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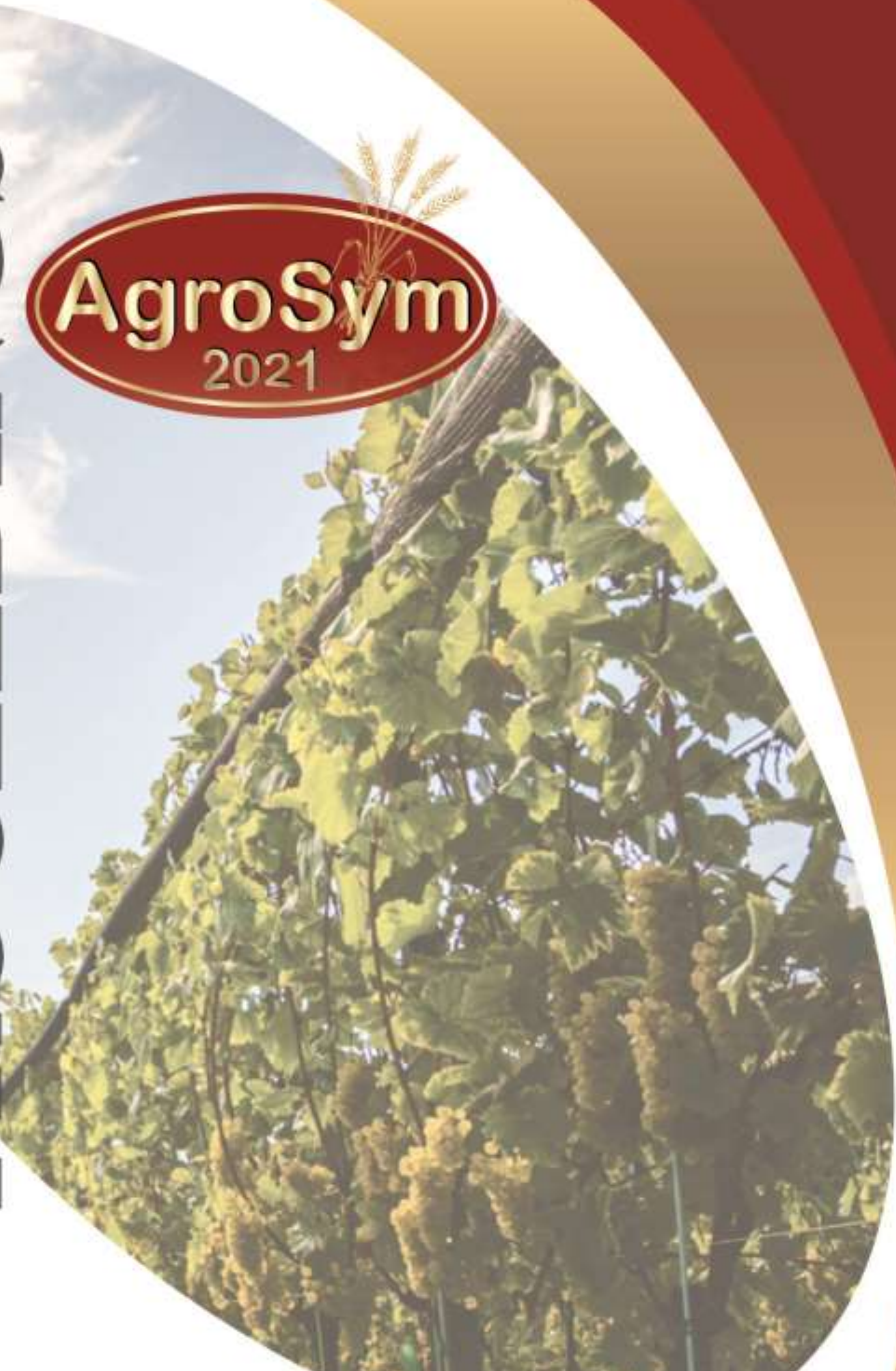

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PREFACE

The Faculty of Agriculture of the University of East Sarajevo (Bosnia and Herzegovina), the Faculty of Agriculture of the University of Belgrade (Serbia) and the International Centre for Advanced Mediterranean Agronomic Studies - Mediterranean Agronomic Institute of Bari (CIHEAM-Bari, Italy) organized the XII International Scientific Symposium “Agrosym 2021” on Jahorina mountain (East Sarajevo, Bosnia and Herzegovina). This year’s edition of Agrosym was organized for the first time in a hybrid format, in-person (250 participants) and virtual via ZOOM (450 participants), because of the prescribed restrictions due to the COVID-19 pandemic.
The 12th Scientific International Symposium "Agrosym 2021" made an important contribution to the agriculture practice in different fields e.g. plant production, animal husbandry, environmental protection, organic farming, forestry, and agro-economy. The Scientific Committee received 750 papers and after review, we accepted 695 papers; 159 for oral presentations and 535 for poster presentations, which addressed all the sessions of the symposium: plant production (43 oral and 167 poster presentations), plant protection and food safety (25 oral and 105 poster), environmental protection and natural resources management (24 oral and 86 poster), organic farming (6 oral and 37 poster), animal husbandry (24 oral and 69 poster), rural development and agro-economy (25 oral and 40 poster), forestry and agroforestry (12 oral and 33 poster presentations). The presented papers were submitted by about 2000 authors representing more than 80 countries worldwide.
We have had the opportunity to share new information on biotechnology, plant breeding and world markets during the COVID-19 pandemic in the plenary keynote session and many interesting research results and findings in parallel sessions. It can be pointed out that sustainable agriculture development must focus on building policies and practices at national and regional levels, with an emphasis on quality and greater diversity, followed by a demonstration of agronomic and economic viability, environmental protection and food safety, and social benefits, while fostering the convergence of rural and urban populations as well as closing the gap between producers and consumers.
AGROSYM 2021 has been a considerable undertaking from scientific, logistical and organizational points of view. Big thanks to all members of the Scientific Committee for their continued efforts and hard work, which made the symposium possible and successful. I would also like to thank my colleagues from the Organizing Committee, for all they have done to bring this event together, particularly the dean of the Faculty of Agriculture of the University of East Sarajevo, prof. Vesna Milic, as a host and chairperson, His Excellency, prof. Sinisa Berjan. Finally, I would like to thank all the authors, reviewers, session moderators and colleagues for their help in preparing and editing this book of proceedings. Special thanks also go to the organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief

Dušan Kovačević

East Sarajevo, 10 October 2021
Academician Dusan Kovacevic, Academy of Engineering Sciences of Serbia
President of the Scientific Committee of Agrosym 2021

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PLANT PRODUCTION

INFLUENCE OF DIFFERENT FERTILIZATION MODELS ON THE MORPHOLOGY OF GALA SHNIGA APPLE LEAF (Malus domestica L.)

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Abstract

In this research, the influence of different fertilization methods on the morphological characteristics of Gala Shniga leaf leaves in the agroecological conditions of Sarajevo during 2019 and 2020 was studied . The studies included dietary treatments : control variant (standard fertilization NPK 15-15-15); variant A foliar feeding (Yara Vita Universal Bio); variant B (KAN + NPK). The Gala Shing apple (Malus Domestica L.) originates from New Zealand and was obtained by crossing Golden Delicious and Kidd's Orange Red varieties. Generally speaking , the results showed that the new generation of fertilizers, as well as the method of fertilization through lists show the best results of morphological characteristics of leaf cultivars of apples. The results of the study showed that proper plant nutrition is one of the measures by which it is possible to achieve high yields and good quality of agricultural products. The leaf is the basic part of the plant that performs three very important functions: photosynthesis, transpiration and gas exchange. It also plays a big role in the nutrition of apple fruits. Of the necessary elements, the quality of apple fruits is most affected by: nitrogen, potassium, phosphorus, calcium, magnesium and boron. Their lack or excess can have a very negative effect on fruit quality.

Key words: *Apple variety, Gala Shniga , fertilization, leaf, morphological characteristics.*

Introduction

Apple (Malus domestica) is the most widespread fruit species in the world and belongs to the group of perennial woody plants from the rose family. It is a very important part of the daily diet. Known as a mother of all fruit, human kind used apple since ancient times in a variety of purposes, but most commonly as food, medicine and as a means of refreshment. Apple as a fruit species, in terms of fruit trade, is on the world market in third place, just behind citrus and bananas. It is one of the more commercial fruit species, which is why great attention is paid to improving its cultivation (Ubavić et al., 2001).

World apple production has increased slightly in recent years and 2018 amounted to 83,139,326 tons (FAO, 2018). The largest producers are China with 44, followed by EU countries with about 10, the United States with 4.7, Turkey with 2.8 and India with 2.3 million tons. In the EU, Poland leads in production with 2.9, Italy with 2.1, France with 1.4 million tons, and Germany with 990,000 and Spain with 550 thousand tons. The quality of fruits, their colour, taste, size, firmness is varietal characteristics of apples. The mentioned properties are also influenced by ecological factors, and especially agrotechnical measures, among which the influence of fertilization and its application in certain phenophases of apple growth and development should be emphasized. Depending on the dose and ratio of different fertilizers, nutrients may improve or in the case of improper application decrease the quality of fruit (Lukić, M. 2010). Thanks to the sunlight and mineral substances, plants produce food, not only for themselves but for all

living beings. The main organ for food production in plants is the leaf. The leaf is the youngest vegetative plant organ, responsible for the process of photosynthesis and the creation of organic matter. It means that development, yield and quality of the fruit and quantity are related to the development of leaf (Denffer and Ziegler, 1991). At one plant can be distinguished three categories of leaves: lower, middle and upper leaves. The middle leaves are the most numerous group of leaves. It includes green leaves in which photosynthesis is carried out. The most important for the plant is the middle (true) leaves (Janjatović, 1989). He says the leaves are composed of three parts: leaf blade (lamina), stems (petioles) and shelf (basis) leaf. The leaflet is the most important part of the leaf. It serves assimilation and transpiration. They have green colour and usually are flattened and expanded (Bacic, 2003). Types rate, size, and even the shape adapted to the exposure to the sunlight (Janjatović, 1989).
The total amount of organic fertilizers are limited, thus today in practice most common is the second type of fertilizer under the name of mineral fertilizers (Bulatovic, 1984). In relation to organic fertilizers, a mineral fertilizers are characterized by increased concentration of the nutritive elements. Thus, for example while 100 kg of manure on average contains 0.5 kg of nitrogen, 0.2 kg of phosphorus and 0.6 kg potassium, therefore a total of 1.3 kg of active substance, long 100 kg of mineral fertilizers eg. 15:15:15 contains 15 kg of nitrogen, 15 kg of phosphorus as P_2O_5 and 15 kg of potassium as K_2O, as a total amount of 45 kg of active nutrient substances (Ubavić et al., 2001). The leaves can develop in the spring, and arise from the leaf buds. Each type of plant has a different shape and size of leaves, but its role is always the same.

Material and method

Studies have been conducted during 2019 and 2020 on experimental test site of the Federal Institute for Agriculture in Sarajevo (Butmir) to the introduced planting of apple varieties Gala Shniga age of 16 years (Malus Domestica L.- cv. "Gala Shniga") per block system (10 trees per treatment). The tested apple cultivar was grafted on a weak vegetative substrate M9. The planting density is 1,300 trees/ha. Row spacing planting is 3,5 m spacing within rows was 1 m.

The study has represented the following treatments fertilization apples:
1) Control, standard variant of fertilization with NPK fertilizer 15-15_15 300kg/ha);
2) Variant A: foliar fertilizer - YaraVitaUniversal Bio (P_2O_5 - 35 g/l, K_2O - 60 g/l, B 0.2 g/l, Cu 1 - g/ l, Mn - 1.1 g/l, Mo - 0.3 g/l, Zn - 0.6 g/l);
3) Variant B: KAN (27% N) 400 kg/ha + NPK (15-15-15) 200 kg/ha.
Each variant of fertilization is covered by 10 trees, set by a random block system.
Control variant Standard- fertilization of NPK fertilizer and variant B, fertilizing with KAN + NPK are applied in early spring - March during both years of research.
Variant A fertilization with foliar fertilizer YaraVitaUniversal was applied four times during the growing season at intervals of after 15 days.
A certain number of leaves (medium - from the middle of the one - year increment) was taken for testing and further measurements.
The obtained results of measurement of parameters statistically the processed two-factorial analysis of variance using the software package SPSS 23.0 by ANOVA procedure, a difference mean of the tested LSD test.

Results and Discussion

Tab.1. The length of the leaf width of the leaf index of form, leaf area, leaf length stems depending on the year and fertilization ± s. deviation

variant of fertilization	Leaf length (cm)		Leaf width (cm)		Index form (L/W)		Leaf area (cm^2)		Leaf stalk length (cm)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	9.94 ± 0.11	10.8 ± 0.32	4.73±0.24	5.81±0.12	2.10±0.12	1.85±0.17	35.59±3.91	37.62±1.21	3.47±0.24	3.55±0.22
Variant A	13.36± 0.21	14.11± 0.16	6.92±0.13	7.54±0.19	1.9 ±0.19	1.87±0.16	67.33±3.71	77.82±2.32	4.15±0.31	4.28±0.19
Variant B	10.21±0.13	11.08± 0.19	5.83±0.19	6.33±0.21	1.75±0.23	1.75±0.11	44.64±5.46	52.60±6.19	4.10±0.33	4.36±0.14

Table 2. Analysis of variance for leaf length (cm) in relation to the method of fertilization and year

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	139,6463333	2	69,82316667	6,14	0,00042	3,168246
Columns	10,25066667	1	10,25066667	4,77	0,08945	8,019541
Interaction	0,044333333	2	0,022166667	4,89	0,00242	3,168246
Total	149,9413333	59				

Observing the results of statistical analysis for leaf length, it is possible to notice that there are statistically significant differences in relation to the method of fertilization ($p < 0.05$). (Table 2.) Foliar fertilization shows the best results in terms of leaf length. When it comes to differences in terms of the year of the survey, we can argue that there are no statistically significant differences. Similar results came and Janjatović 1989, Denffer and Ziegler (1991), Ubavić et al. (2001), Bačić (2003), Lukić , M. (2010).

Leaf width

Table 3. Variance analysis table for leaf width in relation to the method of fertilization and year

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	38.80133	2	19,40067	5,66	0,0001 2	3,168246
Columns	8,066667	1	8,066667	11,1	0,0007 9	4,019541
Interaction	0.937333	2	0.468667	6,42	0,0029 2	3,168246
Total	47.80533	59				

The results of statistical analysis for leaf width , possibly show that there are statistically significant differences in relation to the method of fertilization ($p < 0.05$). (Table 4.) We can claim that the best results are shown by foliar application of fertilizers. When it comes to differences in terms of the year of the survey, we can also argue that there are statistically significant differences in relation to the year of observation. Although Janjatović (1989), Denffer and Ziegler (1991), Ubavić et al. (2001), observed noticeable statistically significant difference,it is not so pronounced as in the recent research carried out by hand Bacic (2003) and Lukic, (2010).

Index form leaf shape

Table 4. Variance analysis table for index leaf form in relation to the method of fertilization and year

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.525	2	0,2625	5,19	0,00065	3.168246
Columns	0.160167	1	0,160167	4,95	1,967	1,019541
Interaction	0.170333	2	0,085167	5,04	0,00021	3,168246
Total	0.8555	59				

Statistical analysis of data related to index form leaf, it shows there are statistically significant differences in relation to the method of fertilization (p <0.05). (Table 6.) We may claim that, unlike other observed traits, the best results are shown by the control group. The difference between the years of observation is not statistically significant. When it comes to research by other authors, similar results were obtained by Janjatović (1989), Denffer and Ziegler (1991), Ubavić et al. (2001). In some recent works of Bačić (2003), Lukić, (2010). different results were noted. Namely, in the works of mentioned authors, it was noticed that foliar fertilization gives the best results in terms of leaf index.

Leaf area

Table 5. Analysis of variance for leaf area in relation to the method of fertilization and year

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	13413.62	2	6706.81	6.52	0.0003 2	3.168246
Columns	699.0507	1	699.0507	6.8	0.0007 9	4,019541
Interaction	188.5623	2	94.28117	9.17	0.0008 2	3.168246
Total	14301,23	59				

The statistical analysis of data on the leaf surface , showing statistically significant differences with respect to the method of fertilization (p <0.05). (Table 8.) We can claim that the best results are shown by foliar application of fertilizers. When it comes to differences in terms of the year of the survey, slightly better results were observed in 2020. Similar results were obtained by Janjatović 1989; Denffer and Ziegler, 1991; Ubavić et al., 2001; Bačić, 2003; Lukić, 2010.

Leaf stalk length

Table 6. Analysis of variance for leaf stalk length in relation to the method of fertilization and year

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-alue*	*F crit*
Sample	6,771	2	3,3855	1,03	0,179	3,168246
Columns	0.368167	1	0,368167	1,12	0,238	4,019541
Interaction	0.086333	2	0,043167	1,31	0,567	3,168246
Total	7,2255	59				

Observing the results of statistical analysis for leaf stalk length , we can claim that there are no statistically significant differences in relation to the method of fertilization ($p < 0.05$). (Table 6.) When it comes to differences in terms of the year of the survey, we can say that there are no statistically significant differences. Similar results were obtained by Janjatović 1989; Denffer and Ziegler, 1991; Ubavić et al., 2001; Bačić, 2003; Lukić, 2010.

Conclusion

Bearing in mind above-mentioned results, we may conclude that there are statistically significant differences in the method of fertilization in terms of length, width, index of form and surface sheet ($p<0,05$). Statistically significant differences between the ways of fertilization were not found when it comes to the length of the leaf stalk ($p>0,05$). The statistical differences between years of observation observed were found only at leaf area. On the basis of these results, we recommend foliar way fertilization and give him an advantage in relation to other modes of apple nutrition.

References

Bačić, T. (2003), Morphology and anatomy of plants. JJ Strossmayer University, Faculty of Education in Osijek. Osijek.

Bubić, S. (1977). Special fruit growing, Sarajevo.

Bulatović, S. (1984). Contemporary fruit growing, Nolit, Belgrade.

Chear, C.B, Faust, M. (1971). Value of various tissue analysis in determining the Ca status of the apple tree and fruit, New York.

Denffer, D., & Ziegler, H. (1991). Textbook of Botany for High Schools , morphology and physiology. Skolska knjiga, Zagreb.

FAO (2018). World Food and Agriculture - Statistical Pocketbook 2018. Rome.

Partridge, Dz., Kurtovic M. (1997). Breeding of fruit trees and grape vines, Edis, Sarajevo.

Kurtović, M. and Maličević, A. (2006). Apple and pear growing technology, Sarajevo.

Kurtović, M., Jusović, H., Salkić, B., Maličević, A. (2009). 100 practical tips for successful fruit growing, Sarajevo.

Lucic, P., Djuric, G., Micic, N. (1996). Fruit growing I, Nolit Belgrade.

Lukic, M., Maric, S., Mitrovic, M., & Milosevic, N. (2010). Application of chemicals for thinning of apple fruits. In Conference on Biotechnology, 15, Cacak (Serbia), 26-27 Mar 2010. Faculty of Agriculture.

Ubavić, M. (1996). Fertilizers and their application, Feljton, Novi Sad.

Ubavić, M., Kastori, R., Oljača, R., Marković, M. (2001). Fruit nutrition, Banja Luka.

THE EFFECT OF POLYMER AND LOCATION ON SOME MORPHOLOGICAL CHARACTERISTICS OF POTATO

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Abstract

Potato is a field crop which has a great significance in Bosnia and Herzegovina, however, the production is according to all indicators rather unstable and unreliable. Two-factorial trials were set up (of different combination of adsorbent and location) for examining the influence of location and super-adsorbents on morphological characteristicsof potato. The morphological characteristics monitored were as follows: plant height (cm) (measured from the ground surface to the top point of the plant); the number of sprouts; leaf surface per plant (m^2) with the help of leaf contour on paper method; new above-ground plant mass (g), leaf mass (g) and potato stem mass (g). The first factor consisted of 6 variants: control variant (A_0), superadsorbent (A_1), superadsorbent enriched with growth stimulants (A_2), superadsorbent enriched with microorganisms (A_3), superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5) in the quantity of 20 kg ha^{-1}. The trials were set up in East Sarajevo and Bijeljina. The main goal of this research was to determine the effect of super-adsorbent and area on morphological characteristics of potato. Through analysis of the results, it was determined that the use of different variants of adsorbent had great influence on the examined, while the influence of area had great significance for all the examined characteristics except for the number of stems per plant. The use of different variants of super-adsorbent in both areas had positive effect on morphological characteristics of potato, where the use of super-adsorbent enriched with growth stimulants, microorganisms and microelements is especially emphasised, while the variant in which super-adsorbents were not used (control variant) had poor results for the examined characteristics.

Key words: *superadsorbent, plant height, plant mass, assimilation surface area, stem number*.

Introduction

Climate change resulted in increased demand for water in agricultural production, affecting the development of various models with a view to preserving soil moisture and decreasing water consumption in agricultural production. Besides the continuous trend of air temperature increase, further rainfall shortage, accompanied by decreased soil moisture, have a significant influence on the uncertainty of agricultural production and considerable crop yield decrease per unit of area. Water conservation in agriculture can be achieved through use of various substances (polymers), which alter water and air properties, but also physical soil properties. The ultimate goal of the abovementioned substances, which started to be used in the 1950s, is the improvement of soil water regime. The use of these polymers improves aggregate stability, decreases soil compaction and erosion, and increases infiltration by 2,5 times (Sepaskhah and Bazrafshan-Jahromi, 2006), positively affects both physical and chemical soil properties, the contents of nutrients in the soil, microorganism activity in the soil, as well as soil productivity (Mann et al., 2011). Polymers, which are insoluble in water (hydrogels), were introduced in agricultural production in the 1980s. The use of hydrogel in soil increases the amount of available water and decreases plant stress, also affecting seed germination, plant growth and yield (Wallace and Wallace, 1986). Previous research demonstrated that not only does the use of polymer prevent agroecosystem pollution, but it also increases economic return for farmers (Islam et al., 2011a). By adding polymers to sandy soils, the efficiency of water consumption and compost in cultivated plants is increased (Bhardvaj et al., 2007; Islam et al., 2011b).

Matherial and methods

Two-factorial trials were set up for examining the influence of location and super-adsorbents on morphological characteristics of potato cultivars Agria. Factor A comprises 6 variants: control variant (A_0), superadsorbent (A_1), superadsorbent enriched with growth stimulants (A_2), superadsorbent enriched with microorganisms (A_3), superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5). in the quantity of 20 kg ha^{-1} (created at the Voronezh State Agrarian University named after Tsar Peter the Great). Factor B: locality B1 - on the territory of the city of East Sarajevo, altitude 550 m (43 ° 49'01 " NW and 18 ° 20'57 " EWD) on alluvial soil (fluvisol) and locality B2 - on the territory of the city of Bijeljina, altitude 90 m (44 ° 41 ′ NW; 19 ° 14 ′ EWD) on semi-glacial land (table 1).
The trials are set up in randomized block system with four replications of 53.333 plants per ha^{-1}. The area of the basic plot is 15 m^2 (four rows 5 m of length with 20 plants per row, at the distance between the rows of 0.75 m and between the plants in a row 0.25 m). Standard agrotechnical measures used in potato production were applied. The morphological characteristics monitored were as follows: plant height (cm) (measured from the ground surface to the top point of the plant); the number of sprouts; leaf surface per plant (m^2) with the help of leaf contour on paper method; above-ground plant mass (g), leaf mass (g) and potato stem mass (g). The trial in Bijeljina was set up on 24 March 2019. Potato started sprouting evenly on 13 April 2019, and flowering on 2 June 2019. The analysis samples were taken on 14 June 2019. The trial in East Sarajevo was set up on 25 April 2019. Potato started sprouting evenly on 15 May 2019, and flowering on 13 July 2019. Meteorological data on mean monthly temperatures, precipitation were taken over from the Republic Hydrometeorological Institute of Republic of Srpska and Federal Hydrometeorological Institute of Bosnia and Herzegovina, from the meteorological stations closest to the areas where field trials were conducted.
Examining the significance of differences between treatments were conducted with the help of analysis of variance (ANOVA). The significance of differences was tested with the help of Fisher's LSD test. STATISTICA 10 (StatSoft, Inc. Corporation, Tulsa, OK, USA), a statistical programme, was used for statistical processing.
Optimal pH value of the soil for growing potatoes is low acidic (from 6.0 to 6.5). Potato requires high soil permeability, as tubers are deformed in compact soil. Furthermore, it requires well-drained soil, while watered soils result in numerous physiological changes in tuber that become watery and are difficult to preserve (storage). In order for potato production to be successful,

organic matter contents, which enhances quality, structure and water-holding capacity, is very important (Baniuniene and Zekaite, 2008).

Table 1. Agrochemical analysis of the soil

Area	Depth (cm)	pH/KCl	Humus %	N %	Soluble P_2O_5 mg $100g^{-1}$	Soluble K_2O mg $100g^{-1}$
East Sarajevo	0-30	6.63	3.62	0.23	15.75	17.59
Bijeljina	0-30	7.16	4.12	0.27	>40	36,41

Besides soil fertility, meteorological factors such as, temperature, rainfall amount and distribution during potato vegetation period are also important. The growth and development, and thus yield and the quality of cultivated plants considerably depend on their effect. Little rainfall in the most critical stages of potato growth and development considerably decrease the yield, ruin tuber quality, which requires constant monitoring of soil moisture with the aim of providing timely soil moisture. When we also take high air temperatures in July and August into consideration, the general conclusion is inevitably drawn that besides high air temperatures and soil, one of the restrictive factors of growth and development of potato plants and tubers in the conditions of arid and semi-arid climate, is precisely insufficient amount and uneven rainfall distribution in vegetative period (Gvozden, 2016). Mean yearly temperature for East Sarajevo and the vicinity in 2019 was 11.7°C and it was higher than the multi-annual average (10.1°C), while the total rainfall amount was 850.5 mm, which is less than the long-term average (932 mm).

Table 2. Mean monthly air temperatures (°C) and rainfall amount (mm) during 2019 and the multi-annual average

	Year / Month		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average/ total
E.Sarajevo	2019	°C	-1.5	2.5	7.7	11.4	12.3	21.1	21	21	16.5	12.9	10.6	3.6	11.7
		mm	79.7	56.7	51.8	98.2	102.3	97.1	67.8	69.7	44.1	38	80.3	64.8	850.5
	1981-2010	°C	-0.1	1.4	5.3	9.9	15.0	17.7	19.8	19.7	15.1	10.9	5.2	1.1	10.1
		mm	67	63	71	78	73	94	72	70	86	85	91	85	932
Bijeljina	2019	°C	0.6	4.7	10.2	13.2	14.9	23.7	23.2	24.3	18.1	13.7	11.3	4.8	13.6
		mm	62.8	34.1	33.7	95.4	121	113	59.2	27	57.2	27.6	52.7	68.1	751.8
	1981-2010	°C	0.3	1.9	6.9	11.8	16.9	19.9	22.1	21.2	17.2	11.6	6.0	2.3	11.5
		mm	54.1	43.5	59.7	66.1	68.2	100	74.6	60.7	55.8	67.6	64	63.9	778.2

Mean yearly temperature for Bijeljina and the vicinity in 2019 was 13.6°C and it was higher than the multi-annual average (11.5°C), while the total rainfall amount was 839.4 mm, which is less than the multi-annual average (778.2 mm). Compared to multi-annual averages, the year of 2019 was characterised by higher temperatures and uneven rainfall distribution. It was only in May that there were lower temperatures in both areas, and which significantly affected slower potato germination in East Sarajevo. Rainfall shortage was reported in Bijeljina in July and August, and in East Sarajevo, the reported values were at the shortage limit in East Sarajevo in these months.

Results and discussion

The table 3 shows analysis of variance of the effect of the use of adsorbent and area on morphological characteristics of potato. The use of different adsorbent variants had a highly significant impact on the examined parameters, while the effect of area had high significance on all the examined characteristics, except for stem number per plant. The interaction adsorbent *x* area had high significance for the plant assimilation surface, plant mass, leaf mass and stem mass.

Table 3. The effect of adsorbent and area on morphological and productive potato characteristics

Factor		Plant height	Stem number	Assimil. surface	Plant mass	Leaf mass	Stem mass
Adsorbent		**	**	**	**	**	**
Area		**	nssd	**	**	**	**
A * B		nssd	nssd	**	**	**	**
A	$LSD_{0.05}$	6.651	1.106	0.0276	41.39	22.370	25.045
	$LSD_{0.01}$	8.936	1.486	0.0370	55.60	30.052	33.647
B	$LSD_{0.05}$	3.840	0.639	0.0159	23.90	12.915	14.460
	$LSD_{0.01}$	5.159	0.858	0.0214	32.10	17.351	19.426
A*B	$LSD_{0.05}$	9.406	1.564	0.0390	58.53	31.635	35.419
	$LSD_{0.01}$	12.637	2.102	0.0524	78.64	42.501	47.584

(*) statistically significant difference, (**) statistically very significant difference, (nssd) no statistically significant difference

Plant height

The average potato plant height, regardless of the application of adsorbent and locality, is 80 cm. The greatest plant height is in the variant where the super-adsorbent enriched with growth stimulants, microorganisms and microelements is used (88 cm).

Table 4. The average potato plant height depending on the adsorbent in chosen areas (cm)

Adsorbent / Area	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	54.25	62.75	69.25	72.25	75.50	78.25	68.71
Bijeljina (B_2)	71.00	87.75	98.50	94.00	98.75	97.75	91.29
Average	62.62	75.25	83.88	83.12	87.12	88.00	80.00

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

The use of different variants of enriched super-adsorbent produced a significantly higher potato plant compared to the control variant (62.62 cm) and the variant in which super-adsorbent was used (75.25 cm). The obtained results are in harmony with the results obtained by Jahan et al. (2013) and Islam et al. (2011b), who, in multi-annual trials, had higher potato plants through use of adsorbents, and compared to control variants. Highly significant differences of plant height between the variant where super-adsorbent was used and the control variant were determined. Plant height in Bijeljina amounts to 91.29 cm, and in East Sarajevo 68.71 cm. These differences are highly significant. The obtained results are in accordance with the results that Lalić et al. (2019) obtained in their research.

Stem number

Stem number per plant varies considerably depending on variety, production conditions, the size of planted tuber, the number of sprouts per tuber and physiological ages of tuber (Khan et al., 2004; Momirović et al., 2016). Stem number per plant is a significant morphological property, as it affects the development of the above-ground mass, that is, assimilation surface area (Van der Zaag, 1992; Struik, 2007), the number of germinated tubers per plant, that is, total yield (Khan et al., 2004; Momirović et al., 2016). The average number of stems per potato plant, regardless of the application of adsorbent and locality is 4.69. The control variant had the smallest number of stems (3.38), and the largest super-adsorbent enriched with growth stimulants, microorganisms and microelements (6.38). The established differences are statistically highly significant, as well as the differences of other variants of super-adsorbents compared to the control variant. The number of stems per plant in Bijeljina is 4.96, and in East Sarajevo 4.42.

Table 5. The average number of stems per potato plant depending on adsorbent in chosen areas

Adsorbent / Area	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	3.75	4.25	5.25	5.25	4.50	6.75	4.96
Bijeljina (B_2)	3.00	4.00	4.25	5.00	4.25	6.00	4.42
Average	3.38	4.12	4.75	5.12	4.38	6.38	4.69

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

These differences had no statistical significance. The obtained results are in accordance with the results that Lalić et al. (2019) obtained in their research.

Assimilation surface area

The average assimilation surface area of potato, regardless of the application of adsorbent and locality, is 0.447 m^2. The assimilation surface area of the plant ranged from 0.340 m^2 per plant (A_0) to 0.553 m^2 (A_5). These differences were statistically highly significant, as well as the differences between the variant A_5 and other combinations of super-adsorbent and differences between enriched super-adsorbents and super-adsorbent.

Potato cultivated in Bijeljina (0.540 m^2) had statistically considerably greater assimilation plant surface area compared to potato cultivated in East Sarajevo (0.354 m^2). These differences are highly significant. The greatest stem mass in both areas was in the variant A_5, and the smallest one in the control variant. The greatest assimilation surface area in both areas was in the variant A_5, and the smallest one in the control variant. The obtained results are in accordance with the results that Lalić et al. (2019) obtained in their research.

The use of various agrotechnical measures can affect the increase of leaf surface. In this research, the use of different adsorbent variant resulted in the increase of assimilation plant surface area. With the increase of assimilation surface area, there was also an impact on the increase of photosynthetic plant production and crop yield increase (Stoimenov and Kirkova, 2009).

Table 6. Assimilation surface area of potato plant depending on adsorbent in chosen areas (m^2)

Adsorbent / Area	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	0,286	0,324	0,344	0,361	0,363	0,449	0,354
Bijeljina (B_2)	0,394	0,464	0,593	0,571	0,558	0,657	0,540
Average	0,340	0,394	0,469	0,466	0,460	0,553	0,447

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

Plant mass

Plant mass ranged from 365.5 to 896 grams. The use of super-adsorbent enriched with growth stimulants, microorganisms and microelements resulted in potato plants which, in comparison with other variants, had statistically considerably greater plant mass, while the control variant compared to the use of different super-adsorbent variants had statistically smallest plant mass. Highly significant variations of plant mass between super-adsorbent (A_1), super-adsorbent enriched with growth stimulants (A_2), super-adsorbent enriched with microorganisms (A_3) and super-adsorbent enriched with microelements (A_4) were determined. Plant mass in Bijeljina is 797.9 grams, and in East Sarajevo 464.3 grams. These differences are highly significant.

Table 7. The average potato plant mass depending on adsorbent in chosen areas (g)

Adsorbent / Area	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	398.3	393.0	381.0	528.9	423.2	661.4	464.3
Bijeljina (B_2)	332.6	642.1	756.7	1023.1	902.1	1130.6	797.9
Average	365.5	517.6	568.9	776.0	662.7	896.0	631.1

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

The greatest plant mass in both areas was in the variant A_5 1130 g; 661.4 g, and the smallest one 332.6 g in the control variant (in Bijeljina), that is, 381 g in the variant A_2 in East Sarajevo. In the area in Bijeljina, and compared to the area in East Sarajevo, plant mass was greater in the variants in which different adsorbent variants were used, while plant mass was greater in the control variant in East Sarajevo, compared to Bijeljina.

Leaf mass

Leaf mass ranged from 215.27 to 492.89 grams. The use of super-adsorbent enriched with growth stimulants, microorganisms and microelements resulted in potato plants which compared to other variants had statistically considerably greater leaf mass, while the control variant compared to the use of different variants had statistically considerably smallest leaf mass. Highly significant leaf mass variations between super-adsorbent (A_1), super-adsorbent enriched with growth stimulants (A_2), super-adsorbent enriched with microorganisms (A_3) and super-adsorbent enriched with microelements (A_4) were also determined. Leaf mass in Bijeljina is 426.01 g, and in East Sarajevo 248.49 g. These differences are highly significant. In both areas, the greatest leaf mass was in the variant A_5 (658.46 g; 327.31 g), and the smallest one 179.60 g in the control variant (in Bijeljina), that is, 213.37 g in the variant A_2 in East Sarajevo. The obtained results are in accordance with the results which Lalić et al. (2019) obtained in their research. The use of different adsorbent variants in the area in Bijeljina resulted in determining a greater potato leaf mass, compared to East Sarajevo, while in the area in East Sarajevo, control variant had greater leaf mass, compared to the control variant.

Table 8. The average potato leaf mass depending on adsorbent in chosen areas (g)

Area \ Adsorbent	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	250.95	220.09	213.37	269.72	209.52	327.31	248.49
Bijeljina (B_2)	179.60	323.33	383.81	513.17	497.69	658.46	426.01
Average	215.27	271.71	298.59	391.45	353.60	492.89	337.25

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

Stem mass

The average potato stem mass, regardless of the application of adsorbent and locality, is 293.84 g. Stem mass ranged from 150.19 grams to 403.12 grams. The use of super-adsorbent enriched with growth stimulants, microorganisms and microelements (403.12 g) and super-adsorbent enriched with microorganisms (384.55 g), resulted in potato plants that compared to other variants had statistically considerably greater stem mass, while the control variant compared to the use of different super-adsorbent variants had statistically considerably smallest stem mass.

Table 9. The average potato stem mass depending on adsorbent in chosen areas (g)

Area \ Adsorbent	A_0	A_1	A_2	A_3	A_4	A_5	Average
East Sarajevo (B_1)	147.38	172.93	167.64	259.15	213.68	334.04	215.80
Bijeljina (B_2)	152.99	318.79	372.89	509.96	404.42	472.19	371.87
Average	150.19	245.86	270.27	384.55	309.05	403.12	293.84

Adsorbent: Control variant (A_0); superadsorbent (A_1); superadsorbent enriched with growth stimulants (A_2); superadsorbent enriched with microorganisms (A_3); superadsorbent enriched with microelements (A_4) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5).

Highly significant variations of stem mass between super-adsorbent (A_1), super-adsorbent enriched with growth stimulants (A_2) and super-adsorbent enriched with microelements (A_4) were also determined. Stem mass in Bijeljina is 371.87 g, and in East Sarajevo 215.80 g. These differences are highly significant. The obtained results are in accordance with the results that Lalić et al. (2019) obtained in their research. In both areas, the greatest stem mass was in the variant A_5, and the smallest one in the control variant.

Conclusions

The use of different variants of super-adsorbent in both areas had positive effect on the morphological characteristics of potato. What is especially emphasised is the use of super-adsorbent enriched with growth stimulants, microorganisms and microelements, where we had the best results for: plant height, the number of stems, plant mass, leaf mass, stem mass and leaf surface area per plant.

Adequate results of the examined characteristics were stated in the use of super-adsorbent enriched with microorganisms, while the results of the variant in which super-adsorbents (control variant) were not used were poor for the examined characteristics.
In the trials conducted in Bijeljina, due to more favourable agro-ecological conditions, better results were realised for the morphological characteristics of potato, and compared to East Sarajevo.

References

Agency for Statistics in Bosnia and Herzegovina (2019): Harvested area and production of late crops, fruits and grapes, pp.1-3.

Baniuniene A., Zekaite V. (2008): The effect of mineraland organic fertilizers on potato tuber yield and quality. Latvian Journal of Agronomy, 11: 202-216.

Bhardwaj A. K., Shainberg I., Goldstein D., Warrington D.N., Levy G.J. (2007): Water retention and hydraulic conductivity of cross-linked polyacrylamides in sandy soils. Soil Science Society of America Journal, 71(2): 406-412.

Гвозден Г. (2016): Испитивање утицаја конвенционалног, интегралног и органског система гајења на продуктивност, квалитет и биолошку вриједност кромпира. Докторска дисертација, Пољопривредни факултет Београд. стр.1-231 (The influence of conventional, integrated and organic farming systems on productivity, quality and biological value of potato. Doctoral Dissertation).

Islam M.R., Xue X., Mao S., Ren C., Eneji A.E., Hu Y.G. (2011a): Effects of water-saving superadsorbent polymer on antioxidant enzyme activities and lipid peroxidation in oat (Avena sativa L.) under drought stress. Journal of the Science of Food and Agriculture, 91(4): 680-686.

Islam M.R., Xue X., Mao S., Zhao X., Eneji A.E., Hu Y. (2011b): Superadsorbent polymers (SAP) enhance efficient and eco-friendly production of corn (Zea mays L.) in drought affected areas of northern china. – African Journal of Biotechnology 10(24): 4887-4894.

Jahan M., Kamayestani N., Ranjbar F. (2013): Assay for applying super absorbent polymer in a low input corn (Zea mays L.) production system aimed to reduce drought stress under Mashhad conditions. – Agroecology 5(3): 272-281.

Khan I.A., Deadman M.L., Al-Nabhani H.S., Al-Habsi K.A. (2004): Interactionsbetween Temperature and yield components in exotic potato cultivars grown in Oman. Plant Breeding Abstracts, Vol. 74, (6): 1011.

Lalić S., Milić V., Lukin A., Govedarica B., Đurđić I., Tomić A. (2019): Influence of superapsorbent „Tverdaya voda" on morphplogical characteristics of potato. Proceedings of the X International Scientific Agricultural Symposium "Agrosym 2019", pp. 471-478.

Mann K.K., Schumann A.W., Obreza T.A., Sartain J.B., Harris W.G., Shukla S.. (2011): Analyzing the efficiency of soil amendments and irrigation for plant production on heterogeneous sandy soils under greenhouse conditions. Journal of Plant Nutrition and Soil Science, 174(6): 925-932.

Momirovic N., Brocic Z., Stanisavljevic R., Strbanovic R., Gvozden G., Stanojkovic Sebic A., Poštić D. (2016): Variability of dutch potato varieties under various agroecological conditions in Serbia. Genetika, Vol. 48, No.1, pp.109-124.

Sepaskhah A. R., Bazrafshan-Jahromi A. R. (2006): Controlling runoff and erosion in sloping land with polyacrylamide under a rainfall simulator. Biosystem Engineering, 93(4): 469-474.

Stoimenov G., Kirkova Y. (2009): Use of Digital Images in Determining the Leaf Surface Area of Plants. Agroinfo, pp.271-276.

Struik P.C. (2007): Responses of the potato plant to temperature. In: Plant Biology and Biotechnology: Advances and Perspectives (Ed. D. Vreugdenhil), 367-393. Elsevier, Amsterdam.

Wallace A., Wallace G.A., (1986): Effects of very low rates of synthetic soil conditioners on soils. Soil Science 141 (5) : 324 - 327. Wallace, A., Wallace, G.A.,(1986a): Effects of soil conditioners on emergence and growth of tomato, cotton and lettuce seedlings. Soil Science 141 (5): 313 - 316.

Van der Zaag D.E. (1992): Potatoes and their cultivations in the Neterlands, ed.NIVAA, The Hague, The Netherlands: 1-76.

CHEMICAL COMPOSITION OF THE FRUIT OF AUTOCHTHONOUS CULTIVARS OF APPLE FROM SARAJEVO AREA (BOSNIA AND HERZEGOVINA)

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Abstract

The paper presents the results of studying the chemical composition of the fruit of autochthonous cultivars of apple from Sarajevo area during an one–year period (2020. year). Determination found that it was the fruit of the next cultivars: Funtaca, Sadicka, Ticimka, Staklara and Car Konstantin. Significant differences were found between cultivars in content of soluble dry matter, total sugar, invert sugar, saccharose, total acids and pH value of the fruit of autochthonous cultivars. The cultivar of Staklara had the highest content of soluble dry matter, total sugar, invert sugar and pH value of fruit. The highest content of saccharose was in fruit of cultivar Sadicka, while the cultivar of Ticiminka had the highest content of total acids. Significant differences were found between cultivars in content of macroelements and microelements (magnesium, manganese, copper, aluminum, and zinc), but significant differences were not found between cultivars in content of trace elements (lead, cadmium, and arsenic). The cultivar Staklara had the highest content of magnesium, manganese, while the highest content of copper, aluminum and zinc was in the fruit of cultivar Car Konstantin. The fruits of autochthonous cultivars of apple had the same content of lead (0.10 mg/kg), cadmium (0.05 mg/kg) and arsenic (0.30 mg/kg). The analyzed autochthonous cultivars of apple from the Sarajevo area can be of great importance for integral and organic production, but also as a hybridization starting material to produce new, better cultivar resistant to pathogens of plant diseases and pests.

Key words: *apple, autochthonous cultivar, chemical composition, Sarajevo area.*

Introduction

Autochthonous cultivars of apple are the result of a long process of selection by human and natural conditions, characteristic of the area in which they originated. Bosnia and Herzegovina is rich with autochthonous cultivars and populations of many species of fruit, thanks to the diversity of its edaphic, climatic and geomorphological conditions. However, old traditional cultivars have been neglected, although they represent an important part of our natural and cultural heritage. Old cultivars represent a rich source of genetic material, but some cultivars, also, have very positive traits (e.g. high and regular yield), which is very important from the aspect of cultivation (*Mitre et al.,* 2009), and can have a significant role in further selection. Autochthonous cultivars are gene donors that are responsible for specific traits: resistance to pathogens and pests, color, aroma, resistance to abiotic environmental factors, storage traits.

Autochthonous cultivars are distributed as individual trees or in smaller plantations in a large number of localities in BiH, especially in the areas of Central and Western Bosnia. Their fruits may not look aesthetically perfect compared to standard cultivars (*Bignami et al.*, 2003), but they are characterized by „specific" pomological properties and characterized by different fullness of taste, as well as the ratio of sugars and acids, while some cultivars have a particularly pronounced fruit aroma. The reason may be that most traditional cultivars have a balanced composition of nutrients and bioactive substances (*Feliciano et al.*, 2010).

On the other hand, the processing industry is interested in production these cultivars due to the possibility of growing them without protection or with reduced use of pesticides. (*Militaru et al.*, 2009; *Tomić et al.,* 2011). Also, some old cultivars of apple are used in breeding programs as gene donors responsible for specific traits: resistance to pathogens and pests, color, aroma, resistance to abiotic factors, storage traits, etc. (*Bignami et al.*, 2003).

The aim of this study was to determine the most important chemical composition of the fruit of autochthonous cultivars of apple in the Sarajevo area.

Material and methods

The study on chemical composition of the fruit of autochthonous apple cultivars in the Sarajevo area in Bosnia and Herzegovina was implemented during an one–year period (2020) on five autochthonous cultivars of apple: Funtaca, Sadicka, Ticimka, Staklara and Car Konstantin. Laboratory tests were performed in the laboratory of the Federal Institute of Agriculture Sarajevo–Butmir. Fruits for chemical characterization were sampled in the period of their full maturity.

The following parameters were determined from the chemical properties of the fruit:

- Content of soluble dry matter („Carl Zeiss" binocular refractometer);
- Content of sugars (total and invert) (Luff−Schoorl method (*Džamić*, 1989));
- Content of total acids (titration with 0.1 N NaOH in the presence of phenol–phthalein as an indicator, (*Džamić*, 1989);
- pH value (pH meter CyberScan 510);
- Content of mineral element (digestion under pressure BAS EN 15763:2011).

The obtained data for chemical properties are represented by the mean value for three replicates ± standard error, and were statistically processed by Fisher's model of analysis of variance (ANOVA), using the F–test (*Fisher*, 1953) for $R \leq 0.05$ and $R \leq 0.01$. When the F−test was significant, the testing of arithmetic mean differences was determined using the *Duncan's* multiple interval test for a significance level of $P \leq 0.05$.

Results and discussion

The quality of the fruit of apple is a combination of a large number of physical and chemical, external and internal properties of the fruit. Also, it depends on the interaction of several factors: genotype (cultivar and rootstock), pollinator, physical and chemical properties of the soil, nutrient content and water regime of the soil (*Tagliavini* and *Marangoni*, 2002; *Militaru et al.*, 2009). According to *Chun et al.* (2005) the most important parameters of fruit quality (dry matter content, content of sugar, content of total acids, organic acids, phenolic compounds), depend on the cultivar, but also on the agro–ecological conditions, growing conditions, agrotechnical and pomotechnical measures, storage. Fruit of apple contain up to 17.0% dry matter, from 6.84% to 8.04% of sugar and from 0.16% to 0.56% of organic acids (*Mratinić and Vulic*, 1988). Table 1. shows the most important chemical composition of the fruit (content of soluble dry matter, content of total sugars, content of invert sugars, content of saccharose, content of total acids and pH value) of the fruit of autochthonous cultivars of apple from sarajevo area. The content of soluble dry matter was from 12.69% (Ticiminka) to 15.82% (Staklara). Significant differences were found between cultivars in content of soluble dry matter (table 1). According to the content of soluble dry matter, the studied cultivars can be classified into cultivars with medium high to high content of soluble dry matter. According to *Božović et al.* (2015) the content of soluble dry matter in fruits was from 9.6% to 15.2%, while *Bostan* (2009) states the content of soluble dry matter in the fruits of the local cultivars of apple from 10.5% to 15.0%. The fruits of the cultivars Busuta and Pazarka had the content of soluble dry matter of 15.2% and 14.7%, respectively, and these cultivars can be good raw materials for industrial processing (*Božović et al.*, 2015). According to *Kulina et al*. (2018) the content of soluble dry matter ranged from 12.93% (Bjeličnik) to 16.15% (Ljepocvjetka). The highest content of soluble dry matter was in the fruits of the cultivars Zlatna zimska parmenka and Citronka (*Skendrović–Babojelić et al.*, 2014). According to *Skender et al.* (2008) the content of soluble dry matter was from 11.0% (Divljakinja) to 17.25% (Muškinja). The highest content of soluble dry matter was in the fruits of the cultivars Aleksandrija (16.0%), Rebraca (15.5%), Jolovaca (14.6%) and Dunjka (14.5%), which indicates that these cultivars can be used as a material in the processing industry (*Božović et al.*, 2013). The lowest content of soluble dry matter was 12.0% and was determined in four cultivars, while the highest content was 17.0%, and it was recorded in fruit samples in only one of cultivar (*Jakobek et al.*, 2020). The content of soluble dry matter is the result of agroecological, primarily climatic conditions, and deviations ours from the results of other authors may be a consequence of cultivar, rootstock, agrotechnical and pomotechnical measures, as well as growing conditions. The content of total sugars in the fruit was from 4.87% (Tičiminka) to 8.83% (Staklara). The lowest content of invert sugars was in the cultivar Car Konstantin (3.79%), while the highest content had the cultivar Staklara (5.97%). The content of sucrose was from 0.88% (Tičiminka) to 2.73% (Šadićka). Significant differences were found between cultivars in total sugar, invert sugar and sucrose (table 1). The lowest content of invert sugars was in the fruits of the cultivar Divljakinja (3.0%), while the highest content was in the fruits of the cultivar Petrovka (6.09%) (*Skender et al.*, 2008). Acids give the fruit a sour taste. During the ripening period, the fruits accumulate sugar and break down the total the fruits tastier. Adequate ratio of sugars and acids gives apple fruits a harmonious and refreshing taste, which is an important criterion when evaluating and consuming fruits (*Mišić*, 2002). The content of total acids was from 0.15% (Staklara) to 0.48% (Ticiminka). Significant differences were found between cultivars in content of total acids (table 1). According to *Kulina et al*. (2018) the content of total acids was from 0.43% (Petrovaca) to 0.71% (Sampanjka). The highest content of total acids had the cultivars Lijepocvjetka and Zlatna zimska parmenka, slightly less the cultivar Bozicnica, while the significantly lowest content of total acids had the cultivars Bobovec and Citronka (*Skendrović–Babojelić et al.*, 2014). The content of total acids in the fruit of cultivar Petrovka was 0.12%, while the highest content of total acids was in the fruit of cultivar Divljakina (1.02%) (*Skender et al.*, 2008). According to *Harker et al.* (2002) high content of total acids is the best indicator of sour taste, while high content of soluble dry matter is an indicator of sweet taste of fruit. The balance between sugar content and organic acids is very important in achieving a harmonized taste of fruit (*Hudina* and *Štampar*, 2000; *Bignami et al.*, 2003).

Table 1. Chemical composition of the fruit of autochthonous cultivars of apple (2020 year)

Cultivar	Soluble dry matter (%)	Total sugars (%)	Invert sugars (%)	Saccharose (%)	Total acids (%)	pH values
Funtaca	14,43 ± 0,01 c	6,48 ± 0,01 c	4,56 ± 0,01 c	1,82 ± 0,01 b	0,41 ± 0,01 c	3,29 ± 0,01 d
Sadicka	15,70 ± 0,01 b	8,36 ± 0,01 b	5,52 ± 0,03 b	2,73 ± 0,01 a	0,43 ± 0,01 b	3,26 ± 0,01 e
Ticiminka	12,69 ± 0,01 e	4,87 ± 0,01 e	3,94 ± 0,01 d	0,88 ± 0,01 d	0,48 ± 0,01 a	3,47 ± 0,01 c
Staklara	15,82 ± 0,01 a	8,83 ± 0,01 a	5,97 ± 0,01 a	2,72 ± 0,01 a	0,15 ± 0,01 d	4,37 ± 0,01 a
Car Konstantin	14,21 ± 0,01 d	5,21 ± 0,01 d	3,79 ± 0,01 e	1,35 ± 0,01 c	0,44 ± 0,01 b	3,70 ± 0,01 b

Mean values followed by the same letter are not significantly differ according to Duncan's test (P≤0.05)

Table 2. Mineral content of the fruit of autochthonous cultivars of apple (2020 year) (mg/kg)

Cultivar	Magnesium	Manganese	Copper	Aluminum	Zinc	Lead	Cadmium	Arsenic
Funtaca	32,54 ± 0,01 e	0,16 ± 0,01 d	0,47 ± 0,01 e	0,18 ± 0,01 e	0,12 ± 0,01 e	0,10 ± 0,01 a	0,05 ± 0,01 a	0,30 ± 0,01 a
Sadicka	39,98 ± 0,01 d	0,16 ± 0,01 d	0,82 ± 0,01 c	0,59 ± 0,01 c	0,33 ± 0,01 c	0,10 ± 0,01 a	0,05 ± 0,01 a	0,30 ± 0,01 a
Ticiminka	46,58 ± 0,01 c	0,22 ± 0,01 c	0,93 ± 0,01 b	0,21 ± 0,01 d	0,20 ± 0,01 d	0,10 ± 0,01 a	0,05 ± 0,01 a	0,30 ± 0,01 a
Staklara	54,30 ± 0,01 a	0,48 ± 0,01 a	0,55 ± 0,01 d	0,45 ± 0,01 b	0,43 ± 0,01 b	0,10 ± 0,01 a	0,05 ± 0,01 a	0,30 ± 0,01 a
Car Konstantin	54,19 ± 0,01 b	0,26 ± 0,01 b	0,96 ± 0,01 a	0,68 ± 0,01 a	0,88 ± 0,01 a	0,10 ± 0,01 a	0,05 ± 0,01 a	0,30 ± 0,01 a

Mean values followed by the same letter are not significantly differ according to Duncan's test (P≤0.05)

The lowest pH value was in the cultivar Sadicka (3.26%), while the highest was in the cultivar Staklara (4.37%). Significant differences were found between cultivars in pH value (table 1). pH value of the fruit of autochthonous cultivars of apple from Sarajevo area was in agreement with earlier studies of autors *Kulina et al*. (2018). According to *Jakobek et al.* (2020) pH value was from 2.4 (Crvenka) to 3.8 (Ivanlija).

The deviation ours results from the results of other authors can be explained as a consequence of the influence of cultivar, rootstock, agrotechnical and pomotechnical measures, meteorological conditions, but also the time of maturity.

The fruits of autochthonous cultivars of apple from Sarajevo area had eight mineral elements divided into three groups: macroelements (magnesium), microelements (manganese, copper, aluminum and zinc) and trace elements (lead, cadmium and arsenic) (Table 2). Magnesium (Mg) was the element with the highest content in the fruit of apple, followed by, in descending order: manganese (Mn) > copper (Cu) > aluminum (Al) > Zinc (Zn) > lead (Pb) > cadmium (Cd) > arsenic (As). The content of magnesium (Mg) was from 32.54 mg/kg (Funtaca) to 54.30 mg/kg (Staklara). The content of microelements was in the following intervals: manganese (Mn) − from 0.16 mg/kg (Funtaca and Sadicka) to 0.48 mg/kg (Staklara); copper (Cu) – from 0.47 mg/kg (Funtaca) to 0.96 mg/kg (Car Konstantin); aluminum (Al) – from 0.18 mg/kg (Funtaca) to 0.68 mg/kg (Car Konstantin) and zinc (Zn)– from 0.12 mg/kg (Funtaca) to 0.88 mg/kg (Car Konstantin). The fruits of autochthonous cultivars of apple had the same content of lead (0.10 mg/kg), cadmium (0.05 mg/kg) and arsenic (0.30 mg/kg).

Significant differences were found between cultivars in content of macroelements and microelements (magnesium, manganese, copper, aluminum and zinc), but significant differences were not found between cultivars in content of trace elements (lead, cadmium and arsenic) (table 2). Heavy metals (lead, chromium, nickel, etc.) are elements whose excessive accumulation in plant products can cause various side effects on human health (*Duran et al.*, 2008). The largest amount of heavy metals is absorbed by the root system, with the mechanism of uptake and accumulation depending on the pH value of the soil, absorption capacity, amount of CaCO3, distance from the source that emits unwanted metals, exposure time, etc. (*Osmanović et al.,* 2014). The accumulation of heavy metals in the soil occurs due to: irrigation with water of inadequate chemical composition, application of mineral fertilizers, pesticides and protective agents based on undesirable elements. According to *Duran et al.* (2008) soil conditions are considered a potential source of accumulation of higher heavy metal content in dried fruits.

The content of mineral elements in fruits also depends on the amount of water in the soil, especially if it is known that there is a difference between mineral elements in their solubility in water. This is the reason why the content of mineral elements in fruits depends on irrigation, precipitation and soil permeability. The uptake of mineral elements is directly related to their availability in the soil, since certain elements can be fixed in clay minerals and as such are not available to plants. On the other hand, it is necessary to know their antagonism, in order to be able to interpret their content in the fruit.

However, analyzing the chemical parameters, we can conclude that we cannot observe the exact regularities, considering that the research was one-year-old and that their content cannot be observed in the context of meteorological conditions. This is especially important if it is known that environmental factors significantly influence the course of physiological and biochemical processes. Also, much more extensive and much more thorough studies are needed for more detailed analysis.

Conclusion

Based on one–year studies of the chemical composition of the fruit of autochthonous cultivars of apple from Sarajevo area, we can be made the following conclusions:
Qualitative properties of the fruit show statistically very significant to significant differences between the analyzed cultivars of apple. The content of soluble dry matter was from 12.63% (Ticiminka) to 15.82% (Staklara). The content of total acids was from 0.15% (Staklara) to 0.48% (Tičiminka). The lowest content of total sugars was in the cultivar Tičiminka (4.87%) and the highest in the cultivar Staklara (8.83%). The lowest content of invert sugars was in the cultivar Car Konstantin (3.79%), and the highest in the cultivar Staklara (5.97%). The saccharose content was from 0.88% (Tičiminka) to 2.73% (Šadićka). The lowest pH value was found in the cultivar Šadićka (3.26), and the highest in the cultivar Staklara (4.37).
The fruits of autochthonous cultivars of apple from Sarajevo area had eight mineral elements divided into three groups: macroelements (magnesium), microelements (manganese, copper, aluminum and zinc) and trace elements (lead, cadmium and arsenic).
Magnesium (Mg) was the element with the highest content in the fruit of apple, followed by, in descending order: manganese (Mn) > copper (Cu) > aluminum (Al) > Zinc (Zn) > lead (Pb) > cadmium (Cd) > arsenic (As).

References

Bignami, C., Scossa, A., Vagnoni, G. (2003). Evaluation of old Italian apple cultivars by means of sensory analysis. *Acta Horticulturae*, 598, 85–90.

Bostan, S. Z. (2009). Pomological traits of local apple and pear cultivars and types grown in Trabzon province (Eastern Black sea region of Turkey). *Acta Horticulturae*, 825, 293–298.

Božović, Đ., Jaćimović, V., Lazović, B. (2013). Old apple varieties in Central Montenegro. *Agriculture and Forestry*, 59(2), 217–223.

Božović, Đ., Jaćimović, V., Lazović, B., Adakalić, M. (2015). Phenological and pomological properties of the fruit of autochthonous cultivars of apple in Northern Montenegro Fenološke i pomološke osobine autohtonih sorti jabuke u sjevernoj Crnoj Gori. *Agro-knowledge Journal*, 16(2), 163–171.

Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T., Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, 85, 1715–1724.

Duran, A., Tuzen, M., Soylak, M. (2008). Trace element levels in some dried fruit samples from Turkey. *International Journal of Food Sciences and Nutrition*, 59 (7-8), 581–589.

Džamić, M. (1989). Biochemistry practicum. *Scientific book*, Belgrade.

Feliciano, R. P., Antunes, C., Ramos, A., Serra, A. T., Figueira, M. E., Duarte, C. M. M., De Carvalho, A., Bronze, M. R. (2010). Characterization of traditional and exotic apple varieties from Portugal. Part 1. *Nutritional, phytochemical and sensory evaluation. Journal of Functional Foods*, 2, 35–45.

Fisher, R.A. (1953). The Design of Experiments. Oliver and Boyd, London, UK.

Harker, F. R., Marsh, K. B., Young, H., Murray, S. H., Gunson, F. A. Walker, S. B. (2002). Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. *Postharvest Biology and Technology*, 24, 241–250.

Hudina, M., Štampar, F. (2000). Sugars and organic acids contents of European (*Pyrus communis* L.) and Asian (*Pyrus serotina* Rehd.) pear cultivars. *Acta Alimentaria*, 29, 217–230.

Jakobek, L., Ištuk, J., Buljeta, I., Voća, S., Šic Žlabur, J., Skendrović Babojelić, M. (2020). Traditional, indigenous apple varieties, a fruit with potential for beneficial effects: their quality traits and bioactive polyphenol contents. *Foods*, 9(52), 1−18.

Kulina, M., Radović, M., Životić, B., Životić, G. (2018). Physico–chemical characteristics the more significant autochthonous cultivars of apple from Majevica area. *XXIII biotechnology consulting*, 146–153.

Militaru, M., Braniste, N., Butac, M. (2009). Fruit quality of some autocthonous and foreign apple cultivars grown in Romania. *Acta Horticulturae*, 825, 547–552.

Mišić, P. (2002). Special fruit breeding. Nolit, 1–502.

Mitre, I., Mitre, V., Ardelean, M., Sestras, R., Sestras, A. (2009). Evaluation of old apple cultivars grown in central Transylvania, Romania. *Notulae Botanicae Horti Agrobotanici Cluj–Napoca*, 37 (1), 235–237.

Mratinić, E., Vulić, T. (1988). Autochthonous cultivars of apple in the Podkopaonicki region. *Yugoslav Pomology*, 86, 31–34.

Osmanović, S., Huseinović, S., Goletić, Š., Šabanović, M., Zavadlav, S. (2014). Accumulation of heavy metals in the fruit and leaves of plum (*Prunus domestica* L.) in the Tuzla area. *Food in health and disease*, 3(1), 44–48.

Skender, A., Jahić, S., Hadžiabulić, S., Kurtović, M. (2008). Evaluation of pomological characteristics of autochthonous cultivars of apple of Cazin region. *Modern agriculture*, 57(1–2), 124–130.

Skendrović–Babojelić, M., Korent, P., Šindrak, Z., Jemrić, T. (2014). Pomological properties and fruit quality of traditional cultivars of apple. *Bulletin of Plant Protection*, 37(3), 20–27.

Tagliavini, M., Marangoni, B. (2002). Major nutritional issues in deciduous fruit orchards of Northern Italy. *HortTechnology*, 12 (1), 26–31.

Tomić, L., Ninić, P., Cvetković, M. (2011). Study of fruit properties of autochthonous cultivars of apple in Western Bosnia. *XVI Consulting on biotechnology with international participation*, 16(18), 353–358.

VASE LIFE OF CUT FLOWERS USING DIFFERENT VASE SOLUTION

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Abstract

The experiment was conducted in the laboratory for post-harvest treatment and pomology of the Faculty of Agriculture, University of Banja Luka, Bosnia and Herzegovina, during 2020. There were one cultivar of gerbera (*Gerbera jamesonii* 'Olympic Gold') and one cultivar of calla (*Zantedeschia aethiopica* 'Captain Ventura') used in this research. Preservative solutions Flower care (0.2%) and sucrose (2%) were used as vase solution to increase vase life of gerbera and calla. Average temperature in cold room, during the experiment was 9°C with relative humidity 65%, without additional light. Statistical data analysis determined a significantdifference in cut flower vase life depending on the vase solution. Vase life of cut gerbera flower kept in sucrose was 28 days (in changing solution) and 21 days (in permanent solution) on average, while vase life of cut gerbera flower kept in Flower care was 24 days (in changing solution) and 16 days (in permanent solution) on average. Vase life of cut calla flower kept in Flower care was 26 days (in changing solution) and 20 days (in permanent solution) on average, while vase life of cut calla flower kept in sucrose was 24 days (in changing solution) and 22 days (in permanent solution) on average. Transpiration intensity also depended on the solution for keeping flowers. The highest transpiration intensity was determined with cut flower which had the longest vase life and vice versa. The research indicates that the application of the preparation Flower care, which contains glucose, biocides and acidifiers, affects the viability of calla flower and prolongs the vase life. Also, the application of the preparation with sucrose prolongs the vase life of gerbera flower.

Keywords: *cut flower, vase life, Gerbera jamesonii, Zantedeschia aethiopica, Flower care, sucrose.*

Introduction

Short postharvest vase life is one of the most important problems on the cut flowers. Longevity of vase life is an important factor in consumer preference (Da Silva, 2003). Maintaining good quality of cut flowers and extending the vase life, is considered important for having acceptable products for the markets. Furthermore, transportation of cut flowers over long distances has increased. These conditions are unsuitable for keeping the quality of cut flowers. Keeping quality is an important parameter for evaluation of cut flower quality, both in export and domestic markets. The major reasons for shorten vase life of cut flowers are nutrient deficiency, bacterial and fungal contaminations (Kazemi *et al.*, 2011) water stress-induced wilting and vascular blockage (Alaey *et al.*, 2011). Bacteria in the vase water can block the vessels in the surface of cut stems (Ferrante *et al.*, 2007). This can have influence on water uptake which is one of the reasons for water deficit and wilting during vase life (Knee, 2000; Van Ieperen et al., 2002).

Gerbera (*Gerbera jamesonii*) and calla (*Zantedeschia aethiopica*) are very popular cut flowers, having short vase life, and mostly are used freshly, so their vase life improvement is one the first floriculture's purposes (Elgimabi and Ahmed, 2009).
Postharvest life of cut flowers could be affected by the application of various chemicals. Different chemicals have been used in vase solution to extend vase life of cut flowers mainly by improving their water uptake and reducing transpiration, inhibiting microorganisms growth, thereby promote the vase life ((Nair *et al*., 2003; Prashanth *et al*., 2010; Lu *et al.*, 2012). Some of these chemicals include silver nitrate, 8-hydroxyquinoline sulfate and 8-hydroxyquinoline citrate, which are expensive and harmful for the environment and human health (Ichimura *et al*., 1999; Solgi *et al*., 2009; Ansari *et al.*, 2011). It is very important to use natural, safe and inexpensive compounds for the application of preservatives improving cut flower vase life (Kilic and Cetin, 2014). The application of different medicinal plants essential oils on increasing the vase life of cut flowers have been studied by many researches. Essential oils (EO) are organic, natural, safe and eco-friendly substances that have strong anti-inflammatory, antibacterial, antifungal, antioxidant and anticarcinogenic effects, and have the high levels of phenolic compounds (Bayat *et al*., 2011; Raut and Karuppayil, 2014).
Carbohydrate is the main food source to maintain the energy requirement for flowers. Sugars play important roles in keeping the quality of cut flowers as substrates for respiration and cell walls as well as osmolytes. Since the amount of sugar contained in cut flowers is limited the addition of sugars such as sucrose to vase water is effective in improving the vase life of some cut flowers (Chaudhary and Khanal, 2018). Addition of sugars to vase water not only extends the vase life of cut flowers but also promotes flower opening.
Cut flower food products provide all the nutrients needed for flowers to bloom naturally and fully. At the same time it keeps the flowers and foliage fresher and healthier longer, and extends the vase life of cut flowers. Flower food is available in gel, powder or liquid forme.
Therefore the aim of this study was to search for the preservative solutions ("Flower care" and sucrose) for extending vase life of cut gerbera and calla flowers.

Material and Methods

The experiment was conducted in the Laboratory for post-harvest treatment and pomology of the Faculty of Agriculture, University of Banja Luka, Bosnia and Herzegovina, during 2020. There were one cultivar of gerbera (*Gerbera jamesonii* 'Olympic Gold') and one cultivar of calla (*Zantedeschia aethiopica* 'Captain Ventura') used in this research. Preservative solutions "Flower care" (0.2%) and sucrose (2%) were used as solution to increase vase life of gerbera and calla. Experiment was laid out with two treatments, under completely randomized design with four replications with three flowers steams in each replicate. Flowers were kept in 750 ml glass jars, containing 300 ml of vase solution. There were two variant, with changing solution (every third day) and with permanent solution. Average temperature in cold room, during the experiment was 9°C with average relative humidity 65%, without additional light. Temperature and relative humidity were recorded in the morning and evening each day. Before setting up the experiment, stems were recut to 35 cm length. The following data were recorded: fresh weight of flowers (g); transpiration intensity of the solution (ml), and flower longevity - vase life. A digital scale, typ KERN, was used to determine fresh weight (g) of the flowers. The transpiration intensity of the solution (ml) was measured by subtracking the vase solution after a certain time from the initial vase solution:

transpiration intensity = $Vu - V_1$
Vu (ml) – initial vase solution (300 ml); V_1 (ml) – the vase solution after a certain time
Flower longevity or vase life of cut flowers was completed when the petals or stem below the flower had lost turgidity. Collected data were analyzed using a statistical program VV-Stat (Vukadinović, 2017). Fisher's LSD test was applied to judge statistical significance of differences between treatments using. Mean values were considered significantly different when $p < 0.05$.

Results and Discussion

The average values of fresh weight of cut gerbera and calla flowers in the cold room statistically depending on the applied and typ of solution.
The highest average value of fresh weight of cut gerbera flower was observed in the flower food solution (A1) in the amount of 30.49 g, in a changing solution. Also, the lowest value was observed in the flower food solution, in a permanent solution, in the amount of 26,14 g (A1). In the sucrose solution (A2), in both solution variant, value of fresh weight of cut gerbera flower was almost the same (27.74 g; 28.71 g).
The highest average value of fresh weight of cut calla flower was observed in the flower food solution (A1) in the amount of 36.23 g, in a changing solution. The lowest value was observed in sucrose solution (A2) in the amount of 28.25 g, in a permanent solution.

Table 1. Fresh weight of cut gerbera and calla flowers (FW g) (means marked with different letters ^{a,b} significantly differ at $p < 0.05$; *ns* = not significant; ** = Ftest 99%; * = Ftest 95%)

		FW (g)		FW (g)	
		Gerbera jamesonii		*Zandetschia aethiopica*	
Type of solution		changing solution	permanent solution	changing solution	permanent solution
Treatment (A1)		30.49^{a}	26.14^{b}	36.23^{a}	29.43^{a}
Treatment (A2)		27.74^{b}	28.71^{a}	28.39^{b}	28.25^{a}
Average		29.12	27.43	32.31	28.84
Analysis of variance - F		*17.13218**	*9.90715**	*51.7518***	*2.087884*
LSD	*0,05*	*1.8444*	*2.2666*	*3.0253*	*ns*
	0,01	*Ns*	*Ns*	*5.0175*	*ns*

The highest transpiration intensity (ml) of cut gerbera flower, in changing solution, was observed in the flower food solution (A1) in the amount of 14.46 ml, and the lowest in the sucrose solution (A2) 11.82 ml. In permanent solution results were different, the highest transpiration intensity (ml) of cut gerbera flower, was observed in the sucrose solution (A2) in the amount of 115 ml, and the lowest in the flower food solution (A1) 75 ml. The same ration was observed in transpiration intensity of cut calla flower, where the highest transpiration intensity was observed in the flower food solution (A1) in the amount of 5.85 ml, and the lowest in the sucrose solution (A2) 3.12 ml. In permanent solution results were almost the same in both vase solution (34 ml; 35 ml) (Table 2.).

Table 2. Transpiration intensity of the solution (TI ml) of cut gerbera and calla flowers (means marked with different letters [a,b] significantly differ at $p < 0.05$; *ns* = not significant; ** = Ftest 99%; * = Ftest 95%)

		TI (ml)		TI (ml)	
		Gerbera jamesonii		*Zandetschia aethiopica*	
Type of solution		changing solution	permanent solution	changing solution	permanent solution
Treatment (A1)		14.46[a]	75.00[b]	5.85[a]	34[a]
Treatment (A2)		11.82[b]	115.00[a]	3.12[b]	35[a]
Average		13.14	95	4.49	34.5
Analysis of variance - F		*17.53128*[*]	*2400*[**]	*29.87903*[**]	*1.5*
LSD	*0,05*	*1.7459*	*2.2666*	*1.3830*	*ns*
	0,01	*Ns*	*3.7592*	*2.2938*	*ns*

Vase life of gerbera and calla varied among the vase solutions (Table 3.). Maximum vase life of cut gerbera flowers, was found in changing sucrose solution (27.78 days) followed by 23.78 days in changing flower food solution. Minimum vase life of cut gerbera flowers was found in permanent sucrose solution with 21.33 days followed by 15.66 days in permanent flower food solution. Maximum vase life of cut calla flowers was also in changing solutions with maximum vase life of 26.33 days in the flower food solution, and minimum with 23.89 days in the sucrose solution. In permanent solutions vase life of cut calla flowers was almost the same in the flower food and in the sucrose vase solution (20.33 days; 21.67days).

Table 3. Vase life of cut gerbera and calla flowers (means marked with different letters [a,b] significantly differ at $p < 0.05$; *ns* = not significant; ** = Ftest 99%; * = Ftest 95%)

		vase life		vase life	
		Gerbera jamesonii		*Zandetschia aethiopica*	
Type of solution		changing solution	permanent solution	changing solution	permanent solution
Treatment (A1)		23.78[b]	15.66[b]	26.33[a]	20.33[a]
Treatment (A2)		27.78[a]	21.33[a]	23.89[b]	21.67[a]
Average		25.78	18.50	25.11	2.693604
Analysis of variance - F		*19.07187*[*]	*48.22528*[**]	*9.848342*[*]	*1.5*
LSD	*0,05*	*2.5426*	*2.2665*	*2.1613*	*ns*
	0,01	*Ns*	*3.7591*	*ns*	*ns*

According to the presented results for flower longevity or vase life is better to changing vase solution than left it constant. Maximum vase life of cut gerbera flowers was in sucrose vase solution, while maximum vase life of cut calla flowers was in the flower food solution. Flower longevity and quality of cut flowers in vase solution depend of numerous number of factors like genetical constituents, pre-harvest conditions, harvesting technique, packaging, post-harvest handling and storage. But for the post harvesting storage different chemicals influences the vase life and floral quality of cut flowers (Khan et al. 2015). The vase life of gerbera and calla mostly depending on the how upright the stem is or "bent neck". Sugar acts as the carbohydrate source and also makes the cells of the flower stem concentrated with sugars that are carried up by the phloem. The hypertonic solutions inside the cells allow water to enter the cells by osmosis and thus make them turgid. This turgidity gives the stem a rigid, upright structure (Khan et al. 2015).

The longest vase life of cut gerbera flower was found in the treatment with sugar, but the longest vase life of cut calla flowers was in the treatment with flower food, in both variant when solution is changing. Flower food has certain antimicrobial properties, which reduce the degree of vascular blockage, thus allowing for optimum solution uptake and reducing stem bending.Treatment with sucrose promoted floret opening and extended the vase life of cut flower, increased anthocyanin concentrations in petals as well as extended the vase life of several cultivars of cut Eustoma flowers and of cut hybrid Limonium (Ichimura, 1998). Longevity of many cut flowers is negatively influenced by the presence of ethylene by inducing various physiological responses like wilting of leaves and petals. Silver thiosulphate is known to suppress autocatalytic ethylene production (Da Silva, 2003). Silver ion available in the silvar thiosulphate had bactericidal property and reduced the frequency of bent necks and improved the vase life of cut roses (Torre and Fjeld, 2001). Citric acid has also been found to play a key role to extended vase life of cut gerbera. Citric acid has an ability to extend vase life of cut flowers in association with inhibition of ethylene production (Srivastava and Dwivedi, 2000).The application of the flower food preparation "Chrysal clear", which contains glucose, biocides and acidifiers, affects the viability of rose flower and prolongs the vase life (Kraljičak et al., 2012).

Conclusions

Based on the results of this study agents such as sugar and "Flower care" had a positive effect on flower vase life. Sugar (treatment A2) was the best chemical treatments for increasing the vase life of gerbera by delayed senescence, while maximum vase life of cut calla flowers was in the flower food solution (treatment A1). Flower food had very effective antimicrobial agents, which inhibited the microbial growth and prevented bacterial plugging in conducting tissues. Additional supply of sugars can increase water balance and osmotic concentration and sucrose had an important role in lengthening the vase life of cut flowers. Flower longevity and quality of cut flowers in vase solution depend of numerous factors, and this reserach showed that vase life of cut flowers also depends on the flower species. According to the presented results for vase life is better to changing vase solution than left it constant. Thus, it is recommended to use sugar or flower food as a vase solution for gerbera and calla to extent shelf life.

References

Alaey M., Babalar M., Naderi R., Kafi, M. (2011). Effect of pre- and post-harvest salicylic acid treatment on physio-chemical attributes in relation to vase-life of rose cut flowers. Postharvest Biology and Technology,Vol. 61, 91-94.

Ansari S., Hadavi E., Salehi M., Moradi P. (2011). Appl ication of microorganisms compared with nanoparticles of silver, humic acid and gibberellic acid on vase life of cut gerbera Good Timing. Journal of ornamental and horticultural plants, Vol. 1(1), 27-33.

Bayat H., Azizi M., Shoor M., Marandi H. (2011). Effect of ethanol and essential oils on extending vase-life of carnation cut flower (Dianthus caryophyllus cv. 'Yellow Candy'). Notulae Scientia Biologicae, Vol. 3(4), 100-104.

Chaudhary G., Khanal A. (2018). Effects of Different Concentrations of Sucrose on Vase Life of Rose (Rosa sps. cv. Dutch Hybrid). Journal of the Institute of Agriculture and Animal Science, Vol. 35(1), 183-190.

Da Silva J.A.T. (2003). The cut flower: Postharvest considerations. Journal of Biological Science, Vol. 3(4), 406-442.

Elgimabi M.N., Ahmed O.K. (2009). Effects of Bactericides and Sucrose-Pulsing on Vase Life of Rose Cut Flowers (Rosa hybirida). Botany Research International, Vol. 2(3), 164-168.

Ferrante A., Alberici A., Antonacci S., Serra G. (2007). Effect of promoter and inhibitors of phenylalanine ammonia-lyase enzyme on stem bending cut gerbera flowers. International Conference on Quality Management in Supply Chains of Ornamentals, Vol. 755, 471-476.

Ichimura K. (1998). Improvement of postharvest life in several cut flowers by the addition of sucrose. Japan Agricultural Research Quarterly, Vol. 32, 275-280.

Ichimura K., Kojima K., Goto R. (1999). Effect of temperature, 8-hydroxyquinoline sulphate and sucrose on the vase life of cut rose flowers. Postharvest Biology and Technology, Vol. 15(1), 33-40.

Kazemi M., Zamani S., Aran M. (2011). Effect of some treatment chemicals on keeping quality and vase life of cut flowers. American Journal of Plant Physiology, Vol. 6, 99-105.

Khan P., Shahrin S., Taufique T., Mehraj H., Jamal Uddin A.F.M. (2015). Prolonging vase life of cut rose (Rosa hybrida L. cv. "Red Pearl") through chemical preservatives. Journal of Bioscience and Agriculture Research, Vol. 5(1), 10-15.

Kilic T., Cetin E.S. (2014). Determination of the effects of sage and balm extracts on vase life in gerbera cv. Rosalin. International Journal of Agricultural and Natural Sciences, Vol. 7(2), 13-15.

Knee M. (2000). Selection of biocides for use in floral preservatives. Postharvest Biology and Technology, Vol. 18(3), 227-234.

Kraljičak J., Parađiković N., Ćosić J., Tkalec M. (2012). Effect of different preparations for prolonged vase life of rose flower. Glasnik zaštite bilja, Vol. 35(6), 14-21.

Lu P., Cao J., He S., Liu J., Li H., Cheng G., Ding Y., Joyce D.C. (2012). Nano-silver pulse treatments improve water relations of cut rose cv. Movie Star flowers. Postharvest Biology and Technology, Vol. 57(3), 196-202.

Nair S.A., Singh V., Sharma T.V.R.S. (2003). Effect of chemical preservatives on enhancing vase-life of gerbera flowers. Journal of Tropical Agriculture, Vol. 41, 56-58.

Prashanth P., Sekhar R.C., Reddy K.C.S. (2010). Influence of floral preservatives on scape bending, biochemical changes and postharvest vase life of cut gerbera (Gerbera jamesonii Bolus ex. Hook.). Asian Journal of Horticulture, Vol. 5(1), 1-6.

Raut J.S., Karuppayil S.M. (2014). A status review on the medicinal properties of essential oils. Industrial crops and products, Vol. 62, 250-264.

Solgi M., Kafi M., Taghavi T.S., Naderi R. (2009). Essential oils and silver nanoparticles (SNP) as novel agents to extend vase life of gerbera (Gerbera jamesonii cv. Dune) flowers. Postharvest Biology and Technology, Vol. 53(3), 155-158.

Srivastava M.K., Dwivedi U.N. (2000). Delayed Ripening of Banana Fruit by Salicylic Acid. Plant Science, Vol. 158, 87-96.

Torre S., Fjeld T. (2001). Water loss and postharvest characteristics of cut roses grown at high or moderate relative air humidity. Scientia Horticulturae, Vol. 89(3), 217-226.

Van Ieperen W., Van Meeteren U., Nijsse J. (2002). Embolism repair in cut flower stems: a physical approach. Postharvest Biology and Technology, Vol. 25(1), 1-14.

Vukadinović V. (2017). VV-Stat - statistical program. http://tlo-i-biljka.eu/Kalkulatori.html.

ENERGY FOR DYNAMIC TEARING OF GYNOPHORS OF BULGARIAN PEANUT VARIETIES

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Abstract

The aim of the research is to determine the energy for dynamic detachment of the fruits of fifteen promising Bulgarian peanut varieties. It was determined that the value and nature of the energy change differ significantly for the studied varieties, but it predetermines scattering losses during the mechanized harvesting. The results shows different dynamics of change of energy for tearing off gynophore in different varieties. In some of them, it varies widely, at the expense of a slight decrease in humidity. In others, the energy changes in a narrower range with a more intensive decrease in the pods' humidity. The strengthening of the gynophore by lowering the moisture content in the fruits is an indicator for increasing the resistance of the beans to mechanical influences during the period of field ripening until reaching technological maturity. The most suitable variety of peanuts for mechanized harvesting is 539_mother. Its energy used to detach fruits from stems decreases with decreasing humidity and mass of the fruit. It reaches a maximum of 0.075 J at an average mass of 2 g and a moisture content of 10%. The results obtained can be used to determine the varieties which give the least scattering losses of peanut fruits at mechanized harvesting as well as in the selection varieties, suitable for mechanized harvesting.

Keywords: *Peanuts, Energy, Gynophors, Bulgarian.*

Introduction

Peanuts are the fifth most important oil crop in the world (Burns, 2010). It is a valuable raw material not only because of its good taste, but also because of its high nutritional value. The seeds contain up to 32% protein and 42% to 52% oil (Putnam et al., 2013). Peanuts in Bulgaria are not the main crop and are grown mainly by small farmers with a lot of manual labor (Bencheva and Gergiev, 1997). Mechanization of peanut harvesting processes makes their cultivation more cost-effective (Bertonha et al., 2014). But with the introduction of mechanization, a significant problem arises, namely the large losses of peanuts fruits on the field (Thomas et al., 1983). Most of the losses are formed during the digging of plants from the soil. According to studies by Santos et al., (2013), these losses range from 3.1% to 47% relative to yield. Other authors report losses of 8%, which reach 40% if harvesting is carried out at a humidity below the optimum (Young et al., 1982; Lamb et al., 2004). Similar results have been achieved in studies conducted in Bulgaria with established Bulgarian peanut varieties. They indicate total losses of 9.7% to 30.6% relative to biological yield (Stamatov et al., 2020).
According to many researchers, the main factor on which the formation of losses in mechanized harvesting of peanuts depends is the retention force of the fruits to the plant (Zerbato et al., 2014; Bencheva et al., 2008). It was found that it is not affected by the diameter of the gynophore and depends most strongly on the anatomical structure of gynophores (Thomas et al., 1983,

Bogomilov et al., 2016). Experiments conducted with Bulgarian peanut varieties - Tsvetelina, Kremena, Kalina, Adata and Hybrid 11 - confirm the individual varietal characteristics in terms of strength of gynophore. The energy for tearing the fruits was studied and was found that of different varieties their values and natures of change are different (Ishpekov, et al., 2021). These results indicate that the selection of Bulgarian peanut varieties is not aimed at strengthening the gynophores so far. The assessment the dynamic strength of gynophores by the energy for their detachment creates conditions for determine the susceptibility of peanut varieties to mechanized harvesting. This energy provides valuable information for selecting peanuts varieties with increased dynamic strength of the bond between the peanuts fruit and the plant in order to minimize losses in mechanized harvesting. The aim of the study is to determine the energy for dynamic tearing of peanut fruits from Bulgarian peanut varieties.

Materials and Methods

Existing methodology and experimental tool are applied to determine the strength of the peanut pod (Ishpekov et al., 2021). The energy consumption for picking a single peanut pods is measured depending on its mass and the moisture content of the shell. The study was performed with 15 genotypes from the early generations of the selection at three levels of the mentioned factors. The mentioned energy is measured by means of an experimental system consisting of a pendulum apparatus, stem attachment tool and an electronic data acquisition system.

At the beginning of each experiment, the stem is cut from the plant with a length of 40 - 50 mm to which a gynophore with peanut beans is attached. The bean is positioned on the base of the pendulum platform so that it touches the pendulum in its equilibrium position, and the stem is fixed between two planes. The electronic system is started, the pendulum is deflected at a set angle and after the pause, it is unlocked and lowered to its equilibrium position, where it detaches the bean from the stem. The energy for tearing and moving the beans is calculated through the difference between the angle of descent and the angle of deflection of the pendulum after tearing the bean off. The measurement is repeated with the same bean to determine the energy for its movement only. The difference between the two measured energies gives that for tearing the bean from the gynophore. The measurement data is visualized using a virtual instrument developed in the environment of the LabView software package (Figure 1).

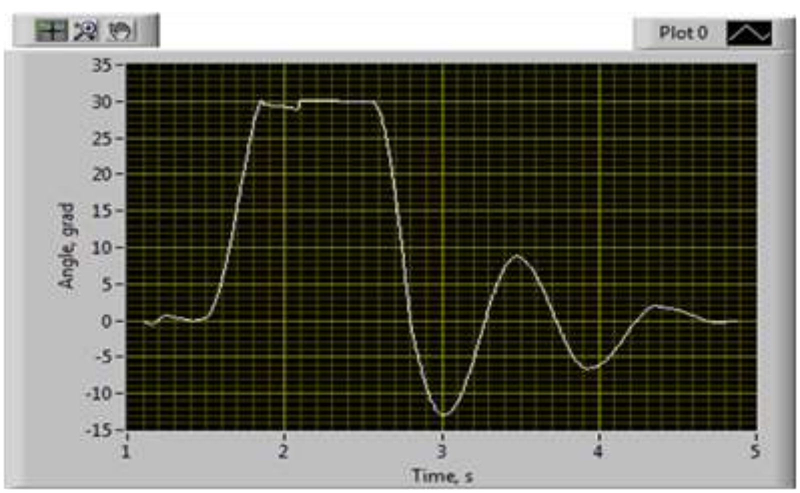

Figure 1. Graph for reading the angles of incidence and deflection of the pendulum after tearing peanuts pods off

Results and Discussion

On figures 2a and 2b are shown the influence of the average mass of bean on the energy for its detachment.

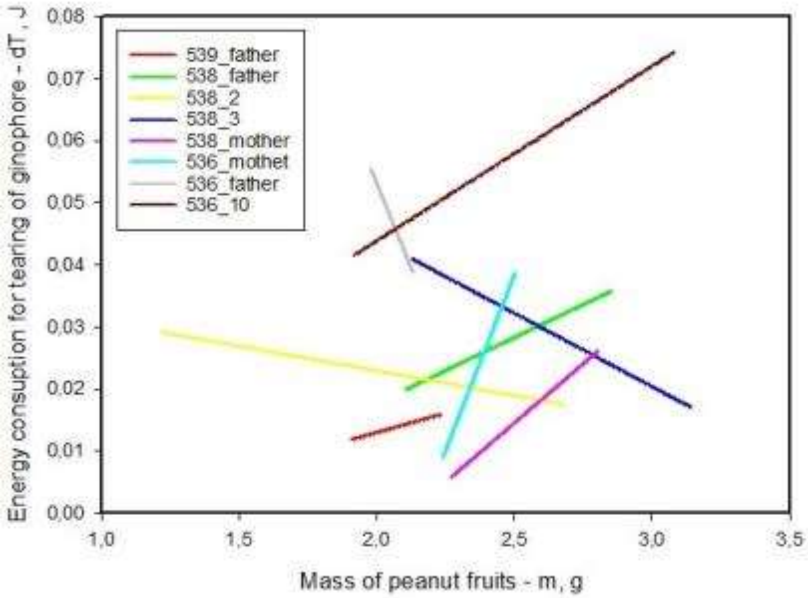

Figure 2a. Energy for tearing off peanut fruit depending on its mass

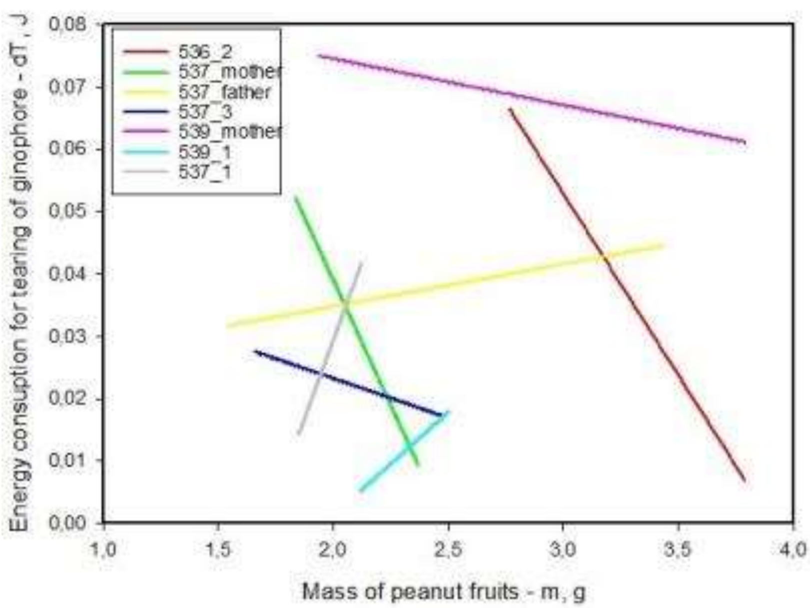

Figure 2b. Energy for tearing off peanut fruit depending on its mass

It is obviously that the nature of the change in the energy for plucking the peanut beans is different in different genotypes. Depending on this, two groups of varieties are distinguished. The first group includes those in which the energy for detachment decreases with the increasing mass of beans. This is observed in genotypes 536_2, 537_mother, 537_3, 539_mother, 538_2, 538_3, and 536_father. The second group includes varieties in which the energy for detachment increases with decreasing bean mass. These genotypes are: 537_father, 539_1, 537_1, 539_father, 538_father, 538_mother, 536_mother and 536_10.

The influence of peanut fruits moisture content on energy consumption is similar (Figure 3 a, b). It is obvious that the nature of the change in energy for dynamic tearing off strongly depends on the specific genotype. This confirms the results from studies conducted by Ishpekov, S., (2021), where the same phenomenon was observed in other varieties.

The results show different dynamics of change of energy for tearing off gynophore in different varieties. In some of them, it varies widely, at the expense of a slight decrease in humidity. This is observed at 536_10, 538_3, 537_1, 537_mother, 536_2, 538_mother and 538_3. In others, the mentioned energy changes in a narrower range with a more intensive decrease in the humidity of the pods. This applies to the varieties 538_father, 539_father, 536_mother, 537_father, 537_3, and 539_mother.

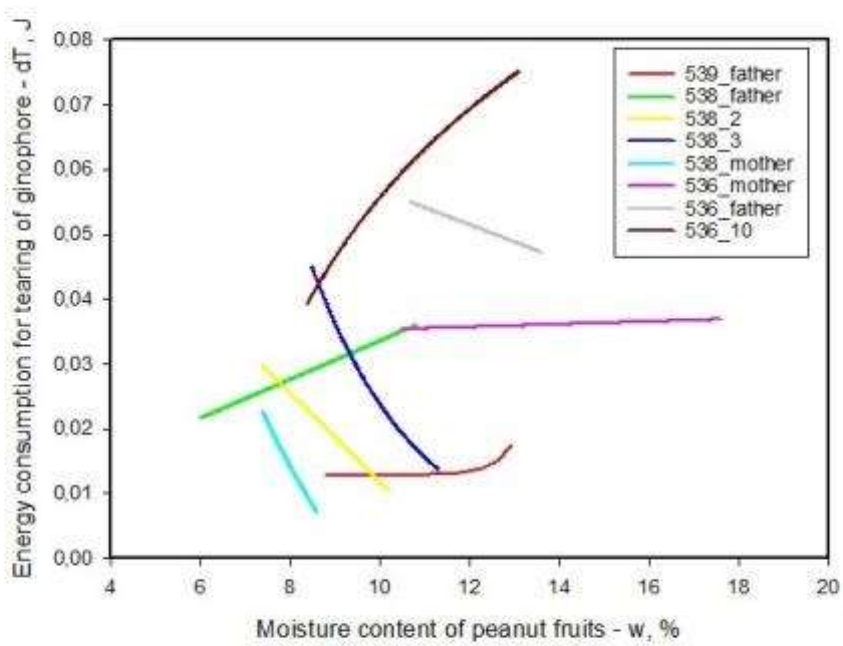

Figure 3a. Energy for tearing beans depending on its humidity

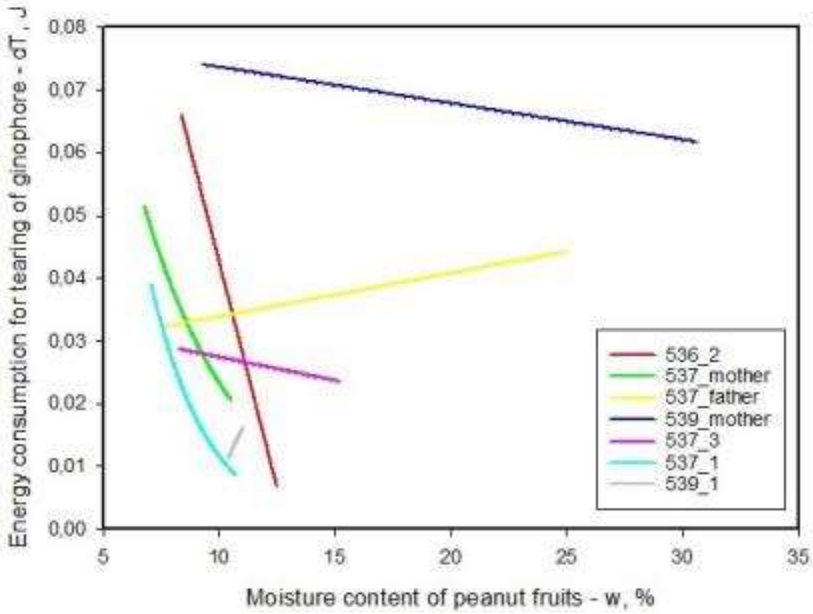

Figure 3b. Energy for tearing beans depending on its humidity

The results provide information for the dynamic strength of the gynophores of 15 Bulgarian peanut varieties. The higher energy release shows a greater dynamic strength between the bean and stem, which is decisive for the losses when digging the fruits out of the soil. The strengthening of the gynophore by lowering the moisture content in the fruits is an indicator for increasing the resistance of the beans to mechanical influences during the period of field ripening until reaching technological maturity.

Within the studied varieties, the best indicators were achieved by variety 539_mother. Its energy expended to detach the beans from the stems decreases with decreasing humidity and bean mass. It reaches its maximum of 0.075 J at an average bean weight of 2 g and shell moisture of 10% (Figure 2b and Figure 3b).

The results obtained can be used to determine the varieties that are suitable for mechanized harvesting, as well as for the selection of varieties with reinforced gynophores, which minimize scattering losses in mechanized harvesting.

Conclusions

The energy for dynamic tearing off of the gynophore, which connects the stem with the fruit of the peanuts, predetermines their resistance to rupture during their mechanized harvesting and reflects on the losses of unharvested production.

The energy for dynamic tearing off of a single bean from promising Bulgarian peanut variety lines has been experimentally determined. These are 536_2, 537_mother, 537_3, 539_mother, 538_2, 538_3, 536_father, 537_father, 539_1, 537_1, 539_father, 538_father, 538_mother, 536_mother and 536_10. The size and nature of its change depending on the mass of the bean and the moisture content of its pods have been established. The energy for tearing the peanut varies in different directions for different varieties. In some, it increases, and in others, it decreases with decreasing moisture content and mass of beans, which confirms that the dynamic strength of the gynophore is a varietal characteristic.

From the point of view of the energy for tearing off the beans, the most suitable for mechanized harvesting is variety 539_mother, followed by 536_10 and 536_2. The varieties 538_mother, 539_father, 539_1, 538_2, and 537_3 show the lowest energy for dynamic tearing off.

References

Bencheva, N., Gergiev, St. (1997). Situation and Prospects of Groundnut Production in Bulgaria, *Agricultural Economics and Management, 7-8*, 43–48.

Bertonha R. S, Silva R. P, Furlani C. E. A, Nascimento J. M, Cavichio li F. A, 2014. Perdas e teor de água das vagens no arranquio mecanizado de a mendoim. Energia na Agricultura 29(1):167-173. DOI: http://dx.doi.org/10.17224/ EnergAgric, 2014v 29n 3p167-173.

Burns S. P (2010). Strategies for enhancing leaf spot (Cercospora arachidicola and Cercosporidium personatum) tolerance in peanut (*Arachis hypogaea* L.). Thesis. University of Florida.

Ishpekov, S., Naydenov, N., Zaykov, R., 2021., in print. Method for measuring the energy consumption for dynamic tearing off the peanut gynophores. *Bulg. J. Agric. Sci., 3: 2021;*

Bogomilov B., Kolev B., Todorov T., 2016. Theoretical background for determining parameters of channel in granulator dies., 55 th Science Conference of Ruse University, Bulgaria, pp. 114 – 120, ISSN 1311-3321.

Lamb MC, Masters MH, Rowland D, Sorensen RB, Zhu H, Blankenship PD, Butts CL (2004). Impact of sprinkler irrigation amount and rotation on peanut yield. Peanut Science. College Station 31:108–113.

Putnam D. H, Oplinger ES, Teynor TM, Oelke EA, Kelling KA, Doll JD (2013). Peanut. Purdue University. Available from: http://www.hort.purdue.edu/newcrop/afcm/peanut.html.

Santos E. P, Silva RP, Bertonha RS, Noronha RHF, Zerbato C (2013) Produtividade e perdas de amendoim em cinco diferentes datas de arranquio. Revista Ciência Agronômica 44:695-702. DOI: http://dx.do i.org/10.1590/S1806-66902013000400005.

Stamatov St., Ishpekov S., Dallev, M., 2020. Losses at mechanized harvesting of Bulgarian peanut varieties. AgroLife Scientific Journal, Scientific Papers. Series A. Agronomy, Issue 1/2020, ISSN 2286-0126, pp 210 - 213.

Thomas, R. J., Pettit R. E., Taber, R. A. and Jones, B. L., 1983. Peanut Science, Vol. 10 p. 97 - 101. The Journal of American Peanut Research and Education Society, ISSN 0095 – 3679.

Young J. H, Person N. K, Donald J. O, Mayfield W. D (1982). Harvesting, curing and energy utilization. In H.E. Pattee and C.T. Young (eds.). Peanut Science and Technology. Amer. Peanut Res. Educ. Society., Inc., Yoakum, TX, USA, pp. 458–485.

NITROGEN FERTILIZATION'S EFFECT ON THE QUALITY CHARACTERISTICS OF VARIOUS SORGHUM VARIETIES

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Abstract

Sorghum is a crop that belongs to the *Poaceae* family and is known for its high yields. A field experiment was conducted in the experimental farm of the University of Thessaly, (coordinates: latitude 39^{o}24'50.95''N, longitude 22^{o}44'0.79''E, altitude 78m ASL), to investigate the quality characteristics of six different sorghum varieties (V_1: Buffalo grain, V_2: Elite, V_3: Big Kahuna, V_4: 25K1009, V_5: 4264 and V_6: 5D61) under different nitrogen fertilization levels (N_1: 0, N_2: 80, N_3: 160 and N_4: 240 kg ha^{-1}, using urinary ammonia 40-0-0). There was used a factorial split-plot design with three replicates and twenty-four plots per replication, where the main factor was the different varieties and the sub-factor the different N-fertilization levels. The climate in the research region is typical of the Mediterranean. During summer months, the average recorded air temperature was 25.8°C and the recorded summer precipitation was just 46 mm. Protein content was not statistically significant different between the tested varieties but only between nitrogen levels with "Buffalo grain" being the variety with the higher protein content, while phosphorus and calcium content were statistically significant different between the tested varieties. Finally, ash, neutral detergent fiber and acid detergent fiber, was also statistically significant different between the tested varieties. The variety "5D61" was the one with higher ash content while the "Buffalo grain" variety lags statistically significantly behind all the others in both measured quality characteristics (neutral detergent fiber and acid detergent fiber).

Keywords: *Sorghum, protein, ash, ADF, NDF, nitrogen.*

Introduction

Agriculture's intensification and over-exploitation of land have resulted a loss of soil fertility and an increase of cultivation costs. Producers began to use more fertilizers to enhance agricultural yield. Nitrogen constitutes the most applied nutrient and nitrogen fertilizers are those with the lowest price. Nitrogen (N) is a key component of enzymes and other proteins essential to all growth functions and when N is suboptimal, growth is reduced (Kiani et al., 2016). Nitrogen is the element that helps crop growth and has been mentioned worldwide for its benefits increasing grain yields in most crops and especially in maize case (Muhammad et al., 2020). Kurai et al. (2015) reported that 90 kg N ha^{-1} is an efficient N fertilization rate for sustainable sweet sorghum cultivation.
Sorghum is a member of the *Poaceae* family and is one of the most important crops used as animal feed in semi-arid, tropical countries because of its capacity to thrive in high temperatures and dry conditions where no other crops can be cultivated (Morris, 2005; McCary et al., 2020). Sorghum is one of the most important cereal crops in the world, coming in fifth place behind

maize, wheat, rice, and barley, with annual production of 57.6 million tons in 2017 (FAO, 2017). Also, sorghum crop is characterized by a high adaptability and a high-water use efficiency (WUE), and a low nutrient requirement compared with corn (Wu et al, 2010; Zhang et al. 2016; Hasan et al., 2017). Sorghum base growing temperature is 13°C (Ferraris and Charles-Edwards, 1986) while the favor temperature growth range is 20-30°C. The above characteristics allow it to grow in a wide variety of climates.
Roby et al., (2017) reported that sorghum cultivation is recommended for silage production under drought scenarios, while there are many studies where it is reported that brown midrib sorghum silage could totally replace maize silage without affecting the milk yield of dairy cows (Oliver et al., 2004; Bernard and Tao, 2015; Cattani et al., 2017). As a result of the circumstances, sorghum output for animal feed has increased in the Mediterranean countries in the previous two decades (Miron et al., 2005).
The aim of this study was to determine which of the tested sorghum varieties could produce an animal feed with higher quality characteristics. In addition, the aim of the present study was to investigate which nitrogen fertilization level will contribute to the improvement of the quality characteristics of the selected sorghum varieties.

Materials and Methods

The experimental site is within the experimental farm of the University of Thessaly, located in the vicinity of Velestino village, Prefecture of Magnesia (coordinates: latitude 39^{o}24'50.95''N, longitude 22^{o}44'0.79''E, altitude 78m ASL).
A field experiment using a factorial split-plot design was used with three replicates (blocks) and twenty-four plots per replication in a particularly fertile (organic matter of 2.91% at a depth of 0-30 cm and 1.86% at 30-60 cm), clayey soil with an alkaline reaction (Table 1; presents the soil properties). The main factor was the different varieties (V_1: Buffalo grain, V_2: Elite, V_3: Big Kahuna, V_4: 25K1009, V_5: 4264 and V_6: 5D61, six varieties in total) and the sub-factor the different nitrogen fertilization levels (N_1: 0, N_2: 80, N_3: 160 and N_4: 240 kg ha^{-1}, using urinary ammonia 40-0-0). The occupied area of each plot was 42 m^2 (6 m wide and 7 m long).

Table 1. Soil properties of surface (0-30 cm) and sub-surface horizons (30-60 cm).

Characteristics / Depth (cm)	***Particle size distribution***			***Bulk Density***	***pH***	***CEC (cmol/kg)***	***Organic Matter (%)***	***CaCO$_3$***	***C/N***
	Sand (%)	***Silt (%)***	***Clay (%)***						
0 - 30	26,8	31,33	41,87	1,27	7,63	26,05	2,91	6,78	8,78
30 - 60	25,93	30,93	43,13	1,27	7,9	23,18	1,86	7,68	8,85

On June 5, 2019, sorghum was sown and on July 20, 2019, the surface nitrogen-fertilization was carried out. Plant distance between lines was 50 cm (for the first three varieties V: 1-3) and 75 cm (for the rest V: 4-6), while plant distance on the line was 8 cm for all types, as per recognized right procedure (and the characteristics of the varieties).
On October 25, 2019, the final harvest took place, by cutting of plants of 1 m^2 from the inner portion of each plot to reduce the boarder impact. Two representative plants were selected and

chopped for further laboratory analysis. Ash, protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium and phosphorus content were determined in crushed plant sub-samples of each sample by near-infrared reflectance (NIR) spectroscopy technique using the DA 7250 NIR analyzer (Perten Instruments, Hägersten, Sweden). NDF and ADF suggest plant quality characteristics that are related to the age and growth stage of the crop. NDF and ADF values estimate the feed content of lignin, cellulose, hemicelluloses, and insoluble minerals.
Complete weather data were recorded by an automated meteorological station, which was installed next to the experimental field.
Finally, the analysis of variance (ANOVA) within sample timings for all measured and derived data was conducted using the statistical package GenStat (7^{th} Edition). The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982) of the main and/or interaction effects.

Results and Discussion

Climatic data

The climate in the research region is typical of the Mediterranean. During the summer months, the average recorded air temperature was 25.8°C, which is nearly identical (26.2°C) to the study area's normal air temperature. Following that, during September, the air temperature dropped by 4°C, the same as the area's normal air temperature (Fig. 1).

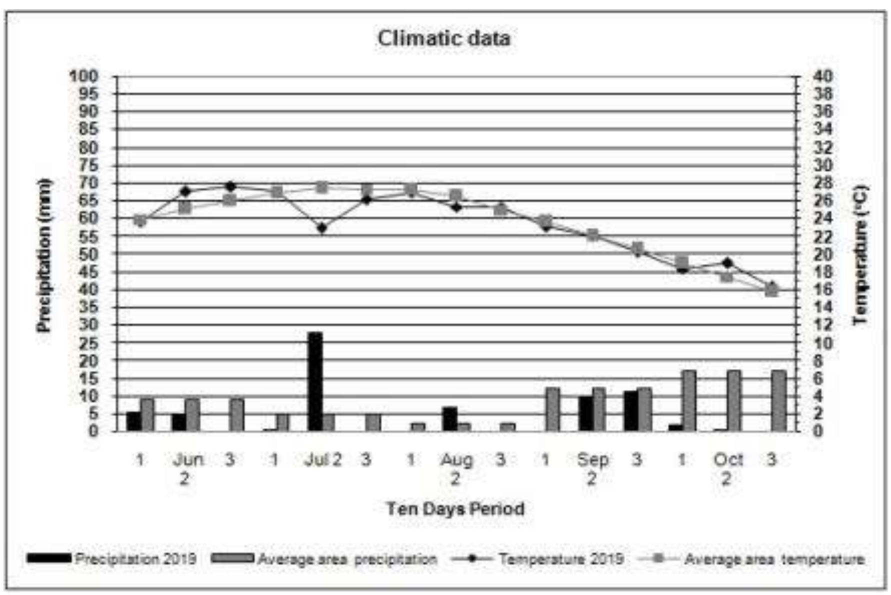

Figure 1. Average air temperature and precipitation occurred during sorghum cultivation.

Summer precipitation was just 46 mm, practically identical to the average precipitation in the study area, and only 5 mm less than the average precipitation (Fig. 1). In addition, it appears that the average precipitation of the area where the experiment is conducted was distributed more smoothly in the summer months, while during the experimental year the 60% of the precipitation occurred in the second ten days of July (Fig. 1). In September there was recorded a precipitation of 21 mm (Fig. 1), which is almost the half (43%) of the average precipitation, making the growing year very dry and adverse. Due to the significant rainfall (28 mm) that recorded in the second ten days of July, there was accompanied a significant drop in temperature.

Biomass quality characteristics

There were not found any statistically significant differences in protein content between the tested varieties (Fig. 2, a). The first three nitrogen levels differed statistically significantly, but not the fourth from the third (Fig. 2, b). It seems that in almost all varieties (except "5D61") the level of 160kg N per hectare had the highest protein content (Fig. 2 c).
In figure 2 (d, e, f) it is illustrated the phosphorus content. The varieties "Big Kahuna" and "5D61" are significantly superior to all others except "Buffalo grain", while "4264" lags significantly behind all except "25K1009" (Fig. 2, d). The different nitrogen levels had not any effect on the phosphorus content (Fig. 2, e), while in figure 2 (f) where are presented the interaction of the factors, the lowest value (0.11) was recorded by the variety "25K1009" at the control treatments and by the "4264". The highest value (0.18) was recorded by the variety "Big Kahuna" at the treatment where 240 kg N ha^{-1} were supplied and by the variety "5D61" for both treatments of 80 and 240 kg N ha^{-1} (Fig. 2, f).
In the case of calcium content, the variety "5D61" is significantly superior to all others, while "Elite" lags significantly behind all except "Buffalo grain" (Fig 2, g). Furthermore, the control treatments (0 nitrogen fertilization) lag significantly behind (Fig. 2, h). Finally, in case of interactions (Fig. 2, i), the lowest calcium content was recorded by the variety "Elite" at 0 nitrogen fertilization per hectare (0.20) and the highest by the "5D61" variety at the fertilization of 80 kg N ha^{-1} (0.33).

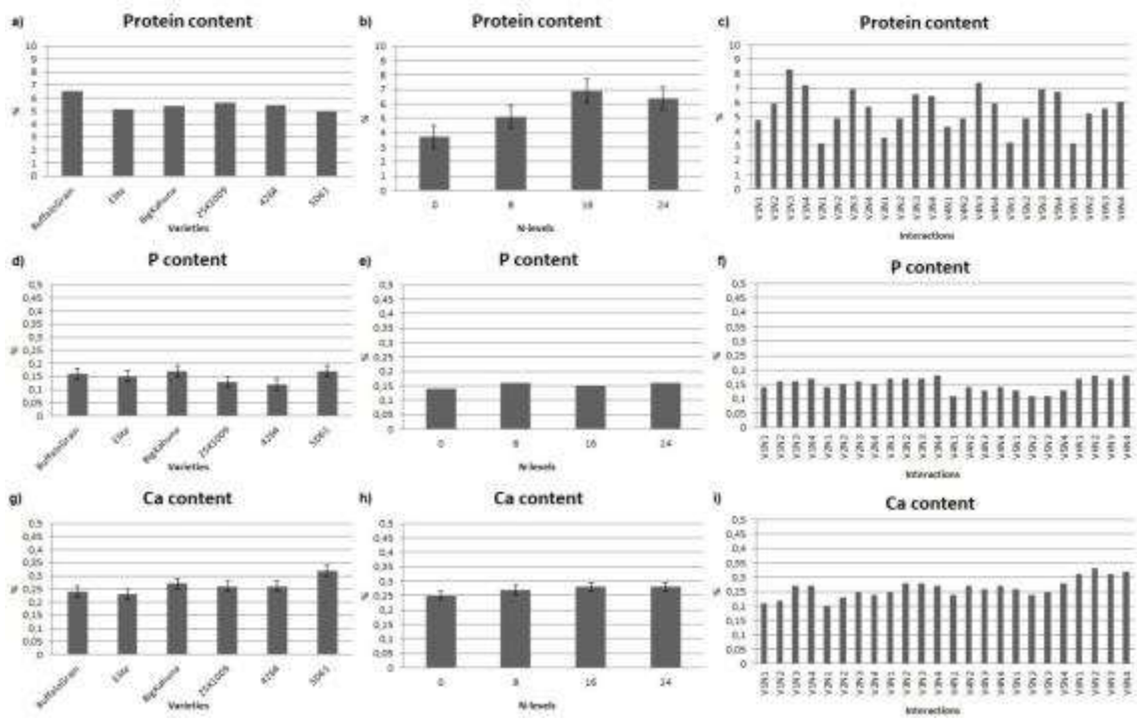

Figure 2. Protein (a, b, c), phosphorus (d, e, f) and calcium (g, h, i) content on the silage of the tested varieties as affected by the different N-fertilization levels (0, 80, 160, and 240 kg N ha^{-1}).
*V1: Buffalo Grain, V2: Elite, V3: Big Kahuna, V4: 25K1009, V5: 4264, V6: 5D61
**N1: 0, N2: 80, N3: 160, N4: 240 kg N ha^{-1}

An additional feature that needs to be measured is the ash content. In this case, the highest percentage was shown by the variety "5D61" (5.6%) followed by "Big Kahuna" (5.1%) and then

the rest with the last being "Elite" variety (4.4%; Fig. 3, a). The results for the ash content at the different nitrogen fertilization levels (Fig. 3, b) were similar as on protein content, while the lowest value was recorded by the variety "Elite" at control and the highest by the "5D61" variety at the treatment where 80 kg N per hectare were supplied.
Finally, high importance features, such as neutral detergent fiber (NDF) and acid detergent fiber (ADF), which are related to the age and growth stage of the crop measured. The NDF index recorded lower values in the first two varieties "Buffalo grain" and "Elite" (Fig. 3, d). "Buffalo grain" variety lags statistically significantly behind all the others except "Elite". "Elite" variety on the other hand, has a significantly lower value than "Big Kahuna", "4264" and "5D61". The results for the NDF content at the different nitrogen fertilization levels (Fig. 3, e) were similar as on protein content where is recorded an increase in percentage as nitrogen increases and a decrease in the fourth level (240 kg N ha^{-1}) compared to the third (240 kg N ha^{-1}). In case of interactions (Fig. 3, f), the lowest value was recorded by the variety "Buffalo grain" at the treatment where 80 kg of nitrogen per hectare were supplied (50.5 %) and the highest by the variety "5D61" at fertilization level of 160 kg N ha^{-1} (59.32 %). In case of the ADF index (Fig. 3, g, h, i), lower values were recorded again by the "Buffalo grain" and "Elite" and the statistical differences were the same as in case of NDF index. The lowest value was recorded by the variety "Buffalo grain" at control treatments (29.8 %) and the highest by the variety "5D61" at the fertilization level of 160 kg N ha^{-1} (36.4 %). ADF values are inversely related to digestion and therefore feed with low ADF concentrations is usually higher in energy.

