of other sites in Greece, Italy and the rest of Europe, demonstrating a process that may be replicated in similar circumstances both in other sites of Greece and Italy, as well as in other Mediterranean countries.

Additional, the project will apply, for the first time in Greece, the method of collecting water during the wet months of the year and then provide them to sustain habitat functions during the dry period, through underground tanks and delivery pipes. The study and implementation methodology, as well as the management of water resources methodology will be demonstrated directly and made available through the project website and social medial to any interested managers of protected areas, related scientists, and other relevant stakeholders in Greece, but also surrounding EU and IPA countries.

The adaptation of the designed management practices according to the results of monitoring and taking into account their cost/efficiency, will demonstrate the optimum way to restore and manage the habitats. This scientific and technical activities carried out during the preliminary, direct and monitoring phases will lead to the definition of good practices that may be applied to other comparable situations regarding the conservation of temporary ponds, floodplain woods, as well as related species of EU interest. Moreover it should be noted the species of the

Mediterranean temporary ponds (3170*) and the species of woodlands (mostly habitat types 91E0* and 91M0), occurring in close spatial and ecological connection, constitute a Mediterranean complex of high ecological value which is at risk, in both Greece and Italy, from a variety of human as well as climate pressures, and so far has been rather neglected in the conservation and restoration efforts. The work that will be carried out through this project will therefore be crucial to identify best practices that are currently limited or even missing for this type of environments, and to demonstrate them to a wide audience.

Figure 1. Temporary ponds (Habitat 3170*) within the Nestos Delta (left figure) and the Palo Laziale Wood (right figure).

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