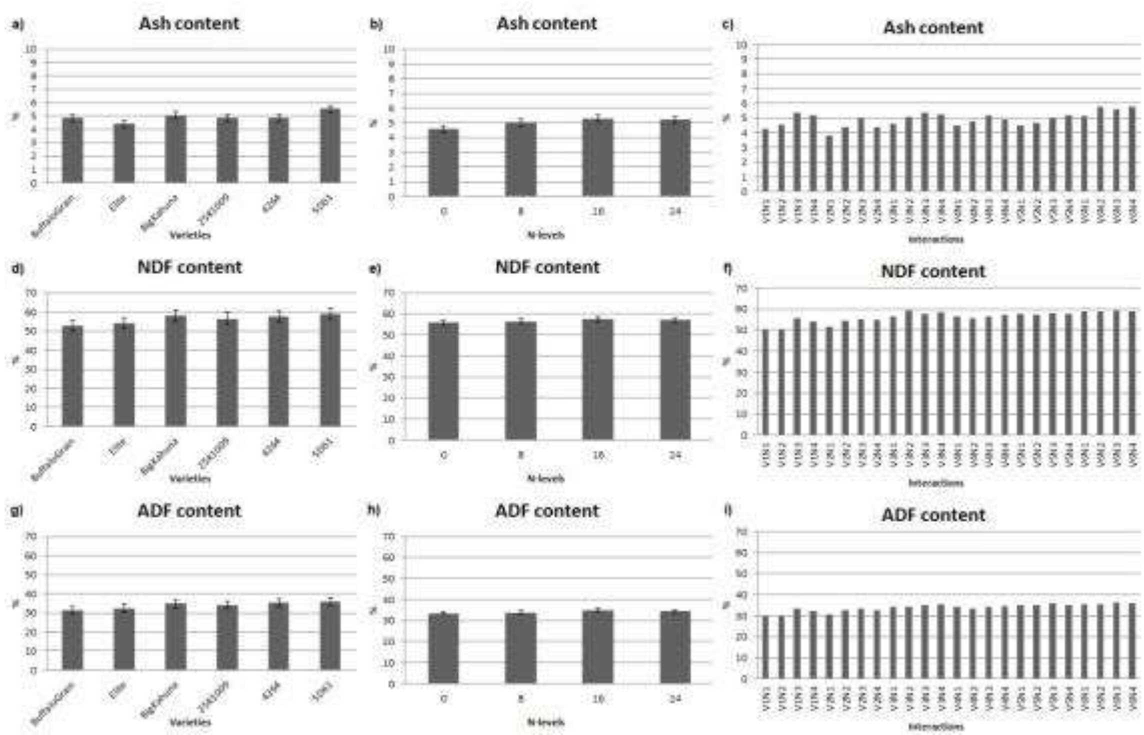

Figure 3. Ash (a, b, c), NDF (d, e, f) and ADF (g, h, i) content on the silage of the tested varieties as affected by the different N-fertilization levels (0, 80, 160, and 240 kg N ha^{-1}).
*V1: Buffalo Grain, V2: Elite, V3: Big Kahuna, V4: 25K1009, V5: 4264, V6: 5D61
**N1: 0, N2: 80, N3: 160, N4: 240 kg N ha^{-1}

However, in our study, P content was lower than the average value of 8.5% which is reported in literature (Miron et al., 2007; Samarappuli and Berti, 2018). The lower content could be explained by the maturity stage at harvest period or may be due to the different species examined since the species in the literature are forage sorghum varieties, which have higher biomass in leaves. Contrary, in case of calcium and phosphorus content the findings of the current study totally agree with the reported in literature (Da Silva Inácio et al., 2018).
The ash content observed in this study was lower than those reported by Mahmood et al. (2013), which may be explained to different soil-climatic conditions in which crops were grown and may be due to the different growing stage when harvest took place. In literature it is clearly reported that there is a decline in ash content according to plant maturity (Filya, 2004).
Finally, in literature has been reported a range in NDF and ADF of 48.3-55.4 % and 21.7 – 37.0 % (Pereira et al., 2017; Da Silva Inácio et al., 2018). The above range totally agrees with the findings of the current study, with values closer to the higher values mentioned above.

Conclusions

There were no statistically significant differences in protein content amongst the examined sorghum varieties, although there were statistically significant differences in phosphate and calcium content. Only the nitrogen levels were shown to have statistically significant differences in protein content. Furthermore, there were found statistically significant differences in ash, neutral detergent fiber, and acid detergent fiber between the studied varieties. Finally, the variety "5D61" has the highest ash content, while the "Buffalo grain" variety lags statistically significantly behind the others in both quality measures (neutral detergent fiber and acid detergent fiber).

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References

Bernard J.K, Tao S. (2015). Short communication: Production response of lactating dairy cows to brachytic forage sorghum silage compared with corn silage from first or second harvest. *Journal* of *Dairy Science*; 98:8994–9000.
Cattani M, Guzzo N, Mantovani R, Bailoni L. (2017). Effects of total replacement of corn silage with sorghum silage on milk yield, composition, and quality. *Journal of Animal Science* and *Biotechnology*; 8:15.
Da Silva Inácio FD, de Rezende CAS, Silva RHP, Melo MM, de Jesus Mendes L, Maruch S, da Costa Barcelos K, Lana ÂQ, Ralston SL. (2018). The use of sorghum silage in feeding weanling horses: Body development. Livestock Science 215:46–48.
FAO (2017) FAOSTAT, Food and Agriculture Organization of the United Nation. Retrieved from http://www.fao.org/faostat/en/#data/QC

Ferraris R, Charles-Edwards DA. (1986). A comparative analysis of the growth of sweet and forage sorghum crops. I. Dry matter production, phenology and morphology. *Australian Journal of Agricultural Research*; 37:495–512.

Filya I. (2004). Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. *Animal Feed Science and Technology*; 116:141-150.

Hasan SA, Rabei SH, Nada RM, Abogadallah GM. (2017). Water use efficiency in the drought-stressed sorghum and maize in relation to expression of aquaporin genes. Biologia Plantarum; 61:127–137.Kiani M, Gheysari M, Mostafazadeh-Fard B, Majidi MM, Karchani K, Hoogenboom G. (2016). Effect of the interaction of water and nitrogen on sunflower under drip irrigation in an arid region. *Agricultural water management*; 171:162–172.

Kurai T, Morey SR, Wani SP, Watanabe T. (2015). Efficient rates of nitrogenous fertilizer for irrigated sweet sorghum cultivation during the post-rainy season in the semi-arid tropics. *European Journal* of *Agronomy*; 71:63–72.

Mahmood A, Ullah H, Ijaz M, Javaid MM, Shahzad AN, Honermeier B. (2013). Evaluation of sorghum hybrids for biomass and biogas production. Australian Journal of Crop Science; 7:1456-1462.

McCary CL, Vyas D, Faciola AP, Ferraretto LF. (2020). Graduate Student Literature Review: Current perspectives on whole-plant sorghum silage production and utilization by lactating dairy cows. *Journal* of *Dairy Science*.

Miron J, Zuckerman E, Adin G, Solomon R, Shoshani E, Nikbachat M, Yosef E, Zenou A, Weinberg ZG, Chen Y, Halachmi I, Ben-Ghedalia D. (2007). Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. *Animal Feed Science* and *Technology*; 139:23-39.

Miron J, Zuckerman E, Sadeh D, Adin G, Nikbachat M, Yosef E, Ben-Ghedalia D, Carmi A, Kipnis T, Solomon R. (2005). Yield, composition and in vitro digestibility of new forage sorghum varieties and their ensilage characteristics. *Animal Feed Science* and *Technology*; 120:17–32.

Morris JB. (2005). Production of sweet sorghum for syrup in Kentucky. University of Kentucky Cooperative Extension Service; 122:6.

Muhammad MUH, Mudassir H, Amjed A, Muhammad A. (2020). Influence of nitrogen application on phenology, growth and yield of sunflower (*Helianthus annuus* L.) International Journal of Biological Sciences; 17(2):9-16.

Oliver AL, Grant RJ, Pedersen JF, O'Rear J. (2004). Comparison of brown midrib-6 and -18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows. *Journal* of *Dairy Science*; 87:637–644.

Pereira DH, Pereira OG, da Silva BC, Leão MI, de Campos Valadares Filho S, Chizzotti FHM, Garcia R. (2017). Intake and total and partial digestibility of nutrients, ruminal pH and ammonia concentration and microbial efficiency in beef cattle fed with diets containing sorghum (Sorghum bicolor (L.) Moench) silage and concentrate in different ratios. Livestock Science; 107:53–61.

Roby MC, Salas Fernandez MG, Heaton EA, Miguez FE, VanLoocke A. (2017). Biomass sorghum and maize have similar water-use-efficiency under non-drought conditions in the rain-fed Midwest U.S. *Agricultural* and Forest *Meteorology*; 247:434–444.

Samarappuli D, Berti MT. (2018). Intercropping forage sorghum with maize is a promising alternative to maize silage for biogas production. Journal of Cleaner Production; 194:515-524.

Steel RGD, Torrie JH. (1982). Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill, Inc (eds); 633.

Wu XR, Staggenborg S, Propheter JL, Rooney WL, Yu JM, Wang DH. (2010). Features of sweet sorghum juice and their performance in ethanol fermentation. Industrial Crops and Products; 31:164–170.

Zhang SJ, Chaudhry AS, Ramdani D, Osman A, Guo XF, Edwards GR, Long C. (2016). Chemical composition and *in vitro* fermentation characteristics of high sugar forage sorghum as an alternative to forage maize for silage making in Tarim Basin, China. Journal of Integrative Agriculture; 15:175–182.

PHOSPHORUS FERTILIZATION EFFECT ON *VICIA FABA*YIELD AND PROTEIN CONTENT

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Abstract

Beans (*Vicia faba*) are a protein-rich food and can be sown in early winter as well as in spring, providing flexibility depending on climatic conditions, soil type and cultivation systems. The micro-sperm bean which is usually used as animal feed is an important legume crop. It was decided to investigate the effect of phosphate fertilization on both seed yield and protein content because the plant performed well even in barren or depleted lands. For the purposes of the study, a field experiment was conducted in the experimental farm of the University of Thessaly (latitude 39°24'50.95''N, longitude 22°44'0.79''E, altitude 78m ASL), using a complete randomized block experimental design with four treatments (F1: control, F2: 30, F3: 60 and F4:90 kg ha^{-1} P_2O_5) and four replications. The soil in the experimental field was clayey and fertile, with 2.91 percent organic matter at a depth of 0 - 30 cm and 1.86 percent at a depth of 30 - 60 cm. Even in such a high-organic-matter soil, it was found that adding phosphorus enhanced seed yield, at the treatment of 90 kg ha^{-1} P_2O_5 having a statistically significant superiority, suggesting the need to add this macronutrient to micro-sperm beans growth. There was no difference in the protein content of the bean seeds across the different phosphate fertilization levels, while the protein content was found to be around 26% in all cases.

Keywords: *Bean, yield, protein, fertilization, phosphorus.*

Introduction

The *Fabaceae* family is one of the largest and most diverse families. Soybeans, beans and lentils are examples of legumes that are commonly consumed by humans and used as animal feed. The fava bean (*Vicia faba*), commonly known as field bean, is one of the oldest and most popular cultivated plants on the planet. It has the highest yielding potential of all *Fabaceae* crops, and it is a particularly ideal high-protein component for the preparation of concentrates, as it contains around 28–30% total protein of high biological value in its seeds (Mekky et al., 2020) and traditionally used as a main source of protein for human and animal nutrition (Vioque et al., 2012; Multari et al., 2015). It can resist bending and hence receives mechanical assistance at all stages of development.

The proportion of fertile land on which grain legumes are cultivated has decreased to 1.5 percent over the last 50 years because of agricultural intensification, primarily for economic reasons (Watson et al., 2017). Of course, due to the environmental and long-term economic benefits of integrating legumes into European cultivation systems, it is now advocated that they be reintroduced into cultivation systems (Reckling et al., 2016), particularly in a decreased influx regime (Nemecek et al., 2008).

Vicia faba can be grown after sorghum and maize crops, taking advantage of the available water remaining in areas where water is not a problem, or precede dry crops (eg. wheat) when water is not available. They can be sown early in winter but also in spring, providing flexibility depending on climatic conditions, soil type and cultivation systems in which they are included (Jensen *et al*., 2010).
They are frequently sown in the winter in locations with mild winter temperatures and heavy clay soils that are difficult to cultivate in the spring, while they can be sown in the early spring in areas with light soils and considerable rainfall. Varieties that are sown in the fall mature faster than those that are sown in the winter.
Creating a healthy symbiotic relationship between bean plants and soil root bacteria is difficult to predict because it is dependent on a variety of environmental factors and growing strategies (Jensen *et al*., 2010). Bean crops bind 290,000 t N of atmospheric nitrogen each year, compared to 22 million t N binded by all other legumes, including soybeans (Herridge *et al*., 2008).
Phosphorous (P) is essential for nodulation, biological nitrogen fixation, photosynthesis, and the nutritional value of legumes like the faba bean (Haling et al., 2016, Makoudi et al., 2018). In P-limited soils, P fertilizer often results in enhanced yield and biomass of faba beans, indicating that P fertilizer is necessary for grain production in faba beans (Nebiyu et al., 2016).
As it has been already mentioned, faba bean is an important source of plant protein for human and animal consumption and can be harvested as dried seeds (Crépon et al., 2010, Singh et al., 2013). It's been suggested that in order to get a bigger seed production, you'll need more biomass accumulation. (Mortimer et al., 2012), while Kubure et al. (2016) reported that faba bean has a strong positive association between seed yield and biomass production. However, because the faba bean is a cool-season grain legume that is susceptible to high temperatures, poor moisture availability, and days to blooming (L'opez-Bellido et al., 2005), earlier maturity may be advantageous for providing the best yield. Seed yields range significantly because to significant variability in faba bean seed size (Etemadi et al., 2017) and a lack of acceptable population density.
Therefore, the aim of the current study was to test the effect of different P-fertilization to the seed yield and its seed protein content in a clayey and fertile soil.

Materials and Methods

The experimental site is within the experimental farm of the University of Thessaly, located in the vicinity of Velestino village, Prefecture of Magnesia (coordinates: latitude 39^{o}24'50.95''N, longitude 22^{o}44'0.79''E, altitude 78m ASL).
A field experiment using a complete randomized design (RCB) with four P-levels in four repetitions (a total of 16 plots) was used. The treatments used were various amounts of phosphate fertilization, with F1 being a control by no fertilization, F2 being phosphate fertilization of 30 kg ha^{-1} P_5O_2, and F3, F4 being 60 and 90 kg ha^{-1} P_2O_5, respectively.
The phosphate fertilizer was manually scattered in the separate treatments after excavating the experimental field, followed by the application of the stomp herbicide and their integration by a rotary cultivator. A quantity of 140 kg ha^{-1} seeds of the "Polykarpi" variety was used. A grain sowing machine was used to sow the seeds on November 21, 2018.
The soil of the experimental area is characterized as a clayey soil with an alkaline reaction. It is particularly fertile with a percentage of organic matter of 2.91% at a depth of 0-30 cm and 1.86% at 30-60 cm. This is initially an indication of mineralization of a higher percentage of organic nitrogen compared to the average of Greek soils.

Seed protein content was determined in crushed plant sub-samples of each sample by near-infrared reflectance (NIR) spectroscopy technique using the DA 7250 NIR analyzer (Perten Instruments, Hägersten, Sweden).
Complete weather data were recorded by an automated meteorological station, which was installed next to the experimental field.
Finally, the analysis of variance (ANOVA) within sample timings for all measured and derived data was conducted using the statistical package GenStat (7th Edition). The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982) of the main and/or interaction effects.

Results and Discussion

Climatic data

Low temperatures have prevailed for the season since the sowing (9/21/2018), causing germination to be delayed (Fig. 1). Normal temperatures with few changes occurred for the region from the end of January 2019 onwards.
In terms of precipitation, a deficit of roughly 50 mm was recorded from sowing to April 2019, compared to the usual climate of the larger area, with 30 mm in March, which is the season of intense plant growth. As a result, their growth was seen to be slowed. Fortunately, the rains that fell in the first few days of April helped to make up for the shortfall and boosted plant growth.

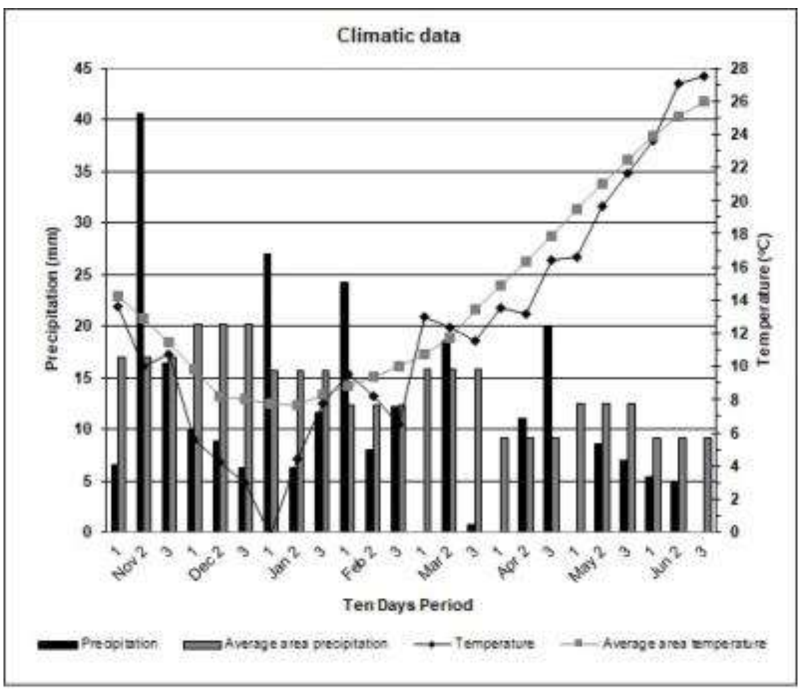

Figure 1. Average air temperature and precipitation occurred during *Vicia faba* cultivation.

Seed yield and quality characteristics

The addition of phosphorus to the field beans appears to have enhanced seed output. Although the addition of 30 and 60 kg P_2O_5 per ha resulted in simply a numerical benefit in yield, the addition of 90 kg P_2O_5 per hectare resulted in a statistically significant advantage, illustrating the

importance of include this macronutrient in legume agriculture (Table 1). Specifically, the yield achieved in control treatments was 2790 kg ha^{-1} which is almost 800 kg ha^{-1} lower compared to the recorded yield in the high p-fertilization treatments (3580 kg ha^{-1}, Table 1).
The reached seed yield is in line with that reported in literature which ranges from 3000 kg ha^{-1} to 5000 kg ha^{-1} under favorable conditions (Kowalczyk et al., 2021) and extremely lower than the recorded yields in Poland (Łabuda, 2012). The average dry yield faba bean var. minor varieties in 2016 was 0.9 t ha^{-1} (FAOSTAT 2016). Faba bean seed yields are highly variable between countries due to many factors (climate, soil, etc.). Narits (2018) reported that the highest seed yield is obtained with varieties which had the longest growing period, while late varieties did not mature and there is a high yield loss due to the small immature seeds.
Maybe higher precipitation concomitant with lower temperatures during the growing season could elevate the harvested yield because faba bean is regarded as a drought-sensitive crop, where water stress decreases the final leaf area and the light use efficiency (Ghassemi-Golezani et al., 2009) and N_2 fixation (Neugschwandtner et al., 2015).

Table 1. Seed yield (kg ha^{-1}) protein content (%).

Treatments \ Measures		Seed Yield (kg ha^{-1})	Protein Content (%)
P_2O_5 (kg ha^{-1})	**0**	2790	26,1
	30	3210	25,7
	60	3250	26,2
	90	3580	25,7
$LSD_{.05}$		596	ns
CV (%)		11,6	1,7

In terms of protein content, there was no difference between the different amounts of phosphate fertilization in terms of seed content, which was around 26% in all cases. The concentration found above is lower than the protein level described in the literature, which is higher than 28% (Iqbal et al., 2006; Rempel et al., 2019; Kowalczyk et al., 2021; Sharan et al., 2021). Furthermore, Vioque et al., (2012) reported that protein content of faba bean ranges from 27% to 34% of dry weight (depending on the variety and growing conditions).

Conclusions

The *Vicia fava* var minor (fodder bean) cultivation seems to lead to satisfactory seed yields. The addition of ninety kg of phosphorus (P_2O_5) increased seed yield by a statistically significant amount without changing seed protein content.

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References

Crépon K., Marget P., Peyronnet C., Carrouee B., Arese P., Duc G. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Research, 115, pp. 329-339.

Etemadi F., Hashemi M., Shureshjani R.A., Autio W.R. (2017). Application of data envelopment analysis to assess performance efficiency of eight faba bean varieties. Agronomy Journal, 109, pp. 1225-1231.

FAOSTAT database (2016). Food and Agriculture Organization of the United Nations.

Ghassemi-Golezani K., Ghanehpoor S., Dabbagh Mohammadi-Nasab A. (2009). Effects of water limitation on growth and grain filling of faba bean cultivars. *Journal* of *Food*, *Agriculture* and *Environment*, 7, pp. 442-447.

Haling R.E., Yang Z., Shadwell N., Culvenor R.A., Stefanski A., Ryan M.H., Simpson R.J., (2016). Growth and root dry matter allocation by pasture legumes and a grass with contrasting external critical phosphorus requirements. Plant and Soil, 407, 67-79.

Herridge D.F., Peoples M. B., Boddey R.M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil, 311(1-2), pp. 1–18.

Iqbal A., Khalil I.A., Ateeq N., Sayyar Khan M. (2006). Nutritional quality of important food legumes. Food Chemistry, 97 (2), pp. 331-335.

Jensen E.S., Peoples M.B., Hauggaard-Nielsen H. (2010). Faba bean in cropping systems. Field Crops Research, 115 (3), pp. 203-216.

Kowalczyk M., Rolnik A., Adach W., Kluska M., Juszczak M., Grabarczyk Ł., Wozniak K., Olas B., Stochmal A. (2021). Multifunctional compounds in the extract from mature seeds of *Vicia faba* var. minor: Phytochemical profiling, antioxidant activity and cellular safety in human selected blood cells in in vitro trials. Biomedicine & Pharmacotherapy, 139, 111718.

Kubure T.E., Raghavaiah C.V., Hamza I. (2016). Production potential of faba bean (*Vicia faba* L.) genotypes in relation to plant densities and phosphorus nutrition on vertisols of central highlands of West Showa Zone, Ethiopia, east Africa. Advances in *Crop Science* and *Technology*, 4, pp. 214.

Łabuda H. (2012). Flowering and characteristics of useful traits of some faba bean (*Vicia faba* L. var. *major* Harz) cultivars and breeding lines. Acta Agrobotanica, 65, pp. 139-148.

L'opez-Bellido F.J., L'opez-Bellido R.J., L'opez-Bellido L. (2005). Competition, growth and yield of faba bean (*Vicia faba* L.). European Journal of Agronomy, 23, pp. 359-378.

Makoudi B., Kabbadj A., Mouradi M., Amenc L., Domergue O., Blair M., Ghoulam C. (2018). Phosphorus deficiency increases nodule phytase activity of faba bean–rhizobia symbiosis. Acta Physiologiae Plantarum, 40, pp. 63.

Mekky R.H., Thabet M.M., Rodríguez-Pérez C., Elnaggar D.M.Y., Mahrous E.A., Segura-Carretero A., Abdel-Sattar E. (2020). Comparative metabolite profiling and antioxidant potentials of seeds and sprouts of three Egyptian cultivars of *Vicia faba* L. *Food Research International*, 136, 109537.

Multari S., Stewart D., Russell W.R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Comprehensive Reviews* in *Food Science* and *Food Safety*, 14, pp. 511-522.

Narits L. (2018). Faba bean (*Vicia faba* L.) variety comparison in Jõgeva during 2015–2017. Agronomy, pp. 63–68

Nebiyu A., Diels J., Boeckx P. (2016). Phosphorus use efficiency of improved faba bean (*Vicia faba* L.) varieties in low-input agro-ecosystems. *Journal* of *Plant Nutrition* and *Soil Science*, 179, pp. 347-354.

Nemecek T., von Richthofen J.-S., Dubois G., Casta P., Charles R., Pahl H. (2008). Environmental impacts of introducing grain legumes into European crop rotations. European Journal of Agronomy, 28, pp. 380-393.

Neugschwandtner R., Ziegler K., Kriegner S., Wagentristl H., Kaul H.-P. (2015). Nitrogen yield and nitrogen fixation of winter faba beans. *Acta Agriculturae Scandinavica, Section B*, 65, pp. 658-666.

Reckling M., Bergkvist G., Watson C.A., Stoddard F.L., Zander P.M., Walker R., Pristeri A., Toncea I., Bachinger J. (2016). Trade-offs between economic and environmental impacts of introducing legumes into cropping systems. Frontiers in *Plant Science*, 7, pp. 1-15.

Rempel C., Geng X., Zhang Y. (2019). Industrial scale preparation of pea flour fractions with enhanced nutritive composition by dry fractionation. Food Chemistry, 276, pp. 119-128.

Sharan S., Zanghelini G., Zotzel J., Bonerz D., Aschoff J., Saint-Eve A., Maillard M. (2021). Fava bean (*Vicia faba* L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. Comprehensive Reviews in Food Science and Food Safety, 20 (1), pp. 401-428.

Singh A.K., Bharati R.C., Manibhushan N.C., Pedpati A. (2013). An assessment of faba bean (*Vicia faba* L.) current status and future prospect. *African Journal* of *Agricultural Research*, 8, pp. 6634-6641.

Steel RGD, Torrie JH. (1982). Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill, Inc (eds), pp. 633.

Vioque J., Alaiz M., Girón-Calle J. (2012). Nutritional and functional properties of *Vicia faba* protein isolates and related fractions. Food Chemistry, 132, pp. 67-72.

Watson C.A., Reckling M., Preissel S., Bachinger J., Bergkvist G., Kuhlman T., Lindström K., Nemecek T., Topp C.F.E., Vanhatalo A., Zander P., Murphy-Bokern D., Stoddard F.L. (2017). Grain Legume Production and Use in European Agricultural Systems. Advances in Agronomy, 144, pp. 235-303.

PEANUT RESPONSE TO PLANTING PATTERN AND ROW SPACING

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Abstract

Peanuts are grown commercially in several Greek regions such as Messinia and Serres. The total cropping area in Greece is around 4500 hectares. However, many farmers have started to grow peanuts at the region of Thessaly and especially at the area of Trikala where the soil texture is medium. Because of the high farmers' interest in growing peanuts in Greece, an experiment carried out at the research farm of the University of Thessaly in Larissa to study peanut response to planting patterns and row spacing. The experiment included four different treatments of planting patterns and row spacing and three replicates of each treatment. Specifically, at the first treatment (control), peanuts were planted 75 cm apart along the rows. At the second treatment, there were twin rows 10 cm apart and the planting spacing was 75 cm along the rows. At the third treatment, there were four rows 50 cm apart and the planting spacing was 75 cm along the rows while at the fourth treatment, peanuts were planted on beds with planting spacing 75 cm along the beds. Peanuts grew in experimental plots of 5 m long, 3 m wide and 1 m apart. The results showed that the yields of the second and fourth treatment were significantly higher by 41.8% and 37% respectively than the yield of the controller. The yield of the third treatment was also higher (no significantly) by 11.2% than the controller. All the above indicate that the use of twin rows of peanuts increases peanut yield because of the use of the double number of seeds compared to the control treatment. However, planting peanuts on beds seems to be an inexpensive solution considering the loamy sand soil type of the peanut plots.

Keywords: beds, twin rows, yield, soil properties, evapotranspiration, fertilizers.

Introduction

Peanut (*Arachis hypogaea* L.) is an important oil seed crop and grown in light, sandy loam soil with a pH of 5.9-7. There are numerous advantages to crop rotation, including weed control, improved soil fertility as well as yield increase. For example, peanuts in a three-year rotation with corn, yielded 50% higher than no rotated peanuts (Baughman et al., 2005). Peanuts can be produced at areas where the annual rainfall varies from 500 mm to 1200 mm and daily average temperature is higher than 20 ^{o}C. (Arioglu et al., 2013; Arioglu, 2014).
Planting density and row pattern play an important role on peanut growth, yield and quality. Thus, the response of peanut to plant density and planting patterns has investigated several years ago. Almost sixty years ago, some experiments resulted that maximum yield could be achieved if plants produce enough leaf area to provide maximum light reception during reproductive growth (Shibles et al., 1966; Tanner and Hume, 1978). Pendleton and Hartwing (1973) resulted that space between plants could affect interplant competition. Duke and Alexander (1964) reported

pod yield that was 14% higher in narrow row plantings compared with traditional wider rows. Norden and Lipscomb (1974) concluded that pod yield was 16% higher when peanut was seeded in rows spaced 46 cm apart compared with 91cm. These results confirmed Perham (1942) who demonstrated that peanuts planted in 46 cm rows yielded higher than those planted in rows spaced 61, 76, 91 or 107 cm apart. On the other hand, Kaushik and Chaubey (2000), noticed that pod yield of 30 cm inter-row spacing was significantly higher than the yield of 45 cm inter-row spacing. Wright and Bell (1992) reported that the ideal peanut plant density was 11-14 plants m^2. Baldwin et al. (1999) demonstrated that peanuts planted in twin-row spacing could produce 381 kg ha^{-1} more than the peanuts in conventional row pattern.
Planting peanuts in twin rows or in beds is a new practice in Greece while the literature does not provide much information on this topic. The objective of this study was to demonstrate the peanut response to different planting patterns and row spacing.

Materials and Methods

This study was conducted at the University of Thessaly research farm in 2019 in Larissa, Greece (Latitude: 39.6246^o, Longitude: 22.380). Peanuts were planted on 18th of May 2019 in plots in plant density of 50 kg/ha. The size of the plots was 5 m long and 3 m wide and the plots were separated with alleys of 1 m wide (Figure 1). Soil analysis showed that the soil type of the plots at the peanut root zone was sandy loam (SL) and sandy clay loam (SCL). More information for the soil profile characteristics is provided at table 1.

Figure 1. Peanut plots at the University of Thessaly research farm

Table 1. Soil texture, pH, $CaCO_3$ and organic material for different depths to understand better the characteristics of the soil profile.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil Type*	pH (H2O 1:1)	CaCO3 (BERNARD) (%)	Organic Material (%)
0-5	71.68	14	14.32	SL	7.74	1.80	2.46
5-26	69.68	18	12.32	SL	7.45	0.10	0.99
26-32	67.68	12	20.32	SCL	7.43	0.00	0.62
32-59	58.36	16.76	24.88	SCL	7.23	0.00	0.60
59-67	62.36	16	21.64	SCL	8.29	9.53	0.52
67-84	63.68	21	15.32	SL	7.44	12.19	0.31
84-102	77.68	10.68	11.64	SL	7.78	8.12	0.18
102-126	74.96	10.08	14.96	SL	7.87	11.75	0.15
126-147	79.68	9.68	10.64	LS/SL	7.89	11.84	0.09
147-165	83.68	6.68	9.64	LS	7.87	11.67	0.06

*LS: Loamy Sand, SL: Sandy Loam, SCL: Sandy Clay Loam

The experiment included four different treatments of planting patterns and row spacing and three replicates of each treatment. Specifically, at the first treatment (control), peanuts were planted 75 cm apart along the rows. At the second treatment, there were twin rows 10 cm apart and the planting spacing was 75 cm along the rows. At the third treatment, there were four rows 50 cm apart and the planting spacing was 75 cm along the rows while at the fourth treatment, peanuts were planted on beds with planting spacing 75 cm along the beds.

Irrigation scheduling

Overhead sprinklers utilized to irrigate each plot. The irrigation scheduling method was based on the crop evapotranspiration (ETc). ETc is calculated with Equation 1 (Allen et al., 1998).

ETc = ETo * Kc (Eq. 1)

Where: ETo is reference evapotranspiration

Kc is crop coefficient

A meteorological station was installed next to the plots to acquire accurate ETo data. Kc data were obtained from other experiments that conducted in the same region in the past. Kc values for peanuts are presented at table 2.

Table 2. Duration (in days) and crop evapotranspiration for each growth stage of peanuts (Allen et al., 1998). The total amount of irrigation used in 2019 growing season was 527.97 mm while precipitation was 73.6 mm.

Growth Stage	1	2	3	4	Total
Duration of each stage (days)	20	35	51	30	136
ET_c (mm)	36,14	233,30	287,55	44,00	601,57

Fertilizer scheduling

Fertilizers were applied by hand uniformly at each plot during the growing season. The first fertilizer application took place on 14th of May 2019 when fertilizers (12-11-18) applied at a rate of 300 kg ha^{-1}. At the second application that occurred on 19th of June 2019 nitrogen (46-0-0) was applied at the rate of 108.6 kg ha^{-1}. Finally, nitrogen (15.5-0-0) applied at the rate of 55 kg ha^{-1} on 7th of August 2019.

Harvest of peanuts

Peanuts were harvested on 30th of September 2019 and the total yield of each plot was weighted manually using a digital scale and big bags.

Results and Discussion

Yield results showed that every treatment gave higher yield than the average peanut yield in the region of Thessaly (~3000 kg ha^{-1}). However, statistical analysis of yield data proved that there were significant differences in yield among the treatments. Specifically, the yield of the control treatment was 2940 kg ha^{-1} and it was significantly lower than the yields of the other treatments. The maximum yield (5047 kg ha^{-1}) was recorded at the treatment with the twin rows of planting pattern. This yield was 41.8% higher the yield of the control. This is similar to (Shibles et al., 1966; Tanner and Hume, 1978) findings who found that the more canopy the higher light perception and consequently the higher peanut yield. The second higher yield (4657 kg ha^{-1}) was achieved at the treatment where peanuts planted on beds and it was 37% higher than the control. Finally, the treatment with four rows as planting pattern gave 3310 kg ha^{-1} and it was 11.2% higher than the yield of the control (Figure 3). The ability of peanuts to adapt to different situations, wide or narrow planting patterns and high seeding rates may be one of the factors that peanuts become famous in Greece.

Even if the current experiment lasted only one growing season, the planting pattern of peanuts and the row spacing played an important role in the productivity of peanuts. However, more experiments should be conducted to confirm the results. Thus, the future plan is to continue the experiment in big commercial peanut fields and record more details such as harvest quality (how many pods stays in the ground after harvest) and soil apparent electrical conductivity.

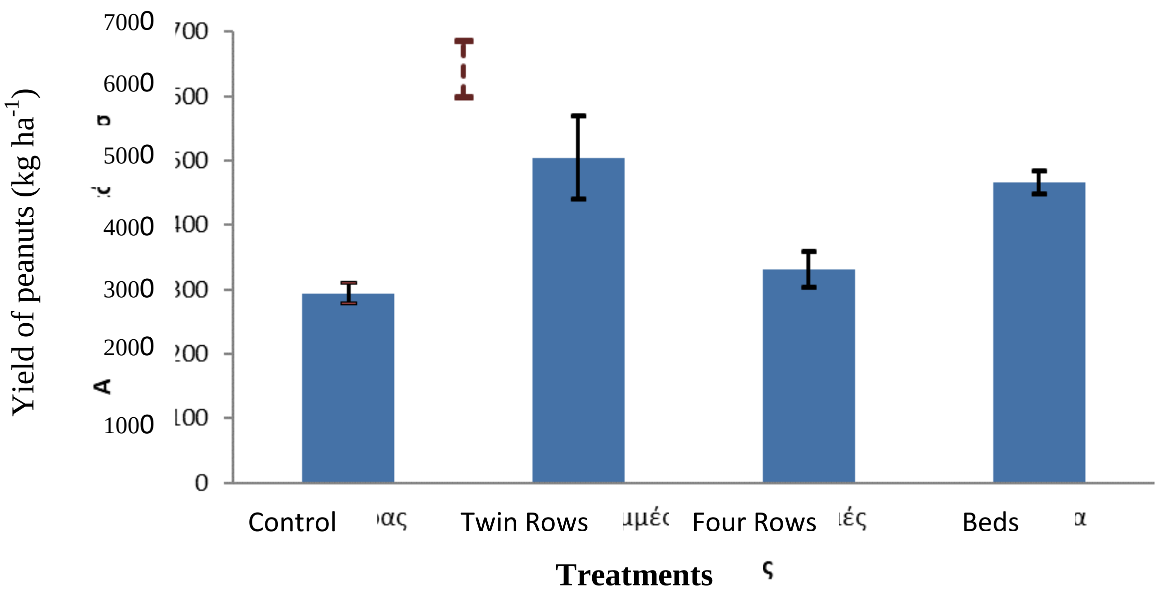

Figure 2. Statistical analysis of yield for each treatment. It is obvious that the control treatment had the significant lowest yield compared to the other treatments of the experiment. On the other hand, the twin rows planting pattern gave the highest peanut yield.

Conclusions

At the current experiment, peanuts were planted in plots. Inputs such as irrigation and fertilizers applied uniformly in every plot. The only difference among the plots is that peanuts were planted in different planting patterns and row spacing to study the peanut response. The results showed that there were significant differences in yield among the different planting patterns and the highest yield was recorded at the treatment where peanut planted in twin rows. Besides the fact that this is one-year study, these results are very valuable for peanut farmer because they can increase yield by changing only the planting pattern.

References

Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. (1998). Crop Evapotranspiration: Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper No 56, FAO, Rome.

Arioglu H., Kurt, C., Bakal, H., Onat, B., Gulluoglu, L. and Sinan, N.S. (2013). The Effects of Pix (Mepiquat-Chloride) Application in Different Growing Stages on Pod Yield and Some Agronomic Characters of Peanut. Turkish Journal of Field Crops, vol. 18(2), pp. 260-267.

Arioglu, H.H. (2014). The oil seed crops growing and breeding. The Publication of University of Cukurova, Faculty of Agriculture, No:A-70, pp. 204, Adana-Turkey.

Baldwin, J.A., Beasley J.P., Brown S.L., Todd J.W., and Culbreath A.K. (1999). Yield, grade, and tomato spotted wilt incidences of four peanut cultivars in response to twin row versus single row planting patterns. In Proceedings of American Peanut Research and Education Society, vol. 31, pp. 54.

Baughman T., Grichar J., Black M, Woodward J, Porter P., New L., Baumann P., McFarland M. "Texas Peanut Production Guide" (PDF). Texas A&M University. Retrieved October 16, 2015.

Duke, G.B., and Alexander M. (1964). Effects of close row spacing on peanut yield and peanut production requirements. USDA Production Research, Bullet: 77, pp 14.

Kaushik, M.K. and Chaubey A.K. (2000). Response of rainy season bunch groundnut (Arachis hypogaea L.) to row spacing and seed rate. Field Crops Research, vol. 20, pp. 407-410.

Norden, A.J., and Lipscomb R.W. (1974). Influence of plant growth habit on peanut production in narrow rows. Crop Science, vol. 14, pp. 454–457.

Perham, S.A. (1942). Peanut production in the Coastal Plain of Georgia. Georgia Coastal Plain. Bulletin. 34.

Pendleton, J.W. and Hartwing E.E. (1973). Crop production and management. In B.E. Caldwell (ed.) Soybean Improvement, production and uses. Vol. 16, pp. 211-237.

Shibles, R.M., Weber C.R. and Byth. D.E. (1966). Effect of plant population and row spacing on soybean development and production. Agronomy journal, vol. 58, pp. 99-102.

Tanner, J.W. and Hume D.J. (1978). Management and production. In: A.G. Norman (Ed.) Soybean Physiology Agronomy and Utilization. Academic Press, New York, pp.157-217.

Wright, G.C. and Bell M.J. (1992). Plant population studies on peanut (Arachis hypogaea L.) in subtropical Australia. 3. Growth and water use during a thermal drought stress. Australian Journal of Experimental Agriculture, vol. 32, pp. 197-203.

COMPARISION OF THE EFFECT OF BIO AND NON-BIO-FERTILIZERS ON YIELD AND ESSENTIAL OIL OF *THYMUS VULGARIS* L.

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Abstract

Since the global approach to the production of medicinal plants is effective in improving the quantity and quality of the material, it seems that the nutrition of these plants through the use of biological fertilizers in different environmental conditions is most in line with the production goals of medicinal plants. Therefore, in order to investigate the effect of biofertilizers and non-biofertilizers on biological yield, flowering branches and thyme essential oil (*Thymus vulgaris* L.), an experiment in a randomized complete block design with 4 replications in a farm in central Yazd, Iran in the crop year 2019 was done. Biofertilizers and non-biofertilizers in five levels including: Control (without fertilizer application), Endomycorrhiza fungi of the genus Glomus (*Glomus mosseae*, *G. intraradics*, *G. etunicatum*), *Azospirillum brasilense*, *Pseudomonas fluorescens* and NPK , were considered as experimental factors. The results of analysis of variance showed that fertilizer treatment had a significant effect on biological yield, flowering branches and thyme essential oil. Mycorrhiza had the greatest effect on biological yield (163%), yield of flowering branches (105%) and essential oil yield (87%) compared to the control. Therefore, the use of biofertilizers, especially mycorrhiza fertilizer, which can greatly increase the yield and amount of active ingredient, is recommended in this plant.

Keywords: *Mycorrhiza, Yield, Essential oil, Thyme.*

Introduction

Thyme (*Thymus vulgaris* L.) is an aromatic and medicinal plant of the Lamiaceae family that has strong antimicrobial, antifungal and antioxidant effects in different parts of the world as a drink, food flavoring and herbal medicine. It has many uses and is very popular due to its high efficiency of essential oil (Safaei-Ghomi et al.,2009).This plant has small flowers, purple color and small seeds with different color variation.The color of the seeds is mostly dark brown (Omidbaigi,2005). Thyme contains 0.8 to 2.6% of essential oil (Imelouane et 2009; Golparvar & Bahari, 2011). Biofertilizers are widely used nowadays to increase crop yields and have largely replaced chemical fertilizers (Afzal & Bano, 2008). These fertilizers not only provide the necessary elements for the plant, but also improve the soil conditions and create a favorable microbial population in the soil (Yadegari, *et al.*, 2010). In order to understand the concepts of sustainable agriculture and create a safe production, as well as a new approach in the use of medicinal plants and fortunately the promotion of biofertilizers on the other hand, it is necessary to combine the two and introduce the best fertilizer composition to help farmers increase production.

Materials and Methods

Time, geographical location and location of the test

This experiment was performed in the 2019 crop year on a farm in the central part of Yazd city, Iran (Longitude 51° 17', Latitude 31° 53', Height 1230.2 m).

Soil characteristics of the test site

Before performing the experiment and preparing the ground to determine the texture as well as some physical and chemical properties of the soil, samples were taken from a depth of 0 to 30 cm and sent to the laboratory for analysis. The typical soil of the area was sandy- loam, which is very good soil for planting thyme.

Table 1. Analysis of soil texture and physical and chemicals characteristics of sample soil**.**

Soil Texture	EC ($dS.m^{-1}$)	(pH)	Sand (%)	Clay (%)	Loam (%)	OC (%)	K (ppm)	P (ppm)	N (%)
Sandy loam	2.05	7.66	75	10	15	0.339	19.08	14.8	0.029

Experimental design specifications and test factors

This experiment was performed as a randomized complete block design with three replications. Experimental factor includes biological and non-biological fertilizers as B_1 = control (without fertilizer application), B_2 = endo-mycorrhizal fungi of three species of the genus Glomus (Glomus mosseae, G.intraradices, G.etunicatum) (in the form of sand inoculum which contained at least 100 active organs fungi, 150 g per plant (used according to the manufacturer's recommendation), B_3 =Azospirillum (Azospirillum brasilense), B_4= Pseudomonas (fluorscens Pseudomonas) (Pseudomonas and Azospirillum were used to immerse the plant roots in them, so that according to the manufacturer's recommendation, 200 g of each The bacteria were dissolved in 10 liters of water and then the roots were immersed in the solution for 15 minutes, then cultured) and B5 = chemical fertilizer NPK (20, 20, 20) (which was 20% of each element(Nitrogen, phosphorus, potassium) in this fertilizer and was used as a granule at a rate of 200 kg per hectare).

Planting and fertilizing

At first, the plant was prepared as a seedling in the greenhouse. After preparing the suitable substrate, blocking and preparing the plots and in the 11-leaves stage, the seedlings were transferred to the field. The subplots had dimensions of 4 meters in length and 3.5 meters in width. Each experimental unit consisted of 4 planting rows with a spacing of 70 cm between rows and 50 cm on the row. After transplanting, the first irrigation in the field was done by flood. Due to the fact that the area had a hot and dry climate, but when the plants were fully established (14-15 leaves), drip irrigation was done once a week with the same volume of irrigation water for all treatments (the same volume of water was used with the contractor). so that the humidity and the amount of water is the same for all treatments. At the flowering stage, the plants were harvested.

Sampling to measure biological yield and flowering branches yield
Sampling was done at the time of full flowering. The plants were harvested and after drying in an oven at 72 ° C for 48 hours, the dry weight of each sample was measured. Flowering branches were also separated from the plants and after drying, they were weighed with an accurate balance and finally the obtained values were calculated in terms of grams per plant.

Essential oil yield measurement
The essential oil was taken from the flowering branches of the plant. In this way, after harvesting and drying the flowering branches, first the mill and then the weight and finally the essential oil extraction operation was done. Essential oil extraction was performed in the laboratory using water distillation by an essential oil extractor (Clevenger). Water distillation is used to separate water-insoluble substances (such as essential oils). Using this method, it is easy to extract essential oils from the desired plants. Finally, the extracted essential oil was poured into small glass containers (previously weighed and the empty weight of the glass was noted and had a strong plastic lid) and weighed with an electric scale. The weight of essential oil was recorded in grams and calculated as a percentage. Finally, the yield of essential oil was obtained by multiplying the yield of flowering branches by the percentage of essential oil.

Data analysis
Finally, the obtained data were statistically analyzed using SAS statistical software (V9.4) and combined analysis of experimental years was performed. Excel¬ software was used to draw the graphs. The means were compared using LSD test at 5% probability level.

Results and Discussion

Biological yield

The results of analysis of variance show a significant effect of fertilizer treatment on biological yield (P <0.01) (Table 2).

Table 2. Analysis of variance of biological and flowering branches yield and essential oil yield in treated Thymus plant with bio and non-bio fertilizers.

		Mean Square		
Source of Variance	df	Biological yield	Flowering Branches yield	Essential oil yield
Repetition(R)	2	297.65 ^{ns}	2.57 ^{ns}	0.39 ^{ns}
Fertilizers (B)	4	49824.39**	138.90 **	9.86 **
Error	8	276.12	2.13	0.14
C.V. (%)		4.85	5.54	5.49

ns, *, **: non-significantly difference and significantly differences at 5 and 1% of probability levels, respectively.

The comparison chart of the mean data showed that mycorrhiza was 163% more effective compared with the control. The highest biological yield (466.83 g /plant) was observed in mycorrhiza (Glomus) and the lowest in control (Figure 1). Regarding the positive effect of application of fungal treatments on biological yield, it can be stated that mycorrhizal fungus through hyphae expansion and root system development, provides more water uptake for the plant and after absorbing more water, more nutrients are absorbed. They lead to the production and accumulation of more dry matter in the plant (Auge, 2001; Casson and Lindsey, 2003). The positive effect of mycorrhiza fungi on increasing the biological performance of various plants including flax, wheat and sorghum has been reported by many researchers (Ansari et al., 2014; Hagh Bahari and Seyed Sharifi, 2014; Hamzei and Sadeghi Meabadi, 2014).

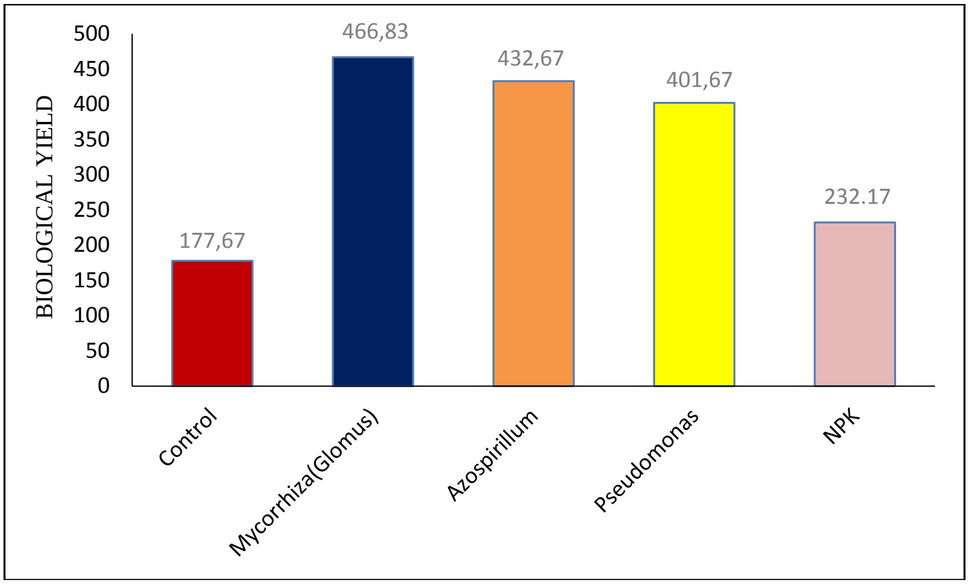

Figure1.The change of biological yield in treated Thymus plant with bio and non-bio fertilizers (Columns with at least one common letter have no statistically significant difference based on LSD test at 5% probability level).

Flowering branches yield

The results of analysis of variance showed that the effect of fertilizer on the flowering branches yield was significant ($P < 0.01$) (Table 2). Comparison of the mean effect of fertilizer on the yield of flowering branches showed that mycorrhiza fertilizer was 105% more effective than the control. The highest yield of flowering branches (36.53 g / plant) was observed in mycorrhiza (Glomus) and the lowest in control (Figure 2). Bacteria in biofertilizers in addition to absorbing the main elements of high consumption and low consumption required by the plant by making and secreting plant growth stimulants as well as the secretion of various amino acids and antibiotics cause root growth and development, aerial destruction and flowering branches (Han and Lee, 2006; Gutierrez-Manero et al., 2001). Khorramdel et al (2008), Observed that the application of mycorrhiza inoculum increased the accumulation of dry matter and flowering branches of black seed compared to the control.

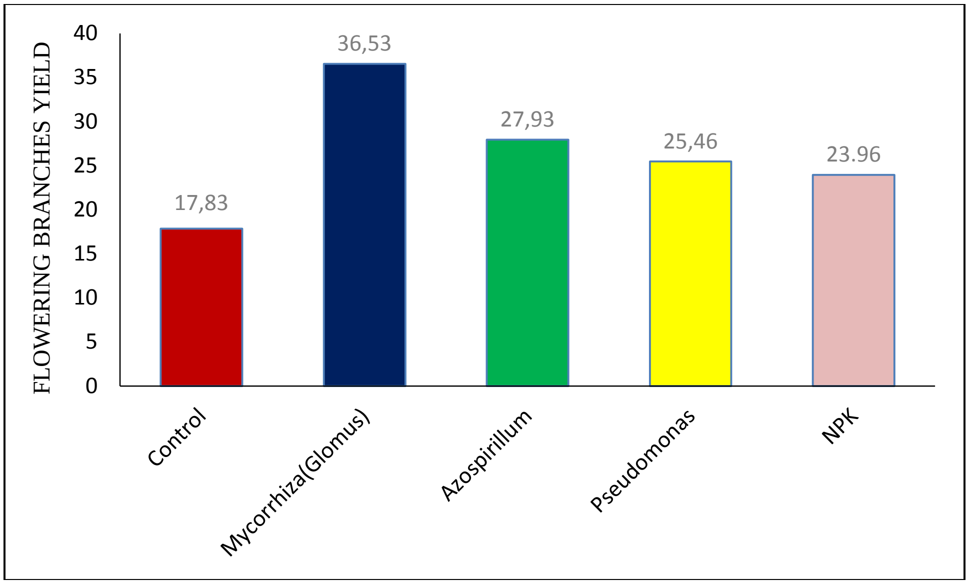

Figure2.The change of flowering branches yield in treated Thymus plant with bio and non-bio fertilizers (Columns with at least one common letter have no statistically significant difference based on LSD test at 5% probability level).

Essential oil yield

The results of analysis of variance showed that fertilizer treatment had a significant effect on essential oil yield (P <0.01) (Table 2). Comparison of mean data showed that mycorrhiza fertilizer was 87% more effective on essential oil yield than the control. The highest yield of essential oil (9.22 g / plant) was observed in mycorrhiza (Glomus) and the lowest in the control treatment (Figure 3).

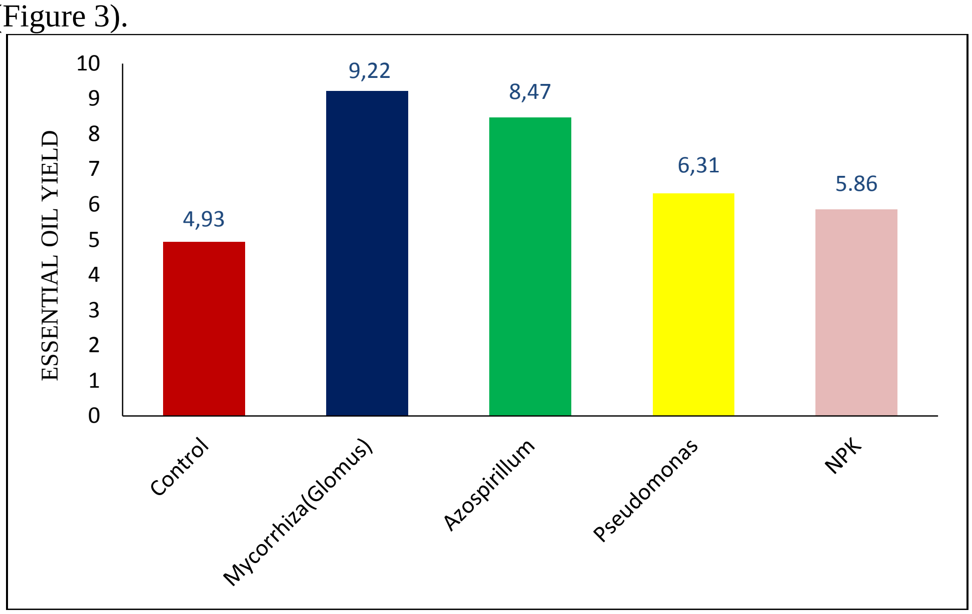

Figure3. The change of essential oil yield in treated Thymus plant with bio and non-bio fertilizers (Columns with at least one common letter have no statistically significant difference based on LSD test at 5% probability level).

Conclusions

Since the ultimate goal of cultivating medicinal plants is to use the essential oils and active ingredients in the essential oils of these plants, and certainly the higher the amount of these effective substances per unit weight of the plant, the greater the economic benefit, Therefore, studying and obtaining the best conditions of the culture environment that can lead to plant production with the highest essential oil yield is one of the most important goals in research related to the cultivation of medicinal plants. According to the results of this study, it is concluded that mycorrhiza biofertilizer has had the greatest impact on biological performance and flowering branches and essential oils of thyme. Therefore, this bioferilizer is recommended to increase the biological yield and essential oil and active ingredient of thyme.

References

Afzal, A., and Bano A. (2008). *Rhizobium* and Phosphate Solubilizing Bacteria Improve the Yield and Phosphorus Uptake in Wheat (*Triticum aestivum*), International Journal of Agriculture and Biology, 101,85–88.

Ansari A, Razmjoo J, Karimmojeni H, Zarei M. (2014). Effect of Mycorrhizal Inoculation and Pre-treatment with Salicylic Acid at Different Levels of Drought on Morphological Traits and Yield of Flax, JCPP,4 (12) ,181-195.

Auge R.M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis Mycorrhiza., 11, 3-42.

Casson S.A. and Lindsey K. (2003). Genes and signalling in root development, New Phytol, 158, 11-38.

Golparvar, A. R. and Bahari B. (2011). Effects of phonological stages on herbage yield and quality/quantity of oil in garden thyme (*Thymus vulgaris* L.), Journal of Medicinal Plants Research, 5(25), 6085-6089.

Gutierrez-Manero F.J. Ramos-Solano B. Probanza A. Mehouachi J. Tadeo F.R. and Talon M. (2001). The plant-growth promoting rhizobacteria Bacillus pumilus and Bacillus licheniformis produce highamounts of physiologically active gibberellins , Physiol. Plant, 111, 206-211.

Hagh Bahari M, Seyed Sharifi R. (2014). Study of quantitative and qualitative yield, chlorophyll content and some growth indices of wheat (*Triticum aestivum* L.) in response to seed inoculation with PGPR at different levels of soil salinity, JSPI, 5 (2) ,51-65.

Hamzei J, Sadeghi Meabadi F. (2014). The Effect of Irrigation Intervals and Arbuscular Mycorrhizal Fungi on Chlorophyll Index, Yield and Yield Components of Grain Sorghum, JCPP,4 (12) ,211-221.

Han H.S. and Lee K.D. (2006). Effect of inoculation with phosphate and potassium co-insolubilizing bacteria on mineral uptake and growth of pepper and cucumber Plant, Soil Environ., 52, 130-136.

Imelouane, B., Amhamdi H., Wathelet J. P. and El Bachiri A. (2009). Chemical Composition and Antimicrobial Activity of Essential Oil of Thyme (*Thymus vulgaris*) from Eastern Morocco, International Journal of Agriculture and Biology, 11 (2), 205–208.

Khorramdel. S., Koocheki S., Nassiri Mahallati A. and Ghorbani R. (2008). Application effects of biofertilizers on the growth indices of black cumin (*Nigella sativa* L.), Iranian Journal of Crop Research,6(2),285-294.

Omidbaigi. R., (2005). Production and processing of medicinal plants. Publications Astan Quds Razavi, Volume III, Second Edition.

Safaei-Ghomi, J., Ebrahimabadi, A.H., Jafari- Bidgoli, Z. and Batooli, H. (2009). GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of Thymus caramanicus Jalas and its main constituent carvacrol, Food Chemistry, 115(4), 1528-1524.

Yadegari, M., Asadi Rahmani H. and Noormohammadi G. (2010). Plant growth promoting Rhizobacteria increase growth, yield and nitrogen fixation in *Phaseolus vulgaris*, Journal of Plant Nutrition, 33, 1733–1743.

GRAPHIC ANALYSIS OF DROUGHT TOLERANCE IN DURUM WHEAT GENOTYPES USING STRESS SELECTION INDICES

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Abstract

The objective of this study was to apply the three-dimensional plots of stress selection indices to screen tolerant genotypes of durum wheat. Fourteen genotypes were grown under both drought stress and potential conditions using a randomized complete block design with four replicates. Eight drought tolerance indices were used as well as yield performance under potential (YP) and stress (YS) conditions for drawing three-dimensional plots. Genotypes G1, G4, G5, G6, and G13 were found the most favorable genotypes according to YP, YS and SSI, while genotypes G3 and G8 displayed the highest performance based on STI. Using modified STI indices, K_1STI and K_2STI did not alter pervious results and produced similar findings. The three-dimensional plots based on average statistics (MP, GMP and HM) as well as YP and YS identified G3 and G8 as the best genotypes, while according to TOL, genotypes G3 and G4 were selected as the most favorable genotypes. Although genotypes performed differently in potential (YP) and stress (YS) conditions, which justifies screening them for both conditions simultaneously, we could find some genotypes which responded good in both conditions like genotypes G3 and G4. Therefore, most of studied indices could discriminate drought tolerant genotypes with high yield using the three-dimensional plots.

Keywords: *the three-dimensional plots, graphic analysis, tolerance indices.*

Introduction

Drought stress may lead to a loss in yield; particularly severe deficit at anthesis stage has serious effects on durum wheat yield, which is the main concern of durum wheat breeders and they emphasize on yield performance under drought stress conditions. Breeding for tolerance to drought stress is complicated by the lack of proper screening methods and the inability to create repeatable water drought conditions where large populations can be evaluated efficiently. To differentiate tolerant genotypes, several indices have been suggested on the basis of a relationship between non-stress and stress conditions which are providing a measure of drought based on loss of yield under drought stress conditions in comparison to non-stress conditions. Fischer and Maurer (1978) proposed the stress susceptibility index, and Rosielle and Hamblin (1981) defined stress tolerance and mean productivity for evaluation of genotypes for drought tolerance. Fernandez (1992) also has been suggested stress tolerance index, geometric mean productivity and harmonic mean for screening breeding materials for stress.

The best index must detect genotypes perform uniform superiority in both stress and non-stress conditions from the genotypes that are favorable only in one condition. Among the different stress tolerance indices, selection based on stress susceptibility index and stress tolerance criteria favors genotypes with low yield potential under non-stress condition and high yield under stress condition and selection based on these indices will be resulted in genotypes with higher stress tolerance and yield potential will be selected. The objectives of this study were to identify tolerant genotype as well as determine the efficiency of screening indices to grouping genotypes into tolerant versus sensitive genotypes.

Materials and methods

14 durum wheat genotypes were studied under dry-land condition in growing season 2012-2013. The experiments were conducted a randomized complete block design with four replicates under non-stress and stress conditions. Each plot was 5 × 6 m, rows with a 20 cm row space and total plot size was 6 m^2 and Plants were fertilized with nitrogen at the rate of 50 kg ha^{-1} urea and phosphorus at the rate of 120 kg ha^{-1} ammonium phosphate and proper agronomic management practices were adopted throughout the growing season to ensure good crop growth. Various drought tolerance indices were calculated as; the stress susceptibility index (SSI) of Fischer and Maurer (1978); the mean productivity (MP) and tolerance (TOL) of Rosielle and Hamblin (1981); the stress tolerance index (STI), geometric mean productivity (GMP) and harmonic mean (HM) of Fernandez (1992); the STI-based indices consist on K_1STI and K_2STI (Naderi *et al*., 1999).

Results and discussion

According to the three-dimensional plot SSI (Fig. 1), four zones, A, B, C and D were identified as A, genotypes with high yield performance in both potential (YP) and stress (YS) conditions; B, genotypes with high yield performance in potential (YP) condition and low yield performance in stress (YS) condition; C, genotypes with low yield performance in potential (YP) condition and high yield performance in stress (YS) condition; and D, genotypes with low yield performance in both potential (YP) and stress (YS) conditions. In each of these conditions, the high values of SSI greater than average can be favorable and thus, genotypes G3 and G8 had the best performance following to G1, G6, G13 and G14. However, genotype G10 was located in this zone with low SSI values and this index could not detect these genotypes as most favorable ones. Genotypes G4, G9 and G12 were located in zone B while only G4 had high SSI values and was identified as the most favorable genotype based on SSI. There is not ant genotype in zone C, and so none of the studied genotypes show high performance only in stress condition. Genotypes G2, G5 and G7 were located in zone D and none of them had high SSI values and was identified as the as worst genotypes.

The pattern of response in STI, K_1STI and K_2STI was similar (Fig. 1), and introduced genotypes G3 and G8 as the most favorable genotypes in zone A. Mevlut and Sait (2011) showed that genotypes with high STI values usually have high difference in yield in two different humidity conditions and also, Ilker *et al*. (2011) and Sabaghnia and Janmohammadi (2014) reported that STI-related indices (K_1STI and K_2STI) are convenient parameters for selecting high yielding genotypes in both stress and non-stress environments whereas relative decrease is observed in yield performance.

The four zones in the three-dimensional plot baes on central tendency indices, MP, GMP and HM as well as TOL were relativity similar and detected introduced genotypes G3 and G8 as the

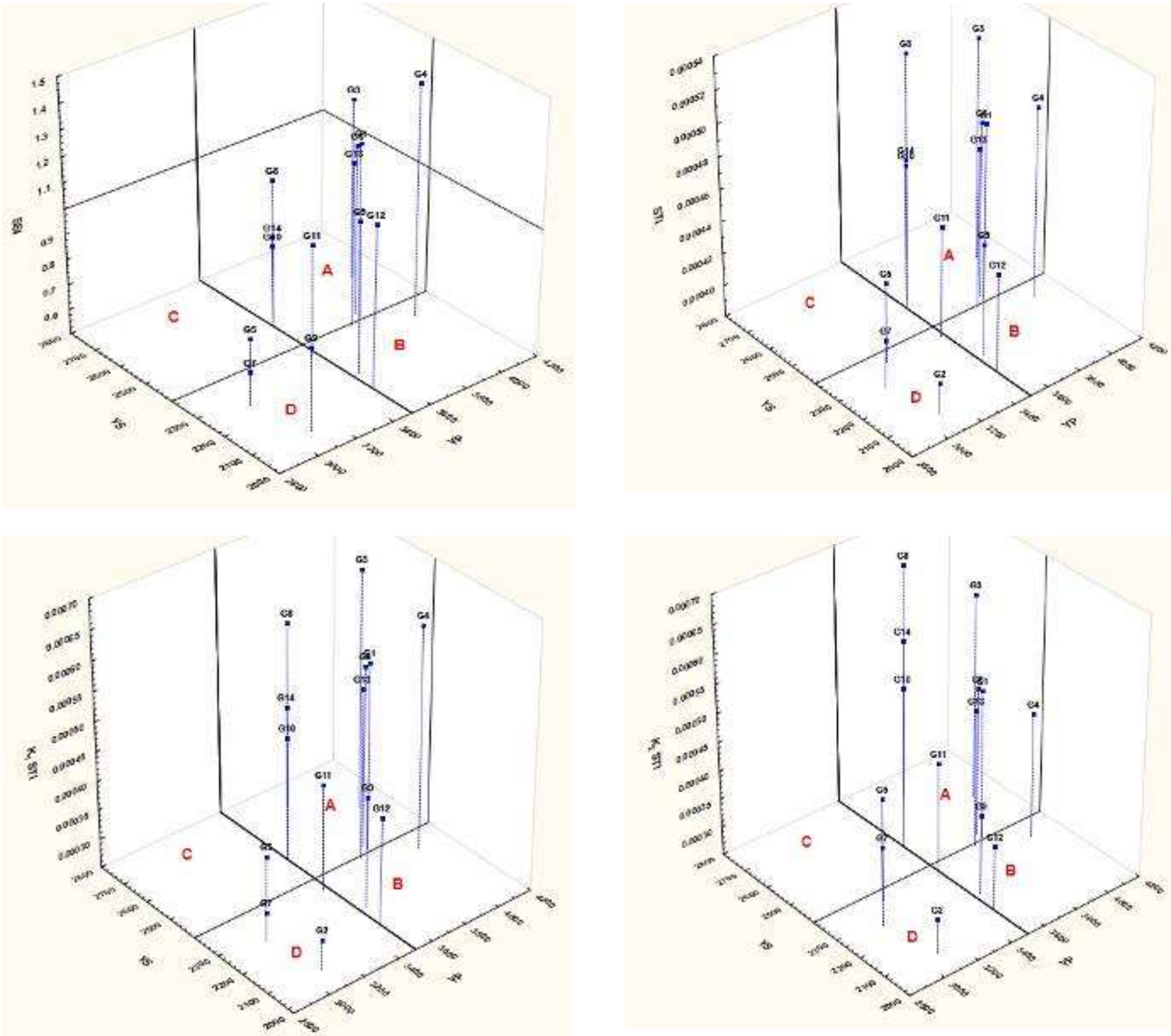

Fig. 1. The three-dimensional plots of YP, YS and drought selection indices (up-left) SSI, (up-right) STI, (down-left) K1STI and (down-right) K2STI.

most favorable genotypes in zone A and these results were in agreement with SSI, STI, K_1STI and K_2STI results which indicates high associations among them.

Similar interrelationships among MP, and GMP indices (Toorchi *et al*., 2012) and among GMP, MP and STI indices (Dehghani *et al*., 2009) were reported. Also, positive correlation of STI, GMP and HM were reported by Sabaghnia and Janmohammadi (2014). In general, the observed relationships in our dataset were consistent with those reported by Khalili *et al*. (2012). Some studies showed that GMP and STI indices are preferred in late drought condition for selecting the most favorable genotypes (Akcura *et al*., 2011) while some other investigation indicated that MP are preferred for selecting the most tolerant genotypes (Sio-Se Mardeh *et al*., 2006; Khalili *et al*., 2012). Relatively, such similar conclusion is reported by Fernandez (1992), and Sabaghnia and

Janmohammadi (2014), which mentioned that priority of STI and K_2STI parameters under level of high to moderate stress. Akcura *et al*. (2011) reported STI was able to differentiate genotypes belong to genotypes with high yield performance in both conditions, from the others. The genotypes G3 and G8 were found to be the best genotypes and advised for future cultivation in semi-arid regions.

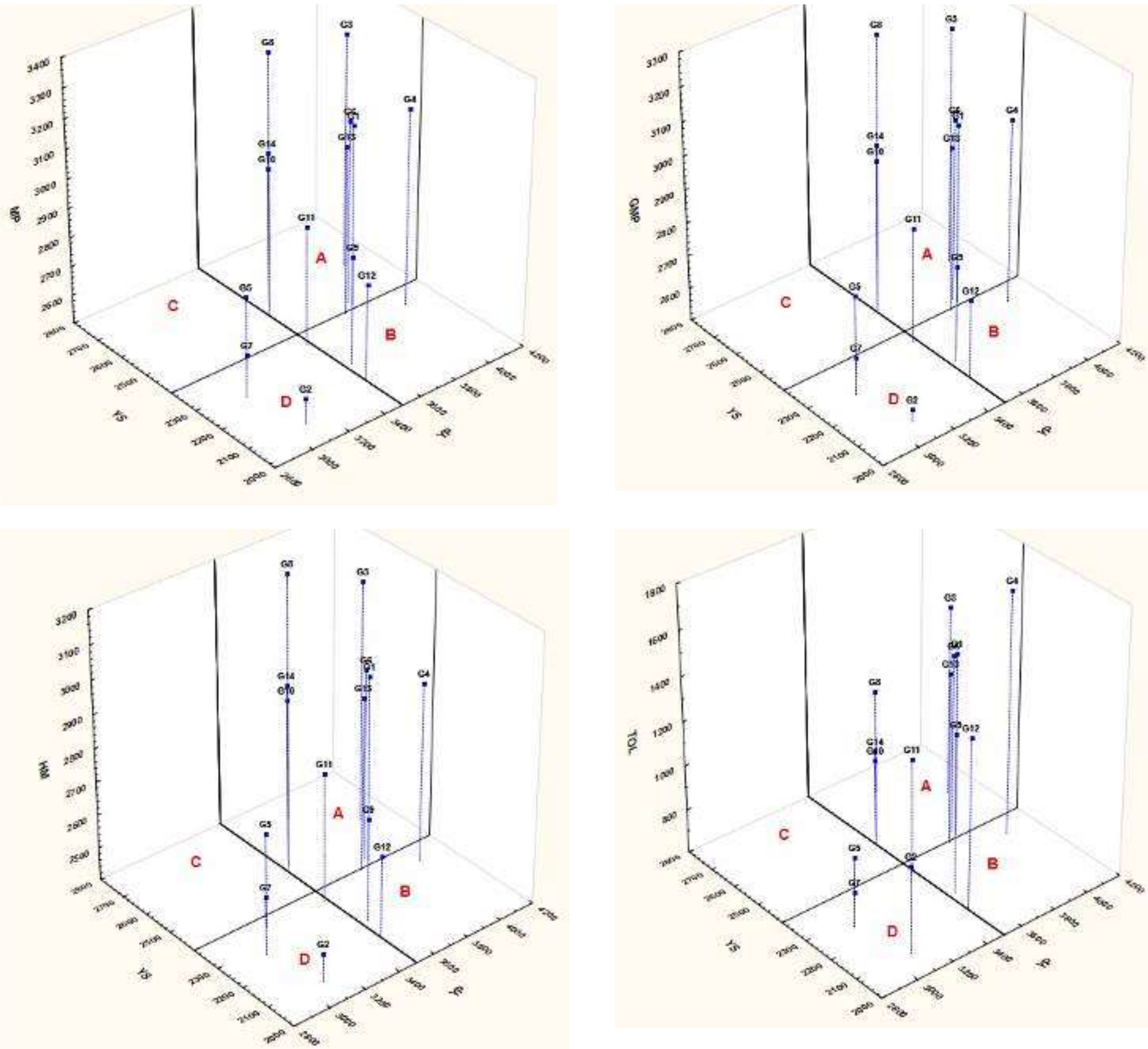

Fig. 2. The three-dimensional plots of YP, YS and drought selection indices (up-left) MP, (up-right) GMP, (down-left) HM and (down-right) TOL.

Conclusion

The GMP and STI indices are found to be best tools in drought condition for selecting the most favorable genotypes and the genotypes G3 and G8 were found to be the best tolerant genotypes in semi-arid conditions.

Acknowledgement

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References

Akcura, M., Partigoc, F., Kaya Y. (2011). Evaluating of drought stress tolerance based on selection indices in Turkish bread wheat landraces. The Journal of Animal and Plant Sciences, 21, 700–709.

Dehghani, H., Malekshhi, F., Alizadeh, B. (2009). A study of drought tolerance indices in canola (*Brassica napus* L.) genotypes. Journal of Science and Technology of Agriculture and Natural Resources, 13: 77–90.

Fernandez, G.C.J. (1992). Effective selection criteria for assessing stress tolerance. In: Kuo, C.G. (Ed.), Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, Publication, Tainan, Taiwan.

Fischer, R.A. Maurer, R. (1978). Drought resistance in spring wheat cultivars. Part 1: grain yield response. Australian Journal of Agriculture Research, 29: 897–912.

Ilker, E., Tatar, O., Aykut-Tonk, F., Tosun, M. Turk, J. (2011). Determination of tolerance level of some wheat genotypes to post-anthesis drought. Turkish Journal of Field Crops, 16: 59–63.

Khalili, M., Naghavi, M. R., Pour-Aboughadareh, A.R., Talebzadeh, S.J. (2012). Evaluating of drought stress tolerance based on selection indices in spring canola cultivars (*Brassica napus* L.). Journal of Agricultural Science, 4: 78–85.

Naderi A., Majidi-Hervan, E., Hashemi-Dezfouli, A., Nourmohammadi, G., Rezaee, A. M. (1999). Performance analysis of indicators evaluating crop tolerance to environmental stress and introducing a new index. – Seed and Plantlet, 15: 390-402.

Rosielle, A. A. Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. Crop Science, 21: 943–946.

Sabaghnia, N., Janmohammadi, M. (2014). Evaluation of selection indices for drought tolerance in some chickpea (*Cicer arietinum* L.) genotypes. Acta Technologica Agriculturae, 12: 6–12.

Sio-Se Mardeh, A., Ahmadi, A., Poustini, Mohammadi, V. (2006). Evaluation of drought resistance indices under various environmental conditions. -Field Crops Research, 98: 222–229.

Toorchi, M., Naderi, R., Kanbar, A. Shakiba, M.R. (2012). Response of spring canola cultivars to sodium chloride stress. Annals of Biological Research, 2: 312–322.

POMOLOGICAL PROPERTIES OF SOME IRANIAN PLUM (PRUNUS DOMESTICA) CULTIVARS

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Abstract

Plums have been known as one of the most diverse fruits in temperate regions, although their variation and diversity also cause differences in the physicochemical features of the fruit. Therefore, the aim of this study is to introduce and investigate the physicochemical and pomological traits of these cultivars. The present study was conducted to investigate the physicochemical properties of 7 Iranian plum. Plum cultivars with the usual names included: 'Ghatretala', 'Pivehzhan', 'Rotaby', 'Ghandy', 'Beygom', 'Torghabeh Sabz' and 'Bokhara'. Analysis of variance demonstrated that all cultivars had significant differences ($p \leq 0.01$) for all measurement parameters of chemical and physical features. The results also showed that in most of the chemical features studied, cultivars 'Beygom' and 'Torghabeh Sabz' were superior to other cultivars and had the highest amount of chemical compounds. The results also showed that there was a high amount of compounds such as antioxidants, flavonoids and vitamin C in the cultivars. Therefore, it can be said that some of the cultivars investigated in this study were rich in bioactive compounds and had high nutritive values.

Keywords: *Antioxidant Capacity, flavonoids, Vitamin C, Physical properties.*

Introduction

Plums belong to the family Rosaceae, subfamily Prunoideae and genus Prunus. Plum is one of the most diverse fruits that grows in a wide range of climatic and geographical conditions. This product is native to Europe and Asia (Karimifar et al., 2012). Some plum cultivars such as Ghandy', 'Black', 'Bokhara', 'Shams' and 'Ghatretala' are native species from Prunus domestica L. The highest provinces of plum production were Khorasan Razavi, Alborz, Golestan and Azarbayejan Gharbi in Iran, respectively (Falati et al., 2018). Plums contain carbohydrates (sucrose, glucose, fructose), organic acids (citric and malic acid), fibers, tannins and aromatic compounds that determine the nutritional value and taste of plums (Arion et al., 2014). It is also a good source of carotene, anthocyanins and natural antioxidants (Kim et al., 2003). Jalili et al. (2011) conducted an experiment on 38 genotypes of Iranian plums and reported that the soluble solids content in plum genotypes was in the range of 16 to 31 ° Brix. The amount of titratable acids in different plum genotypes varied from 0.4 to 0.94. According to Vangdal (1980) studies, the acceptance of plums with a soluble solids content of less than 12 ° Brix is very low for most consumers. The better taste of plum fruit and its greater acceptance depend on less titratable acidity and higher soluble solids content (more than 12%) of the fruit. The quality of fruit plums is also related to external characteristics, such as the size and color of the pulp and skin, which are associated with chemical composition (Yousefi and Emam-Djomeh, 2015).

Material and Methods

Plant material

This research was conducted in Ferdowsi University of Mashhad (Iran) in 2019. Seven important plum cultivars from one of the commercial orchard of Neishabour city in Khorasan Razavi province were picked at full maturity stage and transferred to the laboratory. The experimental design was a randomized complete block design (RCBD) with seven treatments (plum cultivars) and five trees as replications. Due to the fact that the maturity of different cultivars was happened on different days, the cultivars were collected over a period of four months (from June to September).

Physical properties

The weight of the fresh fruit and its stone were determined using a digital laboratory balance (accuracy: 0.001 g). The two linear dimensions of length and width were measured with a digital caliper (accuracy: 0.01 mm). The firmness of fruit texture was determined by penetrometer (8 *mm* Tip) and recorded in N/cm^2 (Davarinejad et al., 2010).

Biochemical properties

The soluble solid content (SSC) of fruit juice was immediately measured using a digital refractometer. The titratable acidity (TA) was determined based on mg of malic acid (major organic acid) in 100 g of fruit tissue by titration of 10 ml of fruit extract with 0.1 N sodium hydroxide solution to reach pH = 8.3 (Najafzadeh et al., 2012). Finally, the SSC/TA ratio was calculated (Davarinejad et al., 2010). The ascorbic acid was measured by titration method and expressed as mg in 100 ml of fruit juice (AOAC, 2005). The antioxidant capacity of plum fruits was determined by the free radical scavenging properties of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate). Finally, the antioxidant capacity of fruit juices was reported as a percentage of inhibition (DPPHsc %). Total flavonoids of fruits were measured according to Gil et al. (2002) at 506 nm.

Statistical analysis

Statistical analysis was performed using JMP 9 statistical software (SAS, Institute, and Cary, NC, USA). The standard error in the tables was also shown as (mean ±SE).

Results and discussion

The results of analysis of variance showed a significant difference between the plum cultivars in terms of physical characteristics including fruit and stone dimensions ($p \leq 0.01$). The results also showed that plum cultivars have significant effects on the soluble solid content, titratable acidity, flavor index, vitamin C, antioxidant capacity and flavonoids.

According to the results of fruit dimensions (Table 1), 'Pivehzhan' cultivar had the highest values of fruit length (36.55 mm) and width (34.71 mm). While 'Ghandy' and 'Beygum' cultivars had the lowest length (30.78 mm) and width (29.30 mm) of fruit, respectively. The maximum and minimum firmness of fruit tissue with values of 2.19 and 1.21 N/cm2 were observed in 'Beygum' and 'Bokhara' cultivars, respectively. Previously, the average firmness in four plum cultivars has been reported by Yousefi and Emam-Djomeh (2015) as 1.87 N/cm^2. Calisir et al., (2005) also studied the physical properties of some wild plums and determined the firmness values of fruit tissue between 0.625-0.499 N/cm^2.

Table 1. Physical properties of plum cultivars.

Cultivar	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	stone weight (g)	Firmness (N/cm^2)
Ghatretala	34.19±0.3 b	34.59±0.3 a	24.52±0.6 b	1.35±0.1b	1.67±0.06 c
Pivehzhan	36.55±0.2 a	34.71±0.3 a	26.24±0.4 a	1.54±0.3a	1.30±0.10 d
Rotaby	35.08±0.3 ab	30.36±0.2 c	21.27±0.4d	0.55±0.1d	1.40±0.02 d
Ghandy	30.78±0.2 d	34.24±0.2 a	25.86±0.3ab	1.27±0.2 c	1.24±0.04 e
Beygom	34.11±0.2 b	29.30±0.2 d	21.37±0.3d	1.29±0.1c	2.19±0.04 a
Torghabeh Sabz	32.68±0.4 c	32.25±0.4 b	22.70±0.8 c	1.40±0.2 b	2.02±0.04 b
Bokhara	34.02±0.3 b	30.03±0.4 cd	21.25±0.8 d	1.32±0.1 bc	1.21±0.03 e

The changes in the TSS/TA ratio of the studied plum fruits ranged from 20.51 ('Ghatretala') to 41.21 ('Ghandy').The highest value of TSS/TA ratio was recorded in 'Torqhabeh Sabz' and 'Ghandy' cultivars. No significant difference was observed between other cultivars.

Vitamin C (ascorbic acid) content in the evaluated cultivars ranged from 2.53 to 9.24 mg per 100 g of fresh weight. This is lower than the amount of ascorbic acid reported by the USDA (2012) for plums at about 9.5 mg per 100 g fresh weight of fruit. Falati et al. (2017) also reported the range of vitamin C changes in plum cultivars and genotypes about 7.04 – 18.5 mg per 100 g of fresh weight. On the other hand, Hajilou et al. (2013) measured lower levels of ascorbic acid for the plum cultivars (4.33 – 4.17 mg per 100 g).

The range of antioxidant activity in the cultivars evaluated in this study was 14.97 to 73.36 %. 'Ghatretala', 'Pivezhan' and 'Rotaby' cultivars had the lowest level of antioxidant activity with significant differences from other cultivars. Previously, the percentage of antioxidant activity of plum cultivars has been reported between 96.3 – 11% (Falati et al., 2017) and 82.8% (Hajilou et al., 2013).

Total flavonoids content was between 452 and 967 mg quercetin per 100 g fresh weight, although there was no significant difference between the cultivars in this experiment. Also, the concentration of total flavonoids in plum cultivars has been reported to be around 118 to 237 (mg/100 g of fresh fruit) (Donovan et al., 1998), which is much lower than values obtained in the cultivars evaluated in this study.

Table 2- Biochemical properties of plum cultivars.

Cultivars	TSS/TA	Ascorbic acid (mg/100 g)	Antioxidant activity (%)	Flavonoid (mg/100 g)
Ghatretala	20.51±0.4d	2.53±0.1e	14.97±2.8e	452.20±8.7d
Pivezhan	27.52±1.9c	9.24±0.5a	23.51±0.d	674.80±15.2c
Rotaby	23.13±0.5cd	4.51±0.5cd	30.97±6.7d	477.80±7.7d
Ghandy	41.21±1.0a	5.94±0.4c	65.23±1.3b	904.80±7.4ab
Beygom	34.97±0.9b	7.15±1.3b	73.36±1.2a	967.00±8.9a
Torghabeh Sabz	41.15±1.1a	8.69±0.1ab	69.78±1.2ab	964.80±14.8a
Bokhara	36.57±1.1b	4.84±0.3d	51.55±3.7c	881.60±24.0b

Conclusion

The high diversity of cultivars studied in this experiment in terms of physical and biochemical properties was one of the most important results that can be used in the introduction, reproduction and breeding projects.

References

AOAC (2005). Official method of analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.

Arion C. M., Tabart J., Kevers C., Niculaua M., Filimon R., Beceanu D., Dommes J. (2014). Antioxidant potential of different plum cultivars during storage. Food Chemistry, 146: 485-–491.

Calisir S., Haciseferogullari H., Ozcan M., Arslan D. (2005). Some nutritional and technological properties of wild plum fruits in Turkey. Journal of Food Engineering, 66: 233–237.

Davarinejad G.H., khorshidi Sh, Nyeki J., Szabo Z., Gal-Remennyik J. (2010). Antioxidant capacity, chemical Composition and physical properties of some Apricot (*Prunus armeniaca* L.) cultivars. Horticulture, Environment, and Biotechnology, 5: 477-482.

Donovan J. L., Meyer A.S. Waterhouse A.L. (1998). Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). Journal of Agricultural and Food Chemistry, 46: 1247–1252.

Falati Z., Fatahi Moghadam M.R., Ebadi A. (2017). The evaluation of phytochemical compounds of fruits in some plum and prune cultivars and genotypes. Iranian Journal of Horticultural Science, 14: 789–802.

Gil M.I., Tomas-Barberan F.A., Hess-Pierce B., Kader A.A. (2002). Antioxidant capacities, phenolic compounds, carotenoids and vitamin C contents of nectarine, peach, and plum cultivars from California. Journal of Agriculture and Food Chemistry, 50: 4976:4982.

Hajilou J., Fakhim Rezaei Sh., Dehghan Gh.R. (2013). Comparison of the quality attributes and antioxidant capacity of plum-apricot interspecific hybrid with some plum and apricots cultivars in harvesting time. Journal of Food Research, 23:107–119.

Karimifar P., Ahmadzadeh R., Ghiafeh-Davoodi M. (2012). Studies physicochemical and organoleptic properties in process of plum fruit, using hurdle technology of Osmo-dehydofreezing. Journal of Food Processing and Preservation, 4: 1-14.

Jalili E., Rabiei V., Azami M.A., Daghestani M. (2011) Genotypic diversity of prune and plum using morphological characteristics in Maragheh region. Seed and Plant Improvement Journal 27: 357–374.

Kim D.O., Chun O.K., Kim Y.J., Moon H.Y., Lee C.Y. (2003). Quantification polyphenolics and their antioxidant capacity in fresh plums. Journal of Agriculture and Food Chemistry, 51: 6509-–6515.

Najafzadeh R., Arzani K., Babayi A. (2012). Investigation of physico-chemical and qualitative properties of some european pear genotypes. Journal of Horticultural Science 26(2): 170–177.

USDA (2012). Food composition data base (www.nal.usda.gov/fnic/foodcomp/).

Vangdal E. (1980). Threshold values of soluble solids in fruits determined for the fresh fruit market. Acta Agriculturae Scandinavica, 30: 445–448.

Yousefi G., Emam-Djomeh Z. (2015). Study of some physical and chemical properties of four plum cultivars (*Prunus domestica* L.). Journal of Innovation in Food Science and Technology, 7: 45–56.

INFLUENCE OF AGRONOMIC TECHNIQUES ON QUANTITATIVE AND QUALITATIVE PRODUCTION OF ESSENTIAL OILS OF SOME OFFICINAL SPECIES

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Abstract

Aromatic plants, in addition to the primary metabolites, fundamental in the life cycle of the plant, produce high levels of secondary metabolites, biologically active compounds that can confer a range of properties such as aromas, fragrances, antioxidant and antimicrobial effects, as well as therapeutic and pharmacological properties. Conditions of stress and alteration of the balance within the plant organism stimulate the production of secondary metabolites, since these substances actively participate in the restoration of normal conditions and the survival of the plant. *Lamiaceae* are characterized by high adaptability and resistance in the most disadvantaged environments, having Mediterranean origin and able to withstand the most diverse climatic conditions; moreover, they are known for high content in active ingredients and essential oils to be used, in addition to the food sector, also for therapeutic uses and the preparation of cosmetics, drugs and pesticides. One of the main aspects distinguishing the quality of officinal species is the content and composition of essential oils, parameters influenced by numerous endogenous and exogenous factors. The aim of this review was to investigate the influence that some cultivation techniques, such as planting density, irrigation regime, fertilization, harvest time, had on the commercial yield, on the percentage content and on the composition of the essential oil extracted from *Origanum. vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., four species belonging to the Lamiaceae family widespread in the Mediterranean area.

Keywords: *Aromatic plants, essential oils, yield, agro techniques.*

Introduction

In recent years, modern society, in addition to requiring food products without any limitation, increasingly feels the need to have "healthy" and "clean" products obtained without the use of chemicals products and with "natural" methods. In this regard, there is an increasing demand for products derived from plants that are proposed, not just as food in the strict sense, but as functional foods capable of providing mineral salts, vitamins and phytocomplexes that can have a beneficial effect on health. (Lucera et al., 2012). In other words, it refers to a serier of compounds that the plant biosynthesizes as part of the so-called secondary metabolism such as aromas, tannins, dyes, etc. (Pichersky et al., 2006).

A group of plants of great interest and able to supply the sector of natural substances with high quality raw materials is represented by medicinal plants. Since ancient times, man has exploited these plants both as food and as a curative product. In addition to the primary metabolites, as highlighted above, which are fundamental in the biological cycle of plants, they also produce high levels of secondary metabolites, biologically active compounds that can confer a range of properties such as aromas, fragrances, antioxidant and antimicrobial effects, and also therapeutic and pharmacological properties. These characteristics allow their use in the food, pharmaceutical, cosmetics and even pesticides sectors, both in pure and mixed form (Piccolella et al., 2018). One of the main aspects that distinguishes the quality of officinal species is the content and the composition of essential oils which are influenced by numerous factors, depend on the plant (endogenous or genetic factors), others depend on the environment in which the plant grows (exogenous or environmental factors and biotic factors), others concern the collection, preparation and conservation of the plant or processed products (Trivellini et al., 2016; Marzi & De Mastro, 2008). Among the medicinal plants, within the Sicilian territory, some species such as *Origanum vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., belonging to the *Lamiaceae* family (ex *Labiatae*), known for their high content in active ingredients and essential oils, to be used, in addition to the food sector, also for therapeutic uses, for the preparation of cosmetics, drugs and pesticides (Lubbe & Verpoorte, 2011). The main purpose of this work, focused on the analysis of the scientific publications of the last twenty years concerning the medicinal plant sector, was to analyze the results obtained by the various authors, in different environmental conditions, in order to establish what the influence that some factors of the agronomic technique, such as planting density, irrigation, fertilization and the time of harvest, exert on the commercial yield, on the percentage content and on the composition of the essential oil extracted from the main medicinal species cultivated in Sicily.

Materials and methods

The activity carried out as part of this work was a thorough bibliographic research on the influence of agronomic techniques (planting density, fertilization, irrigation, harvest time) on the production of essential oils of the four species object of the work. The databases used were the Google Scholar platform, the Discovery Service of the University of Palermo, the Scopus scientific database, and the editorial portal https://www.mdpi.com/. The research took into consideration only the publications of the period 2000 - 2021, using as keywords "aromatic plants", "essential oil", "variability", "influence", "fertilization", "irrigation", "water use", "Plant density", "spacing", "harvesting time", as well as the scientific names of the individual species, *Origanum vulgare* L., *Salvia officinalis* L., *Rosmarinus officinalis* L., *Thymus vulgare* L..
From each publication, data and results regarding the effective influence of agronomic techniques on the qualitative and quantitative production of essential oils have been extrapolated. In the data summarized in the results are highlighted the effects on the yield and content of the main components of the essential oils extracted from each species were highlighted, due to the different plant densities, irrigation strategies, different types of treatments and fertilization, at the different times of harvest.

Results and discussion

The qualitative and quantitative parameters relating to essential oils are fundamental to define the quality and commercial value of a medicinal plant. It is therefore necessary to define the techniques and treatments to be applied to obtain the best results and optimize the cultivation of aromatic and medicinal species. The officinal species accumulate active principles during the entire biological cycle, but it cannot be excluded that, in some cases, temporary stress conditions may cause an increase in the content and yield of active ingredients within the plant species, by appropriately choosing treatments and durations (Trivellini et al., 2016; Kulak et al., 2020).

Influence of plant density

The choice of the planting density is a fundamental aspect to take into consideration to maximize the yields and the quality of the cultivated plants.

A high planting density and, consequently, a high competition between plants, allows to obtain better yields and higher percentages of essential oil in oregano plants (De Falco et al., 2013; Tuttolomondo et al., 2016) and sage (Abu Darwish et al., 2011); also for *Rosmarinus officinalis* L. (Leto et al., 2004) the yield in essential oil increases in conditions of high planting density but it is necessary to deepen the experimentation, since the publications analyzed are a small number and with disagreeing results.

For thyme plants, with low planting densities, were obtained the highest percentages in essential oil, but, in some cases, the higher oil yield per unit area was obtained with a higher planting density, thanks to the increase in the quantity of biomass produced per unit of surface (Badi et al., 2004; Abu-Darwish et al. 2012).

The competition for light, water and nutrients, due to the distances between plants, can play a very important role in increasing or decreasing the synthesis and accumulation of essential oils in the plant organs of medicinal species. It is necessary to optimize plant density to maximize biomass production and essential oil yield, especially when water is a limiting factor, as competition increases and water availability per plant is reduced (Khazaie et al., 2008).

Influence of the irrigation regime

Water is an important resource for plant growth and production. Efficient use of water is important to ensure acceptable production, especially in conditions of scarce availability or in semi-arid areas, where water is not sufficient to meet crop requirements (Bernstein et al., 2009).

Subject to controlled and moderate water stress conditions *Origanum vulgare* L. (Azizi et al., 2009; Morshedloo et al. 2017), *Rosmarinus officinalis* L. (La Bella et al. 2020, Raffo et al. 2020, Pirzad & Mohammadzadeh 2018), *Salvia officinalis* L. (Mohammadi et al. 2020, Vosoughi et al. 2018, Rioba et al. 2015, Govahi et al. 2015) and *Thymus vulgaris* L. (García-Caparrós et al. 2019; Mohasseli & Sadeghi 2019; Arpanahi & Feizian 2019; Mohammadi et al., 2019; Pirzad & Mohammadzadeh 2018; Askary et al. 2018; Alavi-Samani et al. 2015; Pirbalouti et al. 2014; Khazaie et al. 2008) guarantees an increase in yield and content percentage of essential oil extracted.

In thyme plants, severe water stress, accompanied by foliar treatments based on hormones and stimulants, guarantee a significant increase in the yield, percentage content and quantity of thymol (Abd Elbar et al., 2019; Khalil et al., 2018).

The use of wastewater in oregano (Virga et al., 2020; Bernstein et al., 2009) and rosemary (Bernstein et al., 2009) did not cause differences in the quantity and quality of the extracted oils, compared to plants irrigated with fresh water.

Irrigation with waters characterized by a modest content of salts causes an increase in the percentage of oil and carvacrol in oregano plants (Hancioglu et al., 2019); in sage plants, the same treatment causes an increase in the values of camphor, 1.8 cineole, α-thujone and β-thujone (Kulak et al., 2020).

Water stresses greatly influence the growth and productivity of medicinal plants; careful choice of irrigation doses and intervals can cause a significant increase in biomass yield and essential oil production (Virga et al., 2020).

Influence of treatments and fertilization

Nutrition plays a key role in the growth and development of all cultivated plants; in the case of officinal and medicinal herbs that synthesize essential oils, the availability of micro and macro-elements, as essential to produce both primary and secondary metabolites, can influence and effectively increase the yield and quality of essential oils (NurzyĚska-Wierdak, 2013).

Increases in quantitative and qualitative parameters were found by organic fertilization in oregano (Matłok et al. 2020, Bolechowski et al. 2011), sage (El-Haddad et al., 2020), rosemary (Singh and Guleria, 2013) and thyme (Askary et al., 2018; Noroozisharaf and Kaviani, 2018).

Inorganic fertilizations have caused an increase in the oil yield and the percentage of carvacrol in thyme (Abbaszadeh and Layegh Haghighi 2013), an increase in the values of the main constituents of the essential oil in sage (Rioba et al., 2015), an increase in percentages of α-pinene and 1.8 cineole in rosemary essence (Anuradha et al., 2009).

Nitrogen fertilization caused an increase in yield and carvacrol content in oregano plants (Król et al., 2020; Sotiropoulou & Karamanos, 2010; Azizi et al., 2009) and an increase in yield in thyme plants (Baranauskiene et al. 2003), but a decrease in the percentage of essence content in both cases.

Treatments based on phosphorus and potassium on thymus caused an increase in all the parameters considered except for the value of thymol which decreased with the treatment carried out exclusively with potassium (Kılıccedil et al., 2012).

Inoculation with AMF (arbuscular mycorrhizal fungi) and with PGPR (plant growth promoting rhizobacteria) has always caused an increase in quantitative and qualitative parameters in oregano (Kutlu et al. 2020), rosemary (Bidgoli et al., 2019) and sage (Govahi et al., 2015; Geneva et al., 2009), also in combination with organic and inorganic fertilizers.

The administration of hormones and stimulants via foliar treatments resulted in an increase in qualitative and quantitative parameters in oregano (Wahab & Alobady, 2019), rosemary (Nia et al., 2016) and sage (Es‐ sbihi et al., 2020; Vosoughi et al., 2018); also for thyme plants, increases in yield and oil content were obtained with various types of stimulants (Mohammadi et al. 2019, Pavela et al. 2018, Khalil et al. 2018, Pirbalouti et al. 2014), for example exclusion of the treatment with jasmonic acid which caused an increase in the percentages of thymol and carvacrol but a decrease in the yield and content of essential oil (Alavi-Samani et al., 2015).

In addition to the influence of water availability and water stress, the effect linked to the administration of different types of organic and inorganic fertilizers, stimulants, probiotic microorganisms, and mixtures of various fertilizers, is certainly one of the aspects concerning agrotechnics. of medicinal plants which has been most investigated by researchers in the last 20 years.

Influence of the harvest time

The choice of harvest time corresponding to a specific phenological phase is certainly one of the factors that most influences the yield, content and composition of essential oils in all medicinal species (Singh and Guleria, 2013).

The differences in the yield in essential oil, determined by the choice of the different moments of collection, are mainly linked to the structures that secrete essential oils, the glandular trichomes, which, in correspondence with the different phenological stages, have variable densities and dimensions, also in relation at temperature and photoperiod (Miguel et al., 2007).

The optimal harvest time to obtain the best quantitative and qualitative parameters of essential oils from oregano plants seems to be full flowering (Morshedloo et al. 2017, Baranauskienė et al. 2013, De Falco et al. 2013; Napoli et al., 2020).

To obtain the highest yield and oil content, the optimal time to harvest rosemary plants seems to correspond with the initial flowering phase (Yosr et al. 2013); other authors have found an increase in yield at the ripening stage of the seeds but at the same time also a decrease in the percentage content of essential oil (Alipour and Saharkhiz, 2016; Singh and Guleria, 2013).
In the research carried out on sage plants, the authors obtained different results; some authors have obtained the maximum oil content and the highest yield by harvesting at full bloom (Farhat et al. 2016), Nguyen et al. (2019) with harvest taken at the beginning of flowering, and Verma et al. (2015) by harvesting at the last stage of flowering.
Disagreeing results are also found for the thymus. Safaii et al. (2014) found an increase in the content and yield of essential oil by carrying out the harvest in the last phase of flowering and in correspondence with the ripening of the seeds; some authors (Abu-Darwish et al., 2011; Jordàn et al., 2006; Badi et al., 2004), harvesting in correspondence with the vegetative phase and at the beginning of flowering, found only an increase in oil yield.

Conclusions

The quantitative and qualitative parameters of essential oils can be significantly influenced both by exogenous factors such as climate, soil type, altitude, latitude, agronomic technique, post-harvest and extraction treatments, but also by endogenous factors, such as the age of the plant, stage of development, genetic characters.
The synthesis of essential oils can also be stimulated by non-optimal conditions or momentary stress for the plant; to increase the accumulation of secondary metabolites, the so-called crop manipulation or deliberate elicitation is increasingly recognized, a management strategy that consists in provoking, during the crop cycle, temporary conditions of stress for the plant organism.
From the analysis of the literature of the last 20 years, it is noted that the information available for the cultivation techniques analyzed are often conflicting and that it is therefore necessary to develop further research for a better understanding of the mechanisms that regulate the production and quality of essential oils and their interaction with genetic and environmental factors.

References

Abbaszadeh, B., & Layegh Haghighi, M. (2013). Effect of Nutrition and Harvest Time on Growth and Essential Oil Content of *Thymus vulgaris* L. Journal of Medicinal Plants and By-Products, 2(2), 143-151.

Abd Elbar, O. H., Farag, R. E., & Shehata, S. A. (2019). Effect of putrescine application on some growth, biochemical and anatomical characteristics of *Thymus vulgaris* L. under drought stress. Annals of Agricultural Sciences, 64(2), 129-137.

Abu-Darwish, M. S., Al-Fraihat, A. H., Al-Dalain, S. Y., Afifi, F. M. U., & Al-Tabbal, J. A. (2011). Determination of Essential Oils and Heavy Metals Accumulation in *Salvia officinalis* Cultivated in three intra-raw Spacing in Ash-Shoubak, Jordan. International Journal of Agriculture & Biology, 13(6).

Abu-Darwish, M. S., Alu'datt, M. H., Al-Tawaha, A. R., Ereifej, K., Almajwal, A., Odat, N., & Al Khateeb, W. (2012). Seasonal variation in essential oil yield and composition from

Thymus vulgaris L. during different growth stages in the south of Jordan. Natural product research, 26(14), 1310-1317.

Alavi-Samani, S. M., Kachouei, M. A., & Pirbalouti, A. G. (2015). Growth, yield, chemical composition, and antioxidant activity of essential oils from two thyme species under foliar application of jasmonic acid and water deficit conditions. Horticulture, Environment, and Biotechnology, 56(4), 411-420.

Alipour, M., & Saharkhiz, M. J. (2016). Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages. Biocatalysis and agricultural biotechnology, 7, 271-278.

Anuradha, M. N., Srinivasappa, K. N., Raju, B., Farooqi, A. A., & Vasundhara, M. (2009). Influence of seasons and nutrients on the essential oil content and composition of rosemary (*Rosmarinus officinalis*) oil. Biomed, 4(3), 220-227.

Arpanahi, A. A., & Feizian, M. (2019). Arbuscular mycorrhizae alleviate mild to moderate water stress and improve essential oil yield in thyme. Rhizosphere, 9, 93-96.

Askary, M., Behdani, M. A., Parsa, S., Mahmoodi, S., & Jamialahmadi, M. (2018). Water stress and manure application affect the quantity and quality of essential oil of *Thymus daenensis* and *Thymus vulgaris*. Industrial Crops and Products, 111, 336-344.

Azizi, A., Yan, F., & Honermeier, B. (2009). Herbage yield, essential oil content and composition of three oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. Industrial crops and products, 29(2-3), 554-561.

Badi, H. N., Yazdani, D., Ali, S. M., & Nazari, F. (2004). Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. Industrial crops and products, 19(3), 231-236.

Baranauskienė, R., Venskutonis, P. R., Dambrauskienė, E., & Viškelis, P. (2013). Harvesting time influences the yield and oil composition of *Origanum vulgare* L. ssp. *vulgare* and ssp. *hirtum*. Industrial Crops and Products, 49, 43-51.

Bernstein, N., Chaimovitch, D., & Dudai, N. (2009). Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity, and phenolic compounds in oregano and rosemary. Agronomy Journal, 101(1), 1-10.

Bidgoli, R. D., Azarnezhad, N., Akhbari, M., & Ghorbani, M. (2019). Salinity stress and PGPR effects on essential oil changes in Rosmarinus officinalis L. Agriculture & Food Security, 8(1), 1-7.

Bolechowski, A., Moral, R., Bustamante, M. A., Paredes, C., Agulló, E., Bartual, J., & Carbonell-Barrachina, Á. A. (2011). Composition of oregano essential oil (*Origanum vulgare*) as affected by the use of winery-distillery composts. Journal of Essential Oil Research, 23(3), 32-38.

De Falco, E., Mancini, E., Roscigno, G., Mignola, E., Taglialatela-Scafati, O., & Senatore, F. (2013). Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. vulgare L. under different growth conditions. Molecules, 18(12), 14948-14960.

El-Haddad, M. E., Zayed, M. S., El-Sayed, G. A. M., & Abd EL-Satar, A. M. (2020). Efficiency of compost and vermicompost in supporting the growth and chemical constituents of *Salvia officinalis* L. Cultivated in Sand Soil. International journal of recycling organic waste in agriculture, 9(1), 49-59.

Es-sbihi, F. Z., Hazzoumi, Z., Benhima, R., & Amrani Joutei, K. (2020). Effects of salicylic acid on growth, mineral nutrition, glandular hairs distribution and essential oil composition in Salvia officinalis L. grown under copper stress. Environmental Sustainability, 3, 199-208.

Farhat, M. B., Jordán, M. J., Chaouch-Hamada, R., Landoulsi, A., & Sotomayor, J. A. (2016). Phenophase effects on sage (*Salvia officinalis* L.) yield and composition of essential oil. Journal of Applied Research on Medicinal and Aromatic Plants, 3(3), 87-93.

García-Caparrós, P., Romero, M. J., Llanderal, A., Cermeño, P., Lao, M. T., & Segura, M. L. (2019). Effects of drought stress on biomass, essential oil content, nutritional parameters, and costs of production in six *Lamiaceae* species. Water, 11(3), 573.

Geneva, M. P., Stancheva, I. V., Boychinova, M. M., Mincheva, N. H., & Yonova, P. A. (2010). Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition. Journal of the Science of Food and Agriculture, 90(4), 696-702.

Govahi, M., Ghalavand, A., Nadjafi, F., & Sorooshzadeh, A. (2015). Comparing different soil fertility systems in Sage (*Salvia officinalis*) under water deficiency. Industrial Crops and Products, 74, 20-27.

Hancioglu, N. E., Kurunc, A., Tontul, I., & Topuz, A. (2019). Irrigation water salinity effects on oregano (*Origanum onites* L.) water use, yield and quality parameters. Scientia Horticulturae, 247, 327-334.

Jordàn, M. J., Martínez, R. M., Goodner, K. L., Baldwin, E. A., & Sotomayor, J. A. (2006). Seasonal variation of Thymus hyemalis Lange and Spanish Thymus vulgaris L. essential oils composition. Industrial crops and products, 24(3), 253-263.

Khalil, N., Fekry, M., Bishr, M., El-Zalabani, S., & Salama, O. (2018). Foliar spraying of salicylic acid induced accumulation of phenolics, increased radical scavenging activity and modified the composition of the essential oil of water stressed Thymus vulgaris L. Plant Physiology and Biochemistry, 123, 65-74.

Khazaie, H. R., Nadjafi, F., & Bannayan, M. (2008). Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). industrial crops and products, 27(3), 315-321.

Kılıccedil, C. C., Anaccedil, D., & Eryuuml, N. (2012). Effect of potassium and phosphorus fertilization on green herb yield and some quality traits of Thymus vulgaris L. African Journal of Agricultural Research, 7(48), 6427-6431.

Król, B., Sęczyk, Ł., Kołodziej, B., & Paszko, T. (2020). Biomass production, active substance content, and bioaccessibility of Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) following the application of nitrogen. Industrial Crops and Products, 148, 112271.

Kulak, M., Gul, F., & Sekeroglu, N. (2020). Changes in growth parameter and essential oil composition of sage (*Salvia officinalis* L.) leaves in response to various salt stresses. Industrial Crops and Products, 145, 112078.

Kutlu, M., Cakmakci, R. A. M. A. Z. A. N., Hosseinpour, A., & Karagöz, H. (2019). The use of plant growth promoting rhizobacteria (PGPR)'s effect on essential oil rate, essential oil content, some morphological parameters and nutrient uptake of Turkish oregano. Applied Ecology and Environmental Research, 17(2), 1641-1653.

La Bella, S., Virga, G., Iacuzzi, N., Licata, M., Sabatino, L., Consentino, B. B., Tuttolomondo, T. (2020). Effects of Irrigation, Peat-Alternative Substrate and Plant Habitus on the Morphological and Production Characteristics of Sicilian Rosemary (*Rosmarinus officinalis* L.) Biotypes Grown in Pot. Agriculture, 11(1), 1-1.

Leto, C., La Bella, S., Tuttolomondo, T., Licata, M., Carrara, M., Febo, P., Orlando, S. (2004). Effects of plant density on *Rosmarinus officinalis* L. and first tests of mechanical transplanting.

Lubbe, A., & Verpoorte, R. (2011). Cultivation of medicinal and aromatic plants for specialty industrial materials. Industrial crops and products, 34(1), 785-801.

Lucera, A., Costa, C., Conte, A., Del Nobile, M.A., 2012. Food applications of natural antimicrobial compounds. Front. Microbiol. 3, article 287.

Marzi V., De Mastro G., (2008). Piante officinali. Coltivazione, trattamenti post-raccolta, contenuti di principi attivi, impieghi in vari settori industriali ed erboristici. Mario Adda Editore.

Matłok, N., Stępień, A. E., Gorzelany, J., Wojnarowska-Nowak, R., Balawejder, M. (2020). Effects of Organic and Mineral Fertilization on Yield and Selected Quality Parameters for Dried Herbs of Two Varieties of Oregano (*Origanum vulgare* L.). Applied Sciences, 10(16), 5503.

Miguel, M. G., Guerrero, C., Rodrigues, H., Brito, J., Duarte, F., Venâncio, F., & Tavares, R. (2007, July). Essential oils of *Rosmarinus officinalis* L., effect of harvesting dates, growing media and fertilizers. In Int. Conf. on Energy, Environment, Ecosystem and Sustainable Development, Agios Nikolaos, Greece (pp. 24-26).

Mohammadi, H., Akhondzadeh, M., Ghorbanpour, M., & Aghaee, A. (2020). Physiological responses and secondary metabolite ingredients in sage plants induced by 24-epibrassinolide foliar application under different water deficit regimes. Scientia Horticulturae, 263, 109139.

Mohammadi, H., Amirikia, F., Ghorbanpour, M., Fatehi, F., & Hashempour, H. (2019). Salicylic acid induced changes in physiological traits and essential oil constituents in different ecotypes of *Thymus kotschyanus* and *Thymus vulgaris* under well-watered and water stress conditions. Industrial Crops and Products, 129, 561-574.

Mohasseli, V., & Sadeghi, S. (2019). Exogenously applied sodium nitroprusside improves physiological attributes and essential oil yield of two drought susceptible and resistant specie of *Thymus* under reduced irrigation. Industrial Crops and Products, 130, 130-136.

Morshedloo, M. R., Mumivand, H., Craker, L. E., & Maggi, F. (2018). Chemical composition and antioxidant activity of essential oils in Origanum vulgare subsp. gracile at different phenological stages and plant parts. Journal of Food Processing and Preservation, 42(2), e13516.

Napoli, E., Giovino, A., Carrubba, A., How Yuen Siong, V., Rinoldo, C., Nina, O., & Ruberto, G. (2020). Variations of essential oil constituents in Oregano (*Origanum vulgare* subsp. *viridulum* (= *O. heracleoticum*) over cultivation cycles. Plants, 9(9), 1174.

Nguyen, H. T., Radácsi, P., Rajhárt, P., & Németh, É. (2019). Variability of thujone content in essential oil due to plant development and organs from *Artemisia absinthium* L. and *Salvia officinalis* L. J Appl Bot Food Qual, 92, 100-105.

Nia, A. F., Badi, H. N., Mehrafarin, A., Bahman, S., & Sahandi, M. S. (2016). Changes in the essential oil content and terpene composition of rosemary (*Rosmarinus officinalis* L.) by using plant biostimulants. Acta agriculturae Slovenica, 107(1), 147-157.

Noroozisharaf, A., & Kaviani, M. (2018). Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (*Thymus vulgaris* L.) under greenhouse conditions. Physiology and Molecular Biology of Plants, 24(3), 423-431.

Nurzyńska-Wierdak, R. (2013). Does mineral fertilization modify essential oil content and chemical composition in medicinal plants. Acta Sci. Pol., Hortorum Cultus, 12(5), 3-16.

Pavela, R., Žabka, M., Vrchotová, N., & Tříska, J. (2018). Effect of foliar nutrition on the essential oil yield of Thyme (*Thymus vulgaris* L.). Industrial crops and products, 112, 762-765.

Piccolella, S., Crescente, G., Pacifico, F. et al. Wild aromatic plants bioactivity: a function of their (poly)phenol seasonality? A case study from Mediterranean area. Phytochem Rev 17, 785–799 (2018)

Pichersky, E., Noel, J.P., Dudareva, N., 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Sci.,311, 808–811.

Pirbalouti, A. G., Samani, M. R., Hashemi, M., & Zeinali, H. (2014). Salicylic acid affects growth, essential oil and chemical compositions of thyme (*Thymus daenensis* Celak) under reduced irrigation. Plant growth regulation, 72(3), 289-301.

Pirzad, A., & Mohammadzadeh, S. (2018). Water use efficiency of three mycorrhizal *Lamiaceae* species (*Lavandula officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris*). Agricultural Water Management, 204, 1-10.

Raffo, A., Mozzanini, E., Nicoli, S. F., Lupotto, E., & Cervelli, C. (2020). Effect of light intensity and water availability on plant growth, essential oil production and composition in *Rosmarinus officinalis* L. European Food Research and Technology, 246(1), 167-177.

Rioba, N. B., Itulya, F. M., Saidi, M., Dudai, N., & Bernstein, N. (2015). Effects of nitrogen, phosphorus and irrigation frequency on essential oil content and composition of sage (*Salvia officinalis* L.). Journal of Applied Research on Medicinal and Aromatic Plants, 2(1), 21-29.

Safaii, L., Ashoorabadi, E. S., Emami, S. D., & Afiuni, D. (2014). The effect of harvesting stages on quantitative and qualitative characters of essential oil and phenolic yield composition in two thyme species (*Thymus daenensis* Celak & *T. vulgaris* L.) in Iran. International Journal of Agriculture and Crop Sciences, 7(13), 1346.

Singh, M., & Guleria, N. (2013). Influence of harvesting stage and inorganic and organic fertilizers on yield and oil composition of rosemary (*Rosmarinus officinalis* L.) in a semi-arid tropical climate. Industrial Crops and Products, 42, 37-40.

Sotiropoulou, D. E., & Karamanos, A. J. (2010). Field studies of nitrogen application on growth and yield of Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart). Industrial Crops and Products, 32(3), 450-457.

Trivellini, A., Lucchesini, M., Maggini, R., Mosadegh, H., Villamarin, T.S.S., Vernieri, P., Mensuali-Sodi, A., Pardossi, A., *Lamiaceae* phenols as multifaceted compounds: bioactivity, industrial prospects and role of "positive-stress". Industrial Crops and Products, Volume 83, 2016, Pages 241-254

Tuttolomondo, T., La Bella, S., Leto, C., Bonsangue, G., Leone, R., Gennaro, M. C., ... & Licata, M. (2016). Effects of Plant Density on the Number of Glandular Trichomes and on Yield and Quality of Essential Oils from Oregano. Natural product communications, 11(6).

Verma, R. S., Padalia, R. C., & Chauhan, A. (2015). Harvesting season and plant part dependent variations in the essential oil composition of *Salvia officinalis* L. grown in northern India. Journal of Herbal Medicine, 5(3), 165-171.

Virga, G., Sabatino, L., Licata, M., Tuttolomondo, T., Leto, C., & La Bella, S. (2020). Effects of Irrigation with Different Sources of Water on Growth, Yield and Essential Oil Compounds in Oregano. Plants, 9(11), 1618.

Vosoughi, N., Gomarian, M., Pirbalouti, A. G., Khaghani, S., & Malekpoor, F. (2018). Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. Industrial Crops and Products, 117, 366-374.

Wahab, Z. A. A. Z. A., & Alobady, A. F. (2019). Influence of ground and foliar fertilization on vegetative proprieties and volatile oil for *Origanum vulgare* L. Kufa Journal for Agricultural Sciences, 11(1).

Yosr, Z., Hnia, C., Rim, T., & Mohamed, B. (2013). Changes in essential oil composition and phenolic fraction in *Rosmarinus officinalis* L. var. *typicus* Batt. organs during growth and incidence on the antioxidant activity. Industrial Crops and Products, 43, 412-419.

MORPHOLOGICAL AND PRODUCTIVE CHARACTERIZATION OF SEVEN ACCESSIONS OF *CAPPARIS SPINOSA* L. SUBSP. *RUPESTRIS* IN LINOSA ISLAND IN ITALY

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Abstract

The caper is widespread in the Mediterranean basin both spontaneously and in specialized cultivation, showing considerable genetic and phenotypic variations. Although this plant represents a good opportunity for growers in subtropical areas, it still needs innovation in agrotechnical techniques. The aim of the study was to evaluate the morphological and productive behavior of seven accessions of *C. spinosa* L. subsp. *rupestris*, identified on the island of Linosa, in the two-year period 2007-2008. The experimental protocol involved a randomized block design. Not only the main morphological and production parameters were determined but also the phenological phases. In order to broaden the qualitative parameters of the flower buds, in addition to the evaluation of the diameter, the consistency was evaluated using a penetrometer. The results obtained showed variability between accessions and higher levels of production in the second year. Although for most of the parameters examined the best results were provided by the SCP2 access, it is also worth noting that the results for the SCP1 and SCP5 biotypes were also satisfactory. Our results have allowed us to identify accessions of interest for the introduction of new caper fields. Further research is needed to characterize caper biotypes in terms of the chemical composition of flower buds and fruit.

Keywords: *caper plant, island of Linosa, morphological and productive characteristics, growing.*

Introduction

The caper plant belongs to the Capparaceae family, which includes about 40-60 tropical, subtropical and temperate genera, of which 700-900 species belonging to arboreal, shrubby and herbaceous plants (Hall *et al.,* 2002). The genus *Capparis* L. includes about 250 species distributed in tropical and subtropical regions (Fici, 2001).
In Sicily (Italy) and the surrounding islands, *C. spinosa*, with the two intraspecific taxa, subsp. *spinosa* and subsp. *rupestris*, is widespread both spontaneously in natural habitats and as a specialized crop (Gristina *et al.*, 2014).
Caper sprouts, collected from wild and cultivated plants, are mainly used for food and medicinal purposes (Rivera *et al.,* 2005). The immature flower buds, called "capers", the fruits, called "cucunci" or "caperone" and the tender leaves, preserved in salt or vinegar, are widespread in the kitchen, enjoying good levels of world trade (Saadaoui *et al.,* 2014; Chedraoui *et al.,* 2017; Legua *et al.,* 2013; Romeo *et al.,* 2007). Furthermore, different pharmacological properties are attributed to the extracts of leaves, stems, flowers, fruits and roots (Ali *et al.,* 2007; Gadgoli and

Mishra, 1999; Kazemian *et al.,* 2015; Mollica *et al.,* 2017; Trombetta *et al.,* 2005; Zhou *et al.,* 2010).

Among the most abundant phenolic compounds, in fresh bud, rutin is included, while quercetin, now attentive to the possible interference against the replication of the SARS-COV-2 virus, is the most abundant phenolic compound in fermented bud (Francesca *et al.,* 2016; Haslberger *et al.,* 2020).

In addition to its food and medicinal uses, the aesthetic properties of *Capparis spinosa* also make it popular as an ornamental plant for gardens, walls and terraces (Faran, 2014). Furthermore, due to its xerophilic nature, very extensive root system, extremely high root / stem ratio and moderate water consumption (Zuo *et al.,* 2012; Gan *et al.,* 2013), the caper is very suitable as a crop in regions with harsh climatic conditions, such as those of the Mediterranean area.

However, while it contributes to a thriving market, innovation in caper cultivation is low. The specialization of caper cultivation is limited by the absence of improved cultivars and by the lack of studies on the characterization and enhancement of the Sicilian caper germplasm. In Italy this species is of particular interest in the smaller islands of Sicily, in particular in Pantelleria and Salina and recently also in Linosa (La Bella *et al.,* 2021).

The aim of the study was to evaluate the agronomic and productive behavior of seven biotypes of *Capparis spinosa* L. subsp. *rupestris*, identified on the island of Linosa (Italy), in a two-year trial period and to identify the most promising biotypes for cultivation.

Material and Methods

The study was carried out in 2007-2008, on the island of Linosa, Sicily, Italy, (35 ° 51′43″ N 12 ° 52′37″ E: Google Earth) at a local farm, 32 m above sea level. The test site is located on undulating terrain facing north-west. The soil is typical xerorthents; volcanic, shallow, loose and with little organic matter (Fierotti, 1997). Before sowing, the biotypes identified in a previous study (Tuttolomondo *et al.,* 2009), classified as *Capparis spinosa* L. subsp. *rupestris* and marked with the abbreviation SCP1-7. The experimental plot envisaged a randomized block design with three replicates using the plants of the 7 biotypes being evaluated, with a planting spacing of 2.50 × 2.50 m (Figure 1).

In the two-year period (2007-2008), weekly measurements of the main phenological stages were carried out: start of plant growth, flower bud formation, flowering, fruit formation and plant dormancy. Each phenological phase was identified when each parcel presented 70-80% of the plants in the phase considered. The following parameters were also determined for each caper biotype: fresh weight of the flower bud (FW); dry weight of the bud (DW); weight of 100 flower buds; percentage of dry matter of the flower buds; flower bud diameter; texture of the bud; average length of primary branches; number of nodes for cm on the primary branch; number of secondary branches on the primary branch; number of flower buds for primary branch; number of flower buds for secondary branch. The data of all parameters showed a normal distribution. A penetrometric test (FT02, 0–1 kg) with a 2 mm ferrule was used to determine the consistency of the gems; the values are expressed in grams.

All biometric data and production parameters were subjected to variance analysis. The difference between the means was made using the Tukey test. Data analysis was performed using Minitab 19 software for Windows.

Figure 1. A view of the experimental field.

Results and Discussion

The increased demand for caper buds and fruit has prompted farmers to switch from harvesting wild plants to specialized caper plant crops (Tuttolomondo *et al.*, 2009). Of fundamental importance for the creation of new caper plants is undoubtedly the genetic material used for propagation purposes (Gianguzzi *et al.*, 2019; Germanà *et al.*, 2020). Therefore, the identification of biotypes in nature and characterized by high agronomic performances, which can be recommended to breeders or included in genetic improvement programs, is considered an excellent strategy (Tuttolomondo *et al.*, 2020; Perrino *et al.*, 2020; Landucci *et al.*, 2014).
The trend of precipitation and air temperature during the test period is shown in the Figure 2.

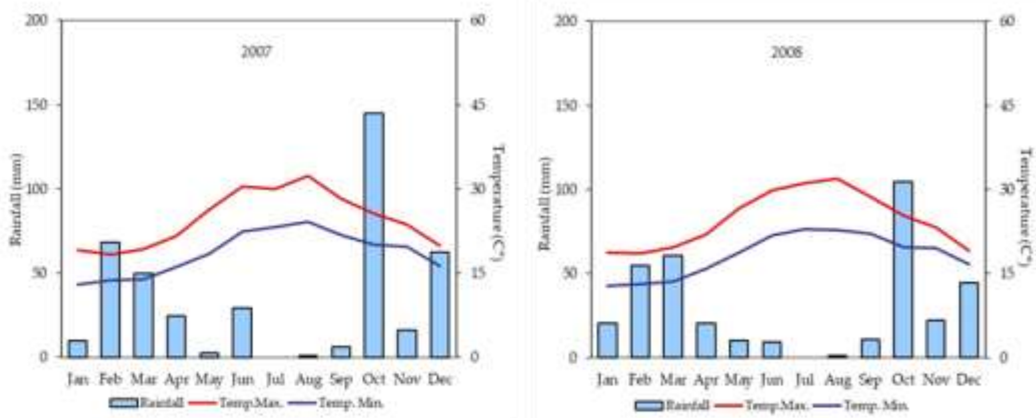

Figure 2. Rainfall and air temperature trends during the test period in the experimental area. The graph on the left refers to the year 2007, the graph on the right to the year 2008.

The levels of precipitation during the 2 years of the experimental test were not always typical of the environment under examination. In 2007, when phenological and production measurements began, rainfall was approximately 100 mm higher than that typical of the test environment (414.40 mm). In 2008, rainfall was consistent with the test environment (359.00 mm). Rainy events have always been concentrated mostly in January and in the months between September and December.
Previous studies (La Bella *et al.*, 2021) have led to the identification of specific characteristics considered of interest for the development of crops; for example, high productivity, long stems, short internodes and high fertility of the nodes, dark green spherical buds with close bracts, non-pubescent and late opening, oval fruits with light green pericarp and few seeds, absence of stipulating thorns, easy separation of the stems to simplify the harvesting and post-harvest operations, suitability for agamic reproduction and resistance to biotic and abiotic stresses.

Table 1. Effects of biotype, year and interaction biotype-by-year on biometric and production parameters. Average values are shown.

Biotypes (B)	Flower bud fresh weight (g)	Flower bud dry weight (g)	Weight 100 flower buds (g)	Flower bud d.m. (%)	Flower bud diameter (mm)	Flower bud consistency (g)	Primary branch average length (cm)	Primary branch nodes/cm^{-1} (n)	Secondary branches /primary branch (n)	Flower buds /primary branch (n)	Flower buds /secondary branches (n)
SCP1	1031.89 ab	187.24 ab	20.63 c	17.79 bc	7.57 c	975.47 a	104.21 d	0.66 b	5.77 c	36.08 b	15.13 f
SCP2	1167.61 a	220.83 a	22.84 b	18.64 ab	8.19 ab	886.67 d	139.19 a	0.70 a	6.37 c	46.58 a	25.51 e
SCP3	1056.70 ab	184.57 ab	22.08 b	17.23 c	7.65 c	855.97 e	127.24 b	0.64 bc	19.07 a	25.58 c	47.01 a
SCP4	1114.43 ab	160.70 b	23.40 b	14.23 d	8.15 ab	842.16 f	117.78 c	0.61 c	18.58 a	24.17 c	43.72 b
SCP5	1099.20 ab	212.70 a	23.04 b	19.11 a	8.49 a	856.59 e	117.92 c	0.65 bc	18.94 a	25.00 c	37.68 d
SCP6	919.16 bc	177.44 ab	28.12 a	19.02 a	7.58 c	922.85 c	99.74 e	0.64 bc	17.37 b	24.46 c	41.41 c
SCP7	745.17 c	147.97 b	27.80 a	19.48 a	7.97 bc	942.10 b	114.77 c	0.66 b	6.62 c	35.94 b	14.16 f
Y	*	*	*	*	*	**	*	**	*	*	**

Means followed by the same letter in the same column are not significantly different according to Tukey's test ($p \leq 0.05$). * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$.

Taking into consideration the biotype factor (Table 1), the highest mean fresh and dry weight values of the bud were found, in SCP2, SCP3, SCP4, SCP5 and SCP1 accessions (FW: 1167.61-1031.89 g; DW: 220, 83–160.70 g), while the lowest averages were recorded in SCP7 (FW: 745.17 g; DW: 147.97 g) which were also distinguished by the maximum weight of 100 flower buds. The dry weight percentage of the gems ranged from 19.84% (SCP7) to 14.23% (SCP4). Among the most sought after features is the diameter. The Official State Gazette distinguishes seven classes of increasing diameter, from the smallest of 7 mm to the largest of 13 mm, highlighting the fact that those most appreciated by consumers are actually less than 7 mm. The diameter of the largest bud (8mm) was recorded in SCP5, SCP2 and SCP4, while that of the smallest bud (7mm) was observed in SCP1, SCP7, SCP6 and SCP3 (Figure 3).

Figure 3. Determination of flower bud diameter.

The consistency of the gems is, without doubt, extremely important in defining the qualitative parameters. In our study, the highest bud consistency (957.47g) was determined in SCP1, while the lowest (842.16g) in SCP4 (Figure 4).

Figure 4. Determination of flower bud consistency using a penetrometer.

Regarding the biometric parameters of the caper accessions in the study, the maximum mean length of the primary branch, the greatest number of nodes / cm of primary branch and the greatest number of flower buds / primary branch were observed in SCP2. According to previous studies (Sozzi e Vicente, 2006; Aytaç *et al.,* 2009) which had found that a longer primary branch determined a greater number of nodes, allowing greater differentiation of flower buds and, therefore, greater productivity. SCP3, SCP5 and SCP4 had the highest number of secondary branches / ratio of primary branches while SCP7, along with the accessions of SCP1 and SCP5, showed the lowest. The highest number of flower buds / secondary branch ratio (47,10) was recorded in SCP3 while the lowest in SCP1 (15,13) and SCP7 (14,16) for which no significant differences were found.

The main results for the productive characteristics of the caper biotypes in two years of study showed that SCP2 and SCP5 achieved the best performance while SCP7 was the least productive biotypes.

The phenological analysis allowed to differentiate the biotypes based on the duration of the single phenological stage (Figure 5). For the SCP2 biotype (Figure 6), shorter plant dormancy and longer plant growth were found and, therefore, longer bud release and fruiting stage (which presumably contributed to the increase in yield). An early production phase inevitably led to a better use of the soil's water resources, accumulated during the autumn-winter period. Plant growth stage (297.02 days), emergence of flower buds (233.02 days), and fruiting (181.51 days) were also longer in adherence to SCP2.

The plant growth phase was shorter in SCP7 adhesion (258.01 days); this accession also recorded the shortest fruiting phase (165.52 days). However, the shortest bud emergence stage was the shortest for SCP6 (208.02). The phenological trend of the various accessions constitutes a further characterization factor of the accessions and is of great interest for the species and for the development of cultivation techniques.

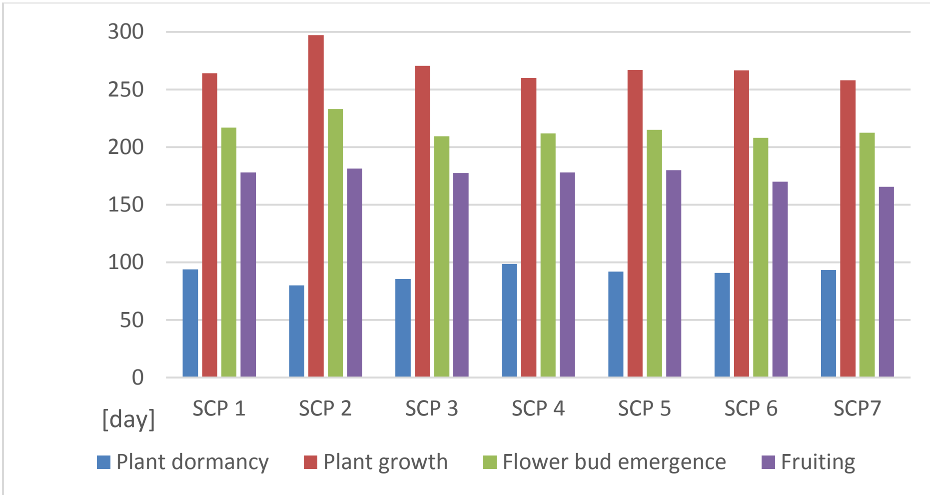

Figure 5. Average length of phenological stages based on accessions of *C. spinosa* subsp. *rupestris*.

Furthermore, the studies conducted by Melgarejo *et al.*, (2009) show that the phenological behavior is fundamental for the improvement of the cultivation techniques of this species as several edible parts are included in the term 'yield', (flower buds, young shoots and fruits) which cover the entire growth cycle annual.

Figure 6. Left: Growth stage of caper plants; Right: Flowering stage of caper biotypes.

Conclusions

The agronomic characteristics linked to resistance to drought and tolerance to high temperatures together with the use of biotypes with good production results, make this species a good candidate for use in marginal land from an environmental point of view. These soils are increasingly fragile due to climate change, which has caused not only a reduction in rainfall levels, but also anomalous intensity and uneven distribution.

The results of this study contribute to deepen the knowledge on the germplasm of the caper found on the island of Linosa. The biotypes that were analyzed showed good adaptability of the test environment and good yield results. Although the best results in terms of flower buds, primary branch length, number of nodes / primary branch and earliness were obtained with the SCP2 biotype, it is also worth noting that the results for the SCP1 and SCP5 biotypes were also satisfactory. Regarding quality parameters, such as average bud diameter and consistency, the best results for both years were obtained with SCP1.

This work can contribute to the ex situ conservation of the species, as the best biotypes can be propagated and cultivated. However, further research is needed to characterize caper accessions in terms of the chemical composition of flower buds, fruits and other parts of the plant with application in the food, cosmetic, pharmaceutical and medicinal sectors.

References

Ali Z.N., Eddouks M., Michel J.B., Sulpice T., Hajji L. (2007). Cardiovascular effect of capparis spinosa aqueous extract. Part III: Antihypertensive effect in spontaneously hypertensive rats. *Am. J. Pharmacol. Toxicol. 2*, 111–115.

Aytaç Z., Kinaci G., Caylan A. (2009). Yield and some morphological characteristics of Caper (*Capparis spinosa* L.) population cultivated at various slopes in Aegean ecological conditions. *Pak. J. Bot. 41*, 591–596.

Chedraoui S., Abi-Rizk A., El-Beyrouthy M., Chalak L., Ouaini N., Rajjou L. (2017). *Capparis spinosa* L. in a systematic review: A xerophilous species of multi values and promising potentialities for agrosystems under the threat of global warming. *Front. Plant Sci.*, *8*, 1845.

Faran M. (2014). Capparis spinosa—The plant on the wall. In *Medicinal and Aromatic Plants of the Middle-East (Medicinal and Aromatic Plants of the World)*; Yaniv, Z., Dudai, N., Eds.; Springer: Dordrecht, The Netherlands, pp. 59–65.

Fici S. (2001). Intraspecific variation and evolutionary trends in *Capparis spinosa* L. (*Capparaceae*). *Plant Syst. Evol. 228*, 123–141.

Fierotti G. (1997). *I Suoli della Sicilia: Con Elementi di Genesi, Classificazione, Cartografia e Valutazione dei Suoli*, 1st ed.; Flaccovio Dario: Palermo, Italy.

Francesca N., Barbera M., Martorana A., Saiano F., Gaglio R., Aponte M., Moschetti G., Settanni L. (2016). Optimised method for the analysis of phenolic compounds from caper (*Capparis spinosa* L.) berries and monitoring of their changes during fermen-tation. *Food Chem. 196*, 1172–1179.

Gadgoli C. and Mishra, S.H. (1999). Antihepatotoxic activity of p-methoxy benzoic acid from *Capparis spinosa*. *J. Ethnopharmacol. 66*, 187–192.

Gan L., Zhang C., Yin Y., Lin Z., Huang Y., Xiang J., Fu C., Li M. (2013). Anatomical adaptations of the xerophilous medicinal plant, Capparis spinosa, to drought conditions. *Hortic. Environ. Biotechnol. 54*, 156–161.

Germanà M.A., Lesto F., Lo Bosco F., Iacuzzi N., Ozudogru E.A. (2020). Encapsulation of in vitro-derived propagules of two genotypes of *Capparis spinosa* (L.) from Pantelleria Island. *Acta Hortic. 1285*, 39-44.

Gianguzzi V., Inglese P., Barone E., Sottile F. (2019). "In Vitro Regeneration of *Capparis spinosa* L. by Using a Temporary Immersion System" *Plants* 8, no. 6: 177.

Gristina A.S., Fici, S., Siragusa M., Fontana I., Garfì G., Carimi, F. (2014). Hybridization in *Capparis spinosa* L.: Molecular and morphological evidence from a Mediterranean island complex. *Flora Morphol. Distrib. Funct. Ecol. Plants*, *209*, 733–741.

Hall J.C., Sytsma K.J., Iltis H.H. (2002). Phylogeny of *Capparaceae* and *Brassicaceae* based on chloroplast sequence data. *Am. J. Bot. 89*, 1826–1842.

Haslberger A.G., Jacob U., Hippe B., Karlic H. (2020). Mechanisms of selected functional foods against viral infections with a view on COVID-19: Mini review. *Funct. Food Health Dis. 5*, 195–209.

Kazemian M., Abad M., Haeri M.R., Ebrahimi M., Heidari R. (2015). Anti-diabetic effect of *Capparis spinosa* L. root extract in diabetic rats. *Avicenna J. Phytomed. 5*, 325–332.

La Bella S., Rossini F., Licata M., Virga G., Ruggeri R., Iacuzzi N., Leto C., Tuttolomondo T. (2021). Four-Year Study on the Bio-Agronomic Response of Biotypes of *Capparis spinosa* L. on the Island of Linosa (Italy). *Agriculture*. 11(4):327.

Landucci F., Panella L., Lucarini D., Gigante D., Donnini D., Kell S., Maxted N., Venanzoni R. Negri V. (2014). A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop. Sci. 54*, 1628–1644.

Legua P., Martínez J., Melgarejo P., Hernández F. (2013). Phenological growth stages of caper plant (*Capparis spinosa* L.) according to the Biologische Bundesanstalt, Bundessortenamt and Chemical scale. *Ann. Appl. Biol. 163*, 135–141.

Melgarejo P., Legua P., Martinez J., Martinez-Font R., Hernandez F. (2009). Preliminary characterization of sixty one caper clones (*Capparis spinosa* L.). *Acta Hortic. 818*, 155–160.

Mollica A., Zengin G., Locatelli M., Stefanucci A., Mocan A., Macedonio G., Carradori S., Onaolapo O., Onaolapo A., Adegoke J., et al. (2017). Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: In vivo and in vitro evaluation of its nutraceutical potential. *J. Funct. Foods*. *35*, 32–42.

Perrino E.V. and Perrino P. (2020). Crop wild relatives: Know how past and present to improve future research, conservation and utilization strategies, especially in Italy: A review. *Genet. Resour. Crop. Evol. 67*, 1067–1105.

Rivera D., Inocencio C., Obón C., Alcaraz F. (2003). Review of food and medicinal uses of *Capparis* L. subgenus *Capparis* (*Capparidaceae*). *Econ. Bot. 57*, 515–534.

Romeo V., Ziino M., Giuffrida D., Condurso C., Verzera A. (2007). Flavor profile of Capers (*Capparis spinosa* L.) from the Eolian Archipelago by HS-SPME/GC-MS. *Food Chem. 101*, 1272–1278.

Saadaoui E., Khaldi A., Khouja M.L., Mohamed E.G. (2009). Intraspecific variation of *Capparis spinosa* L. in Tunisia. *J. Herbs Spices Med. Plants*. *15*, 9–15.

Sozzi O.G., Vicente A.R. (2006). Capers and caperberries. In *Handbook of Herbs and Spices*; Peter, K.V., Ed.; Woodhead Publishing Limited and CRC Press: Boca Raton, FL, USA, pp. 230–256.

Trombetta D., Occhiuto F., Perri D., Puglia C., Santagati N.A., Pasquale A.D., Saija A., Bonina F. (2005). Antiallergic and anti-histaminic effect of two extracts of *Capparis spinosa* L. flowering buds. *Phytother. Res. 19*, 29–33.

Tuttolomondo T., Iapichino G., Licata M., Virga G., Leto C., La Bella S. (2020). Agronomic evaluation and chemical characterization of Sicilian *Salvia sclarea* L. accessions. *Agronomy*, *10*, 1114.

Tuttolomondo T., La Bella S., Cammalleri I., Gaglio G. (2009). Indagine preliminare sul germoplasma di *Capparis spinosa* subsp. *rupestris* (Sm.) Nyman dell'isola di Linosa. *Ital. J. Agron. 4*, 513–517.

Tuttolomondo T., La Bella S., Hornsby L. (2009). Il cappero: Analisi desk sui flussi di commercializzazione in Italia e nei principali Paesi competitors. *Ital. J. Agron. 4*, 789–796.

Zhou H., Jian, R., Kang J., Huang X., Li Y., Zhuang C., Yang F., Zhang L., Fan X., Wu T., et al. (2010). Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J. Agric. Food Chem. 58*, 12717–12721.

Zuo W., Ma M., Ma Z., Gao R., Guo Y., Jiang W., Liu J., Tian L. (2012). Study of photosynthetic physiological characteristics of desert plant *Capparis spinosa* L. *J. Shihezi Univ. 3*, 006.

THE EFFECTIVENESS OF PHYSIOLOGICAL METHODS FOR OPTIMIZING WORK ON THE ARRANGEMENT AND RESTORATION OF OAK FORESTS

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Abstract

In the context of global warming, the need to restore and expand forest areas is becoming an increasingly urgent task. In Moldova, the most suitable afforestation is the oak species *Quercus robur* L., *Quercus petrea* Liebl. and *Quercus pubescens* Willd. To optimize the selection of oak species for afforestation in specific areas, we have determined the resistance of these species to high temperatures. Additional problems appeared due to the slow growth and low viability of seedlings in the first three years after sowing acorns. Having carried out many studies under controlled conditions, we have developed methods that allow us to obtain some plants, which in terms of viability and habitus already in the first year after germination exceeded the plants of the control variant at the age of two to three years. This conclusion is supported by the values of the parameters of annual growth, the number of leaves per seedling, leaf size, and their photosynthetic activity. Another critical factor is that the experimental plants more fully utilize the reserve substances of the cotyledons, which leads to accelerated growth and plant resistance to stress factors. Thanks to these features, the selection of valuable genotypes can already be at the planting stage. Thus the risks of seedlings suppression during the first years after planting and the costs for afforestation are reduced.

Keywords: *oak, physiological methods, afforestation.*

Introduction

The possible consequences of climate change on the state of forests have had a pragmatic impact only after signing the Kyoto Protocol (1997). It explicitly states that one way to mitigate the serum effects is to increase the carbon accumulation in forest ecosystems. Special attention was paid to expanding forested areas with species best adapted to the new environmental conditions (Giurgiu, 2004). The risk of global warming (Hasselman, 1997) has necessitated particular concerns of the Ministerial Conferences for the Protection of Forests in Europe, so the strategies described in the "*Adaptation of Forests and People to Climate Change - a global assessment*" (Seppälä et al., 2009) have been jointly developed. The literature data state that the choice of species for afforestation must be made with great discernment, following their bio-ecological requirements. These species survive in certain environmental conditions with the specific level of excessive temperatures and drought. Scientists' predictions regarding the evolution of forest vegetation in line with alleged global warming foreshadow a significant increase in areas occupied by oak species throughout Europe (Barbu et al., 2016).
Over time, oak species in the temperate zone have adapted to changing environmental factors, especially climatic ones (Reif et al., 2010). In the last century, however, the number of climatic anomalies has increased, such as long periods of drought, precipitation variations and rain

intensity, late spring frosts, etc. (Zhang et al., 2007). As a result, only three spontaneous oak species are widespread in the forests of the Republic of Moldova:

- Pedunculate oak (*Quercus robur* L.)
- Sessile oak (*Quercus petraea* Liebl.)
- White oak (*Quercus pubescens* Willd.)

Low winter temperatures limit the spread of white oak to the north. Because of this, white oak gives way to competition in favor of sessile oak and pedunculate oak (Cuza & Florenta, 2017). Adaptations of different oak species to abiotic stress factors include strategies to avoid (reduce) the influence of stress factors (Cuza, 2017) and functional processes of adaptation (Dascaliuc, Cuza, 2011). Research showed that thermotolerance of the leaves of mentioned oak species differs by the specificity of the mechanisms to avoid the action of the stress factors and the effectiveness of the functional processes. After immersing the leaves in water with different temperatures (intensive factor) and durations (extensive factor) (Levitt, 1980) of heat shock, their ability to maintain electrolyte balance (Dascaliuc & Cuza, 2007) and photosynthetic activity (Dascaliuc & Cuza, 2011), it turned out to be different.

The above mentioned demonstrates the complexity of the problems that must be considered when choosing the suitable oak species for afforestation of different areas. We must also take into account the need to optimize the technologies for installing new oak forests. The low frequency of oak fruiting, acorn damage by parasites and rodents, the low growth rate of young plants are among the significant risk factors for installing oak forests by direct seeding of acorns (Dănescu et al., 2015). In addition to natural regeneration, foresters plant oak seedlings or directly sow acorns. The second procedure is more straightforward, cheaper, and the developed seedlings have deeper pivoting roots, which gives them a high survival rate in the first year and higher resistance to drought over time (Zadworny et al., 2014). Improving cultivating crops in the nursery allows obtaining seedlings of high quality (Villar-Salvador, 2004). When afforestation has used the seedlings of oak species grown in the cultivation room and then transplanted, the cut roots of the seedlings and their prolonged growth determine the slow growth of plantlets, especially in the first years of life (Harris et al. 2008).

The probability of oak seedlings' survival is related to their limited access to soil water during the summer, supplied through solid and deep roots. Among the main risk factors for direct sowing of acorns are rodents, which detect and consume the sown acorns (Bullard et al., 1992). Herbaceous vegetation reduces seedlings' growth due to competition. This situation leads to prolongation of the period in which the seedlings are susceptible to weed overwhelm (Valkonen, 2008). Both factors must be considered when practicing the direct sowing of oak acorns as a viable alternative to that of planting the seedlings.

In this paper, we present the results of initiation of plantation with pedunculate oak by sowing of acorns in autumn (1), sowing in early spring (2), and as well by planting in the field during the spring of seedlings obtained after germination of acorns, cultivation of plantlets in artificially conditions during the autumn and winter period (3). Thanks to the third method of oak plantation initiation, we have eliminated the risk of damage caused to acorns by rodents, substantially reduced the work of caring for plantations, and obtained vigorous and fast-growing seedlings.

Material and Methods

On the territory of the scientific reservation, "Plaiul Fagului" (Ungheni district) and in the Protection Area of the Institute of Genetics, Physiology, and Plant Protection (Chisinau) were established pedunculate oak (Quercus robur) plantations. The acorn was harvested from the trees aged 100-120 years to initiate forest plantations, which grow in the mentioned reservation.
For elaborating the optimal method for initiating oak plantations, three methodical procedures were tested: 1) autumn sowing was performed; 2) during autumn and winter, the acorns were incubated was kept at a temperature of 0-5oC mixed with wood sawdust and sown in spring; 3) immediately after collection the acorns were incubated for germination and plantlets growth in conditions with temperatures and artificial lighting created to expose to moderate doses of heat stress and nutrition, due to which, before planting in the spring, in the obtained seedlings were induced adaptation processes. Sowing of acorns was carried out in plots with a side of 7 x 7 m. In each separate lot, we prepared 64 nests at a depth of 6 - 8 cm and with a width of 30 cm. 5 acorns were evenly sown in each nest. After the first two years of cultivation, the most vigorous brood was selected in each nest, eliminating the others.
During the year, we provided phenological observations and plantation care works. In October of each year, we measured the height of trees with the ruler to the accuracy of ± 1 cm and the diameter of the stem - using the caliper (accuracy ± 0.1 mm). For each sowing period, indices of annual seedling growth were determined. We characterized the morphological parameters and physiological state of plants obtained after direct acorns sowing and those planted with seedlings grown in autumn-winter by the appreciation of total and annual growth in height and diameter of trees at the trunk level, average leaf area (Utkin et al., 2008) and the chlorophyll index using the SPAD apparatus (USA) (Diagnostics, 1988). Based on the experimental results, we calculated the mean and standard deviation of the means (Clewer et al., 2001).

Results and Discussion

Figure 1 shows the curves of the annual growth dynamics at the height of the oak seedlings obtained from acorns sown in autumn and spring. They show that the diversification of the growth rate of seedlings obtained from autumn and spring sowing was manifested only when the age of the plants exceeded three years. Subsequently, the differences between seedling height in the two experimental variants gradually increased until six years of age. Later, the differences between the size of the plants in these two experimental variants remained practically constant. At the age of 11, the average height of trees obtained from acorns sown in autumn and spring reached 560 and 635 cm, respectively. We note that the average diameter of the plant trunk diameters obtained from acorns sown in autumn was also smaller compared to that characteristic for seedlings obtained from acorns sown in spring, being, respectively, equal to 88 and 93 mm. The above results show that incorporating seeds into the soil in spring ensures a significantly faster rate of seedling growth than those obtained after sowing autumn acorns. The more rapid development of oak seedlings since spring sowing has been of practical importance. As a result of the reduction in the number of weeds needed in the plantation obtained after sowing in spring, compared to the number required for plantations brought by autumn sowing, the costs of seedling care work have been substantially reduced. The faster growth of seedlings from spring sowing led to the early formation of well-expressed branches and crowns. Due to this peculiarity of development, in the middle of the fourth vegetation season, the heights of these seedlings united, forming a joint shade at the soil's surface. Insufficient light on the soil surface caused the gradual inhibition of herbaceous species composition that competed with oak seedlings. The reduction of soil cover with accidental vegetation resulting from the union of oak crowns was beneficial for growing seedlings. Initially, this phenomenon caused the decline of competition between seedlings and weeds for mineral substances and unproductive soil moisture losses. The more rapid has also led to a reduction in the volume of seedling care work.

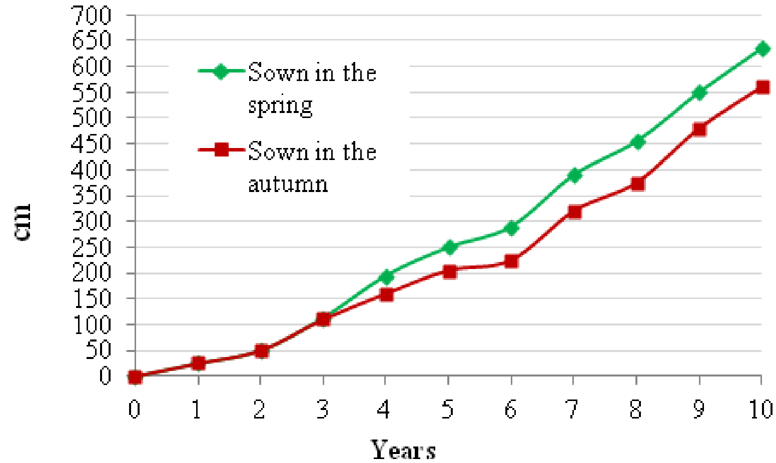

Figure 1. The dynamics of increasing the height of *Quercus robur* seedlings at different years after their initiation through autumn and spring sowing of acorns.

For seedlings that emerged from an autumn sowing, the phenomenon of mass closure was observed only in the sixth year of vegetation, i.e., one year later compared to those obtained in spring sowing. In the first year of life, oaks appeared both after sowing acorns in spring and autumn were cared for five times. In the fourth year after sowing, due to the strong growth of seedlings grown from acorns sown in spring, the crowns of the trees began to unite, and we made only two hoe. In the case of oaks obtained from an autumn sowing, the tendency of the crowns of seedlings to link was manifested only in the sixth year after sowing. In the sixth year after sowing, the oaks from spring sowing were plowed only once. Due to the faster growth of plants starting in the second year and ending in the sixth year after planting, the annual growth rate of seedlings obtained from acorns planted in spring significantly exceeded that characteristic for seedlings obtained from sown acorns. Due to this fact, in four years (years 3-6), the average height of the seedlings increased respectively from 50 cm to 290 cm and 225 cm, the annual growth rate being respectively equal to 60 cm and 44 cm. Over the next four years, the average yearly growth rate of seedlings obtained from acorns sown in spring and autumn was 86 cm and 84 cm, respectively, being practically identical for trees obtained from acorns sown in autumn and spring. It seems that due to the greater vigor of the seedlings obtained at spring sowing; their ability to compete with spontaneous plants for water and minerals has increased more effectively. We consider that due to the influence of these factors, the average annual growth rate of 2-6-year-old seedlings obtained from acorns sown in spring exceeded 1.36 times (60/44) the growth rate of those obtained by sowing acorns in autumn.
The number of necessary care works for the installation of oak plantations can be substantially reduced due to planting seedlings obtained in artificial conditions in autumn, grown during

winter, and planted in the open field in the spring. The data entered in the table 1 supports this view. They show that already at the beginning of the second year of growth, the height and diameter of seedlings planted after artificial germination was about 2.5 times higher compared to those grown after direct sowing in the field, autumn or spring. Due to this, in the first two months of the second year of vegetation in plants grown from planted seedlings, the number of leaves was about five times higher than that determined in plants obtained by direct sowing in autumn. We can also mention that in the plants obtained from the seedlings planted in spring, there is a tendency to increase the leaf surface, the chlorophyll content and decrease the water content in the leaves. These suggest that the vigor and size of seedlings obtained by planting artificially grown seedlings during the winter give them advantages in competition with spontaneous plants over that of the competitive capacity of plants obtained after autumn sowing. In this way, planting seedlings obtained by ensuring acorn germination immediately after their collection, grown artificially during the winter, and planted in the open field in early spring, is advantageous due to accelerating the growth rate of seedlings with high ability to adapt to stressors, such as and due to the decrease in the expenses necessary for the care of the plantations.

Table 1. Morphological and physiological parameters of the white oak plants in the second year of life obtained from acorns sown in the field in autumn and plants grown in laboratory conditions during autumn and winter and planted in field conditions in the spring.

Variant	Plant height, cm	Plant diameter, cm	Number of leaves per plant.	Average growth for May-June, cm	Average leaf area, cm^2	Chlorophyll index	Water content, %
Plants obtained from seedlings	39,7 ± 6,66	0,60 ± 0,07	33,3 ± 11,6	7,04 ± 2,2	32,6 ± 0,54	133,8 ± 13,03	48,5 ± 10,3
Plants obtained from acorns	13,2 ± 2,3	0,24 ± 0,01	5,88 ± 1,17	3,33 ± 1,17	31,35 ± 0,83	126 ± 10,42	52,3 ± 11,8

The conclusions indicated above are supported by the images presented in Figure 2. Analyzing these images, we can see that already in mid-June of the second year of cultivation, the size of plants obtained by planting seedlings grown under artificial conditions exceeds that of plants obtained by direct sowing. It also impresses with a large number of well-developed leaves, characteristic plantlets obtained from the seedlings.

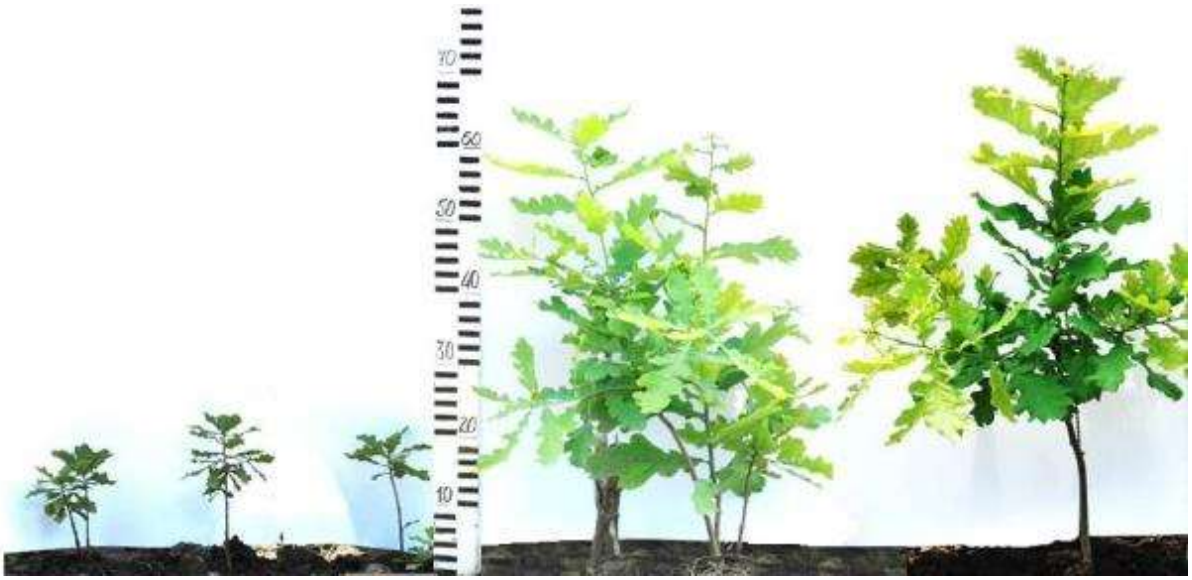

Figure 2. Image of oak seedlings in the second year of growth (made on June 15) obtained from acorns sown in the field in autumn (left) and those obtained from acorns that germinated in autumn, seedlings grown in artificially created conditions during winter and then transferred to open field in spring (right).

In general, the experiments performed to obtain the scientific and practical results presented in this article give the complexity of the biochemical and physiological processes that ensure plant survival during seed germination (Levitt, 1980) and plant development in ontogenesis. Incubation of acorns in ideal conditions during autumn and winter and their sowing in early spring ensured rapid germination, growth of the root system, and adaptation to the gradual increase in temperature in late spring and early summer. In embryos and seedlings obtained from acorns sown in autumn initially induced the adaptation to low temperatures. Later, the processes of reprogramming the expression and metabolism of the genes were performed to adapt the plants to high temperatures. The higher "*cost*" of changing the direction of adaptation processes during the germination of acorns sown in autumn compared to those sown in spring occurred during the first six years of plant growth, a conclusion confirmed by the data presented in Figure 1. To validate these suggestions, we compared the activity of growing and adapting plants obtained after sowing acorns in autumn (general control) with those that were planted after germination and growth in autumn and winter under artificial conditions (experiment). After the germination and growth stage, through hormetic mechanisms (Calabrese, 2006), in plants were induced processes that increase the vigor and adaptive capacity of the plants towards the stress factors. In addition, due to the implementation of this procedure, at the time of planting in the field, the roots of the seedlings were well developed; thus, the plants have better access to water and minerals if compared to the plants of control variant, especially in the first years of life. These priorities have already fully manifested themselves after the first year of life; see the data presented in the table 1 and figure 2.

Conclusions

Taking into account, the faster growth rate of pedunculate oak seedlings (Q. robur L.) obtained after spring sowing with layered acorns, as well as the reduction of the risk of winter damage to autumn seeds by wild boars and rodents, preferred sowing in the spring.
Even more appropriate is the organization of oak plantations by planting seedlings obtained after germination of freshly harvested acorns, cultivated under artificial conditions during autumn and winter, and transferring the seedlings to the open field in the spring.

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References

Barbu I. et al. (2016). Adapting Romania's forests to climate change. Forestry publishing house. Bucharest, 479 pp. (In Romanian)

Bullard S., Hodges J. D., Johnson R. L., Straka T. J. (1992). Economics of direct seeding and planting for establishing oak stands on old-field sites in the south. South. J. Appl. For. Vol. 16, pp. 34-40.

Calabrese, E. (2006). The failure of dose-response models to predict low dose effects: a major challenge for biomedical, toxicological and agingresearch. Biogerontology, Vol 7, No 1, pp. 119–122.

Clewer A.G, Scarisbrick D.H. (2001). Practical statistics and experimental design for plant crop science. Chichester, New York: John Wiley & Sons, LTD. 332 pp.

Cuza P., Florenţă Gh. (2017). Antioxidant activity of substances extracted from buds of the trees of spontaneous oak species. Journal of Botany. Vol 9, No 2 pp. 96-103.

Cuza P. (2017). Installation and maintenance of oak species (theoretical and practical aspects). Mediul ambiant Publishing House. Chisinau, 246 pp. (In Romanian)

Dascaliuc Al., Cuza P. (2011). The capacity of adaptation of the photosynthetic apparatus of oak species (Quercus robur, Q. petraea, Q. pubescens) to the action of high temperatures. Mediul ambiant Vol 2 No 56, pp. 33-36. In Romanian)

Dascaliuc Al., Cuza P. (2007). Determination of thermotolerance to sessile oak and pedunculate oak using the electrolyte leakage method. Mediul ambiant Vol 6, No 36, pp. 27-31. (In Romanian)

Giurgiu V. (2004). Silvologie. Sustainable development of Romania's forests. Academy of Sciences Publishing House. Vol. III B. Bucharest, 230 pp. (In Romanian)

Harris J.R., Day S.D., Kane B. (2008). Nitrogen fertilization during planting and establishment of the urban forest: A collection of five studies. Urban Forestry & Urban Greening 7: 195-206. DOI:10.1016/j.ufug.2008.03.001

Hasselman K. (1997). Climate change research after Kioto. Nature, Vol. 390; No 6657, pp.: 225-226. DOI: 10.1038/36719

Levitt J. (1980). Responses of plants to environmental stresses. Academic Press. New York, 568 p.

Reif A, Bruckner U, Kratzer R, Schmidinger A, Bauhuns J. (2010) Forest management in times of climate change-synergies and potential conflicts between forestry and nature conservation. Naturschutz und Landschaftsplanung, Vol 42; No 2, pp. 261-266.

Seppälä R., Buck A., Katila P.(eds.) (2009) . Adaptation of Forests and People to Climate Change. A Global Assessment Report. IUFRO World Series Volume 22. Helsinki. 224 pp.

Utkin A. I. Ermolova., L. S, Utkina I. A. (2008). The surface area of forest plants. Essence, parameters, use. M. Science. 289 pp. ISBN 978-5-02-034140-1 (In Russian)

Valkonen S. (2008). Survival and growth of planted and seeded oak (*Quercus robur* L.) seedlings with and without shelters on field afforestation sites in Finland. Forest Ecology and Management . Vol 255, no 3-4, 20 March 2008, pp.1085-1094. DOI:10.1016/j.foreco.2007.10.038

Villar-Salvador P., Planelles R., Enríquez E., Peñuelas J. (2004). Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. Forest Ecology and Management Vol 196, No 2-3, 257-266. DOI.org/10.1016/j.foreco.2004.02.061

Zadworny M., Jagodzinski A.M., Łakomy P., Ufnalski K., Oleksyn J. (2014). The silent shareholder in deterioration of oak growth: common planting practices affect the long-term response of oaks to periodic drought. Forest Ecology and Management, Vol 318, No 1, pp. 133-141. DOI.org/10.1016/j.foreco.2014.01.017

Zhang X, Zwiers FW, Hegerl GC, Lambert FH, Gillett NP, Solomon S, Stott PA, Nozawa T. (2007). Detection of human influence on twentieth-century precipitation trends. Nature. Vol. 448 No 7152. Pp. 461-465. DOI: 10.1038/nature06025.

Dănescu F., Ungurean C., Chira F., Horga D., Jung T. (2015). Abnormal drying of oak from O.S. Orchard. Journal of Forestry and Hunting, Vol 37, No 1, pp. 66-79. (In Romanian)

Diagnostics of plant resistance to stress: Methodological guide. (1988). Ed. Udovenko G.V. L .: VIR, 228 pp. (In Russian)

The Intergovernmental Panel on Climate Change (IPCC) (2007). Cambridge University Press, Cambridge, 996 pp.

VARIABLE COMPONENTS DETERMINING THE PRIMARY RESISTANCE TO EXTREME TEMPERATURES OF THE WHEAT SEEDS REPRODUCED IN THE DIFFERENT CLIMATIC ZONE

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Abstract

Specific results of our research have shown that the primary resistance of wheat genotypes varies depending on the conditions of seed reproduction. In laboratory conditions, based on the evaluation of the seeds' germination capacity after their exposure to shock with high or sub-zero temperatures, we determined the resistance of different wheat genotypes reproduced in Moldova and Ukraine. The wheat varieties can be differentiated in the accelerated mode after their primary resistance to extreme temperatures (excluding the influence of the adaptation processes carried out during plant ontogenesis) by exposing them to heat shock or shock with negative temperatures the well-prepared for germination seeds. The resistance of different wheat genotypes seeds to extreme temperatures can vary, being influenced by the environmental conditions of their reproduction. Only a tiny part of genotypes demonstrated at the same time the high resistance to high and sub-zero temperature. As a rule, genotypes with high resistance to negative temperatures have a low level of resistance to high temperatures. In most cases, the primary resistance to negative temperatures of seeds reproduced in Moldova was lower than those produced in Ukraine. Overall, the obtained data support the view that the resistance of seeds of different wheat genotypes to extreme temperatures reflects the interaction between the primary resistance of the genotype and the influence of epigenetic inheritance factors on the seed resistance.

Keywords*: Triticum aestivum* L., seeds, heat and frost tolerance.

Introduction

Plants are usually grown in environmental and agrotechnical conditions suboptimal, so they do not reach the genetic potential for growth and productivity (Rockstrom and Falkenmark, 2000). For example, in the United States, wheat productivity, in some years, was eight times lower than average (Boyer, 1982). Abiotic stressors can reduce crop plant productivity by more than 50% (Wang *et a*l., 2003). Overcoming these factors is detrimental to plant growth and productivity. The simultaneous or consecutive action of different stressors can have additive and even synergic deleterious effects. Therefore, knowledge of the mechanisms of plant response to the changing activity of various factors is essential for selecting genotypes with a broad tolerance spectrum, choosing appropriate conditions for their cultivation, and developing optimal agrotechnical technologies of plant cultivation. The final productive and agrotechnical potential we will achieve only after cultivating the plants in field conditions. Actual models of climate prediction indicate that in the next 50-100 years, the average temperature of the Earth's surface will increase by 3-5°C, which can lead to a drastic impact on global agricultural systems (IPCC, 2007).

The allocation of plant resources for growth and development under stress must be well distributed, as plant growth and productivity will depend on this ratio (Smith and Stitt, 2007). The analytical complexity of the interactions between different factors evaluated in most research programs aims to determine tolerance to one stress factor. It is not necessarily associated with simultaneous testing of susceptibility to other challenges. The result can have unintended consequences, as improved varieties can react unpredictably to interactions between different components when grown in the field. Furthermore, many ordinary stressors during the growing season are acting in combination (Mittler, 2006). The adaptation mechanisms to their action can operate in opposite directions, thus increasing the level of damages. Finally, we have to note that the risk of global warming (IPCC, 2007) also increases the likelihood of widening the range of variation between the extremes of high and low temperatures, indicating the need to select genotypes with increased resistance to high and to negative temperatures, i.e., with high plasticity. When optimizing the selection methods of genotypes resistant to excessive temperatures, it is possible to practice the principles developed for the accelerated determination of the stress resistance of technical devices (Levitt, 1980).

The theoretical generalizations and methods for evaluating plant resistance are summarized (Levitt, 1980; Dascaliuc *et al*., 2013). The effects induced in biological systems by the action of stressors depend on the nature and parameters of the exposure factors (Suzuki N. *et al.,* 2014). They are also specific for plant reactions at the sub-cellular, cellular (Levitt, 1980; Dascaliuc *et al*., 2013), tissue (Ivanov, 2003), organism (Dascaliuc *et al*., 2013), and at ecological levels (Zhuchenko, 1988). To determine the influence of seed reproduction conditions on their primary resistance to high temperatures and frost, we applied the approaches developed for the accelerated assessment of the stress stability of technical systems (Escobar *et al*., 2006). In our research, we chose the seeds as the object of study because the primary resistance of the genotype determines their response to the action of excessive temperatures, the components of adaptation to environmental conditions occurring during plant ontogenesis (Levitt, 1980; Lopes, 2010; Dascaliuc et al., 2013). We exposed the seeds to high temperatures by immersing them in water with different temperatures, thus ensuring the exclusion of the influence of avoidance phenomena on the resistance of the seeds. Our studies used seeds of different hexaploid wheat genotypes reproduced in climatic conditions in the Kharkiv region of Ukraine and the Chisinau area of Moldova. Thus, we developed a method of testing the primary resistance of wheat genotypes to excessive temperatures. As a result, it became possible to elucidate the potential contribution of epigenetic inheritance in modifying the primary wheat resistance to extreme temperatures.

Material and Methods

Our research used the seeds of 10 hexaploid wheat genotypes (lines 21, 111, 466, 517, 542, 1087, and varieties Samurai, Arctis, and Toulouse), reproduced in 2015-2016 in the Kharkiv region, Ukraine, and then reproduced in 2017-2018 in the Chisinau area, Moldova. Initially, the seeds intended for research were calibrated according to volume by passing through a sieve with a hole diameter equal to 2.4 - 2.6 mm. They were then disinfected for 20 minutes by incubation in a 0.1% aqueous solution of potassium permanganate, washed thoroughly with tap water, and then distilled water. After soaking in water at + 4°C for 36 hours, for determining the resistance of the seed to high temperatures, they were exposed to HS by immersion for a certain period in water with specified temperatures, maintained to an accuracy of ± 0,05°C using an ultra -

thermostat U10 (Germany). Finally, to determine the resistance to the action of negative temperatures, the seeds, prepared as mentioned above, were exposed to shock with negative temperature (SNT) by incubation in an air thermostat Rumed 3401 (Germany), maintaining the indicated air temperature with an accuracy of ± 0.5°C.

The first problem solved experimentally was to determine the unique value of the incubation temperature (positive or negative) and the duration of exposure, suitable for separating wheat genotypes according to their resistance to high temperatures or frost. Based on the data obtained in preliminary studies, for the distribution of wheat genotypes according to their primary resistance to frost or heat, we chose the doses of stress provided by exposing the seeds to -7°C or +50°C and the exposure time 8 hours or 30 minutes, respectively.

In these researches, we installed two control variants: the first for experiments determining the wheat genotypes frost resistance and the second for determining their resistance to high temperatures. In mentioned variants, before incubation for germination, the seeds were incubated at a temperature of + 24°C for another 8 hours or 30 minutes, respectively (periods equal to the duration of SNT or HS application in the experimental variants). The seeds of the experimental and control variants were then placed for germination in Petri dishes, 25 seeds each, in three repetitions, in the dark, at a temperature of 25°C and relative humidity of 75-85%. The response of seeds of each wheat genotype to SNT or HS was appreciated by assessing the percentage of seeds that germinated over 5 days after exposure to SNT or HS. During this period, in the control variants sprouted no less than 98% of the seeds. Therefore, we evaluated the seeds of different genotypes' response to SNT or HS directly based on seeds germination rate in experimental variants. Based on the experimental results, we calculated the average percentage of seed germination, the standard deviation of wheat genotype seeds' average response to SNT or HS (Clewer *et al*., 2001).

Results and Discussion

In Figure 1 included the data on the influence of SNT (A) and HS (B) on the germination rate of seeds reproduced in Ukraine and Moldova. The sequence of diagrams presentation in each figure are arranged in the order that correspond to the order of increasing the germination percentage of the genotypes seeds reproduced in Ukraine and exposed to SNT. We considered that the genotypes have low (I), medium (II), or high (II) resistance to SNT or HS, if after seeds exposure to SNT or HS they germinated up to 50% (I), between 50 and 70% (II), and more than 70% (III), respectively. The presented in Figure 1 A data suggests that the reproduced in Ukraine seeds of the genotypes the lines 466, 542, 111, 1108, varieties Samurai, and Arctis are characterized by low resistance; of lines 517 and 1087 - with medium resistance; those of line 21, and variety Toulouse - with high resistance to SNT. At the same time, analyzing the diagrams presented in Figure 1 A, we notice that after reproduction in Moldova, the seeds of all genotypes showed low resistance to SNT.

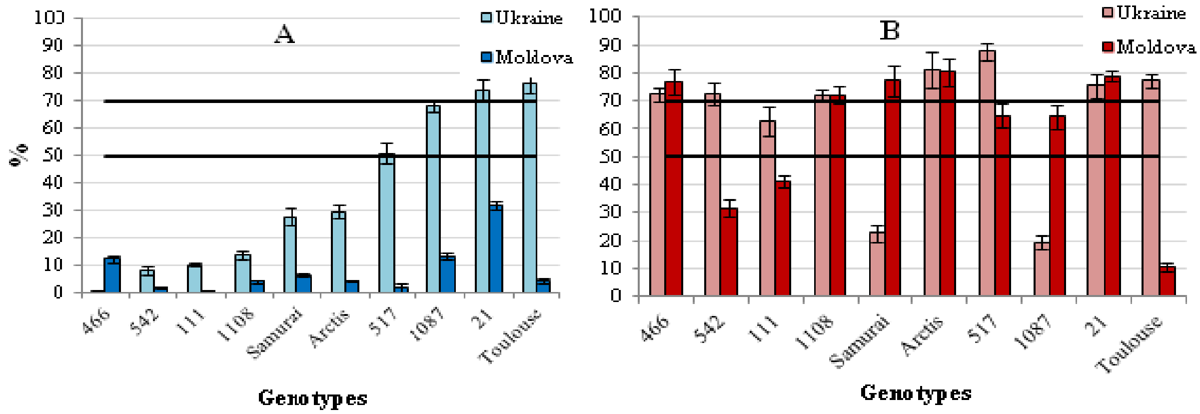

Figure 1. The percentage of the ten winter wheat genotypes, reproduced in Ukraine and Moldova, germinated during five days after exposure to SNT for 8 hours at -7^{o}C (A) and to HS for 30 minutes at 50^{o}C (B).

We analyzed the data from Figure 1 B, and we notice that after reproduction in Ukraine, the seeds of most genotypes have high resistance to HS. Only those of the variety Samurai and of line 1087 were sensitive, and those of line 111 showed medium sensitivity to HS. After reproduction in Moldova, the sensitivity to HS of some genotypes decreased (Toulouse, 111, 142), and of others increased (Samurai, 1087) substantially. The data presented in Figure 1 suggest that the primary resistance of seeds of different wheat genotypes to HS or SNT was influenced differently by the seed reproduction conditions. The direction of these changes was specific to different genotypes. In particular, this trend was manifested in the variation of the response of seeds exposed to HS. This is confirmed by the lower value of the correlation coefficient between the germination percentage after exposure to HS of seeds reproduced in Ukraine and those reproduced in Moldova compared to the correlation coefficient assessed after the application of SNT. The value of this coefficient was respectively equal to -0.017 and 0.481. At the same time, the correlation coefficient between percent of seeds germinated after exposure to HS and SNT, determined for seeds reproduced in Ukraine with those reproduced in Moldova, was equal to -0.058 and 0.434, respectively. Thus, although the primary resistance to SNT of reproduced in Moldova seeds decreased substantially, due to the same direction of changes for different genotypes, the correlation coefficient between seeds of two reproductions reached the value 0.434. At the same time, due to the different directions of changes of the primary resistance to HS of wheat genotypes, although on average the values of HS resistance of genotypes reproduced in Ukraine and Moldova was comparable, the correlation coefficient between resistance to HS of genotypes reproduced in Moldova and Ukraine was much lower than 0.434, being equal to -0.058. In general, obtained data show that for seeds reproduced in Moldova, independently of genotype, there was a tendency of decreasing their primary resistance to SNT. At the same time, for some genotypes reproduced in Moldova, the primary resistance to HS changed in different directions.

To analyze the complex processes of modifying the primary resistance of wheat genotypes to HS and SNT under reproducing their seeds in new environmental conditions, we appreciated the plasticity of genotypes' response to extreme temperatures. We mention that all theoretically possible values of the germination percentage of seeds representing different genotypes exposed to SNT or ST can occupy the range between 0 and 100%. Plasticity is influenced by the resistance to both types of temperature stress. Therefore its values can be estimated by the percent seeds germinated after exposure to HS plus that of those germinated after exposition to SNT. Theoretically, the values of this parameter of plasticity can vary between 0 and 200%. Since seed germination for all genotypes has practically reached 100% in the control variants, the values of the sum less than 200% of germination represent the plasticity of genotype response to extreme temperatures. The lower is the percentage of seeds germination after exposure to SNT, plus that after exposure to HS, the lower is the genotype plasticity. To differentiate the genotypes after their plasticity, we have divided them into three groups: I - the genotypes with low plasticity - the summary seed germination rate did not exceed 100%; II - the genotypes with medium plasticity - the summary seed germination rate lay between 100 and 140%; III - the genotypes with high plasticity - the summary seed germination rate exceeds 140%. Based on the results presented in Figure 2, we can mention that for reproduced in Ukraine seeds; high plasticity was specific to the variety Tuluza and line 21, medium - those of the variety Arctis and line 517, and the seeds of the variety Samurai and lines 11, 466, 542, 1087, and 1108, have the low plasticity. The seeds of line 21 reproduced in Moldova have high plasticity. In contrast, the plasticity of reaction to excessive temperatures of the seeds of nine other wheat genotypes was low.

Considering that the difference of frost resistance between wheat varieties increases with the adaptation processes' accomplishment, the traditional methods of determining the frost resistance of wheat genotypes ordinarily are after performing the adaptation processes (Flower *et al*., 2014). Therefore, it was considered that the resolution power of methods for differentiating genotypes according to their level of frost resistance turns out to be the highest precisely when the plants are in an adapted state. However, we have to consider that the duration of accomplishing the adaptation processes and the specificity of the kinetics of these processes in the plants of different genotypes depend on the environmental conditions (Dascaliuc *et al*., 2013). Although the maximum difference between the primary frost resistance of wheat varieties practically does not exceed 3^oC and that of adapted plants reaches 12^oC (Flowler *et al*., 2014), the relative simplicity and reproducibility of the procedures for determining the primary frost resistance of seeds inspired us to test in more detail the resolving power of this method. Later we posed the problem of determining the primary resistance of wheat genotypes to high temperatures, which ultimately allowed appreciating the plasticity of the reaction of different wheat genotypes to the action of excessive temperatures (positives and negatives). By soaking the seeds in water at a temperature of 4^oC, we brought the seeds to an equivalent pre-emergence physiological state. The effectiveness of synchronization was manifested by the germination in the control variant of practically 100% of seeds during the first 24 hours of incubation under favorable germination conditions. When seeds reach an equivalent physiological state, their reaction to HS or SNT mainly depends on the genetic specificity of the genotype.

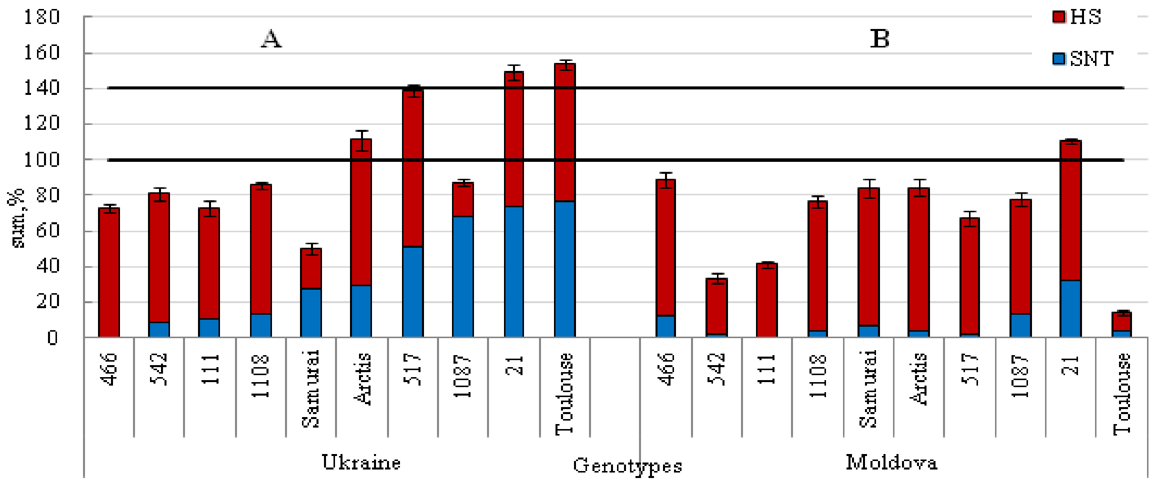

Figure 2. The percentage of seeds germinated during five days of the ten winter wheat genotypes expressed as a sum of the percentage of seeds germinated after 8 hours exposure to -7°C (blue) plus the percentage of those germinated after exposure for 30 minutes to HS at 50°C (red). A and B – the seeds were reproduced in Ukraine and Moldova, respectively.

The applicability of mentioned approaches for studying the primary resistance of plants to extreme temperatures (before the manifestation of adaptation processes) has repeatedly been confirmed in laboratory experiments. Compared to the methods of determining thermotolerance based on the influence of HS on plant growth parameters (Jelev, 2016), the simplicity and advantages of the proposed method are evident. Since the seeds were well prepared for germination but have not yet germinated, we avoided the contribution of ontogenetic adaptations. In this state, seeds represent a unique model to evaluate the primary resistance of wheat genotypes to the action of excessive temperatures. According to their primary resistance to extreme temperatures, the proposed method of wheat genotypes distribution is in strict accordance with the accelerated methodology of testing the resistance of the biosystems to stress factors (Escobar, 2006; Dascaliuc *et al*., 2013). In the accelerated test, the plant exposure temperatures exceeded the natural ones, but the exposure doses were within the tolerance range of the genotypes. This statement is supported by the data in Figures 1 and 2. The data given in these figures provide a qualitative and quantitative characterization of 10 wheat genotype resistance to high temperatures and frost. We mention that the resistance characteristics determined by us depend on genetic, biochemical, and physiological processes that characterize the response of wheat genotypes to the action of extreme temperatures. In our experiments, the reactions were not influenced by the complex mechanisms of avoiding the action of stressors, phenomena that have mainly been taken into account in the amelioration of wheat (Lopes *et al*., 2010; Fowler *et al*., 2014). The obtained data demonstrate that seeds resistance to both types of temperature shock is specific for different wheat varieties and is influenced by the seed reproduction conditions. Due to this adaptive variability of genetic and epigenetic nature (Jaligot, Rival, 2015)), wheat varieties and their descendants are characterized by high resistance and productivity in very different environmental conditions. The possibility of epigenetic inheritance suggests that it may influence wheat embryo primary frost or heat resistance. Their level of influence can be different depending on the conditions of seed reproduction. Because meteorological conditions vary from year to year, they can probably influence genotypes' primary resistance to heat stress factors even when reproduced in the same zone. Recently we obtained data showing that the distribution of seeds of wheat genotypes based on primary resistance to extreme temperatures does not change after one year of storage of seeds. Simultaneously, the data showed that the distribution of genotypes after the primary resistance to extreme temperatures varied when tested seeds reproduced in different years in the same zone.

Conclusion

The primary resistance of different wheat genotypes seeds to HS or SNT can vary, being influenced by the environmental conditions of their reproduction.
In general, the data obtained support the opinion that, due to the influence of epigenetic inheritance factors, the primary resistance of seeds wheat genotypes may vary depending on the cultivation area.

Acknowledgment

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References

Boyer JS. (1982) Plant productivity and environment. Science. Vol. 218, № 4571, pp.:443-8. doi: 10.1126/science.218.4571.443. PMID: 17808529.

Clewer A.G, Scarisbrick D.H. (2001) Practical statistics and experimental design for plant crop science. Chichester, New York: John Wiley & Sons, LTD. 332 pp.

Dascaliuc A., Ivanova R., Arpentin Gh. (2013). Systemic approach in determining the role of bioactive compounds. In Pierce G.N.; Mizin V. I.; Omelchenko A., eds. Advanced Bioactive Compounds Countering the Effects of Radiological, Chemical, and Biological Agents, Strategies to counter biological damage; Series: NATO Science for Peace and Security Series A: Chemistry and Biology. Springer: pp. 121 – 131.

Escobar L. A., Meeker W.Q. (2006) A Review of Accelerated Test Models. Statistical Science, Vol. 21, № 4, pp. 552–577. Doi: 10.1214/088342306000000321

Fowler D. Byrns B. M., Greer K. J. (2014) Overwinter Low-Temperature Responses of Cereals: Analyses and Simulation. Crop science, Vol. 54, № 6, pp. 2395-2405. Doi:10.2135/CROPSCI2014.03.0196

Ivanov V.B. (2003). The problem of stem cells in plants. Russian Journal of Developmental Biology, Vol. 34, № 4, pp. 253–261. Doi.org/10.1134/S1062360407060021 [in Russian]

Jaligot E. Rival A. (2015) Applying Epigenetics in Plant Breeding: Balancing Genome Stability and Phenotypic Plasticity. In Advances in Plant Breeding Strategies: Breeding, Biotechnology, and Molecular Tools.Volume 1, Springer. pp. 159–192. Doi.org/10.1007/978-3-319-22521-0_6

Jelev N. (2016) Diminishing the impact of abiotic stressors on *Triticum aestivum* L. plants by using the natural growth regulator Reglalg. Bulletin of the Academy of Sciences of Moldova. Life sciences. Vol. 330, № 3, pp. 72–79. (In Romanian)

Levitt J. (1980) Responses of plant to environmental stresses. Vol.1. – New York: Acad. Press, 568 p. ISBN: 012445 5018

Lopes M.S., Reynolds M.P. (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Functional Plant Biology. Vol. 37, № 2, pp. 147–156. Doi: org/10.1071/FP09121

Mittler R. (2006) Abiotic Stress, the Field Environment and Stress Combination. Trends in Plant Science Vol. 11, № 1, pp.:15-9. Doi: 0.1016/j.tplants.2005.11.002

Rockstrom J., Falkenmark M. (2000) Semiarid Crop Production from a Hydrological Perspective: Gap between Potential and Actual Yields. Critical Reviews in Plant Sciences Vol. 19, № 4, pp. 319-346. Doi: 10.1080/07352680091139259

Smith A.M., Stitt M. (2007) Coordination of carbon supply and plant growth. Plant Cells and Environment, Vol. 30, №9, pp. 1126-1149. Doi.org/10.1111/j.1365-3040.2007.01708.

Suzuki N., Rivero R.M., Shulaev V. et al. (2014) Abiotic and biotic stress combinations. New Phytologist, Vol. 203, № 1, pp. 32-43. Doi.org/10.1111/nph.12797

The Intergovernmental Panel on Climate Change (IPCC) (2007), Cambridge University Press, Cambridge, 996 pp.

Wang, W., Vinocur, B. & Altman,A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, Vol. 218, № 1, pp. 1–14 Doi.org/10.1007/s00425-003-1105-5

Zhuchenko A. A. (1988) The adaptive potential of cultivated plants: genetic and ecological bases. Publisher: Kishinev: «Shtiintsa» 267 p. [in Russian]

CONTENT OF BIOACTIVE COMPOUNDS IN BIOMASS OF NATURAL LAWN OF ARRHENATHERETALIA

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Abstract

Natural meadows and pastures represent the most important resource in the production of bulky fodder in the hilly and mountainous area of Serbia. Yield and quality of grass biomass mainly depend on a large number of factors, among which are primarily floristic composition, which implies a share of quality grasses and legumes. The paper analyzes the influence of NPK application of mineral fertilizers with different nitrogen levels, where (A0- non-fertilized, A1-N60:P40:K40, A2-N100:P40:K40, A3-N140:P40:K40), on the content of total phenol, flavonoids, chlorophyll a and b and carotenoids in the biomass of natural grassland of the order Arrhenatheretalia in the municipality of Raska. It was observed that the application of increasing doses of nitrogen fertilizer did not significantly affect the phenol content in grasses and other plants. However, in legumes, it is noticed that the variant with the highest dose of nitrogen had a significantly lower phenol content compared to the control variant. The content of flavonoids in grasses and legumes did not change significantly depending on the application of different nitrogen levels. Phenolic compounds are considered to play the greatest role in the biological activity of extracts and their presence contributes to the antioxidant activity of the plant. The content of chlorophyll in grasses on the examined grass did not change with the application of increasing doses of nitrogen in the experiment, while unlike chlorophyll, the highest content of carotenoids in grasses of natural grass Arrhenatheretalia was achieved with the use of the highest amount of nitrogen in experiment N140.

Keywords: *natural grasses, bioactive compounds, order Arrhenatheretalia.*

Introduction

Grasses are one of the most widespread and numerous plant communities. A large number of genera and species are known in the world, and the ability to spread them quickly is attributed to the way they reproduce. The diet of domestic animals is largely based on fodder grasses. Great importance of grasslands also stems from the fact that they are the largest, and often the only source of bulky food in the mountainous area where they are considered to occupy 30-60% of agricultural land (Stošić and Lazarević, 2007). Grasses also participate in the repair and stabilization of soil properties, prevention of erosion and are important in the arrangement of urban areas (Erić et al., 2016). The main factor that affects the quality of natural lawns as well as the achieved yield is the floristic composition. In addition, soil properties, light, amount and distribution of precipitation have a significant impact, but of course also adequate agro-technical measures, which are often the cause of unstable and low yields.
Over 3000 flavonoids were isolated and studied from plants, which, according to the degree of oxidation of the central Piran ring, were divided into twelve classes. In addition to being plant pigments, flavonoids also contribute to the taste, most often bitterness, and astringency of the

plants in which they are found. This class of compounds undoubtedly participates in the defensive chemistry of plants, because the hardness they give to plants is unpleasant to herbivores, and they also protect the plant from the harmful effects of UV radiation (Zhishen et al., 1999; Harborne et al., 2000). Phenolic compounds represent a widespread heterogeneous group of secondary plant metabolites and one of the most important classes of natural antioxidants. They have been shown to be synthesized in response to increased concentrations of some metals in soil, such as aluminum (Petrović et al., 2005).

Chlorophyll is the main photosynthetic pigment present in all photosynthetic organisms. It is one of the most important bio-organic compounds in general, primarily due to its central role in the process of photosynthesis, the most important global physico-chemical process on our planet, in which light energy is converted into chemical energy for use in the final result - synthesis of organic sugar. The arrangement of double bonds along the outer periphery of the porphyrin structure is conjugative (alternating arrangement of single and double bonds), which is crucial for the role it plays in photosynthesis, because it shifts the absorption maximum towards longer wavelengths, in the visible region of the solar spectrum (Woodward et al. , 1960).

Carotenoids are polyunsaturated hydrocarbons composed of isoprene units. The structural formula of carotenoids contains a significant number of conjugated double bonds, the most important being α- and β-carotene, lutein, lycopene, and β-cryptoxanthin (Thurnham, 1994). In addition to being a β-carotene precursor of vitamin A, it is important for carotenoids to point out that they have important antioxidant activity (Nagata and Yamashita, 1992).

The aim of this study was to analyze the effect of NPK application of mineral fertilizers with different nitrogen levels on the content of phenols, flavonoids, chlorophyll a and b and carotenoids in the biomass of natural grassland of the order Arrhenatheretalia.

Material and Methods

Samples of represented plant species from the natural grassland of the order Arrhenatheretalia, previously dried and classified and divided into three groups: grasses, legumes and weeds, were used for the examination. Samples were taken in 2020, from a natural meadow of the order Arrhenatheretalia, located on the slopes of Kopaonik, municipality of Raska. The experiment was set up in three replications according to a completely randomized block system, where the plot size is 7,5 m^2, on black soil on dolomite pH 6.5. Fertilization variants are as follows:

- non-fertilized - control (A0),
- N60:P40:K40 (A1),
- N100:P40:K40 (A2),
- N140:P40:K40 (A3).

Mowing was done in the phase of grading or brooming of the most common grass species, the second mowing, on September 3, 2020. The total amount of green mass per plot was measured, after which a sample of 500 g was taken from each plot. The weight share of grasses (family Poaceae), legumes (family Fabaceae) and other plants (weeds, medicinal plants, etc.) was determined from the sample.

The following analyzes were performed from the prepared material:

- Determination of total flavonoid content by spectrophotometric method (Sharma et al., 2011);
- Determination of total phenol content by Folin-Ciocalte method (Singleton et al., 1999);
- Determination of chlorophyll a and b spectrophotometric method (Wettstein, 1957);
- Determination of carotenoids - spectrophotometric method (Nagati and Yamashiti, 1992).

Results and Discussion

Total phenol content

The content of total phenols was determined by the Folin-Ciocalte method and on the basis of the calibration diagram of the standard, gallic acid, the content of phenolic compounds in the tested extracts was determined (Figure 1).

The absorbances of different concentrations of gallic acid standards were measured at 765 nm and the following values were obtained:

- for a solution with a concentration of 0.05 mg / mL, an absorbance of 0.011 was measured;
- for a solution of concentration 0.1 mg / mL, absorbance 0.028 was measured;
- for a solution with a concentration of 0.25 mg / mL, the absorbance of 0.058 was measured;
- for a solution of concentration 1 mg / mL, absorbance of 0.097 was measured;
- for a solution of 2.5 mg / mL, the absorbance of 0.209 was measured.

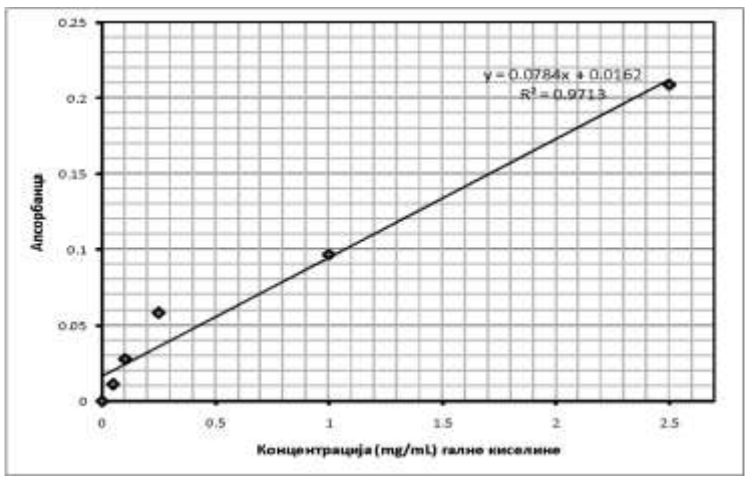

Figure 1. Calibration diagram of gallic acid

Table 1. Phenol content (mg/mL) in natural grassland biomass of the order Arrhenatheretalia

Fertilization	Grasses	Legumes	Other plants	$\bar{x}$
A 0	0,163	0,1117^{a}	0,129	0,134
A 1	0,070	0,0663^{ab}	0,258	0,131
A 2	0,153	0,0913^{a}	0,301	0,182
A 3	0,172	0,0390^{b}	0,065	0,092
$\bar{x}$	0,139	0,07707	0,188	

Values marked with different lowercase letters per column differ significantly (P <0.05), according to the LSD test

The application of increasing doses of nitrogen fertilizer did not have a significant effect on the phenol content in grasses and other plants. However, in legumes, it is noticed that the variant with the highest dose of nitrogen had a significantly lower phenol content compared to the control and A2 variant (Table 1). The phenol content determines the pharmacological properties of the plant and in medicinal plants the phenol concentration is from 0.23 to 2.85 mg GAE/g of fresh sample, while the phenol concentration in culinary plants is from 0.26 to 17.51 mg GAE/g of fresh sample (Zheng, 2001). The highest content of phenolic compounds has a culinary plant from the genus Origanum, about 20 mg GAE/g of fresh sample (Lagouri and Boskou, 1996). The content of total polyphenolic compounds measured by the Folin-Ciocalte method does not provide a complete picture of the quantity and quality of polyphenolic compounds in extracts, due to the possible presence of interfering compounds (sugars, aromatic amines, sulfur dioxide, vitamin C, organic acids and other non-polyester substances).), which affect the unrealistic increase in results (Singelton et al., 1999). Therefore, complete qualitative and quantitative identification of polyphenolic compounds, using the HPLC method, is necessary.

Total flavonoid content

The antioxidant activity of flavonoids depends on the structure and substitution of hydroxyl groups (Sharififar et al., 2008). Flavonoids are present in almost all plants and, thanks to their chemical structure, they have anti-inflammatory and anti-allergic properties. The results of spectrophotometric determination of the content of total flavonoids in the methanol extract of selected species are presented with the help of routine diagrams.

At 430 nm, the absorbances of previously made different concentrations of routine standards were spectrophotometrically measured:

for a solution with a concentration of 0.01 mg/mL, the absorbance of 0.056 was measured;
for a solution with a concentration of 0.05 mg/mL, an absorbance of 0.060 was measured;
for a solution of 0.1 mg/mL, the absorbance of 0.197 was measured;
for a solution with a concentration of 0.25 mg/mL, the absorbance of 0.238 was measured;
for a solution 0.5 mg/mL, an absorbance of 0.756 was measured.

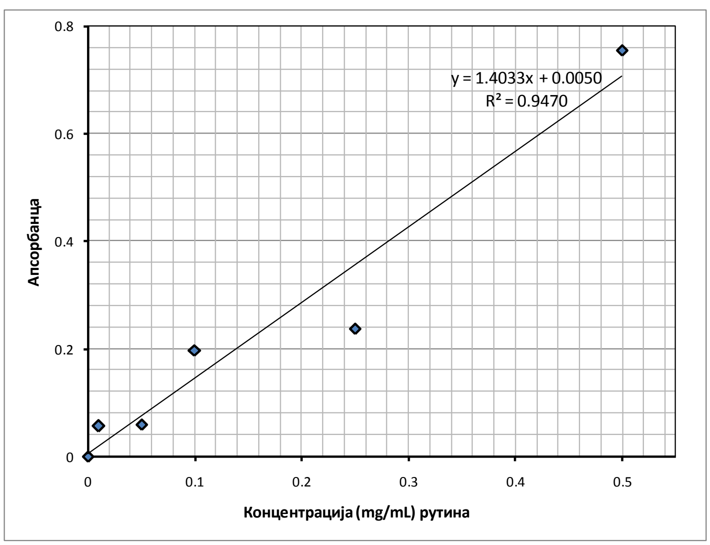

Figure 2. Calibration diagram of routines

Table 2. Flavonoid content in natural grass biomass of the order Arrhenatheretalia (mg/mL)

Fertilization	Grasses	Legumes	Other plants	$\overline{x}$
A 0	0,451	0,692	0,419[ab]	0,520
A 1	0,331	0,373	0,566[a]	0,423
A 2	0,364	0,950	0,524[ab]	0,613
A 3	0,290	0,588	0,095[b]	0,324
$\overline{x}$	0,359	0,651	0,401	

Values marked with different lowercase letters per column differ significantly ($P < 0.05$), according to the LSD test

Comparing all variants of fertilization in relation to the control variant in grasses and legumes, it can be concluded that there are no significant differences in the content of total flavonoids. In other plants, a significantly higher content of flavonoids was achieved on A1 compared to the A3 variant. If we look at the average values, legumes show a higher content of flavonoids than the other two groups, ie grasses and other plants. Phenolic compounds are considered to play the greatest role in the biological activity of extracts and their presence contributes to the antioxidant activity of the plant. A large number of studies indicate that the role of flavonoids, as phenolic compounds, is of special importance. Of the structural elements that are important for the antiradical activity of flavonoids, hydroxyl groups are of the greatest importance (Yang et al., 2001; Heim et al., 2002). In the research conducted within this final master's thesis, the highest average flavonoid content was found in legumes (0.651 mg/mL).

Chlorophyll a, b and carotenoid content

The content of chlorophyll and carotenoids was determined by the procedure with ethanol solution and absorbance measurements at 649 and 665 nm for chlorophyll and 470 nm for carotenoids, respectively (Petrović et al., 2005).

Table 3. Chlorophyll content in the biomass of natural grassland of the order Arrhenatheretalia (mg kg^{-1})

Fertilization	Grasses	Legumes	Other plants	$\overline{x}$
A 0	518	795[b]	1409	907,3
A 1	659	1355[ab]	1523	1179,0
A 2	1665	1497a	1555	1572,3
A 3	1615	1392[ab]	1415	1474,0
$\overline{x}$	1114,2	1259,7	1475,2	

Values marked with different lowercase letters per column differ significantly ($P < 0.05$), according to the LSD test

The values obtained for the share of chlorophyll in grasses show that there are no significant differences between the application of different fertilization variants in relation to the non - fertilized (control) variant. In legumes, the highest chlorophyll content was recorded in variant A2 and differed significantly in relation to the non-fertilized variant (Table 3). In other plants, there are no significant differences between the control variant and the application of different amounts of nitrogen fertilizers.

Table 4. Carotenoid content in natural grass biomass of the order Arrhenatheretalia (mg/mL)

Fertilization	Grasses	Legumes	Other plants	$\bar{x}$
A 0	0,186[b]	0,130	0,400	0,239
A 1	0,432[ab]	1,030	0,170	0,544
A 2	0,383[ab]	0,830	0,950	0,721
A 3	0,812[a]	1,530	0,830	1,057
$\bar{x}$	0,453	0,880	0,730	

Values marked with different lowercase letters per column differ significantly (P <0.05), according to the LSD test

By comparing the values for the content of carotenoids in the biomass of natural grassland of the order Arrhenatheretalia, the fertilization variant A3 shows a significant difference in relation to the control variant (Table 4). By comparing all variants for the obtained values of carotenoids in legumes and grasses, it was also determined that there are no significant differences in relation to the control variant. On average, the highest value was observed in samples of leguminous plants.

Conclusions

On average, the highest phenol content was achieved in grasses. Observing individual values, the highest phenol content in grasses is observed when applying the highest dose of nitrogen, while in legumes the highest content is in the variant without fertilization, which can be related to the fact that legumes, unlike grasses, require the least amount of nitrogen fertilizers due to nitrogen fixation;

Higher content of flavonoids was obtained by using dried plant material, but the content of flavonoids did not change significantly in relation to different levels of nitrogen fertilizers, so it can be concluded that the mineral diet with these doses of nitrogen and constant doses of phosphorus and potassium had no effect on plant antioxidant activity stimulated by flavonoids;

Statistical data processing showed that in legumes only the variant with the applied average dose of nitrogen A2 significantly affected the chlorophyll content in relation to the control variant, while there was no statistically significant difference in the share of carotenoids for legumes and weeds compared to different fertilization variants. Variant A3 differed statistically significantly in terms of chlorophyll content from control and other fertilized variants in grasses of natural grassland of the order Arrhenatheretalia.

References

Erić P., Ćupina B., Krstić Đ., Vujić S. (2016). Lawns. University of Novi Sad, Faculty of Agriculture, pp. 13-30.

Heim K.E., Tagliaferro A.R., Bobilya D.J (2002). Flavonoid antioxidants: chemistry, metabolism and structure activity relationships. *Journal of Nutritional Biochemistry*, Vol. 13, pp. 572-584.

Harborne J.B., Williams C.A. (2000). Advances in flavonoid research since 1992. Phytochemistry, Vol. 55, pp. 481-504.

Lagouri V. Boskou D. (1996). Nutrient antioxidants in oregano. International Journal of Food Science and Nutrition, Vol. 47, pp. 493-497.

Nagata M., Yamashita I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Journal of the *Japanese Society* for *Food Science* and *Technology,* Vol. 39, pp. 925-928.

Stošić M., Lazarević D. (2007). Sustainable systems of production and utilization of fodder plants. Previous results of research on grasslands in Serbia. Proceedings of the XI Symposium on Fodder Plants, May 30 - June 1, Novi Sad, Vol. 44, pp. 333-345.

Petrović S., Mijin D., Stojanović N. (2005). Chemistry of natural organic compounds, Belgrade, Faculty of Technology and Metallurgy.

Nagata M., Yamashita I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Journal of the *Japanese Society* for *Food Science* and *Technology,* Vol. 39, pp. 925-928.

Sharma G.N., Dubey S.K., Sati N., Sanadya J. (2011). Antiinflammatory activity and total flavonoid content of aegle marmelos seeds, The *International Journal of Pharmaceutical Sciences* and *Drug Research*. Vol. 3, pp. 214-218.

Singleton V. L., Orthofer R., Lamuela-Raventós R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods in enzymology, Academic Press, Vol. 299, pp. 152-178.

Singleton V.L., Rossi J.A. (1999). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. The American Journal of Enology and Viticulture. Viticult, Vol. 16, pp. 144-158.

Sharififar F., Nudeh- Dehghn G., Mirtajaldini M. (2008). Major flavonoids withantioxidant activity from *Teucrium polium* L. Food Chemistry Vol. 112, No. 4, 885-888.

Wettstein D. (1957). Chlorophyill-letale und submikroskopische formwechsel der plastiden. Experimental Cell Research, pp. 427- 433.

Woodward R.B., Ayer W.A., Beaton J.M., Bickelhaupt F., Bonnett R., Buchschacher P., Closs G. L., Dutler H., Hannah J., Hauck F.P., Itô S., Langemann A., Le Goff E., Leimgruber W., Lwowski W., Sauer J., Valenta Z., Volz H. (1960). The total synthesisof chlorophyll, *Journal* of the *American Chemical Society* , Vol. 82, pp. 3800-3802.

Yang B., Kotani A., Arai K., Kusu F. (2001). Estimation of the antioxidant activities of flavonoids from their oxidation potentials. Analytical Science, Vol. 17, pp. 599-604.

Zhishen J., Mengcheng T., Jianming W. (1999). The determination of flavonoid contents in mulberryand their scavenging effects on superoxide radicals. Food Chemistry, Vol. 64, pp. 555-559.

Zheng W., Wang S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural* and *Food Chemistry, Vol.* 49, No. 11, pp. 5165-5170.

THE INFLUENCE OF ROOTSTOCK ON WINTER COLD HARDINESS, PRODUCTIVETY AND FRUIT QUALITY OF SWEET CHERRY CULTIVAR 'KORDIA'

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Abstract

The study researched the influence of five clonal rootstocks on the sensitivity of flower buds to frost damage during the period of ecological dormancy and production properties of sweet cherry cultivar 'Kordia'. The study was carried out during five years at an orchard located at the experimental estate "Radmilovac" of the Faculty of Agriculture in Belgrade. Cultivar 'Kordia' was grafted on the following rootstocks: 'Gisela 5', 'Gisela 6', 'Ma×Ma 14', 'Colt' and 'Oblačinska sour cherry'. Winter frosts occurred during the ecological dormancy on March 14th and 15th. The intensity of frost was –7 °C. The percentage of damaged and non-damaged flowers per fruiting branches was determined by counting (100 flower buds per tree were taken from each part of the canopy and fruiting branches). The highest damage of flowers buds had trees grafted on 'Oblačinska cherry', (an average 61.2% of flower buds), while the lowest had trees grafted on 'Ma×Ma 14', (an average 12.3% of total flower buds). The significantly higher sensitivity of flower buds had a short fruiting branch compared to long fruiting branch. Trees grafted on 'Colt' had significantly higher values of TCSA (31.2 cm^2). The highest yield per tree cultivar 'Kordia' had grew on rootstock 'Ma×Ma 14', while the lowest had on 'Oblačinska cherry', 6.5 kg and 2.5 kg respectively. The biggest fruits had trees that grew on 'Colt' (12.5 g), while the smallest had trees that grew on 'Gisela 5' (9.8 g). The highest productivity had trees grafted on 'Gisela 5', 'Gisela 6' and 'Ma×Ma 14'.

Key words: *Dormancy, flower bud, frost damage, yield, fruit quality.*

Introduction

Production of sweet cherries has an increasing trend in Europe. In many countries sweet cherry is one of the most popular summer fruits (Dekena et al., 2020). A satisfactory fruit crop in sweet cherry significantly depends on the quality of flowering and fruit set (Dziedzic et al., 2019). Many authors researched the flowering of sweet cherry, especially the differentiation of generative organs, and the effect of rootstock on fruit setting and quality (Zhang et al., 2015; Radunić et al., 2017). One of the key factors in sweet cherry production is the suitability of cultivar-rootstock combinations to local climate conditions, together with soil and growing technologies (Pal et al., 2017). The rootstock used for growing fruit trees affects many traits of trees and fruits, such as tree vigour, flower bud induction and regular bearing (Koutinas et al., 2010), productivity index (Bielicki and Rozpara, 2010), fruit mass (Gratacós et al., 2008), or cracking susceptibility (Brüggenwirth and Knoche, 2016). In the last two decades, the new dwarfing and semi-dwarfing cherry rootstocks for sweet cherry growing enables obtaining high yields of high quality fruit. Many authors were examined following rootstocks: 'Gisela 5', 'Gisela 6', 'Edabriz', 'Pi-ku 4.20', 'Weiroot 72', 'Weiroot 158', 'Weiroot 13', 'Ma×Ma 14' and 'F 12/1' (Gratacos et al., 2008; Usenik et al., 2008; Bielicki and Rozpara, 2010).

The sweet cherry is vigourously growing tree, and because of that requires the use of dwarf growing rootstocks in modern orchards. Clonal rootstocks are mostly used in intensive cherry production, and the most important of them are 'Gisela 5', 'Gisela 6', 'PiKu 1' (Lanauskas et al., 2012: Milatović et al., 2013). A very strong effect of dwarf growing rootstocks was observed on the growth and yielding of sweet cherry cultivars (Usenik et al., 2008). Also, the rootstocks influence the flowering time (Blažkova et al., 2010), the intensity of flowering (Gratacos et al., 2008), fruits quality of sweet cherry (Balmer, 2008), cracking susceptibility. Nevertheless, growers prefer dwarfing rootstocks for producing hand-picked cherries for fresh markets, which allow planting densities of up to 1.000-5.000 trees per hectare (Musacchi et al., 2015). Also, the influence of rootstocks to grafted cultivars is expressed as differences in the efficiency of water usage, advance or delay of flowering, and harvesting, survival and yield efficiency (Cantin et al., 2010). The rootstocks also influence the response of grafted cultivars to low temperature (Hrotko et al., 2009).

Producing sweet cherry in Serbia has a long tradition. But, most cultivars were grafted onto vigorous seedling rootstocks (Prunus *avium* L. and P. *mahaleb* L.). Nevertheless, one of the prerequisites for the successful production of sweet cherries is the testing of new cultivars and rootstocks in climatic and soil conditions before its use on a large scale. The study aimed to examine the influence of different clonal rootstocks on cold hardiness in the ecological dormancy of sweet cherry cultivar 'Kordia' in Belgrade's environmental conditions. Also, the authors examined the influences of those rootstocks on fruit quality and yield of trees.

Material and methods

The study was carried out at a commercial orchard located at the experimental estate "Radmilovac" of the Faculty of Agriculture in Belgrade, in the five growing years (2020). The area has a temperate continental climate with an average annual rainfall of 650 mm. The orchard was established in spring 2016 with high-quality 1-year-old nursery trees. Sweet cherry cultivar 'Kordia' was grafted on five clonal rootstocks ('Colt', 'Gisela 5', 'Gisela 6', 'Ma×Ma 14' and 'Oblačinska sour cherry'). Trees were planted at a distance of 3.5 m × 1.6 m (1.750 trees/ha).

In the study was researched the influence of winter frost on damages of flower buds of the cultivar 'Kordia' grafted on different clonal rootstocks was researched. Winter frosts occurred during the ecological dormancy of sweet cherry, on March 14th and 15th. During the early morning hours temperature was –5°C, and another day –7^{o}C. The sensitivity of flower buds was represented like the percentage of damage and non-damage flower buds per fruiting branches. The study also included the following generative properties: several of flowers per flowers buds on fruiting branches, time of blooming and harvesting, trunk cross-sectional area (TCSA), yield per tree and yield efficiency, the mass of fruit, a diameter of fruit, soluble solids contents (SSC), total sugars (TS), total acids (TA) and sugars/acids ratio (TS/TA).

The experiment was conducted by a random field with four repetitions (100 flowers buds per tree were taken from each part of the canopy and fruiting branches, five trees were taken for a one repeat). The physical properties of fruits were determined with four repetitions, and each repetition included 50 fruits. Chemical properties were determined with three repetitions. Physical properties were determined used standard morphometrics methods. SSC was determined by refractometer (Atago, pocket PAL-1. Kyoto, Japan). TA was determined by titrating 25 g of fruits with 0.1N NaOH up to pH 7.0 and expressed in %. TS, was determined by the Luff-Schoorl method and expressed in %. Analysis of variance has been done with STATISTICA 9 software package. The significant differences between means determined at P<0.05, were measured with the LSD test.

Results and discussions

Grafted rootstock had a significant influence on the sensitivity of flower buds to winter frost (Table 1). The highest percentage of damaged flowers had trees grafted on 'Oblačinska sour cherry' and 'Colt', (on average 61.7% and 45.8% of total numbers of flowers, respectively) while trees grafted on 'Ma×Ma 14' (12.3%) had the lowest. All trees had a higher percentage of damaged flowers on short fruiting branches (SFB) compared to long fruiting branches (LFB). Average to the all cultivars percentage of damaged flower buds on SFB was 41.5%, while on LFB was 29.3%. Trees on 'Ma×Ma 14' only had less than 10% of damaged flowers on LFB. Further, trees grafted on 'Gisela 5' had similar percentage of damaged flowers on both types of branches. Also, the significantly sensitivity of flower buds of sweet cherries cultivar grafted on 'Gisela 5' to the winter frost was recorded by other authors (Milatović et al., 2013).

Table 1. Percentage of damage and number of flowers per generative buds in different fruiting branches of sweet cherry cultivar'Kordia' grafted on different vegetative rootstocks.

	Frost damaged flower buds (%)			Number of flowers per a flower bud	
Rootstock	*SFB (%)	LFB (%)	Average (%)	SFB	LFB
Colt	57.7 a	42.6 a	45.8 a	2.3 b	2.9 ab
Gisela 5	48.5 ab	44.3 a	44.7 a	2.4 b	2.8 ab
Gisela 6	28.4 b	21.4 b	24.6 b	2.5 b	2.5 b
Ma×Ma 14	19.6 b	8.9 c	12.3 b	2.9 a	3.2 a
Oblacinska sour cherry	65.1 a	45.2 a	61.7 a	2.3 b	2.8 ab
Lsd, p <0.05	21.2	11.5	17.3	0.3	0.4

*SFB – short fruiting branch; LFB – long fruiting branch

The rootstocks had a significant influence on flower buds differentiation. The highest number of flowers per a generative buds on SFB had trees grafted on 'Ma×Ma 14' (2.9), while the lowest had trees grafted on 'Colt' and 'Oblacinska sour cherry' (2.3). On LFB trees grafted on 'Ma×Ma 14' had the highest number of flower per buds (3.2), while the lowest had trees on 'Gisela 6' (2.5). All trees had a higher number of flowers per a generative buds in LFB compared to SFB.
The earliest beginning of the blooming had trees grew on 'Oblačinska sour cherry', while trees grafted on 'Ma×Ma 14' had the latest (Table 2). The earliest full blooming stage had trees grafted on 'Oblačinska cherry and 'Gisela 5'. Fruits of the cultivar 'Kordia' had the earliest ripening on rootstocks 'Gisela 5' and 'Oblačinska sour cherry', while trees grafted on 'Ma×Ma 14' had the latest ripening of fruits. The longest period of full blooming to harvesting had trees grew on 'Colt', while shortest on 'Gisela 5' and 'Ma×Ma 14'. The shortest duration of blooming period had trees grew on 'Oblačinska cherry' (13 days), while trees on 'Ma×Ma 14' had the longest (16 days).

The worldwide trends are driven towards controlling tree vigour with dwarfing rootstocks (Ayala and Lang, 2017). Trees grafted on 'Colt' had significantly higher values of TCSA compared to other rootstocks, only trees grew on 'Ma×Ma 14' had similar values. Significantly higher value of TCSA of sweet cherry trees grafted on 'Colt' compared to 'Ma×Ma 14' rootstocks was recorded by Hrotko *et al*. 2009. Cultivar 'Kordia' had the highest yield per tree when grafted on rootstock 'Ma×Ma 14', while the lowest had on 'Oblačinska sour cherry', 6.5 kg and 2.5 kg respectively.

Table 2. Pomological parameters of sweet cherry cv 'Kordia' grafted on clonal rootstocks.

Rootstock	Beginning of blooming	Full blooming	End of blooming	Time of harvest	TSCA (cm^2)	Yield per tree (kg)	Yield efficiency (kg/cm^2)
Colt	01 April	06 April	13 April	01 Jun	31.2 a	3.5 b	0.11 c
Gisela 5	30 March	05 April	11 April.	28 May	21.2 b	5.2 ab	0.25 ab
Gisela 6	31March	06 April	11 April	30 May	24.6 b	6.1 ab	0.25 a
Ma×Ma 14	03 April	10 April	16 April	01Jun	27.3 ab	6.5 a	0.24 a
Oblacinska sour cherry	29 March	05 April	10 April	28 May	13.9 c	2.5 b	0.18 b
Lsd, p<0.05					5.4	2.3	0.05

The highest yield efficiency had trees grafted on 'Gisela 5' and 'Gisela 6' (0.25 kg/cm^2), while statistical significant lower yield efficiency had trees grafted on 'Oblačinska cherry' (0.18 kg/cm^2) and 'Colt' (0.11 kg/cm^2). Cantin et al. (2010), noticed that the highest yield efficiency of trees grafted on 'Gisela 5' and 'Gisela 6' rootstocks was due to its low trunk cross-sectional area. Similar results were obtained by Rubauskis *et al*. (2014). Also, Hrotko et al. (2009) recorded that trees with higher values of TCSA had significantly lower values of yield efficiency, which confirmed in our results.

Table 3. Physical and chemical properties of fruits of cv 'Kordia' grafted on clonal rootstocks.

Rootstock	Mass of fruits (g)	Diameter of fruits (mm)	Soluble solids (%)	Total sugars (%)	Total acids (%)	Sugar/acid ratio
Colt	12.5 a	30.6 a	17.1 ab	14.8 ab	0.9 a	16.4 b
Gisela 5	9.8 b	26.3 b	16.5 b	14.6 ab	0.8 a	18.3 b
Gisela 6	11.8 ab	29.8 ab	17.6 ab	16.1 ab	0.9 a	17.9 b
Ma×Ma 14	11.6 ab	28.9 ab	18.9 a	17.3 a	0.8 a	21.6 a
Oblacinska sour cherry	10.2 b	27.2 b	16.2 b	14.9 b	0.7 a	21.3 a
Lsd, <0.05	2.1	2.2	2.1	1.9	0.2	2.3

The biggest fruits had trees that grafted on 'Colt' (12.5 g), while the smallest had trees that grafted on 'Gisela 5' (9.8 g). According to Whiting et al., 2005, trees on the dwarfing rootstocks had strong tendency to overcrop, resulting in a much lower leaf-to-fruit ratio, and subsequently smaller fruit size and suppressed vegetative growth. Usenik et al., (2008) noticed that higher leaf area per trees affected significantly fruits mass and diameter. Increased yield efficiency and reduced tree volume of sweet cherry on dwarfing rootstocks affected low fruit quality (Usenik et al., 2006). Aglar and Yildiz (2014) recorded that fruits weight varied according to the rootstock used in a study, and the biggest fruits were produced on trees grafted on 'Ma×Ma 14' and 'SL 64' rootstock, while the trees grafted on 'Gisela 5' had the smallest fruit. Rootstock type had a significant effect on the contents of SSC and TA, and a lowest values were found in fruits from the trees which grew on vigour rootstock (Dziedzic and Blaszczyk, 2019) which is not confirmed in ours study. Trees grown on 'Gisela 5' and 'Oblacinska sour cherry' had fruits with the smallest content of soluble solids. It might be due to the lower leaf-to-fruit ratio which caused a decrease in synthesising of carbohydrates. Also, because of the leaf size variability, a source-to-sink ratio of 200 cm^2 leaf area per fruit is the standard lower limit providing fruit mass of 10–12 g (Ayala and Lang, 2017). The highest values of sugars/acids ratio had the fruit of trees grown on 'Ma×Ma 14' and 'Oblačinska sour cherry'.

Conclusion

In a view of the importance of sweet cherry for fruit growers, the determination of the influence of clonal rootstocks on biological and pomological properties to cultivars is very important. The results indicated that cultivar 'Kordia' grafted on 'Ma×Ma 14' and 'Gisela 6' had the lowest percentage of frost damaged flower buds (less than 25%). Also, trees that grew on those rootstocks had higher yield and better physical properties of fruits. The highest values of yield efficiency had trees grown on 'Gisela 5' and 'Gisela 6'. According to present study and obtained preliminary results, a growing of cultivar 'Kordia' on rootstocks 'Ma×Ma 14' and 'Gisela 6' can be recommended to producers of sweet cherries in similar agroecological conditions.

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References

Ağlar, E. & Yıldız, K. (2014). Influence of Rootstocks (Gisela 5, Gisela 6, Ma×Ma , SL 64) on performance of '0900 Ziraat' sweet cherry. Journal of Basic and Aplied Sciences, 10: 60-66.

Ayala, M. & Lang, G. (2017). 12. Morphology, cropping physiology and canopy training. Quero-García, J., Iezzoni, A., Puławska, J., Lang, G (Eds.), Cherries: Botany, Production and Uses. CABI, Oxfordshire, Wallingford, 269-304.

Balmer, M. (2008). Evaluation of semi-dwarfing rootstocks for sweet cherry orchards in the Rhine river valley (Germany). Acta Horticulturae, 795: 203-207.

Bielicki, P. & Rozpara, E. (2010). Growth and yielding of Kordia sweet cherry trees with various rootstock and interstem combinations. Journal of Fruit and Ornamental Plant Research, 18 (1): 45-50.

Blažkova, J., Drahošova, H. & Hlušičkova, I. (2010). Tree vigour, cropping, and phenology of sweet cherries in two systems of tree training on dwarf rootstocks. Horticultural Science (Prague), 37: 127-138.

Brüggenwirth, M. & Knoche, M. (2016). Mechanical properties of skins of sweet cherry fruit of differing susceptibilities to cracking. Journal of the American Society of Horticultural Science, 141: 162–168.

Cantin, C.M., Pinochet, J., Gogorcena, Y. & Moreno, M.A. (2010). Growth, yield and fruit quality of Van and Stark Hardy Giant sweet cherry cultivars as influenced by grafting on different rootstocks. Scientia Horticulturae, 123: 329-33.

Deķena, D., Feldmane, D. & Laugale, V. (2020). Preliminary results of rootstock evaluation for Estonian sweet cherry cultivar 'Anu'. Agronomy Research, 18(S4): 2693–2700.

Dziedzic, E. & Błaszczyk, J. (2019). Evaluation of sweet cherry fruit quality after short‑term storage in relation to the rootstock. Horticulture, Environment, and Biotechnology, 60: 925–934.

Dziedzic, E., Bieniasz, M. & Kowalczyak, B. (2019). Morphological and physiological features of sweet cherry floral organ affecting the potential fruit crop in relation to the rootstock. Scientia Horticulturae, 251: 127-135.

Gratacos, E., Cortes, A. & Kulczewski, B.M. (2008). Flowering, production and fruit quality of eleven sweet cherry cultivars in central Chile. Acta Horticulturae, 795: 331-338.

Hrotkó, K., Magyar, L. & Gyevik, M. (2009). Effect of rootstocks on growth and yield of 'Carmen'sweet cherry. Bulletin UASVM Horticulture, 66(1): 143-148.

Koutinas, N., Pepelyankov, G. & Lichev, V. (2010). Flower induction and flower bud development in apple and sweet cherry. Biotechnology & Biotechnological Equipment, 24: 1549–1558.

Lanauskas, J., Uselis, N., Kviklys, D., Kviklienė, K. & Buskienė, L. (2012). Rootstock effect on the performance of sweet cherry cv. Lapins. Horticultural Science (Prague), 39(2): 55–60.

Milatović, D., Đurović, D., Đorđević, B., Vulić, T. & Zec, G. (2013): Rodnost novijih sorti trešnje na podlozi Gizela 5. *Voćarstvo*, 47 (181-182): 39-45.

Musacchi, S., Gagliardi, F. & Serra, S. (2015). New training systems for high-density planting of sweet cherry. HortiScience, 50(1): 59-67.

Pal, M.D., Mitre, I., Asanica, A.C., Sestras, A.F., Peticila, A.G. & Mitre, V. (2017). The influence of rootstock on the growth and fructification of cherry cultivars in a high density cultivation system. Notulae Botanicae Horti Agrobotanici *Cluj-Napoca*, 45(2): 451–457.

Radunić, M., Jazbec, A., Ercisli, S., Čmelik, Z. & Goreta-Ban, S. (2017). Pollen-pistil interaction influence on the fruit set of sweet cherry. Scientia Horticulturae, 224: 358-366.

Rubauskis, E., Skrivele, M., Ruisa, S. & Feldmane, D. (2014). Growth and yield of two sweet cherry cultivars on vegetatively propagated rootstocks. Acta Horticulturae, 1058: 657-661.

Usenik, V., Fajt, N. & Stampar, F. (2006). Effects of rootstocks and training system on growth, precocity and productivity of sweet cherry. Journal of Horticultural Science and Biotechnology, 8: 153-157.

Usenik, V., Štampar, F. & Fajt, N. (2008). Sweet cherry rootstocks testing in Slovenia. Acta Horticulturae, 795: 273-276.

Zhang, L., Ampatzidis, Y. & Whiting, M. (2015). Sweet cherry floral organ size varies with genotype and temperature. Scientia Horticulturae, 182: 156-164.

Whiting, M.D., Lang, G. & Ophardt, D. (2005). Rootstock and training system affect sweet cherry growth, yield and fruit quality. HortScience, 40(3): 582-586.

EFFECT OF CALCIUM CHLORIDE ($CaCl_2$) ON THE QUALITY OF APPLE CV 'RED CHIEF' (*Malus × domestica* Borkh.) DURING STORAGE

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Abstract

The paper presents the two-year results of research regarding the impact of calcium chloride ('Stopit') on changes in morphometric properties of the fruit, firmness and the soluble solids content (SSC) in apple cv 'Red Chief', at harvest and during storage of fruits (60 and 120 days) in the regular cold chamber. Foliar treatments were performed in four replications during vegetation (on June 7th, July 2nd, July 20th, August 15th – in 2018; June 14th, July 5th, July 26th, August 16th – in 2019). The obtained results indicate that fruit weight was significantly influenced by the calcium treatment and storage duration, while fruit dimensions varied under influence of storage duration only. The firmness and content of soluble solids was changing under the influence by all tested parameters and interaction effect of the study year and calcium treatment as well as storage duration. In the first year, fruits had higher weight (190.4 g), dimensions and firmness (12.4 N), whereas the SSC content was significantly higher in the second year of the study (16.2 °Brix). The fruits treated with calcium chloride had higher firmness (10.3 N) and soluble solids content (14.2 °Brix), especially after storage of 60 and 120 days (14.9 and 15.1 °Brix) compared to the soluble solids content in fresh fruits (11.5 °Brix). The stated results were confirmed by the highest values of the SSC recorded in the second year of the study after 60 days of fruit storage (17.9 °Brix). Based on the obtained results, it can be concluded that foliar application of calcium chloride during vegetation can be an effective measure with which losses in quality of apple fruit during storage in the regular cold chamber can be avoided with no negative effect on consumer acceptability.

Keywords: *apple, calcium treatment, morphometric properties, soluble solids.*

Introduction

Apple (*Malus × domestica* Borkh.) is one of the most represented and economically most important fruit species. World apple production in 2019 amounted to 87.236.221 t. In the Republic of Serbia, apple has been grown on (an area of) 26.089 ha, with total production of 499.578 t (Faostat, 2019). In the overall structure of fruit production, apple ranks fourth, behind agrumes, grapes and bananas (Milošević et al., 2019), representing the most important temperate fruit species. Fruits of apple can be consumed fresh immediately after harvest or after a pre-defined storage period (Folta and Gardiner, 2009). They are most often stored for a longer period at low temperatures in a controlled atmosphere. However, during storage, there comes to the fruit quality and nutrient deterioration. Losses in quality are mainly caused by relatively high metabolic activity of the fruit during storage. Sams et al. (2008) report that many physiological and pathological disorders of apple fruit during storage are conditioned by low calcium content (Ca^{2+}) in the fruit tissue. Calcium concentration in plant tissue has an extremely significant role in maintaining fruit quality after harvest. Numerous authors (Hossain et al., 2005; Misra and Gupta, 2006; Naeem et al., 2009) emphasize that application of the calcium based product has positive effects on stabilization of the cell membrane and fruit maturation delay, maintain fruit firmness, reduce occurrence of the so called 'bitter pits' and internal fruit decay. (Raese and Drake, 2002; Dierend and Rieken, 2007; Suljevic et al., 2011). In this regard, all ageing processes flow much faster in the lack of calcium, and fruits have lower storage capacity. There are a number of calcium containing products on the market which can be applied before or after harvest and thus delay fruit maturation with no negative effects on consumers. (Lester and Grusak, 1999). One of the products with which lack of calcium can be prevented or removed, and thus, affect the quality of apple fruit, is 'Stopit', where calcium is in the form of calcium chloride. In the last decades, one of economically most important and demanded apple cultivars on the market is 'Red Chief'. Fruit size, flesh firmness and red skin colour are considered the most important characteristics due to which consumers prefer this apple cultivar (Iglesias et al., 2002). Since apple storage in an atmosphere with utterly low oxygen content (ULO) is expensive and to a larger extent, not available to developing countries, it is important to determine the effect of calcium chloride on changes in the fruit quality of apple cultivar 'Red Chief' stored in normal atmosphere (NA).

Material and Methods

The research was conducted in the period 2018–2019 in the production and experimental plantation of apple cultivar 'Red Chief' at the experimental field of Fruit Research Institute in Čačak (43° 89' 40" SGŠ, 20° 43' 42" IGD, altitude 233 m) in Serbia. The plantation was established in 2006, and the seedlings were grafted at rootstock M9. The training system was (slender) spindle bush and the spacing 4 × 1 m (2.500 trees ha^{-1}). During the research period, standard agro and pomotechnical measures were applied. The research included foliar application of the 'Stopit' product, with concentration of calcium chloride 224 g L^{-1}. Foliar application of fertilizer 'Stopit' was applied on 20 trees in four replications (a total of 80 trees per treatment), from the beginning of June until the middle of August (from the beginning of flowering until the end of maturation phenophase, i.e. from the fruit pigmentation phase until harvest). In both years of research, the product was applied four times, of which in 2018 was on June 7th, July 2nd, July 20th, and August 15th and in 2019, on June 14th, July 5th, July 26th and August 16th. Foliar application was done by a motor sprayer SR 420 (STIHL International GmbH Waiblingen, Germany), with usage of 1.000 L ha^{-1}. The product was applied in the amount of 7,5 L ha^{-1} (150 mL on 10 L water). Trees of apple cultivar 'Red Chief' that were not treated with calcium chloride, served as control. Morphometric properties of the fruit (weight, width, height), fruit firmness and the soluble solids content. The stated parameters were determined at harvest (I) and after 60 (II) and 120 days (III) of fruit storage in a cold chamber with normal atmosphere. Testing of weight (g), height and width (mm), fruit firmness (N), as well as the soluble solids content in fruit (°Brix) were done by standard morphometric methods on a sample of 80 fruits (four replications with 20 fruits each). Fruit weight (g) was determined by measuring on a technical scale (Adventurer Pro AV812M, Switzerland), while fruit length and width (mm) were determined using a digital calliper (Carl Roth, Germany). Fruit firmness was determined by a digital penetrometer (Model FHT-803, Italy), and the obtained values were expressed in N. The soluble solids content in apple fruit was determined using a digital refractometer (Carl Zeiss, Jena) at room temperature (20 °C), and the values were expressed in °Brix. The results were presented as mean ± standard error of mean (SE). Differences between mean values were compared by Duncan's test in a three-way analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with p values of ≤ 0.05 were considered insignificant.

Results and Discussion

Results of studying the influence of year, treatment and duration of storage on morphometric properties, firmness and the soluble solids content in the fruit of apple 'Red Chief', depending on calcium chloride application, were shown in Table 1. By analysis of variance, it was found that all studied parameters were under significant influence of fruit storage duration. The fruit firmness and soluble solids content varied significantly under influence of the year and treatment. Fruit height was changing under the impact of the study year, while fruit weight was changing under the impact of calcium chloride application. Interaction effect of year and treatment as well as the year and duration of fruit storage, exhibited a significant impact on the soluble solids content in the apple fruit.

Table 1. Influence of year, calcium chloride treatment and storage duration on morphometric properties and the soluble solids content in the fruit of apple cultivar 'Red Chief'

Treatment	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Firmness (N)	Soluble solids content (°Brix)
Year (A)					
2018	190.4±2.2 a	70.5±0.4 a	75.3±0.3 a	12.4±0.3 a	11.5±0.3 b
2019	188.9±2.5 a	67.9±0.5 b	74.9±0.4 a	7.1±0.3 b	16.2±0.4 a
Treatment (B)					
Treatment	186.5±1.8 b	68.7±0.6 a	75.1±0.3 a	10.3±0.7 a	14.2±0.6 a
Control	192.8±2.6 a	69.7±0.5 a	75.1±0.4 a	9.3±0.6 b	13.5±0.6 b
Storage (C)					
I	196.2±2.2 a	70.5±0.5 a	76.3±0.4 a	11.6±0.7 a	11.5±0.5 b
II	189.0±3.3 ab	69.3±0.7 ab	75.1±0.5 b	9.4±0.7 b	14.9±0.8 a
III	183.7±1.9 b	67.9±0.7 b	73.9±0.4 c	8.3±0.7 c	15.1±0.6 a
ANOVA					
A	ns	*	ns	*	*
B	*	ns	ns	*	*
C	*	*	*	*	*
A × B	ns	ns	ns	ns	*
A × C	ns	ns	ns	ns	*
B × C	ns	ns	ns	ns	ns
A × B × C	ns	ns	ns	ns	ns

Values within each column followed by the same small letter are not significantly different at p≤0.05 by LSD test; ns - non-significant differences.

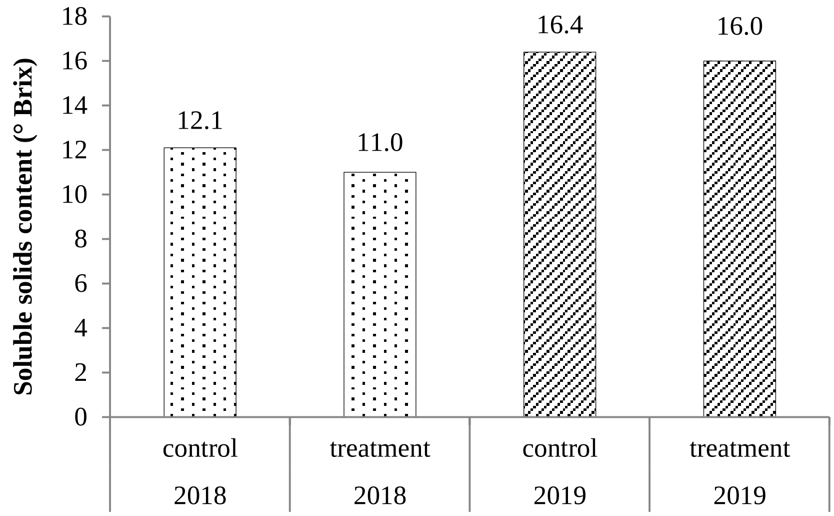
Fruit weight of apple cultivars varies from 70 to 500 g, of which edible part accounts for 98% (Mišić, 1994). According to Mišić (2004), fruits of apple cultivar 'Red Chief' belong to the group of middle large to large (120 – 200 g). Average values of the fruit weight in our research varied from 183.7 to 196.2 g, values of height and width from 67.9 to 70.5 mm, that is, from 73.9 to 76.3 mm, of firmness, from 7.1 to 12.4 N, while the soluble solids content varied from 11.5 to 16.2 °Brix. According to Gvozdenović (1998), fruits of attractive apple cultivars should have fruit dimensions from 65 to 75 mm, which is in accordance with results obtained in our research.

Morphometric properties of apple fruit are genetically conditioned, although their varying could, to a significant extent, be caused by environmental factors (Krgović, 1990; Krpina et al., 2004). In 2018, the fruits had higher values of height (70.5 mm) and fruit firmness (12.4 N) in comparison with those in 2019. On the other side, the soluble solids content was significantly higher in 2019 (16.2 °Brix). Values of fruit weight and width did not vary significantly under the influence of the study year.

Fruit firmness represents one of the significant physical properties, that is, an indicator of fruit ripeness dictating the duration of fruit storage as well as their timely appearance on the market. Apple producers are capable to prolong the time of bringing fruits on the market by keeping them in cold chambers and delaying ripening, i.e. by maintaining the stability of fruit firmness. Fruits treated with calcium chloride had higher firmness (10.3 N) and the soluble solids content (14.2 °Brix) compared to control, while the fruit weight had higher values in the control (192.8 g). Research results obtained in this work are consistent with statements of Asgharzade et al. (2012) that treating apple with calcium chloride before harvest significantly affects the increase of fruit flesh. In addition, Benavides et al. (2002) and Casero et al. (2004) report that their application reduces softening and keeps fruit firmness in apple during storage. Positive effects of calcium chloride application are most likely associated with the calcium content in fractions of covalently bound pectins (Siddiqui and Bangerth, 1995). Moreover, calcium affects stabilization of cell membranes and thus can prevent physiological disorders prescribed to its deficit (Saure, 2005). Influence of the calcium chloride-based product on fruit dimensions was not statistically significant. During the fruit storage, values of the studied parameters were declining and the lowest were recorded after fruit storage for 120 days in a cold chamber with normal atmosphere, except for the soluble solids content. The highest values of weight (196.2 g), height (70.5 mm), width (76,3 mm) and fruit firmness (11.6 °Brix) were recorded at harvest while the highest value of the soluble solids content (15.1 °Brix) was recorded after storage of fruits for 120 days.

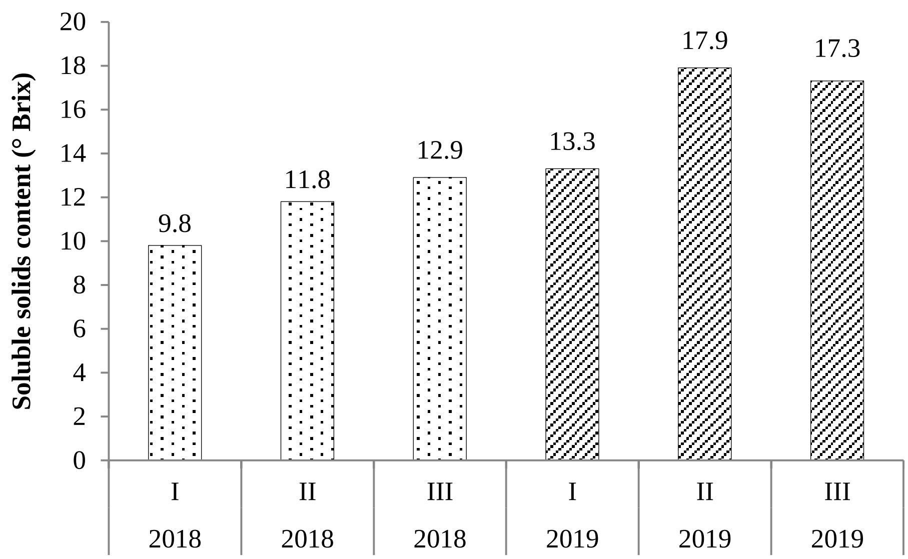
Results of studying the influence of year and calcium chloride treatment as well as the year and duration of fruit storage on the soluble solids content in the fruit of apple cultivar 'Red Chief' were shown in the graphics 1 and 2.

Interaction effects year/treatment and year/storage duration had a significant impact on the soluble solids content in the fruit of apple cultivar 'Red Chief'.



Graph 1. Influence of year and treatment on the soluble solids content in the fruit of apple cultivar 'Red Chief'

Generally, values of the soluble solids content were lower in 2018 compared to those in 2019. Calcium chloride treatment did not significantly affect changes in the content of the soluble solids content compared to control.



Graph 2. Influence of year and storage duration on the soluble solids content in the fruit of apple cutivar 'Red Chief'

In addition, observing the influence of interaction effect of year and storage duration on the soluble solids content, those recorded in the second year of the study can be said to prevail. Fruits analyzed at harvest period had the lowest while the fruits stored for 60 and 120 days in a cold chamber with notmal atmosphere had the highest values of soluble solids in both study years. Also, Netravati et al. (2018) report that after the calcium chloride treatment and apple fruit storage in cold chambers, the soluble solids content increases.

The soluble solids content is one of the most important parameters determining quality, and thus consumer acceptability. It is increased with fruit ripening, representing the basic indicator of apple fruit ripeness. The soluble solids content higher than 11,0% in fruit growing practice is considered the lower threshold of consumer acceptability for an apple cultivar, and if values of this parameter are higher than 13,5%, consumer acceptability is better.

Conclusions

Based on the results obtained, it can be concluded that foliar application of calcium chloride during vegetation can be an efficient measure with which losses in apple fruit quality can be avoided, prior to all in firmness and the soluble solids content, during storage in a cold chamber with normal atmosphere. In this regard, producers may be given some guidelines for enhancement of apple growing technology through application of a calcium-based product, all aimed at obtaining high quality fruits without negative effects on consumer acceptability.

References

Asgharzade, A., Ali Valizade G., and Babaeian M. (2012). Effect of Calcium Chloride ($CaCl_2$) on some quality characteristic of apple fruits in Shirvan region. African Journal of Microbiology Research, Vol. 6, No. 9, pp. 2000−2003.

Benavides, A., Recasens I., Casero T., Soria Y., and Puy J. (2002). Multivariate analysis of quality and mineral parameters on Golden Smoothee apples treated before harvest with calcium and stored in controlled atmosphere. Food Science Technology International, Vol. 8, pp. 139−145.

Casero, T., Benavides A., Puy J., and Recasens I. (2004). Relationships between leaf and fruit nutrients and fruit quality attributes in Golden Smotthee apples using multivariate regression techniques. Journal Plant Nutrition, Vol. 27, pp. 313−324.

Dierend, W. and Rieken S. (2007). Postharvest treatment of apples with Ca chloride. Erwerbs–Obstbau Vol. 49, pp. 51–56.

Faostat, 2019. http//www.fao.org/faostat/en/#data/QC. Available 31st May, 2021.

Folta, K. M. and Gardiner S. E. (2009). Plant genetics and genomics. Genetics and genomics of Rosaceae. Springer Science Business Media, Vol. 6, pp. 162.

Gvozdenović, D. (1998). The apple, Faculty of Agriculture, Novi Sad, Serbia.

Hossain, A. K. M., Koyama H., and Hara T. (2005). Sugar compositions and molecular mass distributions of hemi cellulosic polysaccharides in wheat plants under aluminum stress at higher level of calcium supply. Asian Journal Plant Science, Vol. 4, No. 1, pp. 11−16.

Iglesias, I., Echeverría, G., and Soria, Y. (2008). Differences in fruit colour development, anthocyanin concentration, fruit quality and consumer acceptability of eight 'Gala' apple strains. Scientia Horticulturae, 119: 32–40.

Krgović, Lj. (1990). Important Pomological and Technological Characteristics of Fruit Varieties of Apples Cultivated in Polimlje. Yugoslav Fruit Growing, Vol. 94, pp. 29–33.

Krpina, I., Vrbanek, J., Asić, A., Ljubicić, M., Ivković, F., Ćosić, T. (2004). Fruit Production, Globus publishing house, Zagreb, Croatia.

Lester, G. E. and Grusak M. A. (2004). Postharvest application of calcium and magnesium to honeydew and netted muskmelons: Effects on tissue ion concentrations, quality and senescence. Journal American Society Horticultural Science, Vol. 124, pp. 545−552.

Milošević, T., Milošević, N., Mladenović, J. (2019). Role of rootstock and apple fruit tissue in antioxidant activity. Acta Agriculturae Serbica, XXIV, 48: 97–106.

Mišić, P. (1994). The apple, Nolit, Belgrade, Serbia.

Mišić, P. D. (2004). The apple. Nolit, Belgrade, Serbia.

Misra, N. and Gupta A. (2006). Interactive effects of sodium and calcium on proline metabolism in salt tolerant green gram cultivar. American Journal Plant Physiology, Vol. 1, No. 1, pp. 1−12.

Naeem, M., Masroor M., Khan A., and Morris J. B. (2009). Agro botanical attributes nitrogen fixation, enzyme activities and nutraceuticals of Hyacinth bean (Lablab purpureus L.): A Bio-Functional Medicinal Legume. American Journal Plant Physiology, Vol. 4, No. 2, pp. 58−69.

Netravati, Suresh G.J., and Jagadeesh S.L. (2018). Calcium chloride and wax influences the postharvest behaviour of custard apple fruits. Journal of Pharmacognosy and Phytochemistry, Vol. 7,No. 2, pp. 79-84.

Raese, J. T. and Drake S.R. (2002). Ca spray materials and fruit Ca concentrations influence apple quality. Journal American Pomological Society, Vol. 56, pp. 136−143.

Sams, E. C., Trentham W.R., and Conway E.W. (2008). Histological Effects of Calcium Chloride in Stored Apples. Journal American Society Horticultural Science, Vol. 133, No. 4, pp. 487−491.

Saure, M.C. (2005). Calcium translocation to fleshy fruit: Its mechanism and endogenous control. Science Horticulture, Vol. 105, pp. 65−89.

Siddiqui, S. and Bangerth F. (1995). Effect of pre-harvest application of calcium on flesh firmness and cell-wall composition of apples. Influence of fruit size. Journal Horticultural Science, Vol. 70, pp. 263−269.

ANTIOXIDANT ACTIVITY ESTIMATION OF INNER AND OUTER SEED FRACTIONS OF THE LEGUMES *VIGNA RADIATA* L. AND *GLYCINE MAX* L.

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Abstract

Legumes have multiple functions in sustainable agriculture, but also are a favourable ingredient of functional nutrition. Antioxidants in legumes have several beneficial physiological properties and provide protection against chronic diseases. In this study, we compared the antioxidant activities of the seed fractions (outer and inner) for two different legumes: mung bean (*Vigna radiata* L.) and soybean (*Glycine max* L). The antioxidant activity was estimated using a modified DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay in a 96-well microplate. We showed that mung bean hulls possessed significantly higher ($p<0.05$) DPPH free radical scavenging activity (80.80 % ± 0.19) compared to their inner fraction (10.94 % ± 0.23), as well as to both fractions of the soybean. On the other hand, the soybean seeds' inner fractions (21.00 % ± 0.32) exhibited a significantly ($p<0.05$) higher activity than the hulls (8.78 % ± 0.71) and the inner fraction of the mung bean. The obtained results indicated that in each of the two analysed legume species, inner and outer seed fractions exhibited different antioxidant activities regarding to the elimination of the free radicals. The obtained results indicate that antioxidant capacity may be a useful indicator in the estimation of the quality of legume seeds as food and feed.

Keywords: *Antioxidant activity, Soybean, Mung bean, DPPH, Food quality.*

Introduction

Leguminous seeds are considered as significant source of nutrients (proteins, essential amino acids, starch, fibers, minerals, vitamins) but they also, depending on the biological variety of the plants and their origin, contain different bioactive substances including phenolics. Secondary metabolites are involved in protection of the seeds against plant pathogens, wounds caused by insect pests and herbivores, UV radiation and other biotic and abiotic stress conditions (Yusnawan et al. 2019). Antioxidant activity of various phenolic compounds from legume seeds (flavonoids, alkaloids, tannins, phenolic acids) is important both from nutritional and technological points of view (Amarowicz and Pegg 2008). Legumes are excellent functional food ingredients and a great dietary source of antioxidants due to their nutrient composition (Ganesan and Xu, 2018). Health benefits related to antioxidants contained in edible food sources include reduced risk and prevention of developing major chronic diseases such as cancer, diabetes or cardiovascular diseases (Singh et al. 2017). Phenolic antioxidants and polyphenols, that protect the seeds against the harmful effect of oxygen free radicals, are typically present in high content in seed hulls. Mung bean seeds are consumed as a whole or after separation the hulls while soybean seed hulls are usually discarded as a byproduct. This biowaste has a high polyphenol content which could be extracted and used as nutraceuticals, a source of natural antioxidants, or used as animal feed (Singh et al. 2017).

Mung bean (*Vigna radiata* L.), a summer cultivated legume widely distributed and consumed throughout Asia, has a high nutrient value comparable to that of soybean (*Glycine max* L) (Shi et al. 2016; Orak et al. 2018). Mung bean also contains bioactive food components and polyphenols which possess a high antioxidant capacity (Orak et al. 2018). Soybean is an annual self-pollinated diploid leguminous plant, predominantly grown for use in human and animal diet and as a major source of protein and oil (Gaonkar and Rosentrater 2019). Aside from the nutritional value, soybean contains isoflavones and certain phenolic compounds (Wang and Komatsu 2017). The antioxidant potential of soybean has also proven to have health benefits, especially in their seed coat (Peiretti et al. 2019).

In the present study, differences in antioxidant capacity between two seed fractions (inner and outer) of the two different legume species, soybean and mung bean, were examined. Aqueous-ethanol extracts of both seeds and hulls were examined for their respective radical scavenging activity with DPPH (2,2-Diphenyl-1-picrylhydrazyl) reagent. In a reaction with antioxidants, DPPH is reduced to DPPH-H while the color changes from purple to yellow and the decrease in absorbance maximum at 517 nm is spectrophotometrically detected (Khan et al. 2017).

Material and Methods

Sample preparation

Soybean and Mung bean were purchased from the local market in Belgrade, Serbia. Seed hulls have been separated from the seed endosperm before homogenization, and considered as outer fractions. The rest of the seed (endosperm and embryo) was the inner fraction. Both fractions of the seed were grinded separately in a mill. The samples were further powdered in a mortar with pestle with liquid nitrogen and stored in the dark at 5 ± 3 °C until use.

DPPH Assay

Antioxidant activity (AA %) of both legumes (mung bean and soybean) seeds and hulls, was carried out separately using DPPH (Sigma-Aldrich, St. Louis, MO, USA) test (Khan et al. 2017). Compared to the original method, described by Khan et al. 2017, the procedure was modified in terms of absorbance measurement; smaller sample volumes were required since a microplate reader was used instead of UV/VIS Spectrophotometer. This method enables rapid analysis and possibility of screening a large number of samples.

Powdered samples (8 mg) were extracted with 3 ml of 70 % ethanol. To each sample, 1 ml of freshly prepared 0.4 mM DPPH solution in 96 % ethanol was added to a final concentration of 0.1 mM. After 30 minutes of incubation with constant shaking in the dark, 200 μl aliquots were taken and absorbance at 517 nm was measured using a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Blank consisted of pure deionized water without any additions. Control contained 0.1 mM DPPH in 96 % ethanol solution. Antioxidant activity was calculated using the following equation (Khan et al. 2017):

$$A\ \% = 100 - \left(\frac{A_{sample} - A_{blank}}{A_{control}}\right) \times 100$$

The results were presented as a percentage of depleted DPPH-reagent. Readings were carried out in four replicates for each analysed seed fraction and, based on obtained data, the standard error was calculated. Mann-Whitney sum rank test has been used for statistical analysis of the obtained results.

Results and Discussion

Images of mung bean and soybean seeds are shown in Figure 1. The obtained results indicate that mung bean seed coats possessed significantly higher ($p<0.05$) DPPH radical scavenging activity, according to Mann-Whitney test ($p=0.021$), 80.80 % ± 0.19, compared to their inner fraction of the seed being only 10.94 % ± 0.23 (Figure 2). Our results are consistent with previously reported studies showing that mung bean hull contained higher antioxidant capacity compared to the inner fraction of the seed (Singh et al. 2017). Pigmented grain such as mung bean contains a higher concentration of secondary metabolites especially in the seed coat. These phytochemicals contributing to antioxidant activity, such as phenolic acids and flavonoids (flavones, flavonoids, isoflavone, and isoflavonoids), are distributed unevenly through the seed fractions (Yusnawan et al. 2019). Some of the major polyphenols (caffeic acid, syringic acid, chlorogenic acid, ferulic acid and *p*-coumaric acid) have been detected in mung bean (Singh et al. 2017). Furthermore, water-soluble polysaccharides (containing mannose, rhamnose, and galactose) from mung bean hulls exhibit high DPPH radical-scaveninging activities (Ganesan and Xu, 2018).

Figure 1. Image of mung bean (left) and soybean (right) seeds.

Figure 2 shows antioxidant activity (AA %) of both inner and outer fractions of soybean. Soybean seeds' inner fraction showed a significantly higher antioxidant activity compared to the soybean seed coats, 21.00 % ± 0.32 and 8.78 % ± 0.71, respectively. These results are in accordance with Lim et al. (2021), who showed higher antioxidant activity in cotyledon compared to the seed coat.

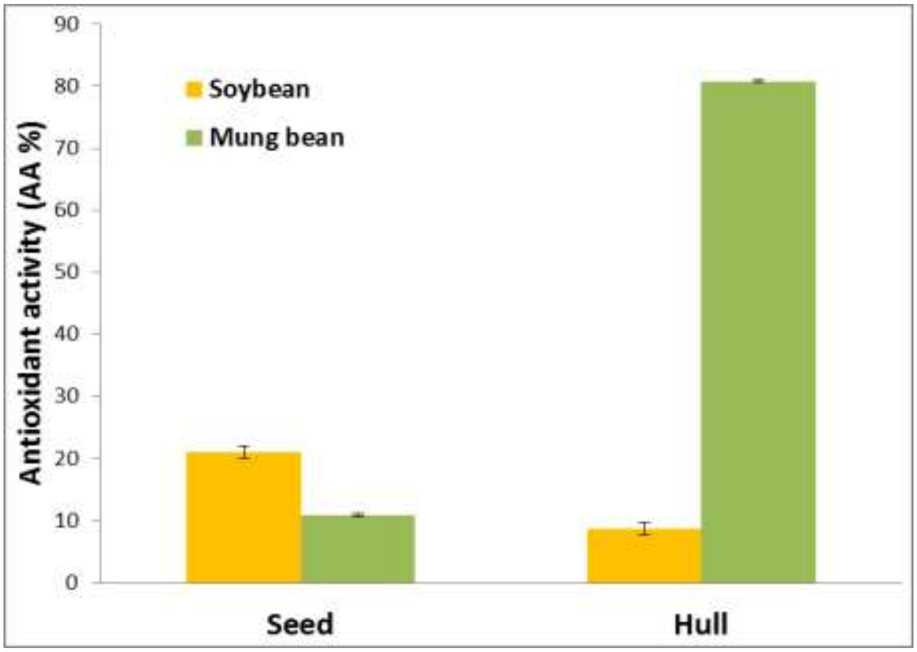

Figure 2. Antioxidant activity (AA %) of both inner and outer fractions of soybean and mung bean. Each value is expressed as the means out of 4 replicates with standard error.

Conclusions

The obtained results indicate that comparing the two analysed legumes, the highest antioxidant capacity was exhibited in the mung bean hulls, almost 10 times higher than the activity of the same seed fraction of soybean. Each of the analysed legume seed fractions had a different antioxidant capacity. Antioxidant activity may be a reliable indicator of seed quality. Efficient radical scavenging activity of the outer fraction of the mung bean suggests that this legume may be evaluated as a functional food ingredient. The seeds possessing higher antioxidant potential could be suggested as a better food source due to their health benefits. The growing interest in the functional properties of mung bean and soybean has focused the attention of research studies towards bioactive compounds and their health benefits.

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References

Amarowicz R, Pegg RB (2008). Legumes as a source of natural antioxidants, European Journal of Lipid Science and Technology, 110 (10), 865-878.

Ganesan K, Xu B (2018) A critical review on phytochemical profile and health promotingeffects of mung bean (Vigna radiata), Food Science and Human Wellness, 7, 11-33.

Gaonkar V, Rosentrater KA (2019). Chapter 4: Soybean, Integrated Processing Technologies for Food and Agricultural By-Products, Elsevier Inc. 73-104.

Khan S, Patel A, Bhise KS (2017). Antioxidant activity of pomegranate peel powder, Journal of Drug Delivery and Therapeutics, 7 (2), 81-84.

Lim YJ, Kwon SJ, Qu S, Kim D-G, Eom SH (2021) Antioxidant Contributors in Seed, Seed Coat, and Cotyledon of γ-ray-Induced Soybean Mutant Lines with Different Seed Coat Colors, Antioxidants, 10, 353.

Orak HH, Karamac M, Orak A, Amarowicz R, Janiak M (2018). Phenolics Content and Antioxidant Capacity of Mung Bean (Vigna radiata L.) Seed. Yüzüncü Yıl Ünv Tarım Bilim Derg (YYU J AGR SCI), 28, 199–207.

Peiretti PG, Karamać M, Janiak M, Longato E, Meineri G, Amarowicz R, Gai F (2019). Phenolic composition and antioxidant activities of soybean (Glycine max (L.) Merr.) plant during growth cycle, Agronomy, 9, 153.

Shi Z, Yao Y, Zhu Y, Ren G (2016). Nutritional composition and antioxidant activity of twenty mung bean cultivars in China, Crop Journal, 4 (5), 398-406.

Singh B, Singh N, Thakur S, Thakur A (2017) Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean, hull and cotyledon, Journal of Food Science and Technology, 54 (4), 921-932.

Wang X, Komatsu S (2017). Chapter four: Improvement of Soybean Products Through the Response Mechanism Analysis Using Proteomic Technique, Advances in Food and Nutrition Research (1st ed.), Elsevier Inc. 82: 117-148.

Yusnawan E, Sutrisno, Kristiono A (2019). Total phenolic content and antioxidant activity of mung bean seed cultivars from optimized extraction treatment, Buletin Palawija 17 (1), 1-9.

AZOTOBACTER, PSEUDOMONAS AND BACILLUS ISOLATES STIMULATE THE GERMINATION AND SEEDLING GROWTH OF MELLISA OFFICINALIS

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Abstract

The objective of this study was the isolation and characterization of *Azotobacter*, *Pseudomonas* and *Bacillus* species from the rhizospheric soil of *Mellisa officinalis* and to examine the effect of isolates application on the seed germination and seedling growth. Isolation, physiological, biochemical, and plant-growth-promoting activity characterization of isolates were done. Monitoring the effects of isolates application on seed germination and seedling growth were evaluated in controlled conditions. The number of germinated seeds, the shoot and root length of seedlings, vigour index and biochemical stress markers (lipid peroxidation intensity and total phenols) were measured. From the rhizosphere of plant 2 *Azotobacter* (denoted as A5 and A6), 5 *Pseudomonas* (denoted as P27, P28, P29, P55 and P56) and 6 *Bacillus* (denoted as B64, B65, B66, B67, B68 and B69) bacteria were isolated. *Azotobacter* isolates showed the ability to live in the condition of low (5) and high (9) pH. The isolates varied in terms of the utilization of carbon sources. Both isolates produced pectinase, hydrogen cyanide (HCN) and utilized organic and inorganic phosphorus. *Pseudomonas* isolates had optimal growth at 10 ºC and 37 ºC, and on medium with pH 5 and 9. All *Pseudomonas* isolates could produce pectinase, lipase, amylose, IAA, siderophores and HCN. All *Bacillus* isolates could grow on a medium containing 7% NaCl. Two isolates (B65 and B67) showed intensive growth on medium with pH 9. Only two isolates (B64 and B67) produced IAA, and all of them siderophores and HCN. *Bacillus* isolates (B64 and B67) increased, while other isolates reduced stress which was observed by lowering the amount of stress molecules in seedlings. The best effect on the seed germination had *Pseudomonas* (P28) and *Bacillus* (B65, B66) isolates. *Azotobacter* isolates had the greatest stimulatory effect on seedling growth.

Key words: *medicinal plants, plant-growth-promoting microorganisms, seedling, total phenols.*

Introduction

The interaction between plants and microbes occurs in three different layers: endosphere, phyllosphere, and rhizosphere. The region of contact between root and soil is rhizosphere. Plant growth-promoting rhizobacteria (PGPR) are bacteria colonizing rhizospheres of plant and enhance plant growth and plant resistance to biotic and abiotic stresses through various mechanisms, such as nitrogen fixation, solubilization of phosphate, production of phytohormones, siderophores, antibiotics, enzymes, etc. (Bhattacharya and Jha, 2012; Etesami and Beattie, 2017). PGPR include many genera and species, among which bacteria belonging to the genera *Bacillus*, *Azotobacter*, and *Streptomyces* are well known for colonizing plant roots and stimulating plant growth (Adesemoye *et al.*, 2008).

Bacillus is the most widespread genus of bacteria in the rhizosphere. In the rhizospheric soil of different plants, *B. subtilis, B. mycoides, B. pumilus, B. megaterium, B. thuringiensis* and *B. firmus* are the most present species of this genus (Garbeva *et al.*, 2003). These bacteria are known to synthesize a large number of secondary metabolites that affect their environment, thus increasing the availability of nutrients to plants (Barriuso and Solano, 2008). Bacteria of the genus *Bacillus* increase the yield and growth of various plants (Mumtaz *et al.*, 2017; Gontia-Mishra *et al.*, 2017). It was reported that *Bacillus* isolates promoted pepper growth and tolerance to drought stress (Lim and Kim, 2013). *Bacillus* sp. isolated from the rhizosphere of green tea (*Camellia sinensis*) had the ability to produce IAA and thus stimulated plant growth (Chakraborty *et al.*, 2006). In addition to providing essential nutrients for plants, some *Bacillus* sp., known as selenobacteria, can remarkably increase the accumulation of selenium in plants (Eswayah *et al.*, 2016; ul Abadin *et al.*, 2017)

Pseudomonas species are gram-negative bacteria, whose PGP activity has been well known for many years (Lucas *et al.*, 2004). The distribution of this genus is very high. Many species have been isolated from the rhizosphere of various plants, from different soils, and also from ponds, wastewater, and even food. Some species of this genus produce metabolites such as antibiotics, hydrogen cyanide (HCN) and IAA (Kim *et al.*, 2014), some produce high-affinity siderophores, and exopolysaccharides (Rolli *et al.*, 2015). All these metabolites strongly influence the environment, by inhibiting the growth of certain harmful microorganisms, and on the other hand, by increasing the availability of nutrients to plants. According to the results of Rokhzadi *et al.* (2008), the application of *Pseudomonas fluorescens* had positive effects on pea growth and yield, while Menhaz *et al.* (2009) found a positive influence of these bacteria on fresh and dry sugar cane mass.

Azotobacter is a group of Gram negative, free-living, nitrogen fixing aerobic bacteria. According to many studies, bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and giberelin acid-like substances that have been found to be directly associated with improved plant growth (Wani *et al.*, 2013). *Azotobacter* species are able to convert atmospheric nitrogen to ammonia, which can be utilized by the plants (Prajapati *et al.*, 2008). In addition to its beneficial impact on plant growth, *Azotobacter* strains are also known to be associated with the suppression of pathogenic diseases of plants (Wani *et al.*, 2013).

The investigation of microorganisms from the rhizosphere of medicinal plants is very significant because these microorganisms have a great impact on plant growth and on the yield quality of the medicinal herbs (Bafana and Lohiya, 2013). For this reason, the objective of this study was the isolation and characterization of *Azotobacter*, *Pseudomonas* and *Bacillus* species from the rhizospheric soil of *Mellisa officinalis* and to examine the effect of isolates application on the seed germination and seedling growth.

Material and methods

Microorganisms were isolated from rhizospheric soil of *Mellisa officinalis*, from chernozem.

A Gram-negative, rod-shaped cell that synthesize pigment fluorescein, were chosen as *Pseudomonas* isolates. A catalase and Gram-positive, rod-shaped cells that create endospores were selected as *Bacillus* isolates. Isolates which were stated as Gram-negative, coccoid-shaped cell that synthesize large quantities of capsular slime and could grow on N-free medium were selected as *Azotobacter* isolates.

The *Pseudomonas, Bacillus* and *Azotobacter* isolates were incubated at different temperatures (3, 13, 37 and 45 °C), pH levels (4, 5, 6, 9), and salt concentrations (5 and 7%), for 4 days, on the following growth media: King-B, Nutrient agar, and N-free medium, respectively. The colony width of each isolate was measured and compared to a colony of the same isolate grown in optimal conditions (at 28 °C, pH 7 and salt concentration 3%). Based on the comparison, the growth of isolates was described as: - absence of growth; + minimal growth; ++ optimal growth; +++ intense growth.

Utilization of carbon sources was tested using Hugh-Leifson medium with different carbon source (glucose, fructose, lactose, sacharose, galactose). In the case of a positive reaction, the colour of medium around the isolates colony changed from greenish to yellow.

Lipase production was performed on medium (pepton 10g l^{-1}, NaCl 5g l^{-1}, $CaCl_2$x H_2O 0,1g l^{-1}, agar 15g l^{-1}) with Tween 80 addition. Formation the cloudy zones around the colony are evidence of lipolytic activity. Starch hydrolysis was assayed on starch agar, following the protocol as reported by Cappuccino and Sherman (1992).

Production of pectinase was tested on pectin agar. Incubation lasted 24 h at 37 °C, after which colonies overflowed with lugol. The appearance of uncolored zones around the colony is evidence of pectinase activity (Soares *et al*., 2001).

Cellulase production was performed by flooding a solution of Congo-red (1mg cm^{-3} H_2O) on isolates grown on CMC agar (Kasing, 1995). The presence of halo zone around the colony was the proof of the cellulose decomposition. Investigation of indol-3-acetic acid (IAA) production by bacterial isolates was observed using Etesami *et al.* (2015) method. Isolates capability to produce the siderophores was examined on chrom-azurol S (CAS) medium (Milagres *et al.*, 1999).

Isolates were screened for PGP activities on HCN induction medium (Tryptic Soy Broth 30 g l^{-1}, Glycine 4.4 g l^{-1}, agar 15 g l^{-1}) for HCN production (Frey-Klett *et al*., 2005).

Bacterial ability to solubilize sparingly soluble Ca_3 $(PO_4)_2$ was examined on Pikovskaya (1948) medium. Phosphate solubilization was confirmed by transparent zone around isolates colonies.

The ability of the mineralization of phosphorus organic compounds was assayed on Menkina medium (Menkina, 1961). The appearance of clear zone around the colony indicate the ability of isolate to dissolve phosphate.

Evaluation of isolates for their plant growth-promoting (PGP) potential on plant

The effect of selected isolates on seed germination and initial growth of *Mellisa officinalis* was examined in controlled conditions. Fifty seeds inoculated with the appropriate bacteria were placed on filter paper at 22ºC on germination. After three days, the number of germinated seeds were determined. The shoot and root length of germinated seeds were measured after seven and ten days. Vigour index and biochemical stress markers (lipid peroxidation intensity and total phenols) were measured after ten days. The vigour Index (VI) was calculated as: VI (%) = (mean root length + mean shoot length) × germination %.

The intensity of LP is determined based on the MDA content extracted from fresh plant material using a mixture of 2-Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) (Jambunathan, 2010). Total phenols in methanol extracts of seedlings were determined based on the reaction of phenols with Folin-Ciocalteu reagent (Makkar *et al.*, 2007).

Statistical analysis

The data were statistically processed using the Statistics 13.3 software (TIBCO Software Inc.). The significance of the difference between the applied treatments was determined using Fisher's LSD test.

Results and discussion

Based on the morphological description of colony and cells, from the rhizospheric soil of *Mellisa officinalis,* five isolates from the genus *Pseudomonas*, six isolates from the *Bacillus* and two isolates from *Azotobacter* genus, were isolated. *Pseudomonas* isolates were denoted by letter P, *Bacillus* isolates by B, and *Azotobacter* isolates by letter A. Physiological and biochemical properties of the isolates are presented in Table 1.

Table 1. Growth of isolates at different temperature, pH, NaCl levels, and with various source of carbon

Isolates	Temperature (°C)					pH				NaCl (%)		Source of carbon				
	3	10	28	37	45	4	5	6	9	5	7	L[b]	F	G	S	Ga
P27	-[a]	+	++	++	-	-	-	++	++	+	+	-	-	+	-	+
P28	-	++	++	+	-	-	-	++	++	-	+	-	-	+	+	+
P29	-	++	++	+	-	-	-	++	++	+	+	-	-	+	-	+
P55	-	++	++	+	-	-	++	++	++	++	-	-	+	+	-	+
P56	-	++	++	++	-	-	+	++	++	-	-	+	+	+	-	+
B64	-	+	++	++	-	-	+	++	+	+	+	-	+	+	+	+
B65	-	+	++	++	-	-	+++	++	+	-	+	+	-	+	-	+
B66	-	+	++	+	-	-	+	++	++	-	+	-	+	+	-	+
B67	-	+	++	+	-	-	+++	++	++	+	+	-	+	+	-	-
B68	-	++	++	++	-	-	+	+	++	+	+	-	+	+	-	+
B69	-	++	++	+	-	-	+	++	++	-	+	-	+	+	-	+
A5	-	-	++	-	-	-	+	+	++	-	-	-	+	+	+	+
A6	-	-	++	+	-	-	+	+	++	-	-	+	+	+	+	+

Legend: [a] - absence of growth; + minimal growth; ++ optimal growth; +++ intense growth; [b] L- lactose; F- fructose; G- glucose; S- sacharose; Ga- galactose

According to Wang *et al.* (2014), temperature is one of the most frequent abiotic stress factors that influence plant growth and microbial activity very strong and adversely. In this study, optimal growth for all isolates was detected at 28 ºC. Almost all isolates could grow at 10 and 37 ºC, but non at 3 and 45 ºC.

The optimum pH for growth of vast majority of the isolates was 6 and 9, while two *Bacillus* isolates, denoted as B65 and B67, had intense growth at pH 5. It is especially interesting that *Azotobacter* isolates had minimal growth in acidic and alkaline conditions. Previous research on *Azotobacter* isolates show that they are very sensitive and not able to survive low and high pH values (Jnawali *et al.*, 2015).

On medium containing 7% NaCl, minimum growth was observed for all *Bacillus* isolates, and three *Pseudomonas* isolates (P27, P28, P29). *Azotobacter* isolates, could not grow on medium containing these amounts of NaCl. The isolates varied in terms of utilization of carbon sources such as lactose, fructose, glucose, sacharose and galactose. The ability of *Pseudomonas* and

Bacillus strains to grow at extreme pH values and temperatures and at high concentrations of NaCl is reported by Karagoz *et al.* (2012). According to the same author, metabolic diversity allows these bacteria to adapt to adverse environmental conditions. The results of this work indicate the adaptability of these isolates, especially *Bacillus* isolates, showing that they have a good ability to survive under different environmental conditions. *Azotobacter* isolates showed the weakest adaptability to different abiotic factors.

Important traits of PGPR, that may directly and indirectly influence the plant growth, is the production of different kind of enzymes, phytohormones, siderophores, hydrogen cyanide (HCN), and ability to solubilize compounds of phosphorus (Beneduzi *et al.*, 2012; Shameer and Prasad, 2018). In this study it was found that all isolates have good PGP potential (Table 2.).

Table 2. Plant-growth-promoting properties of the isolates

Isolates	Lytic enzyme production[a]				Plant growth promoting traits				
	Lipase	Amyl	Pectinase	Cellulase	IAA[b]	Siderophore[c]	HCN[d]	Phosporus[e]	
								organic	inorganic
P27	-	+	+	-	+	8	+	-	++
P28	+	+	+	-	+	6	+	-	-
P29	++	+	+	-	+	3	+	-	-
P55	+	+	+	+	+	5	+	++	+
P56	+	+	+	-	+	3	+	++	+
B64	++	-	-	-	+	7	+	+++	+
B65	-	-	+	-	-	2	+	++	-
B66	++	-	+	-	-	6	+	++	+
B67	++	-	+	-	+	7	+	+	-
B68	+	-	-	-	-	1	+	++	+
B69	++	-	+	-	-	3	+	++	++
A5	-	-	+	-	-	-	+	++	++
A6	-	-	+	-	-	-	+	++	++

Legend: [a] Lipase, amylase (Amyl), pectinase, cellulase activities: + hydrolysis; - no hydrolysis; [b] Production of indole-acetic acid: - not detected; + IAA production; [c] Production of siderophores; width of orange zone; - no zone; [d] Production of HCN; + HCN production, – not detected; [e] Mineralization of phosphorus organic compounds and phosphate solubilization- evaluated according to zone diameter: + represents 4mm/day (weak activity); ++ represents 5-8mm/day (moderate activity) +++ ≥8mm/day (high activity)

All *Pseudomonas* isolates showed the ability to produce amylase and pectinase, IAA, siderophore and HCN. Only one isolate (P55) could produce cellulose. Among *Bacillus* isolates, all isolates had the ability to produce siderophore, HCN and to solubilize phosphorus organic compounds. No isolate had the ability to produce amylase and cellulase. *Azotobacter* isolates could produce pectinase, HCN, and had the ability to do mineralization of phosphorus organic compounds and phosphate solubilization. Similarly to our results, several studies demonstrated the *Bacillus*, *Pseudomonas* and *Azotobacter* isolates ability to produce biologically active substances that promote plant growth (Khan and Joergensen, 2009; Suresh *et al.*, 2010; Ahmad *et al.*, 2012; Alina *et al.*, 2015).

In this study, the applied inoculants had different effects on the seed germination, and on the root and shoot length (Table 3.). The highest percentage of germination (86%) was found in the variant in which isolate *Bacillus* sp. B69 was applied, while in the variant with *Bacillus* sp. B67, germination percentage was the lowest (60%).

Table 3. The effect of selected isolates on seed germination, root and shoot length and vigour index (VI)

Isolates	Seed germination (%)	No. of germinated seeds (out of 50 seeds)	Root length (mm)		Shoot length (mm)		VI (%)
			7 days	10 days	7 days	10 days	
P27	70	35[d]	7,0[gh]	8,0[h]	23,0[cd]	25,0[f]	2310
P28	80	40[b]	6,0[g]	28,0[h]	17,5[f]	25,0[f]	2640
P29	68	34[de]	10,0[d]	14,0[d]	23,5[bcd]	35,0[b]	3332
P55	66	33[ef]	4,0[h]	11,0[ef]	14,0[g]	20,0[g]	2046
P56	64	32[fg]	3,0[h]	4,0[j]	7,0[h]	9,0[h]	832
B64	66	33[ef]	8,0[ef]	9,0[gh]	25,0[ab]	20,0[g]	1914
B65	74	37[c]	10,0[d]	12,0[e]	22,0[de]	27,3[e]	2908,2
B66	82	41[b]	13,0[c]	15,0[cd]	21,0[e]	25,5[f]	3321
B67	60	30[h]	13,0[c]	15,0[cd]	22,0[de]	30,5[d]	2730
B68	86	43[a]	15,5[b]	16,0[c]	24,5[abc]	32,6[c]	4179,6
B69	68	34[de]	17,0[b]	18,0[b]	25,5[a]	28,6[e]	3168,8
A5	82	41[b]	19,3[a]	21,0[a]	23,6[bcd]	35,0[b]	4592
A6	68	34[de]	19,5[a]	22,0[a]	24,0[abc]	38,0[a]	4080
control	62	31[gh]	9,0[ed]	10,0[fg]	15,0[g]	20,0[g]	1860

* The different letter above the number indicates significant difference at $P<0.05$ according to Fisher`s test

Application of all *Bacillus, Pseudomonas* and *Azotobacter* isolates, compared to the control, had a positive effect on the number of germinated seeds. This result is in accordance with Shaukat *et al.* (2006), where the introduction of *Azospirillum*, *Azotobacter* and *Pseudomonas* in the rhizosphere of sunflower and wheat had a positive effect on the germination and the length of the seedlings. Also, Niranjan *et al.* (2004) reported that the application of *P. fluorescens* had a positive effect on germination of millet grains.

After seven days, the greatest impact on the root length had the application of *Azotobacter* isolates, while on the shoot length the best results were achieved with *Bacillus* isolates (B64, B69). Application of *Azotobacter* isolates and *Pseudomonas* sp. P28, after ten days, had the greatest effect on root length. After ten days, all *Bacillus* and *Azotobacter* isolates positively affected shoot length. The highest VI was detected in variant where isolate B68 was applied. *Pseudomonas* isolate, denoted as P56, negatively acted on the root and shoot length. Similarly to this, Heydari *et al.* (2008) determined the inhibitory effect of the *Pseudomonas* isolates on the germination, and seedling growth of wheat and rye. However, there are more studies whose results confirm the positive effect of microorganisms application, especially of the genera *Bacillus*, *Pseudomonas* and *Azotobacter*, on the germination, initial growth and yield parameters of different plants (Khaosaad *et al.*, 2006; Arpanahi *et al.*, 2019; Mohammadi *et al.*, 2018).

According to Yasmin *et al.* (2019), the PGP microorganisms play an important role in promoting tolerance of plants to abiotic stresses. The total phenols and the intensity of lipid peroxidation indicate the plant response to stress. Higher values of these two parameters mean greater possibility that the cell is in oxidative stress. When the values obtained in the variants with the applied isolates were compared with the values obtained in the control variant, it was found that all isolates, except B67 and B64, reduced stress by lowering the amount of stress molecules, while these two isolates increased it (Figure 1.).

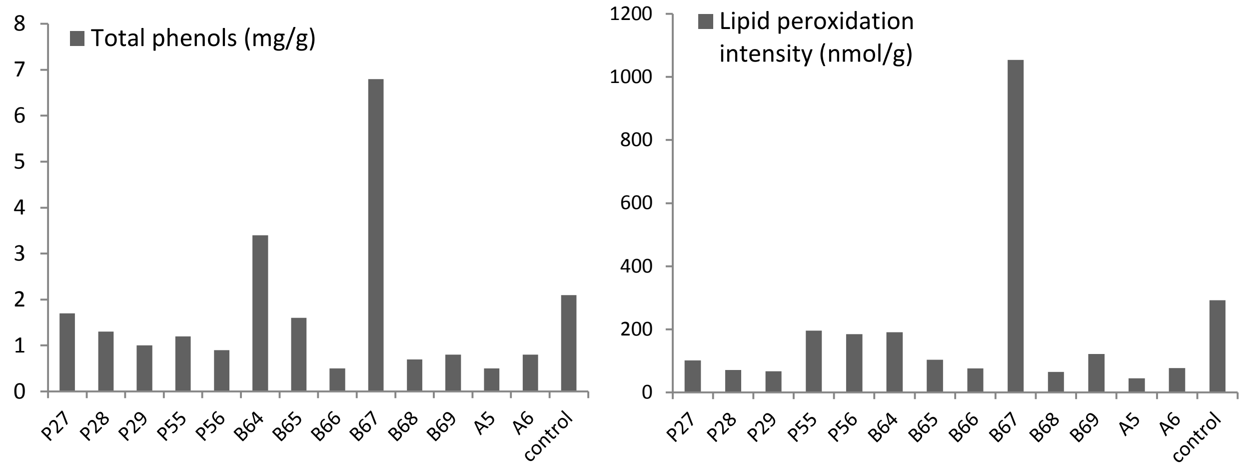

Figure 1. The effect of isolates on biochemical stress markers

Kumar *et al.* (2016) examined the influence of PGPR on the levels of total phenols and the intensity of lipid peroxidation in chickpea. The results showed that the application of PGPR reduced the stress molecules levels in plants, which is in accordance to our study.

Conclusions

Having great PGP traits, all isolates, except one, showed good PGP potential. The isolates that stand out are *Bacillus* isolates B64, B68, B69, and *Pseudomonas* isolates P27 and P29.
Bacillus isolates (B64 and B67) increased, while other isolates reduced stress which was observed by lowering the amount of stress molecules in seedlings.
The best effect on the seed germination had *Pseudomonas* (P28) and *Bacillus* (B65, B66) isolates. *Azotobacter* isolates had the greatest stimulatory effect on seedling growth. Isolate P56 negatively affected root and shoot length.

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References

Adesemoye A.O., Obini M., Ugoji E.O. (2008): Comparation of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Brazilian Journal of Microbiology, 39: 423-426.

Ahmad M., Zahir Z.A., Asghar H.N., Arshad M. (2012): The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. Annals of *Microbiology*, 62: 1321-1330.

Alina, S. O., Constantiscu, F., Calina Petruta, C. (2015): Biodiversity of *Bacillus subtilis* group and beneficial traits of *Bacillus* species useful in plant protection. Romanian Biotechnological Letters. 20 (5): 10737-10750.

Arpanahi, A.A., Feizian, M., Mehdipourian, G. (2019): Plant growth promoting rhizobacteria enhance oil content and physiological status of *Thymus daenensis* Celak. under drought stress. Journal of Herbal Drugs, 9 (4): 223-231.

Bafana, A., Lohiya, R. (2013): Diversity and metabolic potential of culturable root-associated bacteria from Origanum vulgare in sub-Himalayan region. *World Journal of Microbiology* and *Biotechnology*, 29:63–74.

Barriuso, J., Solano, B.R. (2008): Ecology, Genetic Diversity and Screening strategies of Plant Growth Promoting Rhizobacteria (PGPR), Journal of Plant nutrition, 1-17.

Beneduzi, A., Ambrosini, A., Passaglia, L.M.P. (2012): Planth growth- promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Journal of *Genetics* and *Molecular Biology*, 35 (4): 1044-1051.

Bhattacharyya, P., Jha, D., (2012): Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology, 28: 1327–1350.

Cappuccino, J.C., Sherman, N. (1992): Microbiology: A Laboratory Manual (third ed), Benjamin/cummings Pub. Co., New York , 125-179.

Chakraborty, U., Chakraborty, B., Basnet, M. (2006): Plant growth promotion and induction of resistance in Camellia sinensis by Bacillus megaterium, Journal of Basic Microbiology, 46 (Suppl 3): 186 – 195.

Eswayah, A.S., Smith, T.J., Gardiner, P.H.E. (2016): Microbial transformations of selenium species of relevance to bioremediation. *Applied* and *Environmental Microbiology* Journal, 82: 4848–4859.

Etesami H., Alikhani H.A., Hosseini H.M. (2015): Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX, 2: 72–78.

Etesami, H., Beattie, G.A., (2017): Plant-Microbe Interactions in Adaptation of Agricultural Crops to Abiotic Stress Conditions. Probiotics and Plant Health, Springer, 163–200.

Frey-Klett, P., Chavatte, M., Clausse, M.L., Courrier, S., Le Roux, C., Raaijmakers, J., Martinotti, M.G., Pierrat, J.C., Garbaye,J. (2005): Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytologist, 165: 317–328.

Garbeva, P., Van Veen, J. A., Van Elsas, J. D. (2003): Predominant *Bacillus spp*. in agricultural soil under different management regimes detected via PCR-DGGE. *Microbial Ecology*, 45:302–316.

Gontia-Mishra, I., Sapre, S., Tiwari, S., (2017): Zinc solubilizing bacteria from the rhizosphere of rice as prospective modulator of zinc biofortification in rice. Rhizosphere, 3: 185–190.

Heydari, S., Moghadam, P.R., Arab, S.M. (2008): Hydrogen cyanide production ability by *Pseudomonas fluorescence* bacteria and their inhibition potential on weed. Paper presented at Competition for Resources in a changing world: New drive for rural development, Tropentag,Hohenheim.

Jambunathan, N. (2010): Determination and Detection of Reactive Oxygen Species (ROS), Lipid Peroxidation, and Electrolyte Leakage in Plants. In: Sunkar R. (ed). Plant Stress Tolerance, Methods in Molecular Biology (Methods and Protocols), 639.

Jnawali, A.D., Ojha, R.B., Marahatta, S. (2015): Role of *Azotobacter* in soil fertility and sustainability–A review, Advances in Plants & Agriculture Research, 2(6):1–5.

Karagoz, K., Ates,F., Kotan, R., Cakmakci, K. (2012): Characterization of plant growth-promoting traits of bacteria isolated from the rhizosphere of gravine grown in alkaline and acidic soils. The European Journal of *Soil Biology*, 50: 144-150.

Kasing , A.(1995): Cellulase production, Practical Biotechnology

Khan, K.S., Joergensen, R.G. (2009): Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. *Bioresource Technology*, 100: 303-309.

Khaosaad T., Vierheilig H., Nell M., Zitterl-Eglseer K., Novak J. (2006): *Arbuscular mycorrhiza* alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). Mycorrhiza, 15:443–446.

Kim, K., Jang, Y.-J., Lee, S.-M., Oh, B.-T., Chae, J.-C., Lee, K.-J. (2014): Alleviation of salt stress by Enterobacter sp. EJ01 in tomato and Arabidopsis is accompanied by upregulation of conserved salinity responsive factors in plants. *Molecules and Cells,* 37, 109.

Kumar, M., Mishra, S., Dixit, V., Kumar, M., Agarwal, L., Singh Chauhan, P., Nautiyal, C.S. (2016): Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signaling and Behavior, 11(1): 2-10.

Lim, J.-H., Kim, S.-D. (2013): Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in pepper. The Journal of *Plant Pathology*, 29, 201.

Lucas, García, J.A., Domenech, J., Santamaría, C., Camacho, M., Daza, A., Gutiérrez Mańero, F.J. (2004): Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. Environmental and Experimental Botany, 52 (3): 239–251.

Mehnaz, S., Weselowski, B., Aftab, F., Zahid, S., Lazarovits, G., Iqbal, J. (2009): Isolation, characterization, and effect of fluorescent pseudomonads on micropropagated sugarcane. Canadian Journal of Microbiology, 55: 1007–1011.

Menkin, R.A. (1961): Rol *Bacillus megaterium* var phosphaticum b pitanii rastenij. Mikroorganizmi i efektivnoje plodprodije poĉv. Akademii Nauk. SSSR., 238-245 (in Russian).

Milagres, A.F.M., Machuca, A., Napoleao, D. (1999): Detection of siderophore production from several fungi and bacterial by a modiWcation of chrome azurol S (CAS) agar plate assay. Journal of *Microbiological Methods*, 37: 1–6.

Mohammadi, H., Esmailpour, M., Ghorabi, S., Hatami, M. (2018): Physiological and biochemical changes in *Matricaria chamomilla* induced *by Pseudomonas fluorescens* and water deficit stress. Acta Agriculturae Slovenica, 111 (1): 63-72.

Mumtaz, M.Z., Ahmad, M., Jamil, M., Hussain, T., (2017): Zinc solubilizing Bacillus spp. potential candidates for biofortification in maize. *Microbiology Research, 113-120.*

Niranjan-Raj, S., N.P. Shetty, Shetty, H.S. (2004): Seed-bio-priming with *Pseudomonas fluorescens* isolates, enhances growth of pearl millet plants and induce resistance against downy mildew. The *International Journal* of *Pest Management*, 50: 41-48.

Pikovskaya, R.I. (1948): Mobilization of phosphorus in soil in connection with vital activities by some microbial species. Microbiologia, 17: 362-370.

Prajapati, K., Yami, K.D., Singh, A. (2008): Plant growth promotional effect of Azotobacter chroococcum, Piriformospora indica and vermicompost on rice plant. NAST, 9: 85–90.

Rokhzadi, A., Asgharzadeh, A., Darvish, F., Nour-Mohammadi, Majidi, E. (2008): Influence of plant growth-promotiing rhizobacteria on dry matter accumulation and yield of chickpea

(Cicer arietinum L.) under field conditions. Am-Euras, *Journal of Agriculture* and Environmental Sciences, 3(2): 253–257.

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., Gandolfi, C., Casati, E., Previtali, F., Gerbino, R. (2015): Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environmental Microbiology, 17: 316–331.

Shameer S., Prasad T.N.V.K.V. (2018): Plant growth promoting rhizobacteria for sustainable agricultural practices with special reference to biotic and abiotic stresses. *Plant Growth Regulation*, 84:603-615.

Shaukat, K., Affrasayab, S., Hasnain, S. (2006): Growth responses of *Helianthus annus* to plant growth promoting rhizobacteria used as a biofertilizer. *Journal of Agricultural Research*, 1: 573–581.

Soares, M.M., da Silva, R., Carmona, E.C, Gomes, E. (2001): Pectinolytic enzyme production by Bacillus species and their potential application on juice extraction. J Microbiol Biotechnol, 17: 79-82.

Suresh, A., Pallavi, P., Srinivas, P., Kumar, V. P., Chandra, S. J. (2010): Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. African Journal of Microbiology Research, 4: 1491-1494.

ul Abadin, Z., Yasin, M., Faisal, M., (2017): Bacterial-Mediated Selenium Biofortification of Triticum aestivum: Strategy for Improvement in Selenium Phytoremediation and Biofortification. Agriculturally Important Microbes for Sustainable Agriculture. Springer, 299–315.

Wani, S.A., Chand, S., Ali, T. (2013): Potential use of Azotobacter chroococcum in crop production: an overview. *Current Agriculture Research Journal*, *1*(1): 35–38.

Whang, K.S., Lee, J.C., Lee, H.R., Han, S.I., Chung, S.H. (2014): Terriglobus tenax sp. nov., an exopolysaccharide-producing Acidobacterium isolated from rhizosphere soil of a medicinal plant. International Journal of Systematic and Evolutionary *Microbiology*, 64:431–437

Yasmin, H., Nosheen, A., Naz, R., Keyani, R., Anjum, S. (2019): Regulatory role of rhizobacteria to induce drought and salt stress tolerance in plants. Maheshwari D., Dheeman S. (eds). Field Crops: Sustainable Management by PGPR: Sustainable Development and Biodiversity 23. Springer. https://doi.org/10.1007/978-3-030-30926-8_11

AGRONOMIC PERFORMANCE OF ALMOND CULTIVARS IN SERBIA

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Abstract

Almond production in Serbia is very small. The main reason for this is the frequent occurrence of late spring frosts during flowering. Present study was carried out to evaluate phenological characteristics (flowering and maturity times), productivity, and characteristics of the nut and the kernel (nut weight and dimensions, shell thickness, weight of kernel, kernel share, oil and protein contents) in 13 almond genotypes of a different origin growing in the region of Belgrade. The average flowering time was the second half of March and the first decade of April. The flowering began first in 'Nessebar', 'Selection 25', and 'Tétényi kedvenc' (16th of March), and latest in 'Francoli', and 'Texas' (29th of March). Beginning of the fruit maturity was recorded in the range of 13 days, from August 15th ('Tétényi bőtermő' and 'Tétényi keményhéjú') to August 28th ('Glorieta'). The average productivity was the lowest in 'Glorieta' and 'Francoli' and the highest in 'Tuono'. In March 2017 late spring frost that significantly reduced yield in early flowering cultivars was recorded. Nut weight ranged from 1.95 g ('Budatétény') to 5.58 g ('Marcona'). Shell thickness varied from 1.71 mm ('Budatétény') to 5.06 mm ('Marcona'). Kernel weight was the lowest in 'Selection 25' (0.80 g) and 'Tuono' (0.81 g), and the highest in 'Texas' (1.30 g). Kernel share was the lowest in 'Selection 25' (16.03%), and the highest in 'Budatétény' (57.93%). Oil content ranged from 44.35% ('Glorieta') to 57.52% ('Budatétény'), and protein content ranged from 21.72% ('Budatétény') to 30.51% ('Selection 25'). Obtained results indicate that Belgrade region is suitable for growing of late flowering almond cultivars.

Keywords: *Prunus dulcis, flowering, maturation, productivity, nut and kernel characteristics.*

Introduction

Almond [*Prunus dulcis* (Mill.) D.A.Webb syn. *Prunus amygdalus* (L.) Batsch] is a species of genus *Prunus* and subgenus *Amygdalus* (*Rosaceae,* subfamily *Prunoideae*). It originated from Central Asia and dispersed through cold and xeric environments in the mountainous areas and deserts of western China into Iran (Watkins, 1976). Over the time, the almond culture has extended to colder areas. In the winter rest period, the almond can tolerate temperature of -20 to -24°C, thus it is also grown in countries with a more severe climate, such as Hungary, Czech Republic, Bulgaria and Romania (Bulatović, 1985). Almond cultivation in Serbia is limited to a small number of locations, including Slankamen Hill (Čolić and Zec, 2007; Čolić et al., 2009). Due to early flowering and a weak growing tradition, Serbia has very small areas under almonds. Commercial almond production in Serbia is low considering the demand and economic potential. In Serbia, except early-flowering cultivars, in the experimental trials are also present late-flowering cultivars, which bloom after apricot cultivars (Zec et al., 1999). The most intensive studies were performed on the almond population in the area of Slankamen hill (Čolić and Zec, 2007; Čolić et al. 2009, 2012), while Zec et al. (1999) and Milatović et al. (2013, 2017) evaluated agronomic characteristics of introduced almond cultivars grown in the area of Belgrade. The aim of this study was to evaluate phenological traits, yield and fruit characteristics of 13 almond cultivars originated from several European countries. The best performing cultivars will be recommended for growing in the region of Belgrade, as well as in other regions with similar environmental conditions.

Materials and methods

Study was carried out in the almond orchard at the experimental station 'Radmilovac' of the Faculty of Agriculture in Belgrade, in the period 2017 - 2019. The orchard was planted in 2014. The study included 12 introduced almond cultivars: 'Budatétény', 'Tétényi bőtermő', 'Tétényi kedvenc', 'Tétényi keményhéjú' and 'Tétényi rekord' from Hungary; 'Francoli', 'Glorieta', 'Marcona' and 'Masbovera' from Spain, 'Nessebar' from Bulgaria; 'Texas' from USA; 'Tuono' from Italy and 'Selection 25' from Serbia. All cultivars were grafted on the clonal rootstock 'GF 677' and were represented with five trees. Training system is open vase, and planting distance is 5 × 3.5 m. Flowering was monitored according to the recommendations of the International Working Group for Pollination: beginning of flowering - when 10% of flowers open, full flowering - 80% of open flowers, and the end of flowering - when 90% of petals fall off (Wertheim, 1996). The samples of 30 fruits per tree were randomly harvested at full maturity (hulls fully desiccated and opened along the suture). Yield was determined by ranking from 0 to 5. Nut and kernel length, width and thickness were measured with a digital calliper, whereas nut and kernel were weighted on a digital scale. Kernel/nut ratio was expressed as a percentage. The oil content was determined by extraction with petroleum ether in a Soxhlet apparatus (Faithfull, 2002.). The nitrogen content in the kernel was determined by the Kjeldahl method, and the content of crude protein was obtained by calculation, multiplying by a factor of 5.18 (Faithfull, 2002). The data for nut characteristics were statistically analyzed using analysis of variance. The significance of differences between mean values was determined using Duncan's multiple range test at 0.05 level of probability.

Results and discussion

Almonds are characterized by a short period of deep winter dormancy and early flowering time. Almond bloom lasts 5 to 25 days depending on the cultivar, rootstock and the temperature (Bulatović, 1985). In our study significant differences in flowering time were observed (Table 1). Cultivars 'Nessebar' and 'Tétényi kedvenc' as well as 'Selection 25' began to bloom the earliest (March 16th) whereas the cultivars 'Francoli' and 'Texas' had the latest (March 29th) beginning of flowering. Zec et al. (1999) recorded that the cultivar 'Texas' in the conditions of the Pančevo started to bloom on April 5th. A significant difference in the flowering time could be influenced by micro location and climate change. The difference between the earliest and the latest flowering time was 13 days which is five days shorter compared to the results obtained by Milatović et al. (2017). Segura et al. (2017) state that almonds have the widest range of flowering time among of all fruit and nut trees. Almond nuts are ready for harvesting when the hull separates from the shell easily. Hungarian cultivars 'Tétényi bőtermő' and 'Tétényi keményhéjú' had the earliest harvesting time (August 15th). The latest harvesting time was recorded for 'Glorieta' (August 28th). Cultivars 'Marcona' and 'Texas' also had a late ripening time (August 27th), only one day before 'Glorieta'. All the observed cultivars were harvested prior to autumn rains. According to Zec et al. (1999) cultivars 'Marcona' and 'Texas' under the conditions of Pančevo had an average ripening time 7 days later in comparison with the obtained results. Observed differences were caused by the location climate differences.

Table 1. Phenological characteristics and productivity of almond genotypes (2017-2019 average).

Genotype	Origin	Start of flowering	End of Flowering	Duration of flowering (days)	Date of harvest start	Yield (0-5)*
Budatétény	HUN	March, 18	April, 1	14	August, 16	3.3
Francoli	ESP	March, 29	April, 12	14	August, 23	3.0
Glorieta	ESP	March, 26	April, 8	13	August, 28	3.0
Marcona	ESP	March, 23	April, 5	13	August, 27	3.8
Masbovera	ESP	March, 25	April, 8	14	August, 23	3.5
Nessebar	BGR	March, 16	March, 29	14	August, 18	3.8
Selection 25	SRB	March, 16	March, 30	15	August, 18	3,7
Texas	USA	March, 29	April, 11	13	August, 27	4.0
Tétényi bőtermő	HUN	March, 19	April, 2	14	August, 15	3.3
Tétényi kedvenc	HUN	March, 16	March, 29	13	August, 17	3.5
Tétényi keményhéjú	HUN	March, 17	April, 1	14	August, 15	3.8
Tétényi rekord	HUN	March, 20	April, 3	14	August, 18	3.2
Tuono	ITA	March, 24	April, 6	13	August, 24	4.5

*Scale: 0-without yield, 1- very low, 2-low, 3-good, 4-very good, 5-high.

The average yield was the lowest in 'Glorieta' and 'Francoli' (score 3.0) and the highest in 'Tuono' (score 4.5). In March 2017 late spring frost was recorded, which significantly reduced yield in early flowering cultivars. The majority of the tested cultivars (7) had medium yield, above 3.5.

Table 2. Characteristics of the nut of almond genotypes (2017-2019 average)

Genotype	Weight (g)	Length (mm)	Thickness (mm)	Width (mm)	Shell thickness (mm)
Budatétény	1.95 d	35.7 ab	17.8 b	12.3 d	1.71 e
Francoli	3.53 cd	34.3 abc	20.7 b	15.3 bc	3.80 c
Glorieta	4.98 a	38.9 a	24.8 a	17.8 a	4.70 ab
Marcona	5.58 a	31.5 bc	25.4 a	18.4 a	5.06 a
Masbovera	4.30 b	38.2 a	24.8 a	17.8 a	4.06 bc
Nessebar	2.30 d	30.9 bc	18.6 b	12.4 d	2.48 de
Selection 25	5.00 a	35.1 ab	20.8 b	15.2 bc	3.79 c
Texas	3.92 bc	32.6 bc	19.3 b	12.8 cd	3.63 c
Tétényi bőtermő	2.24 d	38.8 a	19.2 b	14.9 bc	2.69 d
Tétényi kedvenc	2.55 d	34.9 ab	18.7 b	13.9 bcd	2.52 de
Tétényi keményhéjú	3.20 d	31.9 bc	20.3 b	13.1 cd	3.61 c
Tétényi rekord	3.44 cd	37.8 a	20.3 b	15.2 bc	3.54 c
Tuono	3.50 cd	29.2 c	20.2 b	16.0 a	3.30 cd

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05.

Data for nut characteristics (Table 2) showed large differences for the weight, dimensions of the nut and shell thickness. The fruit weight varied from 1.95 g ('Budatétény') to 5.58 g ('Marcona'). The highest values of the nut dimensions were recorded in 'Glorieta' and 'Marcona'. The 'Tuono' had the smallest fruit length, while the width and thickness of the nut were smallest in 'Budatétény'. The shell thickness varied from 1.71 mm ('Budatétény') to 5.06 mm ('Marcona').

Table 3 shows characteristics of the kernel of almond cultivars. The kernel weight was lowest in the 'Selection 25' (0.80 g) and highest in the 'Texas' (1.30 g). According to the classification given by Socias i Company et al. (2008) the largest number of examined varieties (eight) had a medium-sized kernel (weight 1.1-1.4 g). Three cultivars ('Francoli', 'Glorieta' and 'Tétényikeményhéjú') were classified to the group of genotypes with a small kernel (0.9-1.1 g), while the cultivars with a very small kernel (below 0.9 g) were 'Tuono' and 'Selection 25'.

Table 3. Characteristics of the kernel of almond genotypes (2017-2019 average)

Genotype	Kernel weight (g)	Kernel share (%)	Oil content (%)	Protein content (%)
Budatétény	1.13	57.93	57.52	21.72
Francoli	0.91	25.88	50.21	25.72
Glorieta	1.07	21.43	44.35	24.48
Marcona	1.16	20.78	55.19	28.00
Masbovera	1.25	29.10	52.87	23.45
Nessebar	1.04	45.35	53.87	23.23
Selection 25	0.80	16.03	48.00	30.51
Texas	1.30	33.08	54.52	22.72
Tétényi bőtermő	1.12	49.73	51.11	21.81
Tétényi kedvenc	1.11	43.26	47.10	27.78
Tétényi keményhéjú	1.00	31.26	55.13	24.62
Tétényi rekord	1.17	34.08	46.60	25.51
Tuono	0.81	23.26	47.57	27.58

The kernel share varied from 16.03% ('Selection 25') to 57.93% ('Budatétény'). Based on the kernel share and according to the classification given by Batlle et al. (2017) five studied cultivars have a very hard shell (kernel share below 30%), four cultivars have a hard shell (kernel share 30-40%), three cultivars ('Nessebar', 'Tétényibőtermő' and 'Tétényi kedvenc') have a semi-soft shell (kernel share 40-50%), while the cultivar 'Budatétény' was characterized by soft shell (kernel share 50-60%).

The oil content in the kernel varied in the range from 44.35% ('Glorieta') to 57.52% ('Budatétény'). According to the classification given by Torabi et al. (2011) only kernels of the 'Budatétény' have a very high oil content (above 55%), five cultivars have a high oil content (50-55%), and four cultivars fall into the category with a medium oil content (45-50%). Our results are in range with data reported by Kodad (2017) and reviewed by Čolić et al. (2019) which showed variability from 20 to 67.5% for commercial and local almond cultivars/selections.

Among the nut fruits, almonds are considered to be a good source of high-quality proteins. Protein contents of the commercial almond cultivars ranged from 13% to 29% on a dry weight basis (Kodad, 2017). Drogoudi et al. (2013) analysed variation in the protein content among 72 almond genotypes found in Greece, France and Italy, and concluded that protein content depends on the genotype rather than the origin. The analysis of results for protein content indicates significant differences among cultivars, varied from 21.72 to 30.51%. This is similar to results of Askin et al. (2007), where in the study of almond selection from Elazig (Turkey), the obtained values varied from 16.07 to 31.46%.

Conclusions

The average flowering time of the examined almond varieties was in the second half of March and the beginning of April. The latest flowering time and good yield had the varieties 'Texas', 'Marcona' and 'Tuono', which were introduced a long time ago and therefore are well adapted to the agro-ecological conditions in Serbia. Other late-flowering varieties ('Francoli' and 'Glorieta') had lower yields, so they cannot be recommended for cultivation. The 'Texas' and 'Masbovera' varieties had the largest kernel weight, but also medium kernel share (about 30%). Varieties 'Budatétény', 'Nessebar', 'Tétényi bőtermő' and 'Tétényi kedvenc' had high kernel share and can be recommended as parents for hybridization with late flowering genotypes.
Based on obtained results 'Texas', 'Marcona' and 'Tuono' can be recommended for growing in the wider surroundings of Belgrade. Due to the limited almond assortment in Serbia, introduction and evaluation of newer late-flowering genotypes should be continued.

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References

Askin, M.A., Balta, M.F., Twekinas, F.E., Kazankaya, A., Balta, F. (2007). Fatty acid composition affected by kernel weight in almond [*Prunus dulcis* Mill. D.A. Webb] genetic resources. Journal of Food Composition and Analysis, 20, 7–12.

Batlle, I., Dicenta, F., Socias I Company, R., Gradziel, T.M., Wirthensohn, M., Duval, H., Vargas, F.J. (2017). Classical genetics and breeding. In: Almonds: Botany, Production and Uses (Socias i Company, R. and Gradziel, T.M., eds.), CAB International, Wallingford, UK, pp. 111–148.

Bulatović, S. (1985). Almond. In: Walnut, Hazelnut, and Almond (In Serbian). Nolit, Belgrade, Serbia, pp. 291–352.

Drogoudi, P.D., Pantelidis, G., Bacchetta, L., De Giorgio, D., Duval, H., Metzidakis, I., Spera, D. (2013). Protein and mineral nutrient contents in kernels from 72 sweet almond cultivars and accessions grown in France, Greece and Italy. International Journal of Food Science and Nutrition, 64, 202–209.

Čolić, S., Zec, G. (2007). Morphological and pomological traits variability of almond genotypes from Slankamen hill population. Genetika, 39: 291–296.

Čolić, S., Zec, G., Janković, Z., Rahović, D. (2009). Selection of almond genotypes [*Prunus dulcis* (Mill.) D.A. Webb.] in the region of Slankamen Hill (In Serbian). Zbornik naučnih radova Instituta PKB Agroekonomik, 15(5): 69–75.

Čolić, S., Rakonjac, V., Zec, G., Nikolić, D., and Fotirić Aksić, M. (2012). Morphological and biochemical evaluation of selected almond [Prunus dulcis (Mill.) D.A.Webb] genotypes in northern Serbia. Turk. J. Agric. For. 36, 429–438.

Čolić, S., Zec, G., Natić, M., Fotirić-Akšić, M. (2019). Almond (*Prunus dulcis*) oil. In: Fruit Oils: Chemistry and Functionality (Ramadan, M.F.). Springer Nature Switzerland, pp. 149–180.

Faithfull, N.T. (2002). Methods in agricultural chemical analysis: a practical handbook. CABI Publishing, Wallingford, UK.

Kodad, O. (2017). Chemical composition of almond nuts. In: Almonds Botany, Production and Uses (Socias i Company, R. and Gradziel, T.M., eds.), CAB International, Wallingford, UK, pp. 428–448.

Milatović, D., Đurović, D., Zec, G., Čoloveić, A. (2013). Pomological characteristics of almond cultivars in the region of Šumadija (In Serbian). Zbornik naučnih radova Instituta PKB Agroekonomik, 19(5): 47–54.

Milatović, D., Zec. G., Đurović, D., Boškov, Đ. (2017). Phenology of flowering and pomological traits of almond cultivars in the region of Belgrade (In Croatian). Pomologia Croatica, 21, 181–188.

Segura, J.M.A., Socias I Company, R., Kodad, O. (2017). Late-blooming in almond: A controversial objective. Scientia Horticulturae, 224: 61–67.

Socias I Company, R., Kodad, O., Alonso, J.M., Gradziel, T.M. (2008). Almond quality: a breeding perspective. Horticultural Reviews, 34: 197–238.

Torabi, A., Imani, A., Rabiei, V. (2011). Oil content of seeds of 25 Iranian, European and American almond genotypes and cultivars. Acta Horticulturae, 912: 367–369.

Watkins, R. (1976) Cherry plum, peach, apricot, and almond. In: Evolution of Crop Plants (Simmonds, N.W., Ed.). Longman, London, pp. 342–347.

Wertheim, S.J. (1996). Methods for cross pollination and flowering assessment and their interpretation. Acta Horticulturae, 423: 237–241.

Zec, G., Čolić, S., Pekić, M., and Marinković, D. (1999). Time of flowering of some almond varieties in the conditions of Padinska Skela (In Serbian). Zbornik naučnih radova Instituta PKB Agroekonomik 5(2), 19–24.

QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF WINTER BARLEY IN CONDITIONS OF SOUTHERN SERBIA

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Abstract

Production of barely is possible even at higher altitudes and wider latitudes, but it is most frequently grown in temperate climate (autumn and spring sowing), as well as in semi-arid subtropical climate (winter sowing). The aim of this paper is to present the quantitative and qualitative characteristics of three varieties of livestock feed winter barley, namely of the varieties *KG Zlatnik, Nonius* and *Amorosa*, as well as to draw conclusions about the best variety which is to be grown under tested conditions on the basis of fertility characteristics of the tested varieties. The experiment was conducted in the area of the city of Niš in the course of 2018 and 2019, according to a random block system with four replications and the basic plot size of 5 m^2. After the harvest, the grain yield from the basic plot was measured and converted into kilograms per hectar. The obtained results were processed by means of variance analysis. In the second year of research, due to favourable climatic conditions, the length of spikes in all varieties was bigger in comparison to the first year. The maximum length of spikes for both research years was measured for the variety *Zlatnik*, indicating an increase in the number of grains per spike, and consequently a higher total yield. On average, for all varieties in the experiment, 39 grains per spike were formed in the second year of research, which is six grains more per spike than in the first year of research. The largest number of grains per spike had the variety *Zlatnik*, which also had the largest spike length.

Keywords: *livestock feed winter barley, agro-ecological conditions, fertility traits, variety.*

Introduction

Due to its widespread use, barley (*Hordeum sativum* L.) is one of the most important plant species in the world, in terms of both human and animal nutrition and industrial use. It is a cereal grain most widely adapted to different growing conditions, exhibiting great tolerance to drought, cold and soil salinity. Production of barley is possible even at higher altitudes and wider latitudes, but it is most frequently grown in temperate climate (autumn and spring sowing), as well as in semi-arid subtropical climate (winter sowing). Currently, 55% to 60% of barley in the world is used for livestock, 30% to 40% for malt production, while the rest is used for the purposes of food production and for other industrial purposes. Regardless of its purpose, the main goals with respect to breeding barley are as follows: an increase in grain yield and its stability, low protein content in malting barely (less than 11,5%), as well as high protein content in livestock barely (over 13%), high content of fine extract (over 80% sm), an increase in the total biological yield while retaining the harvest index (0,40-0,50), an increase in grain-filling

rate, given the current length of grain-filling rate, as well as resistance to major diseases and stress conditions, especially to drought (Pržulj et al., 2000).
The aim of this paper is to present the quantitative and qualitative characteristics of three varieties of livestock feed winter barley, namely of the varieties *KG Zlatnik*, *Nonius* and *Amorosa*, as well as to draw conclusions about the best variety which is to be grown under tested conditions on the basis of productivity traits of the tested varieties.

Materials and Methods

The aforementioned research studies examined fertility characteristics of three genotypes of winter barley (*Hordeum sativum* L.). The experiment was conducted in the area of the city of Niš in the course of 2018 and 2019, according to a random block system with four replications and the basic plot size of 5 m^2. In accordance with agro-climatic conditions, standard production technology was applied in terms of plant care and protection measures. In the full maturity phase, each plot was sampled for the purposes of determining the examined parameters. After the harvest, the grain yield from the basic plot was measured and converted into kilograms per hectar. The evaluation of morphological, production and physiological characteristics was performed by resorting to the usual methods. The obtained results were processed by means of variance analysis, and individual differences in the means were tested by means of Fisher´s least significant difference test (LSD).
The *Amorosa-Delta* seed variety is a six-row winter malting barley for animal nutrition, with excellent amino acid composition. It offers high rust and cold resistance, as well as tolerance to low temperatures. It produces good quality grain with 15% protein content.
The *Nonius-NS* seed variety is a six-row medium-early winter barley intended primarily for animal feed (high percentage of easily digestible protein). It is highly resistant to low temperatures, flattening and diseases. The concentration of protein is over 13%. The variety is recommended for growing in all soil types.
The *KG-Zlatnik* seed variety is a two-row medium malting winter barley intended for animal feed. It is highly resistant to diseases and tolerant to low temperatures. It produces good quality grain with the concentration of protein up to 14%.

Results and Discussion

The basic yield components whose economic product is grain are as follows: number of plants per m^2, number of productive spikes per plant, number of grains per spike and the weight of 1000 grains. Maximum fertility implies the achievement of the most favourable balance between yield components. By analysing the connection between the yield and its components in two-row barely, Barczak and Majcherczak, (2009) indicated that the largest interdependence had been obtained between the grain yield and number of spikes per m^2 and between the grain yield and number of grains per spike, regardless of the level of nutrients in the soil.

Table 1. Spike length, number of grains per spike and number of plants per unit area.

Variety	Spike length (cm)			Number of grains per spike			Number of plants per m^2		
Cultivar	2018.	2019.	average	2018.	2019.	average	2018.	2019.	average
Nonius	6.5	7.1	6.80	29	36	32.5	323	343	333.0
Zlatnik	7.2	7.5	7.35	33	40	36.5	346	359	352.5
Amorosa	6.3	6.8	6.55	31	33	32.0	333	338	335.5
average	6.7	7.1	6.90	33	39	36.0	334	347	340.3
LSD 005	0.8	0.6		3	5		12	15	
001	1.1	0.8		5	8		20	21	

Before the aforementioned authors, the same results had been obtained by Dofing and Knight, (1994) who analysed the grain yield and yield components and stated that an increase in the number of grains per spike had caused a slight reduction in grain weight. By analysing the interdependence between yield and its components Madić et al., (2005) indicated that there was a high positive correlation between harvest index and grain yield (0,68). Positive values of the correlation coefficient were also recorded between harvest index and the number of grains per spike (0,51), biological yield (0,42) as well as the hight of stalks (0,42), while negative value was recorded between harvest index and grain weight per spike (-0,34). The highest significant positive correlation existed between biological yield and grain yield (0,83), then between the hight of stalk and biological yield (0,65). The number of grains per spike was in the highest positive correlation with the hight of stalk and grain yield, as well as with biological yield and harvest index. On the basis of two years of test results, barley varieties have achieved different values for yield components depending on climatic conditions. Thus, the spike length is a trait which mostly depends on climatic conditions during the vegetative stages of growth (Table 1). In the second year of research, due to favourable climatic conditions, the length of spikes in all varieties was bigger in comparison to the first year. The maximum length of spikes for both research years was measured for the variety *Zlatnik*, which indicates an increase in the number of grains per spike, and consequently a higher total yield. Multiple-row barley essentially has a larger number of grains per spike, reaching the number of 100 and more grains, and with two-row barley that number is between 30 and 40. Stojanović, et al., (1998) believe that the number of grains is one of the most important yield components. On average, for all varieties in the experiment 39 grains per spike were formed in the second year of research, which is 6 grain more per spike than in the first year of research. The largest number of grains per spike had the variety *Zlatnik*, which also had the largest spike length. In the second year of research there were a bigger number of plants per m^2 in comparison with the first year. Thus, in the course of two years of research, the largest number of plants was achieved in the variety *Zlatnik*, while other varieties had statistically significant smaller number of plants per m^2. The number of spikes is the most important yield component of all small grains. The amount of seed directly influences the number of spikes. Barely is a small grain which has the largest coefficient of tillering. It depends on genotypes, as well as on plant nitrogen nutrition (Madić et al., 2006). The number of spikes directly influences the number of grains per spike. Paunović, et al., (2007) asserts that greater sowing density affects an increase in the number of spikes. In the first year of research the largest number of spikes per m^2 was found with the variety *Nonius* (481), while in the second year it was with the variety *Zlatnik* (551). On average, in the second year we have achieved 40 spikes more than in the first year.

Table 2. The number of spikes per m^2, the number of stalks per m^2 and the grain weight per spike.

Variety	The number of spikes per m^2			The number of stalks per m^2			The grain weight per spike (g)		
Cultivar	2018.	2019.	average	2018.	2019.	average	2018.	2019.	average
Nonius	481	473	477	777	806	792	1.008	1.035	1.021
Zlatnik	472	551	511	890	900	895	1.040	1.045	1.042
Amorosa	453	502	477	769	879	824	1.032	1.026	1.029
average	469	509	489	812	862	837	1.026	1.035	1.030
LSD 005	18	35		162	76		0.019	0.017	
001	25	42		176	87		0.033	0.023	

The number of stalks per unit area, provided the same sowing norm, the same seed quality and the same production conditions, is not the same in all varieties and depends on their biological characteristics. In the case of winter varieties, the number of plants per unit area mostly depends on their resistance to low temperatures, but also on hereditary characteristics of a variety and the ability of a stronger or weaker tillering. The number of offshoots which are formed in the early stages of ontogeny represents the basis in terms of crop uniformity and directly influences the number of spikes per unit area (Madić, et al., 2006). In the course of both years of research the largest number of stalks was recorded in the variety *Zlatnik* 890 stalks per m^2 in the course of the first year, and 900 stalks per m^2 in the course of the second year. If one considers the average for both years of research, in the second year there were 50 stalks more per m^2 than in the first year.
Production per spike is a yield component which depends on the number of grains, as well as on the size and density of a grain. Two years of research into the weight of grain on a barely spike show statistically significant differences between the examined varieties. The average grain weight for all varieties was 1,030 g. A significantly larger grain weight on a spike in comparison to other varieties was exhibited by the variety *Zlatnik* (1,042 g), while a significantly smaller grain weight on a spike was exhibited by the variety *Nonius* (1,021 g).The weight of 1000 grains (Table 3) depends on the size of a grain, i.e. on its dimensions and density. Full, healthy grain of a regular shape provides a large percentage of the first and second class grains. The weight of 1000 grains, however, is not merely a yield component, but also a very important quality component of malting barley grains (Ullrich, 2002). The results regarding the weight of 1000 grains show that in the course of the first year of research the best results were exhibited by the variety *Amorosa* (53,1 g), while in the second year it was the variety *Zlatnik* (56,2 g).

Table 3. The weight of 1000 grains, hectolitre weight and grain yield.

Variety	1000 grain weight (g)			Hectolitre weight (kg hl^{-1})			Grain yield (kg ha^{-1})		
Cultivar	2018.	2019.	average	2018.	2019.	average	2018.	2019.	average
Nonius	49.10	51.10	50.10	60.20	60.50	60.35	4 480	4 520	4 500
Zlatnik	52.30	56.20	54.25	68.80	69.40	69.10	5 380	5 410	5 395
Amorosa	53.10	54.50	53.80	66.10	67.20	66.65	4 750	4 790	4 770
average	51.50	53.93	52.71	65.03	65.70	65.36	4 870	4 906	4 888
LSD 005	3.75	4.75		2.35	7.56		581	614	
001	4.25	5.45		2.85	8.12		785	865	

The results regarding hectolitre weight of the examined barley varieties indicate that there are statistically significant differences between the examined varieties. In the course of both years of research the highest values were achieved by the variety *Zlatnik*, while the influence of a particular year on the achieved values in the examined varieties is considered inconsequential. Aside from its impact on the grain quality, a great hectolitre grain weight significantly influences the increase in yield, which is in compliance with our results.
With an increase in the planting density and mineral nitrogen nutrition, the number of spikes per m^2 increases as well, and with an increase in the number of spikes, grain yield increases as well (correlation coefficient 0,63). Hesselbach, (1985) indicates that an increase in barley grain yield in recent years is mostly the result of an increase in the number of grains per spike. The number of spikes per unit area remained the same, while the grain weight varied. Barley varieties differed according to the realised average grain yields. The largest grain average yield was prodiced by the variety *Zlatnik* (5.395 kg ha^{-1}), and the smallest by the variety *Nonius* (4.500 kg ha^{-1}). Significant differences have been established between the examined varieties. According to the result by Pržulj et al., (2000), the varieties of malting barely and animal feed barely which we produce have the yield potential up to 11 tons per hectare, i.e. 8 tons per hectare in spring barely. Depending on the year, yields of barley varieties from Kragujevac in macroexperiments moved from 4.23 to 6.20 tons per hectare Madić, et al., (2006), which is consistent with our results.

Conclusions

The results obtained by examining yield components of the three aforementioned varieties of winter barley in agro-ecological conditions of the Niš District indicate that the genotype and growing conditions have significantly influenced the values of the analysed characteristics. From the standpoint of spike length, number of grains per spike, number of plants per m^2, number of spikes per m^2, number of stalks per m^2, grain weight per spike, weight of 1000 grains and hectolitre weight, the variety *Zlatnik* provided the best results, both according to the years of research and according to the average for both years. On the basis of high values for all examined yield components the variety *Zlatnik* has produced a significantly larger grain yield in comparison to other examined varieties. The average grain yield for both years of research in case of the variety *Zlatnik* was 5.395 kg ha^{-1}, in the variety *Amorosa* 4.770 kg ha^{-1}, while the lowest yield was produced by the variety *Nonius* 4.500 kg ha^{-1}. The yield variation per years of research was the lowest in the variety *Zlatnik* which, aside from a high fertility potential, indicates a great genetic stability of the variety.

References

Barczak B. and Majcherczak E. (2009). Effects of varied fertilization with sulfur on selected spring barley yield structure components. *Journal of Central European Agriculture*, 9 (4), 777-784.

Dofing S. and Knight C.W. (1994). Yield Component Compensation in Uniculm Barley Lines. *Agronomy Journal*, 86:273–276.

Hesselbach J. (1985). *Breeding progress with winter barley* (*Hordeum vulgare L.).* Z. Pflanzenzuchtung, 76, 102-112.

Madić M., Paunović A., Đurović D., Kraljević-Balalić M., Knežević D. (2005). The analysis of gene effect in the inheritance of kernel number per spike in barley hybrid. *Genetika,* 37, 3, 261-269.

Madić M., Knežević D., Paunović A., and Bokan N. (2006). Variability and inheritance of tillering in barley hybrids. *Genetika,* 38, 3,193-202.

Paunović A., Madić M., Knežević D., Bokan N. (2007). Sowing density and nitrogen fertilization influences on yield components of barley. *Cereal Research Communications*. 35(2): 901-904.

Przulj N., Momčilović V., Knežević D. (2000). Dry matter and nitrogen accumulation and retranslocation in spring barley. *Proc. of 8th Barley Genetic Symposium,* 3, 23-235.

Stojanović Ž., Dodig D., Stanković S., Petrović R. (1998). Importance of six-rowed spike for increasing in genetic fertility potential of barley. *Breeding of Small Grains. Proceedings. Kragujevac*, 209-215.

Ullrich E.S. (2002). *Genetics and breeding of barley feed quality, p. 115-142*. In Slafer, G.A., Molina-Cano, J.L., R. Savin, R., Araus J.L. and Romagosa, I.156 (eds.). *Barley Science: Recent Advances from Molecular Biology to Agronomyof Yield and Quality*. Haworth Press, Binghamton, NY.

INFLUENCE OF GENOTYPES AND ENVIRONMENT ON EGGPLANT FRUIT LENGTH AND WIDTH

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Abstract

One of the goals in eggplant breeding (*Solanum melongena* L.) is higher yield, as well as adaptation to different environments. Our study included 20 different eggplant genotypes. The experiment was conducted at three different locations (Smederevska Palanka, Vranovo and Kusadak) using a randomized complete block design in three replications. The length and width of fruit were examined, characteristics important both for the shape and the weight of the fruit. Two-factors ANOVA determined a statistically significant influence of genotype and locality for fruit length and width. Average fruit lengths ranged from 134.86 mm (K19) to 246.07 mm (K38). The mean value of fruit length from the Kusadak locality was significantly ($p<0.01$) higher than the general average (181.58 mm), and significantly longer fruit length was recorded in 10 genotypes: K6, K13, K15, K16, K21, K22, K22, K25, K36 and K39. The highest average fruit width (120.05 mm) was recorded in genotype K19 and differed significantly in relation to the average values of other observed genotypes. The lowest average fruit width was recorded in the K38 genotype at Smederevska Palanka and Kusadak localities (54.33 mm and 54.82 mm). According to AMMI analysis, the least stable locality in terms of fruit length was Kusadak, while the most stable was Smederevska Palanka with an AMMI stability coefficient of 7.00. The most stable genotype was K3 (0.76). The locality Smederevska Palanka was the most stable for fruit width (9.50), while the most stable genotype was K7 (0.28). The least stable locality was Vranovo.

Keywords: *eggplant, environment, fruit length, fruit width, AMMI analysis.*

Introduction

Eggplant (*Solanum melongena* L.) is a agronomically and economically important plant member of *Solanaceae* family with a significant foundation source of various vital pharmaceuticals and nutraceuticals compounds (Naeem and Ugur, 2019). In 2018, world production of eggplants was 54 million tonnes on more than 1.8 million ha, led by China with 63% of the total and India with 24% (FAOSTAT, 2019). The presence of good fiber and various vitamins and minerals in fruits (rich source of iron, manganese) is of great benefit to human health. Eggplant also contain high phenolic contents that act as antioxidants (Cao et al., 1996; Stommel and Whitaker, 2003; Caguiat and Hautea, 2014). Foods that contain antioxidants may help prevent a range of diseases. Among the antioxidants in eggplants are anthocyanins, including nasunin, lutein, and zeaxanthin. Eating foods containing certain flavonoids, including anthocyanins, helps reduce inflammatory markers that increase the risk of heart disease (Fallah et al., 2020). The color of purple skin cultivars is due to the anthocyanin nasunin (Noda et al., 2000). The browning of eggplant flesh results from the oxidation of polyphenols, such as the most abundant phenolic compound in the fruit, chlorogenic acid (Prohens et al., 2007). In addition to being used as an important vegetable plant, eggplant is also used in traditional medicine (Khan, 1979). Various research shows that the eggplant extracts have superb healing effects on different disorders like burns, warts, inflammatory infections, gastritis, stomatitis and arthritis (Im et al., 2016). A research conducted by Afshari et al. (2016) proved that extracts from eggplant have a extra toxic result on cancer cells than on normal cells. Chlorogenic acid also shows anticarcinogenic functions by making apoptosis in many human cancer cells, such as leukemia and lung cancer cells (Tajik et al., 2017). One of the goals of the eggplant breeding has a higher yield and better quality fruits, as well as adaptation to various environmental (Borgato et al., 2007). Minor changes in nutrient composition occur with season, environment of cultivation (open field or greenhouse), and genotype (San José et al., 2014). The effect of environmental factors may also affect the divergence. Many of the forms are created as a form of adaptation to environmental conditions. The environments especially significantly influences the form of expression of quantitative properties. Variability is observed even within the same form in terms of plant height, fruit size and yield depending on the climate, exposure, place and method of cultivation (Prodanović et al., 2015). In this experiment the influence of two parameteres: genotype and locality on the length and width of the fruits were examined, characteristics important both for the shape and the weight of the fruit, ie. yield.

Material and methods

The research included 20 different genotypes that are part of the collection of the Institute of Vegetables, Smederevska Palanka. 16 genotypes originated from Serbia, 2 from the Netherlands (K22 and K25), one from Italy (K19) and one from Israel (K38). The experiment was conducted at 3 different localities: Smederevska Palanka (44°21'24.79"N, 20°56'55.70"E, altitude 103 m), Vranovo (latitude 44°36'6,35"N, longitude 20°59'55.47"E, altitude 87 m) and Kusadak (latitude 44°24'6,73"N, longitude 20°56'30,52"E, altitude 175 m). The localities differed in soil type and climatic conditions. In Smederevska Planka, the type of land was alluvial smonica, while in Vranovo and Kusadak was grove. The average air temperatures in all three different localities were higher than the multi-year average. During the period of development of vegetative organs and flowering in eggplant plants, average temperatures ranged from 20.5 - 29.2 °C at the locality Smederevska Palanka, Kusadak 19.8 -26.5 °C and Vranovo 21.0 - 27.8 °C. The total amount of precipitation at the localities Kusadak (152.2 mm) and Vranovno (139.6 mm) was lower compared to the multi-year average precipitation. In Smederevska Palanka, it was 240.7 mm, which is more than the multi-year average. Sowing was carried out in individual pots of 11 cm diameter, filled with sterile substrate and kept in a protected area. The seedling were maintained in a common manner, i.e. every 15 days fertilized with NPK 20:20:20 (25g / 10l of water) and treated with pesticides as needed. Planting was carried out at the beginning of June and during the vegetation, identical agro-technical measures were implemented at all three localities. The area of the basic plot was 56 m^2. In each replication, 10 plants were placed in a row for each genotype. The length of the rows was 4 m, the distance between the rows was 0.70 m, while the distance between the plants in the row was 0.40 m. The length and width of the fruits were examined, characteristics important both for the shape and the weight of the fruit, ie. yield. The trials was conducted in the Randomized Complete Block Design (RCBD) with 3 replications.

The influence of 2 factors on the measured parameters was observed: genotype and locality. All data is statistically processed in StatSoft Inc. STATISTICA, version 8.0 (2007) and shown as the mean value ± standard error. Statistical data processing implied analysis of the variance of the four-factor experiment (ANOVA) and the comparison of mean values with the LSD test (least significant difference) at the level of significance $P \leq 0,05$.
Stability of the observed traits were analyzed using the AMMI (Additive Main Effects and Multiplicative Interaction) analysis (Gauch, 1988; 1992). GxE interaction was interpreted based on the AMMI2 biplot graph - abscissa representing value of PC1, and ordinata representing value of PC2. AMMI stability value (ASV) was calculated according to the formula (Purchace, 2000):

$$ASV = \sqrt{\left[\frac{SS_{PC1}}{SS_{PC2}}\ x\ (PC1\ value)\right]^2 + [PC2\ value]^2}$$

SS = sum of the squares; PC1 = the first major component; PC2 = the second major component.

AMMI analysis was performed using the R software, version 2.15.2 (A Language and Environment, Copyright 2012).

Results and discussion

Fruit length and width are very important features for fruit shape formation, and have been studied by a number of researchers (Chadha et al., 1987; Singh and Singh, 1985). Analysis of variance (ANOVA) revealed statistically significant differences between the studied genotypes, localities and genotype × environment interactions for both traits, fruit length and width (Table 1).

Table 1. Analysis of variance (ANOVA) for fruit length (A) and width (B)

	Source of variation	Df	SS	MS	F value	F – tabular 0,05	F – tabular 0,01
A. fruit lenght	Repetition	2	1,94	0,97	$0,28^{nz}$	3,07	4,80
	Genotype (G)	19	110.455,81	5.813,46	$1.705,77^{**}$	1,68	2,06
	Locality (L)	2	2.500,86	1.250,43	$366,90^{**}$	3,07	4,80
	G × L	38	26.929,71	708,68	$207,94^{**}$	1,51	1,78
	Error	118	402,16	3,41			
	Total	179	140.290,48				
B. fruit width	Repetition	2	7,49	3,75	$1,53^{nz}$	3,07	4,80
	Genotype (G)	19	55.439,00	2.917,84	$1.187,68^{**}$	1,68	2,06
	Locality (L)	2	83,27	41,64	$16,95^{**}$	3,07	4,80
	G × L	38	4.588,65	120,75	$49,15^{**}$	1,51	1,78
	Error	118	289,90	2,46			
	Total	179	60.408,31				

Df- Degrees of freedom; SS - The sum of the squares; MS - The middle of the square

Average fruit lengths ranged from 134.86 mm (K19) to 246.07 mm (K38). In both genotypes, a statistically significant deviation from the mean value of the general average was observed. The highest average fruit width (120.05 mm) was recorded in genotype K19 and differed significantly in relation to the average values of other observed genotypes. Genotype K19 also had the highest average value of fruit width at the localities Smederevska Palanka and Vranovo, 123.50 mm and 117.33 mm respectively. The lowest average fruit width was recorded in the K38 genotype at Smederevska Palanka and Kusadak localities (54.33 mm and 54.82 mm). The mean value of fruit length from the Kusadak locality was significantly ($p<0.01$) higher than the general average (181.58 mm), and significantly longer fruit was recorded in 10 genotypes: K6, K13, K15, K16, K21, K22, K22, K25, K36 and K39.

Table 2. Analysis of AMMI model variance for length (A) and fruit width (B)

	Source of variation	Df	SS	SS (%)	MS	F Value
A.	Genotype (G)	19	110.434,00	78,73	5.812,30	1.782,68**
fruit length	PON	6	31,00	0,02	5,20	1,59nz
	Locality (L)	2	2.499,00	1,78	1.249,30	240,42**
	G × L	38	26.940,00	19,20	709,00	217,44**
	PC1 (59,9%)	20	16.131,39	59,88	806,57	247,38**
	PC2 (40,1%)	18	10.808,93	40,12	600,50	184,18**
	PC3 (0%)	16	0	0	0	0
	Error	114	372,00	0,27	3,30	
	Total	179	140.276,00	100,00		
B.	Genotype (G)	19	55.416,00	91,78	2.916,62	1.288,30**
fruit width	PON	6	39,00	0,07	6,44	2,85*
	Locality (L)	2	82,00	0,13	40,76	6,33*
	G × L	38	4.585,00	7,59	120,65	53,29**
	PC1 (84,2%)	20	3.859,32	84,17	192,97	85,23
	PC2 (15,8%)	18	725,49	15,83	40,31	17,80
	PC3 (0%)	16	0	0	0	0
	Error	114	258,00	0,43	2,26	
	Total	179	60.380,00	100,00		

Df- Degrees of freedom; SS - The sum of the squares; MS - The middle of the square

AMMI analysis of eggplant fruit length and width showed significant differences between localities, genotypes, and their interactions (Table 2). 78.73% of the total sum of squares refers to the effect of genotype in the case of length (Table 2A) and even 91.78% in the case of fruit width (Table 2B). A large sum of squares of genotypes indicates a very pronounced divergence between the observed genotypes for the examined traits. For the length of the fruit, the sum of the squares G × L is ten times higher than the sum of the squares of the locality (Table 2A), while for the width of the fruit it is even 58 times higher (Table 2B). Based on the large sum of the squares of the interaction, we can conclude that there were significant differences between the reactions of the genotypes within different environments.

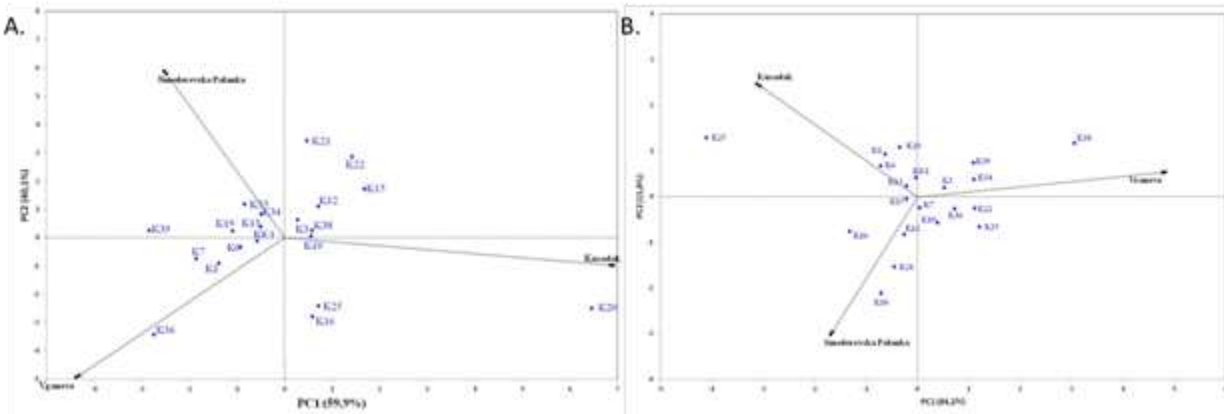

Figure 1. AMMI2 biplot for 20 genotypes of eggplant at three localities for the fruit length (A) and fruit width (B)
Legend: Locations: Smederevska Palanka, Kusadak, Vranovo; Genotypes: K1, K3, K6, K7, K8/1, K10, K12, K13, K15, K16, K19, K20, K21, K22, K25, K34, K35, K36, K38, K39

Figure 1. shows the relationship between the first and second main components - the G × L interaction is shown graphically. A smaller angle between the vectors represents a greater similarity in their interaction (Babić et al., 2010). The genotypes grouped on the graph have similar adaptability (Balalić, 2010), those located near the center of the section can be considered the most stable, while those furthest from the center of the section are the least stable.
The least stable locality, in terms of fruit length, was Kusadak, while the most stable was Smederevska Palanka, which means that there was the least variation of this trait at that locality. The least stable genotype K20 corresponded to the conditions of the locality Kusadak, where the most stable results for the observed trait were recorded. In addition, the conditions in this locality also affected the genotypes: K3, K10, K38. The most stable results for fruit length at the locality Vranovo were recorded in genotypes K36, K1, K6, K7, K8/1, while in Smederevska Palanka genotypes K13, K34 and K35 stood out in terms of stability (Figure 1A).
For the fruit width, the least stable locality was Vranovo, while the most stable was Smederevska Palanka. Environmental conditions at the Kusadak site favored genotypes K1, K6, K13, and they had the greatest stability in the conditions of this locality. The least stable genotype K25 was affected by the environmental conditions that characterized Kusadak, and the genotype K38, which was also unstable, was affected by the environmental conditions in Vranovo. The most stable results for fruit width at the locality of Smederevska Palanka were observed in genotypes K7, K12, K10, K21, while in Vranovo genotypes K3, K22, K34, K36 were singled out (Figure 1B).

Conclusion

Analysis of variance determined a statistically significant influence of all examined factors (genotype, locality and genotype × locality interaction) on the observed traits: fruit length and width. Also based on AMMI analysis for all analyzed traits in this experiment, there were significant differences between genotypes, localities and their interactions.

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References

Afshari F., Serah H., Hashemi Z.S., Timajchi M., Olamafar E., Ghotbi L., Asadi M., Elyasi Z., Ganjibakhsh M. (2016). The cytotoxic effects of eggplant peel extract on human gastric adenocarcinoma cells and normal cells. Mod Med Lab J., 1: 42-48.

Babić V., Babić M., Ivanović M., Kraljević-Balalić M., Dimitrijević M. (2010). Understanding and utilization of genotype-by-environment interaction in maize breeding. Genetics 42(1): 79-90.

Balalić I. (2010). Multivariate analysis of the influence of hybrid interaction and sowing dates on oil content, yield and yield components of sunflower. Doctoral dissertation. Faculty of Agriculture, Novi Sad.

Borgato L., Conicella C., Pisani F., Furini A. (2007). Production and characterization of arboreous and fertile Solanum melongena and Solanum marginatum somatic fertile hybrid plants. Planta 226: 961-969.

Caguiat X., Hautea D. (2014). Genetic diversity analysis of eggplant (Solanum melongena L.) and related wild species in the philippines using morphological and SSR markers. Sabrao J. Breed. Genet., 46(2): 183-201.

Cao G., Sofic E., Prior, R. (1996). Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44(11): 3426-3431.

Chadha M.L., Hegde R.K. (1987). Graphic analysis of some agronomic characters in brinjal. Ind. Jour. of Horg. 44(3-4): 220-225.FAOSTAT (2019). http://www.fao.org/faostat/en/#data/QC/

Fallah A.A., Sarmast E., Fatehi P., Jafari T. (2020). Impact of dietary anthocyanins on systemic and vascular inflammation: Systematic review and meta-analysis on randomised clinical trials. Food Chem Toxicol., 135: 110922.

Gauch H.G. (1988). Model selection and validation for yield trials with interaction. Biometrics 44: 705-715.

Gauch H.G. (1992). Statistical analysis of regional yield trials: AMMI analisys of factorial desings. Elsevier science publishers B.V., Amsterdam, The Netherlands.

Im K., Lee J.Y., Byeon H., Hwang K.W., Kang W., Whang W.K., Min H. (2016). *In Vitro* antioxidative and anti-inflammatory activities of the ethanol extract of eggplant (*Solanum melongena*) stalks in macrophage RAW 264.7 cells. Food Agr Immunol., 27: 758-771.

Khan R. (1979). Solanum melongena and its ancestral forms. In: Hawkes J.G., Lester R.N., Skelding A.O. (Eds) The biology and taxonomy of the Solanaceae. Academic Press, London, pp 629-635.

Naeem M.Y., Ugur S. (2019). Nutritional Content and Health Benefits of Eggplant. Turkish Journal of Agriculture - Food Science and Technology, 7(sp3): 31-36.

Noda Y. Kneyuki T., Igarashi K., Mori A., Packer L.(2000). Antioxidant activity of nasunin, an anthocyanin in eggplant peels. Toxicology, 148 (2–3): 119–23.

Prodanović S., Šurlan-Momirović G., Rakonjac V., Petrović D. (2015). Genetic resources of plants. Editor - Dušan Radivojević, Publisher - Faculty of Agriculture, University of Belgrade, Belgrade, p. 1-248.

Prohens J., Rodríguez-Burruezo A., Raigón M.D., Nuez F. (2007). Total Phenolic Concentration and Browning Susceptibility in a Collection of Different Varietal Types and Hybrids of Eggplant: Implications for Breeding for Higher Nutritional Quality and Reduced Browning. J. Am. Soc. Hortic Sci., 132 (5): 638–646.

San José R., Sánchez-Mata M.C., Cámara M., Prohens J. (2014). Eggplant fruit composition as affected by the cultivation environment and genetic constitution. J Sci Food Agric., 94 (13): 2774–84.

Singh S.N., Singh H.N. (1985). Studies on overage degree of dominance and heritability estimates in eggplant. Hor. Jour. of Hort. Sci., 14(3-4): 240-242.

Stommel J.R., Whitaker B.D. (2003). Phenolic acid content and composition of effplant fruit in a germplasm core subset. J. Am. Soc. Hortic. Sci., 128: 704-710.

Tajik N., Tajik M., Mack I., Enck P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Eur. J. Nutr., 56: 2215-2244.

BIOPRIMING: A SUSTAINABLE SUPPORT FOR CROP ESTABLISHMENT

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Abstract

Crop yields are highly depended on germination and early stages of plant growth. Numerous priming techniques are being developed aimed to improve germination. Biopriming represents a sustainable approach based on seed treatment in bacterial suspension of selected plant growth promoting strains. One of the most promising plant growth promoting bacteria is *Azotobacter chroococcum*. The aim of the research was to evaluate the effects of *A. chroococcum* F8/2 as a biopriming agent on germination of various cultivable plants: basil, white mustard, cucumber, tomato, wheat, canola, and soybean. After surface sterilization, seeds were bioprimed in the bacterial suspension (10^7CFU/ml). Uninoculated seeds represented control treatment. Germination test was conducted with 100 seeds per treatment and the germination was monitored for 7 days. Following germination parameters were determined: germination percentage, germination index, mean germination time, vigor I, vigor II, length and dry biomass of the seedlings. The bacterial inoculation caused higher germination percentages of cucumber, tomato, wheat and soybean. The highest increase in germination index was observed in wheat (an increase of 19.8%). Tomato and basil were the only plants where vigor I was not increased by inoculation. Generally, the most favorable effects of *A. chroococcum* biopriming were observed in wheat where vigor I was increased more than twice, and vigor II was higher by 75.4% in inoculated seeds. The results indicate a significant potential for *A. chroococcum* use in biopriming. The observed effects of seed priming on germination parameters were crop-specific, with the most prominent potential in wheat biopriming.

Keywords: *Azotobacter chroococcum, Biopriming, Germination, Seedlings' growth.*

Introduction

The current agricultural, plant production concerning the needs of the world human population is out of step. Limited soil resource, its increased compaction, salinity, absence of organic matter, presence of pollutants are just some of the abiotic factors that compromise this production. However, the disturbed balance in the agroecosystem reflects its destructive impact on all other ecosystems and their living components. The introduction of new technologies that respect natural laws are and will be the key to sustainable crop production. Among them, bioprimig technology contributes to increasing the yield and quality of crops by using natural potentials while respecting the principles of sustainability. Biopriming involves the use of bioagents in seed treatment by various methods. Microbiological inoculation is the most desirable and most frequently used biopriming method based on the application of rhizosphere microorganisms as bioagents in the form of bacterial suspension (Ashraf and Foolad, 2005). This significant population of microorganisms from the soil of the narrow root zone (rhizosphere) has properties that in the interaction with the plant promote physiological events resulting in the good yield.

Therefore, they are defined as plant growth bacteria (Plant Growth Promoting Bacteria / Rhizobacteria, PGPR) (Kloepper and Schroth, 1978). They are active participants in the plant's nutritional cycle, phytohormonal modulation, biocontrol and elimination of toxic substances (Sumbul *et al.*, 2020).
Since the seeds are the starting material in plant production and carry a huge potential responsible for plant growth and development, biopriming is focused on the seeds and the initiation of their potential, which occurs in germination. Germination is defined as a crucial and critical phase in the life of a plant and its good start is a prerequisite for the promotion of yield and quality (Sanchez *et al*., 2014; Houle et al., 2001**).** The yield can be increased in the range of 25-65% (Ahmad *et al.,* 2016) with an improvement in crop quality at the same time (Revillas *et al.,* 2000). By microbiological inoculation, selected PGPR strains will successfully colonize the seed structure **(**Mahmood and Kataoka, 2018) and after stimulating germination and seedlings' growth, will continue to actively participate by various mechanisms in the plant life in the rhizosphere (Yadav *et al*., 2015). Among the most common inoculants is *Azotobacter* due to its prestigious PGP traits such as the ability to provide nutrients to plants, especially nitrogen through the process of nitrogen fixation, synthesis of phytohormones, siderophores, antibiotics, participation in biocontrol and degradation of toxic compounds (Nosrati *et al.*, 2014; Sumbul *et al*., 2020; Kumari *et al*., 2017). However, the positive effect is far greater and reflected in the improvement of the quantitative and qualitative properties of the soil while preserving its diversity (Jeffries *et al.*, 2003).
Bearing in mind the importance of biopriming, the aim of this work was focused on the bioagent, *Azotobacter chroococcum* F8/2 and its influence on germination and seedlings´ condition. The success of germination was determined by germination parameters such as final germination percentage (FGP), germination index (GI), average germination time (MGT), vigor I and II, seedling length, and their dry weight. In addition to the choice of inoculant, the results of germination are influenced by numerous factors, among which the plant species is of exceptional importance. Thus, different plant species are used to test the effect of the proposed biopriming.

Material and methods

Bacterial strain

The isolated strain belongs to the collection of the Department for Ecological Microbiology, the Faculty of Agriculture. Growing on the selective Fiodorov medium, based on cell morphology, colony appearance, and biochemical profile the isolate was identified as *Azotobacter chroococcum.*

Plant species and seed pre-treatment

Seven plant species are selected, important for national and worldwide agricultural production: basil (*Ocimum basilicum*), mustard (*Synapsis alba*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum*), canola (*Brassica napus* L.), and soybean (*Glycine max.* L.). All seeds were uniform, compact in structure, and subjected to sterilization with 70% alcohol (v/v) / 2 min and 0.02% NaOCl (v/v)/ 2 min. After thorough rinsing with sterile deionized water, they were set to dry in sterile conditions. Ten seeds were selected for each plant species, placed on MPA medium, and incubated for 24 hours at a temperature of 30 °C to check the success of sterilization.

Inoculum preparation and seed inoculation

48h-old bacterial culture of *A. chroococcum* F8/2 was "scratched" from solid media, and resuspended in the sterile saline (0,9% NaCl) until the inoculum suspension of 10^7 CFU /ml was reached. Previously prepared seeds were immersed in the inoculum suspension and incubated in a rotary shaker (130 rpm) for 1 hour at the temperature of 28±2 °C.

Germination assay

Germination test was performed by applying the slightly changed filter paper method (ISTA; 1999). Sterile Petri dishes were used in which sterile filter papers were placed on the bottom. Before placing the seeds, the filter paper was moistened with sterile water. 100 seeds for each plant species were arranged in 4 replicates of 25 seeds, except for soybeans, where due to the size of the seeds, the number of replicates was 10 with 10 seeds each. Non-inoculated seeds were set up in a completely identical way as control. Previously dried and inoculated seeds were placed on Petri dishes and left at natural light and an average room temperature of 25°C for 7 days. Sterile water was added when needed to maintain optimal humidity.

The number of germinated seeds was recorded daily based on a radical emergence and its length of 2 mm. After 7 days, representative seedlings from each replicate were chosen, randomly for measuring their length. Thereafter, they were dried overnight at 90 °C and their dry weight was recorded. Advanced germination measurement tool (Argon Info-Tech) was used to calculate germination parameters such as the final germination percentage, germination index (GI), mean germination time (MGT), vigor I, and vigor II.

Statistical analysis

The analysis was conducted by Tukey's test comparing the differences between means of obtained results from the inoculated seeds vs control at the 5% level of probability.

Results and discussion

Data obtained from the conducted experiment show differences in plant species response to the applied biopriming with A. chroococcum F8/2. Significant increases in FGP were achieved in cucumber (12.70%), tomato (8.79%), soybean (14.58%), and wheat which showed the largest increase of 19.58% compared to the control (Tab. 3, 4, 5, and 7). Although a desirable reduction in MGT was observed in some plants, it did not indicate a significant difference between biopriming and control treatments. A slight increase in germination index (GI) was observed in canola, tomato, and wheat, while GI of inoculated treatments was significantly higher compared to control in cucumber for 28.30%, and soybeans for 56.72% (Tab. 3 and 7).

Table 1. Germination parameters of basil (Ocimum basilicum) bioprimed by Azotobacter chroococcum F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry mass (g)
Inoculation	58 ± 4	5.01 ± 0.13	3.16 ±0.16	293.90 ± 13.79	0.0392 ±0,0040	5.1 ±0.23	0.0007 ±0.0001
Control	69 ± 4	5.16 ± 0.09	3.74 ±0.25	321.45 ± 24.73	0.0355 ±0.0027	4.63 ±0.29	0.0005 ±0.0001

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 2. Germination parameters of white mustard (*Sinapsis alba* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry mass (g)
Inoculation	100 ± 0	1.06 ± 0.02	24.52 ±0.34	1266.4 ±77.8	0.5192 ±0.0298*	12.65 ±0.67	0.0052 ±0.0010*
Control	100 ± 0	1.08 ± 0.05	24.57 ±0.37	1186.8 ±56.2	0.3967 ±0.0252*	11.87 ±0.67	0.0040 ±0.0008*

*Data show means ±Std.Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 3. Germination parameters of cucumber (*Cucumis sativus*) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	71 ± 1*	2.82 ± 0.07	7.40 ±0.36*	1293.09 ±48.43*	1.3242 0.0789	18.19 ±0.62*	0.0186 ±0.0011
Control	63 ± 2*	3.14 ± 0.17	5.76 ±0.08*	549.40 ±30.05*	1.0991 0.0842	8.71 ±0.42*	0.0174 ±0.0012

*Data show means ±Std. Err; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 4. Germination parameters of tomato (*Solanum lycopersicum* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	99 ± 1*	3.97 ± 0.09	6.64 ± 0.21	991.75 ± 93.54	0.1931 ± 0.0125*	9.25 ± 0.29*	0.0020 ± 0.0001*
Control	91 ± 2*	3.78 ± 0.07	6.48 ± 0.08	1076.17 ± 33.741	0.143 ± 0.0065*	11.82 ± 0.33*	0.0016 ± 0.0001*

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 5. Germination parameters of wheat (*Triticum aestivum*) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	89 ± 3*	1.97 ± 0.10	14.38 ± 0.76	1883.44 ± 69.81*	1.8277 ± 0.0573*	21.18 ± 0.69*	0.0204 ± 0.0005*
Control	77 ± 1*	1.84 ± 0.08	12.00 ± 0.62	894.52 ± 48.66*	1.0421 ± 0.0396*	11.64 ± 0.66*	0.0135 ± 0.0005*

*Data show means ±Std.Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 6. Germination parameters of canola (*Brassica napus* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	43 ± 6	1.66 ± 0.20	8.48 ± 0.87	591.84 ± 36.32*	0.1303 ± 0.0119*	13.77 ± 0.46*	0.003 ± 0.0002
Control	44 ± 0	1.75 ± 0.25	8.36 ± 0.91	397.99 ± 36.22*	0.1397 ± 0.0119*	9.04 ± 0.46*	0.0031 ± 0.0002

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.0*

Table 7. Germination parameters of soybean (*Glycine max* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	% FGP	MGT (day)	GI (seed/day)	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	55 ± 2*	4.64 ± 0.24	3.73 ± 0.25*	500.87 ± 46.37*	10.4019 ± 0.5542*	9.26 ± 0.98*	0.1811 ± 0.0106
Control	48 ± 2*	5.20 ± 0.05	2.38 ± 0.25*	222.64 ± 29.50*	8.4321 ± 0.5201*	4.63 ± 0.60*	0.1759 ± 0.0108

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Vigor I was determined as the common value of the germination percentage and the length of seedlings. Statistically significant increases were observed in wheat of 110%, soybean (124%), and canola (48.71%). However, a significant decrease of 46.61% was presented in cucumber. Analyzing the data of Vigor II, increases were recorded in soybeans (23.36%), followed by mustard (30%), tomatoes (37.39%), and wheat of 75.39%, which was the most significant increase achieved by Azotobacter biopriming. Considering the seedlings' length, the promotion was noted in cucumber (108.84%), soybean (100%), and wheat (81.96%). The inhibitory effect of inoculation treatment was evident in tomatoes where a reduction of the seedlings length was 27.78%. Although the inoculation had an inhibitory effect on the length of seedlings in tomatoes, it contributed to an increase in their dry matter by 25%. Also, an increase of 30% in dry mass was recorded in mustard. However, according to the results, the most prominent dry mass increase was in the wheat of even 51.11%.

Since germination is a complex physiological event that determines the entire future plant development and its final production, all of the studied parameters were in purpose for a better assessment of germination success influenced by Azotobacter biopriming. Biopriming stimulates pre-germinative processes in the seed contributing to better germination (Bradford, 1986). The selected bioagent, A. chroococcum F8/2, initiated significant promotion of the germination percentage in most of the plants, as the best in wheat. Although the germination percentage is an important determinant of germination success it is not crucial. Previous studies have given priority to vigor as a powerful seed potential in the realization of final yield under given conditions (TeKrony, 2003). The phytohormonal activity of A. chroococcum is obvious by analyzing seedling length. The ability to modulate hormonal balance among ethylene, GAs, and auxin is most likely the reason for the increase in seedling length and the direct reason for the improvement of vigor I. The stimulating effect of Azotobacter inoculation on seedlings' development is reflected through dry weight increase that affects vigor II as an indicator of seedlings' ability to continue to develop successfully in the later life stages. Also, germination and vigor are genetic traits (Wu et al., 2017). It is known that germination depends largely on the vitality of the embryo and the depth of dormation (Martinez, 2013). The result of germination may be influenced by the different strain affinity for a certain plant species and its exudates (Lugtenberg et al.,2001; Kumar et al., 2007; Compant, 2010) that are released in the imbibition phase, significantly affecting the colonization process and metabolic activity of biopriming agents (Brelwey and Black, 1993).

Conclusion

The effect of biopriming using *Azotobacter chroococcum* F8/2 is specifically dependent on plant species, with best results in germination promotion in wheat. *A. chroococcum* biopriming of soybean, wheat, tomato and cucumber seeds may trigger the promotion of germination, but also could help the creation of a compatible connection between inoculant and plants providing useful activities in the rhizosphere in later crop development stages. The final results of the application of *Azotobacter* biopriming could be more far-reaching, including agronomic, ecological, and economic aspects.

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References

Ahmad P., Abdel Latef A.A., Hashem A., Abd_Allah E.F., Gucel S., Tran L.S.P. (2016). Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Frontiers in *Plant Science. vol. 7, pp. 347.*

Ashraf M., Foolad M. (2005). Pre Sowing Seed Treatment—A Shotgun Approach to Improve Germination, Plant Growth, and Crop Yield Under Saline and Non Saline Conditions. Advances in Agronomy. vol. 88, pp. 223-271.

Bewley, J.D., Black, M. (1978). Physiology and Biochemistry of Seeds in Relation to Germination. Springer-Verlag. New York. pp. 305-375.

Bradford K.J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience. vol. 21, pp. 1105–1112.

Compant S., Clément C., Sessitsch A. (2010). Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry. vol.* 42(5), pp. 669–678.

Houle G., More L., Reynolds C.E., Siége J. (2001). The effect of salinity on different developmental stages of an endemic annual plant *Aster laurentianus* (*Asteraceae*). American Journal of Botany. vol. 88, pp. 62-67.

Jeffries P., Gianinazzi S., Perotto S. et al (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology* and *Fertility* of *Soils.* vol. 37, pp. 1–16.

Kloepper J.W., Schroth M.N. (1978). Plant growth promoting rhizobacteria on radishes. In, Proceedings of 4th International Conference on Plant Pathogenic Bacteria. vol. 2, pp. 879-882.

Kumar J., Singh D., Ghosh P., Kumar A. (2017). Endophytic and epiphytic modes of microbial interactions and benefits. In, Singh D.P., Singh H.B., Prabha R. "Plant-microbe interactions in agro-ecological perspectives, Fundamental mechanisms, methods and functions". Springer. vol. 1, pp. 227–253.

Kumar R., Bhatia R., Kukreja K., Behl R.K., Dudeja S.S., Narula N. (2007). Establishment of *Azotobacter* on plant roots: chemotactic response, development and analysis of root exudates of cotton (*G. hirusitum L.*) and wheat (*T. aestivum L.*). *Journal of Basic Microbiology. vol.* 47, pp. 436–439.

Lugtenberg B.J.J., Dekkers L.C., Bloemberg G.V. (2001). Molecular determinants of rhizosphere colonization by Pseudomonas. The *Annual Review* of *Phytopathology*. vol. 39, pp. 461-490.

Mahmood A., Kataoka R. (2018). Potential of biopriming in enhancing crop productivity and stress tolerance. In, Rakshit A., Singh H.B. Advances in seed priming. Springer. pp. 127–145.

Martinez E., Carbonell M.V., Amaya J.M. (2000). A static magnetic field of 125 mT sitimulates the initial growth stages of barley (*Hordeum vulgare* L.). Electro- and Magnetobiology. vol. 19(3), pp. 271-277.

Nosrati R., Owlia P., Saderi H., Rasooli I., Malboobi M.A. (2014). Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. I*ranian Journal of Microbiology*. vol. 6, pp. 285–295.

Revillas J.J., Rodelas B., Pozo C., Martinez-Toledo M.V., Gonzalez-Lopez, J. (2000). Production of B-group vitamins by two *Azotobacter* strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. Journal of Applied Microbiology. vol. 89, pp. 486-493.

Sanchez P.L., Chen M.K., Pessarakli M., Hill H.J., Gore M.A., Jenks M.A. (2014). Effects of temperature and salinity on germination of non-pelleted and pelleted Guayule (*Parthenium argentatum* A. Gray) seeds. *Industrial Crops* and *Products*. pp. 55: 90.

Sumbul R., Ansari A., Rizvi R., Mahmood I. (2020). *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi Journal of Biological Science. https//doi.org/10.1016/j.sjbs.2020.08.004.

TeKrony D.M. (2003). Precision is an essential component in seed vigour testing. *Seed Science* and *Technology*. vol. 31, pp. 435-447.

Wu C., Zeng A., Chen P., Florez-Palacios L., Hummer W., Mokua J., Cheng Y. (2017). An effective field screening method for flood tolerance in soybean. Plant Breeding. vol. 136, pp. 710–719.

Yadav A., Sharma D., Gulati S. *et al.* (2015). Haloarchaea Endowed with Phosphorus Solubilization Attribute Implicated in Phosphorus Cycle. *Scientific Reports. vol.* 5**,** 12293.

SUITABILITY OF FIELD PEA: OAT AND COMMON VETCH - OAT MIXTURES FOR ENSILING

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Abstract

The practice of preserving green crops by fermentation as silage has increased dramatically in recent years. Successful ensiling can be difficult due to numerous problems such as: high moisture content, high feed buffer capacity, insufficient amount of fermentable carbohydrates etc. The aim of this investigation was to determine the suitability for ensiling field pea:oat and common vetch:oat mixtures grown at different seeding rate and harvested at different stages of growth. Field pea, common vetch and oat were grown in pure stands and in the mixtures of 75:25, 50:50 and 25:75 ratios and harvested at three stages of plant development: beginning of field pea and common vetch flowering, forming the first pods in field pea and common vetch and at the forming green seeds in 2/3 field pea and common vetch pods. The most favorable ratio of sugar and buffer capacity for the established level of dry matter in the examined crops was stated in pure oat crop. The results obtained in the mixtures of field pea and oat are based on the ratio of sugar and buffer capacity and can successfully ensile in all three examined relations of germinating grains of field pea and oats. The highest sugar content (182.1 g kg^{-1} DM) and the highest buffer capacity were determined in the 50:50 field pea:oat mixture at the pea flowering stage of development. The least favorable ratio of sugar and buffer capacity was found in pure common vetch.

Keywords: *field pea, common vetch, ensiling.*

Introduction

In the conditions of an increasingly pronounced deficit and high food prices, Đorđević et al. (2000) found out that ensiling is the most acceptable solution in order to obtain cheap and quality animal feed. However, succesful ensiling can be difficult due to numerous problems such as: high moisture content, high feed buffer capacity, insufficient amount of fermentable carbohydrates, soil pollutions, etc. The most important factors that should be taken into account before preparing silage is to ensure adequate dry matter content in the biomass for ensiling as well as adequate sugar content, buffer capacity, and acidity coefficient (Knicky, 2005). According to Đorđević et al. (2001), the dry matter content in plant material should be above 35%, to ensure succesful fermentation. In these circumstances the work of most plant and bacterial enzymes is inactivated, which reduces nutrient losses matter. Sugars are the main source of fermentable compounds during the preperation of silage and together with buffer capacity act on the process of silage preparation. That is why they are expected to be useful indicators of crop conservation potential. Sugars necessary for ensiling include mainly monosaccharaides – glucose and fructose, disaccharides – sucrose and polysaccharides -

fructans. The concentration of thes sugars depends on the plant species and the stage of growth of plant exploatation. The sugar content of grasses and cereals increases as the proportion of the stem increases. On the other hand, legumes contain low sugar concentrations. One of the very important parameter for the success of ensiling process represents the buffer capacity of the plant biomass. The objective of this study was to determine the content of dry matter, sugar content, buffer capacity and sugar:buffer capacity ratio in investigated mixtures of pea – oat and common vetch – oat depending on the stage of growth and seeding rate in the mixtures in order to determine the suitability this crop mixtures for ensiling.

Materials and methods

Field pea and oat as well as common vetch and oat were grown in binary mixtures at the experimental field of the Institute for forage crops Kruševac, Serbia (21° 19' 35" E, 43° 34' 58" N). The study area was situated at altitude of 166 above sea level in South East Serbia. Soil type was with humus content of approximately 3.5% and a pH in H_2O 6.87, pH in 1N KCl 5.85; nitrogen content of 0.176%; Al-soluble P_2O_5 and K_2O 3.6 and 28.6 mg 100 g^{-1}, respectively. Experiment was established in autumn in 2015, on October the 20th, and plant samples were taken in 2016. The experiment was designed with three replication according to a randomized complete block. All mixtures were sown on plots of 20 m^2. The experiment was performed using three different cutting stages: A_1 – beginning of pea and common vetch flowering – 10% of flowering; A_2 – forming the first pods on 2/3 pea and common vetch plants and A_3 – forming green seeds in 2/3 pods of pea and common vetch plants. The pea-oat and common vetch-oat mixtures were tested in the pure stands and at three different mixture rates: P:O 25:75 – 25% pea + 75% oat, P:O 50:50 – 50% pea + 50% oat; P:O 75:25 – 75% pea + 25% oat; CV:O 25:75 - and 25% common vetch + 75% oat; CV:O 50:50 - 50% common vetch + 50% oat; and CV:O 75:25 - 75% common vetch + 25% oat. The ratio was formed according to the number of germinating grains of legumes and oat in the mixtures, where the germinating legumes grain per m^2 was taken as a reference for determining the ratio in the mixtures. The reference parameter for pea was 120 germinating grains per m^2, while 250 germinating grains per m^2 was taken for common vetch. Based on this values, the ratio of germinating grains in mixtures were formed. Forage was cut and a sample of the whole plants was collected. Dry matter was determined by the drying out samples at 65° C. Buffering capacity of the samples was assessed according to the procedure of Prohászka and Baron (1980) and expressed as the quantity (Meq) of 0.1N lactic acid required to acidify 100 g of the sample to pH 4. Fermentable coefficient was calculated using formula: FC = DM% + 8 x S/BC by Olt et al. (2005). Total carbohydrates and mosaccharides were determined by spectrophotometric method with phenol-sulfuric acid according to Dubois et al. (1956) and Hall et al. (1999). All results are calculated in order to dry matter basis determined by drying samples to a constant weight at 105°C. The data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmetic means was tested by Fisher test ($p < 0.05$).

Results and discussion

The obtained results for the dry matter content, buffer capacity, content of monosaccharides and total carbohydrates, the ratio of sugar and buffer capacity and fermentable coeficient of oat, pea and common vetch pure stands and investigated mixtures of pea:oat and common vetch:oat depending on the stage of plant development are presented in the Table 1.

The stage of plant development significantly influenced ($p < 0.05$) the dry matter content in oat, pea and common vetch pure stand, as well as in the investigated pea:oat and common vetch:oat mixtures. The highest dry matter content was established at the third stage of plant development – at forming seeds in 2/3 pods of pea and common vetch in allmost all investigated mixtures. Only in the mixture contained 75% of pea dry matter decreased with plant growth and development from 40% to 31%. The most dominating and therefore most important factor in controlling the fermentation in a given crop is the dry matter content of the crop. Increased dry matter content also favours the growth of lactic acid bacteria, and in particular, restricts the development of *Clostridia* (Spröndly and Pauly, 2008). The earlier works by Silliker et al. (1980) showing that dry matter contents above 40-45% inhibits *Clostridia* growth in silage and well confirmed by recent experiments by Kaiser et al (2005) and Pauly et al. (2008), both clearly showing that a high dry matter content inhibit the development of *Clostridia*.

Table 1. Dry matter content, buffer capacity, content of Monosaccharides and Total Carbohydrates, the ratio of sugar and buffer capacity and Fermentable Coeficient of oat, pea and common vetch pure stands and investigated mixtures of pea:oat and common vetch:oat depending on the stage of plant development

	SG	DM	BC	Monosac.	TC	TC / BC	FC
Oat	A_1	37.0^b	67.6^b	104.9^b	115.1^b	1.70^b	50.60^b
	A_2	31.0^c	79.3^a	126.8^a	127.7^a	1.60^c	43.80^c
	A_3	44.0^a	52.3^c	95.0^c	102.3^c	1.95^a	59.60^a
Pea	A_1	33.0^b	66.7^b	202.1^a	207.9^b	3.11^b	57.88^b
	A_2	35.0^a	52.6^c	190.9^b	205.4^c	3.90^a	66.20^a
	A_3	26.0^c	92.3^a	200.0^{ab}	213.5^a	2.31^c	44.48^c
Common vetch	A_1	27.0^b	86.7^b	61.9^b	75.2^b	0.86^b	33.88^b
	A_2	25.0^c	100.0^a	66.0^a	91.2^a	0.91^a	32.28^b
	A_3	32.0^a	78.7^c	42.2^c	46.9^c	0.59^c	36.72^a
P:O 25:75	A_1	33.0^a	78.8^a	146.4^a	154.8^b	1.96^b	48.68^a
	A_2	27.0^b	70.4^b	149.3^a	164.8^a	2.34^a	45.72^b
	A_3	36.0^a	53.3^c	84.7^b	90.3^c	1.69^c	49.52^a
P:O 50:50	A_1	33.0^b	75.1^a	160.9^a	182.1^a	2.42^a	52.36^a
	A_2	26.0^c	70.0^b	156.2^a	163.5^b	2.33^a	44.64^b
	A_3	39.0^a	56.4^c	73.1^b	84.9^c	1.50^b	51.0^a
P:O 75:25	A_1	40.0^a	60.0^b	119.5^a	129.8^{ab}	2.16^{ab}	57.28^a
	A_2	32.0^b	62.5^a	118.1^a	119.1^b	1.90^b	47.20^b
	A_3	31.0^b	61.3^{ab}	113.9^b	139.7^a	2.28^a	49.24^b
CV:O 25:75	A_1	24.0^b	87.5^a	124.2^a	134.2^a	1.53^{ns}	36.24^b
	A_2	25.0^b	76.0^b	78.0^b	117.6^b	1.54^{ns}	37.32^b
	A_3	33.0^a	56.9^c	69.7^c	83.3^c	1.46^{ns}	44.68^a
CV:O 50:50	A_1	29.0^b	86.2^a	148.6^a	169.7^a	1.96^a	44.68^a
	A_2	24.0^c	81.6^b	110.4^b	143.3^b	1.75^a	38.00^c
	A_3	36.0^a	62.7^c	44.7^c	45.3^c	0.72^b	41.76^b
CV:O 75:25	A_1	19.0^c	90.0^a	107.9^a	120.0^a	1.33^a	29.64^c
	A_2	26.0^b	86.5^b	78.1^b	80.4^b	0.93^b	33.44^b
	A_3	37.0^a	51.9^c	46.5^c	54.9^c	1.06^b	45.48^a

SG – Stage of growth; DM – Dry Matter, %; BC – Buffer Capacity, mEq lactic acid; Monosac. – Monosaccharides content, g kg^{-1} DM; TC – Total Carbohydrates content, g kg^{-1} DM; TC/BC – Total Carbohydrates/Buffer capacity; FC – Fermentable

Coeficient; A_1 – beginning of pea and common vetch flowering – 10% of flowering; A_2 – forming the first pods on 2/3 pea and common vetch plants and A_3 – forming green seeds in 2/3 pods of pea and common vetch plants; P:O 25:75 – 25% pea + 75% oat, P:O 50:50 – 50% pea + 50% oat; P:O 75:25 – 75% pea + 25% oat; CV:O 25:75 - and 25% common vetch + 75% oat; CV:O 50:50 - 50% common vetch + 50% oat; and CV:O 75:25 - 75% common vetch + 25% oat; Values denoted by the different letter are significantly different according to Fisher's protected LSD values; $LSD_{0.05}$ – least significant difference at $P_{<}$ 0.05

Intercropping of cereals with legumes is an alternative for monocropping and has a number of advantages, for example lower inputs, lower costs of production and better silage quality than monocrop systems (Dewitto et al., 2008). Successful fermentation depends on a sufficient content of carbohydrates – mainly glucose, fructose, fructosans, saccharose and maltose – and has a positive impact on palatability of ensiled forages.

The obtained results of this study for the ratio of sugar and buffer capacity of pure crops oat, pea, common vetch and investigated mixtures of pea with oat and common vetch with oat depending on the stage of development are presented in the Table 1. The most favorable ratio of sugar and buffer capacity for the established dry matter level in the tested crops was found in pure oat crop. Optimal content of fermentable sugars in pea:oat mixtures conditioned this ratio to be over 2.0 in almost all tested stages of plant development and in all investigated pea:oat mixtures. The lowest value of sugars and buffer capacity was observed at the second stage of oat monocrop, which could be explained by lower dry matter content.

Fermentable coeficient calculated according to the previously mentioned formula given by Olt et al. (2005) in the pure oat, pea and pea:oat mixtures had values greater than 45.0, which showed that the ratio of sugar and buffer capacity of this crops was optimal and that these mixtures can be successfully ensiled. The problem would be represented in a low dry matter content of common vetch:oat mixtures and lower values of sugar and buffer capacity. The lowest values for ratio of sugar and buffer capacity, as well as fermentable coeficient were observed in pure common vetch crop.

Fermentable coeficient ranged from 32.28 at the second stage of plant development to 36.72 at the third stage of plant development in pure stand of common vetch show that the common vetch belongs to a group of plants that are difficult or imposible to ensile on their own. This observation confirmed by the results obtained for 75:25 and 25:75 common vetch:oat mixtures for fermentable coeficient at all investigated stage of plant development. High buffer capacity and low monosaccharides and total carbohydrates content in pure common vetch stand at all three stages of plant development influenced low TC/BC ratio and low fermentable coeficient. On the other hand, the pure stand of common vetch contained the lowest dry matter, which also may affect inadequately fermentable processes during ensiling.

It is necessary to emphasize that in relation to the small suitability of this plant species for ensiling, a much bigger problem is the pronounced lodging feature of the plant, and it is necessary to grow common vetch in a mixture with cereals. This practically solves the biological problem of this plant species in relation to lodging, and at the same time the necessary sugar minimum would be achieved (Đorđević and Dinić, 2003).

Conclusions

According to the results obtained in this investigations we can conclude that the annual forage legumes, especially common vetch, is not suitable for ensiling process, mostly due to the low sugar content and high buffer capacity, which can be compensated by their cultivation in a mixture with cereals. The effect of maturity at harvest and increasing the pea content of bi-crop on suitability for ensiling have been evaluated in this study. Due to the low dry matter content at the cutting and the low sugar content and high buffer capacity, the common vetch can be successfully ensiled only with the high proportion of cereals in the mixtures.

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References

Dewitto, K., Latre, J., Wambacq, E., Haesaert, G. (2008). Culture, harvest and ensiling of some grain legumes. 13th ICFC, Forage Conservation, 54-55.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analitical Chemistry, 28 (3), 350-356.

Đorđević, N., Koljajić, V., Grubić, G., Glamočić, D. (2000). Specifics of different types of silage used in cow nutrition. Journal of Scientific Agricultural Research, 61, 51-62.

Đorđević, N., Koljajić, V., Dinić, B., Grubić, G. (2001). Conservation procedures and effects of lucerne use. Journal of Scientific Agricultural Research, 62, 285-292.

Đorđević, N., Grubić, G., Jokić, Ž. (2003). Basics of domestic animal nutrition – Handbook. Faculty of Agriculture, Belgrade.

Đorđević, N. and Dinić, B. (2003). Siliranje leguminoza. Institut za istraživanja u Srbiji, Srbija.

Hall, M. B., Hoover, W. H., Jennings, J. P., Webster, T. K. M. (1999). A method for partitioning neutral detergent soluble carbohydratres. Journal of Food Science and Agriculture, 79, 2079-2086.

Kaiser, E., Weiss, K., Polip, I. (2005). New results on inhibition of *Clostridia* development in silages. In: Silage production and utilization (eds. Park & Shong). Proceedings of the XIV International Silage Conference, Belfast, Northern Ireland, 213.

Knicky, M. (2005). Possibilities to improve silage conservation. Doctoral Thesis, Swedish University of Agricultural Science, Uppsala, 62.

Olt, A., Kärt, O., Kaldwäe, H., Ots, M., Songiseep, E., Smidt, I. (2005). The effect of additive and dry matter content on silage protein degradability and biogenic amine content. Journal of Agricultural Science, 16 (2), 117-123.

Pauly, T., De Paula Sansa, D., Spröndly, R., Christiansson, A. (2008). Inoculation of experimental silages with different *Clostridium* spores. In: Biodiversity and animal feed (eds. Hopkins et al.). Proceedings of the 22nd General meeting of the European Grassland Federation, Uppsala, Sweden, 678-680.

Prohászka, L. and Baron, F. (1980). The predisposing role of high dietary protein supplies in enteropathogenic *E. coli* infections of weaned pigs. Zentralbl. Veterinarmed B., 27 (3), 222-232.

Silliker, J. H., Elliot, R. P., Baired-Parker, A. C., Bryan, F. L., Christian, J. H. B., Clark, D. S., Olsen, J. R., Roberts, T. A. (1980). Microbial ecology of foods. ICMSF, Academic Press, New York.

Spröndly, R. and Pauly, T. (2008). Control of the fermentation process at the conservation of feeds. 13th ICFC, Forage Conservation, 27-33.

STABILITY OF WHEAT CULTIVARS FOR YIELD AND QUALITY COMPONENTS IN DIFFERENT AGROECOLOGICAL CONDITIONS

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Abstract

Stability of grain yield, 1000 kernel weight and sedimentation value were analyzed in 10 winter wheat cultivars (Perfekta, Toplica, KG-52/3, Merkur, Vizeljka, Talas, NS 40S, Zvezdana, Javorka and Pobeda), created in different breeding institutions in Serbia. The experiment was carried out during 2019/2020 in two localities: Centre for Small Grains in Kragujevac and Institute for Forage Crops in Kruševac, Serbia. The analyses of variance showed highly significant differences in grain yield, between genotypes, investigated localities, as well as their interaction. The influence of the locality did not show statistical significance on the expression of 1000 kernel weight and sedimentation value. The highest average values were recorded by Perfekta for grain yield, Zvezdana for 1000 kernel weight and KG-52/3 for sedimentation value at both localities. The AMMI model was used for analysis of genotype × environment interaction. The most stabile cultivars were Vizeljka, NS 40S and Perfekta with values above the average for grain yield. Cultivars Talas, KG-52/3 and Javorka showed the highest stability for the 1000 kernel weight. Cultivar Javorka had the highest, while Talas and KG-52/3 had the lowest average values for this trait. Vizeljka and KG-52/3 were the most stable genotypes for sedimentation value, with KG-52/3 achieving the highest values of the observed trait at the level of the entire experiment. Both analyzed locations had high interaction value for all three analyzed traits.

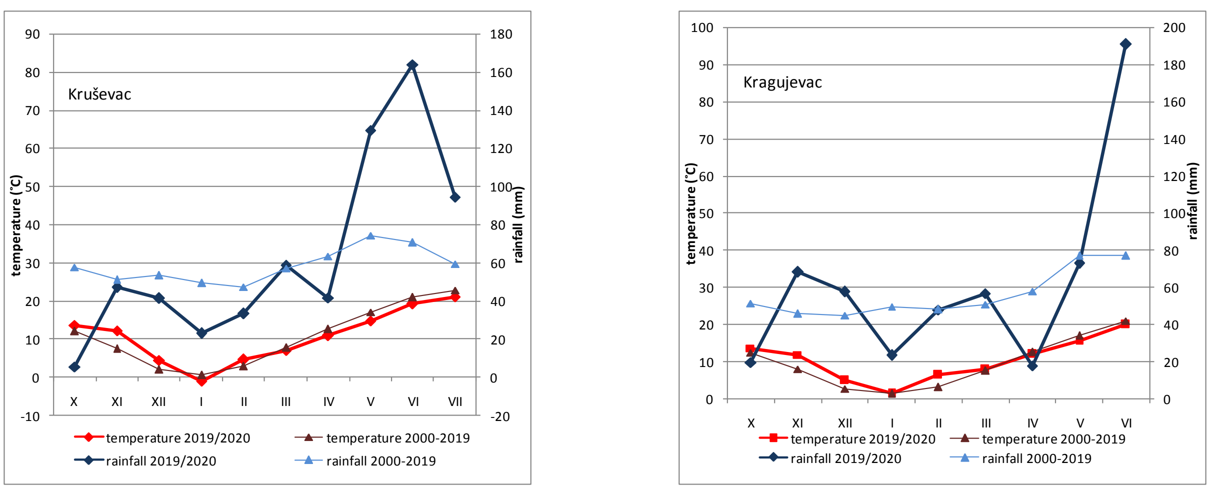
Keywords: *wheat, yield, quality, AMMI, stability.*

Introduction

Varieties of winter wheat represented in agricultural production have genetic potential for grain yield over 10 t ha^{-1} and good or satisfactory quality of grain and flour. However, average yields and grain quality, from year to year are extremely unstable. This variability can be largely attributed to unfavorable climatic conditions that accompany changes in the intensity and distribution of precipitation in critical phases of plant growing, as well as inadequate production technology (Luković et al., 2019; 2020; Ruiz et al., 2019; Senapati and Semenov, 2020). Poor soil preparation, late sowing, weeds, insufficient mineral nutrition of plants and inadequate protection significantly reduce grain yield and directly affect the unsatisfactory utilization of the genetic potential of the variety. In addition to climatic characteristics and applied production technology, the adaptation of varieties to agroecological conditions of a certain area has a significant impact in achieving high yields and grain quality. Yield stability and grain quality are crucial for agricultural producers when deciding on the choice of variety for sowing, especially in agroecological areas with less favorable climatic and edaphic factors. The aim of this study was to evaluate the stability and adaptability of yield and quality of divergent wheat varieties, based on the results of experiments performed in central Serbia, where soils are heterogeneous and less fertile.

Material and Methods

As research material in this paper, 10 varieties of winter wheat were used, which were produced in three breeding institutes in Serbia: Center for Small Grains in Kragujevac (Perfekta, KG-52/3 and Takovčanka), Institute PKB Agroekonomik in Belgrade (Merkur, Vizeljka and Talas) and Institute for Field and Vegetable Crops in Novi Sad (NS 40S, Zvezdana, Javorka and Pobeda). The field experiments were performed during 2019/2020 at two locations: Center for Small Grains in Kragujevac and Institute for Forage Plants in Kruševac. The experiments were set in field conditions according to a completely random block system, in three replications with the size of the basic plot of 5 m^2. After the harvest, grain yield was measured for each plot and then converted to yield in t ha^{-1}. The 1000 kernels weight and sedimentation value were determined according to international standard methods (JUS E.B1.200; Zeleny, ICC No. 116/1, 1972, respectively). The AMMI model (Gauch and Zobel, 1996) was used to assess genotype × environment interaction. Statistical analysis of the data was performed using the computer statistical program GenStat 12th (GenStat, 2009).
The average values of the monthly air temperatures and the sum of precipitation by individual months, in the period of conducting the experiment, are shown in graph 1.



(a) (b)

Graph. 1. Average monthly air temperatures and total amount of precipitation for Kruševac (a) and Kragujevac (b) during the vegetation period 2019/2020

The year of the experiment was marked by a pronounced deficit of precipitation in October at both localities. The winter was mild with a lower amount of precipitation compared to the multi-year average. The periods of the intensive vegetative growth and anthesis of wheat, during April and May, had similar temperature conditions in both localities, with the amount of precipitation differing significantly by localities. The dry period with an extremely low amount of precipitation was characterized by April in Kragujevac (17.8 mm compared to 57.9 mm), while in May the amount of precipitation was around the multi-year average. In May, 129.4 mm of precipitation occurred in Kruševac which was 50 mm higher than the multi-year average for this time of the year. Finally, the stage of grain filling and grain maturity during the June were under extremely unfavorable weather conditions. During this period 163.8 mm of precipitation was recorded in Kruševac and 191 mm in Kragujevac, which represents twice and three times higher amount of precipitation compared to the multi-year average.

Results and Discussion

Analysis of the variance of the AMMI model showed that all sources of variation (genotype, environment and G × E) had statistically significant effects (p <0.01) on grain yield expression, while the influence of locality was not statistically significant on the expression of 1000 kernel weight and sedimentation value (Table 1). Significant genotype / environment interaction indicates the existence of differences in stability between the examined genotypes, which justify application of AMMI analysis.

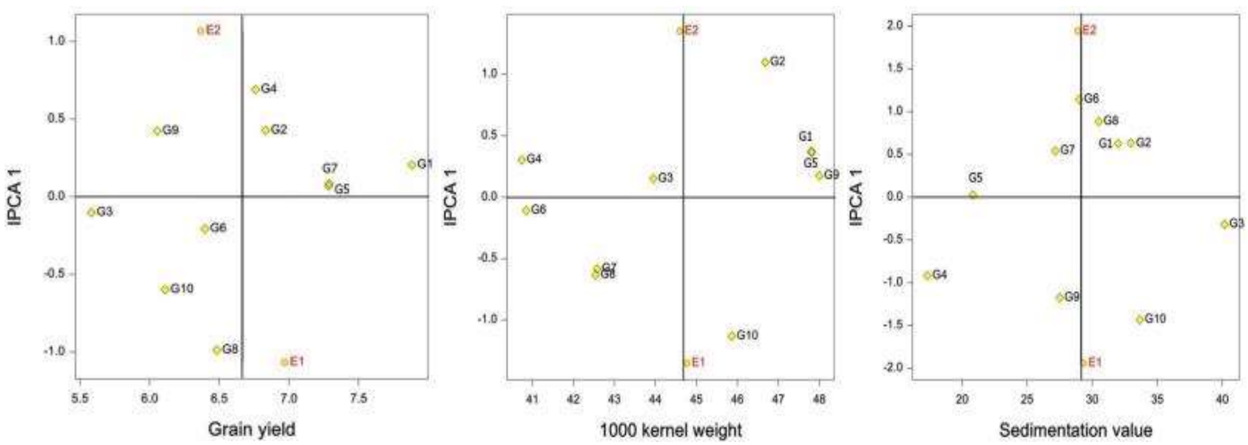
Table 1. AMMI analysis of variance for 1000 kernel weight, grain yield and sedimentation value

Source of variation	df	1000 kernel weight (g)		Grain yield (t ha^{-1})		Sedimentation value (ml)	
		MS	F	MS	F	MS	F
Genotypes	9	50.52	41.50**	2.83	102.51**	254.67	169.78**
Block	4	0.92	0.76^{ns}	0.01	0.51^{ns}	1.67	1.11^{ns}
Environment	1	0.43	0.47^{ns}	5.47	387.49**	2.82	1.69^{ns}
IPCA	9	4.46	3.66**	1.73	62.62**	18.96	12.64**
Error	36	1.22	-	0.03	-	1.50	-
Total	59	9.20	-	0.81	-	42.82	-

** Significant at P = 0.01 level, ^{ns} Non significant

The AMMI 1 biplot (Graph 2 a) shows the ratio of the average grain yield value and the value of the first main component (IPCA1). According to the value of the first main component (IPCA1), the highest stability was recorded in the varieties Vizeljka, NS 40S and KG-52/3 (G5, G7 and G3), followed by Perfekta and Talas (G1 and G6). At the same time, the variety Perfekta achieved the highest grain yield at the level of the whole experiment (7.88 t ha^{-1}) and stood out as the most productive variety. Varieties NS 40S and Vizeljka also had high yield (7.28 t ha^{-1}; 7.29 t ha^{-1}, respectively), while Talas and KG-52/3 although showed high stability, achieved a lower grain yield than the average of the experiment (6.4 t ha^{-1}; 5.58 t ha^{-1}, respectively). Zvezdana (G8) proved to be the least stable variety. Both environments showed a high value of interaction where by Kragujevac standing out as a more productive environment with higher average values compared to Kruševac. The most stable varieties for the 1000 kernel weight were Talas, KG-52/3 and Javorka (G6, G3 and G9) according to the values of the first main component (Graph 3 b), of which Javorka achieved higher, and Talas and KG-52/3 lower values

of the analyzed trait than the general average. The greatest effect of interaction and thus the least stability was observed in the cultivars Takovčanka and Pobeda (G2 and G10).
In the analysis of expression stability for sedimentation value, Vizeljka and KG-52/3 (G5 and G3) stood out as the most stable cultivars, followed by NS 40S, Perfekta and Takovčanka (G7, G1 and G2). However, although they expressed high stability, the cultivars Vizeljka and NS 40S achieved lower than average values (20.8 ml; 27.7 ml, respectively), while Perfekta, Takovčanka and KG-52/3 had above average values of the observed traits. At the same time, KG-52/3 proved to be superior in comparison with other varieties, achieving the highest value of sedimentation value at the level of the overall experiment (40.2 ml). This genotype also stood out in terms of quality traits in 2015, characterized as a year with extremely high rainfall during the periods of flowering and the beginning of grain filling, achieving high average values of sedimentation value at three sites in Serbia (Lukovic, 2020). The variety Pobeda (G 10), which represents a quality standard in experiments of Commission for the approval of new varieties, had the lowest stability (Graph 2 c).



(a) (b) (c)

Graf. 2. Average grain yield (a), 1000 kernel weight (b) and sedimentation value (c) in relation to the value of the first principal component of interaction for 10 wheat cultivars in two localities (Kragujevac, Kruševac);
Legend: 1-Perfekta; 2-Takovčanka; 3-KG-52/3; 4-Merkur; 5-Vizeljka; 6-Talas; 7- NS 40S; 8-Zvezdana; 9-Javorka; 10-Pobeda

Grain yield and quality depend on genetic differences between varieties, environmental factors as well as their interaction (Zečević et al., 2013; Đurić et al., 2020). Aktas (2020) emphasizes the predominant influence of the external environment on the variability of yield and grain quality. Similar results were published Mut et al. (2010) who state that the most stable variety (Bezostaya) gave the highest value to protein sedimentation. In the research of Gómez-Becerra et al. (2006) the most productive genotype was also the least stable, i.e. adapted to specific agroecological conditions. Hristov and Mladenov (2005) emphasized the Kremna variety as a stable genotype, with above-average values of protein sedimentation. In research of Khazratkulov et al. (2015) in addition to stable and high grain yields, singled out wheat genotypes which possess stable and good grain quality and can serve as desirable parents in wheat breeding programs. The year 2020 is characterized by a dry period in April in Kragujevac and exceptionally large amounts of precipitation in June at both localities. In Kruševac alone, 163.8 mm of precipitation was recorded in June, and 191 mm in Kragujevac. Such unfavorable weather conditions negatively affected the processes of grain filling and maturity of grains, causing the formation of the poorly filled grains with lower quality. In such an unfavorable year, wheat genotypes without standing high stability of grain yield and quality were determined, so they can be considered as adapted to different agroecological conditions. In addition to stable yield, the stability of quality traits is an important characteristic of varieties, especially in years with unfavorable climatic conditions. Such varieties represent a valuable raw material in the milling and baking industry necessary for obtaining a quality end product.

Conclusion

Using the AMMI model, high stability of the varieties Vizeljka, NS 40S and Perfekta for grain yield, and KG-52/3, Perfekta and Takovčanka for grain sedimentation was established. These cultivars achieved higher values than the overall average cultivar Perfekta for grain yield and KG-52/3 for sedimentation value. The obtained results in this research indicate that, in central Serbia where the soils are heterogeneous, with pronounced acidity and poorer fertility, an advantage should be given to varieties with high adaptability and stability of yield and quality such as the varieties Perfekta and KG-52/3. These varieties can be considered highly adapted and desirable for cultivation in central Serbia.

Acknowledgement

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References

Aktas B. (2020): Evaluation of yield and agronomic traits of new winter bread wheat cultivars. GENETIKA, 52 (1), 81-96.

Đurić N., Cvijanović G., Dozet G., Rajičić V., Branković G., Poštić D. (2020): The influence of year and location on grain yield and yield components in winter wheat. Plant Breeding and Seed Production, 26 (1), 9-18.

Ebadi A., Mehreban A., Kamrani M., Shiri M. (2020): Evaluation of grain yield stability and selection of bread wheat (*Triticum aestivum* L.) genotypes under different irrigation regimes. GENETIKA, 52 (2), 453-464.

Gauch H., Zobel W. (1996): AMMI Analysis of yield trials. In: Genotype-by environment interaction, Chapter 4, edited by Kang M., Gauch H. CRC Press.

GenStat (2009): GenStat for Windows (12th Edition) Introduction. VSN International, Hemel Hempstead.

Gómez-Becerra H. F., Morgounov A., Abugalieva A. (2006): Evaluation of grain yield stability, reliability and cultivar recommendations in spring wheat (*Triticum aestivum* L.) from Kazakhstan and Siberia. Journal of Central European Agriculture, 7 (4), 649-660.

Hristov N., Mladenov N. (2005): Wheat technological quality indicators in space and time. Proseeding of the Institute of Field and Vegetable Crop, Institute of Field and Vegetable Crop, Novi Sad, 41, 221-234.

Khazratkulova S., Sharma R. C., Amanov A., Ziyadullaev Z., Amanov O., Alikulov S., Ziyaev Z., Muzafarova D. (2015): Genotype × environment interaction and stability of grain yield and selected quality traits in winter wheat in Central Asia. Turkish Journal of Agriculture and Forestry, 39, 920-929.

Luković (2020): Characterization of KG-lines of wheat by morphological characteristics and technological quality of grain. Doctoral Dissertation, University of Belgrade, Faculty of agriculture.

Luković K., Prodanović S., Perišić V., Milovanović M., PerišićV., Raičić V., Zečević V. (2020): Multivariate analysis of morphological traits and the most important productive traits of wheat in extreme wet conditions. APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 18 (4), 5857-5871. http://www.aloki.hu. DOI: http://dx.doi.org/10.15666/aeer/1804_58575871

Luković K., Zečević V., Perišić V., Perišić V., Rajičić V., Bratković K., Matković Stojšin M. (2019): Variability of quality and rheological properties in winter wheat under the influence of ecological factors. Proceedings of X International Scientific Agriculture Symposium "Agrosym 2019", Jahorina, Faculty of Agriculture, East Sarajevo, 379 - 384.

Mut Z., Aydin N., Bayramoglu H. O., Ozcan H. (2010): Stability of some quality traits in bread wheat (*Triticum aestivum*) genotypes. Journal of Environmental Biology, 31, 489-495.

Ruiz, M., Zambrana, E., Fite, R., Sole, A., Tenorio, J.L., Benavente, E. (2019): Yield and quality performance of traditional and improved bread and durum wheat varieties under two conservation tillage systems. Sustainability 11 (17), 4522.https://doi.org/10.3390/su11174522

Senapati N., Semenov M. A. (2020): Large genetic yield potential and genetic yield gap estimated for wheat in Europe. Global Food Security, 24, 100340.

Zečević V., Bošković J., Knežević D., Mićanović D., Milenković S. (2013): Influence of cultivar and growing season on quality properties of winter wheat (*Triticum aestivum* L.). African Journal of Agricultural Research, 8 (21), 2545-2550.

EFFECT OF ROOTSTOCKS ON GRAFTING WATERMELON PLANT GROWTH, YIELD AND QUALITY

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Abstract

During the last two decades, the production of watermelon in Serbia is based on the use of grafted seedlings. Grafting aims to increase the yield and maintain the quality of fruits, as well as resistance to stress caused by biotic and abiotic factors. The watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) cultivar Top Gun F1 was grafted onto Emphasis F1 and Strong Tosa F1 commercial rootstock hybrids and *Lagenaria siceraria* as simple rootstock accepted by many local farmers, for late open field growing conditions. Nongrafted and selfgrafted plants were used as control. The percentage of grafting in the seedling period ranged from 81% (Strong tosaF1 / Top Gun F1) to 91% (*Lagenaria siceraria* / Top GunF1). Grafted seedling plants had a higher height and thickness of the stem, as well as a larger leaf area compared to nongrafted and selfgrafted. It has been confirmed that grafting watermelons produce a larger number of larger fruits. The highest number of fruits per plant (2,2) was recorded in the variant *Lagenaria siceraria* / Top Gun F1, with an average weight of 6.510 g. Hybrid rootstocks contributed to a uniform number of fruits per plant 1,7-1,8 with an average weight of 7.450-7.700g. Stress caused by unfavorable climatic conditions and pest attacks affected the number of fruits per plant 0,9-1,1 of nongrafted and selfgrafted plants, with a weight of 3.200-3.910 g. Detrimental effects were not determinates in fruit quality such as fruit index, rind thickness and sensory properties (has been proven by the results of panel tasting) on grafted plants, but differences according to the rootstock being used were evident. Grafted plants improved plant growth and yield without any harmful effects on fruit quality.

Keywords: *watermelon, grafting, rootstocks, yield, quality*.

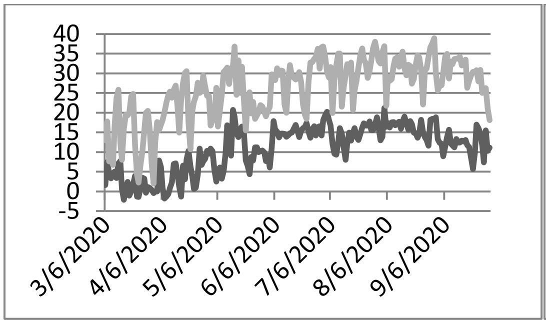
Introduction

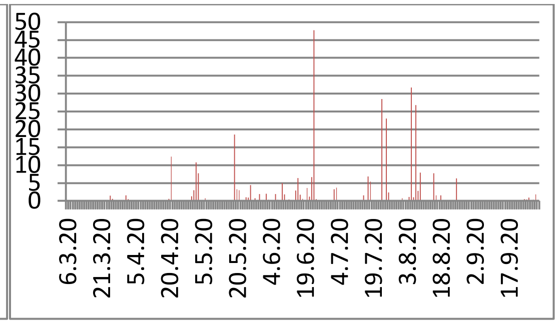
Grafting can be defined as the natural or deliberate fusion of plant parts so that vascular continuity is established between them (Pina and Errea, 2005) and the resulting genetically composite organism functions as a single plant (Mudge *et al*., 2009). By grafting vegetable species the problems caused by biotic and abiotic stress are overcome. Grafted watermelon has been reported to resist infection by pathogens, as well as nematodes (Lee *et al*., 2010). The root systems of grafted plants are stronger and more efficient in the uptake of water and nutrients, which indirectly improves yield (Miskovic *et al*., 2016). The use of rootstocks can enhance plant vigor through vigorous attainment of soil nutrients, avoidance of soil pathogens and tolerance of low soil temperatures, salinity and wet-soil conditions (Davis *et al*., 2008). However, rootstock/scion combinations affect the final size, yield and quality of fruits from grafted plants , both immediately postharvest and during prolonged storage (Falik and Ilic, 2014). The quality of grafted fruit must be equal to or better than that of nongrafted watermelon to ensure market acceptability (Colla *et al*., 2010). Grafting can enhance fruit quality of watermelon by increasing

the synthesis of endogenous hormones and the acquisition and transport of mineral nutrients (Lee *et al*., 2010; Wang *et al*., 2017) and also by altering secondary metabolites (Lee *et al*.,2010; Kakizaki *et al*., 2017). There are many conflicting reports on changes in fruit quality due to grafting and whether grafting effects are advantageous or deleterious. The differences in reported results may be attributable in part to different production methods and environments, type of rootstock/scion combinations used, and harvest date (Rouphael *et al*., 2010). Despite the challenges, grafting is becoming a common practice in several countries, such as Spain, Italy, Turkey, Greece, and Israel (Edelstein *et al.*, 2017).
The objectives of this study were to assess the survival rate and morphological characteristics of grafted and nongrafted seedlings of watermelon. After transplanting, plant growth, yield and quality of grafted watermelon and control (nongrafted and selfgrafted plants) were monitored and compared.

Material and methods

Production of a grafted, nongrafted and selfgrafted watermelon was conducted in randomized complete blocks design with three replications during 2020 in an experimental garden located in the village of Moravac near Aleksinac (21∘ 42 ′ E, 43∘ 30 ′ N, altitude 159 m) in the central area of Serbia. The watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) cultivar Top Gun F1 was grafted onto Emphasis F1 (*Lagenaria type*) and Strong Tosa F1 (interspecies type: *C. maxima X C. moschata*) commercial rootstock hybrids and *Lagenaria siceraria* as simple rootstock accepted by many local farmers. Nongrafted and selfgrafted plants were used as control. The cleft graft (CG) applied in our research, was the initial grafting method used for cucumber and watermelon (Ishibashi, 1959). The sowing of the rootstock was done in pots with a diameter of 10 cm, and the sowing of the scion in boxes, at the end of March 2020. Morphological characteristics of scion and rootstocks cotyledons, grafted and nongrafted seedling plants were recorded: plant mass (g/plant), root mass (g), leaf area by conture paper method (cm^2/plant), and the survival rate of grafted plants (%).
When plants developed true five-six leaves (6 weeks after sowing) they were transferred into the experimental field to soil with spacing between rows 240 cm and 120 cm in a row, with a plant density of 0,35 plants m^{-2}. Growing technology involved the primary soil preparation before planting and fertilization with formulation 12: 11: 24 + ME (Yara Mila Complex) applied through a regular supplemental feeding drip system and foliar application. Vegetative growth characters were recorded in samples of three plants randomly chosen from each plot as follows. The fruits were harvested after 70-80 days (depending on the rootstock) from transplanting and at harvest the following characteristics were evaluated: total yield (t/ha^{-1}), number of fruits/plant, average fruit weight (g)**.** Fruit characteristics were determined by measuring: fruit length (cm), fruit diameter (cm), index of fruit, fruit rind thickness (mm). Panel test was determined by panel testing flavor (sweet aroma, flesh color, texture, juiciness, sweet taste, fermented flavor, after taste) by 5 persons per replication: Scale system from 1 (week) to 10 (strong). Meteorological data from March to September 2020 were used from the local meteo-station in Moravac.





Graf.1Max and min. temperature (III-IX 2020) Graf.2 Rainfall (III-IX 2020)

The data were analyzed by analyses of variance (ANOVA) follow by Tukeyś HSD test, using the Statistica 6.1 software (Statsoft, Tulsa, OK, USA). All analyses were performed at a 95 % and 99% level of confidence ($p<0.05$; $p<0.01$).

Results and Discussion

One of the most important factors in the process of grafting is the correct choice of rootstock and scion, as a prerequisite for compatibility. Grafting compatibility is related to taxonomic affinity (Davis *et al*., 2008). The result of compatibility is the establishment of vascularization between the rootstock and the scion, and the formation of callus, so it is important to determine the moment when grafting is approached, especially in species that have hollow hypocotyls.

Tab. 1 Morphological characteristics of watermelon seedling plants

Cultivar	Rootstocks and scions ready for grafting			Rootstock/ Scion	Grafted, nongrafted, selfgrafted seedlings of watermelon (phase true 3-4 leaves)			
	Plant mass g	Root mass g	Leaf area cm²/plant		Survival rate (%)	Plant mass (g)	Root mass (g)	Leaf area cm²/plant
Emphasis F1	2,20	0,63	23,8	Emphasis/ Top gunF1	85	18,1	3,48	215,1
Strong tosa F1	2,84	0,75	28,5	S.tosa F1 /Top gunF1	81	18,7	3,18	244,3
Lagenaria siceraria	2,02	0,51	24,2	L.siceraria/ Top gunF1	91	19,4	4,60	240,1
Top gunF1	0,91	0,11	18,9	TopgunF1/ Top gun F1	82	7,72	0,78	114,3
-	-	-	-	Top gun F1	-	7,30	0,65	93,9
LSD 0,05	0,501	0,167	3,891			1,131	0,618	11,91
LSD 0,01	0,759	0,253	5,895			1,646	0,900	17,33

The too young plant is difficult to manipulate. Optimum seedling age varies for species and different grafting methods. The cleft graft (CG) method applied in our research and results indicate that the scion should be in the cotyledons phase up to 48 hours of age from the beginning of sprouting, and the rootstocks 60-96 hours, according to the vigor of the species, with a true leaf just conceived. Thus, hybrid Strong tosa F1 is most often included in grafting 2-3 days, and *L. siceraria* 4-5 days after sprouting. Uniformity in both germination and growth of the rootstock and scion are critical for grafting survival. Graft healing is dependent on hormonal

signaling originating in the cotyledons to successfully heal the wounded region (Daley and Hassell, 2014; Melnyk, 2017), therefore, traditional commercial methods of grafting watermelon depend on retaining at least one rootstocks cotyledon. The period of acclimatization was under the conditions of increased air humidity and moderate temperature (VV% 92-96%, daily temperature 23-28˚C) with shading of plants for five days. Turgor of the scion cotyledons indicates that vascularization has been established. Callus is formed 5-6 days after grafting and then the plant returns to natural microclimate. Similarly, Kubota *et al*. (2016), indicate that the success of callus bridge formation is reported to take 4–5 days under optimum temperatures.
The availability of seeds of the species *L. siceraria*, the high survival rate received during grafting and the influence on the increase of watermelon yield, make these rootstock very popular among the producers of grafted watermelon seedlings. In our study, the survival rate seedlings grafted on the rootstock of *L. siceraria* was 91%, 5-10% higher than compared to the commercial rootstock hybrids of Emphasis F1 (85%) and Strong tosa F1 (81%) (tab.1). The survival rate of grafted plants depends on compatibility between scion and rootstock, quality and age of seedlings, quality of the joined section, and post-grafting management (Hang *et al*. 2005). In contrast to solanaceous crops, it is rare to get 100% graft survival for watermelon, so it is recommended to graft up to 20% more plants than needed, depending on the grafting method and environmental factors (Miles *et al*., 2017). Watermelon seedlings grafted onto *L. siceraria* have the best morphological characteristics with significant differences compared to the plants grafted on commercial rootstocks hybrids. Control seedlings of watermelon have a significantly lower root mass, plant mass and leaf area compared to grafted plants. Đurovka *et al*. (2002) note that the average weight of grafted watermelon seedlings, depending on the rootstocks, is higher by 54.5-99.3% compared to nongrafted seedlings.
The grafting of watermelon onto different rootstocks increases plant growth. Data in tab.2, indicated that there were significant increments in plant vigor of the grafted plants compared to nongrafted and selfgrafted. Yetisir and Sari (2004) reported that out of 11 rootstocks tested on watermelon, all the grafted plants produced more biomass than control plants. Grafting watermelon positively affected plant vigor, height and disease control (El-Eslamboly, 2010; Alan *et al*., 2007) which is associated with the vigor and hormonal initiation of the root system.

Table 2 .Effects of the grafting on the vegetative growth characteristics of watermelon plants

Rootstock / Scion	Branch length to the first fruit (cm)	Stem diameter (cm)	Number of brunches	Number of leaves / plant
Emphasis/ Top gunF1	136,8	2,4	6,17	296
Strong tosa F1/ Top gunF1	214,2	2,7	6,82	360
L.siceraria/Top gunF1	89,5	2,5	6,34	310
TopgunF1/ Top gun F1	74,6	1,3	4,6	212
Top gun F1	82,3	1,2	4,4	194
LSD 0,05	32,05	0,693	0,837	64,74
LSD 0,01	46,64	0,541	2,508	94,20

Commercial rootstocks are vigorous and will quickly overtake the scion and therefore after receiving the grafted plants, it is necessary to monitor the appearance of lateral shoots of the rootstocks and remove them. Daley and Hassell (2014) apply fatty alcohol treatments for these purposes. Hagihara (2004) classified the species and cultivars of the rootstocks used in Japan on the basis of plant vigor. In general, 'Shin-tosa' rootstocks are more vigorous than the bottle gourd rootstock cultivars, which is confirmed by our research by comparing the rootstocks of Strong tosa F1 and *L. siceraria*. The total yield of watermelon grafted on *L. siceraria* was higher compared to the watermelon grafted on Emphasis F1, with statistically significant differences. However, the control watermelon plants formed a very significantly lower yield compared to the grafted plants, which is caused by climatic conditions. During May 2020, intense precipitation was recorded followed by hail that caused damage to plants, and June 2020 is recorded as the fifth rainiest in Serbia in relation to measurements in the last 70 years (RHMZ, 2020). Grafted plants regenerated more intensively in wet soil conditions, which resulted in significantly higher yields compared to the control.

Table 3. Effects of the grafting on total yield, fruits characteristics and quality of watermelon

Rootstock/ Scion	Total yield (t/ha^{-1})	Average mass fruit (g)	N° fruit/ plant	Fruit characteristics			Rind thickness (mm)	Panel test - flavor
				Length (cm)	Diameter (cm)	Index of fruit		
Emphasis F1/ Top gunF1	44,95	7.450	1,7	24,2	22,9	1,06	16,51	8,5
Strong tosaF1 /Top gun F1	49,01	7.700	1,8	24,7	23,6	1,03	17,10	7,06
L.siceraria/ Top gun F1	50,86	6.510	2,2	25,1	22,2	1,07	16,46	7,65
Top gun F1/ Top gun F1	15,65	3.910	1,1	18,7	18,3	1,02	15,90	7,3
Top gun F1	11,08	3.200	0,9	17,9	17,8	1,005	15,79	7,1
LSD 0,05	5,197	1253	0,618	2,016	2,387	0,0842	1,350	0,347
LSD 0,01	7,562	1824	0,9003	2,934	3,473	0,1225	1,964	0,505

In watermelon, two primary quality-related characteristics are fruit weight and flesh properties (Fredes *et al*., 2017). The positive effect of grafting on the average weight, number of fruits per plant and physical characteristics of the fruit is evident (tab.3). The exception is the fruit index, with no significant differences between variants and control. Rind thickness is significantly higher only in the fruits of watermelon grafted on Strong tosa F1 compared to nongrafted plants. Increasing the rind thickness in grafted plants affects the strength of the fruit and transportability. Quality fruits of control and plants grafted on hybrid Strong tosa F1 are balanced, thus achieving the basic postulation of grafting, but Panel tasting results indicate a significant to very significant improvement in taste in watermelon grafted on *L. siceraria* or Emphasis F1. Rootstocks influence the sensory properties of the fruit watermelon without detrimental effects on quality. Watermelon is a non-climacteric vegetable, so the moment of harvest is reflected in the quality. The traditional approach to assessing maturity may not apply to grafted plants. The harvesting of watermelon fruits grafted on *L. siceraria* and Emphasis F1 started about 7-10 days earlier compared to control or plants grafted on Stong tosa F1 (data not shown). Most literature relies on the assumption of synchronous ripening in grafted and nongrafted plants, potentially leading to confounding reports for fruit maturity and fruit quality resulting from grafting (Davi *et al*., 2020). Reduced quality may be a factor of improper (early) harvest timing (Soteriou *et al*., 2014). Simultaneous harvest of grafted and self-rooted plants assumes a synchronized maturation, which overlooks the effect of grafting on fruit ripening behavior (Davis *et al*., 2008). Future research should focus on this issue.

Conclusions

Grafted watermelon with a good rootstock/scion combination has better abiotic stress tolerance, higher yield and good quality. Grafting success depends on compatibility between scion and rootstock, quality and age of seedling, and post-grafting acclimatization. In our study, the survival rate of grafted seedlings was: 81% Strong tosa F1, 85% Emphasis F1 and 91% *L. siceraria*. Control watermelon seedlings have a significantly lower root mass, plant mass and leaf area compared to grafted seedlings. Grafted watermelon positively affected plant vigor. The grafted plants produced more biomass than control plants (nongrafted, selfgrafted). Grafting increased fruit size and the number of fruit per plant, and this causes higher yield than control. The fruits quality of grafted plants was equal to or better than control plants. Rootstocks affect the sensory properties of the fruit but without any harmful effects on quality.

References

Alan, O., N. Ozdemir and Y. Gunen, (2007). Effect of grafting on watermelon plant growth, yield and quality. Journal of Agronomy, 6,(2): 362-365.

Colla, G.; Rouphael, Y.; Leonardi, C.; Bie, Z. (2010). Role of grafting in vegetable crops grown under saline conditions. *Sci. Hortic. 127*, 147–155.

Daley, S.L.; Hassell, R.L.(2014). Fatty alcohol application to control meristematic regrowth in bottle gourd and interspecific hybrid squash rootstocks used for grafting watermelon. HortScience ,49, 260–264.

Davi, P., Perkins-Veazie, P., Miles, C. (2020). Impact of Grafting on Watermelon Fruit Maturity and Quality. Horticulturae , 6, 97.

Davis, A.R., Perkins-Veazie, P., Sakata, Y.; Galarza, S.L.Ó.; Maroto, J.V.; Lee, S.G.; Huh, Y.C.; Miguel, A.; King, S.R.; Cohen, R.. (2008). Cucurbit grafting. *Crit. Rev. Plant Sci. , 27*, 50–74.

Đurovka, M., Ilin,Ž.(2002). Bostan-gajenje dinje i lubenice, Poljoprivredni fakultet, Novi Sad.

Edelstein M.,Cohen R., Baumkoler F.,Ben-Hur M.(2017). Using grafted vegetables to increase tolerance to salt and toxic elements. Isr J Plant Sci 64, 3–20

El-Eslamboly, A.A.S.A.(2010). Studies on Propagation of Watermelon Plants. . Ph.D. Thesis Agric. Sci. (Vegetable rops), Fac. Agric., Univ., Cairo, Egypt, 150p.

Fallik E., Ilic S.Z.,(2014). Grafted vegetables - the influence of rootstock and scion on postharvest quality, Folia Hort. 26, 2:79-90.

Fredes, A., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-de Castro, A. Gisbert, C.,Pico M.B. (2017). Fruit quality assessment of watermelons grafted onto citron melon rootstock *J. Sciense of Food and Agriculture,* 97, 1646-1655

Hagihara,T.(2004). Rootstock cultivars and their characteristics. In:Watermelon and Pumpkin 5.147–154. Yasai-engei Hyakka, Ed., Noubunkyo, Japan.

Hang, S.D., Y.P. Zhao, G.Y. Wang and G.Y. Song, (2005). Vegetable Grafting, China Agriculture Press, Beijng, China.

Ishibashi, K. (1959). Cucumber cultivation using grafting.Agr. Hort.4,343–347.

Kakizaki, T.; Kitashiba, H.; Zou, Z.; Li, F.; Fukino, N.; Ohara, T.; Nishio, T.; Ishida, M.(2017). A 2-oxoglutarate-dependent dioxygenase mediates the biosynthesis of glucoraphasatin in radish. *Plant Physiol.*, *173*, 1583–1593.

Kubota,C., Miles, C.A. (2016). Healing and acclimatization methods and design principles. In Grafting Manual: How to Produce Grafted Vegetable Plants; Kubota, C., Miles, C., Zhao, X., Eds.; National Institute of Food andAgriculture: Washington, DC, USA; Chapter 2.4.

Lee, J.M.; Kubota, C.; Tsao, S.J.; Bie, Z.; Echevarria, P.H.; Morra, L.; Oda, M. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci.Hortic.*, *127*, 93–105.

Melnyk, C.W. (2017).Monitoring vascular regeneration and xylem connectivity in Arabidopsis thaliana. Methods Mol. Biol., 1544, 91–102.

Miles,C., Devi, P., Zhao, X., Guan,W. (2017). Watermelon and melon grafting. In Grafting Manual: How to Produce Grafted Vegetable Plants; Kubota, C., Miles, C., Zhao, X., Eds.; National Institute of Food and Agriculture: Washington, DC, USA; Chapter 3.2.1.

Miskovic A., Ilic O., Bacanovic J., Vujasinovic V., Kukic B.,(2016). Effect of eggplant rootstock on yield and quality parameters of grafted tomato. Acta Sci. Pol. Hort Cultus., 15, 149–159.

Mudge K., Janick J., Scofield S., Goldschmidt E.E.(2009). A history of grafting. Horticultural Reviews., 35, 437-493.

Pina, P., Errea, P. (2005). A review of new advances in mechanism of graft compatibility - incompatibility. Scientia Hort., 106, 1–11.

RHMZ (2020). http://www.hidmet.gov.rs/data/klimatologija/ciril/Jun.pdf

Rouphael Y., Schwarz D., Krumbein A., Colla G., (2010). Impact of grafting on product quality of fruit vegetables. Sci Hortic., 127, 172–179.

Soteriou, G.A.; Kyriacou, M.C.; Siomos, A.S.; Gerasopoulos, D. (2014). Evolution of watermelon fruit physicochemical and phytochemical composition during ripening as affected by grafting. *Food Chem.*, *165*, 282–289.

Wang, Q.; Men, L.; Gao, L.; Tian, Y.(2017). Effect of grafting and gypsum application on cucumber (*Cucumis sativus* L.) growth under saline water irrigation. *Agric. Water Manag.*, *188*, 79–90.

Yetisir, H., Sari N. (2004). Effect of hypocotyls morphology on survival rate and growth of watermelon seedlings grafted on rootstocks with different emergence performance at various temperatures. Turk J Agric For., 28 , 231-237.

GERMINATION OF *DIANTHUS SEROTINUS* SEED ORIGINATING FROM PLANTS PRODUCED BY MICROPROPAGATION

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Abstract

Dianthus serotinus Waldst.& Kit. is important endemic, endangered and decorative species. It has a status of extremely endangered species in Serbia and it is under legal protection. *D. serotinus* can also be used as an ornamental, drought tolerant, low-maintenance species. To enable its *ex situ* and *in situ* conservation, it has been successfully propagated *in vitro*, in the Laboratory at the Faculty of Forestry in Belgrade. The obtained plants were acclimatized, flowered and produced seeds. The seed germination was tested immediately after collecting and after two years of storage. The effect of different disinfection methods on seed germination was also evaluated: treatment with 3.5% NaOCl solution for 15 minutes, treatment with *Allium sativum* L. extract containing bioactive allicin, and treatment with a fungicide Previcur (control). Immediately after collection, the seed germination (92.2 - 94.3%) and germination energy (90.0 – 91.1%) were high, and NaOCl and *A. sativum* extract had no effect on germination. However, after two years of storage, treatment with NaOCl significantly increased germination rate (90.0%) compared to control (81.1%), while *A. sativum* extract had an adverse effect (68.9%) on seed germination.

Keywords: *endangered plants, generative propagation, NaOCl, Previcur, allicin, Allium sativum.*

Introduction

Dianthus serotinus Waldst. et Kit. is a species included in category V (vulnerable taxa) of the IUCN Red List of endangered species. This endemic Pannonian species has the status of extremely endangered species in Serbia and it is under legal protection (Law on Environmental Protection, Rulebook on protected species, 2010). In Serbia, it grows in the area of Subotica - Horgoš Sands (Boža, 1999). *D. serotinus* is also an ornamental perennial species with long flowering period, scented white flowers, and bluish-green leaves. This drought tolerant species grows well on alcaline (pH 7.0 - 8.1) sandy soils with a low level of humus. With the aim to enable its *ex situ* and *in situ* conservation, *D. serotinus* has been successfully propagated using *in vitro* method (Marković *et al*., 2013, 2014a,b) and obtained microplants flowered and produced seeds.

Taking into account that *D. serotinus* is an endangered species, generative propagation is a convenient method for its *ex situ* conservation. In this way, propagation is simple and fast, and genetic diversity of obtained plants can be preserved. Thus, the seed obtained from microplants can be sown enabling further propagation and conservation of this species. Therefore, besides seed germination, the effects of storage length on seed germination should also be evaluated.

The use of pesticides is prohibited in the Subotica Sandlands protected area that includes the habitat of *D. serotinus* ("Official Gazette of RS", no. 127/2003) and hence propagation of *D.*

serotinus should be based on the principles of organic production, excluding the use of artificial pesticides. Lately, many research reporting the use of plant extracts with antibacterial and antifungal activities in plant production were published (Mbega *et al*., 2012; Bello and Sisterna, 2010; Rajendra *et al*., 2014; Sarfraz *et al*., 2020). A lot of researchers proved the inhibitory effect of *Allium sativum* L. extracts (containing bioactives allicin, thiosulfate and other constituents) on germination of spores of different species of fungi (*Penicillium* sp., *Aspergillus* sp., *Fusarium oxysporum*) that cause plant diseases (Irkin and Korukluoglu 2007; Ogbebor and Adekunle 2008; Portz *et al*., 2008; Perelló *et al*., 2013; Slusarenko *et al*., 2008; Okigbo *et al*., 2009; Nisa *et al*., 2011; Sarfraz *et al*., 2020; Borlinghaus *et al*., 2021). These studies were based on the need to point out the potential of the natural products that might be used to develop strategies in plant protection, especially for seeds treatments against seed decay or damping-off. The use of *A. sativum* extracts as a biopesticide has been promoted by many associations and organizations engaged in organic production, including Henry Doubleday Research Association (HDRA). Slusarenko *et al*. (2008) reported that *A. sativum* extracts can be used in pre-sowing disinfection of carrot seeds. Taking into account the recommendations and EU regulations related to organic production (Council Regulation 2092/91 on Organic Farming) and the positive effect of *A. sativum* extract on germination and further growth of seedlings of seeds infected with different fungi species (Slusarenko 2008; Perelló *et al*., 2013), we can consider that the *A. sativum* extract can be used as a pretreatment of *D. serotinus* seed. However, the question arose how the *A. sativum* extract may affect seed germination, and whether it could have inhibitory or stimulating effect.

Therefore, the aim of this research was to assess germination rate of *D. serotinus* seed immediately after collection and after two years of storage, as well as the effect of *A. sativum* extract on seed germination.

Material and methods

The seeds were collected from micropropagated plants produced in the Laboratory for tissue culture at the Faculty of Forestry in Belgrade. Part of the collected seeds was used for experiments immediately after collection and the remaining seeds were kept at room temperature, in dry conditions during 2 years. After 2 years of storage, the seeds were chilled for 4 weeks at the temperature of 4 - 5°C before setting the experiment.

The effect of different disinfection methods on seed germination was evaluated. First treatment included immersion of seed in a 3.5% NaOCl solution for 15 minutes. The seeds were rinsed three times for 2 minutes using sterile distilled water. The concentration of NaOCl and duration of treatment was set according to the method successfully used by Marković et al (2007) during establishing sterile *in vitro* culture from seed.

Second treatment consisted of soaking the seeds with water extract from *A. sativum* bulbs. The extract was made using 200 g bulbs that were chopped in a mortar, mixed with 200 ml of distilled water, homogenized for 5 minutes and then filtered through sterile gauze. This method of making *A. sativum* water extract was already reported by Nisa *et al*. (2011).

In the third treatment (control) the seeds were soaked in 1.5% (v/v) Previcur N fungicide solution for 5 minutes (Bayer AG Germany, active ingredient Propamocarb hydrochloride) according to method described by Baker *et al*. (2005) for testing seed germination.

After these disinfection treatments, the seeds were placed on the top of two layered filter paper in the petri dishes for germination. The seed testing was conducted according to the recommendations of the International Seed Testing Association (ISTA, 2010) for testing seed germination of the *D. plumarius* and *D. deltoides*, but a smaller amount of seeds was used. Humidity was controlled regularly by adding the distilled water if needed. Temperature was 20 - 26°C and germination was tested in long days conditions. First count (germination energy) was recorded on 4^{th} day and final count of germinated seeds was performed on 14^{th} day after placing seeds on germination.

Each treatment consisted of three replicates with 30 seeds. Obtained data were statistically analyzed and significance of differences between mean values was determined by analysis of variance (ANOVA, $p<0.5$) and the LSD (least significant difference) method. Prior to analysis, arcsin data transformation was performed.

Results and Discussion

The seed disinfection method did not influence germination of seed tested immediately after collection, the obtained differences were not statistically significant (Table 1) and all treatments had high germination rate (92.2 – 94.3%) and germination energy (90.0 – 91.1%). After two years of seed storage, germination energy was significantly reduced (60.0 – 75.6%), and germination rate was lower (68.9 – 90.0%). Change in germination rate after seed storage was studied for other *Dianthus* species. For example, Mikulik and Vinter (2002) examined germination of *Dianthus superbus* subsp. *superbus* immediately after collection, or after long-term storage at the temperature of 0°C. However, their results were probably influenced by storage temperature because germination energy (30%) and germination rate (80%) of seed tested after collection were lower than germination energy (32%) and germination (86%) of seed tested after 5 months of storage, but the germination rate was additionally reduced after two years (78%) and three years (71%) of storage.

In our research, the germination after two years of seed storage differed depending on disinfection method used. The highest germination rate was obtained after NaOCl treatment (90%), lower germination was achieved after Previcur treatment (81.1%) and the considerably lower germination was recorded after *Allium* extract treatment (68.9%). The influence of NaOCl on seed germination has already been reported for other plant species, because NaOCl is an oxidizing agent which may have a stimulatory effect on seed germination due to its influence on partial degradation of the seed coat and solubilization and oxidation of the inhibitors of seed germination (Marković *et al*., 2016; Lee *et al*., 2007). However, the effect of NaOCl on germination depends on its concentration and the length of treatment. For example, after 11 months of storage, the seed of *Sorghastrum nutans* were soaked in 5.25% NaOCl for 20 or 60 minutes, and germination was the lower after 60 minutes treatment (51%) compared to germination after 20 minutes treatment (53%) but in a control treatment (without NaOCl) only 47% of seeds germinated (Watkinsonand Pill, 1998). However, gibberellic acid (GA_3) had better effect on germination *S. nutans* (67%) and moreover Watkinson and Pill (1998) achieved the best results (71%) by soaking the *S. nutans* seed in NaOCl solution for 20 minutes, followed by GA_3 treatment.

Addition of NaOCl sometimes can have inhibitory effect on seed germination. For example, although treatment with 1% or 3% NaOCl for 5 or 40 minutes did not have a significant effect on germination of *Capsicum annuum* seed immediately after collection compared to a control, NaOCl showed inhibitory effect on germination of the seed stored at the room temperature for 10 months, and germination rate decreased with increased exposure to NaOCl solution (5, 10 or 20

minutes) (Khah and Passam, 1992). These findings are in a contrast to our results where NaOCl had a stimulating effect on the germination of seed after storage.
Beside having an effect on seed germination, NaOCl has also a fungicidal action (Macnish *et al.*, 2010; Sitara and Akhter, 2007) which is important for germination and seedlings survival (Mikulik *et al.*, 2002). The presence of pathogens on seed can affect seed germination, and for example the seed of *D. superbus* ssp. *superbus* infected with pathogens had a low germination rate (60%) compared to pathogen-free seed (84%), but germination rate of the infected seed increased considerably (88%) after NaOCl treatment (Mikulik *et al*., 2002).

Table 1. Germination of *D. serotinus* seed after different disinfection treatments

Storage length	Treatment	Germination energy (%)	Germination rate (%)
fresh seed	NaOCl	90.0^{d}	92.2^{cd}
fresh seed	*A. sativum*	87.8^{d}	93.3^{cd}
fresh seed	Previcur	91.1^{d}	94.3^{d}
2 years old seed	NaOCl	68.9^{bc}	90.0^{c}
2 years old seed	*A. sativum*	60.0^{a}	68.9^{a}
2 years old seed	Previcur	75.6^{c}	81.1^{b}

Values followed by different letters are significantly different at the $P<0.05$ level according to the LSD test

Although *A. sativum* extract did not have a significant effect on germination energy and germination rate of seeds that were sown immediately after collection, it had inhibitory effect on seed germination after two years of storage, because the obtained values were significantly lower than control treatment with Previcur (Table 1.). This indicates that *A. sativum* extract can be used in the disinfection of *D. serotinus* seeds for sowing immediately after collection, but it cannot be used as a pretreatment of the seed after long-term storage. However, it is necessary to conduct additional research to determine the effect of different concentrations of *A. sativum* extract on germination of *D. serotinus,* especially because allicin can induce necrosis if it is applied at higher doses (Borlinghaus *et al*., 2021).

Conclusions

After *D. serotinus* has been successfuly micropropagated, the obtained plants produced viable seed that can be used for further propagation of this species. The seed has high germination rate (above 90%) immediately after collection and the seed viability was preserved after 2 years of storage. However, to achieve high germination rate after 2 years of storage at the room temperature, the seeds should be chilled for 4 weeks at 4 - 5°C, following by immersion in a 3.5% NaOCl solution for 15 minutes. *A. sativum* extract can be used for disinfection of fresh seed, but it has inhibitory effect on germination of long-term stored seed.
Long-term seed storage at lower temperatures should be considered.

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References

Baker K.S., Steadman K.J., Plummer J.A., Merritt D.J., Dixon K.W. (2005). The changing window of conditions that promote germination of two fire ephemerals, Actinotus leucocephalus (Apiaceae) and Tersonia cyathiflora (Gyrostemonaceae), Annals of Botany, 96: 1225–1236.

Bello G., Sisterna M. (2010). Use of plants extracts as natural fungicides in the management of seedborne diseases. In: Arya A, Perelló A (eds) Management of fungal plant pathogens. MPG Books Group, UK

Borlinghaus J., Foerster J.; Kappler U., Antelmann H., Noll U., Gruhlke M., Slusarenko A. (2021). Allicin, the Odor of Freshly Crushed Garlic: A Review of Recent Progress in Understanding Allicin's Effects on Cells, Molecules 26, no. 6: 1505. https://doi.org/10.3390/molecules26061505

Boža P. (1999). *Dianthus serotinus* Waldst. & Kit., "Red Book of Flora of Serbia", Ministry of Environment of the Republic of Serbia, Faculty of Biology, University of Belgrade, Institute for Nature Protection of the Republic of Serbia, Belgrade (252-254) (in Serbian)

Council Regulation 2092/91 on Organic Farming, organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. (http://ec.europa.eu/agriculture/organic/eu-policy/legislation_en) (pristupljeno/ accessed, 2012).

Gajić M. (1986). Flora and vegetation of the Subotica-Horgos sands, Faculty of Forestry, University of Belgrade, Belgrade - Forestry, Subotica (in Serbian)

Irkin R., Korukluoglu M. (2007). Control of Aspergillus niger with garlic, onion and leek extracts. African Journal of Biotechnology 6 (4): 384-387.

ISTA (2010). Rules Proposals for the International Rules for Seed Testing 2011 Edition, International Seed Testing Association.

Khah E.M., Passam H.C. (1992). Sodium Hypochlorite Concentration, Temperature and Seed Age Influence Germination of Sweet Pepper. HortScience 27(7): 821-823.

Macnish A.J., Morris K.L., deTheije A., Mensink M.G.J., Boerrigter H.A.M., Reid M.S., Jiang C.Z., Woltering E.J. (2010). Sodium hypochlorite: A promising agent for reducing Botrytis cinerea infection on rose flowers. Postharvest Biology and Technology 58: 262-267.

Marković M., Grbić M., Djukić M. (2013). Micropropagation of the Endangered and Decorative Species *Dianthus serotinus* Waldst. et Kit. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41(2): 1-8.

Marković M., Grbić M., Đukić M. (2014a). Effect of sugar alcohol sorbitol on in vitro shoot development of Dianthus serotinus Waldst. et Kit., Glasnik Šumarskog fakulteta 109: 113-124.

Marković M., Grbić M., Skočajić D., Đukić M. (2014b). Effect of explant type on the rooting and acclimatization of Dianthus serotinus Waldst. & Kit., Glasnik Šumarskog fakulteta 109: 125-136.

Marković M., Grbić M., Skočajić D., Đunisijević-Bojović D. (2007). Effect of phytohormone balance on shoot multiplication and rooting of the species Dianthus serotinus Waldst. & Kit., Glasnik Šumarskog fakulteta 95: 83-94. DOI:10.2298/GSF0795083M

Markovic M., Grbić M., Djukic M. (2016). Micropropagation of Endangered and Decorative Species *Dianthus pinifolius* Sibth. et Sm.. Brazilian Archives of Biology and Technology. 59. 10.1590/1678-4324-2016150320.

Mbega E.R., Mortensen C.N., Mabagala R.B. (2012). The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. Journal of General Plant Pathology 78(4): 277–286.

Mikulik J., Sedlarova M., Vinter V. (2002). Pathogenic fungi on *Dianthus superbus* ssp. *superbus* and their influence on host plants germination and survival. Acta Universistatis Palackianae Olomucensis Facultas rerum Naturalium. Biologica, 39/40: 19-25.

Mikulík J., Vinter V. (2002). Evaluation of factors affecting germination of *Dianthus superbus* L. subsp. *superbus*, Acta Universitatis Palackianae Olomucensis, Biologica 39/40: 13-18.

Natural Pesticides No. TNP3, Garlic, Allium sativum. Henry Doubleday Research Association (HDRA), Ryton Organic Gardens Coventry, UK. (http://www.gardenorganic.org.uk/pdfs/international_programme/TNP3-Garlic.pdf) (accessed 2012).

Nisa T., Wani A.H., Bhat M.Y., Pala S.A., Mir R.A. (2011). *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. Journal of Biopesticides 4 (1): 53 - 56.

Ogbebor O.N., Adekunle A.T. (2008). Inhibition of *Drechslera heveae* (Petch) M. B. Ellis, causal organism of Bird's eye spot disease of rubber (*Hevea brasiliensis* Muell Arg.) using plant extracts. African Journal of General Agriculture 4/1: 19-26.

Okigbo R.N., Okorie R.E., Putheti R.R. (2009). In vitro effects of garlic (*Allium sativum* L.) and african basil (*Ocimum gratissimum* L.) on pathogens isolated from rotted cassava roots. Interciencia 34/10: 742-747.

Perelló A., Gruhlke M., Slusarenko A. (2013). Effect of garlic extract on seed germination, seedling health, and vigour of pathogen-infested wheat. Journal of Plant Protection Research. 53. 10.2478/jppr-2013-0048.

Portz D., Koch E., Slusarenko A. (2008). Effects of garlic (Allium sativum) juice containing allicin on Phytophthora infestans and downy mildew of cucumber caused by *Pseudoperonospora cubensis*. European Journal of Plant Pathology 122: 197–206.

Rajendra K.S., Shah A., Shukala D.N. (2014). Efficacy of plant extracts on the germination of wheat seeds. IOSR Journal Agriculture and Veterinary Science, 7(8): 72-76

Sarfraz M. Nasim J. Jacob C. Gruhlke M. (2020). Efficacy of Allicin against Plant Pathogenic Fungi and Unveiling the Underlying Mode of Action Employing Yeast Based Chemogenetic Profiling Approach. Applied Sciences. 10. 2563. 10.3390/app10072563.

Sitara U., Akhter S. (2007). Efficacy of fungicides, sodium hypochlorite and neem seed powder to control seed borne pathogen of maize. Pakistan Journal of Botany 39(1): 285-297.

Slusarenko A., Patel A., Portz D. (2008). Control of plant diseases by natural products: Allicin from garlic as a case study. European Journal of Plant Pathology 121:313–322.

Watkinson J.I., Pill W.G. (1998). Gibberellic Acid and Presowing Chilling Increase Seed Germination of Indiangrass (Sorghastrum nutans (L.) Nash.). HortScience 33(5): 849-851.

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EFFECTS OF RED AND FAR-RED LIGHT ON SEED GERMINATION OF PLATANUS × ACERIFOLIA (AITON) WILLD.

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Abstract

Platanus × acerifolia is a widely planted ornamental tree, suitable for planting in cities, especially as a roadside tree because it is very tolerant of atmospheric pollution and root compaction. Despite producing large amounts of seeds, it is usually propagated by cuttings. However, the London plane produces a large amount of seeds and generative propagation is also possible, thus helping in a preservation of genetic variability which enables plane tree populations to adapt and to survive in heterogeneous environments in changing climate conditions. The aim of this study was to evaluate the effects of red and far-red light on seed germination, considering that seeds are small and possibly photoblastic. The seeds were collected in April and imbibed for 72 or 103 hours, followed by exposure to white light, red light, far-red light or their combinations before placing seeds on germination. The seeds in the control treatment were kept in dark. Despite expectations, obtained results show that London plane seeds are not photoblastic. Furthermore, the highest germination rate (41.0%) was obtained in a control treatment (seeds kept in dark). However, dark conditions did not promote germination because there was no significant difference in a germination rate among seeds exposed to white light, red light or far-red light.

Keywords: *London plane, photoblastic seed, germination, photosensitivity, far-red light.*

Introduction

Platanus × acerifolia (London plane) is large deciduous tree growing up to 40 m, which is widely planted in cities for its hardiness and ability to survive in harsh urban street and soil environments (Zion, 1994; Vukićević, 1996; Plotnik, 2000). The London plane is very tolerant of atmospheric pollution and it can endure root compaction when planted in tree row with a small space for root development (Dixon *et al*. 2013). It is also very decorative tree, with its large crown and changing colors of the bark (Plotnik, 2000). In Serbia, London plane is very popular urban roadside or parkland tree. Besides its ornamental use, the plane tree wood can be used in carpentry, for plywood production, in a paper industry, or it can be used to produce attractive veneers (Dixon *et al*., 2013).

London plane can be propagated by seeds or cuttings (Hartmann *et al*., 2014; Grbić, 2004). The cultivars are usually propagated by hardwood cutting, but softwood cuttings can also be used. Some cultivars (e.g. 'Bloodgood', 'Columbia', 'Liberty') can be propagate by grafting. Generative propagation is simple, but germination percentage is usually low. The seeds can overwinter in the seedballs on tree before collection. If the seeds are collected in autumn, then stratification at about 4°C is necessary.

The aim of this study is to investigate the possibility to use seed collected from elite trees growing in Belgrade for generative propagation of this species. In this way, propagation is fast and easy, and obtained plants will preserve genetic variability thus enabling to obtain plane trees adapted on local conditions, capable to survive in heterogeneous environments in changing climate conditions. Besides, *P.* × *acerifolia* seeds are very small, without endosperm which indicates that seeds can be photoblastic. However, there is no data available regarding photodormancy of the London plane seed. For this reason, we evaluated the effects of white, red and far-red light on seed germination in order to assess phytochrome regulated seed germination i.e. if the plane tree seeds are photoblastic or not.

Material and Methods

The seeds were collected from *P.* × *acerifolia* elite tree growing in park Kalemegdan, in April. *Upon bringing in* the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia), the seeds were stored in a dry and dark place few days before setting an experiment.
To test the effect of red and far-red light on germination, two types of filters and two types of lamps were used. Red light was obtained using a red plastic filter which was 3 mm thick and a fluorescent lamp (Philips 100w). Far-red light was obtained using a dark red plastic filter which was 4 mm thick and an incandescent lamp (Tesla 100w).
Before light treatments, the seeds were imbibed for 72 hours or 103 hours in distilled water. The seeds were exposed to red or far-red light for 5 minutes and put on germination in a dark conditions, at 25˚C. Two other light treatments included germination of seed in dark (24 h) or natural white light conditions (16h light / 8 h dark) (table 1).

Table 1. Germination treatments

	Treatment
R72	72 h imbibing + red light
R-FR72	72 h imbibing + red light + far-red light
R-FR-R72	72 h imbibing + red light + far-red light + red light
D72	72 h imbibing + 24 h dark
L72	72 h imbibing + 16h/8h white light
R103	103 h imbibing + red light
R-FR103	103 h imbibing + red light + far-red light
R-FR-R103	103 h imbibing + red light + far-red light + red light
D103	103 h imbibing + 24 h dark
L103	103 h imbibing + 16h/8h white light

The seeds (three replicates with 100 seeds per treatment) were placed on germination on the top of two layers of filter paper in the Petri dishes. The number of germinated seeds was recorded daily during the period of 21 days. After that, the number of remaining viable seeds that did not germinate was determined in order 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

The germination percentage was low in all treatments (Tables 2, 3) ranging mostly between 30% and 35%. This can be expected because low germination percentage of P. × acerifolia has already been reported, varying from 20-30% (Dixon et al., 2013) to 48% (Falleri and Pacella, 2011).
The light or dark conditions and treatments with a red and far-red light did not affect germination because there was no statistically significant difference among obtained values (Tables 2, 3). The highest germination rate (41%) was obtained in a dark conditions with seeds that imbibed for 72 h before germination. However, dark conditions did not promote germination because there was no significant difference in a germination rate among seeds exposed to white light, red light or far-red light. Moreover, germination of seeds in dark conditions after imbibing of 103 h was only 32%.

Table 2. Germination of 72 h imbibed seed

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
R72	22.7[a]	18.7[a]	23.7[a]
R-FR72	23.3[a]	18.7[b]	24.1[a]
R-FR-R72	33.3[a]	24.0[a]	32.7[a]
D72	41.0[a]	23.0[a]	42.3[a]
L72	30.7[a]	21.0[a]	31.2[a]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Table 3. Germination of 103 h imbibed seed

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
R103	28.3[a]	22.3[a]	28.9[a]
R-FR103	31.3[a]	26.0[a]	32.5[a]
R-FR-R103	32.0[a]	26.3[a]	33.8[a]
D103	32.0[a]	23.0[a]	32.3[a]
L103	33.7[a]	16.7[a]	34.5[a]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Comparing obtained germination rates between seed imbibed for 72 h and 103 h, it can be observed that germination rate was more uniform after 103 h, ranging from 28.3% to 33.7%, while germination rate after 72 h varied from 22.7% to 41.0%. This indicates that after 72 h seed probably did not imbibe enough water and hence the difference in germination, although it was not significant.
The real germination rate expressed as a percentage of full seeds that germinated was slightly higher than germination rate in all treatments, indicating that there were no many empty seeds in these samples. However, there are reports that full seeds can be dead and not able to germinate (Donald, 1985; Barnett and Dumroese, 2006). For this reason, Falleri and Pacella (2011) used IDS (incubation, drying, separation) technique which was developed on the principle that water imbibed by live seeds is lost at a slower rate than water imbibed by dead seeds when both are

subjected to uniform drying conditions, and they succeed to increase germination rate of *P.* × *acerifolia* from 48% (control) to 86% using petroleum ether as a separation medium.
Taking this into account there is a possibility that we could achieve better results by removing the dead seeds from the samples. However, it is also possible that germination rate and quality of seed was affected by seed source and that collecting seeds from other trees could give different results, especially because poor cross-pollination in isolated trees can cause a production of large numbers of empty seeds (Hartmann *et al*., 2014). For example, Hishyar and Gailan (2016) showed that seed collected from *P. orientalis* plants growing in different altitude exibited significant differences in germination rate (46.3% - 66.5%) and further seedlings growth.

Conclusions

The results obtained in this research showed that *P.* × *acerifolia* seeds don't respond to light treatments of white, red and far-rad light which indicate that phytochrome is not involved in regulation of germination i.e. seeds are not photoblastic. However, germination rate was low, ranging from 30 – 40% which can be caused by a large percentage of dead seeds in a samples or poor choice of mother trees. For this reason, different elite trees growing in Belgrade area should be examined as a seed source for further propagation of this species. Also, different techiques for removing dead seeds from seeds samples, including IDS method, should be examined.

Acknowledgments

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References

Barnett J., Dumroese R. K. (2006). Separating live from dead longleaf pine seeds: good and bad news. General Technical Report SRS-92, Asheville (NC): USDA Forest Service, Southern Research Station.

Dixon C., Fyson G.F., Pasiecznik N., Praciak A., Rushforth K., Sassen M., Sheil D., Correia C.S., Teeling C., Van Heist M., (eds.). (2013). CABI Encyclopedia of Forest Trees CABI Publishing.

Donald D.G.M. (1985). The separation of full dead seed from live seed in Pinus elliottii. In: South, D.B., ed. Proceedings of the international symposium on nursery management: practices for the southern pines. Auburn, AL: Auburn University School of Forestry: 83-87.

Falleri E. and Pacella R. (2011). Applying the IDS method to remove empty seeds in *Platanus* × *acerifolia*. Canadian Journal of Forest Research. 27. 1311-1315. 10.1139/x97-107.

Grbić M. (2004). Vegetativno razmnožavanje ukrasnog drveća i žbunja. (Vegetative propagation of ornamental trees and shrubs). I.P "Tragovi", I.K.P. "Ne&Bo", Beograd. (in Serbian)

Grbić M., Skočajić D., Obratov-Petković D., Bjedov I., Đukić M., Đunisijević-Bojović D. (2010). Presowing treatments to breaking seed dormancy of *Pterocarya stenoptera* C. DC as an indicator of potential invasiveness. Bulletin of the Faculty of Forestry 103: 29-40.

Hartmann H. T., Kester D. E., Davies F. T., Geneve R. L. (2014). Hartmann and Kester'splant propagation. Principles and practices. Eigth edition, Pearson Education Limited, Essex, UK, pp. 928.
Hishyar H. S. and Gailan B. A. (2016). Effect of altitudinal variation on seed and seedling growth characters of *Platanus orientalis* L. in Duhok province. Journal of University of Duhok, 17(1): 108-120. 10.13140/RG.2.1.4796.6320.
Plotnik A. (2000). The Urban Tree Book an Uncommon Field Guide for City and Town. Three Rivers Press, New York.
Vukićević E. (1996). Dekorativna dendrologija (Ornamental dendrology) Šumarski fakultet, Beograd (in Serbian)
Zion R. L. (1994). Trees for Architecture and Landscape, 2nd ed. John Wiley & Sons, 384 pp

GERMINATION OF BUDDLEJA DAVIDII FRANCH. SEED EXPOSED TO RED AND FAR-RED LIGHT TREATMENTS

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Abstract

Buddleja davidii a vigorous shrub with an arching habit and honey-scented lilac to purple flowers, widely used as an ornamental plant. There are many varieties and cultivars of this species, and they are propagated vegetatively. However, it produces viable seed and it has been recorded as naturalized or even invasive species in many countries. For this reason, testing germination of the seeds collected from the plants growing in the Belgrade area in Serbia can help to evaluate their potential to spread by self-sowing. The seeds of summer lilac are positively photoblastic and the aim of this study was to investigate the effect of red and far-red pulse of 5 minutes on seed germination. The obtained results show that a reversible effect of red and far red light on seed germination is partially expressed. The highest germination rate was recorded after 5 minutes of red light pulse (29.3%), red light treatment followed by far-red light resulted in much lower germination (9.3%). However, treatment with far-red light followed by red light reversed the effect of far-red light, but germination percentage was lower, only 14.7%.

Keywords: *summer lilac, butterfly bush, photoblastic seed, photosensitivity, far-red light.*

Introduction

Buddleja davidii (summer lilac, butterfly bush) is a deciduous, fast growing, shrub native to China. It is widely grown as an ornamental drought tolerant plant. Summer lilac can reach height of 2 – 3 m, with a spread of 1 – 3 m, and it is popular garden plant because of its bushy habit, arching stems and lilac to purple fragrant flowers that blooms from late summer to late autumn, sometimes to first frost if overblown flowers are regularly removed (Vukićević, 1996). It thrives in the full sun, in medium moisture, well-drained soil and it is slightly salt tolerant. Butterfly bush is suitable for borders or mass planting, usually grown in parks, cottage gardens or butterfly gardens, because flowers are attractive to butterflies and bees). Besides, it is a popular fresh cut flower (Wasson, 2003). There are many varieties and cultivars of this species with white, pink, blue or reddish flowers, and they are propagated vegetatively by softwood or semi-hardwood cuttings (Vukićević, 1996; Wasson, 2003; Hartmann *et al*., 2014). Generative propagation is also possible, and seeds can be sown in spring without pretreatment, but plants are not genetically true by seed (Hartmann *et al*., 2014).
However, this species has escaped gardens and naturalized in the United States, Canada, New Zealand and in central Europe (Invasive Species Compendium, 2021). Summer lilac produces large number of small seeds that are easily distributed by wind, or by water. Considering that self-seeding is a cause of spreading, removal of spent flower clusters prior to formation of seed is recommended. However, summer lilac still has the potential for further expansion and it can invade and dominate disturbed natural areas. Since the seeds of summer lilac are positively

photoblastic (Tallent-Halsell and Watt, 2009; Thompson and Abbott, 2013), the aim of this study was to investigate the effect of red and far-red pulse of 5 minutes on seed germination.

Material and methods

Healthy, vigorous shrub growing in park-forest Kosutnjak (Belgrade, Serbia) was chosen as a seed source for this research. The seeds were collected in autumn, brought to the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia). The effect of 5 minutes exposures to red and far red light on seed germination was evaluated (Table 1). The control treatment was set in dark conditions. Three types of light filters were used. A fluorescent lamp (Philips 100w) with 3 mm thick red plastic filter was used to obtain a red light. An incandescent lamp (Tesla 100w) with 4 mm thick dark red plastic filter was used to obtain far-red light. Green fluorescent tube and a green plastic filter were used for handling the seed in dark conditions.
The seeds were imbibed for 72 hours at the temperature of 20°C before being exposed to light treatments. After light treatments the seeds were put in a germination chamber in dark conditions. The seeds in a control treatment were put in a germination chamber without any light pretreatment.

Table 1. Germination treatments

	Treatment
R	5 minutes red light
R-FR	5 minutes red light + 5 minutes far-red light
R-FR-R	5 min. red light + 5 min. far-red light + 5 min. red light
R-FR-R-FR	5 min. red light + 5 min. far-red light + 5 min. red light + 5 min. far-red light
D	24 h dark conditions

Three replicates with 75 seeds per treatment were placed on germination on the top of two layers of filter paper in the petri dishes. The seeds were examined daily. After 7 days, the number of germinated seeds was recorded (germination energy) and germinated seeds were counted daily during next 14 days. Last count was on 21st day after placing the seeds on germination. After that, the seeds were examined with the aim to determine the percentage of viable seeds which is needed to calculate the real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić, 2003).
Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1. The significance of differences between the means was calculated by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test.

Results and Discussion

The obtained results showed red light and far-red light treatments influenced seed germination significantly (Table 2). Also, our results confirmed that seeds of *B. davidii* are positively photoblastic because there were no germinated seeds in dark conditions. The highest germination rate was recorded after 5 minutes of red light pulse treatment (29.3%), and red light treatment followed by far-red light resulted in much lower germination (9.3%). However, red light treatment after far-red light reversed the effect of far-red light, and germination percentage was higher (14.7%) than after far-red treatment, but lower compared to seeds that were only treated with red light.

The 5 minutes pulse of red light was sufficient to induce germination, despite seeds being placed after this treatment in a germination in dark conditions. Tang *et al*., (2008) showed that duration of seed exposure to red light can influence germination rate and they obtained higher germination of *Chenopodium album* after 20 minutes of red light treatment compared to 1 or 2 minutes treatments. However, their research showed that the treatment with red light 3 times for 1 minutes at the intervals of 12 h was more effective than a single 20 minutes treatment with red light.

Table 2. Germination of seed exposed to different light pretreatments

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
R	29.3[a]	21.3[a]	29.3[a]
R-FR	9.3[bc]	8.0[ab]	9.3[bc]
R-FR-R	14.7[b]	12.0[ab]	14.7[b]
R-FR-R-FR	8.0[bc]	4.0[b]	8.0[bc]
D	0.0[c]	0.0[b]	0.0[c]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Germination rate obtained in our research was low, not exceeding 29.3%. However, Ebeling *et al*., (2008) also obtained relatively low values (32-42%) by testing germination of seeds collected from 10 *populations of B. davidii in China and 10 populations in Germany. They germinated seeds under 12 h light / 12 h dark regime and under* alternating temperature from 10°C to 20°C. *There is a possibility that germination of B. davidii seeds in our research could be higher if the seeds were exposed to natural light. Also,* Tang *et al*., (2008) reported that there was significant interaction of temperature changes and red light treatments, and light treatments were more effective if temperature alternated between 25°C and 5°C than if the temperature were maintained at 25°C constantly. This indicates that germination of *B. davidii could be higher with different duration of red light pretreatments, or with change in a temperature regime during germination.*

Conclusions

The results presented in this research confirmed that B. davidii seeds are positively photoblastic. The treatments with red and far-red light affected significantly germination. Seeds did not germinate in the dark and also far-red treatment resulted in low germination rate. However, the red light treatment following a far-red light treatment reversed the effect of far-red light increasing germination rate. Taking into account that germination of B. davidii can be higher with seed exposed to natural light and kept in alternating temperature conditions, there is a possibility that this species can easily spread by seed in Serbia. For this reason, special attention should be paid on removal of spent flower clusters prior to formation of seeds and use of alternative non-invasive species instead of B. davidii is recommended.

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References

Ebeling S.K., Hensen I., Auge H. (2008). The invasive shrub *Buddleja davidii* performs better in its introduced range. Divers Distrib, Vol. 14: 225–233.

Grbić M. (2003). Dormantnost i klijanje semena - mehanizmi, klasifikacije i postupci. Glasnik Šumarskog fakulteta Vol. (87): 25-49.

Hartmann H. T., Kester D. E., Davies F. T., Geneve R. L. (2014). Hartmann and Kester'splant propagation. Principles and practices. Eigth edition, Pearson Education Limited, Essex, UK, p. 928.

Thompson, R., & Abbott, J. (2013). History, Dispersal, and distribution of *Buddleja davidii* (Scrophulariaceae) in Kentucky. Journal of the Botanical Research Institute of Texas, 7(1), 495-505.

Tallent-Halsell N.G., Watt M. (2009). The invasive *Buddleja davidii* (Butterfly Bush). The Botanical Review. Vol 75(3):292-325.

Tang D.S., Hamayun M. Ko Y.M., Zhang Y.P., Kang S.M., Lee I.J. (2008). Role of Red Light, Temperature, Stratification and Nitrogen in Breaking Seed Dormancy of *Chenopodium album* L. Journal Crop Sci. Biotech.. Vol 11. 199-204.

Vukićević E. (1996). Ornamental dendrology. Šumarski fakultet, Beograd. p. 580 (in Serbian)

Wasson E. (2003). The Complete Encyclopedia of Trees and Shrubs. Thunder Bay Press, p. 816.

Invasive Species Compendium https://www.cabi.org/isc/datasheet/10314 (accessed June 2021)

GERMINATION OF PAULOWNIA FORTUNEI (SEEM.) HEMSL. SEED AFTER DIFFERENT RED AND FAR-RED LIGHT TREATMENTS

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Abstract

Paulownia fortunei is a fast-growing, ornamental deciduous tree with large, fragrant pink flowers that has recently become popular for growing in green areas in Serbia, as well as on plantations for timber production. Dragon tree paulownia tolerates a wide range of soils, including soils contaminated with heavy metals pollution and it endures urban conditions well. The elite trees of dragon tree paulownia growing in the Belgrade area, well adapted to local conditions, were selected as a source for production of planting material for urban coenoses and specific-purpose plantations of this species. The dragon tree paulownia can be propagated by seed or vegetatively by cuttings. It produces a light-sensitive seed that requires light to germinate. For this reason, the aim of this research was to evaluate the effects of a red and far-red light pulse of 5 minutes on seed germination. Obtained results showed that red light (R) pulse had a positive effect on germination compared to far-red (FR) light treatments and that effect of red light could be reversed by far-red light and vice versa. The germination was higher after red light treatment (R – 10.5%, R-FR-R – 29.0%) than after far-red treatment (R-FR – 1.0%, R-FR-R-FR – 0.3%). However, the best result (77.5%) was recorded with seeds that germinate in white light conditions, without R or FR treatments.

Keywords: *dragon tree paulownia, Fortune's empress tree, photoblastic seed, photosensitivity, far-red light.*

Introduction

Paulownia fortunei (dragontree, Fortune's empress tree) is a deciduous fast-growing tree native to China and Taiwan (Viet, 2001; Radošević and Vilotić, 2010). The heart-shaped leaves are large reaching length of 25 cm. In the spring, dragon tree produces numerous large purple tubular flowers and it has been used as an ornamental tree. It is a melliferous species (Shankar, Abrol, 2015). Besides, it is used in agroforestry for growing in plantations for wood production. Dragon tree has light timber of fine grain, quite resistant to insect attacks and it is suitable for wood based panels, paper production and utility furniture industries (musical instruments, boxes, chests, furniture, picture frames, toys, doors and windows) (Rafighi, Tabarsa, 2011; Navroodi, 2013). *P. fortunei* has been used in a traditional Chinese medicine for treatment of bronchitis, enteritis, hypertension, dysentery, and its flowers are also edible, used as an ingredient for making some local delicacies (Grosso, 2018).
Dragon tree paulownia tolerates a wide range of soils, including soils contaminated with heavy metals pollution and it is used for removing heavy metals in contaminated soils, to decrease air pollution and for reforestryzation (Wang *et al.*, 2009; Viet, 2001; Madejón *et al.*, 2015). *P. fortunei* also endures urban conditions well.

The dragon tree paulownia can be propagated by seed or vegetatively by cuttings (Stuepp *et al*., 2014, 2015). Successful micropropagation of *P. fortunei* has been also reported (Clapa *et al*., 2014; Chunchukov and Yancheva, 2015). However, generative propagation is simple, enabling preservation of the genetic variability which is important for obtaining large number of different genotypes for selection of individuals with enhanced features and adapted well to heterogeneous local environments. Hence, the elite trees of dragon tree paulownia growing in the Belgrade area, well adapted to local conditions, were selected as a seed source. Dragon tree produces a large amount of small, light-sensitive seed that requires light to germinate (Grubišić *et al*., 1985). The aim of this research was to evaluate the effects of a red and far-red light 5 minutes pulse on seed germination, and to determine possibility of using selected trees as a seed source for production of planting material for urban coenoses and specific-purpose plantations of this species.

Material and Methods

The seeds of *P. fortunei* were collected from trees growing in Belgrade, brought in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia) and used for testing the effect of red and far-red light on germination.

Red light was obtained using 3 mm thick red plastic filter and a fluorescent lamp (Philips 100w). Far-red light was obtained using 4 mm thick dark red plastic filter and an incandescent lamp (Tesla 100w). Green fluorescent tube and a green plastic filter were used for handling the seed in dark conditions.

Before light treatments, the seeds were imbibed for 72 hours in distilled water, at the temperature of 20˚C. After imbibition, the seeds were exposed to red or far-red light treatment, 5 minutes each (Table 1) and put on germination in a dark conditions, at 25˚C. Two other light treatments included germination of seed in dark (24 h) or natural white light conditions (16h light / 8 h dark) (Table 1).

Table 1. Germination treatments

	Treatment
R	5 minutes red light
R-FR	5 minutes red light + 5 minutes far-red light
R-FR-R	5 min. red light + 5 min. far-red light + 5 min. red light
R-FR-R-FR	5 min. red light + 5 min. far-red light + 5 min. red light + 5 min. far-red light
D	24 h dark conditions
L	16h white light / 8 h dark conditions

The seeds (four replicates with 100 seeds per treatment) were placed on germination on the top of two layers of filter paper in the Petri dishes. The number of germinated seeds was recorded daily, first count was on 7th day (germination energy) and the number of germinated seeds was recorded during next 14 days. The last count was on 21st day after placing the seeds on germination. After that, the number of remaining viable seeds that did not germinate was determined in order to calculate the real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić et al., 2010). Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1. The significance of differences between the means was calculated by the analysis of variance (ANOVA, $p < 0.05$) and LSD (the least significant difference) test.

Results and Discussion

Results obtained in this research showed that red light and far-red light treatments influenced seed germination significantly and confirmed that seeds of *P. fortunei* are positively photoblastic (Table 2). The far-red treatments inhibited germination, and obtained germination rate was very low and the same as the germination of the seed kept in dark, up to 2.5%. However, although seed exposed to red light had higher germination rate, it was low (10.5% and 29%) compared to seed that germinated in white light conditions (77.5%). Grubišić *et al*. (1985) obtained positive effect of red light on germination of *P. fortunei* but only after 6 hours of exposure, which was in contrast to their results obtained with other *Paulownia* species. Namely, while testing *P. tomentosa* response to red light treatments only one minute of exposure to red light was enough to obtain satisfactory germination of *P. tomentosa* seed.

Table 2. Germination of *P. fortunei* seed after exposure to different light pretreatments

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
R	10.5[c]	9.2[c]	10.5[c]
R-FR	1.0[d]	0.3[d]	1.0[d]
R-FR-R	29.0[b]	21.2[b]	29.0[b]
R-FR-R-FR	0.3[d]	0.3[d]	0.3[d]
D	2.5[d]	0.75[d]	2.5[d]
L	77.5[a]	69.3[a]	77.5[a]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The obtained germination rate (77.5%) in a white light conditions was satisfactory and collected seed can be used for *P. fortunei* production. Similarly, Viet (2001) reported that expected germination of *P. fortunei* is 70%, which is in accordance with results obtained in our research, but Viet (2001) also stated that germination of *P. fortunei* can be increased considerably (90 - 95%) if the seeds are sown in *in vitro* conditions. The higher germination rate *in vitro* than *ex vitro* has already been reported for other plant species (Marković and Popović, 2012). However, there are reports of higher germination rate of *P. fortunei* (89%) obtained in standard *ex vitro* conditions (Aliarab *et al*., 2018) indicating that a seed source, i.e. genotype of mother trees can also affect germination rate. Besides, Grubišić *et al*. (1985) showed that gibberellic acid (GA_3) can induce germination which was previously inhibited by a far-red light pulse. Hence, there is a possibility that GA_3 could also have a promoting effect on germination of *P. fortunei* seed which should be investigated additionally.

Conclusions

The results obtained in this research confirmed that *P. fortunei* seeds are positively photoblastic and that far-red pulse can inhibit germination. The germination can be restored after additional red light treatment, but it is still low compared to a germination of the seed kept in white light conditions. However, maximum germination obtained in this research was high (77.5%) indicating that the seeds collected from selected trees have high germination rate in white light conditions and that these trees can be used as a seed source for production of *P. fortunei*. However, additional research should be conducted, and effect of gibberellic acid should be tested with the aim to increase germination rate.

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References

Aliarab A., Sadati E., Sheikh H. (2018). Effect of salinity on seed germination, growth and survival of *Paulownia fortunei* seedlings under laboratory and greenhouse conditions. 10.22059/jfwp.2017.238432.862.

Chunchukov A., Yancheva S. (2015). Micropropagation of Paulownia species and hybrids. Annuaire de l'Universitéde Sofia "St. Kliment Ohridski" Faculte de Biologie 100, 223–230.

Clapa D., Fira A., Simu M., Vasu L., Buduroi D. (2014). Improved in Vitro Propagation of *Paulownia elongata*, *P. fortunei* and its Interspecific Hybrid *P. elongata* x *P. fortunei*. Bulletin UASVM Horticulture Print ISSN 1843-5254. Electronic ISSN 1843-5394. 71. 6-14.

Grbić M., Skočajić D., Obratov-Petković D., Bjedov I., Đukić M., Đunisijević-Bojović D. (2010). Pre-sowing treatments to breaking seed dormancy of *Pterocarya stenoptera* C. DC as an indicator of potential invasiveness. Bulletin of the Faculty of Forestry 103: 29-40.

Grosso G. (2018). Effects of Polyphenol-Rich Foods on Human Health. Nutrients, 10(8), 1089. https://doi.org/10.3390/nu10081089.

Grubišić D., Nešković M., Konjević R. (1985). Changes in light sensitivity of *Paulownia tomentosa* and *P. fortunei* seeds. Plant Science. Volume 39, Issue 1. Pages 13-16.

Madejón P., Dominguez M., Díaz Blanco M., Madejón E. (2015). Improving sustainability in the remediation of contaminated soils by the use of compost and energy valorization by *Paulownia fortunei*. The Science of the total environment. 539. 401-409. 10.1016/j.scitotenv.2015.09.018.

Marković M., Popović M. (2012): Germination of *Dianthus deltoides* L. and *Dianthus pinifolius* Sibth. & amp. Sm. in ex vitro and in vitro conditions. Forestry Science and Practice for the Purpose of Sustainable Development of Forestry. Twenty Years of the Facultyof Forestry: Proceedings: Banja Luka. Republic of Srpska. ISBN 9993856274.

Navroodi H. I. (2013). Comparison of growth and wood production of *Populus deltoides* and *Paulownia fortunei* in Guilan province (Iran). Indian Journal of Science and Technology. 6. 84-88. 10.17485/ijst/2013/v6i2.14.

Radošević G., Vilotić D. (2010). The Influence of Climate Factors on Radial Growth in the Species of the Genus Paulownia. *Forestry (Belgrade)* 1-2: 57-58.

Rafighi A., Tabarsa T. (2011). Manufacturing High Performance Wood composite Panel from Paulownia. Key Engineering Materials. 471-472: 1091-1094.(11) (PDF) Improved In Vitro Propagation of *Paulownia elongata*, *P. fortunei* and its Interspecific Hybrid *P. elongata* x *P. fortunei*.

Shankar U., Abrol D. P. (2015). Plants for Bees: *Paulownia fortunei*. Bee World. 92. 90-91. 10.1080/0005772X.2015.1118974.

Stuepp C., Zuffellato-Ribas K., Koehler H., Wendling I. (2015). Rooting mini-cuttings of *Paulownia fortunei* var. Mikado derived from clonal mini-garden. Revista Árvore. 39. 497-504. 10.1590/0100-67622015000300010.

Stuepp C., Zuffellato-Ribas K., Wendling I., Koehler H.S., Bona C. (2014). Vegetative propagation of mature dragon trees through epicormic shoots. Bosque (Valdivia), 35(3), 337-345. https://dx.doi.org/10.4067/S0717-92002014000300008.

Viet T. Q. (2001). Technology and Practice Development Research for the cultivation of *Paulownia fortunei*. Forest Science and Technology Research Results, PP: 5. http://www.mekonginfo.org/assets/midocs/0001637-environment-technology-and-practice-development-researchfor-the-cultivation-of-paulownia-fortunei.pdf.

Wang J., Zhang C.B., Jin Z.X. (2009). The distribution and phytoavailability of heavy metal fractions in rhizosphere soils of *Paulowniu fortunei* (seem) Hems near a Pb/Zn smelter in Guangdong. PR China, Geoderma.Volume 148. Issues 3–4. Pages 299-306.

GERMINATION OF RUDBECKIA FULGIDA VAR. SULLIVANTII 'GOLDSTURM' SEED UNDER DIFFERENT TREATMENTS

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Abstract

Rudbeckia fulgida var. *sullivantii* 'Goldsturm' is a low-maintenance perennial species that produces numerous flower heads with golden-yellow rays and brownish-black center disks, from July to October. It can grow on different soils and can endure drought once established. It can also be used as a cut flower. It is often grown in green spaces in Serbia. Usually it is propagated vegetatively, by division or cuttings. Also, it produces a large amount of seed and generative propagation is possible, but plants do not come true from seed. However, the seed is small and it can easily be dispersed at long distances by wind, spreading this plant in surrounding natural habitats. For this reason, the aim of this study was to examine the germination of *Rudbeckia* 'Goldsturm' seeds and thus determine the probability of its naturalization. Treatments included pre-chilling for 5 days at 3°C, followed by the exposure to white light, far-red light, red light or their combinations, considering that seeds were small and possibly photoblastics. Although pre-chilling treatment was recommended for *R. fulgida* according to ISTA rules, our results showed that germination of seed in all treatments that included pre-chilling was very low (below 3%). The highest germination rate of 14% was obtained in a control treatment where seed was not pre-chilled and germination was performed in white light conditions. The seed kept in dark did not germinate. Obtained results indicate that *Rudbeckia* 'Goldsturm' have no potential to establish its population in natural habitats by seed dispersion, and it can be included in a Green list of non-invading alien taxa that can be grown in Serbia without a risk of becoming invasive.

Keywords: *Black-eyed Susan, far-red light, photoblastic seed, photosensitivity, germination.*

Introduction

Rudbeckia fulgida var. *sullivantii* 'Goldsturm' (Black-eyed Susan, gloriosa daisy, orange cone-flower) is a rhizomatous, clump-forming perennial plant which can reach a height of up to 60 cm. It produces numerous, large, daisy-like flowers with deep yellow rays and dark brownish-black center disks. Black-eyed Susan blooms during summer and autumn (till October). It can grow on different soils, but prefers average, moist, well-drained soils in full sun, although it tolerates light shade. Regular watering is preferred throughout the growing season, but it can endure drought once established (Kellum 2008). The cultivar 'Goldsturm' is worldwide popular low-maintenance perennial plant, often used in a perennial borders, cottage gardens, or as a cut flower. It is also grown in green spaces in Serbia.

Usually, orange cone-flower is propagated vegetatively, by division or cuttings. Also, plants slowly spread in the garden by rhizomes. However, *R. fulgida* produces a large amount of seed and generative propagation is also possible, even plants often re-seed naturally, but they do not come true from seed (Hartmann et al. 2014; Fay et al. 1994). *R. fulgida* is native in North

America and it is not recorded as an invasive plant in Europe (https://www.cabi.org). However, there is a possibility of its naturalization because the cypselae are small and they can easily be dispersed at long distances by wind, spreading this plant in surrounding natural habitats.
For this reason, the aim of this study was to examine the germination of *Rudbeckia* 'Goldsturm' seeds and thus determine the probability of its naturalization. According to ISTA (1985) prechilling and light are necessary for germination of *R. fulgida*, but according to Hartmann et al. (2014) prechilling is not needed. Also, small-seeded species often require light for germination and seeds are mostly photoblastic (Milberg et al. 2000; McDonald and Kwong 2005). Hence, the effect of red ligh and far-red light pulse was also investigated in this reseach.

Material and Methods

The seedheads were collected in a private garden in Belgrade in August and brought in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia). Dimensions and weight of seedheads were measured as well as weight of achenes. These data were used for calculation of an *extraction factor (the weight of cleaned achenes per given weight of* seedheads, *expressed in percent),* number of achenes in 1 kg and *absolute weight of achenes (weight of 1000 achenes).* The achenes were stored in a dry and dark place before setting the experiment 3 months later, in November.
Two types of filters and three types of lamps were used to test the effect of red and far-red light on germination, Red light was obtained using a red plastic filter which was 3 mm thick and a fluorescent lamp (Philips 100w). Far-red light was obtained using a dark red plastic filter which was 4 mm thick and an incandescent lamp (Tesla 100w). White fluorescent lamp (Philips 100w) was used to obtain white light (control treatment).
The seeds (three replicates with 50 seeds each) were placed on germination on the top of two layers of filter paper in the petri dishes, after being exposed to the different pretreatments (Table 1). Part of the seed were prechilled at 3˚C *and then exposed to light treatments. Part of seeds were placed on germination without prechilling. Germination was conducted* at 25˚C, except two treatments where seeds were placed on germination at 3˚C.

Table 1. Germination pretreatments

	Treatment
CL	control in light conditions, no prechilling, 16h light / 8 h dark
CD	control in 24 h dark conditions, no prechilling
D+3˚C	24 h dark conditions, no prechilling, germination at 3˚C
L+3˚C	16h light / 8 h dark, no prechilling, germination at 3˚C
L	prechilling 5 days at 3˚C, germination at 25˚C, 16h light / 8 h dark
P 5 + R	prechilling 5 days at 3˚C + 5 minutes of red light pulse, germination in dark at 25˚C
P 5 + R + FR	prechilling 5 days at 3˚C + 5 minutes of red light pulse + 5 minutes far-red light, germination in dark at 25˚C
P 5 + R + FR + R	prechilling 5 days at 3˚C + 5 minutes of red light pulse + 5 minutes far-red light + 5 minutes red light, germination in dark at 25˚C
R	no prechilling, seeds kept in dark for 5 days at 25˚C, then exposed to 5 minutes of red light pulse and returned in dark for germination
R + FR	no prechilling, seeds kept in dark for 5 days at 25˚C, then exposed to 5 minutes of red light pulse + 5 minutes far-red light, returned in dark for

	germination
R + FR + R	no prechilling, seeds kept in dark for 5 days at 25˚C, then exposed to 5 minutes of red light pulse + 5 minutes far-red light + 5 minutes red light, returned in dark for germination
P 7 + R	prechilling 7 days at 3˚C + 5 minutes of red light pulse, germination in dark at 25˚C
P 7 + R + FR	prechilling 7 days at 3˚C + 5 minutes of red light pulse + 5 minutes far-red light, germination in dark at 25˚C
P 7 + R + FR + R	prechilling 7 days at 3˚C + 5 minutes of red light pulse + 5 minutes far-red light + 5 minutes red light, germination in dark at 25˚C

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. The significance of differences between the means was calculated by the analysis of variance (ANOVA, $p < 0.05$) and LSD (the least significant difference) test.

Results and Discussion

R. fulgida var. *sullivantii* 'Goldsturm' seedheads collected in this experiment were averagely 13.5 mm long and 13.4 mm wide. The *extraction factor was* 23.82%, 1 kg contains 945 358 achenes and *absolute weight of achenes is 1.05 g*.
Germination was low in all treatments, and the highest germination rate (14%) was obtained in a control treatment where seed was not pre-chilled and germination was performed in white light conditions (Table 2). The germination of the seeds that were prechilled 5 days at 3˚C and placed on germination at 25˚C in light (16/8h) was only 2.7% indicating that germination was inhibited by prechilling and that recommendations by ISTA (1985) for *R. fulgida* cannot be used for this cultivar. Chilling time of 5 or 7 days also did not affected germination.
The seed kept in dark did not germinate, and there were no significant difference in the germination of seeds exposed to any of treatments with red light and far-red light. Nevertheless, the length of exposure to red light and far-red light treatments can also affect germination and for some species positive effect of red light on germination can be achieved only after 6 hours or of exposure (Grubišić *et al*. 1985).
It can be noticed that real germination rate was also low (Table 2) because a large number of sound, viable seeds did not germinate. Furthermore, the germination energy was 0% in all treatments, which means that none of the seeds germinated in the first 7 days.

Table 2. Germination of *R. fulgida* var. *sullivantii* 'Goldsturm'

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
CL	14.0[a]	0.0[a]	15.4[a]
CD	0.0[b]	0.0[a]	0.0[b]
D+3˚C	0.0[b]	0.0[a]	0.0[b]
L+3˚C	0.0[b]	0.0[a]	0.0[b]
L	2.7[b]	0.0[a]	2.9[b]
P 5 + R	0.7[b]	0.0[a]	0.7[b]
P 5 + R + FR	1.4[b]	0.0[a]	1.4[b]
P 5 + R + FR + R	1.4[b]	0.0[a]	1.4[b]
R	0.0[b]	0.0[a]	0.0[b]
R + FR	0.7[b]	0.0[a]	0.7[b]
R + FR + R	1.3[b]	0.0[a]	1.4[b]
P 7 + R	0.0[b]	0.0[a]	0.0[b]
P 7 + R + FR	0.7[b]	0.0[a]	0.7[b]
P 7 + R + FR + R	2.0[b]	0.0[a]	2.1[b]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Low germination in our research can be explained with relatively low germination temperatures. Although temperature of 25˚C has been commonly used for testing seed germination of majority of plant species, and Hartmann et al. (2014) recommend temperature of 21 - 24˚C for *Rudbeckia* spp., Fay et al. (1993) showed that germination of *R. fulgida* can increase considerably with increasing germination temperature. They used *R. fulgida* seeds from different sources and in all samples there were no germinated seeds at the temperature of 18˚C, but germination was 16-41% (depending on seed source) with increasing temperature to 25˚C, reaching 83-90% at the temperature of 32˚C. Further increase of germination temperature caused decreasing of germination rate (Fay et al. 1993). Thus we can expect that germination of *R. fulgida* var. *sullivantii* 'Goldsturm' could be higher if the achenes were placed on germination at higher temperatures (28-32˚C). Fay et al. (1993) conducted their research in a laboratory conditions at the constant temperatures, while in the field conditions temperatures would be different depending on a time of day, dropping considerably during night. For this reason, we can expect that *R. fulgida* var. *sullivantii* 'Goldsturm' germination will not be high enough for its expanding and naturalization in Serbia. Thus we can preliminary include this cultivar in the Green list of non-invading alien taxa in Serbia. This list comprises non-native species that do not have a potential to become invasive, according to Dehnen-Schmutz (2011). However, additional research should be conducted in order to assess the germination rate of *R. fulgida* var. *sullivantii* 'Goldsturm' in field conditions, as well as survival of seedlings.

Conclusions

The germination rate of *R. fulgida* var. *sullivantii* 'Goldsturm' was low, not exceeding 14%. The light was necessary for germination and seeds kept in dark did not germinate. The treatments with 5 minutes of red and far-red light pulse had no effect on germination. Prechilled seeds did not germinate or the germination was very low, not exceeding 3% in all treatments. Obtained results indicate that *Rudbeckia* 'Goldsturm' have no potential to establish its population in natural habitats by seed dispersion, and thus it can be included in a Green list of non-invading alien taxa that can be grown in Serbia, but further research of germination of *Rudbeckia* 'Goldsturm' in the field conditions is recommended.

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References

Dehnen-Schmutz K. (2011). Determining non-invasiveness in ornamental plants to build green lists. Journal of Applied Ecology. 48. 1374 - 1380. 10.1111/j.1365-2664.2011.02061.x.

Fay A.M., Bennet M.A., S.M. Still. (1994). Osmotic seed priming of RudbecIkia fIulgida improves germination and expands germination range. HortScience 29:868-870

Fay A.M., S.M. Still., Bennet M.A. (1993). Optimum germination temperature of *Rudbeckia fulgida*. HortTechnology 3: 433-435.

Grbić M., Skočajić D., Obratov-Petković D., Bjedov I., Đukić M., Đunisijević-Bojović D. (2010). Presowing treatments to breaking seed dormancy of *Pterocarya stenoptera* C. DC as an indicator of potential invasiveness. Bulletin of the Faculty of Forestry 103: 29-40.

Grubišić D., Nešković M., Konjević R. (1985). Changes in light sensitivity of *Paulownia tomentosa* and *P. fortunei* seeds. Plant Science. Volume 39, Issue 1. Pages 13-16.

Hartmann H. T., Kester D. E., Davies F. T., Geneve R. L. (2014). Hartmann and Kester'splant propagation. Principles and practices. Eigth edition, Pearson Education Limited, Essex, UK, pp. 928.

ISTA - International Rules for Seed Testing (1985). Adopted at the 20th International SeedTesting Congress, Canada.

Kellum J. (2008). Southern Shade: A Plant Selection Guide, University Press of Mississippi, pp. 160.

McDonald M.B., Kwong F.Y. (2005). Flower seeds Biology and technology, CABI publishing, Oxfordshire, UK.

Milberg P., Andersson L., Thompson K. (2000). Large-seeded spices are less dependent on light for germination than small-seeded ones. Seed Science Research 104, 99-104.

https://www.cabi.org/isc/datasheet/110306 (accessed June 2021)

EFFECTS OF THE SOWING DATE ON RELATIONSHIPS OF MORPHOLOGICAL PROPERTIES OF MAIZE EARS

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Abstract

The aim of this study was to observe effects of the sowing date on the relationships among morphological properties of maize ears. The trial was set up in Zemun Polje in 2019 and encompassed five sowing dates with the initial one on April 1, and then on every 10 days until May 10 (S1, S2, S3, S4, S5). Three inbred lines (L1, L2, L3) were used as a material. During sowing, seeds were classified according to their size into small (6.5-8.4 mm), large (8.5-11 mm) and the primary seed fraction (6.5-11 mm). The parameters for the following morphological ear traits were determined under laboratory conditions: ear weight, ear length, ear thickness, cob weight and the grain yield. Obtained results indicated the significant contribution of all factors in expression of observed traits, as well as the significance of the interactions. The inbred L1 was the most stable genotype for the ear weight and the cob weight. The ear length and the ear thickness varied the least in all genotypes. The initial sowing dates (S1 and S2) were the most important for the ear weight. The third sowing date was the most important for the ear length (19.81cm) and the ear thickness (5.94cm). The highest cob weight was recorded in plants sown on the fifth sowing date (S5). The LSD tests showed that the differences in the morphological traits of ears of different sowing dates were significant between S5 and the remaining four sowing dates ($p<0.05$). Various fractions used in sowing affected all traits.

Key words: *agroecological conditions, cob, trait variability.*

Introduction

Due to the increasingly frequent changes occurring in environmental conditions in local regions, a need to reconsider the suitability of conditions for crop production has arisen. The oscillation of soil and air temperatures and precipitation sums during the maize growing season affects the yield and the yield components. Intra-seasonal climate variability can affect crop production during all stages of the crop growing cycle: directly through the effects of temperature, water availability, radiation interception, and carbon fixation; indirectly by modulating nutrient availability and the occurrence of diseases and pests (Olesen et al., 2000, Ceglar et al., 2016). The sensitivity of the optimal crop growth and development to specific weather conditions depends on the crop and the growth stage (Cantelaube and Terres, 2005). The properties of the produced hybrid maize seed depend on many factors: genotypic combination of parental inbreds, synchronicity of flowering, duration of pollination, duration of grain filling, length of maturation, grain moisture content at harvest. During the expression of the stated factors, agroecological

conditions also change. The variability of these conditions can change the growth and development of maize (Chen et al., 2011; Asare et al., 2011; Zhou. et al.; 2016).
Although maize yield increased, there was a significant seasonal yield variation due to the variation of weather factors (Ray et al., 2015; Sun et al., 2016; Farmaha et al., 2016).
The aim of this study was to determine the significance of the sowing adapted to the conditions of local climatic regions on the formation of ear, morphological traits and grain yield of maize.

Material and Methods

Three maize inbred lines L1 (FAO 400) L2 (FAO 600) and L3 (FAO 600), developed at the Maize Research Institute, Zemun Polje (Belgrade, Serbia), were used in the study.
The one-year trial was set up according to the randomised block design in the location of Zemun Polje in 2019. Sowing was performed on five dates starting from April 1 to May 10, every 10 days (S1: April 1, S2: April 10, S3: April 20, S4: April 30, and May 10).
Seeds used in sowing were classified according to their size into three fractions by using sieves with mesh sizes of 8.5-11(mm) (F1); 6.5-8.4(mm) (F2); 6.5-11(mm) (F3). The elementary plot encompassed nine rows of one inbred, 3x3 rows of seeds of the equal size.
Ears were harvested by hand. Five ears per a row were taken for testing. Morphological traits: ear weight (EW), ear length (EL), ear thickness (ET), cob weight (CW) and grain yield (GY) were determined after harvest under the laboratory conditions using the method of arithmetic mean of measured values obtained by measuring instruments for weight, length, and width. The results were processed using descriptive statistics, mean values according to research variants. The effect of factors on trait variability was determined by the factorial analysis of parametric tests, ANOVA and LDS, using the statistical package SPSS 20.

Results and Discussion

According to results of the trial set up in 2019, S3 was the most favourable sowing date. The highest mean values of EL, ET and GY (22.803 g, 6.930 cm and 5.670 t ha^{-1}, respectively) were obtained in inbreds sown on this date. S2 was the most optimal sowing date for EW (149.797 g), while the largest cob percentage (18.750%) was in S4, which was the most unfavourable grain to cob ratio. The sowing date S5 did not favour the development of morphological traits. With regard to S5, all traits lagged in the development. The cob percentage was the only trait with the positive result (11.185%). This phenomenon can be explained by insufficiently filled grain due to unfavourable conditions during the grain filling, and therefore the seed to ear ratio was low. The sowing date, as a beginning of the production, determines the crop uniformity, first of all a stable yield, which depends on various (?) moisture, structure and temperature of the soil, method and dates of sowing and seed quality (Pommel et al., 2002). These changes limit the established cropping practices (Lobell et al., 2013; Cicchino et al., 2010; Mayer et al., 2014), due to which seeds from different sowing dates differ in quantitative and qualitative traits. The resulting differences among various sowing dates were significant between S5 and remaining dates for all traits (EW, EL, ET, GY, CW). The difference between S1 and S4, i.e. S1 and S3 was significant for the trait EW, i.e. ET, respectively (Table 1). Values of observed traits were uniform for sowing dates S1 and S2.

Table 1. Mean values of ear morphological traits over sowing dates (EL-ear length, ET- ear thickness, GY-rain yield, CW-cob weight)

Dependent Variable	(I)	Mean Difference (I-J) (J) S1	S2	S3	S4	S5		Mean values
EW	S1		-2.352	4.082	12.330*	69.715*	S1	147.445
	S2	2.352		6.433	14.681*	72.067*	S2	149.797
	S3	-4.082	-6.433		8.248*	65.633*	S3	143.363
	S4	-12.330*	-14.681*	-8.248*		57.385*	S4	135.115
	S5	-69.715*	-72.067*	-65.633*	-57.385*		S5	77.730
EL	S1		-0.526	-3.306	0.435	7.637*	S1	19.496
	S2	0.526		-2.781	0.961	8.162*	S2	20.022
	S3	3.306	2.781		3.741	10.943*	S3	22.803
	S4	-0.435	-0.961	-3.741		7.202*	S4	19.062
	S5	-7.637*	-8.162*	-10.943*	-7.202*		S5	11.860
ET	S1		-0.019	-1.548*	0.018	2.035*	S1	5.382
	S2	0.019		-1.529*	0.037	2.054*	S2	5.400
	S3	1.548*	1.529*		1.566*	3.583*	S3	6.930
	S4	-0.018	-0.037	-1.566*		2.016*	S4	5.363
	S5	-2.035*	-2.054*	-3.583*	-2.016*		S5	3.347
GY	S1		-0.144	-0.166	0.015	3.223*	S1	5.504
	S2	0.144		-0.021	0.160	3.367*	S2	5.648
	S3	0.166	0.021		0.181	3.388*	S3	5.670
	S4	-0.015	-0.160	-0.181		3.207*	S4	5.489
	S5	-3.223*	-3.367*	-3.388*	-3.207*		S5	2.282
CW	S1		0.155	0.062	-0.598	6.967*	S1	18.152
	S2	-0.155		-0.094	-0.753	6.811*	S2	17.997
	S3	-0.062	0.094		-0.659	6.905*	S3	18.090
	S4	0.598	0.753	0.659		7.564*	S4	18.750
	S5	-6.967*	-6.811*	-6.905*	-7.564*		S5	11.185

Maize is the plant that is most variable in terms of the seed shape and size. The importance of the seed size in sowing is presented in Table 2. The formation of morphological traits of seeds differed among seeds of various fractions that were used in sowing. Stress is one of the main reasons that can lead to a decrease in a germination rate and uniformity (Kranner et al., 2010), which is related to the composition of the seed material. The ears with the highest EW (131.831 g), EL (17.630 cm) and GY (4.912 tha^{-1}) were developed from seeds that had not been classified according to their size (F3) in sowing. This is a result of the highest number of seeds that were adapted to the conditions of reduced moisture, that were capable to emerge rapidly and to establish a uniform crop. The F1 fraction favoured ET (4.616 cm), but also the most unfavourable cob percentage (16.822%). The results show that the seed selection according to their size was an important aspect of the production. The differences among seeds of various sizes were significant. The ear weight (EW) significantly differed over all fractions, while grain yield (GY) significantly differed over F1 and F2. The cob weight (CW) was significantly lower in F2 seeds than in F1 and F3 seeds. The seed size did not significantly affect traits ET and EL (Table 3).

Table 2. Effect of seed fractions on the expression of seed morphological traits (EL-ear length, ET- ear thickness, GY-rain yield, CW-cob weight)

Dependent Variable	(I)	Mean Difference (I-J) (J) fraction F1	F2	F3	Mean values	
EW	F1		10.602*	-3.889	F1	127.942
	F2	-10.602*		-14.491*	F2	117.340
	F3	3.889	14.491*		F3	131.831
EL	F1		0.403	-2.746	F1	14.884
	F2	-0.403		-3.149	F2	14.481
	F3	2.746	3.149		F3	17.630
ET	F1		0.875	0.345	F1	4.616
	F2	-0.875		-0.530	F2	3.742
	F3	-0.345	0.530		F3	4.271
GY	F1		0.088	-0.407*	F1	4.504
	F2	-0.088		-0.495*	F2	4.416
	F3	0.407*	0.495*		F3	4.912
CW	F1		2.109*	0.087	F1	16.822
	F2	-2.109*		-2.022*	F2	14.713
	F3	-0.087	2.022*		F3	16.735

Three inbreds (L1, L2, L3) were used in the trial. The diversity in genetic potential resulted in the relationship to the application of different sowing dates (Figure 1). L1 had approximately the same values for EL and ET in all four sowing dates (S1→S4). Based on the variability of traits in relation to the sowing date, L1 was the most stable inbred, while L3 expressed the greatest variability. All three inbreds had the lowest values of EL, ET and EW in S5.

With regard to the seed size used in sowing, L1 differed from remaining two inbreds. The F2 seeds of this inbred were the most optimal for the formation of morphological traits. On the other hand, F3 seeds were the most optimal for L2 and L3 in 2019 sowing. The application of different side sizes in sowing depends on the precipitation sum and temperature conditions during sowing. The classification of seeds over fractions homogenises endosperm mass. Seeds with small endosperms are more susceptible and a dysfunction of reserve/storage substances occur (Styer and Cantliffe, 1983) adversely affecting seed vigour, but on the other hand, such seeds emerge faster due to insufficient rainfall.

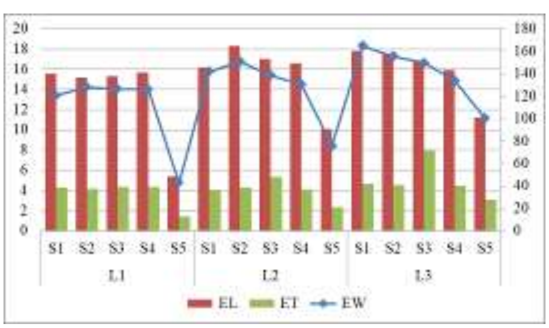

Figure 1. Effect of sowing dates on morphological traits (EW-ear weight, El-ear length, ET-ear thickness) over genotypes (L1, L2, L3)

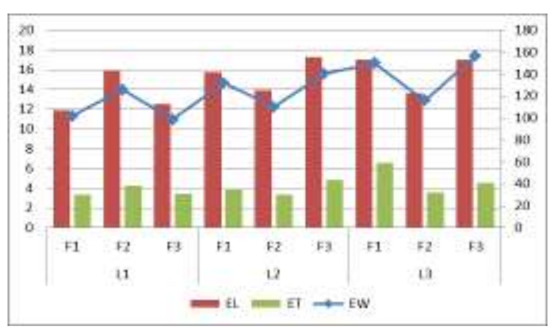

Figure 2 Effect of seed sizes on morphological traits (EW-ear weight, El-ear length, ET-ear thickness) over genotypes (L1, L2, L3)

The change of morphological traits and yield under the influence of observed factors (sowing date, genotype and seed fraction) was of the different level of significance. The ear weight (EW) and the cob weight (CW) significantly changed under the impacts of factors and their interactions. The action of both individual and combined factors was important for these traits. In contrast, ET changed the least in relation to factors. A significant change in this trait was observed with the change in sowing dates and the seed size x genotype interaction (FxG). The ear length (EL) is a trait that was also changed significantly under effects of sowing dates, genotypes and the interaction of all three factors (SxFxG). The highest effect of factors on the change of variance was on the cob weight (R = 0.818, Table 3).

Table 3. Effects of factors on the expression of seed morphological traits, ANOVA

Source	Dependent Variable (F value)				
	EW	EL	ET	GY	CW
Sowing date (S)	142.833**	7.263**	5.495**	89.915**	70.910**
Genotype (G)	68.203**	3.328*	2.228ns	30.553**	70.874**
Fraction (F)	14.754**	2.150ns	1.096ns	4.804*	16.729**
SxG	5.184**	1.205ns	0.648ns	1.846ns	4.804**
SxF	7.275**	0.957ns	0.818ns	3.294*	8.306**
GxF	33.384**	3.007*	2.492*	9.307**	23.106**
SxGxF	25.971**	2.015*	1.547ns	8.865**	21.879**

a. R Squared = 0.911 (Adjusted R Squared = 0.882)
b. R Squared = 0.429 (Adjusted R Squared = 0.243)
c. R Squared = 0.357 (Adjusted R Squared = .148)
d. R Squared = 0.828 (Adjusted R Squared = 0.772)
e. R Squared = 0.882 (Adjusted R Squared = 0.843)

EL- -ear length, ET- ear thickness, GY-rain yield, CW-cob weight

Conclusions

According to presented results it can be concluded that all three factors (genotypes, sowing dates, seed fractions) significantly contribute to the quality of seed production. Producers decide on the application of appropriate technology based on traits of the genotype. In addition to genotypes, ecological conditions are also an important decisive factor in the choice of growing practices. The choice of the proper sowing date is one of the main prerequisites for successful production. Our results show that there were significant differences in the application of different sowing dates. The best conditions for the majority of the traits were achieved by the application of S3.
The seed size, as the third analysed factor, was important for the achieved results. The F3 seed resulted in the largest ear weight, longer ears, and in the end, this resulted in the highest grain yield. The successfulness of the seed material, which was of the widest range in size, is a consequence of the fact that the largest number of seeds had enough heat and moisture for germination and emergence.

References

Asare D.K., Frimpong J.O., Ayeh E.O., Amoatey H.M. (2011). Water use efficiencies of maize cultivars grown under rain-fed conditions. Agricultural Sciences, Vol.2, 125–130. 10.4236/as.2011.22018

Cantelaube P., Terres J.M. (2005). Seasonal weather forecasts for crop yield modelling in Europe. Tellus A Vol.57, 476–487, http://dx.doi.org/10.1111/j.1600-0870.2005.00125.x.

Ceglar A., Toreti A., Lecerf R., Van der Velde M., Dentener F. (2016). Impact of meteorological drivers on regional inter-annual crop yield variability in France. Agricultural and forest meteorology, Vol. 216, 58-67.

Chen, C., Lei C., Deng A., Qian C., Hoogmoed W., Zhang W. (2011). Will higher minimum temperatures increase corn production in Northeast China? An analysis of historical data over 1965–2008. Agricultural and Forest Meteorology, Vol.151, 1580–588.doi:10.1016/j.agrformet.2011.06.013

Cicchino M., Rattalino Edreira J.I., Uribelarrea M., Otegui M.E. (2010). Heat stress in field-grown maize: Response of physiological determinants of grain yield. Crop Science, Vol.50, 1438–1448. doi:10.2135/cropsci2009.10.0574

Farmaha B.S.,Lobell D.B., Boone K.E., Cassman K.G., Yang H.S., Grassini P.(2016). Contribution of persistent factors to yieldgaps in high-yield irrigated maize. Field Crops Research. Vol.186, 124–132.doi:10.1016/j.fcr.2015.10.020

Kranner I., Minibayeva F.V., Beckett R.P., Seal C.E. (2010).What is stress? Concepts, efinitions and applications in seed science. New Phytologist, Vol.188, 655–673. doi:10.1111/j.1469-8137.2010.03461.x

Lobell D.B., Hammer G.L., McLean G., Messina C., Roberts M.J., Schlenker W.(2013). The critical role of extreme heat for maize production in the United States. Nature Climate Change, Vol.3, 497–501. doi:10.1038/nclimate1832

Mayer L.I., Savin R., Maddonni G.A. (2016). Heat stress during grain filling modifies kernel protein composition in field-grown maize. Crop Science, Vol.56, 1890–1903. doi:10.2135/cropsci2015.09.0537

Olesen J.E., Jensen,T., Petersen,J. (2000). Sensitivity of field-scale winter wheatproduction in Denmark to climate variability and climate change. Climate Research, Vol.15,221–238.

Pommel B., Mouraux D., Cappellen O., Ledent J.F.(2002). Influence of delayed emergence and canopy skips on the growth and development of maize plants: A plant scale approach with CERES maize. European Journalof Agronomy.Vol.16, 263–277. doi:10.1016/S1161-0301(01)00130-7

Ray D.K., GerberJ.S. G., Macdonald K., West P.C. (2015). Climatevariation explains a third of global crop yield variability. Nature Communications, Vol.6, 5989. doi:10.1038/ncomms6989

Styer R.C., Cantliffe D.J. (1983): Relationship between environment during seed development and seed vigor of two endosperm mutant of corn. Journal of the American Society for Horticultural Science Vol.108, 717–720

Sun H., Zhang X., Wang E., Chen S.,Shao L., Qin W.(2016). Assessingthe contribution of weather and management to the annualyield variation of summer maize using APSIM in the North ChinaPlain. Field Crops Research, Vol.194, 94–102. doi:10.1016/j.fcr.2016.05.007

Zhou B., Yue Y., Sun X., Wang X., Wang Z., Ma W., Zhao M. (2016). Maize Grain Yield and Dry Matter Production Responses to Variations in Weather Conditions. Agronomy Journal, Vol.108, 196–204 (2016). doi:10.2134/agronj2015.0196

THE EVALUATION OF THE STABILITY OF SOME ZP MAIZE HYBRIDS BASED ON THE GENOTYPE × ENVIRONMENT INTERACTION

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Abstract

Stability of yield or of any other trait depends on the ability of a given cultivar to response to changes in the environment, which is also referred to as phenotypic plasticity. The analysis of a genotype × location interaction is necessary to obtain information on the stable performance of genotypes. This paper presents results of the genotype (G) main effect and the genotype by environment (GE) interaction, genotype plus genotype by environment interaction (G × GE) biplot analysis of a multi-environmental trial with eight maize hybrids of the FAO maturity group 600 and six different locations in Serbia conducted in 2018. The objective was to determine the effects of genotypes, six environments and their interactions on grain yield and to identify stable maize genotypes. The trial was set up in a randomised complete block design with three replications in each environment. The combined ANOVA indicated that the main effects of environments, genotypes and the genotype by environment interaction were highly significant. The G × GE effects were further partitioned using a GGE biplot model. In the FAO maturity group 600, the first mega-environment contained environments E6 (Požarevac), E5 (Pančevo) and E2 (Bečej) with a check G7 (ZP EXP1) producing the highest yield. The mega-environment contained environments E4 (Sremska Mitrovica) and E1 (Zemun Polje) with the most yielding hybrid ZPSC 606. Genotypes G8 (ZP EXP2) and G7 (ZP 707) are considered to be the "ideal" genotype in terms of performance and stability.

Key words: *AMMI stability value, GGE biplot, genotype, environment, maize.*

Abbreviations: AMMI (additive main effects and multiplicative interactions), E (environment), G (genotype), GE (genotype × environment), GEI (genotype × environment interaction), GGE (genotype and genotype × environment), MET (multi-environment trial), PCA (principal component analysis), IPCA (interaction principal component analysis).

Introduction

Global forecasts indicate that by 2025, maize will be the crop with the highest production in the world, and that the need for maize will double in the developing countries by 2050 (Rosegrant et al., 2008). Maize, together with wheat and rice, is the most important crop in global agricultural production. In terms of the total sown areas in the world, maize ranks second just behind wheat. The basic economic importance of maize results from the traits of the plant itself, diversity of the use and the production volume. In addition, maize is greatly important in terms of cropping practices, as the plant requires intensive growing conditions. Almost all parts of maize plants may be processed, which is exactly what gives maize a special economic significance.

The development of high-yielding and stable varieties and hybrids is the ultimate goal set in the majority of breeding programmes. The ideal maize genotype should have a high average yield together with a low degree of variation under different environmental conditions (Epinat-LeSignor et al., 2001). In addition to the variable environmental conditions, crop yields are greatly affected by genetic factors.

The yield is a multidimensional trait that encompasses several different properties affected by a number of factors. Maize yield depends on a genotype and its yielding and quality potential, agroecological conditions, levels of applied cropping practices. Certain genotypes express various performances when grown in many different environments. If a certain genotype shows good performance in one environment, it does not imply that it will express the same performances in other environments in which it is grown (Stevanović et al., 2012). Therefore, multi-environment trials are of a great importance for the majority of scientific breeding programmes. Multi-environment trials (METs) performed for the yield, involve testing of a larger number of genotypes in different environments and are trials most frequently carried out in agriculture (Huhn, 2000; Yan et al., 2000; Crossa et al., 2004;Yan and Tinker, 2006;Gauch, 2006;Čamdžija et.al.2011; Andrade et al., 2016).

Stability of yield or any other trait depends on the ability of a given cultivar to respond to the environmental changes, which is also referred to as phenotypic plasticity, Eberhart and Russell (1966). Moreover, stability is one of the most desirable traits of a genotype being introduced into production. However, activities of the identification, breeding and selection and the recommendation of superior genotypes are complex and limited by the genotype × location interaction that is inevitable in the multi-location environments (Asfaw et al., 2009).

The aim of this study was to determine effects of a genotype, environment, and their interaction on grain yield and to identify stable maize genotypes. Results of this and similar studies can potentially be a good basis for making recommendations in the future maize production in our country.

Material and methods

Plant material and the method of trial setting up

In 2018, the study was carried out in six different locations used by the Maize Research Institute, Zemun Polje (Table 1) and they were the first factor in this study. The locations were mainly in the lowlands of Serbia, which were intended for the successful cultivation of observed maize hybrids of the FAO maturity group 600.

Table 1. Various locations in which trials were performed in 2018

Location		Latitude	Longitude	Altitude (m)
E1	Zemun Polje	44°, 51′ N	20°,19′ E	83
E2	Bečej	45°, 36′ N	20°,02′ E	78
E3	Bajša	45°, 47′ N	19°,34′ E	101
E4	Sremska Mitrovica	44°, 58′ N	19°,37′ E	81
E5	Pančevo	44°, 53′ N	20°,41′ E	74
E6	Požarevac	44°, 37′ N	21°,09′ E	76

Maize hybrids of the FAO maturity group 600 are the basic plant material used in the study. Eight genotypes of the FAO maturity group 600, developed at the Maize Research Institute, Zemun Polje, were tested. The genotype G7 was a check in the FAO 600.
The three-replicate traits with 20 plants within each replicate were set up according to the randomised complete block design. Each genotype was sown in eight 5-m long rows. The row spacing was 0.75 m, thus the elementary plot size amounted to 30 m^2. The common maize growing practices were applied in the trial. Sowing and harvesting was done mechanically. Only four inner rows were harvested due to the border row effect, and the yield was determined in these four rows and then it was computed to one hectare. The genotypes (hybrids) were the second factor.

Table 2. Data on tested maize genotypes in multi-environment trials in 2018

Genotypes of the FAO maturity group 600	Hybrid designation
G1	ZP SC 6037
G2	ZP SC 6078
G3	ZP SC 6707
G4	ZPSC 6708
G5	ZPSC 6710
G6	ZPSC 606
G7 (check)	ZPSC EXP.
G8	ZPSC 666

Statistical data analysis

The assessment of differences among the analysed maize genotypes in six locations during one year as well as their interaction was performed by the analysis of variance for the factorial trial set up according to randomised block design. The additive main effects and multiplicative interaction (AMMI) model is characterised by the fact that it combines two methods: analysis of variance and the principal component analysis into the unique model with additive and multiplicative parameters. This characteristic of the AMMI model was applied in this assessment with the aim to separate the total phenotypic variance into additive (genetic) and non-additive (ecological) components, in order to assess the significance of the effect of the source of variation on the expression of traits. The last step in the AMMI analysis of stability is certainly the graphical presentation in the form of a biplot, which enables the perception of the dispersion of the observed genotypes, locations, treatments and their interaction. The horizontal x-axis of the AMMI1 biplot shows the main additive effects of a genotype, location, i.e. genotype, treatments, while the vertical y-axis shows the multiplicative effects of the genotype × environment interaction, and the genotype × treatment interaction, contained in the first two PC components.
In order to further and more accurate evaluate maize genotypes and locations, as well as the genotypes and the treatments, the GGE bioplot analysis according to Yan and Kang, 2003, (Gauch, 2013) was applied. The characteristic of the GGE biplot method is that, when studying yield stability, it does not separate but it jointly analyses the variability resulted from the effects of the genotype and the genotype × environment interaction. The statistical data analysis was performed using the computer statistical program GenStat 12th (GenStat, 2009).

Results and discussion

In recent years, GEI quantifications and studies on yield stability of several cereal crops have been performed. The different methods using non-parametic and paremetic statistics in the estimation of the stability of genotypes of cultivated crops are very important (Delić et al., 2009). The GEI analysis can be derived by using various methods, such as the stability analysis and additive main effects and multiplicative interaction (AMMI) model, principal component analysis or the linear regression analysis, analysis of variance (ANOVA) and the GGE biplot analysis. ANOVA explains only main effects and does not provide any information about individual genotypes and locations that are an integral part of the interaction. AMMI provides a large set of technical interpretations and uses a main component (autovector) to interpret cultivar performances by integrating the use of ANOVA and PCA. AMMI analyses combine additive components into a single model for main effects of a genotype and an environment, as well as multiplicative components for the interaction effect (Gauch, 2006).

Table 3. The average yield of maize grain yield (t ha^{-1}) of eight genotypes of FAO maturity group 600 (G1 → G8) tested in six locations (E1→ E6) in 2018

Genotype (G)	Tested locations (E)						Average E
	E1	E2	E3	E4	E5	E6	
G1	10.718	15.344	11.501	11.607	15.156	13.118	12.907
G2	10.710	16.033	10.206	9.478	15.187	13.952	12.594
G3	12.437	15.649	9.945	11.548	17.180	14.455	13.524
G4	13.360	14.695	11.017	13.238	15.650	14.965	13.820
G5	13.227	15.982	11.728	11.757	16.129	15.179	14.000
G6	14.195	15.830	11.037	12.512	16.832	14.701	14.184
G7	12.706	18.110	11.158	13.009	16.971	16.734	14.781
G8	13.913	16.022	11.301	11.782	16.833	14.115	13.994
Average G	12.658	15.958	10.986	11.866	16.242	14.652	13.726

The maize yield ranged between 10.986 t ha^{-1} and 16.242 t ha^{-1} over different environments or locations and between 12.594 t ha^{-1} and 14.781 t ha^{-1} over various genotypes (Table 3). The total average maize grain yield amounted to 13.726 t ha^{-1}. The raw data on the maize yield revealed that E5 (Pančevo), i.e. E3 (Bajša) was the most, i.e. least productive environment for all genotypes, respectively. On average, the genotypes G7 (check) and G2 (ZP 6078) were the most and the least productive, respectively.

ANOVA for the AMMI model of the maize grain yield (Table 4) points out that the effect of the location/environment (E), genotype (G) and their interactions (GEI) was very significant ($p<0.01$) in the investigation year (2018) (Table 2).

The environment or locations significantly affected the maize grain yield and this factor accounted for 70.6% of yield variability in 2018. The G effect on maize production was lower (8.09%), since that year was more favourable for maize cultivation. The AMMI1 analysis of variance showed that the effects of the source of variation, genotype, environment and GEI were important for the maize yield (Table 4).

This analysis of variance also showed that 72.5 %, 8.095% and 8.647% of the total SS were attributed to effects of environments, genotypes and GEI, respectively.

Table 4. Combined AMMI analysis of variance for the grain yield of observed maize genotypes of FAO 600

Source of variation	df	SS	MS	SS (%)	GxE explained (%)	Cumulative (%)
Total	143	795.6	5.56			
Block	12	16.4	1.36ns	2.061		
G	7	64.4	9.20***	8.095		
E	5	577.0	115.41***	72.523		
G×E interaction	35	68.8	1.96**	8.647		
IPC1	11	31.1	2.82**		45.203	45.203
IPC2	9	17.5	1.94*		25.436	70.639
IPC3	7	12.6	1.81*		18.314	88.953
IPC4	5	5.8	1.16ns		8.430	97.383
IPC5	3	1.8	0.59ns		2.616	99.999
Error	84	69.1	0.82			
Mean =13.726 t ha^{-1}						
Coefficient of variation (CV%)= 69						

***, ** and * significant at 0.1%, 1%, and 5%, respectively; ns = not significant (p > 0.05)

The large SS for the environment points out that locations were different with great differences in environments that caused the majority of variations in maize yields. In addition to the heterogeneity of the environments, or locations, significant differences were determined among genotypes.

Graphical analyses revealed phenotypic stability, genotypic behaviour of cultivars and performance-optimising environments. The AMMI model points out to the main effects of genotypes (G), environments (E) and their interactions (G×E). Furthermore, this model contributes to a better assessment of the cultivar, and based on that, recommendations for the selection of the test site (location). Analysed data based on the these models can be very useful for making better conclusions.

The GGE biplot analysis is another method that combines the genotype and the genotype under effects of environments in the evaluation of cultivars. The GGE model uses graphic axes and identifies superior genotypes in mega-environments. The mega-environment encompasses the groups of locations that consistently share the same genotypes tested. It also combines ANOVA and IPCA by partitioning the sum of squares of genotypes and the sum of squatters for the genotype × environment (G×E) interaction using the IPCA method. It is also used to present and assess genotypes in various environments (Gomes Machad et al., 2019).

The results showed the importance of testing and comparison of genotypes so that only the specific ones in terms of broad adaptability and stability and accordingly the location for which they are representative could be selected. Higher IPCA1 indicates a higher discriminatory ability of the environment (Yan et al., 2000; Gauch, 2006). This contributes to the determination of the discriminatory ability to improve partitioning through performances of different genotypes (Yan&Tinker, 2006; Yan, 2011). For instance, E6 (Požarevac) although the location with lower yields (only third in terms of average values), provided more information about tested genotypes than remaining locations. Thus, this study provides important information on the selection of the

best and ideal genotype or genotypes of the observed FAO maturity group 600 that are good for production in specific and widely adapted locations (Figures 1 and 2). Two mega-environments were revealed in the study of hybrids of FAO 600. The first group encompassed three locations (E5 - Pančevo, E6 - Požarevac and E2 - Bečej), while the second one consisted of two locations (E4 - Sremska Mitrovica and E1 - Zemun Polje). The G7 (check) and G3 (ZP 6707) are genotypes with the best performances. These genotypes are the most adapted to the mentioned environments (Figure 3).
With regard to "which-won-where", the hybrids with the best performances in the mega-environment or the sector were identified. The genotype G7 (Figure 3) was the hybrid with the best performance in the multi-location trial. At the same time, this genotype can be considered close to ideal in observed locations in 2018, including the assessment based on the GGE biplot with concentric circles (Figure 4). Furthermore, this was the best-ranked hybrid in all tested locations and among all genotypes (Figure 5). This fact provided researchers with the information, which hybrids should be recommended for the given and specific location, i.e. environment.

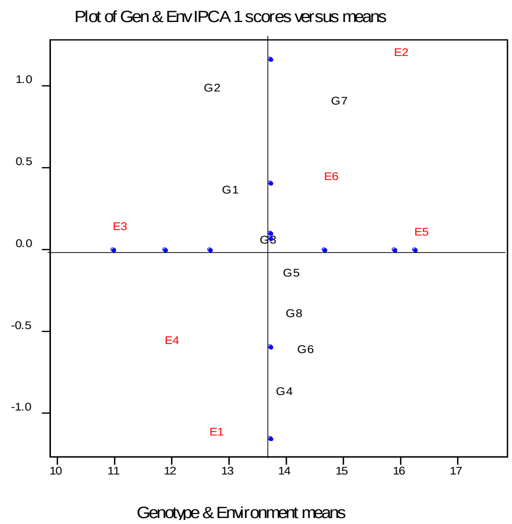

Figure 1. AMMI1 biplot (main effects of PC1) far data on maize yield

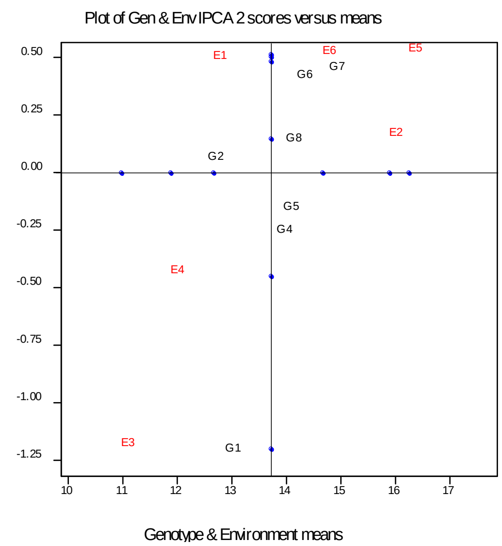

Figure 2. AMM1 biplot of grain yield (IPCA2) in 6 environments

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GGE biplot analysis

Polygonal view of the mega-environment (which-won-where)

The important feature of the GGE biplot is that it predicts performances of hybrids by locations. In the mega-environment during the identification process, the farthest genotypes were linked together in the form of the polygon. Sectors, making visualisation of the mega-environment easy, were formed with lines vertical to the straight line segments of the polygon. Environments in one sector within which the best performing genotype was located could be considered a mega-environment for that genotype. Biplots in the Figure 3 are divided into five sectors. Genotypes belonging to the same sector with locations are better adapted to agroecological conditions of those locations. Two mega-environments are clearly observable: one consists of three locations

(E5, E6 and E2) and the other encompasses two locations (E4 and E1). Therefore, the best performing hybrids in each mega-environment are located at the top of the polygon. The genotype G7 (check) had best performances for the mega-environment consisting of three locations (E2, E5 and E6). The genotype G6 performed best in the mega-environment consisting of two locations (E4 and E1). The genotypes G8 and G4 best performed in the third location. These genotypes are most adapted to the stated environments.

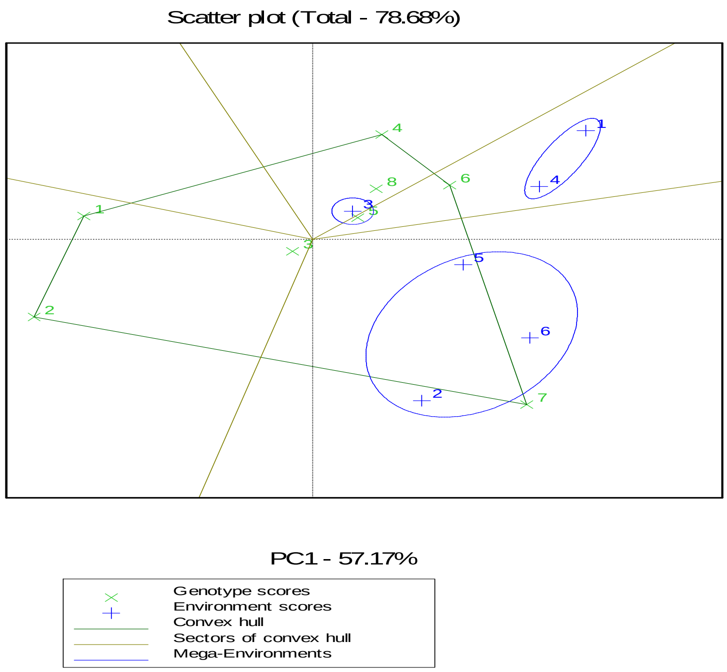
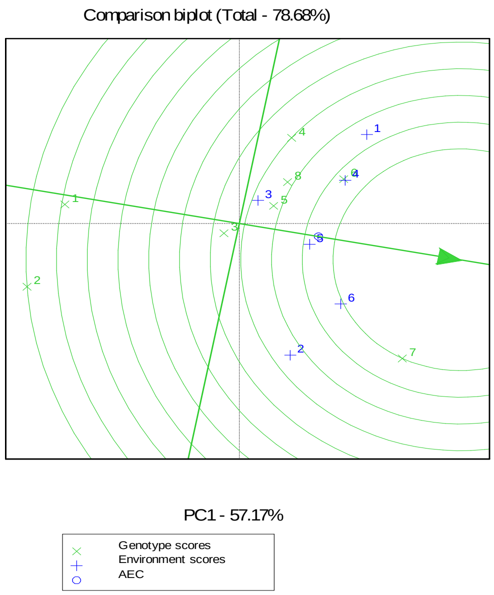


Figure 3. The "which-won-where" view of the GGE biplot

The ideal environment or a location is the one placed in the inner circle (Figure 4). Thus, E6 is considered the ideal environment or location. However, E3 and E2, being at the furthest circle from the centre of the concentric circles, are consider the least ideal environments.
The ideal genotype is the one in the centre or in the vicinity of concentric circles. According to the analysis, the genotype G7 is close to the ideal genotype, as shown by its position in the centre of the concentric circles.
The GGE biplot (Figure 5) presents the ranking of genotypes by yields and stability in locations. The line passing through the bioplot origin is called the average environment coordinate (AEC). Hybrids are ranked in the direction of a tester axis, while parallel lines help in the visualisation of the hybrid ranking.

The comparison biplot for genotypes based on concentric circles and the evaluation of efficiency of different genotypes in observed locations



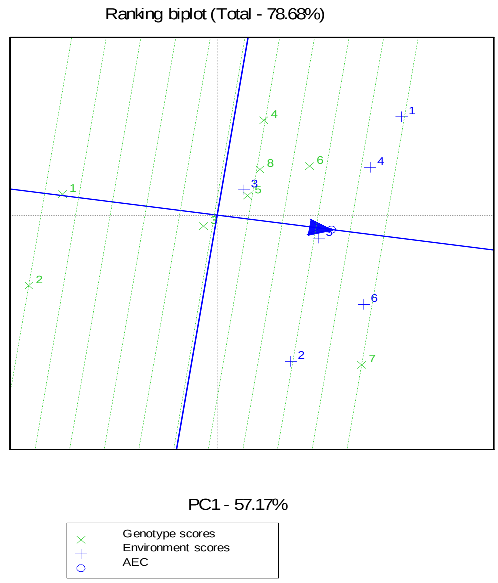


Figure 4. View of genotypes based on concentric circles

Figure 5. Ranking of environments based on the highest yielding genotype

In this example, the highest yield was determined in G7 in locations E6, E5 and E2. This genotype was followed by G6, G5, G8 and G4 in locations E1, E4 and E3, while genotypes G3, G1 and G2, having the lowest grain yield in 2018, were ranked last in all locations.

Table 5. Explanation of variations of two components PCI1 and PCI2

Models	Explanation of variations (%)	
	Hybrids of FAO maturity group 600	
	PCI1	PCI2
AMMI	45.23	25.44
GGE biplot	57.21	21.52

The GGE biplot also provided data that are important if the researcher has to make decisions and conclusions about specific correlations between locations and genotypes. The used AMMI and GGE biplot models were efficient in the observation of the genotype by environment (location) interaction. Satisfactory results were obtained by both methods - the difference was minimal, which only confirms the fact that these two models represent standards for data interpretation in this type of research, while interpretations of the GGE biplot graphics are considered the most reliable.

A comparative data analysis (Table 5) shows that the GGE biplot model was more efficient, especially in interpreting the variation of the first interaction axis, because it explained a greater variation on the first axis (57.21%), which was almost two thirds of the total variation unlike the AMMI model by which 45.23% were explained. Similar results have been obtained by other authors in the world and in our country (Rao et al., 2011; Mitrović et al., 2012).

Conclusion

Based on the detailed statistical analysis of results on the grain yield of eight maize genotypes of the FAO maturity group 600, obtained in trials in six locations in Serbia performed in 2018, the following can be concluded:

Grain yields of these hybrids were significantly affected by environments (or locations) (E), genotypes (G) and their interactions (G×E).

The contribution of the interaction of the environment (E), genotype (G) and the environment by genotype (G×E) interaction in the total variation of the hybrid grain yield amounted to approximately 72.52%, 8.09% and 9.64%, respectively, which was statistically very significant.

Effects of G × GE were further divided using the GGE biplot model.

The first mega-environment based on the GGE biplot contained locations E6 (Požarevac), E5 (Pančevo) and E2 (Bečej) with the genotype G7 (check), which had the highest yield of all genotypes of the FAO maturity group 600. The second mega-environment contained tow locations E4 (Sremska Mitrovica) and E1 (Zemun Polje), and the winning genotype was G6 (ZP 606). These genotypes were best adapted to these environments.

Based on the analysis, the genotype G7 (check) is the "ideal" genotype within FAO maturity group 600. This genotype is stable with a high mean value of the grain yield. This genotype was followed by genotypes G6 (ZP 606) and G8 (ZP 666) that were close to the ideal type on concentric circles. Then, these two genotypes were followed by genotypes G5 (ZP 6710) and G4 (ZP 6708). Based on their stability and yields these hybrids can be recommended immediately after the check.

References

Andrade, M. I., Naico A., Ricardo, J., Eyzaguirre, R., Makunde, G.S., Ortiz, R., Grüneberg,W.J.(2016): Genotype × environment interaction and selection for drought adaptation in sweetpotato (*Ipomoea batatas* L.) Lam.) in Mozambique. Euphytica. International Journal of Plant Breeding. Springer.

Asfaw, A., Alemayehu, F., Gurmu, F., Atnaf, M. (2009): AMMI and sreg GGE biplot analysis for matching varieties onto soybean production environments in Ethiopia. Scientific research and essay 4: 1322-1330.

Čamdžija, Z., Mladenović-Drinić, S., Filipović, M., Stevanović, M., Pavlov, J., Božinović, S. (2011):Genetic variability among maize hybrids for yield and yield components. 46thCroatian and 6th International Symposium on Agriculture, Opatia, Croatia. Proceedings, pp 388-390.

Crossa, J., Yang, R., Cornelius, P. (2004): Studying Crossover Genotype x Environment Interaction Using Linear-Bilinear Models and Mixed Models. Journal of Agricultural, Biological, and Environmental Statistics, Vol. 9, No 3, pp: 362–380.

Delić, N., Stanković, G., Konstatinov, K. (2009):Use of non-parametric statistics in estimation of genotypes stability. Maydica 54: 155-160.

Eberhart, S. A., Russell, W. A. (1966): Stability parameters for comparing varieties. Crop Sci., 6: 36-40.

Epinat Le Signor,C., Dousse, S., Lorgeou, J., Denis, J. B., Bonhomme, R., Carolo, P., Charcosset, A., (2001): Interpretation of genotype x environment interactions forearlymaize hybrids over 12 years. Crop Science, 41: 663-669.

Gauch, G.H. (2006): Statistical Analysis of Yield Trials by AMMI and GGE. Crop Science, 46: 1488-1500.

Gauch, H.G. (2013): A simple protocol for AMMI analysis of yield trials. Crop Science, 53(5):1860-1869.

GenStat (2009): GenStat for Windows (12th Edition) Introduction. VSN International, Hemel Hempstead.

Gomes Machad, N.,Lotufo-Neto, N.,Hongyu, K. (2019): Statistical analysis for genotype stability and adaptability in maize yield based on environment and genotype interaction models. Ci. e Nat., Santa Maria v.41, e25, p. 01-09, 2019 DOI:10.5902/2179460X32873 ISSN 2179-460X

Huhn, M. (1979): Beitrage zur Erfassung der phanotypischen stabilitat. I. Vorschlag einiger auf Ranginformationnen beruhenden stabilitats parameter," EDV in Medizin Und Biologie, vol. 10, pp. 112–117.

Mitrović, B., Stanisavljević, D., Treskić, S., Stojaković, M.,. Ivanović, M., Bekavac, G., Rajković, M. (2012): Evaluation of experimental maize hybrids tested in multilocation trials using AMMI and GGE biplot analyses. Turk. J. Field Crops, 17: 35-40.

Rao, PS, Reddy, P.S., Ratore, A., Reddy, B.V.S., Panwar, S. (2011) Application GGE biplot and AMMI model to evaluate sweet sorghum (*Sorghum bicolor*) hybrids for genotype 9 environment interaction and seasonal adaptation. Indian J Agric Sci 81:438–444.

Rosegrant, M.W., Msangi, S., Ringler, C. T. Sulser, B., Zhu, T.S., Cline, A. (2008): International Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT): Model Description. International Food Policy Research Institute: Washington, D.C. www.ifpri.org/themes/impact/impactwater.

Stevanović, M., Mladenović-Drinić, S., Stanković, G., Kandić, V., Čamdžija, Z., Grčić, N., Crevar, M. (2012): Analyses of variance and correlation among yield and yield componentsof maize hybrids and their parental inbred lines. Proceedings. 47th Croatian and 7thInternational Symposium on Agriculture. February 13-17. Opatija, Croatia, pp: 327–330.

Yan, W. K.; Hunt, L. A.; Sheng, Q. L.; Szlavnics, Z. (2000): Cultivar evaluation and mega-environment investigation based on the GGE Biplot. Crop Science, 40: 597-605.

Yan, W., Kang, M.S. (2003): GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists and Agronomists. 1st Edn., CRC Press, Boca Raton, FL., USA., Pages: 288.

Yan, W., Tinker, N. (2006): Biplot analysis of multi-environment trial data: Principles and applications. Can. J. Plant Science, 86, pp: 623–645.

Yan, W. 2011. GGE biplot vs. AMMI graphs for Genotypes-by-Environments Data Analysis. Journal of the Indian Society of Agricultural Statistics, 65 (2): 181-193.

GRAIN YIELD IN MAIZE HYBRIDS OF DIFFERENT FAO MATURITY GROUPS

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Abstract

A two year field trial with ten promising native maize hybrids for grain production (Maize Research Institute Zemun Polje, Institute of Field and Vegetable Crops, Novi Sad), FAO maturty groups 300-600, was set up in the vicinity of Kragujevac (Lužnice site, 44 ° 06 ′ N, 20 ° 49 ′ S, 240 m a.s.l.), on brown forest soil type. The aim of this study was to recommend hybrids for specific agroecological conditions. In both years, sowing was performed at the end of April, of eight rows per elementary plot, at a row distance of 70 cm, and 18 to 25 cm within row, depending on recomended number of plants, in three replicates. During the experiment, standard agricultural measures were applied, excluding irrigation. Owing to a much larger amount and more favorable distribution of precipitation, especially in the critical developmental phases (tasseling - fertilization), the average grain yield of all maize hybrids in 2018 was almost three times higher than in 2017. The most noticeable reaction of hybrids to environmental factors was recorded in the hybrid NS 548 which, as one of the hybrids with the highest yield in 2017, in 2018 had a yield significantly lower than other hybrids. Hybrids ZP 560, ZP 606 and ZP 666 in both years belonged to the group of hybrids with the highest grain yield.

Keywords: *maize, hybrid, grain yield.*

Introduction

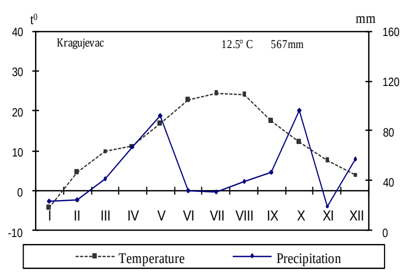
Maize, together with wheat, has the most important place in field production in the Republic of Serbia. In 2020, maize was sown on 996,527 ha, which is 3.6% more than in 2019, or 0.1% more than the ten-year average (2010–2019) (https://www.stat.gov.rs/sr-latn/oblasti/poljoprivreda-sumarstvo-i-ribarstvo/biljna-proizvodnja). Maize production in the Republic of Serbia is characterized by large variations in yield over the years. Insufficient precipitation and above-average air temperature during maize vegetation presents a major problem in its cultivation, both in Serbia and in the world, because in such conditions the potential for grain yield of high-yielding hybrids is not fully expressed (Kovačević et al., 2010; Markulj et al. 2010; Paunović et al. 2013, Pejić et al., 2020). A similar effect of weather conditions on maize yields is also in US Western Corn Belt (Shaw, 1988, Gaffney et al, 2015). Ummenhofer et al. (2015) indicate that historically high (low) yields in Iowa usually occur during years with an abnormally wet (dry) vegetation season. In the last 25 years, maize grain yield has increasingly depended on meteorological conditions during the growing season, which are very often characterized by the occurrence of "extreme climatic events" (Bekavac et al. 2010, Pavlov et al. 2011). One way to reach a compromise and satisfy the interests of both producers and breeders is to divide the area of cultivation of a crop into regions based on geographical, climatic and soil conditions that characterize them, and recommend hybrids for each region (Babić et al., 2013). The field experiment with 10 native maize hybrids for grain production, FAO maturity groups 300-600, was set up in the vicinity of Kragujevac for two years with the aim to recommend hybrids for specific environmental conditions.

Material and methods

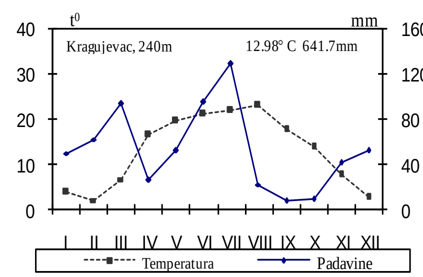
A field experiment with maize hybrids set up during 2017 and 2018 at the Lužnice site, municipality of Kragujevac (44 ° 06 ′ N, 20 ° 49 ′ S, 240 m a.s.l.), on brown forest soil type. Ten promising native maize hybrids of different FAO maturty groups (300-600) were selected: ZP 434, ZP 548, ZP 560, ZP 606, ZP 666 (Maize Research Institute Zemun Polje) NS 3022, NS 4051, NS 5051, NS 6030 and NS 6102 (Institute of Field and Vegetable Crops, Novi Sad). Sowing was performed in both years at the end of April. All hybrids were sown in three replicates of 8 rows per elementary plot, with the recommended number of plants, at a row distance of 70 cm, and 18 to 25 cm within row. In both years, along with primary tillage 20 t ha^{-1} of manure was incorporated; 400 kg ha^{-1} NPK (16: 16: 16) in pre-sowing preparation and 250 kg ha^{-1} KAN (27 % N) in top-dressing (phase 5-6 leaves) was added. During the vegetation, the crops were not irrigated. Harvesting was done manually at physiological maturity. After harvesting, the yield of cobs (60 plants) was measured from each plot and the grain yield ha^{-1} with 14% moisture was calculated. The obtained data were processed by the method of analysis of variance of two-factorial experiment (hybrid/year).

Meteorological conditions

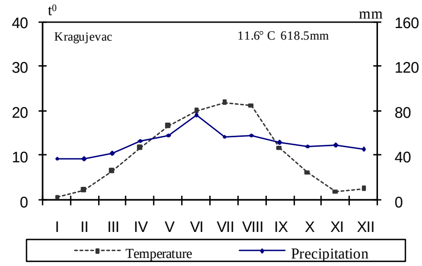
From April to September 2017, about 20% less precipitation than the multi-year average was recorded (Graph 1). The spring of 2017 (March - May) started with much higher temperatures than usual. Summer (June-August) was warmer and with less amount of precipitation (100.9 mm) compared to the multi-year average. Very warm weather continued in September, the last month of vegetation, when air temperatures were often around and above 30 ° C, with very little rainfall.



Graph 1. Climate diagram for Kragujevac in 2017



Graph 2. Climate diagram for Kragujevac in 2018



Graph 3. Climate diagram for Kragujevac in 1981-2010

In June and July 2018, in the phase of fertilisation and grain filling, a total of 224.9 mm of precipitation was recorded, while August and September were warm with a significantly lower amount of precipitation (29.5 mm) (Graph 2). The summer of 2018 (June - August) was warmer, with a higher amount of precipitation compared to the multi-year average (Graph 3).

Results and discussion

The maize vegetation period (April - October) in 2018 (Graph 2) was characterized by much higher amounts and a more favorable distribution of precipitation compared to 2017 (Graph 1), especially in the critical stages of maize development (tasseling - fertilization) so that the grain yield of all hybrids, on average, was almost three times higher (Table 1). The highest yield, on average for both years, was recorded in the hybrid ZP 560, significantly higher compared to other hybrids (Table 1). Hybrids ZP 606, ZP 666 and NS 4051 also had a significantly higher grain yield than other hybrids, while the lowest yield, significantly lower than others, was recorded in the hybrid ZP 548. Observed by years (Graph 4), a different reaction of hybrids to water supply is noticed (hybrid/year interaction). In 2017, the highest grain yields were recorded in hybrids NS 5051, followed by ZP 548 and NS 6102, while the lowest yield, significantly lower than others, was observed in hybrid NS 3022. The most noticeable reaction of hybrids to environmental factors was recorded in hybrid NS 548, which, as one of the hybrid with the highest yield in 2017, in 2018 had a yield significantly lower than other hybrids. In contrast, the hybrid NS 3022, with the lowest yield in 2017, in 2018 achieved grain yield at the same level, or even significantly higher than some hybrids belonging to later maturity groups (FAO 500 and 600).

Table 1. Grain yield (t ha^{-1}) of maize hybrids

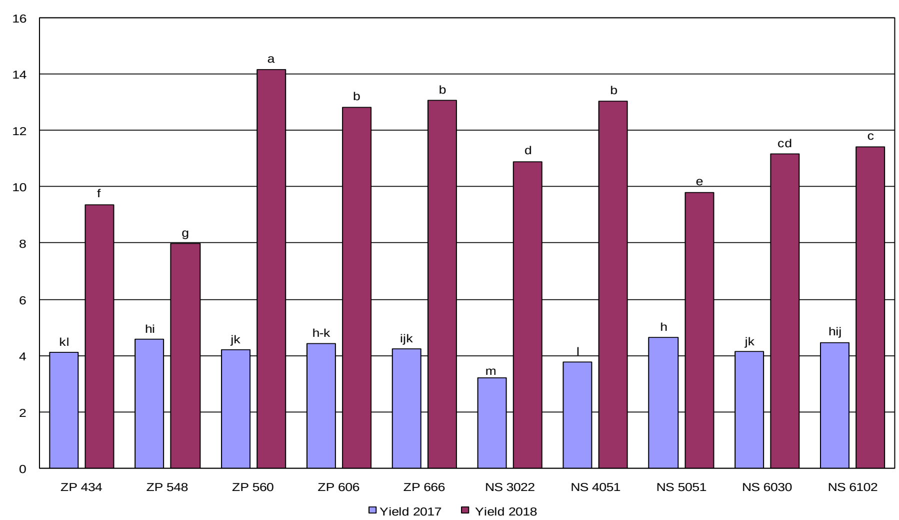
		Grain yield
Hybrid	ZP 434	6.73$^{f'''}$
(A)	ZP 548	6.27^{g}
	ZP 560	9.18^{a}
	ZP 606	8.63^{b}
	ZP 666	8.65^{b}
	NS 3022	7.08^{e}
	NS 4051	8.40^{b}
	NS 5051	7.22^{e}
	NS 6030	7.65^{d}

	NS 6102	7.95[c]
Years	2017	4.17[b]
(B)	2018	11.38[a]
ANOVA	Hybrid(A)	**
	Year (B)	**
	AxB	**

‴Means followed by different lowercase letters in columns for cultivars and years indicate significant differences according to LSD test ($P \leq 0.05$).

** F - test significant at the 99% level

Significantly higher grain yield compared to other hybrids in 2018, achieved the hybrid ZP 560, which in 2017 also belonged to the group of hybrids with the highest yield. In this regard, it should be also pointed out the hybrids ZP 606 and ZP 666, which in both years belonged to the group of hybrids with the highest grain yield. Weather conditions in the summer months (June, July and August) have a significant impact on maize grain yield (Boyer et al. 2013, Simić et al. 2018, Pejić et al. 2020). Filipović et al. (2015) state that the average annual yield reduction caused by drought, and related stress factors, in relation to the expected yields in the given conditions, ranges from 10% to 20%, even up to 50%. Drought periods in critical growth phases can negatively affect yield, even if soil moisture is not a limiting factor in other developmental stages (Roth et al., 2013)



Graph 4. Grain yield (t ha^{-1}) of maize hybrids in 2017 and 2018

Mean values marked with different lower case letters differ significantly at the level of $p = 0.05$ according to the LSD test.

Based on the results of macro-experiments with 15 maize hybrids at 30 different localities in the Republic of Serbia, Stojaković et al.(2010) state that hybrids from FAO 500 and 600 maturity groups had higher yield and lower grain moisture content than hybrids of FAO group 700, which suggests that, when selecting hybrids, the sum of temperatures is the determining factor. Growing drought-resistant maize hybrids and appropriate agronomic measures as components of improved technology could mitigate the effects of stress caused by adverse weather conditions (Kovačević et al. 2010, Simić et al. 2018).

Conclusion

Owing to a much larger amount and more favorable distribution of precipitation, especially in the critical developmental phases (tasseling - fertilization), the average grain yield of all maize hybrids in 2018 was almost three times higher than in 2017. The most noticeable reaction of hybrids to environmental factors was recorded in the hybrid NS 548, which, as one of the hybrids with the highest yield in 2017, in 2018 had a yield significantly lower than other hybrids. Hybrids ZP 560, ZP 606 and ZP 666 in both years belonged to the group of hybrids with the highest grain yield.

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References

Babić, V., Prodanović, S., Babić, M., Deletić, N., Anđelković ,V. (2013). The identification of bands related to yields and stability in maize hybrids and their parental components. Genetika, 45(2): 589-599.

Bekavac, G., Purar, B., Jocković, Đ., Stojaković, M., Ivanović, M., Malidža, G., Đalović, I. (2010). Maize production in terms of global climate changes. Field and Vegetable Crops Research, 47(2): 443-450.

Boyer, J. S., Byrne, P., Cassman, K. G., Cooper, M., Delmer, D., Greene, T., et al. (2013). The US drought of 2012 in perspective: A call to action. Glob. Food Sec. 2: 139–143.

Filipović, M., Jovanović, Ž., Tolimir, M. (2015). New ZP hybrid selection trends. XX Savetovanje o biotehnologiji sa međunarodnim učešćem (zbornik radova). Univerzitet u Kragujevcu, Agronomski fakultet Čačak, 13. -14. mart 2015. 7-15.

Gaffney, J., Schussler, J., Löffler, C., Cai, W., Paszkiewicz, S., Messina, C., et al. (2015). Industry scale evaluation of maize hybrids selected for increased yield in drought stress conditions of the U.S. Corn Belt. Crop Sci. 55: 1608–1618. doi: 10.2135/cropsci2014.09.0654

https://www.stat.gov.rs/sr-latn/oblasti/poljoprivreda-sumarstvo-i-ribarstvo/biljna-proizvodnja.

Kovačević, V., A. Paunović, D. Knežević, M. Biberdžić, M. Josipović (2010): Uticaj vremenskih prilika na prinose kukuruza u periodu 2000-2007. godine. XV Savetovanje o biotehnologiji, Agronomski fakultet, Čačak, 26-27. mart 2010.godine, Zbornik radova 15 (16): 13-19.

Markulj, A., Marijanović, M., Tkalec, M., Jozić, A., Kovačević, V. (2010). Effects of precipitation and temperature regimes on maize (*Zea mays* L.) yields in Northwestern Croatia. Acta agriculturae Serbica, 15(29): 39-45.

Paunović, A., Madić, M., Đurović, D., Živić, M., Bokan, N., Knežević, D., Jelić, M. (2013). Grain yield variability in maize hybrids of various FAO maturity groups. 48th Croatian &

8th International Symposium on Agriculture, Faculty of Agriculturae Osijek. 17th-22th February 2013., Dubrovnik, Croatia, 530-534.

Pavlov, J., Delić, N., Stevanović, M., Čamdžija, Z., Grčić, N., Crevar, M. (2011). Grain yield of ZP maize hybrids in the maize growing areas in Serbija. Proceedings 46th Croatian and 6th International Symposium on Agriculture, February 14 – 18, 2011. University of Zagreb, Faculty of Agriculture, Opatija, Croatia, 395-398.

Pejić, B., Mačkića, K., Simić, D., Bajić, I., Sikorac, V., Bekavac, G, Kresović, B. (2020). Effect of irrigation on yield, water productivity and leaf area index of maize. Annals of agronomy, 44(2): 187-193.

Republički Hidrometeorološki Zavod Srbije

Roth, J. A., Ciampitti, I. A., Vyn, T. J. (2013). Physiological Evaluation of recent drought-tolerant maize hybrids at varying stress levels. Agron. J. 105: 1129–1141. doi: 10.2134/agronj2013.0066

Shaw, R.W. (1988). Climatic requirement. In: Sprague G.F. (ed.) Corn and corn improvement, Agronomy Monograph No 18 ASA-CSSA-SSSA, -Madison, Wisconsin, USA, 609-638.

Simić, M., Kresović, B., Dragičević, V., Tolimir, M., Brankov, M. (2018). Improving cropping technology of maize to reduce the impact of climate changes. Proceedings of the IX International Scientific Agriculture Symposium "Agrosym 2018", Jahorina, October, 3-7, BiH: Faculty of Agriculture in East Sarajevo. 631-639.

Stojaković, M., Ivanović, M., Jocković, Đ., Bekavac, G., Purar, B., Nastasić, A., Stanisavljević, D., Mitrović, B., Treskić, S., Lajšić, R. (2010). NS maize hybrids in production regions of Serbia. Field and Vegetable Crops Research, 47 (1): 93-102.

Ummenhofer, C. C., Xu, H., Twine, T. E., Girvetz, E. H., McCarthy, H. R., Chhetri, N., Nicholas, K. A. (2015). How climate change affects extremes in maize and wheat yield in two cropping regions. J. of Climate, 28 (12): 4653-4687.

BIOFUNGICIDES IN THE PRODUCTION OF HEALTHY SEEDLINGS

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Abstract

In order to introduce proper use of new disease-fighting agents into a country, certain relevant principles, requirements and criteria prescribed by the Forest Stewardship Council (FSC) must be observed, primarily with respect to measures of assessment and mitigation of risks, the list of dangerous and highly dangerous pesticides with the possibility of alternative protection. One of the main goals of the research is adjustment of the protective measures to the FSC policy through selection of eco-toxicologically favourable fungicides. We study alternative protection on the occurrence of mass dieback in oak forests seedlings in Central Serbia (caused by *Microsphaera alphitoides* Griff. et Maubl.) with various dosages of AQ-10 biofungicide, which is a pelleted formulation of conidia of *Ampelomyces quisqualis* Ces. ex Schlechtend. Simultaneous testing has been conducted on the efficacy of a chemical sulphur-based preparation. The results of the research have demonstrated that AQ-10 biofungicide can be used as a part of integrated disease management programmes as an alternative. The best results in suppression of oak powdery mildew have been attained through use of sulphur SC in the concentration of 0.5%, while very satisfactory results have been obtained by use of AQ-10 biofungicide in the highest dosage of application (70 g/ha). The number of treatments has been proven to have no significant impact on increased efficacy of the bio-preparation, or in other words, it shows that besides the application dosage, the high efficacy of the bio-preparation depends primarily on proper timing of the application.

Keywords: *Alternative protection, Quercus robur, Central Serbia.*

Introduction

One of the most spread pathogens that endangers forest survival and process of sustainable forest management in Serbia is *Microsphaera alphitoides*, fungi that causes oak powdery mildew. Without appropriate protection measures taken, this disease can destroy all seedlings in nurseries, or can significantly reduce height increment due to the sprout death.

High numbers of pesticides being used on a regular basis seriously endanger human health and environment. The facts that 60000 chemicals are regularly used, and even 1000 new ones are produced every year, illustrate adequately the seriousness of the problem (Jankovic *et al*., 1981).

Development of modern, non-dangerous sustainable strategies such as usage of biopesticides, effective microorganisms or products of their metabolism (as the alternative to chemical, synthetic compounds), is caused by global demand for reduction of usage of pesticides that are considered harmful. Biofungicides have more specific influence on the target organism comparing to chemical compounds – they impact exclusively the target organism not causing toxic effects on environment and therefore increase biodiversity.

Materials and methods

For treatment we were using new biofungicide AQ-10 which contains spores of *Ampelomyces quisqualis* Ces. ex Schlechtend. – hyper-parasite that kills the fungi causing powdery mildew. This biofungicide has good efficacy, biological protection, it is not selective for specific breeds of *Microsphaera alphitoides*. Chemical preparation used during research was sulphur. Sulphur is the first fungicide that was successfully applied in powdery mildew control (in 1848) and after 150 years there is no sign of the pathogen's resistance. It acts both as prevention and as treatment and its efficacy is due to the external factors, temperature in the first place (it is inefficient on low temperatures and it is phytotoxic on high temperatures). Appearance and development of fungi are examined when first signs of disease are evident on control plots, that is, when the clear difference between control plots and plots treated with fungicide and biofungicide becomes evident. Experiments are designed according to the instructions of the Method PP 1/152 (2) (EPPO, 1997b), as a random block design with four replications, and are placed on two localities in Central Serbia during 2010. The size of a replication (plot) was 8 trees (1 x 3m), 25 m^2.

In the first experimental plot the investigations were carried out on the oak seedlings *Q. robur* L. of seed origin, aged 6 years. The seedlings were between 0.30 cm and 1.70 m in height (with the majority roughly 1.20 m tall). The seedlings were planted densely in rows approximately 60 m long (8 rows in total, an average of 6 seedlings per m^2), with about 40 cm space between the rows. On the second site, the experiments were conducted on oak seedlings *Q. robur* L. aged 6 years. The Pedunculate oak seedlings aged (2+0) were planted in 2006 on the total of 24 plots, in 4 blocks with 6 rows each.

<u>The assessment</u> of the secondary infection on leaves was performed on 100 leaves with four repetitions per each variant. The assessment scale of each leaf was as follows: 0 = no infection; 1 = very low infection; 2 = partial attack (scattered spots affected by powdery mildew); 3 = moderate to severe disease (up to half of the leaf surface is affected by powdery mildews); 4 = very severe disease (more than half of the leaf surface under the powdery mildew; edges of the leaves begin to crumple and dry up). Regarding the method of application and amount of water per unit surface, the fungicides were applied using the backstroke sprayer with the consumption of 1000 l/ha of water.

<u>Statistical analysis:</u>

The intensity of infection was performed according to methods EPPO, PP 1/69 (2): 100-102 and PP 1/152 (2): 37-51. Phytotoxicity was monitored according to method PP 1/135 (2): 31-36. The data were analyzed by statistical methods - the intensity of infection according to method Towsend and Heuberger (1943), efficacy according to Fleming and Retnakaran (1985), and method PP/181 (2): 52-58. The differences in the intensity of infection were determined through analysis of variance and LSD test.

Results and discussion

Results of testing the effects of the chemical and biological preparation on oak powdery mildew control, presented in tables 1 and 2, demonstrate that on both sites the chemical preparation had somewhat higher efficacy than the biopreparation. It is also apparent that the efficacy of the biopreparation on both sites increased with the increase of the active matter dosage.

Table 1**.** Efficacy of the preparation on control of causers of oak powdery mildew (site I)

Number of Variants	Fungicide	Concentration/Doses (%), g, l/ha	Infection (%)	**Efficacy** (%)	Standard (%)
1.	AQ-10	30 g	15.35 d	48.05	56.91
2.	AQ-10	50 g	7.15 bc	75.80	89.78
3.	AQ-10	70 g	6.15 abc	**79.19**	93.79
4.	Sulphur SC	0.5 %	4.60 a	**84.43**	100.00
5.	Untreated	-	19.90 b	0.00	0.00
lsd 005			5.36		
lsd 001			7.35		

Table 2**.** Efficacy of the preparation on control of causers of oak powdery mildew (site II)

Number of Variants	Fungicide	Concentration/Doses (%), g, l/ha	Infection (%)	**Efficacy** (%)	Standard (%)
1.	AQ-10	30 g	10.60 a	61.21	65.03
2.	AQ-10	50 g	12.42 a	54.53	57.93
3.	AQ-10	70 g	4.45 a	**83.71**	88.94
4.	Sulphur SC	0.5 %	1.61 a	**94.13**	100.00
5.	Untreated	-	27.33 b	0.00	0.00
lsd 005			10.34		
lsd 001			14.61		

Statistical processing of the obtained results has shown that the differences are significant in all tested variances as compared to the control variance. Based on the variance analysis of a random block system, the difference between mean repetitions was found to be statistically significant at the probability of 95%, since $F_0 > F_{0.05}$.

A statistically significant difference was also found between mean treatments at the probability of 99%, since $F_0 > F_{0.01}$. Between the mean values of the control and the variance AQ-10 (application dosage 50 g) and all other tested variances there is a statistically significant difference at the probability of 99%.

There is no statistically significant difference between the mean treatments of other variances and all other treatments, and the differences are incidental.

Application of comparative analysis Duncan test (Duncan, 1955) aimed at determination of significant differences resulted in identification of a homogenous group in which a statistically significant difference between the mean treatments was found at the probability of 99%, since $F_0 > F_{0.01}$.

No adverse effects of the biological and chemical preparations on the treated plants and other organisms were observed at the tested sites. Moreover, the chemical preparation had no adverse effects (phytotoxicity on plants), since the application was conducted according to the prescribed procedure and at the temperature below 28°C, which is the critical temperature in application of sulphur compounds.

In the reference literature, multiple authors have confirmed and proved in different climatic regions that the intensity of infection with powdery mildew on a variety of plant species is directly dependent on environmental conditions, primarily temperature and air humidity. The intensity of infection of Pedunculate oak seedlings with powdery mildew in the nursery was therefore examined in connection with temperature and air humidity, as shown in Table 4, for the period from 13 May through 24 June.

Table 3. Infection of Pedunculate oak seedlings with powdery mildew depending on outside temperature and air humidity

Date	Rh (%)	Temperature (^{0}C)	Infection (%)
13. May	100	21	51
19. May	97	11	10
25. May	90	23	33
03. June	95	17	63
10. June	91	28	41
16. June	94	30	32
24. June	85	20	51

For germination of spores, the relative air humidity need not be exceedingly high. The presence of the pathogen is thus distinct in dense plantings with poor air circulation and damp and dark places. The frequency of infection increases with the increase of relative humidity of up to 90%, but the infection does not occur when the leaf surface is wet, or when it rains. Rain showers wash off the leaf inoculum and reduce the intensity of infection since the pathogen is epiphytic, while prolonged and notable periods of high temperature favour the development of pathogens.

The presented Table 3 clearly shows that low and high air temperatures (11° and 30°C) had a direct impact on reduction of the infection, which in this period amounted to 10% and 32% out of the total number of tested seedlings. At a time when the air temperature was 11°C and the relative air humidity was extremely high at 97%, which does not favour the development of pathogens, hence this was the probable reason why the infection was three times lower than at the temperature of 30°C - at that time when air humidity was favourable for the pathogen and amounted to 94%.

Very high rate of infection on seedlings was observed at temperatures of 17° to 21°C at the relative air humidity from 85% to 100% (the infection in this period amounted to 51% to 63% of all the tested seedlings).

Table 3 clearly shows that the negative effect on the pathogen of one of these two factors (humidity and temperature) can be greatly mitigated by the positive influence of other factors. Thus, a very favourable temperature of 21°C for pathogen development directly reduced the negative effect of maximum air humidity of 100% and led to a high degree of infection of seedlings of 51%, which was also registered under very good conditions of 85% humidity and temperature of 21°C. The effect of slightly lower temperature than the optimal one for the development of pathogen (17°C) was hence probably mitigated by the corresponding relative humidity of 94%, and therefore the infection of seedlings in that period was maximal and reached 63%.

The comparative analysis of the effects of temperature and air humidity on the occurrence of secondary infections and spread of powdery mildew infection with the results obtained by other authors (Kothari and Verma, 1972; Whipps and Budge, 2000; Guzman-Plazola *et al.*, 2003) shows that high relative humidity reduces the intensity of infection, which can help in controlling this pathogen in the future.

Temperatures of 30°C and above are fatal for the development of pathogens. The growth of the fungus is thus significantly higher at 20°C than at 25°C. High levels of relative humidity (80-

90%) are favourable for pathogen development in the short term, but longer exposure to these conditions leads to a restricted infection. According to the research of Guzman-Plazola *et al.*, (2003), short daylight period (two or three exposures of at least 2 hours at high temperatures of 35°C) suppressed the development of the disease as much as 70-92%.
Results of testing the effects of the chemical preparation have shown the efficacy of 84.43% (site I) and 94.13% (site II). Compared to the chemical preparation, biopreparation AQ-10 has also demonstrated very high efficacy. Moreover, fewer treatments with the biopreparation (carried out in the nursery "Rogot", with the application of the highest dosage of the biopreparation – 70 g/ha) have shown similar efficacy as a larger number of treatments with the same dosage of the biopreparation (applied in 7-day intervals in the nursery "Barosevac", following numerous secondary infections), which amounted to 79.19 and 83.71% respectively.
In practice, this means that the number of treatments is not a deciding factor in achieving high efficacy of the biopreparation. If the treatment is carried out at the suitable time, fewer treatments will accomplish a very satisfactory level of efficacy. This is obviously quite significant from the economic point of view, and this experience may be applied in nurseries for control of powdery mildew through use of biopreparations.

Conclusion

The chemical preparation Sulphur SC is still efficient to a satisfactory degree, with the advantage that resistance of pathogen cannot occur during its application.
The bio-fungicide AQ-10 can be used as part of an integrated disease management program, as an alternative to reduce the use of standard fungicides for control of powdery mildew in oak nurseries. The best results in suppressing the oak powdery mildew were achieved in variances in which the biofungicide AQ-10 was applied in higher dosages (50 and 70 g/ha).
When AQ-10 is applied prior to disease infection, it may significantly reduce powdery mildew compared to the untreated variant. In view of the fact that biopreparation AQ-10 is non-toxic to humans and warm-blooded animals as opposed to the chemical preparation, has no negative effects on the environment, ensures long-term control and is favourably priced, it can be concluded that it should be introduced in control of oak powdery mildew in nurseries. Future scientific research should certainly focus on testing the effect of AQ-10 on powdery mildews of other major forest species.
Within the scope of repressive measures, development and introduction of alternative methods of forest protection against harmful organisms is carried out with the purpose to generate suitable alternative preparations and protection methods, so as to overcome the problem of exclusion of unwanted pesticides. It is therefore necessary to support the scientific institutions in conducting the research aimed at producing alternative methods and pesticides with less adverse effects on the environment and biodiversity in forest ecosystems.

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References

Duncan, D.B. (1955). Multiple-range and multiple F test. Biometrics, (11): 1-42

EPPO (1997a). Guideline for the efficacy evaluation of fungicides – Podosphaera leucotricha, No. PP 1/69 (2) in Guideline for the efficacy evaluation of Plant Protection Products, pp. 100-102.

EPPO Guidelines for the efficacy evaluation of plant protection products: Design and analysis of efficacy evaluation trials – PP 1/152(2), in EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 1, EPPO, 1997b; Paris, 37-51.

EPPO Guidelines for the efficacy evaluation of plant protection products: Phytotoxicity assessment – PP 1/135(2), in EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 1, EPPO, 1997c; Paris, 31-36.

EPPO Guidelines for the efficacy evaluation of plant protection products: Conduct and reporting of efficacy evaluation trials – PP 1/181(2), in EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 1, EPPO, 1997d; Paris, 52-58.

Fleming, R., Retnakaran, A. 1985. Evaluating Single Treatment Data Using Abbott's Formula With Reference to Insecticides, Journal of Economic Entomology, 78(6):1179-1181.

Guzman-Plazola, R., A., Davis, R.M., and Maroisc, J.J. 2003. Effects of relative humidity and high temperature on spore germination and development of tomato powdery mildew (*Leveillula taurica*), Crop Protection, 22 (10):1157-1168.

Jankovic, M., Djordjevic, V. 1981. Applied Ecology, Scientific Book, Belgrade

Kothari, K.L.,Verma, A. C. 1972. Germination of conidia of poppy powdery mildew (*Erysiphe polygoni* DC) Journal Mycopathologia, Springer Netherlands, 47 (3): 253-260.

Towsend, G.R. and Heuberger, J.W. 1943. Methods for estimating losses by diseases in fungicide experiments. Plant Dis. Rep.; 24, 340-343.

Whipps, J.M. and Budge, S.P. 2000. Effect of Humidity on Development of Tomato Powdery Mildew (*Oidium lycopersici*) in the Glasshouse. European Journal of Plant Pathology, 395-397.

HYDROXYCINNAMICACID DERIVATIVES: POTENTIAL ANTIOXIDANTS IN RARE GROWN *ALLIUM* SPECIES FROM SERBIA

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Abstract

The modern lifestyle imposes the need to discover and introduce foodstuffs that have nutritional and medicinal value into the food chain. The genus *Allium* includes a great number of species. The most studied species are onion, garlic and leek, for which there is evidence of beneficial effects on human health. Investigation of other, rare grown species of this genus, offers the possibility of expanding the range of food to vegetable species with potentially enriched chemical composition. Bearing in mind that the antioxidant properties of food are an important parameterofits quality, this paper aimed to examine the content of hydroxycinnamic derivatives (HCAs), as natural antioxidants, in two *Allium* species - *A. schoenoprasum* (chives) and *A. nutans* (blue chives), grown under different foliar application of selenium - Se (0, 10, 20, 30 g per ha). The total HCAs content was determined by spectrophotometric method, measured the absorbance at 525 nm. The obtained results are expressed as mg equivalent of chlorogenic acid (CGAE) per g of fresh weight. HCAs was in range 0.18 to 0.39 mg/g CGAE for *A. schoenoprasum*, and from 0.18 to 0.94 mg/g CGAE for *A. nutans*. The presence of HCAs indicates the potential antioxidant activity of the investigated species and justifies further detailed research with the aim of identifying other bioactive components that manifest beneficial effects on human health.

Key words: *Allium nutans, Allium schoenoprasum, antioxidant properties, HCAs.*

Introduction

The genus *Allium* includes a great number of species, which are spread throughout the Northern hemisphere (*Khassanov*, 2018). The best examined species of this genus which also have the greatest economic and nutritional significance are onion (*Allium cepa*), garlic (*A. sativum*) and leek (*A. ampeloprasum*). Although interest of researchers in studying other *Allium* species is constantly increased, data on their morphological characteristics, cultivation technologies as well as more detailed phytochemical analyzes are still modest, especially in the domestic literature.
Researches have shown that alliums have a number of beneficial effects on the human health, which are associated with a high content of various bioactive compounds. *Zeng et al.* (2017) indicated that introducing alliums into the diet can reduce the risk for various cancers. The same authors suggested that alliums extracts and oils have cardioprotective, antibacterial, anti-inflammatory and antioxidant effects. *Wan et al.* (2019) also indicated that consumption of

alliums had a preventive effect on the occurrence of cancer of the organs of the digestive tract. There is also evidence that garlic show effect in reducing blood pressure, regulating lipid and glucose status, etc. Given that these pharmacological effects are generally associated with the use of garlic or onion, there is a need to test other, rare grown species belonging to this genus. In this way, it is possible to expanding the range of food onto vegetable species with a potentially enriched chemical composition. For this purposes, current research was conducted on the species *A. schoenoprasum* L. (chives) and *A. nutans* L. (blue chives), which are rarely grown in Serbia, mainly at backyards or hobby gardens.

A. schoenoprasum L. and *A. nutans* L. are perennial species whose leaves can be used either fresh or dried. They are differed in terms of morphological properties, while had taste similar to onions. The natural habitats of *A. schoenoprasum* are cold regions of Europe and Asia. *A. schoenoprasum* has small, thin, dark green, tubular leaves, 0.3 to 0.5 cm in diameter (Figure 1). *A. nutans* L. also originates from colder regions, and is a widespread species in Siberia and Tibet. Leaves of *A. nutans* are dark green, lanceolate shape, flattened, 0.5 to 2.5 cm in diameter (*Lazić et al.*, 1997) (Figure 2).

Figure 1. Appearance of *A. schoenoprasum* L.

Figure 2. Appearance of *A. nutans* L.

Organosulfur compounds and polyphenols are the main bioactive components in the *Allium* species which have been studied so far (*Putnik et al.*, 2019). Organosulfur compounds, in addition to giving a characteristic smell and taste to alliums, are also responsible for their biological activities. However, the highly-volatile and thermally-unstable nature of these compounds, according to *Kathori et al.* (2020) reduce their effects on human health. In more recent studies, the pharmacological effects of alliums has been attributed to polyphenols that are more stable than organosulfur compounds, as well as due to its proven antioxidant activity (*Tanase et al.,* 2019; *Lorenzo et al.,* 2021). Polyphenols represent a large class of secondary metabolites in plants and more than 8000 have been identified so far (*Pandey and Rizivi*, 2009; *Neveu et al.,* 2010). Their presence in food improves its quality, nutritional and healing properties (*Vlase et al.,* 2013). In addition to exhibited pharmacological effects, polyphenols are also recognized as biomolecules that play crucial role for many physiological processes in plants, such as growth, development, reproduction, response to stress conditions (*Tanase et al.,* 2019; *Šamec et al.,* 2021).

The main polyphenolic compounds in the genus *Allium* are: phenolic acids, flavonoids and lignans (Figure 3) (*Kathori et al.,* 2020).

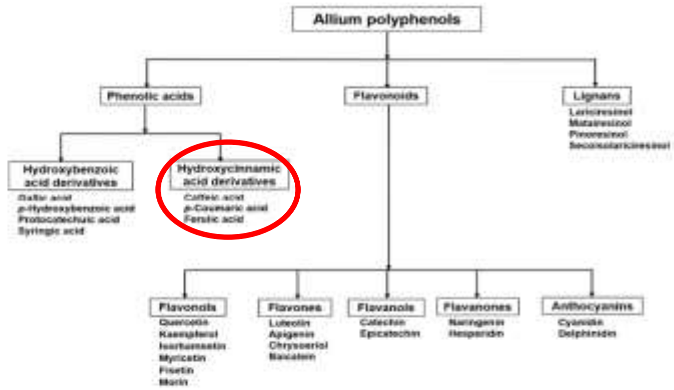

Figure 3. Polyphenolic compounds of the genus *Allium* (Source: *Kathori et al.*, 2020)

Hydroxycinnamic acid derivatives (HCAs) - caffeic, *p*-coumaric, ferulinic and sinapic acids are plant polyphenols from the group of phenolic acids and are defined as hydroxyl derivatives of cinnamic acid (*McCarthy et al.*, 2013). They are found in a large number of foods, including fruits, vegetables, cereals, spices and various beverages (tea, cocoa, wine, beer, coffee) (*El-Seddi et al.*, 2018; *Fási et al.,* 2019). It is assumed that of the total amount of polyphenolic compounds ingested with food, one third represents these acids (*Teixeira et al.*, 2013). The antioxidant properties of HCAs have been well described, especially in medical research. The role of HCAs in physiological processes in the human body is analogous to the role of polyphenols, so the literature emphasizes their potential ability to protect against cardiovascular disease, cancer, infectious diseases, as well as mitigate the adverse effects of chemotherapy (*El-Seddi et al.*, 2018). Considering the overall importance of polyphenols, but also HCAs as subgroups of polyphenolic compounds, various methods for determining their content have been developed. By using the standard Folin–Ciocalteu spectrophotometric method the total content of polyphenolic compounds can be obtained, which also including content of HCAs. Therefore, methods based on the specific quantification of HCA provide more reliable data on the presence of these derivatives, indicating the potential antioxidant effect of the tested samples. Literature data indicate that, although these acids were the subject of study back to the 1970s, during the recent years they are starting to gain an importance again and finding their place in scientific research. Given that the antioxidant properties of food are a valuable parameter of quality, and that mineral nutrition can affect the content of secondary metabolites in plants, the aim of this study was to determine the content of HCAs, as natural antioxidants, in the above mentioned rare *Allium* species from Serbia, grown under different foliar application of Se.

Material and methods

Production of *A. schoenoprasum* L. and *A. nutans* L. was carried out during two vegetation seasons, from October 2019 to September 2020, in open field conditions, at the location of Kovilovo, Palilula Municipality, Belgrade (Serbia). The experiment was conducted in three replicates. Selected species were grown under different foliar application of selenium fertilizer - sodium selenate (Na_2SeO_4), which was applied as a aqueous solution, in doses of 0 g, 10 g, 20 g and 30 g per ha, in the phase of intensive growth and development of plants (end of April).

In June, several samples (leaves) of fresh plant material were taken from plants grown under each treatment and each replicate, and were used to prepare a representative sample. The plant material was then cleaned from the ground and washed, chopped in an electric mill and used to

prepare extracts. Plant extracts were prepared by pouring 1 g of chopped samples with 20 ml of 80% methanol (MeOH). After that samples were intensively shacked for 2x90 min in plastic cuvettes protected from the light. Upon completion of the extraction, the precipitate was separated from the supernatant by centrifugation (4000 *g*). The supernatants were stored in a cool and dark place until they were analyzed. The determination of the total hydroxycinnamic acid derivatives (HCAs) content was evaluated using the spectrophotometric method as described by *Fraisse et al.* (2011), with some modification. The reacting mixture (RM) contained 0.2 ml of the plant extract, 0.4 ml of 0.4 M HCl, 0.4 ml of Arnow's reagent, 0.4 ml of 2.125 M NaOH solution and 0.6 ml of distilled water. The Arnow's reagent was prepared by dissolving 10 g of $NaNO_2$ and 10 g of Na_2MoO_4 in 100 ml of distilled water. The total volume of the RM was 2 ml. After preparation, the samples were left in the dark for 20 minutes, and then the absorbance of the obtained colored solutions was measured at 525 nm with a blank containing 0.2 ml of distilled water instead of plant extract. The content of HCAs derivatives was determined using a calibration curve with chlorogenic acid (CGA) as a standard. The obtained results are expressed as mg of CGA equivalents (CGAE) per kg of fresh weight.
For each sample, measurements were performed in three replicates, and the results were presented as the mean of three replicates ± standard deviation (SD).

Results and discussion

The obtained results showed that the selected species differ in terms of the content of HCAs. Also, within both species, there was a difference in the content of HCAs according to the examined Se treatments (Table 1). In *A. schoenoprasum*, the highest content of HDAs was found in plants treated with 10 g Se per ha, while the plants treated with the maximum concentration of Se (30 g per ha) had the lowest content. The lowest content of HDAs, in *A. nutans*, was observed in plants with application of Se at a concentration of 20 g per ha, while the highest content was recorded in untreated plants (Table 1).

Table 1. Content of HCAs in *A. nutans* and *A. schoenoprasum* according to different concentrations of foliar applied Se.

Se - treatment	***Allium SCHOENOPRASUM***	***Allium NUTANS***
	$\bar{X}$ (mg/g CGAE) ± SD	**$\bar{X}$ (mg/g CGAE) ± SD**
Control	0.25 ± 0.005	**0.94 ± 0.01**
10 g per ha	**0.38 ± 0.005**	0.19 ± 0.004
20 g per ha	0.23 ± 0.01	**0.18 ± 0.01**
30 g per ha	**0.19 ± 0.002**	0.19 ± 0.02

In this study, it was found that foliar application of Se does not affect on the increase in the content of HDAs in a clear and obvious way, compared to untreated samples. On the other hand, the research of *Vuković et al.* (data are not presented) showed that in other alliums, which are rare grown in our country, the presence of HDAs was not detected in either untreated or Se-treated plants. In contrast to these results, research conducted on basil (*Ocimum basilicum* L.) by *Skrypnik et al.* (2019) suggested that the application of Se, either foliar or by its addition to the nutrient solution, significantly increases the content of total polyphenols and HDAs. The same authors pointed out that the content of total flavonoids - compounds from the group of polyphenols, did not significantly differ between untreated and treated basil plants with selenium.

The observed differences between plant species and treatments can be explained by the still poorly investigated and understood influence of Se on the synthesis of secondary metabolites.

In general, the presence of HDAs indicates potential antioxidant capacity of *A. schoenoprasum* and *A. nutans*, and justifies further detailed research on these species in order to identify other bioactive components that have beneficial effects on human health.

Conclusion

Antioxidant properties are an important indicator of food quality. Previous research indicates that species of the genus *Allium* represent a rich source of bioactive molecules with potential antioxidant activity. Considering that the genus *Allium* has been consisted of about 1000 species of which a few have been examined in more details, numerous possibilities are provided for conducting both agronomic and phytochemical research. By introducing new plant species into production, but also by expanding the production of rare grown species of this genus, it can contribute to expanding the food range for vegetable species that, in addition to being nutritious, also have medicinal properties. The nutritional and medicinal properties of these species, could be further improved by the planned enrichment of alliums with microelements that are essential for human health, by implementing the process of biofortification, which is an increasingly popular agrotehnical measure in the world. Therefore, this research can be useful as a basis for more detailed studies of the process of biofortification over vegetable plants that are characterized by a potential therapeutic effect.

References

Aseervatham, G. S. B., Sivasudha, T., Jeyadevi, R., Arul Ananth, D. (2013). Environmental factors and unhealthy lifestyle influence oxidative stress in humans—an overview. Environmental Science and Pollution Research, 20(7), 4356–4369. doi:10.1007/s11356-013-1748-0

El-Seedi, H. R., Taher, E. A., Sheikh, B. Y., Anjum, S., Saeed, A., AlAjmi, M. F., AlAjmi F.M. Moustafa S.M, Al-Mousawi, M. S., Farag, A. M., Hegazy, F.M.E., Khalifa, A.M.S., Goransson, U. (2018). Hydroxycinnamic Acids: Natural Sources, Biosynthesis, Possible Biological Activities, and Roles in Islamic Medicine. Studies in Natural Products Chemistry, 269–292. doi:10.1016/b978-0-444-64068-0.00008-5

Fási, L., Di Meo, F., Kuo, C.-Y., Stojkovic Buric, S., Martins, A., Kúsz, N., Béni, Z., Dékány, M., Tibor Balogh, G., Pešić., M., Wang H.-C., Trouillas, P., Hunyadi, A. (2019). Antioxidant-inspired drug discovery: antitumor metabolite is formed in situ from a hydroxycinnamic acid derivative upon free radical scavenging. Journal of Medicinal Chemistry. doi:10.1021/acs.jmedchem.8b01994

Fraisse, D., Felgines, C., Texier, O., & Lamaison, J.-L. (2011). Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the *Asteraceae* family. Food and Nutrition Sciences, 02(03), 181–192. doi:10.4236/fns.2011.230025

Khassanov, F.O. (2018). Taxonomical and ethnobotanical aspects of *Allium* species from Middle Asia with particular reference to subgenus *Allium*. In: Shigyo M, Khar A, Abdelrahman M, editors. The *Allium* genomes. Switzerland: Springer Nature, 11–21. doi:10.1007/978-3-319-95825-5

Kothari, D., Lee, W.-D., Kim, S.-K. (2020). *Allium* Flavonols: Health Benefits, Molecular Targets, and Bioavailability. Antioxidants, 9(9), 888. doi:10.3390/antiox9090888

Lazić, B., Cuprudija, N., Tomičić, M., Gvozdanović-Varga, J. (1997). Agrobiological characteristics of rare onion *A.fistulosum* L., *A. nutans* L. and *A. schoenoprasum* L. Acta Horticulturae, 462, 577-583.

Lorenzo, D.C, Colombo, F., Biella, S., Stockley, C., Restani P. (2021). Polyphenols and Human Health: The Role of Bioavailability. Nutrients, 13(1), 273. doi: 10.3390/nu13010273.

McCarthy, A. L., O'Callaghan, Y. C., Neugart, S., Piggott, C. O., Connolly, A., Jansen, M. A. K., Krumbein, A., Schreiner, M., FitzGerald, J. R., O'Brien, N. M. (2013). The hydroxycinnamic acid content of barley and brewers' spent grain (BSG) and the potential to incorporate phenolic extracts of BSG as antioxidants into fruit beverages. Food Chemistry, 141(3), 2567–2574. doi:10.1016/j.foodchem.2013.05.048

Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D. Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database, 1-9. doi:10.1093/database/bap024

Pandey, B.K. and Rizvi I.S. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2(5), 270-278.

Putnik, P., Gabrić, D., Roohinejad, S., Barba, F. J., Granato, D., Mallikarjunan, K., Lorenzo, J.M., Kovačević, D. B. (2019). An overview of organosulfur compounds from *Allium* spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chemistry, 276, 680–691. doi:10.1016/j.foodchem.2018.10.068

Šamec, D., Karalija, E., Šola, I., Vujčić, B. V., Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: the influence of molecular structure. Plants, 10, 118. https://doi.org/10.3390/ plants10010118

Skrypnik, L., Novikova, A., Tokupova, E. (2019). Improvement of phenolic compounds, essential oil content and antioxidant properties of sweet basil (*Ocimum basilicum* L.) depending on type and concentration of selenium application. Plants, 8(11), 458. doi:10.3390/plants8110458

Tanase, C., Bujor, O.-C., Popa, V. I. (2019). Phenolic Natural Compounds and Their Influence on Physiological Processes in Plants. Polyphenols in Plants, 45–58. doi:10.1016/b978-0-12-813768-0.00003-7

Teixeira, J., Gaspar, A., Garrido, E. M., Garrido, J., & Borges, F. (2013). Hydroxycinnamic Acid Antioxidants: An Electrochemical Overview. BioMed Research International, 1-11. doi:10.1155/2013/251754

Vlase, L., Parvu, M., Parvu, E. A., Toiu, A. (2013). Phytochemical analysis of *Allium fistulosum* L. and *A. ursinum* L. Digest Journal of Nanomaterials and Biostructures, 8(1), 457-467.

Wan, Q., Li, N., Du, L., Zhao, R., Yi, M., Xu, Q., Zhou, Y. (2019). *Allium* vegetable consumption and health: An umbrella review of meta-analyses of multiple health outcomes. Food Science & Nutrition, 1-20. doi:10.1002/fsn3.1117

Zeng, Y., Li, Y., Yang, J., Pu, X., Du, J., Yang, X., Yang, T., Yang, S. (2017). Therapeutic Role of Functional Components in Alliums for Preventive Chronic Disease in Human Being. Evidence-Based Complementary and Alternative Medicine, 1–13. doi:10.1155/2017/9402849

RESULTS OF TESTING OF SEEDERS WITH DIFFERENT SOWING MECHANISMS IN MAIZE SOWING

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Abstract

Sowing of maize is a very important agro-technical intervention, bearing in mind that the omissions during sowing cannot be corrected later by other agro-technical measures. The quality of sowing maize, in addition to the quality of agro-technical works before sowing, also depends on the quality of work of sowing aggregates. Sowing aggregates for sowing maize must ensure the correct horizontal and vertical distribution of seeds because only the correct arrangement provides the plants with optimal vegetation space and the possibility to achieve high yields. A successful sowing unit, in addition to satisfactory quality of work, should also have high work productivity, i.e. achieved effects, because optimization is successful only with operational tests. The paper presents the results of testing the quality of work and performance indicators of units for sowing maize with different sowing mechanisms. The tests were performed in the agroecological conditions of central Serbia - Kragujevac area and included the assessment of the distribution of corn seeds along the length as well as the achieved effects concerning the projected depending on the defined parameters for mechanical sowing unit mechanism, under-pressurized sowing unit mechanism, and sowing unit with an over-pressurized mechanism. The obtained results show that the best longitudinal seed distribution was achieved when sowing maize with sowing mechanism type because the percentage of distances that were > 0.5 <1.5 of the theoretical grain spacing within the row was over 80% and the worst with mechanical sowing mechanism type A (mechanical) when these values were the lowest. The achieved effects concerning the projected ones varied in the range from 73.52% of the sowing unit type B to 83.41% as measured with the sowing unit type C. The increase in the speed of movement during sowing significantly affected the quality of the longitudinal distribution of corn seeds.

Keywords: *Seed drill, Quality, Longitudinal seed distribution, Performance.*

Introduction

Sowing is one of the most important operations in the production of maize because the omissions during sowing cannot be corrected later by other agro-technical measures. Proper horizontal and vertical seed placement allows for better germination and sprouting of maize and increased yields (Karayel *et al.*, 2002; 2006; Parish *et al.*, 2003). The introduction of "precision agriculture" enables a much better distribution of seeds by area and depth (Auernhammer, 2004; Meši *et al.*, 2008). Numerous field and laboratory methods have been developed to test and evaluate seeder performance (Navid *et al.*, 2011). Seeders that are traditionally used for sowing maize have the function of copying the terrain, but the height of seed ejection is conditioned by the construction of the sowing mechanism and the investor himself (Tao *et al.*, 2012). Sowing

quality is measured and calculated after crop emergence, bearing in mind that the composition of plants is the main factor of successful and profitable production (Raheman *et al.*, 2003). The value of the time utilization coefficient decreases with increasing working speed, which affects the reduction of labor productivity (Vičacki *et al.*, 2013). The highest percentage of obtained effects to the projected ones was recorded in variant S2 and amounted to 89.39%, and the lowest in variant S3, namely 71.34% (Barać *et al.*, 2006). The development and improvement of sowing mechanisms are conditioned by the requirements for a wider range of continuous dosing, increasing the performance of seeders and the precision of seed distribution of different sizes (Findura *et al.*, 2012). The quality of maize sowing is the most important factor influencing high and quality yield and is expressed as a percentage of the obtained sowing intervals according to ISO 7256/1 and 7256/2 (Bozdogan *et al.*, 2006; 2008; Turan *et al.*, 2015). At a sowing speed of 3.6 km h^{-1} seeder with a mechanical apparatus 68.5% of seeds were in the group of 0.5-1.5 required distance, at a speed of 4.3 km h^{-1} 65.3% of seeds, and at a speed of 5.4 km h^{-1} 62.4%. (Ivančan *et al.*, 2007). The same authors state that when sowing with a pressurized seed drill at a sowing speed of 3.6 km h^{-1}, 65.4% of seeds were in the group 0.5-1.5, at a speed of 4.3 km h^{-1} 63.2 % of seeds, and a speed of 5.4 km h^{-1} 61.0% of seeds. The share of realized gaps from 0.5 to 1.5 in the pressurized seed drill is higher concerning the pressurized seeder 81.43 to 88.57 to 68.89 to 77.78% (Turan *et al.*, 2011). The pressurized seeder obtained a very good quality of maize sowing, bearing in mind that in the group of 0.5-1.5 required spacing, there were 94.34 - 98.79% of plants (Banaj *et al.*, 2018).
The research is aimed to determine the effects of seeders with different sowing mechanisms when sowing maize and point out the advantages and disadvantages of the applied system in terms of quality of longitudinal seed distribution under different operating modes, with emphasis on reliability and quality of sowing aggregates under tested conditions.

Material and Methods

In the production conditions of central Serbia – at the vicinity of Kragujevac in Serbia during 2018/19 (44°07'15.9"N 20°50'04.3"E) tests of seeders with different sowing mechanisms were performed. The distribution of maize seeds by length and the achieved effects in relation to the projected ones depending on the defined parameters in four-row seeders: with mechanical sowing mechanism - Type A, seeder with underpressure sowing mechanism with - Type B- and seeder with overpressure mechanism - Type C Seeds of ZP 600 maize hybrids with a germination rate of 96% were used for sowing and met the basic requirements of seeds in terms of germination and dimensions (Vitazek *et al.,* 2005). The theoretical sowing distance was set differently and was for seed drill Type A 250 mm, Type B 245 mm, and Type C 240 mm. Based on the known theoretical sowing intervals, empty and double places are defined, where the double place in the case of sowing at a distance is less than 0.5 of the theoretical distance and the empty place in the case of sowing at a distance is greater than 1.5 than the theoretical distance. In the Type A seed drill, a seed plate 11 was installed, in the Type B seed drill the seed plate had 22 holes and a diameter of 190 mm, and in the Type C seed drill a sowing disc with 24 holes with a diameter of 250 mm. The required power for towing the tested seeders is 40 kW. The preparation of the land was done in a timely manner and included autumn plowing at 25 cm, while the pre-sowing preparation was done in the spring with a seed drill at a depth of 15 cm, so a well-scattered and crushed sowing layer was created. All seeders operated in similar conditions that were satisfactory, sowing was done at the end of April, and adjustments were made depending on the characteristics of the seeds. Tests and evaluation of the quality of work of seeders with different sowing mechanisms in maize sowing was performed according to ISO standards 7256/1 and 7256/2. The achieved effect was determined chronometrically, and all values during the test were read in 5 replicates. The experiment was performed in a completely random plan variant. The obtained results were processed using Microsoft Office Excel 2007.

Results and Discussion

Figures 1-3 illustrate the results of testing the longitudinal distribution of maize by the tested variants. The obtained results show that with the change of the defined parameters, the quality of the longitudinal distribution of maize seeds in sowing significantly changes. The results in Graph 1 indicates that the share of realized sowing distances in the group <0.5 of the theoretical distance obtained when sowing with a Type A seeder was in the range of 5.43 - 20.25%, working speed of 9.43 and 4.21 km h^{-1}, which are the largest measured values for all tested variants. The share of realized distances from 0.5 to 1.5 from the theoretical sowing with this type of seeder varied in the range of 54.32–78.53% (the lowest measured values of realized distances in this group according to all examined variants). When it comes to the share of open distances > 1.5 from the theoretical when sowing maize with this type of seeder, it is noticed that it was in the range of 4.03 - 39.81%, working speed of 4.21 and 9.43 km h $^{-1}$ (Figure 1).

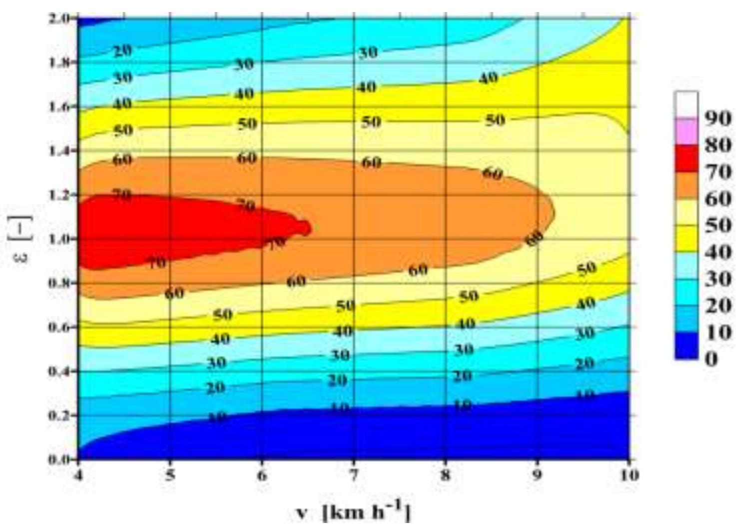

Figure 1. Obtained distance of maize along the length when sowing with seeders type A

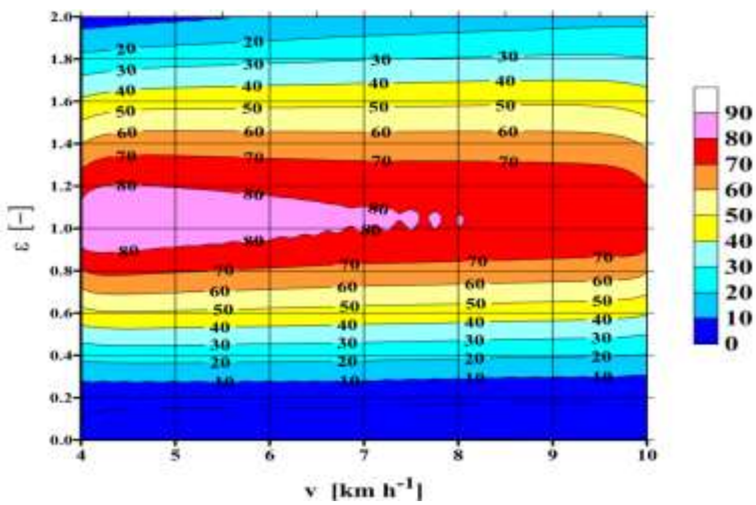

Figure 2. Obtained distance of maize along the length in sowing seeders Type B

The results in Graph 2 show that when sowing maize with a Type B seeder, the share of realized sowing intervals in the group <0.5 of the theoretical distance varied in the range of 3.99 - 8.72%, working speed of 9.83 and 4.47 km h^{-1}. The share of the obtained distances from 0.5 to 1.5 to the theoretically measured with the Type B seeder ranged from 74.35 to 88.67% (working speed from 9.83 or 4.47 km h^{-1}). The share in the group of realized distances >1.5 from the theoretical sowing with this seeder was in the range of 4.34 - 19.47%, the speed of movement was 4.47 or 9.83 km h^{-1} (Figure 2). Analyzing the results in Graph 3, it is noticed that when sowing with a Type C seeder, the smallest share of realized sowing intervals in the group <0.5 of the theoretical distance was measured and was in the range of 2.40 - 8.61% at an operating speed of 8.78 and 4.95 km h^{-1}. The share of realized intervals from 0.5 to 1.5 of the theoretical when sowing with this type of seeder varied in the range of 78.96 - 88.98% (the highest measured values of realized distances in this group according to all examined variants). The values of the share in the group of realized distances > 1.5 from the theoretical one were in the range of 3.24 - 16.63%, the speed of movement was 4.95 or 9.95 km h^{-1} (Figure 3).

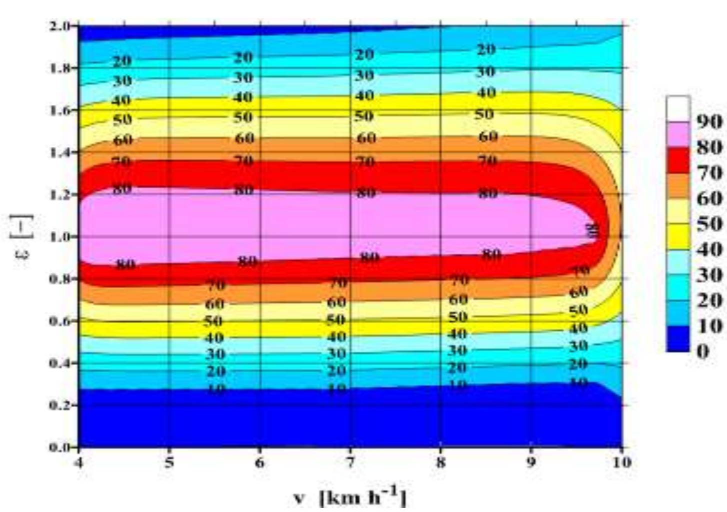

Figure 3. Obtained distance of maize along the length when sowing with seeders type C

The increase in working speed had a significant effect on the reduction of the quality of the longitudinal distribution of maize at sowing, bearing in mind that the share of realized distances from 0.5 to 1.5 concerning the theoretical distance decreased according to all examined variants. All three drills obtained a higher share of empty places concerning the double places, except in the case of the Type C seed drill, where the lowest values of 2.40% were measured .

The results we obtained in our research coincide with the statements of other authors (Auernhammer, 2004; Ivančan *et al.*, 2007; Bozdogan, 2008; Meši *et al.*, 2008; Findura *et al.*, 2012; Turan *et al.*, 2015; Banaj *et al.*, 2018).

The results in Graph 4 show that there is a significant impact of increasing the speed of movement on the values of the obtained effects because the values of the obtained effects increase with the increase of the speed of movement. According to all examined variants, smaller effects were obtained than projected. The lowest average values of the obtained effects concerning the projected ones were measured with the Type B seeder, namely 73.52% (0.77 ha h^{-1} concerning the projected ones 1.05 ha h^{-1}, working speed of 4.21 km h^{-1}), and the highest for the Type C seeder 83.41% (1.86 ha h^{-1} to the projected yield of 2.23 ha h^{-1}). With the Type A seeder, the obtained effects to the projected ones varied in the range from 74.45% (projected 0.94 and obtained 0.73 ha h^{-1}) to 82.65% (projected 2.11 obtained 1.74 ha h^{-1}) (Figure 4). Others came to similar results in their research (Barać *et al.*, 2006; Turan *et al.*, 2011; Findura *et al.*, 2012; Vičacki *et al.*, 2013).

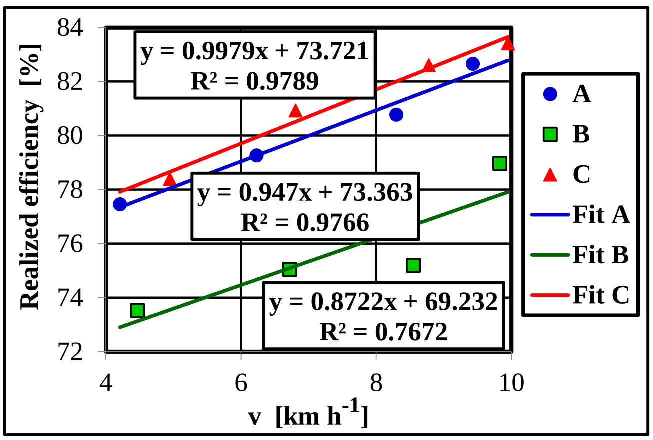

Figure 4. The obtained effects of tested seeders concerning the designed ones

Conclusions

Based on the obtained results, it can be concluded that the change of the defined parameters had a significant impact on the quality of the longitudinal distribution of maize seeds in sowing and the obtained effects concerning the projected ones. The smallest share of realized distances in the group <0.5 of the theoretical distance was measured when sowing corn with a Type C seeder and was 2.40%, and the largest with a Type A seeder - 20.25%. The highest values of the share of realized intervals from 0.5 to 1.5 from the theoretical one were measured when sowing corn with a Type C seeder, namely 88.98%, and the lowest when sowing corn with a Type A seeder, 54.32%. The lowest value of the share in the group of realized intervals >1.5 from the theoretical of 3.24% was measured when sowing with a Type C seeder, and the highest of 39.81%. in the case of a Type A seeder. With the increase of the working speed in all tested seeders, the quality of work deteriorated because the share of seed spacing in the longitudinal distribution decreased from 0.5 to 1.5 of the given spacing, and the share of corn blanks increased. All three tested seeders obtained a higher share of empty places to double places, except in the case of the Type C seeder, where the lowest values of 2.40% were measured. As the speed of movement increases, so do the values of the obtained effects in all variants. The lowest average values of the obtained effects to the projected ones were measured with the Type B seeder, namely 73.52%, and the highest with the Type C seeder, 83.41%. According to all examined variants, smaller effects were obtained than projected ones.

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References

Auernhammer H. (2004). Praziser Ackerbau-Precision Crop Farming. Jahrbuch. Agrartechnik-Yearbook Agricultural Engineering. Vol.16, pp. 31-38.

Barać S., Milenković B., Biberdžić M. (2006). The results of maize seeding machines exploitational trials in Rasin county. Agro – knowledge Journal, Vol. 7(1), pp. 35-42. (In Serbian)

Banaj A., Banaj Đ., Petrović D., Knežević D., Tadić V. (2018). Imact of seeding system on the maize grain yield. Agronomski Glasnik, Vol. 80(1), pp. 35-48. DOI:10.33128/ag.80.1.3

Bozdogan A. M. (2006). Uniformity of within-row distance in precision seeders: laboratory experiment. Journal of Applied Sciences, Vol. 6(10), pp. 2281-2286. DOI: 10.3923/jas.2006.2281.2286

Bozdogan A. M. (2008). Seeding uniformity for facum precision seeder. Scientia Agricola, Vol. 65(3), pp. 103-116. DOI:10.1590/S0103-90162008000300013

Findura P., Turan J., Jobbagy J., Angelovič M., Kuruc O. (2012). Influence of the physical attributes of seeds on the sowing quality within selected sowing mechanism. Contemporary Agricultural Engineering. Vol. 38(1), pp. 1-8.

Ivančan S., Sito S. (2007). Sowing sugar beet with mechanical and pneumatic drills. Proceedings the 42nd Croatian & 2nd International Symposium on Agriculture. Opatia, Croatia, pp 328 - 331

Karayel D., Ozmerzi A. (2002). Effect of tillage methods on sowing uniformity of maize. Can. Biosyst. Eng. Vol. 44, pp. 23–26.

Karayel D., Wiesehoff M., Ozmerzi A., Müller J. (2006). Laboratory measurement of seed drill seed spacing and velocity of fall of seeds using high-speed camera system. Computers and Electronics in Agriculture, Vol. 50(2), pp. 89-96.

https://doi.org/10.1016/j.compag.2005.05.005
Meši M., Malinović N., Kostić M. (2008). Quality parameters of seed maize planting. Tractors and power machines, Vol. 13(2), pp. 14-19. (In Serbian)
Navid H., Ebrahimian S., Gassemzadeh H.R., Mousavinia M.J. (2011). Laboratory evaluation of seed metering device using image processing method. Australian Journal of Agricultural Engineering, Vol. 2(1), pp. 1-4.
Parish R. L., Bracy R.P. (2003). An attempt to improve uniformity of a Gaspardo precision seeder. HortTechnology, 13(1), pp 100-103; DOI:10.21273/HORTTECH.13.1.0100
Raheman H., Singh U. (2003). A sensor for seed flow from seed metering mechanisms. Biosystems IE (I) Journal-AG, Vol. 84, pp. 6–8.
Tao C., Dongxing Z., Li Y., Nana G. (2012). Design and experiment of collocated-copying and semi-low-height planting-unit for corn precision seeder. Transactions of the Chinese Society of Agricultural Engineering, Vol. 28(2), pp. 18-23.
Turan J., Findura P., Zemanek P., Bugarin R., Sedlar A. (2011). Parameters of corn seeding with different seeding mechanisms. Contemporary Agricultural Engineering. Vol. 37(3), pp. 277-282. (In Serbian)
Turan J., Višacki V., Sedlar A., Pantelić S., Findura P., Machal P., Mareček J. (2015). Seeder with Different Seeding Apparatus in Maize Sowing. Acta Universitatis Agriculturae Silviculturae Mendelianae Brunensis. Vol. 63(1), pp. 137-141
DOI: 10.11118/actaun201563010137
Vitazek I., Havelka J., Piršel M. (2005). Aerodynamic Porosity Measurement. In: Agriculture, Vol.51(7), pp 368 – 371.
Višacki V., Turan J., Sedlar A., Zoranović M., Findura P. (2013). Comparative tests of pneumatic seed drills on sunflower sowing. Contemporary Agricultural Engineering. Vol. 39(3), pp. 159-166. (In Serbian)

'LEDA' A NEW SOUR CHERRY CULTIVAR

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Abstract

Sour cherry is one of the most economically important temperate fruit species worldwide, which is widely used both fresh and processed. Due to the consumer demand for antioxidant-rich products, the development of new tart cherry selections with desirable quality characteristics is critical for the sustainability of cherry producers and processors. 'Leda' is a new sour cherry cultivar released from the sour cherry breeding program at the Institute PKB Agroekonomik. It was selected in mixed sour cherry orchard, from spontaneus seedlings population, and was recognized as cultivar in 2018 by the Serbian Ministry of Agriculture, Forestry and Water Management. The evaluation of this cultivar was done in comparison with the control cultivar 'Oblačinska' during a two-year period (2015 and 2017). Averagely, it bloomed a day or two after the control cultivar, while the ripening time was a day earlier than 'Oblačinska'. The productivity is higher than the standard cultivar. The attractive bright red coloured fruits are larger (3.8 g), compared to 'Oblačinska' (3.3 g), with shorter fruit stalk length (2.9 and 3.4 cm, respectively). It has rich, mild flavour, sweet-acidic and harmonic taste, having slightly higher value of soluble solids/total acidity ratio (10.4) compared to the standard cultivar (9.5). 'Leda' is highly resistant to economically important diseases and pests (*Monilinia laxa*, *Monilinia fructigena*, *Blumeriella jaapii*, *Wilsonomyces carpophilus* and *Rhagolethis cerasi)*. This cultivar could be used both for processing and fresh consumption.

Keywords: *Prunus cerasus, new realised cultivar, light red colour.*

Introduction

Sour cherry (*Prunus cerasus* L.), originated from around the Caspian Sea, is one of the most economically important temperate fruit species worldwide. It is one of the most popular fruits, which is widely used both fresh and processed. Sour cherries are excellent functional foods due to their high antioxidant capacity (Ferretti et al., 2010). Health benefits of cherries and their polyphenols against human diseases such as heart disease, cancers, diabetes are reviewed by Farretti et al. (2010). Also, there has been a great interest in sour cherry by-products due to the increasing production rate of sour cherry worldwide and the increasing efforts on seeking bioactive compounds from natural sources as functional food (Yilmaz et al., 2019).

Molecular analysis such as isozyme analysis, genomic in situ hybridization and karyotyping strongly suggests that *P. cerasus* is segmental allotetraploid obtained from natural hybridization between ground cherry (*Prunus fruticosa* L.) and sweet cherry (*Prunus avium* L.). (Hancock and Iezzoni, 1987; Santi and Lemoine, 1990; Beaver and Iezzoni, 1993; Schuster and Schreiber, 2000). Furthermore, it has also been established that majority of the chloroplast genome of sour cherry is likely inherited maternally from ground cherry, thus validating an inter-specific origin for sour cherry (Brettin et al., 2000). It is a small tree, or more often a deciduous bush, which suckers profusely from the root (Dirlewanger et al., 2007). Sour cherry growing is limited to a very small number of cultivars, mostly regional. In Central Europe the main sour cherry cultivar is the 'Schattenmorelle', a self-compatible and highly productive cultivar with dark red fruits and juice. In North America, 'Montmorency' – a self-compatible variety with bright red fruits and clear juice is the cultivar of choice. Among the other notable varieties, 'Pandy' (and its derivatives) are popular in Hungary and Romania, although it is self-sterile. In the structure of fruit growing in Republic of Serbia, sour cherry has an important place with 19,114 ha and the production of 101,568 tonnes (average for the period 2010-2019; FAOSTAT database, 2019). Together with raspberry, sour cherry represents the country's most important exporting fruit (Radičević et al., 2016). In assortment structure, different clones of 'Oblačinska' are predominant, accounting for 85% of the total crop along with spontaneously spread 'Cigančica' (Cerović and Radičević, 2008), while the rest make large fruit sour cherries, i.e. 'Rexelle', 'Heimanns Konservenweichsel', 'Kelleriis 14' and 'Šumadinka' (Milatović et al., 2015).

Regular yields and superior fruit quality are the two main objectives in sour cherry breeding programs. Besides that, improvement priorities include suitability for mechanical harvesting and processing, late flowering to avoid spring frost damage, round and small pit, resistance to *Blumeriella jaapii* and *Monilinia laxa*, self-compatibility, and a wide range of ripening dates (Iezzoni, 2008). Since the variability in sour cherry germplasm presents a wealthy source of diversity for breeders (Radičević et al., 2012), the vast majority of sour cherry breeding programs are in Europe. According to some estimates, there is about 500 sour cherry cultivars in the world. Sour cherries assortment is less dynamic compared to most other fruit species. Also the number of new cultivars that are developed worldwide is relatively small. In the last 30 years more than 200 new sour cherry cultivars have been released. About half of those were bred in Russia, followed by Ukraine, Poland, Romania, Hungary and Germany (Milatović and Nikolić, 2011). Breeding programs in Serbia so far have released ten sour cherry cultivars. 'Čačanski Rubin' ('Shasse Morello' × 'Köröser Weichsel') and 'Šumadinka' ('Köröser Weichsel' × 'Heimanns Konservenweichsel'), both from the Fruit Research Institute, Čačak, which were recognized in 1973 and 1984, respectively; 'Lara' ('Kelleriis 14' × 'Rexelle'), from Institute PKB Agroekonomik, Belgrade, which was recognized in 1993. 'Prima', selection from domesticated regional landrace of sour cherry, locally called 'Feketićka' was released in 2013; 'Lenka' is the first sour cherry cultivar from the breeding program at the Faculty of Agriculture, released in 2014. Cultivars 'Nevena' ('Köröser Weichsel' × 'Heimanns Konservenweichsel'), 'Iskra' ('Köröser Weichsel' × 'Heimanns Rubin') and 'Sofija' ('Čačanski rubin' × 'Heimanns Konservenweichsel') were released in 2015 from the sour cherry breeding program in the Fruit Research Institute, Čačak. 'Ivo 45' (clone of 'Oblačinska') released by Superior doo, and 'Leda' (spontaneous seedling) developed in the Institute PKB Agroekonomik were released in 2018. The main objective of sour cherry breeding program was to obtain new genotypes which would be well adapted to agro-ecological conditions of the Republic of Serbia., and which would produce typical dessert fruit or fruit for use by the food processing and frozen-food industries.

The aim of this study was to provide most important information on the new sour cherry cultivar 'Leda' developed in the Institute PKB Agroekonomik and to compare it with the control cultivar

'Oblačinska', with respect to tree characteristics, productivity, fruit characteristics and quality attributes, so as disease tolerance.

Materials and methods

Study of a new sour cherry cultivar 'Leda' was done at the Ljubić facility of the Fruit Research Institute, Čačak (43°53'N, 20°20'E, 220 m a.s.l.) near Čačak city in Serbia. The control cultivar for comparison was 'Oblačinska'. The experimental plot was planted in 2012. The rootstock was *Prunus avium* L. seedling and the tree spacing was 5×3 m. The experimental design was the randomized block with five trees. Study was carried out over a period of two years (2015 and 2017). The evaluations presented in this paper were carried out according to UPOV (2006). The flowering time of each individual tree was recorded visually, considered as the day when approximately 10-20% of the flowers were open (beginning), and when more than 90% of petals were dropped (end). Ripening time was recorded from date when more than 20% of fruits were for fresh consumption. Yield was determined by weighting all the fruits in the tree and was expressed in kg per tree. Fruit characteristics were measured on fruits harvested in full maturity stage. Fruit length, width and thickness were measured by caliper, while fruit stalk length was measured by a ruler. Fruit and stone weight were measured by scale. Mesocarp ratio was determined as a ratio between flesh weight and fruit weight. Soluble solids content was analyzed by using a digital refractometer Pocket PAL-1 (Atago, Japan). Titratable acidity was measured by neutralization to pH 7.0 with 0.1 N NaOH and expressed as percent of malic acid equivalent. Total and invert sugars contents were determined according to Luff-Schoorl method (Egan et al., 1981). Resistance of several tree organs to low temperatures and to economically important diseases and pests, such as *Monilinia laxa*, *Monilinia fructigena*, *Blumeriella jaapii* and *Rhagoletis cerasi* was done through field observation. For all traits mentioned a nine-score scale (from 1, representing very resistant, up to 9, representing very susceptible) was used.

Results and discussion

Phenological characteristics

The flowering time of 'Leda' was two days after 'Oblačinska' and lasted one day longer. Fruits ripen in the first decade of June, one day before a control (Table 1).

Table 1. Comparisons of phonological and tree characteristics, as well as field resistance between 'Leda' and 'Oblačinska' (average 2015 and 2017).

	'Leda'	'Oblačinska'
Flowering (start)	Apr-10	Apr-08
Flowering (end)	Apr-18	Apr-17
Flowering (duration)	9	10
Ripening (start)	Jun-09	Jun-10
Ripening (end)	Jun-16	Jun-17
Ripening (duration)	8	8
Tree habit	Spreading	upright
Tree vigor	Low	Low
Disease and pest resistance		

Monilia laxa	Resistant	Resistant
Monilia fructigena	Tolerant	Tolerant
Blumeriella jaapii	Resistant	Resistant
Rhagolethis cerasi	Resistant	Resistant

Tree, leaf and fruit

'Leda' is characterized by spreading tree habit and weak vigor. One-year-old shoot length of internode is short. Leaf blade color on the upper side is light green. Orange yellow nectaries are present. All fruit dimensions, goes in the favor of 'Oblačinska' which showed higher values. Contrary, the average fruit weight of 'Leda' was 3.8 g and it was slightly higher than in the control cultivar (Table 2, Figure 1). The data concerning fruit weight is somewhat lower than those reported by Fotirić-Akšić et al. (2016) and Radičević et al. (2010, 2018) for newly sour cherry cultivars originated from Serbia. According to the UPOV descriptor for sour cherry (2006) 'Leda' has a circular fruit. Fruit stalk length differs between 'Leda' and 'Oblačinska'. 'Leda' had a shorter fruit stalk of 4.53 cm, while in the control cultivar it was 3.63 cm. Altogether they are in the range reported by Perez-Sanchez et al. (2008). The stone was medium sized, with a share of ~7% in the fruit weight. Yield of cultivar 'Leda' was a 600 g higher than for the 'Oblačinska.

Table 2. Comparisons of fruit characteristics and yield between 'Leda' and 'Oblačinska' (average 2015 and 2017).

	'Leda'	'Oblačinska'
Fruit weight (g)	3.8	3.3
Fruit length (mm)	14.4	14.9
Fruit width (mm)	15.4	16.6
Fruit thickness (mm)	13.8	14.9
Stone weight (g)	0.28	0.33
Mesocarp ratio (%)	92.9	89.7
Stalk length (cm)	2.9	3.4
Fruit cracking (%)	Resistant	Resistant
Tree habit	Spreading	Upright
Tree vigor	Low	Low
Skin colour	Light red	Dark red
Juice colour	Colorless	Dark red
Yield (kg/tree)	3.03	2.43

Figure 1. Fruits of 'Leda' (left) and 'Oblačinska' (right).

Disease and Pest Reaction

Field examination (Table 1) showed that both 'Leda' and control cultivar exhibited high resistance of economically important fungal diseases and pest (*Monilinia laxa, Monilinia fructigena*, *Blumeriella jaapii* and *Rhagolethis cerasi*).

Fruit quality

Fruits of 'Leda' are characterized with a relatively high content of soluble solids, 16.95% in average (Table 3), which was higher than in 'Oblačinska' (15.55%). Also, 'Leda' showed slightly higher acid content (1.71%) compared to the control. Differences were also determined between soluble solids/total acids ratio, where 'Leda' (10.40) showed higher value than the control cultivar (9.49). The data on the chemical composition of fruits goes in the favor of 'Leda'. These results are lower than those reported in the literature (Fotirić-Akšić et al. 2016; Radičević et al., 2018) probably due to environmental factors.

Table 3. Comparisons of fruit quality between 'Leda' and 'Oblačinska' (average 2015 and 2017).

	'Leda'	'Oblačinska'
Soluble solids	16.95	15.55
Total acids	1.71	1.69
Soluble solids/Total acids	10.40	9.49
Total sugars	10.45	9.92
Inverted sugars	9.80	9.00
Sucrose	0.62	0.87

Conclusions

New Serbian sour cherry cultivar 'Leda', developed at the Institute PKB Agroekonomik, has attractive bright red coloured fruits with rich, mild flavour, sweet-acidic and harmonic taste, high productivity and resistance to economically important fungal diseases and pests. It surpasses the 'Oblačinska' cultivar in most traits studied. This cultivar could be used both for fresh consumption and processing. Given the consumer demand for antioxidant-rich products, the development of new tart cherry selections with desirable quality characteristics is critical for the sustainability of cherry producers and processors.

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References

Beaver, J.A., Iezzoni, A.F. (1993). Allozyme inheritance in tetraploid sour cherry (*Prunus cerasus* L.). Journal of American Society for Horticultural Science, 118, 873–877.

Brettin, T.S., Karle, R., Crowe, E.L., Iezzoni, A.F. (2000). Chloroplast inheritance and DNA variation in sweet, sour, and ground cherry. Journal of Heredity, 91, 75–79.

Cerović, R., Radičević, S. (2008). Sour cherry research and production in Serbia and Montenegro. Acta Horticulturae, 795, 493–496.

Dirlewanger, E., Claverie, J., Wünsch, A., Iezzoni, A.F. (2007). Cherry. In Genome Mapping and Molecular Breeding in Plants, Vol. 4, Fruits and Nuts, C. Kole, ed. (Berlin, Heidelberg, Germany: Springer-Verlag), p.103–118.

Egan, H., Krik, R., Sawyer, R. (1981). The Luff Schoorl method. Sugars and preserves. In Pearson's Chemical Analysis of Foods, 8th edn (Harlow, UK: Longman Scientific and Technical), p.152–153.

Ferretti, G., Bacchetti, T., Belleggia, A., Neri, D. (2010). Cherry antioxidants: from farm to table. Molecules (Basel, Switzerland), 15(10), 6993–7005.

FAOSTAT Databases (2019): http://www.fao.org/faostat/en/#data/QC.

Fotirić-Akšić, M., Nikolić, T., Zec, G., Cerović, R., Nikolić, M., Milivojević, J. and Radivojević, D. (2016). 'Lenka', a new sour cherry cultivar from Serbia. Acta Horticulturae 1139, 95–100.

Hancock, A.M., Iezzoni, A.F. (1987). Malate dehydrogenase isozyme patterns in seven *Prunus* species. HortScience, 23, 381–383.

Iezzoni, A.F. (2008). Cherries. In Temperate Fruit Crop Breeding: Germplasm to Genomics, J.F. Hancock, ed. (Berlin, Heidelberg, Germany: Springer-Verlag), p.151–176.

Milatović, D., Nikolić, D. (2011). Cherry breeding in the world. proceedings of 3rd Conference "Innovations in Fruit Growing" (Belgrade, Serbia), p.21–47.

Milatović, D., Nikolić, M., Miletić, N. (2015): Sweet and sour cherry - second edition. Scientific Pomological Society of Serbia, Čačak, Republic of Serbia.

Perez-Sanchez, R., Gomez-Sanchez, M.A., Morales-Corts, R. (2008). Agromorphological characterization of traditional Spanish sweet cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.) and duke cherry (*Prunus gondouinii* Rehd.) cultivars. Spanish Journal of Agricultural Research, 6 (1), 42–55.

Radičević, S., Cerović, R., Glišić, I., Karaklajić-Stajić, Ž. (2010). Promising sour cherry hybrids (*Prunus cerasus* L.) developed at Fruit Research Institute Čačak. Genetika 42 (2), 299–306.

Radičević, S., Cerović, R., Lukić, M., Paunović, S.A., Jevremović, D., Milenković, S., and Mitrović, M. (2012). Selection of autochthonous sour cherry (*Prunus cerasus* L.) genotypes in Feketic region. Genetika, 44 (2): 285–297.

Radičević, S., Cerović, R., Marić, S., Đorđević, M. (2016). Cherry breeding work at Fruit Research Institute Čačak (Republic of Serbia). Book of Proceedings, VII International Scientific Agriculture Symposium 'Agrosym 2016', Jahorina (Bosnia and Herzegovina), pp. 472–478.

Radičević, S., Cerović, R., Marić, S., Milošević, N., Glišić, I., Mitrović, O., Korićanac, A. (2018). Biološke osobine novijih genotipova višnje (Prunus cerasus L.) stvorenih u Institutu za voćarstvo, Čačak. Voćarstvo, 52 (202), 59–66.

Santi, F., Lemoine, M. (1990). Genetic markers for *Prunus avium* L. 2. Clonal identification and discrimination from *P. cerasus* and *P. cerasus* × *P. avium*. Annales des Sciences Forestières, 47, 219–227.

Schuster, M., Schreiber, H. (2000). Genome investigation in sour cherry, *P. cerasus* L. Acta Horticulturae, 538, 375–379.

UPOV (2006). Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability. Sour cherry, Duke cherry (Geneva, Switzerland: International Union for the Protection of New Varieties of Plants), pp.1–35.

Yılmaz, F.M., Görgüç A., Karaaslan, M., Vardin, H., Ersus Bilek, S., Uygun, Ö., Bircan, C. (2019). Sour Cherry By-products: Compositions, Functional Properties and Recovery Potentials - A Review. Critical Reviews in Food Science and Nutrition, 59 (22), 3549–3563.

THE EFFECT OF ACTINOMYCETES APPLICATION ON GREEN MASS YIELD OF RED CLOVER

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Abstract

Red clover (*Trifolium pratense* L.) is one of the most important and widespread leguminous forage species in temperate agriculture, which is characterized by a high yield of quality biomass. Actinomycetes produce antibiotics and vitamins that act favourably on physiological processes in plants. These microorganisms are present in the soil, however the application of selected microorganisms for seed inoculation leads to better results in crop production. The experiment was carried out in vegetation pots in semi-controlled conditions. This research consisted of investigating the effect of actinomycetes (*Streptomyces* sp.) application on the green mass per plants of 12 cultivars of red clover of different geographical origin: K-27, K-32, K-38, K-39, Una, Kolubara, Viglana, Manuela, Wilo, Repio, Diana and Longevo. The effect of inoculation was determined out at the onset of flowering. The results obtained showed that the application of microorganisms could affect plant mass yield. The highest value for green mass per plant was achieved in cv. K-39 using inoculum that contained *Streptomyces* sp. The application of actinomycetes had a positive effect in the most cultivars of red clover and there was a statistically significant difference between inoculated treatments and control, indicating the justification of the use of these microorganisms in plant production.

Keywords: *Red clover, Cultivar, Actinomycetes. Green mass.*

Introduction

Red clover (*Trifolium pratense* L.) is grown in Serbia in monoculture on 120.000 ha and it can successfully substitute alfalfa on soils of increased acidity in lowland and hilly regions. It is a very important perennial forage legume that is grown in various forms in agroecological conditions from subtropical to subpolar regions (Lugić *et al*., 2006). Its economical significance is based on high potential for production of biomass with high content of crude protein in biomass (Tomić *et al*., 2007) and red clover has quality and nutritive hay (Acikgoz, 2001). The chemical composition and quality of red clover forage depends on the stage of development, i.e. age of plants (Marković *et al*., 2011). It tolerates lower pH, less fertility and poorer drainage than alfalfa (Vasiljević *et al.*, 2005) and domestic populations are characterized by good resistance to drought and cold (Lakić and Vojin, 2010). Thank to developed root and intensive biological nitrogen fixation and favourable effect on soil, it is very important in crop rotation (Lugić *et al*., 2010).

Great importance of red clover is in the fact that it lives in symbiosis with *Rhizobium leguminosarium* bv. *trifoli* and it is beneficial to the agro-ecosystem two-fold; first through nodule transfer, and second through biomass decomposition (Wyngaarden *et al*., 2015). Next to rhizobia in the rhizosphere of red clover live numerous microorganisms. such as actinomycetes

(Andjelković *et al.,* 2014). Approximately 60 % of biologically active compounds used in agriculture are derived from the genus *Streptomyces* (Ilic *et al*. 2007). These genera have shown over time great potential in improving the future of agriculture (Olanrewaju and Babalola, 2019). Actinomycetes produce different type of growth factors like auxin and gibberellin substances, antibiotics and vitamins, which act favorably on the physiological processes of plants (Berg *et a*l., 2001; Kumar *et al.* 2010). Also, actinomycetes are known for their production of wide variety of enzymes which are important in order to degrade the cellulose, pectin and chitin and other substances in order to maintain the structure of soil (Priyadharsini and Dhanasekaran, 2015). These microorganisms are present, in higher or lesser abundance in the soil, buit the application of selected microorganisms for seed inoculation leads to can aid in increasing the potential of growth promotion in sustainable agriculture (Mitra *et al.,* 2019).
The aim of this study was to examine the influence of actinomycetes (*Streptomyces* sp.) on the green mass per plants of different cultivars of red clover.

Materials and methods

The experiment was set up in the semi-controlled conditions, in a greenhouse. For the purposes of research twelve commercial red clover cultivars were used: K-27, K-32, K-38, K-39 (Institute for forage crops, Kruševac, Serbia), Una and Kolubara (Institute of Field and Vegetable Crops, Novi Sad, Serbia), Viglana, Manuela (Research Institute of Plant Production in Piestany, Slovakia,) Wilo, Repio and Diana (US Dairy Forage Research Dairy Farm, Wisconsin, USA) and Longevo (Istituto Sperimentale per le Colture Foraggere, Lodi, Italy).
The inoculation was done using *Streptomyces* sp. (actinomycetes) concentration $7.8x10^{10}$ cells per ml were grown on the substrate by Krasiljnikov (Jarak and Djurić, 2006).
Red clover seed was sterilized with 0.2% solution of $HgCl_2$ and 70% ethanol, rinsed several times with sterile tap water and then immersed in the inoculum. After that, ten seeds of each cultivar from inoculum were planted in the 8 dm^3 volume vegetation pots filled with about 10 kg soil (five seeds per pot). Five milliliter of the inoculum was added to each pot. The experiment was conducted in ten repetitions, 10 plants per cultivar with inoculum. Also, ten plants per cultivar were without inoculation – control. Green mass per plant (g) was measured at the beginning of flowering.
The results were processed using the statistical package STATISTICA 8.0. The significance of the difference between the investigated treatments was determined by analysis of variance and LSD tests.

Results and Discussion

The results obtained showed that the application of actinomycetes thereof can affect on green mass per plant red clover. Thanks to use of inoculation with effective strains of microorganisms can intensify certain microbiological processes which can increase available nutrients for plants. The highest value for green mass per plant – 19.80 g was achieved in cv. K-39 using inoculum that contained *Streptomyces* sp, while the lowest value is recorded in cv. Longevo – 12.20 g (Tab.1).
The application of actinomycetes had a positive effect in the most cultivars of red clover and there was a statistically significant difference between inoculated treatments and control. Actinomycetes help the plant in the utilization of nutrient available in the soil for their growth, differentiation and used as a biocontrol agent against different pathogen (Persello-Cartieaux *et*

al., 2003). By usinginoculum of these microorganisms, Jarak *et al*. (2007) reported better germination and height and weight of red clover than recorded on the control variant. Application of the inoculation also gave positive effects on the length of red clover plants (Miličić and Jarak, 2008).
Horewer the response of plants three cultivars (K-32, Repo and Longevo) to inoculation was negative (Tab.1). Similar results were obtained by Andjelković *et al*. (2014). The authors reported that the application of individual cultures of *Streptomyces* sp. on alfalfa cv. Syntéza 1 has had a positive impact, but to cv. K-28 did not result in a statistically significant increase in green mass per plant compared to the control .The effect of inoculation depends on many factors: host plant activity, species, cultivar, strain and cell concentration of microorganisms in the inoculum (Walker *et al*. 2003; Bizos *et al.*, 2020). Each cultivar had a specific composition of root secretions, different conditions in the rhizosphere and specific interaction between microorganisms and the plant is formed (Andjelković *et al.*, 2020).

Table 1. The effect of inoculation by *Streptomyces* sp. on the green mass different cultivar red clover

Cultivar	Variant	Green mass per plant (g)
K-27	Control	13.10 ^{n}
	With inoculum	13.90 ^{l}
K-32	Control	16.70 ^{f}
	With inoculum	15.90 ^{h}
K-38	Control	17.37 ^{d}
	With inoculum	18.20 ^{b}
K-39	Control	18.30 ^{b}
	With inoculum	19.80 ^{a}
Una	Control	13.50 ^{m}
	With inoculum	13.93 ^{l}
Kolubara	Control	13.60 ^{m}
	With inoculum	14.30 ^{k}
Manuela	Control	16.30 ^{g}
	With inoculum	17.00 ^{e}
Viglana	Control	14.30 ^{k}
	With inoculum	14.80 ^{j}
Repio	Control	17.10 ^{d,e}
	With inoculum	16.30 ^{g}
Wilo	Control	17.30 ^{d}
	With inoculum	17.70 ^{c}
Diana	Control	14.30 ^{k}
	With inoculum	15.30 ^{i}
Longevo	Control	12.50 ^{o}
	With inoculum	12.20 ^{p}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p<0.05$)

Conclusion

This study showed that the response of plants in the most cultivars of red clover to application of actinomycetes was positive. Only in three cultivars (K-32, Repo and Longevo) inoculation was negative effect on green mass per plant. The results suggest use of actinomycetes as effective biocontrol agent and should be considered a good way in the search for viable plant growth promoters for sustainable agriculture.

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References

Acikgoz, E. (2001) Forage crops. University of Uludag, Bursa, Turkey, University of Uludag Press, 584.

Andjelković S., Radović J.. Babić S., Vasić T., Djurić S., Stamenov D., Hajnal-Jafari T.(2020). Response of microorganisms in alfalfa rhizosphere to microbial inoculation. Legume research- An International Journal, 43,706-710.

Andjelković S., Vasić T., Lugić Z., Babić S., Milenković J., Jevtić G., Živković S. (2014). The Influence of individual and combined inoculants on development of alfalfa on acidic soil. In: Soković D., Huyghe C., Radović J. (eds) Quantitative Traits Breeding for Multifunctional Grasslands and Turf. „30th Meeting of the Eucarpia Fodder Crops and Amenity Grasses Section", Springer,12-16 May, Vrnjačka Banja, Serbia, 353-357.

Berg G., Marten P., Minkewity A., Brukner S. (2001). Efficient biological control of fungi plant diseases by *Streptomyces* sp. Zeitschrift fur Pflanyenkrankheiten und Pflanzenschuty, 108, 1-10.

Bizos G., Papatheodorou E.M., Chatzistathis T., Ntalli N., Aschonitis V.G., Monokrousos N. (2020).The role of microbial inoculants on plant protection, growth stimulation, and crop productivity of the olive tree (*Olea europea* L.). Plants, 9, 743. doi.org/10.3390/plants9060743

Ilic S.B., Konstantinovic S.S., Todorovic Z.B., Lazic M.L., Veljkovic V.B., Jokovic N., Radovanovic B.C. (2007). Characterization and antimicrobial activity of the bioactive metabolites in *Streptomycete* isolates.Microbiol, 76, 421–428.

Jarak M., Djurić S., Djukić D. (2007). The effect of inoculation on the emergence and early growth of alfalfa and red clover. Field and Vegetable Crops Research, 42(1), 415-421.

Jarak M., Đurić S. (2006). Practicum of Microbiology. Faculty of Agriculture, Novi Sad. (Sr)

Kumar N., Singh R.K., Mishra S.K., Singh A.K., Pachouri U.C. (2010). Isolation and screening of soil actinomycetes as source of antibiotics active against bacteria. Int J Microbiol Res 2(2), 12–16.

Lakić Ž., Vojin S. (2010). Variability of agronomic traits of red clover genotypes (*Trifolium pratense* L.). Biotechnology in Animal Husbandry, 26, 35-40,

Lugić Z., Lazarević D., Erić P., Mihajlović V., Vučković S. (2010). The state of forage crops production in Serbia. Biotechnology in Animal Husbandry, 26, 29-47.

Lugić Z., Radović J., Sokolović D., Jevtić G. (2006). Forage yield and quality of some new cultivar of red clover (*Trifolium pratense* L.) in Serbia. Proceedings of 26th Eucarpia Fodder Crops and Amenity Grasses Section, 3–7 Sept, Perugia, Italy, 179-181.

Marković J., Štrbanović R., Terzić D., Stanisavljević R. Đokić D., Vasić T., Anđelković B. (2011). Estimation of red clover (Trifolium pratense l.) forage quality parameters depending on the stage of growth. Biotechnology in Animal Husbandry, 27 (4), 1563-1569.

Miličić D., Jarak M. (2008). The improvement of acidic soils by means of microbe inocula application and liming. Annals of agronomy, 32 (1), 51-56.

Mitra D., Anđelković S., Panneerselvam P., Senapati A., Vasić T., Ganeshamurthy A.N., Verma D.P., Radha T.K., Jain D. (2019). Plant growth promoting microorganisms (PGPMs) helping in sustainable agriculture: current perspective. International Journal of Agricultural Sciences and Veterinary Medicine, 7 (2), 50-74.

Olanrewaju O.S., Babalola O.O. (2019). *Streptomyces:* implications and interactions in plant growth promotion. Appl Microbiol Biotechnol, 103, 1179–1188.

Persello-Cartieaux F., Nussaume L., Robaglia C. (2003). Tales from the underground: molecularplantrhizobacteria interactions. Plant Cell and Environment, 26, 189-199.

Priyadharsini P, Dhanasekaran D (2015). Diversity of soil allelopathic Actinobacteria in Tiruchirappalli district, Tamilnadu, India. J Saud Soci Agri Sci. 14(1), 54-60.

Tomić Z., Lugić Z., Radović J. , Sokolović D., Nešić Z., Krnjaja V. (2007). Perennial legumes and grasses stable source of quality livestock fodder feed. Biotechnology in Animal Husbandry, 23 (5-6), 559–572.

Vasiljević S. Lazarević B., Mihailović V., Mikić A., Katić S., Milić D. (2005). Possibility of growing red clover (*Trifolium pratense* L.) on acid soil. Proceedings of the 2nd CyST 852 Workshop Sward Dynamics, N-flows and Forage Utilisation in Legume-Based Systems, 10-12 November 2005, Grado, Italy,181–184.

Walker H., Grotewold E., Vivanco J.M. (2003). Root exudation and rhizosphere biology. Plant Physiology 132, 44-51.

Wyngaarden L.S., Gaudin A.C.M., Deen W., Martin C.R. (2015). Concept paper expanding red clover (Trifolium pratense) usage in the corn–soy–wheat rotation Sustainability, 7, 15487-15509.

ANALYSIS OF THE RELATIONSHIP OF THE MOST IMPORTANT TRAITS IN MEADOW FESCUE

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Abstract

Meadow fescue (*Festuca pratensis* Huds.) is one of the most important perennial grasses for animal feed production on grasslands. It is characterized by high and stabile yield potential and good biomass quality. The aim of this study was to determine genetic and phenotypic correlation coefficients of seven most important traits of two synthetic cultivars (Kruševački 21 and Pradel). Individual plants for hybridization, in order to obtain progeny by the method of Comstock and Robinson, were selected completely randomly. In order to examine the obtained progeny, the study was performed in an experimental field of the Institute for Forage Crops, in two replications. The experiment was set up according to the Nested Design I, with sets within the replications. In Kruševački 21, 60 full-sib progeny were studied in two sets, while in the Pradel a total of 39 full-sib progeny were studied. Within each full-sib progeny, 60 plants were analyzed. Statistically and high statistically relation was obtained between some traits and presented as genetic (r_g) and phenotypic (r_f) correlation coefficients. In cultivar K-21 very high statistically significant genetic correlation coefficients were determined between heading date and leaf length (r_g=0.873**), plant height and number of generative tillers per plant (r_g=0.893**) and dry matter yield in the first cut and annual dry matter yield (r_g=0.988**). In cultivar Pradel very high statistically significant relationship was obtained between heading date and plant height (r_g=0.978**), heading date and number of vegetative tillers per plant (r_g=0.926**) and annual dry matter yield with one side, and plant height (r_g=0.912**) and dry matter yield in the first cut (r_g=0.978**), on the other side.

Keywords: *meadow fescue, Nested Design I, genetic correlation coefficients, phenotypic correlation coefficients.*

Introduction

Meadow fescue (*Festuca pratensis* Huds.) as important perennial grass species, with high yield potential and biomass quality has great significance for animal feed production on grasslands and pastures. Meadow fescue is well adapted to continental climates where it can be an alternative to *Lolium perenne*, having in mind the fact that meadow fescue can be compared to species of the genus *Lolium* by quality and digestibility (Kölliker, 1998). Also, this species is of increasing importance in the northern regions (Casler and Santen, 2000). Meadow fescue is caenobiont of plant associations from the class *Molinio-Arrhenatheretae*, from lowlands to valleys in hilly areas (Vučković, 2004). It is mostly frequent in associations *Cynosuretum cristati*, *Festucetum pratensis* and anthropogenic association *Arrhenatheretum elatioris*. Meadow fescue is one of the most productive forage perennial grasses in extreme environmental conditions, like summer drought or winter hardiness. Also, meadow fescue is very tolerant to grazing; therefore pastures

where it dominates are long lasting. It can be grown as a pure crop, but more often in mixtures with other grasses and legumes.
Some forage grass characteristic (perennial species with anemophily mode of pollination, presence of high self-incompatibility and inbreeding depression) make breeding of this group of plants very difficult and complex. Basic condition for successful grass breeding process is the determination of genetic variability in breeding material. The existence of genetic variability in available breeding material allows success in breeding process. Variability within wild populations and cultivars of meadow fescue were documented for important agronomical traits, forage yield and forage quality traits (Babić, 2009; Babić et al., 2010; Babić et al., 2018).
The main goal in breeding program of any one forage grasses is to increase the dry matter yield, or maintain the yield through improving of other traits. The yield is very variable trait, influenced by a large number of genes, and also depends on the present agroecological conditions. Dry matter yield increase is part of all breeding schemes in perennial grasses, but small number of study consisted of improving the dry matter yield *per se* (Burton, 1982, Ceccarellii et al., 1980). Lot of breeding programs is aimed to improve different forage grasses traits which have high influence on DMY and showing high correlation coefficients with yield (Carlson, 1990; Babić et al., 2013). Determining in which way one trait affects another, makes it easier to determine criteria and predicts the course of breeding (Falconer and Mackay, 1996). In breeding process of one trait in the desired direction, the values of some other traits will change in size and direction in proportion to the size of the correlation coefficients between the given traits. The value of correlation coefficients depends on the frequency of genes in the studied collection (Falconer and Mackay, 1996), which means that the obtained correlation coefficients refer only to the studied collection in the given agroecological conditions.

Material and methods

The study was conducted in an experimental field of the Institute for Forage Crops Kruševac. Initial plant material were cultivars Kruševački 21 (K-21) from Serbia and Pradel from Switzerland. In the first phase of the research, 3000 plants per cultivar were sown in containers. At the beginning of tillering, individual plants were randomly selected and planted to obtain progeny according to the method of Comstock and Robinson (1948). The selection of full-sib families for the field experiment was carried out based on the obtained sufficient amount of seeds from hybridization (60 full-sib families were selected from the K-21 cultivar and 39 full-sib families from the Pradel cultivar). In order to examine the obtained full-sib families, the experiment was set up according to the Nested design I in two replications, with two sets within each replication. In K-21 were analyzed 60 full-sib families in total (30 full-sib families per set), while in the Pradel were studied 39 full-sib families (21 full-sib families within one set and 18 in the second set). Within each full-sib family, 60 plants were studied.
Genetic and phenotypic correlation coefficients were calculated based on the ratio of covariance and corresponding variances as follows:

$r_{g_{xy}}$ - Genetic correlation coefficient

$$r_{g_{xy}} = \frac{COV_{g_{xy}}}{\sqrt{\sigma^2_{g_x} \times \sigma^2_{g_y}}}$$

$r_{f_{xy}}$ - Phenotypic correlation coefficient

$$r_{f_{xy}} = \frac{COV_{f_{xy}}}{\sqrt{\sigma^2_{f_x} \times \sigma^2_{f_y}}}$$

Testing the significance of the correlation coefficient was performed by T-test using the ratio t=r/SEr for n-2 degrees of freedom (n is the number of analysed full-sib families).
The expected genetic gain from indirect selection (ΔGe) was also calculated as follows:

$$\Delta Ge = r_{gxy}\, k\, h_x\, h_y\, \sigma_{fx}$$

r_{gxy} - genetic correlation coefficient of trait x and trait y to which selection is performed
k - standardized selection differential (tabular value that depends on the selection pressure which in these studies was 1%)
h_x and h_y - square roots of heritability in the narrower sense of traits x and y
σ_{fx} - standard phenotypic deviation of trait x

Results and discussion

Table 1 shows the values of genetic and phenotypic correlation coefficients between the important traits for cultivar K-21 in the second productive year. In Table 2 presented values for these parameters for cultivar Pradel. Statistically significant and highly significant correlation coefficients were determined between some analyzed traits. In cultivar K-21 very high statistically significant genetic correlation coefficients were determined between heading date and leaf length (r_g=0.873**), plant height and number of generative tillers per plant (r_g=0.893**) and dry matter yield in the first cut and annual dry matter yield (r_g=0.988**).

Table 1. Genetic (above diagonal) and phenotypic (below diagonal) correlation coefficients in K - 21

Traits	HD	PH	LL	NVTP	NGTP	DMY I cut	Anual DMY
HD		0.008	0.873**	0.217	-0.660*	0.345	0.357
PH	-0.118		-0.674*	0.431	0.893**	-0.491	-0.459
LL	0.874**	-0.645		0.124	-0.021	0.633	0.643
NVTP	-0.105	0.149	-0.119		0.158	0.339	0.140
NGTP	-0.936**	0.549	-0.365	0.014		0.641	0.612
DMY I cut	0.007	-0.544	0.319	0.188	0.482		0.988**
Anual DMY	0.079	-0.519	0.389	0.078	0.486	0.919**	

*p< 0.05; **p< 0.01
HD - heading date, PH - plant height, LL - leaf length, NVTP - number of vegetative tillers per plant, NGTP - number of generative tillers per plant, DMY - dry matter yield per plant

In cultivar Pradel very high statistically significant relationship was obtained between heading date and plant height (r_g=0.978**) and heading date and number of vegetative tillers per plant (r_g=0.926**). Also, very high statistically significant genetic correlation coefficients were determined between plant height and leaf length (r_g=0.794**), dry matter yield in the first cut (r_g=0.875**) and annual dry matter yield (r_g=0.912**). Number of generative tillers per plant is highly correlated with leaf length (r_g=0.834**), number of vegetative tillers per plant (r_g=0.874**), dry matter yield in the fistr cut (r_g=0.771**) and annual dry matter yield (r_g=0.866**). Majidi et al. (2009) found strong correlations between plant height and dry matter yield (r_g=0.86**) and number of generative tillers and dry matter yield (r_g=0.58**) in half-sib families of tall fescue.

In cultivar K-21 statistically significant negative relationship was found between plant height and leaf length (r_g=-0.674*) and between heading date and number of generative tillers (r_g=-0.660*), while in Pradel negative genetic correlation coefficient was determined between leaf length and number of vegetative tillers (r_g=-0.830**). According to this results Babić et al. (2013) were found negative relationship between heading date and number of tillers per plant (r_g=-0.707).

Table 2. Genetic (above diagonal) and phenotypic (below diagonal) correlation coefficients in Pradel

Traits	HD	PH	LL	NVTP	NGTP	DMY I cut	Anual DMY
HD		0.978**	0.584	0.926**	0.711**	-0.098	0.127
PH	0.958**		0.794**	0.532	0.238	0.875**	0.912**
LL	0.548	0.695*		-0.830**	0.834**	0.398	0.269
NVTP	0.372	0.496	-0.265		0.874**	0.711*	0.514
NGTP	0.411	0.185	0.762**	0.793**		0.771**	0.866**
DMY I cut	-0.067	0.503	0.357	0.333	0.885**		0.978**
Anual DMY	-0.068	0.871**	0.116	0.322	0.833**	0.847**	

*p< 0.05; **p< 0.01

HD - heading date, PH - plant height, LL - leaf length, NVTP - number of vegetative tillers per plant, NGTP - number of generative tillers per plant, DMY - dry matter yield per plant

In both studied cultivars, strong correlations were observed between dry matter yield in the first cut and annual dry matter yield (in K-21 r_g=0.988**; in Pradel r_g=0.978**). This is a consequence of the high share of dry matter yield in the first cut in the total annual yield, which ranged from 62.91% in K-21 to 87.34% in Pradel. A high share of yield in the first cut in the total annual yield of meadow fescue from 68.95 to 76.89% was recorded in the research of Babić et al. (2010). Also, Kanapeckas et al. (2005) and Lemežiene et al. (2004) reported that the share of dry matter yield in the first cut in the total yield of dry matter ranged from 52 to 55%.

Having in mind the previously calculated correlations between the most important agronomic traits, it is noticed that often by improving one, a negative response of the other trait is encountered. For this reason, the expected genetic gain from indirect selection was calculated, to determine what effect breeding of one trait has on other ordered traits.

Table 3. Expected genetic gain from indirect selection in K-21

Traits	HD	PH	LL	NVTP	NGTP	DMY I cut	Anual DMY
HD		0.02	2.23	0.59	-2.28	1.09	0.89
PH	0.07		-5.68	3.91	10.17	-5.14	-3.80
LL	3.81	-3.14		0.58	-0.12	3.39	2.73
NVTP	4.35	9.24	2.46		4.24	8.35	2.74
NGTP	-30.98	44.79	-0.98	7.90		36.95	27.97
DMY I cut	17.86	-27.16	32.43	18.70	44.48		49.44
Anual DMY	20.37	-27.99	36.31	8.51	46.81	69.73	

HD - heading date, PH - plant height, LL - leaf length, NVTP - number of vegetative tillers per plant, NGTP - number of generative tillers per plant, DMY - dry matter yield per plant

In K-21, achieving the expected genetic gain in plant height would lead to a decrease in the dry matter yield in the first cut and annual dry matter yield, but also the length of the leaves (Table 3). The obtained results are in accordance with the results obtained in Sokolović (2006). By breeding the meadow fescue in the direction of later maturity, the number of generative tillers per plant would be reduced. In Pradel, a positive effect was observed for most of the studied traits by improving other traits. Only the realization of the expected genetic gain at the heading date would lead to a decrease in the dry matter yield in the first cut. Also, an increase in leaf length would result in a decrease in the number of vegetative tillers per plant (Table 4).

Table 4. Expected genetic gain from indirect selection in Pradel

Traits	HD	PH	LL	NVTP	NGTP	DMY I cut	Anual DMY
HD		5.42	1.74	5.30	4.03	-0.55	0.704
PH	11.50		4.94	6.35	2.81	10.18	10.55
LL	1.52	2.03		-2.20	2.19	1.03	0.69
NVTP	39.14	22.07	-18.55		37.11	29.71	21.35
NGTP	81.93	26.93	50.84	102.21		87.84	98.09
DMY I cut	-9.12	80.02	19.60	67.20	72.14		89.55
Anual DMY	12.66	89.22	14.18	51.97	86.69	96.35	

HD - heading date, PH - plant height, LL - leaf length, NVTP - number of vegetative tillers per plant, NGTP - number of generative tillers per plant, DMY - dry matter yield per plant

Conclusion

The most important trait, which requires the most attention in the process of breeding and creating new cultivars of meadow fescue for forage, as well as other perennial grasses, is dry matter yield. The results obtained in these studies suggest that indirect selection for dry matter yield is possible. In cultivar K-21, breeding on increased plant height affects obtaining genotypes with a larger number of generative tillers per plant, which leads to an increase in dry matter yield. In cultivar Pradel, breeding on increased plant height and number of generative tillers per plant, could lead to the creation of more productive genotypes. In both studied cultivars, K-21 and Pradel, a positive effect was observed for most of the studied traits by improving other traits.

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References

Babić S. (2009): Genetic variability of meadow fescue populations and cultivars (*Festuca pratensis* Huds.). Doctoral dissertation, Faculty of Agriculture, Zemun - Belgrade

Babić S., Sokolović D., Šurlan-Momirović G., Vasić T. and Simić A. (2010): Variability of forage yield components of meadow fescue (*Festuca pratensis* Huds.) populations and cultivars. XII International Symposium on Forage Crops of Republic of Serbia, 26-28. May, Kruševac-Serbia, Biotechnology in Animal Husbandry, 26 (Spec. issue), Book 2, 93-100.

Babić S., Sokolović D., Anđelković S., Vasić T., Zornić V. (2013): Correlative relations of meadow fescue important traits. Proceedings of XVIII Advising on Biotechnology, 18. (20), 15-16. march, Čačak, 537-542.

Babić S., Sokolović D., Radović J., Anđelković S., Lugić Z., Vasić T., Simić A. (2018): Analysis of variability of meadow fescue (*Festuca pratensis* Huds.) populations and cultivars. Proceedings of IX International Agricultural Symposium "Agrosym 2018", Jahorina, October 04-07, 419-424.

Burton G.W. (1982): Improved recurrent restricted phenotypic selection increases bahiagrass forage yields. Crop Sci., 22, 1058-1061.

Casler M. D. and Santen E. V. (2000): Patterns of variation in a collection of meadow fescue accessions: Crop Science, 40, 248-255.

Carlson I.T. (1990): Direct and indirect selection for forage yield in reed canarygrass. Agronomy abstracts, ASA, Madison, WI. 83-84.

Ceccarelli S., Falcinelli M., Damiani F. (1980): Selection for dry matter yield in *Lolium perenne* L. I Direct response to selection. Can. J. Plant Sci., 60, 491-500.

Falconer D.S. and Mackay T.F.C. (1996): Introduction to quantitative genetics. (4th Ed) Longman, UK.

Kanapeckas J., Tarakanovas P. and Lemežiene N. (2005): Variability, heritability and correlations of genetic resources in meadow fescue. Biologija, No. 3, 10-14.

Kölliker R. (1998): Genetic variability in *Festuca pratensis* Huds.: Effect of management on natural populations and comparison of cultivars to other species. A disertation submited to the Swiss Federal Institute of Technology, Zurich.

Lemežiene N., Kanapeckas J., Tarakanovas P. and Nekrošas S. (2004): Analysis of dry matter yield structure of forage grasses. Plant Soil Environmental, Vol. 50, No. 6, 277-282.

Majidi M. M., Mirlohi A. and Amini F. (2009): Genetic variation, heritability and correlations of agro-morphological traits in tall fescue (*Festuca arundinacea* Schreb.). Euphytica, 167, 323–331.

Sokolović D. (2006): Genetic gain in perennial ryegrass breeding process (*Lolium perenne* L.). Doctoral dissertation, Faculty of Agriculture, Zemun - Belgrade

Vučković, S. (2004): Grasslands. Faculty of Agronomy University in Belgrade. Belgrade.

RESEARCH OF HUMUS REPRESENTATION IN AGRICULTURAL LAND

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Abstract

Humus is a stable form of organic matter in the soil, and at the same time it is most important form. Maintaining humus levels in the interval of 3-5% is the most important obligation of land users. However, the percentage of humus in soils has decreased in the last 20-30 years. Special emphasis should be placed on the harmfulness of burning crop residues on the plot for constant reduction of organic matter content and humus. As for the role of humus in the soil, its useful properties directly depend on which organic substances were the basis for it. In any case, it is a universal fertilizer suitable for different types of plants and soil. Moreover, it is permanently stored in the soil, it saturates it with useful elements. Humus and fertility - interconnected thing. In laboratory conditions, Agriculture Extension Service "Sombor" examined the humus content at two depths, of 0-30 cm and 30-60 cm, which consists of the oxidation of soil organic matter with 0.1N $KMnO_4$ solution. The average humus content in this study on agricultural land in the layer of 0-30 cm was 3.18%, while the maximum content was determined at the site near the settlement of Telečka and amounted to 5.94%. The land where this humus content was recorded belongs to the type of meadow black carbonate on a light plateau. The lowest humus content was recorded on saline near the settlement of Stanišić. At a depth of 30-60 cm, the humus content in the soil is significantly lower and averages 2.28%, which is 27.3% less than the content in the layer of 0-30 cm. The highest humus content in the layer of 30-60 cm was also in the sample from point 102 in the area of Telečka and was 5.12%. The lowest humus content at the examined depth of 30-60 cm was in the sample from point no. 46 which is located in the cadastral municipality of Kolut and amounted to 0.13%.

Keywords: humus, organic matter, oxidation, agricultural land.

Introduction

Humus is a dark, organic substance, formed by the humification of plants and animals. Humus is a high molecular weight organic compound in the soil formed as a product of partial decomposition of dead organic matter (plant and animal residues). Humus primarily has a very beneficial effect on soil structure. Heavy and compacted soils become looser and lighter by humanization, and thus other soil properties are improved, primarily water regime, airiness and heat (Živković,1972). In terms of chemical properties, humus contains all the necessary plant nutrients. Humus also has a beneficial effect on the biological properties of the soil by being a source of carbon necessary for the life and reproduction of microorganisms. Microorganisms dissolve organic matter and break it down into mineral ingredients that are used by plants for nutrition. During the decomposition of humus, CO_2 is created, which the plant needs for assimilation and which activates chemical compounds inaccessible to the plant in the soil. It follows that one should know the forms of humus and its quantity in the soil (Petrović N., Kastori, R, Rajčan, I. (1991).

Materials and Methods

In modern agriculture, all measures should be taken to promote the formation of mild humus and its increase in the soil. There are several methods for determining humus, and they are all based on the same principle - strong oxidants act on organic matter, which leads to the oxidation of carbon from humus. According to the humus content, soils are classified into the following groups. Very low humus soils contain up to 1% humus, low humus 1-3% humus, very humus 3-5% humus, very humus soils with 5-10% humus and very very humus soils containing more than 10% humus. Since there are several projects, and one biogas plant is under construction in the city of Sombor, there is a possibility of using the substrate after fermentation and biogas production, in order to use it to increase organic matter and thus the humus content in the soil. It should be emphasized that constant quality control of the produced substrate is necessary because it depends on the raw materials from which biogas is produced, and in addition to increasing the content of organic matter, it can also contain harmful substances that can negatively affect the quality of agricultural land. In this experiment, the amount of humus is determined by oxidizing the humus substance from the soil with a strong oxidant, $KMnO_4$ - the amount of humus present can be determined from the consumption of $KMnO_4$.

Laboratory procedure in PSS "Sombor": 1. Weigh 200-500 mg of air-dried soil on an analytical balance. If the soil is very humus, a smaller mass is weighed, and if it is less humus, a larger mass is weighed. 2. Transfer the soil completely to a larger Erlenmeyer flask (500 ml). Using a beaker, add 130 ml of distilled water and 20 ml of H_2SO_4 (previously diluted 1: 3 to 1 by volume of acid and 3 by volume of distilled water) to the flask. Then 50 ml of 0.1 N $KMnO_4$ solution are added with a burette. 3. Cook the contents of the zucchini by simmering for 10 minutes. A funnel should be placed on the opening of the flask to prevent a drop of the solution from splashing during cooking. Since the reduction of manganese from oxidation number +7 to number +2 is possible only in a strongly acidic solution, H_2SO_4 is added to the flask. If oxidation took place without H_2SO_4, manganese would be reduced only to the number +4. The oxygen released in this reaction oxidizes the organic matter in the humus to CO_2, which is released from the flask. The more humus in the soil, the higher the consumption of $KMnO_4$. 5.After the contents of the flask were boiled for 10 min, remove from the heat and immediately titrate the excess $KMnO_4$ using 0.1 N oxalic acid solution $H_2C_2O_4$, from the burette until decolorized. Since the decolorization reaction is slow, excess $H_2C_2O_4$ will be added to the flask, which is then re-titrated with 0.1 N $KMnO_4$ solution from the burette, until a pink color appears. This reaction is quite fast, so care must be taken when adding $KMnO_4$, as the contents of the flask will turn pink by adding just one extra drop of $KMnO_4$.

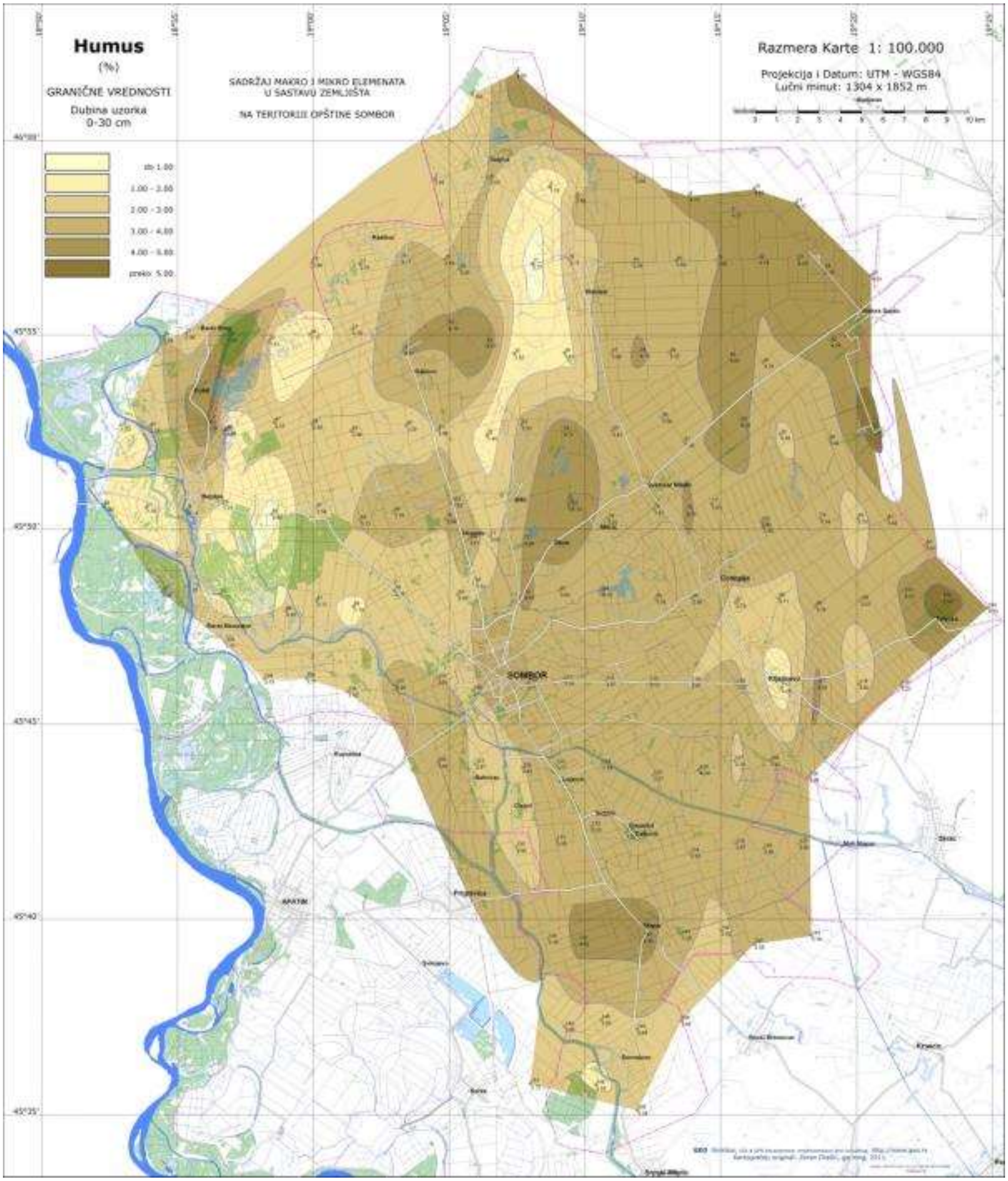
The humus content at a depth of 30-60 cm is significantly lower than the content in the soil layer of 0-30 cm. 75.52% of agricultural land in a layer of 30-60 cm has a lower humus content of 3%.

The content of humus in the soil, in addition to human influence, also depends on the type of agricultural land, and the following results can be stated: chernozem on a light plateau, part of the land in the "upper terrain" which includes cadastral municipalities Kljajićevo, Čonoplja, Svetozar Miletić, Stanišić and Riđica, has an average humus content in the layer of 0-30 cm, 3.73%, while in the layer of 30-60 cm, the humus content is 2.86%.

Photo 1.Transition from the upper to the lower terrain in the area of the inhabited place Svetozar Miletić

Results and Discussion

Meadow black carbonate on a light plateau, which includes areas on the "upper terrain" has a significant production capacity due to the content of humus and other production characteristics of the soil. In the case of meadow black soils, as well as chernozems on the loess plateau, the limiting factor of production capacities of these soils is the lack of soil moisture and low groundwater levels, and in dry years yields on the "upper terrain", ie on areas belonging to plateaus as part of the production area of the city of Sombor. The average humus content of this type is 3.03% in the soil layer of 0-30 cm, while the humus content in the soil layer of 30-60 cm is significantly lower and amounts to 2.32% according to these studies. On the type of soil (carbonate chernozem on the light terrace), which is located on the "lower ground", the humus content is average, 3.03% in the soil layer of 0-30. It is significantly lower in the soil layer of 30-60 cm and according to these studies is 2.32%. The type of agricultural land meadow black on the light terrace has an average humus content of 3.31% in the layer of 0-30 cm, and 2.50% in the layer of 30-60 cm. According to the manner of use of agricultural land, the highest average content of humus in the layer of 0-30 cm has orchards and arable land (3.30% and 3.23%), while the lowest average content of agricultural land that has been converted into landfills and it is 2 , 51%. At the second examined depth of 30-60 cm, the highest average humus content is in the lands used as gardens, and that is 2.67%. In this way of using agricultural land, the smallest difference in the average humus content is between the first and the second layer and it is 0.30%. The state-owned agricultural land of agricultural enterprises in restructuring has the highest humus content in both examined layers and it averages 3.82% in the layer of 0-30 cm, and 2.96% in the layer of 30-60 cm. It is the lowest in both examined layers with state land, which is rarely or never leased due to its weaker potential. Such lands can also be used primarily for grazing livestock, as well as for establishing artificial meadows, raising perennial orchards of fruits, vineyards, woody plants and alternative crops for the production of renewable energy sources. Such lands are also favorable for the cultivation of autochthonous plant species from the area of Sombor and Vojvodina, such as chamomile, dog rose, zova and other lesser-known species.



Map number 1: Values of humus content of agricultural land at a depth of 0-30 cm in the city of Sombor

Conclusion

Humus is not only a land, but also a community of living beings. Organic matter is the agricultural soil for many types of microbes and other microorganisms, which help the plant root absorb nutrients. The goal of every farmer is to increase the content of organic matter in the soil.This can be done by using compost, green manure, sowing annual forage crops, reduced or completely omitted cultivation, and afforestation. In an effort to meet the food needs of a growing population, more and more farmers are switching to intensive agriculture. Such a way of growing plants negatively affects the humus, intensifying the process of humus mineralization. Therefore, it is necessary to constantly add organic matter (organic fertilization) and maintain the optimal humus content in the soil by appropriate crop rotation and regulation of the soil reaction. These organic fertilizers can be livestock, chicken, horse or some other manure. Each crop has different requirements for organic fertilizer. The system instructs you, for more than 100 different crops, when to apply fertilizer and in what amount. Because humus is a product of the decomposition of organic matter, each time you add organic matter to the soil, the amount of humus will increase. It is a slow process, but if organic matter is added every year, the amount of humus will slowly increase.

References

Bonfiglioli, O., (1957): Fertilizers - what they are and how they are used.Agricultural Institute, Zagreb

DP "Agroinstitut" Sombor. (2004): Salt and saline soils in the area of Sombor (possibility and ways of use). Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Sombor.

Hadžić, V., et al., (1996): Basis of protection, use and arrangement of agricultural land in the municipality of Bački Petrovac ("pilot"). Republic of Serbia Republic Fund for Protection, Use, Improvement and Arrangement of Agricultural Land. Belgrade.

Cartographic Contributions, (1972): Lands of Vojvodina. Novi Sad.

Kastori, R., (2005): Nitrogen - agrochemical, agrotechnological, physiological and ecological aspects. Scientific Institute of Field and Vegetable Crops, Novi Sad.

Lazić, B., Babović, J., (2008): Organic agriculture. Institute of Field and Vegetable Crops, Novi Sad.

Petrović, N., Kastori, R, Rajčan, I. (1991): Land and plants.

Vasin, J., Sekulić, P., Kurjački, I. (2004): The state of soil fertility in Vojvodina. Proceedings, Scientific Institute of Field and Vegetable Crops, Novi Sad, vol. 42.

Živković, B., et al., (1972): Land of Vojvodina. Institute for Agricultural Research Novi Sad, Novi Sad.

GENERAL COMBINING ABILITY AND HETEROSIS OF SEX EXPRESSION TRAITS IN MELON

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Abstract

Melon is an annual, cross-pollinated species belonging to the family of *Cucurbitaceae*. Melon plants are usually monoecious: male and female flowers develop on one plant, but most commercial varieties and hybrids are andromonoecious, and on their plants develop male and hermaphrodite flowers. On melon plants, male flowers appear first, and then develop flowers with female reproductive organs. In this experiment, eight melon genotypes (parents), of which four were monoecious (Sesame, ED-3, ED-4, Pobeditel), and four andromonoecious (Chinese muskmelon, Anannas, Honey dew, A2-3lb), and their 20 hybrids (monoecious) were used. The experiment was conducted in Smederevska Palanka, during two vegetative seasons. Seven characteristics related to sex expression were observed. The aim was to determine the parents with the best general combining abilities (GCA) and to determine the heterosis in their hybrids for all seven observed traits. Negative heterosis for the trait period from sowing to the emergence of the first perfect/pistillate flower was recorded in 16 of 20 genotypes. A reduction of the period between the appearance of male (positive heterosis) and perfect/pistillate flowers on plants (negative heterosis) was found in 12 hybrid combinations. It was found that in as many as 17 hybrid combinations, the fruits ripen earlier than their parents. All eight observed parental genotypes showed significant GCA values for most of the observed traits. The results showed that the Sesame was the best general combiner, and represented a potential source of the desired alleles required for the melon breeding programs.

Keywords: *Cucumis melo L., monoecious, andromonoecious, hybrid, flowering.*

Introduction

Melon is an economically important, annual, cross-pollinated species belonging to the family of *Cucurbitaceae*. Melon (*Cucumis melo* L.) is the most diverse species of the genus *Cucumis*, and besides of a rich diversity of shapes and sizes of fruits, there is great variability in the morphology of the flower (Zhang et al., 2014).
Sexual reproduction is considered a turning point in the evolution of plant species (Rodriguez-Granados et al., 2016). Flower plays a major role in ensuring the continuation of the species in flowering plants. The flower, as a most important reproductive organ of the plant, is the least susceptible to changes under the influence of the external environment. The classification of flowering plants used today is, for this reason, based on parts of flowers or reproductive organs (Barret, 2010).
In order to increase the intensity of crossbreeding, plants have developed a number of different mechanisms to encourage sex determination, a process in which flowers become male or female.

In the majority of angiosperm species hermaphrodite flowers are formed on the plants, in which the male and female reproductive organs are formed within the same flower. Approximately 10% of angiosperm species develop unisexual flowers, flowers with only male or only female reproductive organs (Ming et al., 2011). Melon is especially interesting because of the biology of its flower. Three types of flowers can develop on melon plants: male - staminate, female - pistillate and hermaphrodite - perfect (Pitrat, 2008). Melon wild relatives are monoecious - male and female flowers are formed on the same plant, while about 70% of all commercial melon varieties and hybrids are andromonoecious - male and hermaphrodite flowers occurred on the same plant (Abdelmohsin and Pitrat, 2008). On melon plants, male flowers develop first, on the main stem, and on the first three nodes of side branches are formed flowers with female reproductive organs (Rodriguez-Granados et al., 2016).

Before choosing parental lines, it is very important to determine GCA and heterosis in the pre-breeding programs for all observed traits in all lines that are planned to be included in further hybridization (Olfati et al., 2012). General combining abilities for the observed characteristic are an important biometric parameter in determining the contribution of one parent to the positive or negative heterosis of his progeny (Fareghi et al., 2018). Heterosis is an agricultural phenomenon where, for some observed traits, hybrid genotypes demonstrated the phenotypic superiority compared to their parents (Yilmaz and Sari, 2012).

Many researches have studied and reported heterosis in melon for earliness (Abou Kamer et al., 2015; Choudhary et al., 2018), fruit yield (Abou Kamer et al., 2015; Selim, 2019), number of fruit per plant (Abou Kamer et al., 2015). Also, the combining ability for all this important traits in melon breeding programs was often determined by many researchers and breeders (Shashikumar et al., 2016). The aim of this study was to single out parental genotypes with the best general combining abilities, and to determine the heterosis of 20 hybrids for all seven observed traits.

Materials and methods

Four monoecious (Sesame, ED-3, ED-4, Pobeditel) and four andromonoecious (Chinese muskmelon, Anannas, Honey dew, A2-3lb) melon genotypes and their 20 hybrids were used as material in this study. Experiment was set up in Smederevska Palanka in Serbia (latitude 44°21'22.46"N, longitude 20°57'08.97"E, elevation 101 m), during two growing seasons (2011 and 2012). Melon plants were grown in clay pots (diameter 10 cm), and at the stage of 5-7 leaves they were transplanted to an open field. The trial was organized in three replications using complete randomized block design of 28 plots in one block (one plot for each genotype). Each plot consisted of 10 plants with a 150 cm distance between rows, and a 100 cm distance between plants. The soil type at this location is vertisol. Seven characteristics of sex expression in melon were monitored, measured and analyzed: total number of flowers per plant, number of pistillate/perfect flowers per plant, number of staminate flowers per plant, number of fruits per plant, period from sowing to the appearance of the first perfect/pistillate flower (in days), period from sowing to the appearance of the first staminate flower (in days), and period from sowing to full fruit ripening (in days). The aim of this study was to single out parental genotypes with the best general combining abilities, and to determine the heterosis of 20 hybrids for all seven observed traits.

Determination of general combination abilities was performed by the M x N hybridization method (Table 1).

Table 1. A method for determining the GCA by MxN hybridization

Genotype	5	6	7	8	T_N
1	F_{15}	F_{16}	F_{17}	F_{18}	Σ
2	F_{25}	F_{26}	F_{27}	F_{28}	Σ
3	F_{35}	F_{36}	F_{37}	F_{38}	Σ
4	F_{45}	F_{46}	F_{47}	F_{48}	Σ
$\mathbf{T_M}$	Σ	Σ	Σ	Σ	Σ

General average: $\boldsymbol{G_M = \frac{\sum T_M}{NxM}}$

For parents 1, 2, 3, 4: $\boldsymbol{GCA = \left(\frac{T_N}{M}\right) - G_M}$

For parents 5, 6, 7, 8: $\boldsymbol{GCA = \left(\frac{T_M}{N}\right) - G_M}$

To determinate heterosis in broader sense, the following formula was used:

$$\boldsymbol{H = F_1 - \left(\frac{(P_1 - P_2)}{2}\right)}$$

where F_1 is a mean value of F_1 generation, and P_1 a mean value of first parent, and P_2 a mean value of second parent. All the obtained results were statistically analyzed, using the Fisher's Least Significant Difference (LSD) test (Fisher, 1935).

Results and discussion

Based on the results for all 7 observed traits variety Sesame was single out as the best general combiner (Table 2).

Table 2. General combining ability for seven sex expression traits in melon

Genotype	Parent	A	B	C	D	E	F	G
Sesame	Mother	8.64	0.67	-0.67	-5.52	-1.91	-5.24	0.43
ED-3	Mother	-8.85	-0.15	0.15	2.93	0.25	1.21	-0.29
ED-4	Mother	-6.61	-1.09	1.09	3.80	2.40	2.87	-0.20
Pobeditel	Mother	6.82	0.57	-0.57	-1.21	-0.74	1.16	0.06
Chinese muskmelon	Father	-3.60	-0.80	0.80	1.21	0.86	0.47	-0.08
Anannas	Father	-1.53	0.30	-0.30	-1.62	-0.28	-4.63	-0.05
Honey dew	Father	-3.08	-0.51	0.51	1.24	0.73	3.90	0.05
A2-3lb	Father	8.21	1.01	-1.01	-0.83	-1.31	0.26	0.08
$lsd_{0.05}$		0.46	0.10	0.10	0.55	0.54	3.06	0.15
$lsd_{0.01}$		0.62	0.14	0.14	0.74	0.73	4.11	0.21

A – Total number of flowers per plant, **B** - number of pistillate/perfect flowers per plant, **C** - number of staminate flowers per plant, **D** - period from sowing to the appearance of the first perfect/pistillate flower (in days), **E** - period from sowing to the appearance of the first staminate flower (in days), **F** - period from sowing to full fruit ripening (in days), **G** - number of fruits per plant

For five of seven traits genotype ED-4 was singled out as the worst combiner with all negative GCA values. Beside Sesame, A2-3lb was excellent general combiner for trait number of pistillate/perfect flowers per plant, with a high significant value of GCA. Earliness is one of the important traits in melon breeding programs (Glala et al., 2010). Melon growers prefer high-yielding hybrids that will be placed on the market at a time when supply of market is still low and price is high (Duradundi et al., 2018). In order to produce early melon hybrids, it is necessary that pistillate or perfect flowers on mother plants and male flowers on father plants occurred as early as possible. As the best general combinators for this trait were single out the monoecious genotype Sesame and the andromonoecious genotype Anannas, and it may be recommended to cross these varieties in order to produce an early-growing hybrid. It was concluded that the best general combiner for breeding late-maturing hybrids is the variety Honey dew. For the trait number of fruits per plant, based on the results of GCA, best general combiner was Sesame, while genotypes ED-3 and ED-4 were single out as bad general combiner for this trait (Table 2). The estimates and direction of GCA for earliness, number of flowers with female reproductive organs, number of fruits per plant are similar with those obtained by Duradundi et al. (2018), and Shajari et al. (2021).

Table 3. Heterosis for seven sex expression traits in melon

Genotype	A	B	C	D	E	F	G
1 x 2	26.28	1.80	-1.80	-9.22	-3.08	0.31	0.20
1 x 4	14.86	1.87	-1.87	-5.67	0.56	-3.78	0.20
1 x 5	-10.61	-2.50	2.50	-4.78	1.81	-2.47	-0.15
1 x 6	4.19	1.00	-1.00	-4.06	5.36	-4.64	0.50
1 x 7	17.56	1.49	-1.49	-5.00	-6.86	-17.39	0.80
1 x 8	-3.17	-1.14	1.14	-1.78	3.28	-1.94	-0.25
2 x 4	2.09	1.54	-1.54	-0.33	6.56	-4.97	1.15
2 x 5	-12.33	-1.15	1.15	-0.11	4.03	-1.56	-0.10
2 x 6	4.86	4.28	-4.28	-2.56	2.36	-7.39	0.40
2 x 7	1.56	3.79	-3.79	0.22	-5.31	-14.92	0.25
2 x 8	-23.16	-1.16	1.16	-1.33	3.11	-0.03	-0.95
3 x 4	6.58	1.00	-1.00	-0.28	8.33	-4.25	-0.05
3 x 5	-15.22	-2.81	2.81	1.94	5.19	-3.00	-0.40
3 x 6	-10.42	0.89	-0.89	0.94	8.25	-3.72	0.30
3 x 7	8.22	0.99	-0.99	-0.33	-2.36	-9.80	0.10
3 x 8	-11.83	1.99	-1.99	1.11	5.72	2.64	-1.05
4 x 5	-5.92	-0.02	0.02	-5.22	9.53	-1.42	-0.45
4 x 6	15.22	3.56	-3.56	-1.83	9.14	-3.97	0.20
4 x 7	11.53	3.39	-3.39	-2.94	-2.08	-14.94	0.45
4 x 8	5.53	1.80	-1.80	-8.33	3.67	-2.72	0.20
$lsd_{0.05}$	2.27	0.37	0.37	0.44	0.48	0.94	0.16
$lsd_{0.01}$	2.71	0.45	0.45	0.52	0.57	1.12	0.19

1 – Sesame, **2 –** ED-3, **3 –** ED-4, **4 –** Pobeditel, **5 –** Chinese muskmelon, **6 –** Anannas, **7 –** Honey dew, **8 –** A2-3lb
A – Total number of flowers per plant, **B** - number of pistillate/perfect flowers per plant, **C** - number of staminate flowers per plant, **D** - period from sowing to the appearance of the first perfect/pistillate flower (in days), **E** - period from sowing to the appearance of the first staminate flower (in days), **F** - period from sowing to full fruit ripening (in days), **G** - number of fruits per plant

On melon plants, male flowers are formed first, and then flowers with female reproductive organs. The more uniform time of their formation on the plant, the greater the possibility that fertilization and fruit formation will occur. Negative value of heterosis for the trait period from sowing to the appearance of the first perfect/pistillate flower represents decreasing of this period of F_1 generation compared to their parents, and was observed in 16 hybrids. Negative values of heterosis for the trait period from sowing to the appearance of the first perfect/pistillate flower and positive values of heterosis for the trait period from sowing to the appearance of the first staminate flower were recorded in 12 genotypes (Table 3). In these genotypes, an earlier appearance of flower with female reproductive organs and a later appearance of male flowers was occurred, compared to their parents, that is, a shortening of the period between the first male and the first pistillate/perfect flower on the plant. These results are similar with those obtained by (Yilmaz and Sari, 2012; Choudhary et al., 2018). Earliness is complex but important trait in melons. This trait is correlated with number of days to first female and first male flower, number of nodes to first female flower, days to first harvest, and negative values of heterosis for these traits are desirable (Duradundi et al., 2018). Seventeen of the twenty hybrids had a negative heterosis value for trait period from sowing to full fruit ripening (Table 3). The hybrid combinations Sesame x ED-3, ED-4 x Fiat, and ED-4 x A2-3lb were the only ones whose fruits matured later as compared to their parents. Regarding the trait number of pistillate/perfect flowers per plant, positive heterosis was recorded in 14 of 20 melon genotypes (Table 3). For this trait, the highest positive value of heterosis was observed in the combination ED-3 x Anannas, and the highest negative value of heterosis was observed in the ED-4 x Chinese muskmelon combination. On the plants of ED-4 x Chinese muskmelon were detected the largest decrease in the formation of flowers with female reproductive organs, and the highest increase in the formation of male flowers, compared to the parents.

Conclusion

Some of the primary objectives in melon breeding programs include earliness, high female reproductive organs flower to male flower ratio, high number of fruits per plant, high fruit yield per plant, etc. Hybrids often demonstrate superiority of some economically important traits compared to their parents (heterosis). All eight observed parental genotypes showed significant GCA values for most of the observed traits. From 8 studied genotypes monoecious variety Sesame, used in experiment as mother line, was single out with best combining abilities for all 7 observed traits. This genotype represented a potential source of the desired alleles required for the melon breeding programs. Combination of monoecious variety Sesame (mother) and andromonoecious variety Anannas (father) may be recommended for future production of an early-growing hybrid. A reduction of the period between the appearance of male (positive heterosis) and perfect/pistillate flowers on plants (negative heterosis) was found in 12 hybrid combinations. These results are of great importance for organization of further steps in melon breeding programs in our country.

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References

Abdelmohsin M.E., Pitrat M. (2008). Pleiotropic effect of sex expression on fruit shape in melon. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of *Cucurbitaceae*, Avignon, France, pp 551-555.

Abou Kamer M.E., Yousry M.M., El-Gamal A.M. (2015). Heterosis and heritability studies for fruit characters and yield in melon (*Cucumis melo* L.). Middle East Journal of Applied Sciences, 5(1): 262-273.

Barret S.C.H. (2010). Understanding plant reproductive diversity. Philosophical Transactions of the Royal Society. B: Biological Sciences, 365(1537): 99-109.

Choudhary B.R., Haldhar S.M., Maheshwari S.K. (2018). Identification and possibility of monoecious inbred of muskmelon (*Cucumis melo* L.) for heterosis breeding. Vegetable Science, 45(1): 118-120.

Duradundi S.K., Gasti V.D., Mulge R., Masuthi M.K.D. (2018). Heterosis studies in muskmelon (*Cucumis melo* L.) for growth, earliness and yield traits. International Journal of Chemical Studies, 6(4): 3079-3086.

Fareghi S., Mirlohi A., Saeidi G. (2018). Relationship among combining ability, heterosis and genetic distance in maize (*Zea mays* L.) inbred lines under water-deficit conditions using line x tester and molecular analysis. Bioscience Biotechnology Research Communications, 11(2): 216-223.

Fisher R.A. (1935). The design of experiments. Oliver and Boyd, London, pp. 251.

Glala A.A., Abd-Alla A.M., El-Dessouky S.E.I., Obiadalla-Ali H.A. (2010). Heterosis and combining ability for earliness, yield, and fruit quality of some Egyptian melon inbred lines via line× tester analysis. Acta Horticulturae, 918: 491-500.

Ming R., Bendahmane A., Renner S.S. (2011). Sex chromosomes in land plants. Annual Review of Plant Biology, 62: 485-514.

Olfati J.A., Samizadeh H., Rabiei B., Peyvast G. (2012). Griffing's methods comparison for general and specific combining ability in cucumber. The Scientific World Journal, pp. 1-4.

Pitrat M. (2008). Melon. In: Vegetables I. Springer, New York, pp. 283-315.

Rodriguez-Granados N.Y., Lemhemdi A., Choucha F.A., Latrasse D., Benhamed M., Boualem A., Bendahmane A. (2016). Sex determination in *Cucumis*. In: Genetics and genomics of *Cucurbitaceae*. Springer, Cham, p. 307-320.

Selim M.A. (2019). Heterosis and combining ability for some fruit quality traits of egyptian melon inbred lines using line× tester analysis. Egyptian Journal of Agricultural Research, 97(1): 317-342.

Shajari M., Soltani F., Reza Bihamta M., Alabboud M. (2021). Genetic analysis and inheritance of floral and fruit traits in melon (*Cucumis melo*) in the full diallel cross. Plant Breeding, 140(3): 486-496.

Shashikumar K.T., Pitchaimuthu M. (2016). Heterosis and combining ability analysis of quantitative and qualitative traits in muskmelon (*Cucumis melo* L.). International Journal of Agricultural Science, 6: 341-348.

Zhang J., Boualem A., Bendahmane A., Ming R. (2014). Genomics of sex determination. Current Opinion in Plant Biology, 18: 110–116.

Yilmaz N., Sari N. (2012). Heterosis effect on plant growth fruit, yield and quality in single, triple and double crosses of melon (*Cucumis melo* var. *cantalupensis*) hybrids. Proceeding of the Xth EUCARPIA meeting on genetics and breeding of *Cucurbitaceae*, Antalya, Turkey, pp. 535-543.

THE IMPACT OF INTEGRATED GROWING SYSTEM AND TOP DRESSING IN PRODUCTIVITY OF WINTER WHEAT

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Abstract

The examination of the effects of sustainable growing systems on the grain yield of winter wheat was conducted at the research and study field "Radmilovac" of Faculty of Agriculture (44°45' N, 20°35' E Serbia, 130 m above mean see level). Investigations was conducted in 2016/17 and 2017/18 on the luvic chernozem soil type, in completely randomized blocks. In intergrated growing system (IGS), based on low inputs, tillage was performed using a chisel plough at 25 cm with ≥30% of maize crop residues retaining on the soil surface and the pre-sowing tillage using a disc harrow and a harrow, basic fertilization in autumn with 600 kg ha^{-1} NPK and top dressing in spring with 60, 120 kg ha^{-1} N and control treatment, without top dressing. Two common winter wheat cultivars (*Triticum aestivum* ssp. *vulgare*) Ilina and Zvezdana grew in this growing system. Statistical analysis confirmed that year, variety and top dressing had a significantly greater impact on grain yield and year and top dressing on weight of 1000 grains. Interaction of year*cultivar and year*top dressing had a significant effect on investigation parameters. More favorable meteorological conditions in the first year led to obtaining statistically significantly higher grain yields (5885.56:5585.56 kg ha^{-1}). A higher yield per unit area was found in the Ilina variety (6012.22 kg ha^{-1}) compared to the Zvezdana variety (5458.89 kg ha^{-1}). With increasing N dose in top dressing, the weight of 1000 grains and the grain yield of winter wheat increased in both tested varieties (5437.5; 5704.17; 6065.00 kg ha^{-1}). An integrated cultivation system on heavier soils has less positive effects than on soils with more favorable characteristics, especially in the higher dose of N.

Key words: *integrated management, winter wheat, grain yield, top dressing.*

Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant cultivated species and the staple food for more than 50% of the global population (Rizwan et al., 2016) and special attention must be paid to the farming system of this species. Integrated farming system is sustainability and it combine the best practices from organic and conventional systems have potential to reduce negative environmental impacts while maintaining yield levels. Special attention in this growing system is paid to soil tillage, selection of genotypes, fertilization and application of chemical protection means. The main trend in tillage for winter wheat growing the past 3-4 decades has been the introduction and increased use of pot-powered harrows for secondary tillage. A comprehensive survey of soil characteristics in many countries winter wheat fields showed that the use of rotary harrows significantly affects soil structure. At present, new technologies for the

cultivation of grain crops based on the use of minimum tillage are becoming more widespread (Korchagin and Chudanov, 2002).
With different reductions in soil tillage in our country, there are numerous results that are related, mainly, to better soils such as chernozem, but also to something more difficult such as eutric cambisol. According to some results, with the application of herbicides and special planters on chernozem, intensive production of wheat without plowing and pre-sowing preparation is possible (Konstantinović, 1997), while on heavier soils the advantage is mainly related to soil moisture preservation and soil protection from erosion. Compared to the conventional one, the conservation system of tillage achieved lower grain yields of winter wheat (25–35%), spring barley (5.72–51.85%), maize (24.90–24.62%) and soybeans (34.95–39.41%) as stated by Kovačević et al. (2020). Conservation technologies are more rational, however, since they give lower biomass yields than the conventional system, there is a need to examine their economic efficiency from the energy and soil protection point from degradation. Insisted conservation tillage systems are far more rational, but since they have resulted in slightly lower yields, there is a need to test their economic efficiency based on the ratio of additional energy input and the degree of soil conservation in each individual case. The introduction of a system of conservation tillage in the future must be in line with changes, both in crop rotation and in plant breeding (Momirović et al., 2021).
In addition to soil tillage systems, the optimal application of fertilizers is an important factor for obtaining high yield and high quality products. Tillage system and level of nitrogen fertilization in top dressing, as well as interaction between these two factors have important influence on grain yield of winter wheat (Dolijanović et al., 2019). Among the elements of mineral nutrition, nitrogen has the most important role in forming the yield (Litke et al., 2018), quality and utilizable value of the wheat grain (Blandino et al., 2016).
The objective of this study was to evaluate an effect of conservation tillage system and level of nitrogen top dressing on 1000 grain weight and grain yield of winter wheat in agroecological condition in Belgrade (Radmilovac research field).

Material and methods

Study site

The field experiment was conducted at the research field "Radmilovac" of Faculty of Agriculture ($44^o45'$ N, $20^o35'$ E Serbia, 130 m above mean see level). Since 1990 up to now, this experimental field has witnessed decades-long examinations of the impact of conventional, integrated and organic growing systems crop properties. The soil type is a luvic chernozem.

Experimental design

A three-factor field experiment (year, top dressing and variety) was designed as a randomised block with three replications, with the elementary plot of 6 m^2. The crop rotation was included four crops (maize → winter wheat → spring barley + red clover → red clover). In intergrated cropping system (ICS), based on low-inputs, tillage was performed using a chisel plough at 25 cm with ≥30% of maize crop residues retaining on the soil surface and the pre-sowing tillage, using a disc harrow and a harrow, basic fertilization in autumn with 600 kg ha^{-1} NPK and top dressing in spring with 0, 60 and 120 kg ha^{-1} N. Two common winter wheat cultivars 'Ilina' and 'Zvezdana' were used with 550 seeds m^{-2}. Cv. 'Ilina' is an excellent winter hardiness cultivar of medium-late maturity, which belongs to the B1 quality group. Cv. 'Zvezdana' is a very good winter hardiness cultivar of medium early maturity, which belongs belongs to the A1 - A2 quality group. Both varieties are very resistant to *Erysiphe graminis* DC. and well-resistant to

Puccinia striiformisf. sp. *tritici* (Pst). All used varieties are winter resistant and morphologically very similar, produced in the same seed company (Institute of Field and Vegetable Crops, Novi Sad, Serbia).

Sowing was conducted on October 22th and 25th, 2016 and 2017 and harvest was done on July 03rd, and June 28th. For weed control in the studied cropping systems used the preparation Maton (2,4D) for treating from the middle of tillering to the beginning of jointing (stages 25-30 of the BBCH scale) in the amount of 0.5 l ha^{-1}.

Statistical analysis

Sampling of wheat grains was performed in June, at the stage of full maturity and measuring 1000 grain weight and grain yield. The data were statistically processed using statistical package, IBM SPSS Statistics Version 25 (ANOVA). The level of significance was set at $P < 0.05$.

Results and discussion

The long-term mean precipitation in this area is on average 531.2 mm and the mean annual temperature is 10.3°C. However, in the research years (2016/17 and 2017/18), significant differences in meteorological conditions could be noticed, particularly regarding precipitation (Table 1). The annual precipitation in the first year was lower than the long-term average, with the extreme values in December (as low as 2.6 mm). Smaller amounts of precipitation were recorded in January, February, March and June compared to long-term averages. In this year, the average air temperature was also lower than the long-term average by 0.5°C. Higher temperature deviations from the long-term average were registered in October, November, December, January, February and May. On the other hand, significantly higher temperatures than the long-term average were recorded in June (+ 2.4°C). In the second year, precipitation was on level long-term average with more favourable precipitation schedule by months. The average months and annual temperatures were very higher than first year investigation (Table 1). In general, in terms of meteorological conditions, the second year of investigation was less favourable, primarily due to higher precipitation in June and higher air temperatures during April and May, which affected the 1000 grain weight and grain yield, especially in the Zvezdana variety.

Table 1. Mean monthly air temperatures and precipitation sums from October to June in Belgrade

Months	Temperature (°C)			Precipitation (mm)		
	2016/17	2017/18	1991-2016	2016/17	2017/18	1991-2016
October	11.1	13.9	13.3	76.8	65.9	56.2
November	7.7	8.4	8.4	71.8	41.2	46.6
December	0.9	5.1	3.1	2.6	45.2	53.9
January	-3.3	5.3	2.3	23.4	39.3	52.8
February	5.4	2.3	3.8	23.5	58.1	48.6
March	11.5	6.9	8.2	27	64.8	54.1
April	12.7	18.2	13.8	51.8	39.7	48.2
May	18.4	21.5	18.1	86.1	56.2	84.0
June	24.3	22.3	21.9	53	121.6	86.8
Average/Sum	9.9	11.5	10.3	416	532	531.2

The results of the study of the influence of the observed factors on the weight of 1000 grains and the grain yield of winter wheat are shown in Table 2. The weight of 1000 grains was statistically significantly influenced by the year of investigating and the level of top dressing (individual factors), and interaction of all investigation factors. The grain yield of the examined winter wheat cultivars changed statistically significantly under the influence of all examined individual factors, as well as under the influence of the year * variety interaction (Table 2).

When we look at the obtained yield of winter wheat by years, it can be seen that the lower yields were obtained in 2017/18, which was with more unfavorable meteorological conditions. By growing the Zvezdana variety, lower grain yields and a weight of 1000 grains were achieved in relation to the Ilina variety. The absolute highest grain yields in both cultivars were recorded in conservation cultivation technology with protective treatment and intensive top-dressing of 120 kg ha^{-1} nitrogen (6597 and 6080 kg ha^{-1}). When it comes to the interaction between the year and top-dressing, it is interesting that with the increased amount of nitrogen in top-dressing, the negative effects of weaker meteorological conditions can be eliminated.

The results show that top-dressing in the first and second year of investigating, with the amount of 60 kg ha^{-1} nitrogen in the cultivar Ilina increased the yield by 6.5 and 4.6%, and with 120 kg ha^{-1} by 11.5 and 11.3% compared to the control. The increases in the variety Zvezdana were smaller, ie they were 1.7 and 6.7% for the dose of 60, respectively 9.0 and 14.5% for the second dose of nitrogen compared to the control. The relatively smaller increase in yield observed by the years of testing is the result of the weak effect of nitrogen in the first year of testing, primarily caused by bad meteorological conditions. However, the small difference between the two fertilization levels examined suggests that the Ilina and Zvezdana cultivars we used in these studies were cultivars with relatively lower nitrogen requirements. This fact recommends them as suitable for rational cultivation technologies based on reduced investments. It should be noted that increased nitrogen doses in top dressing are most justified in intensive winter wheat gay systems, especially in the case of intensive varieties (high input) (Kovačević et al., 2009).

Table 2. Yield and 1000-grain weight of the studied wheat cultivars in integrated cropping systems

Top dressing (kg ha^{-1} N) (C)	Variety (B)/ Year (A)	1000-grain weight (g) 2016/17	1000-grain weight (g) 2017/18	Yield kg ha^{-1} 2016/17	Yield kg ha^{-1} 2017/18
0	'Ilina'	41.2	39.8	5917	5463
	'Zvezdana'	41.4	39.4	5310	5060
	Average	41.3	39.6	5614	5262
60	'Ilina'	40.9	40.3	6300	5717
	'Zvezdana'	41.4	40.4	5400	5400
	Average	41.2	40.3	5850	5559
120	Ilina	40.7	40.9	6597	6080
	Zvezdana	42.1	40.7	5790	5793
	Average	41.4	40.8	6194	5937

ANOVA - LSD (0.05) 1000-grain weight				Yield			
A	0.249	AC	0.431	A	156.3	AC	270.7
B	0.249	BC	0.431	B	156.3	BC	270.7
C	0.305	ABC	0.610	C	191.4	ABC	382.9
AB	0.352			AB	221.1		

Conclusions

Based on the results of examining the impact of important agro-technical measures as elements of growing technology based on the basic postulates of sustainable agriculture on the weight of 1000 grains and the grain yield of winter wheat, it can be concluded:
The examined parameters had higher values in the first, meteorologically more favorable, year of investigating. Top dressing with a higher dose of nitrogen was more efficient than the rational dose in both years. The choice of variety is extremely important for rational (low-input) technology of growing winter wheat. The chosen degree of reduction in soil tillage, mineral nutrition and protection corresponded to the selected varieties, which showed increased adaptability to reduced tillage conditions, achieving optimal grain yields and a more modest mineral diet.

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References

Blandino, M., Marinaccio, F., Reyneri, A. (2016): Effect of late-season nitrogen fertilization on grain yield and on flour rheological quality and stability in common wheat, under different production situations. - Italian Journal of Agronomy. 11(745): 107-113.

Dolijanović, Ž., Roljević-Nikolić S., Kovačević, D., Djurdjić S., Miodragović, R., Jovanović-Todorović M., Popović-Djordjević, J. (2019): Mineral profile of the winter wheat grain: effects of soil tillage systems and nitrogen fertilization. Applied ecology and environmental research 17 (5): 11757-11771.

Korchagin, V.A., Chudanov, I.A. (2002): Modern technological complexes for the cultivation of grain crops in adaptive farming systems Samara Research Institute of Agriculture Volga MIS 28–39.

Litke, L., Gaile, Z., Ruža, A. (2018): Effect of nitrogen fertilization on winter wheat yield and yield quality. - Agronomy Research. 16(2): 500-509.

Rizwan, M., Ali, S., Abbas, T., Zia-ur-Rehman, M., Hannan, F., Keller, C., Ok, Y. S. (2016): Cadmium minimization in wheat: a critical review. - Ecotoxicology and environmental safety, 130: 43-53.

Kovačević, D., Dolijanović, Ž., Oljača, S. (2009): Influence of tillage system, nitrogen supplementation and cultivars on winter wheat yield. Agricultural Engineering, Year XXXIV, No. 2, 9-13.

Kovačević, D., Oljača, S., Momirović, N., Broćić, Z., Dolijanović, Ž., Milić, V. (2020): Systems of conventional, integral and organic production of cultivated plants. In: Janjić V, Pržulj N (editors) Limitations and challenges in plant production. Academy of Sciences and Arts of the Republic of Srpska, Banja Luka, Monograph LXII: 91-133.

Konstantinović, J. (1997): Soil tillage in field science production. Institute of Field and Vegetable Crops Novi Sad, Novi Sad.

Momirović, N., Kovačević, D., Dolijanović, Ž. (2021): Technology of plant production in field and vegetable science production from the humanistic aspect of protection of man and nature. Scientific-professional gathering: Djordje Radic - the founder of modern agriculture in Serbia, 180 years since the birth of the first doctor of agricultural sciences among Serbs. SANU, Belgrade. Book CXSIV, Department of Chemical and Biological Sciences Book 18. Proceedings, 127-165.

INFLUENCE OF VINEYARD GRASS COVER ON TECHNOLOGICAL CHARACTERISTICS OF WINE GRAPE CULTIVARS

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Abstract

Research was performed in vineyard of "Radovanović" winery, Serbia. Experimental vineyard is located at altitude of 220 meters, GPS coordinates N 44º 25' 47"and E 21º 02' 14". Row spacing is 2.4 m and 0.9 m between vines in row. The inter-row space was sown with a following grass-legume mixture: 60% *Festuca rubra,* 30% *Lolium perenne* and 10% *Trifolium repens*. Experiment with grass-legume mixture was set with two factors, fertilization with two nitrogen fertilizers (AN and KAN) and rates of 0, 50 and 100 kg N ha^{-1}. The yield of fresh and dry biomass and visual assessment of grass quality under influence of nitrogen fertilization were monitored. For soil fertility analysis samples were collected from 3 depths: 0-30, 30-60 and 80-100 cm. The most important grapes and berries structural indicators, sugar and total acids content in must were determined. The aim of the research is to determine influence of grass inter-row cover maintained according to criteria of optimal turf management, on structural indicators and quality of grapes. Results show that soil pH generally increases with depth, concentrations of organic C, total N and available P_2O_5 and K_2O decrease with depth. Grass groundcover and fertilization have a clear influence on examined soil fertility parameters. Sugar content in must varied from 17.6–25.2%. Large variations were found for bunch weight (103-259 g), epicarp weight from 100 berries (2.23-9.62 g) and average seeds number from 100 berries (110-177).

Keywords: *wine grape, soil, grass cover, structural indicators, grape quality.*

Introduction

The promotion of the environmental sustainability in viticulture requires increasing knowledge in all the issues related to vineyard ecosystems; as well as the need to reconsider management techniques for plants and their hosts, for soil and topsoil, paying an increasing attention to actions aiming at safeguarding the structure and fertility of the cultivation sites. The degradation of soil quality may lead to serious problems for wine production, and a cultural damage as soil is unanimously considered a key component of the terroir (Marques et al., 2010). Among strategies to increase environmental sustainability in vineyards, cover cropping with grasses has been recognized as an effective practice to promote soil conservation and protection (erosion control, aggregate stabilization, soil porosity enhancement) (Keesstra et al., 2018). Perennial cover crops offer a wide range of agronomic and environmental benefits to agroecosystems. Recent studies on intercropping in vineyards have shown that in some situations, water stress may not be greater than that prevailing in bare soil vineyards (Celette et al., 2008). Also, Celette et al. (2005) found that grape vine predawn leaf water potential and stomatal conductance did not differ significantly in vines where weeds were controlled in each inter-row by applying glyphosate during the growth period or sown with tall fescue (*Lolium arundinaceum* Schreb/Darbysh.). Despite this, the authors have highlighted, in the plants managed by covering a lower length of the canes and a lower number of leaves, suggesting that vine and tall fescue were competing for nutritional resources. Ingels et al. (2005) underlined that with an adequate distance between the row and the cover crop, the short time effects of grass cover on the vineyard performance are negligible. Moorlat and Jacquet report that grass covering afect decrease in the number of vine roots in the upper soil layers interrow and increase amount of organic matter, N, K_2O and pH. On cover soils temperature is higer, soil erosion is reduced (Napoli et al., 2017), water use is higer, primarly in spring, vine vigor and shoot lenght, must acidity it is reduce and did not affect grape yield. Total phenol content in berry skin can be incrisead. Absolute and relative percent of individual components of bunch (berries and stem) and berries (skin, mesocarp and seeds) is important in assessing grape quality as processing materials for wine. On average, 100 kg of grapes contain 3-5% of stem and 95-97% berries, while berry composition include 8-11% of skin, 2-4% of seeds and 85-95% of mesocarp (Marković et al., 2017). Values of mechanical composition of grapes and berries parameters can vary under influence of temperature, insolation, microclimate in grapevine trunk (Keller, 2020), but also under application of different agrotechnical and ampelotechnical measures like irrigation, fertilization, grassing, defoliation etc. (Cataldo et al., 2021). This paper is intended to provide useful indications on sustainable management for vineyards growing in southeastern Europe at Balkan peninsula. Our objectives were to analyze the effects of grass intercrops on grapevines quality.

Material and Methods

Research was performed in vegetation season 2020, in vineyard of "Radovanović" winery, Serbia on Cabernet auvignon cv, clones VCR 169 and 191. Experimental vineyard is located at altitude of 220 m, GPS coordinates N 44º 25' 47"and E 21º 02' 14". Row spacing is 2.4 m and 0.9 m between vines in row. Vineyards is characterized training system with height of 90 cm on which is Guyot pruning used. All experimental vines were uniformly pruned where was left spur with two buds and one arc with eight buds. For the analysis of basic fertility, soil samples were collected with a drill from depths: 0-30, 30-60 and 80-100 cm. Using standard agrochemical methods it was determined: pH, (both actual pH_{H2O} and exchangeable pH_{KCl}), total organic carbon, total nitrogen content, available phosphorus (P_2O_5) and potassium (K_2O). The effect of different rates (N1- 0 kg N/ha; N2 - 50 kg N/ha; N3- 100 kg) of applied nitrogen fertilizers (AN and KAN) on sown grass-legume mixture: 60%, *Festuca rubra* 30% *Lolium perenne* and 10% *Trifolium repens* was investigated. The trial was set up in a random block design with plot size 10 m^2 in three replications. The grass cover in clone 169 was cut once, while cover in clone 191 was cut two times, and fresh, dry biomass and visual assessment of grass quality under influence of nitrogen fertilization were monitored. Visual assessment was monitored by scale from 1 (bad conditions, weak cover) to 10 (complete cover, compact sod). Data were analyzed were analyzed through analysis of variance and LSD test, in order to recognize significant effects of fertilization treatments. Analysis of collected grape samples was done at laboratory of Faculty of Agriculture University of Belgrade. Berries were harvested at full maturity (fenolic maturity). Mechanical analysis of grape bunches and berries was done by Prostoserdov (1946) and Markovic and Przić (2015). Bunches were measured for their weight, length and width, and rachis (pedicel) from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on stem. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each variety 100 berries were selected for purpose of mechanical analysis and after measuring mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on analytical balance, and number of seeds in 100 berries was determined by counting. Other parameters were obtained by computation. Ripening parameters are shown through content of accumulated sugar in grape juice-must, total acids content and pH. Sugar content was determined by physicochemical methods using Oeshle mostwage, values were calculated using Dujardin-Salleron tables. The total acid content was determined by titration with n/4 NaOH and pH using pH-meter.

Results and Discussion

The surface (0-30 cm) and subsurface layer of the soil (30-60 cm deep) is acidic, while at a depth of 80-100 cm it is neutral to alkaline (Table 1). In relation to soils under other cultivated crops and natural stands, the difference between the measured values of pH (so-called ΔpH) in the surface layer is very large. According to the content of total organic carbon, the surface layer of the soil is weakly in humus, the subsurface is very weakly to slightly, while the layer at a depth of 80-100 cm is very weakly in humus. The nitrogen content in the soil under the vineyard is generally low, while in the available P_2O_5 this soil is poor. In available K_2O, the land under the vineyard is generally well supplied.

Table 1. Agrochemical properties of soils

Treatment (kg N/ha)	Depth (cm)	pH in H_2O	pH in 1M KCl	Total organic C (%)	Total N (%)	Available (mg/100 g)	
						P_2O_5	K_2O
0	0-30	6.98	4.87	0.589	0.054	1.82	19.76
	30-60	6.48	4.78	0.954	0.069	2.19	24.62
	80-100	7.84	6.84	0.154	0.040	2.92	19.75
50	0-30	6.71	5.34	0.938	0.107	3.95	22.78
	30-60	6.85	4.95	0.866	0.103	1.37	22.06
	80-100	8.29	7.20	0.315	0.042	4.39	20.64
100	0-30	7.19	5.12	0.618	0.088	1.87	19.93
	30-60	6.58	4.78	0.966	0.117	2.57	21.32
	80-100	8.51	7.39	0.361	0.043	6.21	21.29

The observations showed that the grass covers were different in examined vineyards. The grass cover in clone 169 was poor which resulted in one cutting (beginning of May), while another grass cover (in clone 191) was more productive, compact, generally in better condition (table 2). Fresh and dry matter yield was much better under clone 191, without effect of applied amount or fertilizer type, similar to results Krga et al. (2015) without effect of nitrogen application on dry matter yield in sown legume grass mixture. The visual assessment of grass cover followed yield and turf under clone 191 was in much better conditions then turf under clone 169. Both clones (169 and 191) had the highest values of bunch length and width in treatment with a concentration of 50 for AN and KAN. Clone 191 had lower cluster mass with the lowest value of this parameter determined for KAN 50 (103 g) and AN control (129 g). Clone 169 had higher values of cluster mass, with treatment AN 50 (259 g) and KAN control (225 g).

Table 2. Fresh and dry biomass, visual assessment of grass cover, bunch and berry mechanical composition of Cabernet Sauvignon, clones 169 and 191

Clone	169						191					
Fertilazer	**AN**			**KAN**			**AN**			**KAN**		
Doses	**0**	**50**	**100**	**0**	**50**	**100**	**0**	**50**	**100**	**0**	**50**	**100**
Fresh biomass of grass cover (t/ha)	1.90	1.76	2.06	0.82$^{\oplus}$	1.25	1.90	6.42	7.55	7.66	7.01	7.71	7.87*
Dry biomass of grass cover (t/ha)	0.71	0.63	0.78	0.28$^{\oplus}$	0.64	1.13	1.92	2.19	2.53	2.36	2.34	2.68*
Visual assessment of grass quality	2.70	3.30	4.00	2.70$^{\oplus}$	4.00	4.70	7.30	7.00	8.30*	6.30	6.70	7.30
Bunch length (cm)	13.6	16.6*	16.3	15.0	15.4	15.2	12.0	13.6	11.6	12.0	9.60$^{\oplus}$	11.2
Bunch width (cm)	8.60	11.8*	10.1	10.4	8.40	7.60	6.80$^{\oplus}$	10.4	7.80	7.80	7.80	8.00
Bunch mass (g)	164	259*	217	225	197	203	129	164	137	160	103$^{\oplus}$	145
Average berries number in bunch	121	183*	157	162	156	156	106	142	125	118	84.0$^{\oplus}$	94.0
Bunch stem mass (g)	5.2	9.6*	6.6	8.6	8.6	8.6	6.4	8.2	6.8	7.8	5.2$^{\oplus}$	9.2
Berries mass (g)	158	246*	212	214	187	194	122	153	130	150	96.0$^{\oplus}$	129
Mass of berry skin from 100 berries (g)	7.87	5.48	7.46	9.62*	7.19	6.88	5.08	6.81	5.35	6.19	4.61$^{\oplus}$	6.23
Mass of seeds from 100 berries (g)	4.54	3.08$^{\oplus}$	4.35	3.98	4.20	4.83*	3.85	4.37	4.17	3.90	3.40	3.85
Average seed number in 100 berries	142	115	158	110$^{\oplus}$	153	170	151	167	177*	145	149	157
Average berry skin mass from one bunch (g)	9.48	10.0	11.7	15.6*	11.2	10.7	5.41	9.69	6.70	7.32	3.86$^{\oplus}$	5.83
Average seed mass from one bunch (g)	5.47	5.62	6.84	6.45	6.54	7.54*	4.10	6.22	5.22	4.61	2.85$^{\oplus}$	3.61
% of stem in bunch	3.18	3.71	3.04$^{\oplus}$	3.83	4.36	4.23	4.96	5.00	4.98	4.87	5.04	6.35*
% of berries in bunch	96.8	96.3	97.0*	96.2	95.6	95.8	95.0	95.0	95.0	95.1	95.0	93.6$^{\oplus}$
% of epicarp in berry	5.66	4.28$^{\oplus}$	5.49	7.63*	5.29	5.55	4.58	5.32	5.05	4.65	4.31	4.61
% of seed in berry	3.26	2.41$^{\oplus}$	3.20	3.16	3.09	3.90	3.47	3.41	3.93*	2.93	3.18	2.85
% of mesocarp in berry	91.1	93.3*	91.3	89.2$^{\oplus}$	91.6	90.6	92.0	91.3	91.0	92.4	92.5	92.5
Berries indicator	73.7	70.6	72.4	72.1	78.8	76.7	82.5	86.8	91.7*	73.8	81.2	64.6$^{\oplus}$
Bunch structure indicator	7.11	9.24*	7.63	6.33	6.49	6.56	7.11	5.80$^{\oplus}$	6.30	7.12	7.66	6.77
Sugar content	24.2	22.8	25.2*	24.9	25.2	23.7	21.8	21.8	21.0	20.8	19.9	17.6$^{\oplus}$
Acid content	7.2	7.9	7.2	8.0	7.2	8.1	7.2	6.3$^{\oplus}$	7.7	9.0	8.7	9.9*
pH	2.54	2.55	2.51$^{\oplus}$	2.59	2.68	2.64	3.06	2.99	3.07*	2.97	2.92	2.88

*Maximum values; $^{\oplus}$minimum values.

The same trend of variation was determined for average number of berries in the bunch, weight of stem and the weight of all berries in bunch. Mechanical composition of grape and berry represents ampelographic and technological characteristic of each variety. This elements significantly affect technological properties of cluster and eventually physical and chemical properties of wine (Blackford et al., 2021). Through these indicators can be made conclusion about oenological potential of variety or clone, bearing in mind that these elements are important for synthesis and accumulation of polyphenols (Pržić, 2015). A higher percent of berry epidermis was recorded for clone 169 in control treatment of fertilization with KAN (7.63%), while slightly lower values were found in treatment with AN. The lowest value of percent of berry epidermis was determined for AN 50 (4.28%). Clone 191 had higher values for all three KAN treatments for % of mesocarp in berry, while in all three AN treatments had the highest values of % seeds in the berry.In treatment with AN 100 for clone 191, the highest values of berry indicator (91.6) were determined, while for the AN treatment 50 for clone 169, was determined the lowest value of this indicator (70.6). Sugar content in must varied from 17.6–23.2% (AN 100) and 20.8–25.2% (KAN 100). According to total acids content expressed as tartaric acid, clone 191 figure in treatment of fertilization with KAN. Acording to Marković et al. (2017) and Keller (2020), acid content varies widely, which leads to the conclusion that acid content was influenced by treatment fertilization with KAN (treatments with 50 and 100). In AN treatments was found a lower content of total acids expressed as tartaric. This variation trend was founded for both clones. Clone 169 had lower pH must values in all AN and KAN fertilization treatments.
Gontier et al. (2011), in an experiment carried out in three field cases, comparing chemical weeding with natural grass and sown grass, observed reduction of vigor and yield, fall in nitrogen content of the must and increase in sugar and polyphenolic content using cover crops. However, those effects were not the same on all the experimental fields (water reserve of the soil, variety) and on all the studied treatments (species sown). Muscas et al. (2016) report that legume mixture reduces bunch weight, whereas grass mixture affect lower bunch weight and number. Also, grass mixture increase sugar content, content of anthocyanina and other ploypheno ls, whereas legume mixture reduce content of this components. Marques et al. (2020) report that that cover crop reduce vine vigor, does not affect yield and increase grape quality present throe total anthocyanins content.

Conclusions

Based on the research results, it can be concluded that the vineyard inter-row grass cover gave different yields of fresh and dry biomass, as well as grass quality was different in clones. The applied of different levels of mixture fertilization did not significantly affect the cover in individual clones. On the other hand, the parameters of the mechanical grape composition depended on clone, but also on the levels and types of applied nutrients to mixture in the inter-row distance. Namely, in clone 169, by applying 50 kg of AN on the mixture, the largest width, length and weight of the bunch, the largest number and weight of berries are achieved. Chemical characteristics of grapes depend on clone and fertilization. Sugar content is higher in clone 169, while the acid content is highest in clone 191.

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References

Blackford, M., Comby, M., Zeng, L., Dienes-Nagy, Á., Bourdin, G., Lorenzini, F., Bach, B. (2021). A Review on Stems Composition and Their Impact on Wine Quality. Molecules. 26(5):1240.

Cataldo, E., Salvi, L., Mattii, G.B. (2021). Effects of irrigation on ecophysiology, sugar content andthiol precursors (3-S-cysteinylhexan-1-ol and 3-S-glutathionylhexan-1-ol) on *Vitis vinifera* cv. Sauvignon Blanc. Plant Physiol Biochem. 164:247-259.

Celette, F., Wery, J., Chantelot, E., Celette, J., Gary, C. (2005). Belowground interactions in a vine (*Vitis vinifera* L.) tall fescue (*Festuca arundinacea* Shreb.) intercropping system: water relations and growth. Plant Soil 276, 205–217.

Celette, F., Gaudin, R., Gary, C. (2008). Spatial and temporal changes to the water regime of a Mediterranean vineyard due to the adoption of cover cropping. European Journal of Agronomy, 29(4), 153–162.

Gontier, L., Dufourcq, T., Gaviglio, C. (2011). Total grass cover in vineyards: an innovating and promising soil management alternative to reduce the use of herbicides. In: 17th international GiESCO Symposium, August 29th–September 2th, pp. 95–98.

Ingels, C.A., Scow, K.M., Whisson, D.A., Drenovsky, R.E. (2005). Effects of cover crops on grapevines, yield, juice composition, soil microbial ecology, and gopher activity. Am. J. Enol. Vitic. 56, 19–29

Keesstra, S., Nunes, J., Novara, A., Finger, D., Avelar, D., Kalantari, Z., Cerda, A. (2018). The superior effect of nature based solutions in land management for enhancing ecosystem services. Sci. Total Environ. 610–611, 997–1009

Keller, M. (2020). *The science of grapevines*, Elsevier.

Marković, N., Pržić, Z., Rakonjac, V., Todić, S., Ranković-Vasić, Z., Matijašević, S., Bešlić, Z. (2017). Ampelographic characterization of *Vitis* cv "Prokupac" clones by multivariate analysis. Romanian Biotechnological Letters, Vol. 22(5): 12868-12875.

Marković, N., Pržić, Z., Todić, S., Ličina, V., Niculescu, M., Tešević, V. (2017). Variation of structural bunch and berries indicators and physico-chemical wine properties of Cabernet sauvignon cv. under influence of defoliation and harvest time in agroecological conditions of central Serbia. Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series Vol. XLVII 2017/2:176-187.

Marković, N., Pržić, Z. (2020). Grapevine growing technology. University of Belgrade Faculty of agriculture and Foundation of Holy Monastery Hilandar, Belgrade, Serbia.

Marques, M.J., García-Munoz, S., Munoz-Organero, G., Bienes, R., (2010). Soil conservation beneath grass cover in hillside vineyards under Mediterranean Climatic conditions (Madrid, Spain). Land Degrad. Dev. 21 (2), 122–131.

Marques, M. J., Ruiz-Colmenero, M., Bienes, R., Garcia-Diaz, A., Sastre, B. (2020). Effects of a Permanent soil cover on water dynamics and wine characteristics in a steep vineyard in the central Spain. Air, Soil and Water Research, 13:1-10.
Muscas, E., Cocco, A., Mercenaro, L., Cabras, M., Lentini, A., Porqueddu, C., Nieddu, G. (2017). Effect of vineyard floor cover crops on grapevine vigor, yield, and fruit quality and the development on the mealybug under a Mediterranean climate. Agriculture, Ecosystems and Environment, 237:203-212.
Napoli, M., Marta, A. D., Zanchi, C. A., Orlandini, S. (2017). Assesment of soil and nutrient losses by runoff under different soil management practices in an Italian hilly vineyard. Soli & Tilage Research, 168:71-80.
Pržić Z. (2015). Influence of partial defoliation on content of major aromatic and flavonoid complex compounds in grapes and wine. Doctoral dissertation. University of Belgrade Faculty of agriculture. Belgrade, Serbia.

FRUITING PATTERNS OF SOME STRAWBERRY CULTIVARS IN OPEN FIELD AND GREENHOUSE CONDITIONS

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Abstract

For the last few decades, the most crucial goal in strawberry cultivation has been to extend the harvest season. Day-neutral varieties are increasingly being used to extend the season. The study aimed to determine the fruiting patterns of some day-neutral and short-day strawberry cultivars grown in open field (OF) and greenhouse conditions (GH). Frigo plants of some day-neutral ('Monterey', 'Albion', 'Sweet Ann', 'San Andreas') and short-day ('Benicia', 'Fortuna', 'Rubygem', 'Festival', 'Camarosa', 'Amiga') strawberry cultivars were planted on 7-8th July 2014 on raised beds covered with silver-grey polyethylene mulch in plastic greenhouse and open field in Samsun, Black Sea Region, Turkey. In OF condition, harvest commenced in May and terminated in October. Day-neutral (DN) cultivars extended the harvest season up to October, two months longer than short-day cultivars. On the contrary to GH, there was no harvest of any variety in April in OF. In OF conditions, intensive harvest periods were May and June for all cultivars. In OF, all cultivars continued fruiting in July and August, but yield of day-neutral cultivars were higher; however, yield of short-day (SD) cultivars 'Fortuna', 'Rubygem' and 'Festival' were also remarkable. Generally, yields were not obtained from short-day cultivars in September in OF. In GH conditions, harvest commenced in April and continued until September with day-neutral and some short day cultivars 'Festival', 'Fortuna' and 'Rubygem' with insufficient yields, none of the cultivars had fruit in October. In GH, the intensive harvest period was May for almost all cultivars. However, Aril was intensive harvest time for 'Benicia', June for 'Festival' and 'Rubygem', April and June for 'Fortuna'. In general, there were no apparent differences between short-day and day-neutral varieties in terms of fruiting patterns in GH, however in June and July, it was noted that short-day cultivars had higher yield than day-neutral. Yield decreased gradually from the beginning of July to the end of August in all cultivars in GH.

Keywords: *Strawberry, Cultivar, Greenhouse, Open Field, Harvest Period, Yield.*

Introduction

Changing production systems, reduced chemical control options, and rising demand for summer-fall strawberries and high-quality strawberries drove strawberry breeding efforts to develop some cultivars to meet these diverse needs (Hokanson and Finn, 2000; Baruzzi and Faedi, 2002; Gu et al., 2017). It was found that high tunnels offer many advantages, including environmental modification, season extension, higher yields, quality improvement, crop protection from severe weather, and the ability to achieve premium prices compared to open field production (Carey et al., 2011). Growers are very interested in high tunnel berry production. Where land is expensive or limited and inclement weather makes production risky, growers are certainly aware of the value of the protected crop (Demchak and Hanson, 2011). The fruit yield (weight, average size and number) of june-bearing strawberry cultivars grown in the high tunnel was higher compared to the open field production system (Kadir et al., 2006). In open-field production systems, there are several studies highlighting the importance of environment for growing strawberry successfully. Temperatures above 29 °C encourage excessive crown development, which will potentially reduce overall plant growth, subsequent fruit size and fruit weight (Hellman and Travis, 1988; Kumakura and Shishido, 1994; Wang and Camp, 2000; Masaru et al., 2016). The strawberry industry in Turkey achieved an expansion and is progressing steadily. Turkey is the 4th largest strawberry production after China, USA and Mexico (FAO, 2019). Main production is provided from southern regions. In the coastal regions of Black Sea, where the weather is relatively cool in summer, cultivar adaptation experiments performed recently have had very promising results with cultivars producing fresh fruits in summer months (Kandemir, 2016; Mısır, 2016). Cultivar selection is one of the key factors to ensure success in strawberry production and selected cultivars should be able for fruiting in early or late season. Short-day cultivars have long been popular in many parts of the world (Pritts and Handley, 1998; Black et al., 2002), but these cultivars prevent growers from participating in the commercial strawberry market except for the short fruiting period of 4 to 6 weeks each year (Pritts and Handley, 1998). Day-neutral strawberry cultivars are less affected by day length and typically continue to flower as long as temperatures remain between 4-26 ºC, resulting in a substantially longer fruiting period compared with short-day plants (Pritts and Handley, 1998; Rowley et al., 2010). Furthermore, day-neutral plants produce ripe fruit 10 weeks after planting, not the following year as with short-day plants (Pritts and Handley, 1998), reducing the period of crop management before financial return (Bornt et al., 1998). The objectives of this study were to determine fruiting patterns of different strawberry cultivars grown in greenhouse and open field conditions and to assess their potential for early and summer-fall season strawberry production.

Material and Methods

The experiment was carried out during 2014-2015 in the greenhouses (GH) and open field (OF) production systems in the experimental area of Agriculture Faculty at Ondokuz Mayis University, Black Sea coastal area, Samsun, Turkey. Elevation at research station is 115 m; the soil was clayey with a soil pH of 6.96 and organic matter 2.29 % in open field and sandy with a soil pH of 7.41 and organic matter 5.43 % in greenhouse. The experiment was arranged as a completely randomized plots design with four replications and 20 plants per plot. Greenhouses sized 6x20x3 m and covered with 1 cm thick polycarbonate were used. To avoid from high temperature over 26 ˚C in greenhouses, passive top and side ventilation as well as shade cloth with 50% light transmittance were used. On 7-8 July 2014, strawberry plants of day-neutral ('Albion', 'Monterey', 'San Andreas', 'Sweet Ann') and short-day cultivars ('Camarosa', 'Amiga', 'Rubygem', 'Fortuna',' Festival') were planted on raised beds (0.70 m wide, 0.40 m high) covered with silver-grey polyethylene mulch. Each bed had two rows of staggered plants with a distance of 0.3 m between the rows and the plants. In each crop environment (greenhouse and open field), single drip lines per bed was used for irrigation. During the soil preparation, manure (0.5 t/ha) was applied to soil. Fertilization and pest control were achieved following current crop recommendations. The monitoring of the air temperature was carried out in each experimental area with data logger (Kistock KH 200), registering the information hourly. The blossomed flowers after planting were continuously removed until autumn. During harvest period, fruit was collected from each plot, two times per week. Monthly yields were calculated by dividing number of plants of fruit amount obtained each month.

Results and Discussion

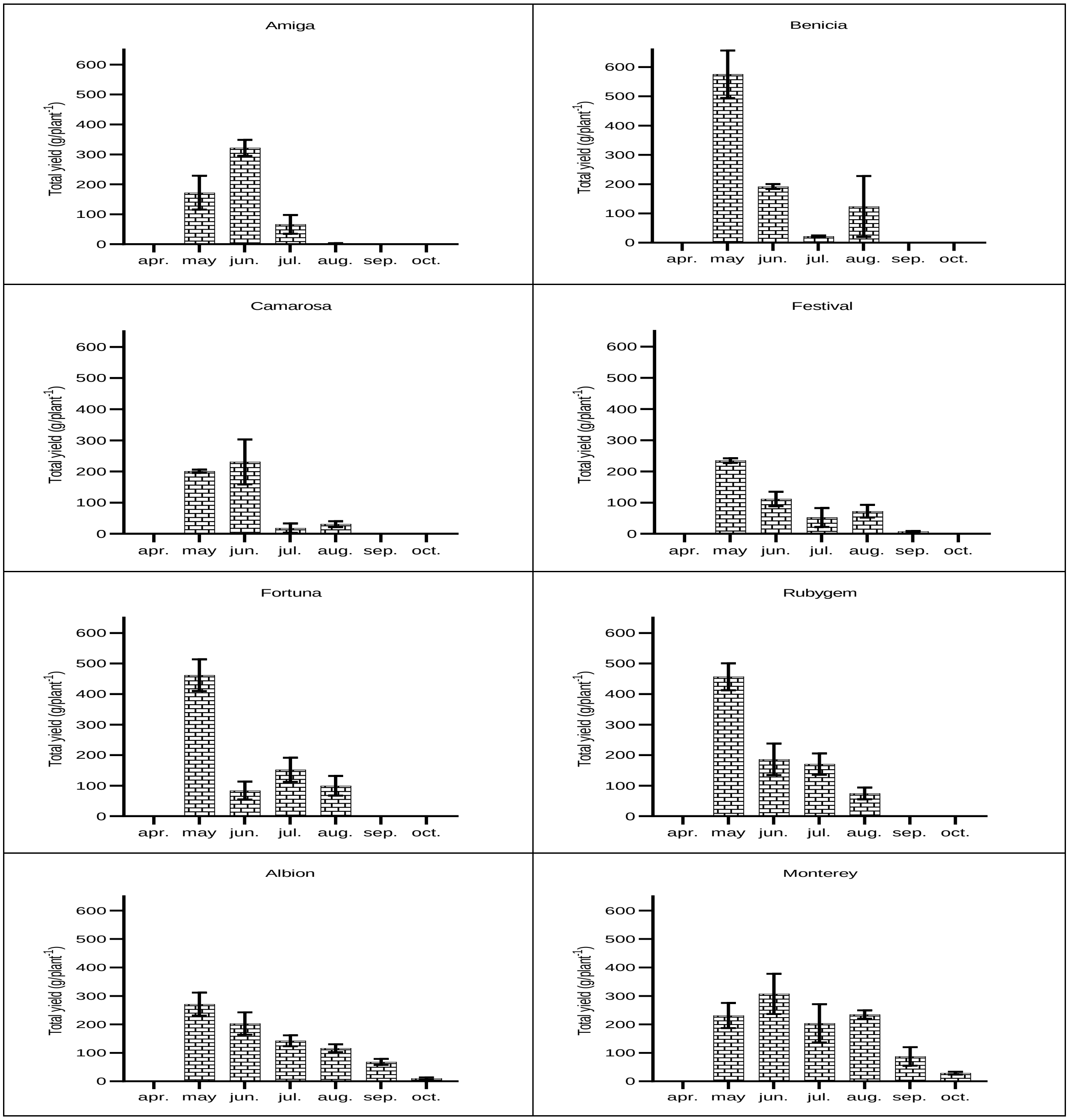
Open Field

In the open field condition, depending on the SD and DN strawberry variety, the harvest continued about 5-6 months from early May to early October. On the contrary to GH, there was no harvest of any variety in OF in April. There was also no harvest after August for all SD cultivars, except for the 1% yield in September at 'Festival' (Figure 1, Table 1). Finally SD cultivars performed 4 months of harvest period except 'Amiga' and 'Festival' (3 and 5 month, respectively), which is contrary to that SD cultivars producing fruit for several weeks in early summer (Solomon et al., 2001; Hoover et al., 2014). The cool summer temperatures in the Black Sea Region seem to have resulted in longer flowering and fruiting in the SD varieties, with the average temperatures during the 2015 harvest season being 16.2, 21.1, 24.7 and 24.4 ºC in May, June, July and August, respectively. Although the intensive harvest period of the SD varieties as general was May and June, as specifically intensive harvest time for 'Benicia' (68%), 'Festival' (48%), 'Fortuna' (58%) and 'Rubygem' (51%) was May, and for 'Amiga' (58%) was June (Figure 1, Table 1). These findings indicate that 'Benicia', 'Festival', 'Fortuna' and 'Rubygem' are early and 'Amiga' is late variety. Kandemir et al. (2019) confirmed this indication in their study. SD cultivars continued harvest season in July and August, however a remarkable yields were obtained from 'Fortuna', 'Rubygem', 'Festival' (totaly 31, 29, 28%) (Table 1).

Table 1. Monthly distribution of yield in strawberry cultivars grown under open field and greenhouse conditions (%).

	Open Field						Greenhouses					
Cultivar	May	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.	May	Jun.	Jul.	Aug.	Sep.
Amiga	30	58	11	1	-	-	13	48	27	10	2	-
Benicia	68	22	2	8	-	-	48	38	9	5	-	-
Camarosa	42	47	4	7	-	-	15	40	29	13	3	-
Festival	48	23	12	16	1	-	17	31	30	16	5	1
Fortuna	58	11	19	12	-	-	26	25	30	15	3	1
Rubygem	51	20	20	9	-	-	17	31	34	14	3	1
Albion	33	25	18	14	8	1	26	35	22	11	4	2
Monterey	21	28	19	20	8	4	17	42	20	13	7	1
San Andreas	35	28	14	15	8	-	29	38	19	11	2	1
Sweet Ann	13	42	17	22	4	2	9	54	17	17	2	1

No yield was obtained from SD cultivars in September, except very insignificant yield of 'Festival' (1%).Harvest period was longer about 2 month in DN cultivars ('Albion', 'Monterey', 'San Andreas' and 'Sweet Ann') than in SD cultivars (Figure 1). In addition, while the intensive harvest period of 'Albion', 'San Andreas', 'Monterey' DN cultivars was May and June (totaly 58, 63 and 49%, respectively), main harvest period for 'Sweet Ann' was June. This suggests that 'Sweet Ann' is a late-flowering cultivar. Kandemir et al. (2019) found that 'Sweet Ann' is the latest variety among ten SD and DN examined varieties. The yields in July and August of all DN cultivars (totally 32% for 'Albion', 39% for 'Monterey', 29% for 'San Andreas', 39% for 'Sweet Ann') were significantly higher compared to SD varieties. Yield sharply declined in September in all DN cultivars, 'San Andreas' terminated harvest period in the end of September. A negligible yield were recorded in October from 'Albion', 'Monterey' and 'Sweet Ann'.



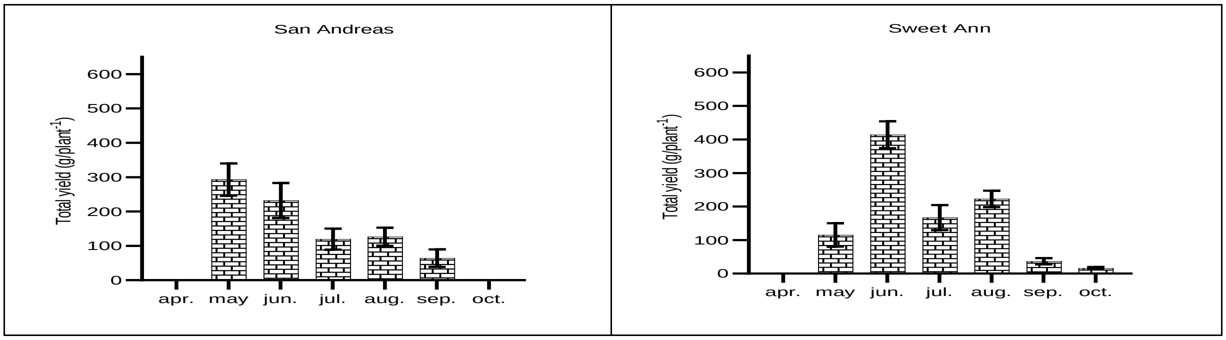


Figure 1. Effect of cultivar on monthly yield under open field conditions during 2014-15 cropping season. Vertical lines through data points are standard errors.

DN cultivars bear fruit in a short period of 3-4 months, they are suitable for summer and autumn fruit production in areas having cool summers (Galletta and Bringhurst, 1990). In mild climate, it was reported that DN cultivars 'Albion' and 'San Andreas' produced fruit up to 2.42 and 3.29 kg/plant, respectively, in a 6-month harvest from April to October in Korea (Ruan et al., 2011). Reitmeier and Nonnecke (1991) reported that DN strawberries have potential to produce fruit throughout growing season, which increases the harvesting periods, potential to increase yields, and income to the producer as it provides the opportunity to grasp off-season price.

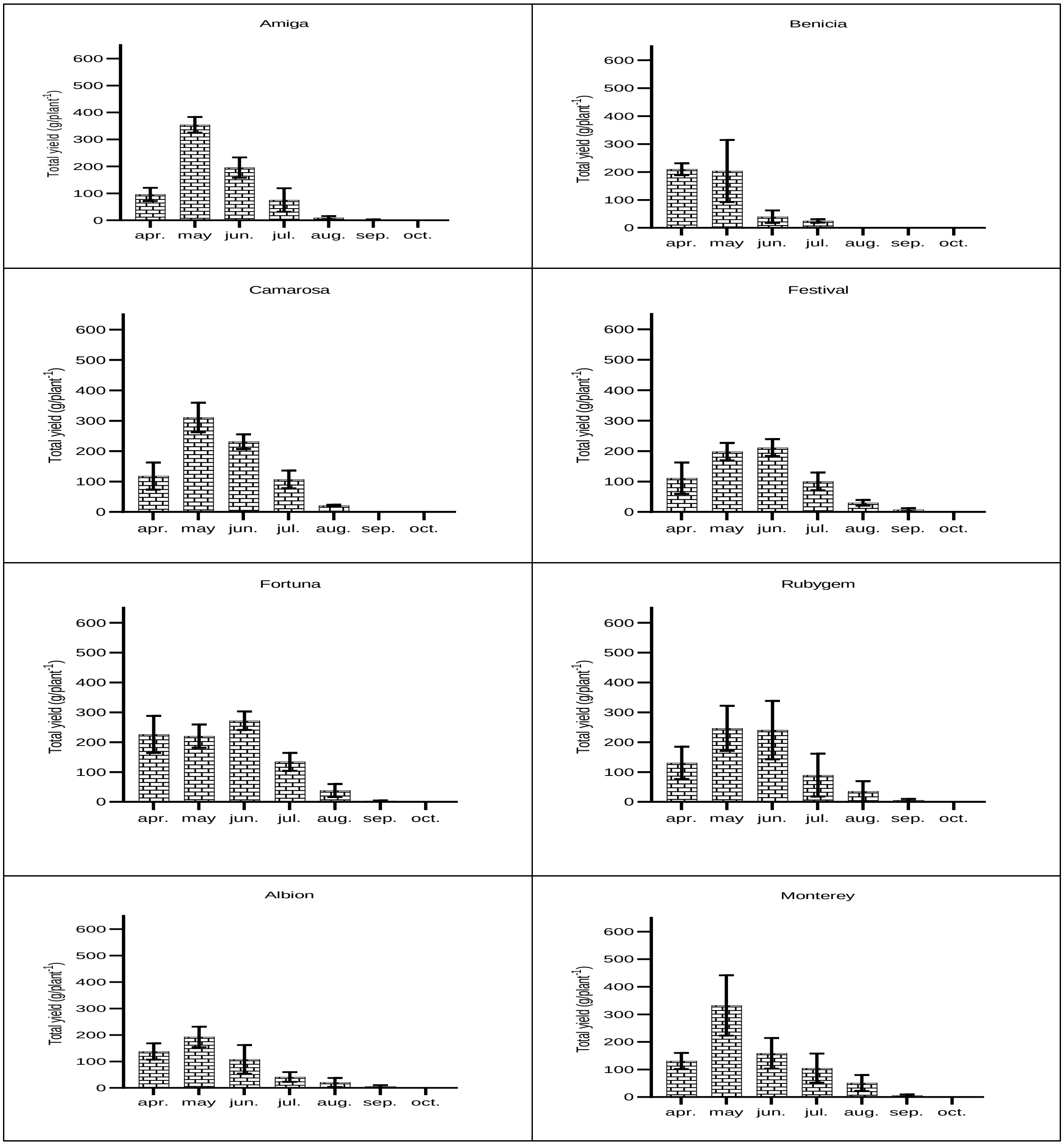
Greenhouse

During the early spring, warm temperatures in GH provided early plant growth, flowering and fruit production of all strawberry cultivars. During the spring, temperatures were 11.0, 12.6, 16.1, 20.5 °C in GH, 8.5, 8.7, 9.9, 16.2 °C in OF, respectively for February, March, April and May. As a result, the harvest in GH started one month earlier than in OF, at the beginning of April. Kadir et al. (2006) found that high tunnel strawberries were 5 weeks earlier than field grown strawberries in Kansas. Many researchers reported earliness of strawberry varieties grown in greenhouse conditions (Önal, 2000; Gündüz and Özdemir, 2003; Öztürk and Demirsoy, 2004; Gündüz and Özdemir, 2008; Crespo, 2010; Rowley et al., 2010; Gündüz and Özdemir, 2012; Kandemir et al., 2019).

Depending on the variety, harvest in GH commenced in April and lasted about 5-6 months through September. The intensive harvest period of almost all cultivars was May, however specifically intensive harvest time was April (48%) and May (38%) for very early cultivar 'Benicia'; May (31, 31%) and June (30, 34%) for 'Festival' and 'Rubygem', respectively; April (26%), May (25%) and June (30%) for 'Fortuna' (Table 1, Figure 2). In April, 'Sweet Ann' and 'Amiga', the late varieties, produced the lowest yields, while the yields of other varieties in April were sufficient. Kandemir et al. (2019) reported the latest harvest dates for this two cultivar among ten strawberry cultivars in GH condition. Late harvest explains their low yields in April in our study. Also, it is noteworthy that remarkable yields in June and July were obtained from SD cultivars except 'Benicia', compared to DN cultivars 'Monterey', 'Albion', 'Sweet Ann' and 'San Andreas'.

Considering all cultivars, SD and DN, their yields decreased except 'Sweet Ann' in July. Yields of 'Sweet Ann' in June and July were identical. 'Benicia' terminated the harvest period in July. Compared to the OF, harvest in GH terminated early in 'Benicia', likely due to its peculiarity that it is an early variety. In the GH, yields also were low in Benicia due to severe black root rot (Kandemir, 2016). In August, most of cultivars considerably decreased yield, simultaneously

'Amiga' and 'Camarosa' terminated harvest period. All DN varieties extended the harvest period into September, however, their yields were insufficient. Also low yields were obtained from SD cultivars 'Festival', 'Fortuna' and 'Rubygem' in September. In GH, unlike in OF, none of the examined cultivars had fruit in October.



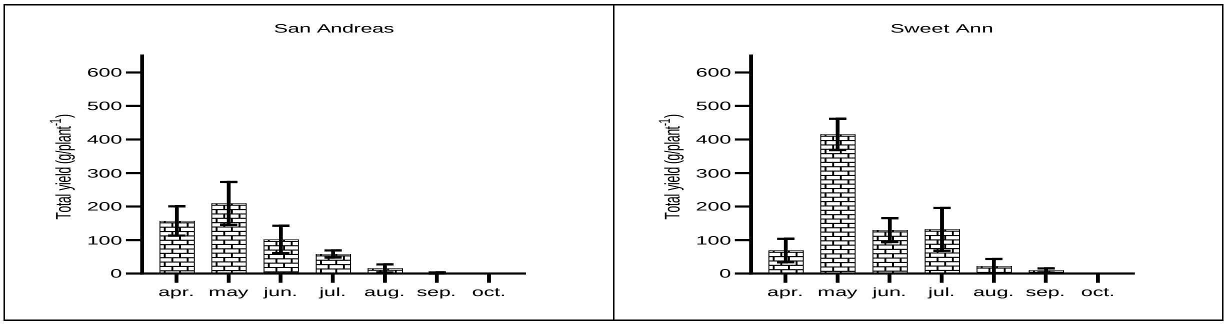


Figure 2. Effect of cultivar on monthly yield under greenhouse conditions during 2014-15 cropping season. Vertical lines through data points are standard errors.

Conclusions

The length of the strawberry harvest season has gained importance in recent years. Turkey has just encountered the demand for locally produced off-season strawberries. In this regard, inner regions with high altitude and Northern areas seem promising. However, using proper DN and SD strawberry cultivars is a key factor to extend the harvest season and obtain performance of high-yield in northern parts of the country where growing conditions are more challenging. In our study harvests commenced in April (GC) and May (OF), continued until September (GH) and October (OF) respectively, mainly with DN cultivars. However, in both growing conditions 'Festival', 'Rubygem', and 'Fortuna' appeared to be suitable SD varieties for extending the harvest season. This result is explained by fact that in Black Sea Region weather conditions are ideal for harvest period extension (early and out-season) with DN and some SD cultivars in combination with GH conditions. Average temperatures below 26 ºC in summer in Black Sea Region seem to encourage flowering and fruiting in DN and SD varieties in summer.

Acknowledgement

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References

Baruzzi, G., Faedi, W. (2002). Innovazioni nelle techniche di coltivazione della fragola. Rivista di frutticoltura e di ortofloricoltura (Innovations in strawberry cultivation techniques). Journal of fruit growing and horticulture, 64(6), 19-28.

Black, B.L., Enns, J.M., Hokanson, S.C. (2002). A comparison of temperate-climate strawberry production systems using eastern genotypes. HortTechnology, 12, 670-675.

Bornt, C.D., Loy, J.B., Lord, W.G., Wells, O.S. (1998). Annual strawberry production in New England, National Agricultural Plastics, 27:99–105.

Carey, R., Krumholz, F., Duignan, K., McConell, K., Browne, J. L., Burns, C., Lawrence, M. (2011). Integrating agriculture and food policy to achieve sustainable peri-urban fruit and vegetable production in Victoria, Australia. Journal of Agriculture, Food Systems, and Community Development, 1(3), 181-195.

Crespo, P. V. A. S. (2010). Variability of health and taste promoting compounds in strawberry (Fragraria x ananassa) fruits (Doctoral dissertation, ETH Zurich).

Demchak, K., Hanson, E. J. (2011, October). Small fruit production in high tunnels in the US. In International Symposium on High Tunnel Horticultural Crop Production 987 (pp. 41-44).

FAO (2019). (http://www.fao.org/faostat/en/?#data/QC).

Galletta, G. J., Bringhurst, R. S. (1990). Strawberry management. In: Small Fruit Crop Management. Prentice-Hall Inc., New York, USA. 83–156.

Gu, S., Guan, W., Beck, J. E. (2017). Strawberry cultivar evaluation under high-tunnel and organic management in North Carolina. HortTechnology, 27(1), 84-92.

Gündüz, K., Özdemir, E. (2003). Yüksek tünel ve açıkta yetiştirilen çileklerde meyve kalite sınıfları (Fruit quality categories in high tunnel and open-grown strawberries). Bahçe, 15(2), 10 – 15.

Gündüz, K., Özdemir, E. (2008). The effect of some strawberry cultivars cultivated in field and high tunnel in Amik plain conditions for earliness, yield and quality. Mustafa Kemal University, Agriculture Faculty, 13(1/2), 35-42.

Gündüz, K., Özdemir, E. (2012). Farklı yetiştirme yerlerinin bazı çilek genotiplerinin erkencilik indeksi, verim ve meyve kalite özellikleri üzerindeki etkileri (The effects of different production places on earliness index, yield and fruit quality characteristics of some strawberry genotypes). Journal of Agriculture Faculty of Ege University, 49(1), 27-36.

Hellman, E. W., Travis, J. D. (1988). Growth inhibition of strawberry at high temperatures. Advances in strawberry production (USA).

Hokanson, S. C., Finn, C. E. (2000). Strawberry cultivar use in North America. HortTechnology, 10(1), 94-106.

Hoover, E. E., Tepe, E. S., Martin, E. F., Wold-Burkness, S., Petran, A., Poppe, S., Kildegaard, A. (2014). Developing an e-book to teach cold climate strawberry farming. In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): II 1117 pp. 297-302.

Kadir, S., Carey, E., Ennahli, S. (2006). Influence of high tunnel and field conditions on strawberry growth and development. HortScience, 41(2), 329-335.

Kandemir, A. (2016). Bazı çilek çeşitlerinin örtü altında performanslarının belirlenmesi (Determining of performance of some strawberry cultivars in plastic greenhouse). Master thesis, Ondokuz Mayis University, Institute of Science, Samsun.

Kandemir, A., Mısır, D., Demirsoy, L., Soysal, D., Demirsoy, H. (2019). Harvest season and some fruit quality characteristics of strawberry under protected and open field conditions. In XXX International Horticultural Congress IHC2018: III International Berry Fruit Symposium 1265 pp. 195-202.

Kumakura, H., Shishido, Y. (1994). The effect of daytime, nighttime, and mean diurnal temperatures on the growth of'Morioka-16'strawberry fruit and plants. Journal of the Japanese Society for Horticultural Science, 62(4), 827-832.

Masaru, S., Uenishi, M., Miyamoto, K., Suzuki, T. (2016). Effect of root-zone temperature on the growth and fruit quality of hydroponically grown strawberry plants. 8(5). doi:10.5539/jas.v8n5p122.

Mısır, D. (2016). Bazı çilek çeşitlerinin adaptasyonu (Adaptation of some strawberry cultivars). Master thesis, Ondokuz Mayis University, Institute of Science, Samsun.

Önal, K. (2000). Investigations on the performance of some strawberry sultivars (Fragaria x AnanassaDuch.) grownin open fields and under high tunnel conditions. Turkish Journal of Agriculture and Forestry, 24(1), 31-36.

Öztürk, A., Demirsoy, L. (2004). Değişik gölgeleme uygulamalarının Camarosa çilek çeşidinde verim ve büyüme üzerine etkileri (The effects of different shading treatments on yield and growth in Camarosa strawberry variety). Bahçe, 33(1).

Pritts, M., Handley, D. (1998). Strawberry production guide. Northeast Regional Agricultural Engineering Service, 88.

Reitmeier, C. A., Nonnecke, G. R. (1991). Objective and sensory evaluation of fresh fruit of day-neutral strawberry cultivars. HortScience, 26(7), 843-845.

Rowley, D., Black, B. L., Drost, D., Feuz, D. (2010). Early-season extension using June-bearing 'Chandler'strawberry in high-elevation high tunnels. HortScience, 45(10), 1464-1469.

Ruan, J., Yeoung, Y. R., Larson, K. D. (2011). Influence of cultivar, planting date, and planting material on yield of day-neutral strawberry cultivars in highland areas of Korea. Horticulture, Environment, and Biotechnology, 52(6), 567-575.

Solomon, M. G., Jay, C. N., Innocenzi, P. J., Fitzgerald, J. D., Crook, D., Crook, A. M., Cross, J. V. (2001). Natural enemies and biocontrol of pests of strawberry in northern and central Europe. Biocontrol Science and Technology, 11(2), 165-216.

Wang, S. Y., Camp, M. J. (2000). Temperatures after bloom affect plant growth and fruit quality of strawberry. Scientia Horticulturae, 85(3), 183-199.

THE EFFECT OF CLIMATE CHANGE AND THE INFORMATION SOURCES USED IN THE ENTERPRISES PRODUCING SUNFLOWERSEED

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Abstract

Sunflower, a member of the Asteraceae family; It is one of the world's strategic products. In the world, oilseed production is of great importance due to the economic value it creates. For this reason, it is very important for countries that the seed sunflower is affected by climate change. The increasing impact of climate change on agricultural production has a wealth of literature focusing on the potential value of weather and climate information to help farmers prepare and respond to these impacts. However, while the impact of climate change on the agricultural sector is growing, there is no clear de facto practice to reduce this impact. In this study, the effect of climate change and the information sources used on climate change in the enterprises producing Sunflower seed were examined. The sample volume was calculated as 62 with a 99% Confidence Interval and 10% error margin for the stratified sampling method, which was one of the simple random sampling methods. The effect of climate change on sunflower production was investigated. As a climate change effect, 1st group enterprises showed drought (4.54), 2nd group enterprises showed insufficient precipitation (441) and 3rd group enterprises showed drought (4.65). The information source used on climate change is the internet (4.36). The internet, agricultural engineers, pesticide dealers, leading farmers, visual and written media should be used to raise awareness about climate change for businesses producing seed sunflowers.

Keywords: *Sunflower, Climate Change, İnformation Sources.*

Introduction

Oilseed production is of great importance due to the economic value it creates (Acaravcı and Ergüven, 2015). Oilseeds, which are the raw material of the vegetable oil sector, are also the raw material of many different sectors (Gürkan et al., 2016). Oilseed meal, which has a very high protein content, is also preferred in animal nutrition (İlkdoğan 2008). Vegetable oils are strategic products that are used extensively in the food, energy, and chemical sectors (Taşkaya Top and Uçum, 2012). For this reason, it is very important that seed sunflower production is affected by climate change. The effects of climate change on agriculture are likely due to many other subtle changes, possibly from the effects of increased CO2 on plant growth, warmer and drier conditions, changes in wind speed, insect and plant disease pressures, and altered interactions between crops components. agro-ecosystems (Smith & Almaraz, 2004); however, effects that cause crop yield losses have often been overlooked (Lim et al., 2004, Scherm, 2004). The increase in CO2 and other greenhouse gases has had serious effects on global climate systems (Koocheki et al., 2006). It is likely to cause a number of new environmental changes, predominantly through anthropogenic activities (Allen, 1990; IPCC 2001; McMichael, 2001, Centritto & Loreto 2005). Increasing CO2 and thus temperature are likely to affect all aspects of plant growth, development, and function differently depending on plant species and geographic location (Centritto & Loreto, 2005). The increasing impact of climate change on agricultural production has generated rich literature focusing on the potential value of weather and climate information to help farmers prepare and respond to these impacts (Solis and Letson, 2013). In this context, calls have been made for a stronger extension role to support climate change adaptation in all areas, particularly in the agriculture sector (Brugger and Crimmins, 2014). But while the impact of climate change science on agriculture is growing and becoming more robust (Hatfield et al. 2014), the evidence for the actual application of extension studies to readiness and adaptation is low (Prokopy et al., 2013; Haigh et al., 2015; Lemos et al., 2014). In this context, organizations that act as intermediaries between climate-related information producers and users have an important role in raising awareness of climate change (Lemos et al., 2012; Prokopy et al., 2015). For this reason, the effect of climate change and the information sources used on climate change in enterprises producing seed sunflowers were examined in this study.

Material and Method

The main material of the study; The original data collected through a questionnaire from the enterprises producing sunflowers in the Çumra district of Konya province in Turkey, which was selected as the research region, constitutes the year 2020. In addition, previous research on the subject and the data of the relevant institutions and organizations were also used. In the research, the Stratified Random Sampling Method, which is one of the simple random sampling methods, was used in order to increase the accuracy of the data collected from the enterprises and to ensure adequate representation of different parts of the population. (Yamane, 1967; Güneş and Arıkan, 1988). Layer widths are grouped as 0-20 decare, 21-60 decare, 61 decare, and above. Sample volume was calculated as 62 at 99% Confidence Interval, with 10% margin of error, according to the Neyman Method. (Yamane, 1967).

$$n = \frac{\sum(Nh.Sh)^2}{N^2.D^2 + \sum Nh.(Sh)^2}$$

$$D^2 = (\frac{d}{t})^2$$

n: Number of samples,
N: Total unit number belonging to the sampling frame,
Nh: Number of enterprises in layer h,
Sh: Standard deviation of layer h,
d: Allowable margin of error from the population average,
t: t table value corresponding to the anticipated 99% confidence limit (Yamane, 1967).
The distribution of sample volume by business groups is given in Table 1.

Table 1. Sample volume by enterprises size groups in the research area (n)

Group	Sample Volume (n)
0-20 da (1. Group)	13
21-60 da (2. Group)	32
61-+ da (3. Group)	17
Total	62

Results and Discussion

The average land amount of the enterprises is 209.64 decares. 98.98% of this land belongs to enterprises and 1.02% is rented land. The main reason why most of the lands are property lands is the habit of the landowners in our country to inherit their lands from father to son. When we look at the production pattern table of the enterprises, we have considered 5 different products that the enterprises generally produce. These products are corn, sugar beet, sunflower, wheat, and barley. Among these products, corn ranks first with a rate of 25.47%. Sugar beet is the second crop with 24.31% and sunflower is the third crop with 24.23%.

In addition to machine power, human power is also very important in sunflower cultivation in the research area. The workforce presence of the enterprises was 3.02-2.64-2.62 MLA, respectively, and the family workforce potential was also found to be 846.3-739.62-732.76, respectively. The average of the workforce used was calculated as 2.71 MLA and 760.11 MLD. In addition to family labor, foreign labor was also used in the enterprises. The foreign labor force employed was calculated as 617,17. The total workforce used in the enterprises was calculated as 1,588.14 MLD.

As a result of the survey studies, corn provided the highest return of 44,386.57 $ and 36.60% of the 5 main products that are the source of income for the enterprises. While sugar beet constituted 26.96% of all production with 32696.65 $, sunflower, which is the subject of the article and contracted in the region, provided the operators with the third return with 25,075.10 $ and a share of 20.67%. These are followed by wheat with a share of 17,024.27 $ and 14.04%, and barley with a share of 2,107.52 $ and 1.74%.

In line with the results prepared with the data obtained by calculating the variable costs such as Seed, Fertilizer, Medicine, Fuel, Tools, Equipment, Money, Water and Electricity fees included in the survey, the costs naturally increase as the decare increases. As a result of the survey, it is seen that Sugar beet is the product with the highest variable cost among the enterprises with 6,979.49 $ and a share of 35.46%. Corn crop, on the other hand, takes second place with 5698.04 $ and 28.95% share. Sunflower is in third place with a share of 4.244.20 $ and 21.57%, and the seed cost is 0 TL because the sunflower is contracted here.

The average of the processing average of the gross production value of the contracted sunflowers is 25075.10 $ and the variable costs are 4,244.20 $. By subtracting these values, gross profit is obtained. Its gross net profit is 20,830.90 $. Gross profit per decare is 289.69 $.

Table 2. Climate change impact on enterprises

Variables	0-20	21-60	61-+	Enterprises Average
Drought	4.54	4.38	4.65	4.48
Untimely Rain	4.46	4.34	4.53	4.42
Insufficient Precipitation	4.46	4.41	4.41	4.42
Wind Storm	2.69	2.97	3.00	2.92
Frost	2.54	2.78	2.76	2.73
Full	2.31	2.31	2.41	2.34
Excessive Precipitation	1.00	1.00	1.00	1.00

5: Always 4: Often 3: Sometimes 2: Rarely 1: Never

The effect of climate change is frequently seen as drought, untimely rain, and insufficient precipitation in the enterprises producing seed sunflower. As an effect of climate change, drought is seen frequently in Group 1 enterprises (4.54), insufficient precipitation in Group 2 enterprises (4.41), and always Regular Precipitation in Group 3 enterprises (4.65).

Table 3. Information sources used by enterprises on climate change

Variables	0-20	21-60	61-+	Enterprises Average
Agricultural Engineers	3.71	4.86	3.69	4.29
Television	4.00	3.90	3.15	3.68
Counselor	1.86	2.71	2.31	2.44
Agriculture Province And District	2.29	3.57	2.46	3.00
Veterinary	1.14	1.57	1.15	1.37
Agricultural Credit coop.	2.43	3.67	2.46	3.07
Internet	4.00	4.71	4.00	4.36
Book Magazine Brochure	2.57	4.24	2.69	3.46
Agriculture Fair	1.71	2.81	2.00	2.37
Technicians	2.14	2.62	2.15	2.39
Pesticide Dealer	4.00	4.19	4.43	4.23
University	2.00	2.52	1.92	2.24
Leader Farmers	2.29	4.81	3.00	3.80
Congress and symposium	2.00	2.71	2.15	2.41
Unions and Cooperatives	3.00	4.05	2.69	3.44

5: Always 4: Often 3: Sometimes 2: Rarely 1: Never

The source of information used on climate change is the internet (4.36). The most widely used information sources in the 1st group businesses are the internet (4.00), television (4.00) and pesticide dealers (4.00). In the 2nd group enterprises, agricultural engineers (4.86), leading farmers (4.81) and the internet (4.71). In the third group enterprises, pesticide dealers (4.43), internet (4.00) and agricultural engineers (3.69). The internet, agricultural engineers, pesticide dealers, leading farmers, visual and written media should be used to raise awareness about climate change for businesses producing seed sunflowers.

Results and Discussion

In the research area, 98.98% of the total land area of 208.85 da of enterprises is private property. Sunflower is produced on 65.49% of the total farm land. 61.14% of the workforce used in businesses is family workforce. Sunflower is a very important product for the research region and all members of the family are involved in production. For this reason, it is necessary to raise awareness of all family members, taking into account the intensity of the family workforce, that is, those who work on climate change. In the research area, climate change is often seen as drought, untimely precipitation and insufficient precipitation. Sunflower is one of the irrigated crops. It is highly affected by drought, untimely rain and insufficient rain and decreases in yield occur. In this case, it affects the socio-economic structures of the farmers. In order to reduce the impact of climate change in enterprises producing seed sunflowers, it should be ensured that the necessary warnings are made in a timely manner. When looking at the information sources used by the farmers, the internet is used by all groups. In addition, agricultural engineers, drug dealers, leading farmers, visual and written media should be used.

References

Acaravcı S.K., Ergüven O.C. (2015). Yağli Tohumlar Ve Bitkisel Yağ Sektörünün Finansal Analizi: Hatay İlinde Bir Uygulama. Journal of Mustafa Kemal University Institute of Social Sciences, *12*(29), 258-282.

Allen L.H. (1990). Plant response to rising carbon dioxide and potential interactions with air pollutants. J Environ Qual 19:15–34

Brugger J., Crimmins M. (2014). Designing institutions to support local level climate change adaptation: insights from a case study of the U.S. Cooperative Extension System. Weather, Climate, and Society

Centritto M., Loreto F. (2005). Photosynthesis in a changing world: photosynthesis and abiotic stresses. Agric Ecosyst Environ 106:115–117

Güneş T., Arıkan R. (1988). Tarım Ekonomisi İstatistiği, A. Ü. Z. Faculty Publications, No: 1049. textbook, *305*.

Gürkan H., Bayraktar N., Bulut H., Koçak N., Eskioğlu O., Demircan M. (2016). İklim Değişikliğinin Ayçiçeği (Helianthus Annuus L.) Verimine Olasi Etkilerinin İncelenmesi: Marmara Bölgesi Örneği. Vii. National Agricultural Economics Congress, Isparta.

Haigh T., Morton L.W., Lemos M.C., Knutson C., Prokopy L.S., Lo Y.J., Angel J. (2015). Agricultural advisors as climate information intermediaries: Exploring differences in capacity to communicate climate. *Weather, Climate, and Society*, *7*(1), 83-93.

Hatfield J., Takle G., Grotjahn R., Holden P., Izaurralde R.C., Mader T., Marshall E., Liverman D. (2014). Ch. 6: Agriculture. Climate change impacts in the United States: The third national climate assessment. In: J. M. Melillo, T. C. Richmond, and G. W. Yohe, editors, U.S. Global Change Research Program, p. 150–174.

İlkdoğan U. (2008). Dünya ve Avrupa Birliği'nde Yağlı Tohum Ticaretinde Gelişmeler Türkiye Bağlamında Değerlendirme. EU Specialization Thesis, Ministry of Agriculture and Rural Affairs, Department of Foreign Relations and European Union Coordination, 128, Ankara.

IPCC (Intergovernmental Panel on Climate Change) (2001). Climate Change 2001: the scientific basis. IPCC Third Assessment Report, Geneva

Koocheki A., Nassiri M., Soltani A., Sharifi H., Ghorbani R. (2006). Effects of climate change on growth criteria and yield of sunflower and chickpea crops in Iran. *Climate Research*, *30*(3), 247-253.

Lemos M.C., Kirchhoff C.J., Kalafatis S.E., Scavia D., Rood R.B. (2014). Moving climate information off the shelf: boundary chains and the role of RISAs as adaptive organizations. Weather Clim Soc 6: 273–285.

Lemos M.C., Kirchhoff C.J., Ramprasad V. (2012). Narrowing the climate information usability gap. *Nature climate change*, *2*(11), 789-794.

Lim C., Kafatos M., Megonigal P. (2004). Correlation between atmospheric CO2 concentration and vegetation greenness in North America: CO2 fertilization effect. Clim Res 28:11–22

McMichael A.J. (2001). Impact of climatic and other environmental changes on food production and population health in coming decades. Proc Nutr Soc 60:195–201

Prokopy L.S., Carlton J.S., Arbuckle J.G., Haigh T., Lemos M.C., Mase A.S., Power R. (2015). Extension′ s role in disseminating information about climate change to agricultural stakeholders in the United States. *Climatic Change*, *130*(2), 261-272.

Prokopy L.S., Haigh T., Mase A.S., Angel J., Hart C., Knutson C., Lemos M.C., Lo Y., McGuire J., Wright Morton L., Perron J., Todey D., Widhalm M. (2013). Agricultural advisors: a receptive audience for weather and climate information? Weather Clim Soc 5(2):162–167

Scherm H. (2004). Climate change: can we predict the impacts on plant pathology and pest management? Can J Plant Pathol 26(3):267–273

Smith D.L., Almaraz J.J. (2004). Climate change and crop production: contributions, impacts, and adaptations. Can J Plant Pathol 26(3):253–266

Solis D., Letson D. (2013). Assessing the value of climate information and forecasts for the agricultural sector in the Southeastern United States: multi-output stochastic frontier approach. Reg Environ Chang 13(1):S5–S14

Taşkaya Top B., Uçum İ. (2012). Türkiye'de Bitkisel Yağ Açığı. Institute of Agricultural Economics and Policy Development. TEPGE Publications.

Yamane T. (1967). Elementary sampling theory. Englewood Cliffs, New Jerse: Prentice-Hall Inc.

ROLE OF DIFFERENT PHOSPHORUS LEVELS ON THE SEED YIELD AND SOME YIELD COMPONENTS OF CLUSTER BEAN (*Cyamopsis tetragonoloba* (L.) Taub.)

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Abstract

Phosphorus (P) is responsible for a number of functions which its major importance to physiological and biochemical function of plant in sustainable agriculture system. However to maximize metabolic functions, legumes need more phosphorus as it is required for energy transformation in nodules. Therefore, an experiment was conducted to understand the yield and phosphorus demand of cluster bean. This trial was arranged as a completely randomized block design with four replications from March to July of 2020 in the outdoor conditions at Izmir, Turkey. An application of phosphorus influences symbiotic nitrogen fixation yield and quality of cluster pods. For this reason, a pot experiment was carried out to investigate the effect of phosphorus levels (0, 60, 90, 120 and 150 kg P ha^{-1}) on the seed yield and some yield components of cluster bean (Pusa Nevbahar cv.). Results shown that rate of phosphorus has a significant effect on plant height, number of pod, biomass weight, seed yield and thousand grain weight of cluster bean. Application of phosphorus at the rate of 120 kg ha^{-1} can be recommended for obtaining the maximum seed yield in cluster bean under Mediterranean climate conditions.

Keywords: *Cluster bean, P level, seed yield.*

Introduction

Cluster bean or guar [*Cyamopsis tetragonoloba* (L.) Taub. (2n=14)] is a valuable crop belongs to the family *Leguminosae*. It is also thought to be developed from African species *Cyamopsis senegalensis* (Kumar and Rodge, 2012). Plant has a deep taproot system, 20-100 cm tall, erect and unbranched stems, trifoliate leaves, white or pink flowers, large and glabrous pods and a life cycle of 80 to 160 days (Chauhan et al., 2020). Guar is an annual legume that can be used for multiple purposes (fresh vegetable, animal feeding, green manure or cover crop) (Batirca et al., 2017). Under the water stress conditions, clusterbean is generally produced semi-arid and arid, tropical-subtropical regions due to its high drought tolerance. It is also a good soil restorative crop, which can fix about 40% N in the soil (Dougles, 2005). The cluster bean includes about 30-33% gum in the endosperm part of seed. The gum is used in many different types of industries like products of food, textile, paper and cosmetics (Pawlik and Laskowski, 2006). Cluster bean gum is biologically made non-toxic, eco-friendly and safe agro-chemical product (Sabahelkheir et al., 2012). This all reasons, guar is as a novel industrial crop with high exchange gaining potential. Phosphorus is the backbone of balanced fertilizer use in the intensive agro-system. P performs early root development, growth, blooming and aids in seed generation when applied to legumes. The supply of phosphorus to legumes is more important than that of nitrogen because, latter is being fixed by symbiosis with rhizobium bacteria (Yadav et al., 2020). This study targets to test the effect of different phosphorus levels on cluster bean seed yield and some yield components under the climates of Mediterranean.

Material and Methods

A pot study was conducted in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University (Turkey), from March to July of 2020 with typical Mediterranean climate conditions. Some meteorological data from the experimental area in Bornova-Izmir are presented in Table 1. The experiment soil was analyzed for pH: 5.83, soil texture: loamy, avaiable Ca:1300 ppm, avaiable P: 2.54 ppm, avaiable K: 40 ppm, $CaCO_3$: 0.82%, organic matter: 2.27%, total nitrogen: 0.092%, total salt: 0.03%, sand: 80.2%, clay: 1.8%, silt: 18.0%.

Table 1. Some meteorological data of research area in Bornova, Izmir, Turkey in 2020

	2020		**Long Year Average**	
	Temperature (ºC)	**Precipitation (mm)**	**Temperature (ºC)**	**Precipitation (mm)**
April	14.7	27.6	16.1	46.4
May	20.6	17.7	21.0	25.4
June	23.2	5.2	26.0	7.5
July	26.7	1.0	28.3	2.1
August	26.0	1.0	27.9	1.7
Total-Mean	**22.2**	**52.2**	**23.8**	**83.1**

Pusa Nevbahar cultivar of cluster bean (*Cyamopsis tetragonoloba*) was used as plant material from India. The seeds were sown in multipode on 17th March 2020, under the laboratory conditions. After the one month, the growing plants were planted in the pots of outdoor conditions at trial area. Five rates of phosphorus (0, 60, 90, 120, and 150 kg P ha^{-1}) were used in a completely randomized block design with four replicates. Total dose of phosphorus (triple superphosphate) fertilizer, 30 kg ha^{-1} nitrogen (urea) and 100 kg ha^{-1} K_2O (potassium sulphate) were applied with planting (Gresta et al., 2019). Weed control was provided by hand. No exposed crop diseases or pests were found.

In our study, the pods in the plants were expected to mature for grain harvesting, in other words, it was done when the moisture content of the grains in the lower pod of the plant reached around 13%. The crops were harvested by hand from the pot. The samples were dried to a constant weight at 65ºC during 48 h and then were weighed (g plant^{-1}). The distance between above soil level and the tip point of the dry plant was measured with a ruler for the plant height (cm). All pods on the plant were counted for the number of pods. The weight of the completely dried plant in the laboratory was weighed with digital scales for the biomass weight (g plant^{-1}). The pods in the dried plants were threshed manually and the grains was weighed with a digital scale for seed yield (g plant^{-1}). Cluster bean seeds (100) were weighted and converted for calculation of 1000-seed weights. The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD test (0.01) was performed to separate them (Stell et al., 1997).

Results and Discussion

In this study, plant height was statistically affected by phosphorus level (Table 2). The maximum plant height was recorded in P120 (60.1 cm) but the minimum plant height (50.3 cm) was recorded in the control pots (P0). Moreover, P120 and P150 levels was found non-different group in the statistical analysis. Increasing P levels increased plant height of cluster bean compared to the control. Singh and Tiwana (1995) reported that 60 kg ha^{-1} P level significantly enhanced plant height by 19.5 cm over the 0 kg ha^{-1} P level. On the similar study, the 40 kg ha^{-1} P application positively affected on plant height of cluster bean and also this fertilizer level was higher than compared the control level (P0) (Parihar, 2003). These results are in similar associated with our findings. The P levels significantly affected number of pod of cluster bean (Table 2), in the study. Numerically, the highest number of pod was recorded in P120 (39.3 $plan^{-1}$), whereas the lowest number of pod was recorded in P0 (30.6 $plant^{-1}$) level. In addition, there were no significant differences between P120 and P150. The number of pod of cluster bean enhanced with the application of P, because this element increases flowering and fruit setting. Numerous researchers pointed out that, increased P levels significant rise number of pod compared the control level for cluster bean (Singh and Tiwana, 1995; Shivran et al., 1996; Naagar and Meena, 2004).

Table 2. Effects of different P levels on the seed yield and some yield components of cluster bean

N levels	Plant height (cm)	Number of pod ($plant^{-1}$)	Biomass weight (g $plant^{-1}$)	Seed yield (g $plant^{-1}$)	Thousand grain weight (g)
P0	50.3 d	30.6 b	22.6 c	4.0 c	33.6 b
P60	55.7 c	31.3 b	26.6 b	4.7 bc	39.0 a
P90	56.1 bc	33.4 b	28.5 b	5.5 ab	36.5 ab
P120	60.1 a	39.3 a	32.7 a	6.6 a	35.5 b
P150	59.2 ab	38.6 a	29.5 ab	6.5 a	34.6 b
Mean	56.3	34.6	28.0	5.5	35.8
LSD (1%)	3.16	5.06	3.31	1.34	3.46
CV (%)	2.7	7.01	5.68	11.82	4.65

Same trend has been monitored in biomass weight. (Table 2). P120 gave highest biomass weight (32.7 g $plant^{-1}$) which was followed by P150. There was no statistical difference between these two doses. Moreover, P0 gave lowest biomass weight (22.6 g $plant^{-1}$). Biomass weight of cluster bean progressively increased with the increasing levels of phosphorus up to 120 kg P ha^{-1}. P is a fragment of DNA and RNA, related in cell division and is momentous for biomass yield (Brady and Weil, 2004). Our findings are in similar line with the results of Shivran et al. (1996) who noticed that the high biological yield (5.2 t/ha) of cluster bean was obtained by application of 40 kg P ha^{-1} which was 19% higher as compared to 20 kg P ha^{-1} level. Bahadur (2003) stated that the maximum biological yield (5.8 t/ha) of cluster bean was observed in 30 kg N ha^{-1} & 60 kg P ha^{-1} fertilizer combination.

There were significant differences among P levels for seed yield in our study (Table 2). The lowest seed yield was found (4.0 g $plant^{-1}$) in the control (P0), and P120 gave the highest seed yield (6.6 g $plant^{-1}$) which was followed by P150 (6.5 g $plant^{-1}$) and P90 (5.5 g $plant^{-1}$). Moreover, P90, P120 and P150 levels statistically were observed at the same group. Increasing doses of P significantly increased seed yield of cluster bean. Use of P fertilizer is critical for influential nodulation and contributes to enhance grain yield (Lokose, 2005). A study was conducted by Raiger et al. (2017) to determine the effects of different P levels (0, 20, 40 and 60

kg P ha^{-1}) on cluster bean. The researchers stated that seed yield (1.2 t/ha) was significantly increased with the application of 40 kg P ha^{-1} level. There also has been reported by several researchers that the application of P increased seed yield of guar (Meena and Sharma, 2001; Shivran, 2004). Our findings are in totally agreement with the results of these researchers.
ANOVA indicated that the 1000-grain weight was significantly impressed by the effect of P levels (Table 2). The maximum 1000-grain weight was determined in P60 (39.0 g) whereas the minimum 1000-grain weight was found in P0 (33.6 g). Increasing P levels increased the weight of seed up to P60 level, but after the weight of seed decreased. P60 and P90 were in the same group statistically as well. As it widely known, ATP formed during photosynthesis has P in its structure and processes from the beginning of seedling growth to the genesis of seed shape and weight (Nesme et al., 2014). Contrary to present study, Hago and Guma'a (2001) stated that phosphorus (50 kg ha^{-1}) had no effect on 1000-seed weight of guar. On the other hand, Patel et al. (2010) applied that the same dose on guar but they found that the best fertilizer level was 50 kg ha^{-1} phosphorus for the maximum thousand grain weight (49.1 g). These results are in conformity with our findings.

Conclusions

In the study, higher phosphorus level produced significantly higher yield and yield components of cluster bean compared to the control treatments. As a result, application of 120 kg P ha^{-1} confirmed to be the best fertilizer level for enhanced seed yield and some yield components under Mediterranean agro-ecological conditions. Since the results are based on the research laid out in single year as a pot study, only it may be corroborated with more than one location and year.

References

Bahadur, S. (2003). Production of potential of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) as influenced by sowing time, plant density and fertilizer levels. M.Sc. Thesis, Department of Agronomy, MPUAT, Udaipur.

Batirca, M., Gökkuş, A., Alatürk, F., Birer, S. (2017). Effect of Fertilization on the Yield and Quality of Hay of Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub.). KSU Journal of Natural Science. 20:130-134.

Brady, N.C., Weil, R.R. (2004). The Nature and Properties of Soils. 13th Ed.. Pearson Educ. Inc. 580p.

Chauhan, P.S., Tomar, J., Chhabra, K. (2020). Cluster Bean Agronomy Leguminous Crops (A Regional Overview), Agronomy legumious crops Publisher: Sea wave book world, Chapter 4: 65-77.

Dougles, C.A. (2005). Evaluation of guar cultivars in Central and Southern Queensland. Rural Industries Research and Development Corporation, 11p.

Gresta, F., Trostle, C., Sortino, O., Santonoceto, C., Avola, G. (2019). Rhizobium inoculation and phosphate fertilization effects on productive and qualitative traits of guar (*Cyamopsis tetragonoloba* (L.) Taub.). Industrial Crops & Products. 139:1-6.

Hago, T.E.M., Guma'a, A.H.A. (2001). Response of three Guar (*Cyamopsis tetragonoloba* (L.) Taub) lines to nitrogen and phosphorus fertilization under irrigation Uof K. The Journal of Agricultural Science. 9 (2): 225-235.

Kumar, D., Rodge, A.B. (2012). Status, scope and strategies of arid legumes research in India: A Review. Journal of Food Legumes. 25: 255-272.

Lokose, Y.P.L. (2005). Effect of phosphorus fertilization on yield and yield components of three lines of guar (*Cyamopsis tetragonoloba* L.). M.Sc. Thesis, Department of Agronomy, University of Khartoum. 49p.

Meena, H., Sharma, O.L. (2001). Effect of phosphorus and sulphur on growth, yield and quality of cluster bean (*Cymopsis tetragonoloba* (L.) Taub.). M.Sc (Ag.) Thesis, Rajasthan Agricultural University, Bikaner.

Naagar, K.C., Meena, N.L. (2004). Effect of phosphorus, sulphur and phosphate solubilizing bacteria on yield components, yield and quality of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.). Legume Research. 27:27-31.

Nesme, T. Colomb, B., Hinsinger, P., Watson, C.A. (2014). Soil phosphorus management in organic cropping systems: from current practices to avenues for a more efficient use of P resources. In: Organic farming, prototype for sustainable agricultures. Springer, Berlin, p 23–45.

Parihar, C.M. (2003). Effect of plant density and graded levels of phosphorus on the productivity pf cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). M.Sc. Thesis, MPUAT, Udaipur.

Patel, C.S., Patel, J.B., Suthar, J.V., Patel, P.M. (2010). Effect of integrated nutrient management on clusterbean [*Cyamopsis tetragonoloba* (L.) Taub] seed production cv. PUSA NAVBAHAR. International Journal of Agricultural Sciences, 6(1): 206-208.

Pawlik, M., Laskowski, J.S. (2006). Stabilization of mineral suspensions by guar gum in potash ores flotation systems. Canadian Journal of Chemical Engineering. 84:532-538.

Raiger, R., Kumawat, B.L., Sanwal, R.C., Kumawat, S.R., Kumawat, S.R. (2017). Response of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] to phosphorus and sulphur in torripsamments of Rajasthan. International Journal of Current Microbiology and Applied Sciences. 6(6): 2003-2008.

Sabahelkheir, M.K., Abdalla, A.H., Nouri, S.H. (2012). Quality assessment of guar gum (endosperm) of guar (*Cyamopsis tetragonoloba*). International Research Journal of Biological Sciences. 1: 67-70.

SAS Institute (1998). INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Shivran, A.C., Khangarot, S.S., Shivran, P.L., Garg, O.R. (1996). Response of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) varities to sulphur and phosphorus. Indian Journal of Agronomy. 41:340-342.

Shivran, B.S. (2004). Effect of phosphorus and potassium on growth yield and quality of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] in Western Rajasthan. M.Sc. Thesis Rajasthan Agriculture University, Bikaner.

Singh, H., Tiwana, U.S. (1995). Response of guar (*Cyamopsis tetragonoloba* (L.) Taub.) varieties to varying levels of phosphorus and spacing. Indian Journal of Agriculture Research. 29:48-52.

Stell, R.G.D., Torrie, J.A., Dickey, D.A. (1997). Principles and Procedures of Statistics. A Biometrical Approach 3rd Edi, Mc Graw Hill Book. INC. NY.

Yadav, K., Naga, S.R., Yadav, S. (2020). Impact of phosphorus and sulphur on nutrient content and uptake by clusterbean. International Journal of Chemical Studies. 8(1): 2396-2400.

GRAIN YIELD AND SOME AGRONOMICAL CHARACTERISTICS OF TEFF [*Eragrostis teff* (Zucc.) Trotter] AS AFFECTED BY SOWING DATES

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Abstract

The sowing date defines the environmental conditions to which the crop will be exposed in important key moments of its developmental cycle that critical period for yield and quality components. Early or late planting dates on crop causes an array of morphological, physiological and biochemical changes which affect growth and development and such changes may lead to a drastic reduction in yield. This research was carried out to determine the potential of teff as a staple crop for the Mediterranean climate during 2015 and 2016 in Soke-Aydin (Turkey). The main goal of the study was to establish the optimal teff planting date for the highest grain production. The effects of different sowing dates on the grain yield and some yield characteristics were investigated. Four different dates of sowing (15th April, 15th May, 15th June and 15th July) were arranged in a Randomized Complete Block Design with three replications. The teff genotype "Dessie" was used as plant material and number of plants, plant height, panicle length, thousand-grain weight, biological yield and grain yield were tested. Two-year average results indicated that there were significant differences in mentioned characteristics among the sowing dates. The experiment has shown that the influence of sowing date on grain yield is mainly due to day-length and temperature differences that are associated with delaying the planting from April to July. Delayed sowing dates reduced the grain yield. The highest total grain yield, 2780 kg ha^{-1}, was obtained for the teff seeds sown on the 15th April under Mediterranean ecological conditions.

Key words: *Grain yield, Mediterranean climate, sowing date, teff.*

Introduction

Teff or lovegrass as a field crop is an ancient staple crop for human consumption and originates from Ethiopia dating back to 4000 BC to 1000 BC (Ketema, 1997). Similarly, it is an annual dual-purpose cereal crop grown in Australia, India, and South Africa as a forage crop. It is a self-pollinated (2n=40), a short-day plant, warm-season grass that uses the C_4 photosynthetic pathway. It belongs to the family *Poacea*, subfamily *Eragrostoidae*, tribe *Eragrosteae* and genus *Eragrostis*. There are about 350 *Eragrostis* species of which *E. teff* is the only species cultivated for human consumption. In the systematic, varieties of its are classified according to grain colour, plant habitus, inflorescence and form of spike clusters, but only grain colours (white, red/brown and mixed) in commercial marketing. The oval-shaped its grain is not glume like wheat or rye. It is the grain of the smallest grain size (less than 1 mm) in the world. A thousand-grain weight is usually 200–400 milligrams (NRC, 1996). Unlike other cereals, the seeds of teff are gluten-free and they are considered a rich source of proteins and nutrients, which can be used as an alternative food source for people with celiac disease and diabetes. It is an excellent food security crop because it is highly resistant to various biotic and abiotic stresses (Spaenij-Dekking et al., 2005).

Teff possesses desirable storage properties without losing viability since the grains are resistant to storage pests compared to the other cereals under the local storage conditions.

One of the key components of plant production is the determination of the sowing date. To obtain high yield and quality products in teff cultivation, it is very important to determine the most adequate growing period where the plant could reach physiological maturity by selecting the most suitable cultivar. Many researchers have overemphasized the effect of sowing time on the grain yield of its. Teff is a newly introduced plant to Turkey, and therefore, knowledge on the productivity and yield of the teff crop under the intensive farming management in the Mediterranean environment has not been well documented so far. Hence, this paper aims to investigate the relationship between yield and yield contributing characters of teff regarding the sowing date under Mediterranean climatic conditions.

Materials and Methods

A field experiment was conducted in two consecutive growing seasons (2015 and 2016) on a private farm in Soke-Aydin (Turkey), located about 35 m above sea level with typical Mediterranean climate conditions. The experimental area is located in the Mediterranean zone of the country with temperate and rainy winters and hot and dry summers. Average temperature and total precipitation data through experimental period (from April to November) were 22.4, 22.9 ºC and 322.2, 144.3 mm, respectively. Average temperature was generally in accordance with long-term average (21.8ºC) but total precipitation of the first year was higher than long-term average (262.5 mm). The soil had sandy texture (80.1% sand, 1.8% clay and 18.1% silt) with a pH of 7.2, organic matter 1.27%, salt 0.05%, total N 0.06%, available phosphorus 2.54 $mg \cdot kg^{-1}$ and available potassium 403 $mg \cdot kg^{-1}$. There are no limiting factors for the establishment and growth of the teff crop in climate and soil conditions of the experimental site.

The Dessie teff cultivar from South Idaho/USA was used as crop material. Four different dates of sowing (15th April, 15th May, 15th June and 15th July) were employed in a randomized complete block design with three replications. Experimental plots were 10.5 m^2 (5 m x 2.1 m), having 12 rows with a row spacing of 17.5 cm. Seeds were sown by hand at a depth of 1 cm on all sowing times at a rate of 20 kg ha^{-1}. The overhead sprinkler system was installed in the field during both growing seasons. The soil was cultivated before sowing, 50 kg ha^{-1} of nitrogen (urea form) and 50 kg ha^{-1} of P_2O_5 were applied before the soil was cultivated again to produce a seedbed suitable for planting and the rest of nitrogen (50 kg ha^{-1}) in the form of $(NH_4)_2SO_4$ was applied at 30–40 cm plant height (Giday et al., 2014). No herbicides were used to control weeds. The weeds were controlled by three hoe weeding regimes throughout the experiment. No diseases or pests were observed in teff plants during the trial.

Plants were harvested at the physiological maturity stage (~13% moisture) from mid-August to late November in both years, collecting the middle 10 rows of plots in order to avoid border effects (Net: 8.75 m^{-2}). Two harvests were done in every experimental year. In each plot, cuttings were made with a hand-sickle at 5 cm above the ground level. The samples were dried under shaded-open conditions for one week, after which the dried samples were threshed by a seed threshing machine. In the study, the following characteristics were investigated: number of plants at grain harvest (plant m^{-2}): The plants in the quadrate of ½ square meter (50 x 100 cm) put in two different sites of the plot before harvesting were counted. Plant height (cm): 10 plants from the ground level to the top of the stem at the time of harvesting were measured. Panicle length (cm): the panicle of each selecting plant was measured from the node (the first panicle branch started) to the tip of the panicle. Thousand-grain weight (mg): the grains were taken from each plot and 1000 grains were counted by hand and then weighed. Biological yield (kg ha^{-1}): after the harvesting, total above-ground biomass (with grains) was measured. Grain yield (kg ha^{-1}): the grains of all the crops of each separated plot were weighed and the grain yield/plot was then converted to kg per hectare. All data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 1998). The Least Significant Difference (LSD) test at $P \leq 0.05$ was used to separate means whenever there were significant differences (Stell et al., 1997).

Results and Discussion

Number of plants: The number of plants at grain harvest was affected by the year x sowing date interaction (Table 1). The maximum number of plants being 2574 plant m^{-2} was recorded on the 15th April in 2016 and the minimum number of plants (1593 plants m^{-2}) was recorded on the 15th July in 2016. The delaying of sowing date (from mid-April to mid-July) decreased the number of plants in both experimental years. Hence, prolonged growing season causes more plant numbers. This could be associated with the facts that rising temperatures and weed competition in late sowings (mid-June and July) was the main effect on the decreasing number of plant. In North Gondar, Ethiopia, Zucca (2016) reported that differences among the sowing dates with regard to the number of tillers per plant were highly significant. Similar results have been reached in research by Geren et al. (2019), with the highest number of teff plants (1734 plants m^{-2}) obtained from the sowing date of mid-April and at 17.5 cm row spacing under typical Mediterranean climatic conditions. It was stated that the number of tillers per plant of teff was affected by the sowing date from early July to late July being 3.8 per plant to 6.9 per plant. In the present study, the delaying sowing date caused the decreasing of the plant number per unit area. In addition, it is widely known that the number of plants at grain harvest reacts positively and directly with the grain yield.

Table 1. The effect of different sowing dates on the grain yield and some yield characteristics of teff crop

Sowing dates	2015	2016	2 yrs average	2015	2016	2 yrs average
	Number of plants (m^{-2})			**Plant height (cm)**		
15/04	2253	2574	2414	109.5	105.7	107.6
15/05	2341	2215	2278	112.3	101.0	106.7
15/06	2066	1805	1936	101.8	92.1	97.0
15/07	1618	1593	1606	96.2	86.8	91.5
Mean	2070	2047	2058	105.0	96.4	100.7
LSD, .05	Y: ns SD: 102 int: 144			Y: 3.7 SD: 5.3 int: ns		
	Panicle length (cm)			**Thousand-grain weight (mg)**		
15/04	28.8	26.7	27.8	208	216	212
15/05	28.7	24.8	26.7	220	223	222

15/06	27.3	23.2	25.2	273	281	277
15/07	22.6	20.4	21.5	150	158	154
Mean	26.8	23.8	25.3	213	219	216
LSD, .05	Y: 0.6 SD: 0.8 int: 1.2			Y: 2.1 SD: 2.9 int: ns		
	Biological yield (kg ha^{-1})			**Grain yield (kg ha^{-1})**		
15/04	9390	8820	9110	2890	2670	2780
15/05	9370	8630	9000	2670	2580	2620
15/06	9020	8150	8580	2370	2300	2340
15/07	6890	6110	6500	1580	1450	1520
Mean	8670	7930	8300	2380	2250	2310
LSD, .05	Y: 130 SD: 180 int: ns			Y: 70 SD: 100 int: ns		

Y: year, SD: sowing date, int: interaction, ns: not significant

Plant height: ANOVA results showed that plant height was significantly affected by sowing date and year, but the interaction had no significant effect on plant heights (Table 1). The highest plant height (107.6 cm) was obtained on the 15th April, which was followed by the 15th May (106.7 cm). However, there was no statistical difference between April and May. The lowest plant height was determined in planting on the 15th July being 91.5 cm. There were significant differences between the two years in terms of plant height. The average plant height of the first year (105.0 cm) was higher than that of the second year (96.4 cm). Variability of precipitation recorded during the trial years caused this result. In experimental conditions, the delaying of sowing date in teff crops led to a decrease in plant height in both years. This can be a result of the shortened vegetative growth period available to the crop plants due to changes in the photoperiod that enhanced the rate of development towards the reproductive phase (Assefa et al., 2015). Juraimi et al. (2009) investigated different sowing dates (SDs) on teff plants (the 1st SD [the second week of July], the 2nd SD [delayed by 7 days after the 1st SD] and the 3rd SD [delayed by 15 days after the 1st SD], respectively). They reported that plant height decreased by delaying sowing dates (89, 84 and 78 cm, respectively).

Panicle length: Year effect was also significant, and average teff panicle length of the first year (26.8 cm) was higher than that of the second year (23.8 cm) due to higher total precipitation in the first year. The year x sowing date interaction was significant for the panicle length (Table 1). The highest panicle length (28.8 cm) was obtained on the 15th April in 2015, whereas the lowest was 20.4 cm on the 15th July in 2016. The panicle length of teff decreased noticeably with the late sowing (the 15th July) in both trial years. Some of the previous researchers recommended early sowing for high panicle length during production seasons (Juraimi et al., 2009; Zucca, 2016), and they also stated that an increase in panicle length was a corresponding increase in teff grain yields, provided that there was no delay in sowing.

1000-grain weight: ANOVA results showed that 1000-grain weight was significantly affected by sowing date and year, but not by the interaction. The year effect was also significant for this feature and the average value in the first year (213 mg) was lower than in the second year (219 mg). Thousand-grain weight significantly increased with the delay in sowing date from the 15th April to the 15th July. The heaviest grains (277 mg) were produced in plots sown on the 15th June and, thousand-grain weight on the 15th April, 15th May and 15th July were not statistically at par with each other (Table 1). Numerically, the least grain weight (154 mg) was obtained when teff was sown on the 15th July. In the present study, delaying sowing dates (from mid-April to mid-

July) caused a limited but significant increase in thousand-grain weight in the first harvest in both growing seasons. Nevertheless, we did not obtain any grain from the second harvest of mid-July sowing in the experimental area; therefore, the means of thousand-grain weights were lower. In addition, thousand-grain weight increased due to the decrease of fertile inflorescence per plant. Many researchers (Kebede, 2012; Asefa et al., 2014; Giday et al., 2014) reported that timely sowing gave higher thousand-grain weight as compared to delayed sowing.

Biological yield: The year effect was also significant for this treat and the average value in the first year (8670 kg ha^{-1}) was higher than in the second year (7930 kg ha^{-1}) (Table 1). The highest biological yield was recorded for the first sowing date (9110 kg ha^{-1}) and the lowest biological yield was recorded for the last sowing date (6500 kg ha^{-1}). In addition, there was no statistical difference between April (9110 kg ha^{-1}) and May (9000 kg ha^{-1}) sowings time in terms of biological yield. The sowing date x year interaction was not significant for the biological yield. Delaying of sowing time caused a decrease in biological yield. A single year field experiment at Alem Tena (Central Ethiopia) showed that when sowing dates were delayed by 1 or 2 weeks, the biological yield was reduced by 35% (Juraimi et al., 2009). Contrarily, some studies in other countries showed that the sowing date had no effect on total biological yield (Vos et al., 2013). Roseberg et al. (2007), in Oregon, mentioned that the total biological yield increased (8627, 10728, 10927 and 12567 kg ha^{-1}) remarkably as sowing time was delayed (the 16th May, the 30th May, the 13th June, and the 27th June), respectively.

Grain yield: The ANOVA results indicated that the grain yield was significantly affected by year, sowing date. The average grain yield in the first year (2380 kg ha^{-1}) was higher than in the second year (2250 kg ha^{-1}) (Table 1), most probably due to providing better humidity and precipitation for the maturation of crops in 2015 compared to 2016. According to two-year results, the maximum total grain yield (2780 kg ha^{-1}) was recorded for plants established at the sowing date of the 15th April. However, the minimum total grain yield (1520 kg ha^{-1}) was recorded for plants established at the sowing date of the 15th July. The year effect was also significant. Delaying of sowing time consistently decreased the grain yield. In other words, delays in sowing date also reduced yield components such as the number of plants per unit area, panicle length and the effective duration of grain filling compared with earlier sowings. It is reported that low temperature reduced grain yield as a result of a reduction in basal tiller numbers, grain number and individual grain weight (Fussell and Norman, 1980; Ong and Squire, 1984). In the present study, it was also found that 80% of the total yield was obtained from the first harvest. In both years, teff grain yield could not be obtained in the second harvest of the crop, which was sown late (on the 15th July) under the experimental area conditions. This indicates that regrowth after grain harvest can also be used as a different alternative like grazing.

Grain yield per unit area is a function of the integrated effect of the yield components that are affected differently by growing conditions (Khan et al., 2000). Sherif (2004) reported a similar relationship among different sowing dates (15, 22 and 28 July) for grain yield of teff. When sowing was delayed for 7 and 15 days, grain yields were reduced by 60% and 68%, respectively. Mengitsu et al. (2001) informed that early sowing in teff cultivation affect positively grain yield at the rate of 19% compared with late sowing. In addition, Juraimi et al. (2009) wrote that timely sown teff produced 18% and 19% higher yield compared to sowing delayed for 7 and 15 days, respectively. Conversely, Dent and Reid (2009) expressed that teff grain yield increased (54, 119, 610, 1168 and 94 kg ha^{-1}) with the delaying sowing dates but later decreased (12th October, 25th October, 11th November, 22th November, 7th December, respectively) in South Tasmania. Vos et al. (2013) conducted a field experiment in Wageningen (Netherland), in 2006 for two sowing dates (April 28th and May 16th) with four teff cultivars. They found that there was no difference in grain yield among cultivars and between the 28th April (1075 kg ha^{-1}) and the 16th of May (1068 kg ha^{-1}).

Conclusion

The objective of the study was to determine the optimal teff planting date for maximising grain production. The vast agro-climatic variation among teff producing countries and the seasonal difference within regions do not allow the allocation of a specific date of sowing. Maximum total grain production was achieved from the 15th April planting, which gave yields of 2780 kg ha^{-1} for the 2015/2016 season. As a conclusion, the 15th April can be recommended as a suitable sowing date in terms of maximum grain production under Mediterranean-type ecological climate conditions.

References

Asefa, F., A. Debela and M. Mohammed. 2014. Evaluation of Teff [*Eragrostis tef* (Zuccagni) Trotter] responses to different rates of NPK along with Zn and B in Didessa District, Southwestern Ethiopia. World Applied Sciences Journal 32(11):2245-2249

Assefa K., G. Cannarozzi, D. Girma, R. Kamies, S. Chanyalew, S. Plaza-wüthrich, R. Blösch, A. Rindisbacher, S. Rafudeen and Z. Tadele. 2015. Genetic diversity in tef [*Eragrostis tef* (Zucc.) Trotter]. Frontiers in Plant Science 6:177-209.

Dent, R. and R. Reid. 2009. Agronomic evaluation of teff in Tasmania, Australian Government, Rural Industries Research and Development Corporation. Project No: PRJ-007907 Pub No: 15/082, 4p

Fussell, L.K. and M.J.T Norman. 1980. Effect of temperature during various growth stages on grain development and yield of *Pennisetum americanum*. Journal of Experimental Botany 3:621-33

Geren, H., Y.T. Kavut and B. Kir. 2019. Effect of different row spacings on the yield and some yield characteristics of Teff (*Eragrostis teff*) crop grown under Soke ecological conditions. Journal of Agriculture Faculty of Ege University 56(2):231-239 (in Turkish)

Giday, O., H. Gibrekidan and T. Berhe. 2014. Response of teff (*Eragrostis tef*) to different rates of slow release and conventional urea fertilizers in Vertisols of Southern Tigray, Ethiopia. Advances in Plants & Agriculture Research 1(5):1-8

Juraimi, A.S., M. Begum, A.M. Sherif and A.A. Rahim. 2009. Competition effects of date of sowing and nutsedge removal time on yield and yield contributing characters of tef [*Eragrostis tef* (Zucc.) Trotter]. American Journal of Applied Sciences 6(10):1820-1825.

Kebede, T. 2012. Response of Tef (*Erogrostis tef* (Zucc.) Trotter) cultivars to nitrogen and phosphorus fertilizer rates at Menzkeya district, North Shewa, Ethiopia. Haramaya University MSc Thesis 66p

Ketema, S. 1997. Tef (*Eragrostis tef* (Zucc.) Trotter), Promoting the conservation and use of underutilized and neglected crops. 12. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute Rome Italy 50p

Khan, H., M.A. Khan, I. Hussain, M.Z. Khan and M.K. Khattak. 2000. Effect of sowing methods and seed rates on grain yield and yield components of wheat variety PAK-81. Pakistan Journal of Biological Science 3(7):1177-1179.

Mengistu, T.K., H.G. Kidan and K. Georgis. 2001. Effect of sowing date and nitrogen fertilization on yield and related traits of teff [*Eragrosris teff* (Zucc) Trotter] on Vertisols of Kobo Area. Orth Wollo Haramaya University Library and Information Services p:10

National Research Council (NRC). 1996. Lost Crops of Africa: Volume I: Grains, Washington DC The National Academies Press

Ong, C.K. and G.R. Squire. 1984. Response to temperature in a stand of pearl millet (*Pennisetum typhoides* S & H), VII Final numbers of spikelets and grain. Journal of Experimental Botany 35:1233

Roseberg, R.J., B.A. Charlton and R.A. Shuck. 2007. Yield and forage quality of four Teff seed brands as affected by planting date and soil/air temperatures in the Klamath Basin. Klamath Basin Research and Extension Center pp:30-41

SAS Institute. 1998. INC SAS/STAT user's guide release 7.0 Cary NC USA.

Sherif, A.M. 2004. Competition effects of purple nutsedge (*Cyperus rotundus* L.) on growth and yield of tef (*Eragrostis teff*) in Ethiopia, Universiti Putra Malaysia, School of Graduate Studies PhD Thesis 234p.

Spaenij-Dekking, L., Y. Kooy-Winkelaar and F. Koning. 2005. The Ethiopian cereal tef in celiac disease. The New England Journal of Medicine 353:1748–1749.

Stell, R.G.D., J.A. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach 3rd ed INC NY Mc Graw Hill Book

Vos, J., S.H.V. Delden and T.J. Stomph. 2013. Some ecophysiological characteristics of Tef, including mechanics of lodging. Achievements and Prospects of Tef Improvement. Proceedings of the Second International Workshop, November 7-9 2011, Debre Zeit Ethiopia pp: 141-160

Zucca, C. 2016. Response of tef row planting to sowing dates on the highland heavy clay soils. Technical Report of Experimental Activities. Project Agreement No 100202

'KSU-46' WALNUT CULTIVAR

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Abstract

This study aimed to determine the phenological and pomological traits of new walnut cultivar named 'KSU-46'. This cultivar was obtained from crossing between 'Maras 18' x 'Franquette'. The reason why these cultivars were chosen as parents in the breeding program was that Maraş-18 had very superior fruit traits and 'Franquette' was the latest leafing cultivar. 'KSU-46' was registered and patented by the Republic of Turkey Ministry of Agriculture and Forestry, Variety Registration, and Seed Certification Center in 2021. In this study, the phenological and pomological traits of 'KSU-46' are presented by comparing them with 'Chandler' and 'Maras 18'. 'KSU-46' have late season leafing same as 'Chandler' and important nut traits. In addition, the cultivar has earlier harvest and defoliation than the 'Chandler'. Time of male flowering compared to female flowering of 'KSU-46' is protandrous. The nut weight, kernel weight, and kernel percentage of 'KSU-46' are 12.53 g, 6.35 g and 50.72%, respectively. The nuts of the 'KSU-46' have medium size, broad eliptic fruit shape, moderately grooved shell structure and yellowish white kernel. In this study, results indicate that this walnut cultivar have good performance considering some phenological and pomological traits.

Keywords: *Walnut, Juglans regia L., Phenology, Pomology, New Cultivar.*

Introduction

Walnut (*Juglans regia* L) is the most produced nut species in the world. Walnut kernel is a nutrient-rich food that has many beneficial contents (unsaturated fatty acid , proteins, vitamins and antioxidant etc.) in terms of human health (Martinez et al., 2010; Xiaoying et al., 2014).
Asia, America and Europe continents stand out in walnut production. The countries that produce the most walnuts in the world are China, USA, Iran and Turkey, respectively. There has been a significant increase in world walnut production after 1984. In 2019, the world production of walnut is approximately 4.5 million tons (FAO, 2021).
The demand for walnuts is increasing due to the increasing world population and the demand for healthy food in people's diets. However, due to the decrease in agricultural areas, the development of more productive and quality cultivars from the existing standard cultivars is one of the primary objectives of breeding in order to meet the increasing demand. In addition, pomological traits such as fruit size, shell strength, ease of removal of kernel halves and kernel percentage are among the other targets of walnut breeding programs. Recently, the adaptation of walnuts to the climatic changes caused by global warming is another important topic in breeding programs (Bernard et al., 2020).

The most important factors limiting walnut cultivation as climatic conditions are late spring and early autumn frosts. In most breeding studies that have been carried out for this purpose, breeders have aimed to identify new genotypes with late leafing and early deciduous. (McGranahan and Lesile, 2009; Ebrahimi et al., 2015; Sütyemez et al 2018; Bükücü et al., 2020). When the walnut breeding studies are examined, one could see that they aimed to collect all or most of the superior traits such as resistance to biotic and abiotic stress conditions, as well as yield and quality criteria into one cultivar. It has been observed that there are significant differences in many studies conducted to determine the phenological and nut traits of walnut populations in different parts of the world (Sharma and Sharma 1998, 2001; Yarilgac et al. 2001; Kazankaya et al. 2001; Solar and Stampar 2003; Zeneli et al. 2005; Cosmulescu and Botu 2012; Cosmulescu 2013; Sütyemez, 2016; Yıldız and Sümbül, 2019). Among the local walnuts with high genetic variation, there is a high possibility of promising genotypes having good fruit quality, late leafing, low chilling requirement and disease resistance. However, finding a genotype that combines all of these traits is very unlikely (Germain, 1997; Zeneli ve ark. 2005). Cultivars or genotypes obtained by selection breeding are very important in terms of both revealing genotypes with superior traits existing in nature and preserving genetic diversity. In recent years, cross-breeding studies have become preferable over selection breeding because of the possibility of collecting more than one heritable trait in one genotype. It should be noted that this process takes longer time than selection breeding. To date, many cultivars have been developed using these breeding methods in the world and Turkey. However, fruit breeding is a long journey that need to be kept up to date and struggled to identify genotypes superior to the old cultivars. This long journey is a major problem that hinders the development of new cultivars.

In the last decades, the number of walnut breeding programs and studies aiming to preserve genetic diversity have increased significantly in the world. In this sense, very important steps are being taken in Turkey as well. Anatolia is a region having significant genetic richness, where walnuts can be found in almost every region. To date, many studies have been conducted and continue to be conducted in Turkey for the screening of genotypes with superior traits. In recent years, there has been a significant increase in the number of new cultivars obtained by crossbreeding.

In this study, some phenological and nut traits of the new cross-breed walnut cultivar 'KSU-46', patented in 2021 by Sütyemez (2005) within the scope of the cross-breeding program, are presented. In addition, performance evaluation was made by comparing the phenological and pomological characteristics of 'Maras 18' and 'Chandler' cultivars planted in the same period with the cultivar 'KSU-46'.

Material and methods

This research was carried out on 'KSU-46' walnut cultivars, which were obtained from cross-breeding program initiated by Prof. Dr. Mehmet Sütyemez in 2005 and patented on 19.03.2021. In the study, phenological and pomologicala comparisons were made with 'Chandler' and 'Maras 18' planted at the same age. 'Chandler' and 'Maras 18' cultivars were also evaluated and compared with 'KSU-46'. The study was located in the Nut Application and Research Center (SEKAMER), Kahramanmaras, Turkey. SEKAMER is located at 37° 35' 27" N latitude, 37° 03' 28" E longitute and 930 m above sea level. The region has a mild climate - an average between Mediterranean and Continental climates with average 727 mm yearly precipitation and 16.9°C yearly temperature. The soil structure in general is suitable for walnut cultivation, and the plants in this study have been irrigated regularly. The trees of each cultivar, were propagated on *Juglans regia* L. seedling rootstocks in 2011. Five plants with similar growing qualities were used for each genotype. The average of the values of the pomological and phenological traits taken for two consecutive years (2019-2020) were presented. The definitions of 14 phenological and 12 pomological traits examined in the study are given in Table 1 and Table 2 (IPGRI, 1994; UPOV, 2014).

Table 1. Descriptors of the phenological traits

Traits	***Description***
Leafing date	Date when 50% of terminal buds had enlarged and the bud scales had split exposing the green leaves
First male bloom date	When first pollen shedding occurred
Last male bloom date	When last pollen shedding occurred
First female bloom date	Date of initial pistillate flower receptivity
Last female bloom date	Date of last pistillate flower receptivity
Male flowering times	Catkins receptive duration
Female flowering times	Female flower receptive duration
Female abundance	Female flower abundance: 3 light; 5 intermediate; 7 high
Catkin abundance	Male flower abundance: 3 light; 5 intermediate; 7 high
Lateral bud flowering	Percent of lateral buds with female flowers
Dichogamy	Female flowers and catkins receptive duration overlap status: 1 Protandrous; 2 Protogynous; 3 Unknown
Yield	The yield per tree of 8 and 9-year-old trees was determined as kg.
Harvest date	When nuts are harvestable
Defoliation date	Date of defoliation

Table 2. Descriptors of the pomological traits

Traits	***Description***
Nut shape	1: Round; 2: Triangular; 3: Broad ovate; 4; Ovate; 5: Short trapezoid; 6: Long trapezoid; 7: Broad eliptic; 8: Eliptic; 9: Cordate
Shell texture	1: Very smooth; 3: Smooth; 5: Medium; 7: Rough; 9: Very rough
Shell colour	1: Very light; 3: Light; 5: Medium; 7: Dark; 9: Very dark
Shell strength	1: Paper; 3: Weak; 5: Intermediate; 7: Strong
Shell thickness (mm)	Near center of half shell was measured with a digital caliper.
Shelled nut weight (g)	Average of total 50 nuts
Kernel weight (g)	Average of total 50 nuts
Kernel percentage (%)	Kernel weight/nut weight × 100
Kernel colour	1: Extra light; 2: Light; 3: Light amber; 4: Amber
Kernel fill	3: Poor; 5: Moderate; 7: Well
Kernel flavour	1: Satisfactory; 2: Unsatisfactory
Ease of removal of kernel halves	1: Very easy; 3: Easy; 5: Moderate; 7: Difficult; 9: Very difficult

Results and Discussion

In this study, information about some pomological and phenological traits of the 'KSU-46' is presented. The leafing date of 'KSU-46' was determined as April 24. While 'Chandler' had a date of April 23, 'Maras 18' had a leafing date 10 days before 'KSU-46'. In order to protect the walnut from late spring frosts, it is desired the leafing date is late. 'Chandler' is classified as a late leafing cultivar among standard walnut cultivars. In terms of this phenological trait, 'KSU-46' showed the same traits with 'Chandler' (Tablo 3). Therefore, it can be said that the 'KSU-46' is suitable for cultivation in regions where late spring frosts are observed frequently. Luedeling and Gasner (2012) reported that the average leafing date of the 'Chandler' was April 4th in California between 1988 and 2007. Türemiş et al. (2017) stated that the leafing date of 'Maras 18' occurred between March 31 and April 24 in 2015. The differences in the findings of the research are due to climatic changes and ecological differences over the years.

It is important to know the flowering traits of a plant species such as walnut with a high dichogamy tendency. In various studies, it has been reported that 'Chandler' and 'Maras 18' have protandrous flowering traits (Sütyemez, 2016; Soleimani, 2019; Sütyemez et al., 2019). As a result of phenological observations made to determine the dichogamy status of 'KSU-46', it was determined that the first and last male bloom date of the cultivar were 22 April and 29 April and the first and last female bloom dates were May 2 and May 11, respectively. In this study, it was determined that KSU-46 had protandrous flowering trait. 'Chandler' and 'Maras 18' cultivars examined in the study had protandrous flowering trait, which were consistent results obtained with the previous studies on these cultivars. The findings of the flowering traits of the cultivars are presented in Table 3.

Lateral bud flowering and female abundance are important parameters to determine the yield in walnut. In addition to these two traits, male abundance was determined in the study. Lateral bud flowering of 'KSU-46' was determined as 80.8%. 'Chandler' is a walnut cultivar with high lateral bud flowering (Manthos and Rouskas, 2021).We found that the lateral bud flowering rate was 90.5% for 'Chandler' and 70.8% for Maraş 18. In terms of lateral bud flowering, 'KSU-46' had a percentage between the standard cultivars. The female abundace of the cultivars were determined as medium for 'KSU-46' and 'Maras 18', and high for 'Chandler'. The male flower abundance determined as light for 'KSU-46', medium for Maraş-18 and high for 'Chandler'. Suyemez et al. (2019) stated that the 'Chandler' has a high male flower abundance. If the maintenance conditions and fertilization status of a cultivar with high lateral bud flowering are at optimum level, the yield per tree would be high. In this study, the average yield per tree at the 8th and 9th years of 'KSU-46' was determined as 4.97 kg. While this value was 7.38 kg for 'Chandler', it was 4.34 kg for 'Maras 18' (Table 3).

In this study, the harvest date of the cultivars were determined, and we found that the 'KSU-46' was harvested on the same date as 'Maras 18' (Sep. 17). 'Chandler' reached harvest maturity on October 6. Manthos and Rouskas (2021) reported that the 'Chandler' reached harvest maturity on October 2. The findings are consistent with our study. Early defoliation dates are an important parameter that is effective in protection from early autumn frosts. 'Maras 18' is classified among early deciduous cultivars (Sütyemez, 2016). In terms of this trait, the defoliation date of the 'KSU-46' was 5 days after one of its parents, 'Maras 18'. For this reason, 'KSU-46' is recommended for regions where frost is observed intensely in the early autumn periods (Table 3).

Table 3. Phenological traits of 'KSU-46', 'Chandler' and 'Maras 18'.

Phenological Traits	***Cultivars***		
	KSU-46	**Chandler**	**Maras 18**
Leafing date	April 24	April 23	April 14
First male bloom date	April 22	April 19	April 14
Last male bloom date	April 29	April 27	April 23
First female bloom date	May 2	April 29	April 22
Last female bloom date	May 11	May 11	April 29
Male flowering times	7	8	9
Female flowering times	9	12	8
Female abundance	Intermediate (5)	High (7)	Intermediate (5)
Catkin abundance	Light (3)	High (7)	Intermediate (5)
Lateral bud flowering	80.8%	90.5%	70.8%
Dichogamy	Protandrous	Protandrous	Protandrous
Yield (kg/tree) (6.-7. Years in tree)	4.97 kg	7.38 kg	4.34 kg
Harvest date	September 17	October 6	September 17
Defoliation date	November 7	December 5	November 2

It is very important to determine the pomological traits of fruits in breeding studies. Fruits with superior pomological traits are always highly demanded in the market and therefore sold at higher prices. The most important traits that determine the nut quality of the walnut are the shelled nut weight, kernel weight, kernel percentage, kernel colour, ease of removal of kernel halves and shell strength. As a result of the pomological analysis, it was determined that 'KSU-46' had a broad eliptic nut shape, smooth shell structure and a light shell color. 'KSU-46' differed from 'Chandler' and 'Maras 18' in terms of fruit shape. Sütyemez (2016) reported that the 'Chandler' and 'Maras 18' has an oval shape. In this study, similar findings were obtained in terms of this trait.

While the shell strength of 'KSU-46' and 'Maras 18' cultivars was intermediate, shell strength of 'Chandler' was weak. As expected, the shell thickness was 1.32 mm for 'KSU-46', 1.22 mm for 'Chandler' and 1.39 mm for Maraş-18. A weak strength is a desirable feature in walnut cultivars. Mahmoodi et al. (2016), in a study conducted in Iranian ecological conditions, stated that the shell thickness of the 'Chandler' was 1.28 mm. It is thought that the difference between these values is due to the age of the plants and the maintenance conditions.

Shelled nut weight is another trait that increases the cultivar's appeal. The shelled nut weight of 'KSU-46' was determined as 12.54 g. The shelled nut weight of Chandler's was 12.51 g, and it was determined that it had close values with 'KSU-46' in terms of this trait. 'Maras 18' had a higher value than the other two cultivars with 14.71 g shelled nut weight. Erturk et al. (2017), found that the shelled nut weight of the 'Chandler' was 12.58 g and the 'Maras 18' was 14.01 g. In addition, Sütyemez (2016) stated that the shelled nut weight of 'Maras 18' varies between 13-15 g and the shelled nut weight of 'Chandler' varies between 12-14 g. The findings obtained in the study showed consistency with our findings.

In walnut breeding, it is desired that the kernel percentage of a cultivar should be higher than 50%. It is seen that the kernel weight changes according to the population used in the studies and ecological conditions. In various studies, it has been determined that the kernel weight in walnuts varies between 1.69 g-14.00 g, and the kernel percentage varies between 28.18%-63.21% (Asma, 2012; Sütyemez, 2016; Ipek et al., 2019; Ergün and Bulduk, 2020; Bujdoso and Cseke, 2021; Kavosi and Khadivi, 2021). In our study, the kernel weight of 'KSU-46' was 6.34 g and its kernel percentage was 50.56%. While these values were 6.04 g and 48.50% for 'Chandler', 7.80 g and 53.05% for 'Maras 18'. 'KSU-46' showed a outstanding performance with its kernel percentage. Generally, there is a corelation between the kernel percentage and kernel fill (Khadivi-Khub et al., 2015). In this study, 'KSU-46' had a well kernel fill in parallel with its kernel percentage. The ease of removal of kernel halves was easy for 'KSU-46' and 'Maras 18', while it was very easy for 'Chandler'. In addition, it all cultivars examined in the study had a satisfactory kernel flavour. The image of the nuts of the 'KSU-46' is presented in Figure 1.

Table 4. Pomological traits of 'KSU 46', 'Chandler' and 'Maras 18'.

Pomological Traits	***Cultivars***		
	KSU-46	**Chandler**	**Maras 18**
Nut shape	Broad eliptic (8)	Ovate (5)	Ovate (5)
Shell texture	Smooth (3)	Medium (5)	Smooth (3)
Shell colour	Light (3)	Light (3)	Very light (1)
Shell strength	Intermediate (5)	Weak (3)	Intermediate (5)
Shell thickness	1.32 mm	1.22 mm	1.39 mm
Shelled nut weight	12.54 g	12.51 g	14.71 g
Kernel weight	6.34 g	6.04 g	7.80 g
Kernel percentage	50.56%	48.30%	53.05%
Kernel colour	Light (2)	Light (2)	Light (2)
Kernel fill	Well (7)	Well (7)	Well (7)
Ease of removal of kernel halves	Easy (3)	Very easy (1)	Easy (3)
Kernel flavour	Satisfactory (1)	Satisfactory (1)	Satisfactory (1)

Figure 1. Visual of the nuts of the 'KSU-46'.

Conclusions

As a result, 'KSU-46', which was obtained from the cross-breeding program in which 'Maras 18' and 'Franquette' were used as parents, is a walnut cultivar that stands out due to its late leafing. The cultivar also reach to early harvest maturity and it has an early defoliation period. In addition, 'KSU-46' has superior pomological traits in terms of some nut quality criteria. The nut weight of 'KSU-46' was determined as 12.54 g, and it is seen that it is a higher than 'Chandler'. This cultivar can be easily recommended for regions where 'Chandler' is not affected by late spring frosts. The authors can be contacted for the supply of scion wood materials and saplings of the cultivar.

References

Asma B.M. (2012). Pomological and phenological characterization of promising walnut (*Juglans regia* L.) genotypes from Malatya, Turkey. Acta Scientiarum Polonorum Hortorum Cultus, 11(4), 169-178.

Bernard A., Marrano A., Donkpegan A., Brown P.J., Leslie C.A., Neale D.B., Lheureux F., Dirlewanger E. (2020). Association and linkage mapping to unravel genetic architecture of phenological traits and lateral bearing in Persian walnut (*Juglans regia* L.). BMC genomics, 21(1), 1-25.

Bujdoso G., Cseke K. (2021). The Persian (English) walnut (*Juglans regia* L.) assortment of Hungary: Nut characteristics and origin. Scientia Horticulturae, 283, 110035.

Bükücü Ş, Özcan A, Sütyemez M., Yildirim E. (2020). Determınation in the phenological difference levels of seedlings of some walnut genotypes (*Juglans regia* L.). Applied Ecology and Environmental Research, 18(3), 4807-4815.

Cosmulescu S. (2013). Phenotypic diversity of walnut (*Juglans regia* L.) in Romania opportunity for genetic improvement. South West J Hortic Biol Environ 4:117–126.

Cosmulescu S., Botu M. (2012). Walnut biodiversity in South-Western Romania resource for perspective cultivars. Pak J Bot 44:307–311.

Ebrahimi A, Khadivi-Khub A, Nosrati Z, Karimi R. (2015). Identification of superior walnut (*Juglans regia*) genotypes with late leafing and high kernel quality in Iran. Scientia Horticulturae, 193, 195-201.

Ergün M., Bulduk A. (2020). Nut and kernel properties of five promising walnut (*Juglans regia* L.) genotypes adopted to high altitudes. International Journal of Naturaland Engineering Sciences, 14(3): 40-49.

Ertürk Ü., Utku Ö., Mert C., Kaya O. (2017). Evaluation of Fruit Properties of Native and Foreign Walnut Cultivars in Bursa Conditions. Bahçe, 46 (Special Issue 2), 47-52.

Germain E. (1997). Genetic improvement of the Persian walnut (*Juglans regia* L). Acta Horticulturae, 442:21–31.

Ipek M., Arıkan Ş., Pırlak L., Eşitken A. (2019). Phenological, morphological and molecular characterization of some promising walnut (*Juglans regia* L) genotypes in Konya. Erwerbs-Obstbau, 61(2), 149-156.

IPGRI. 1994. Descriptors for walnut (Juglans spp.). International Plant Genetic Resources Institute, Rome, Italy.

Kavosi H., Khadivi A. (2021). The selection of superior late-leafing genotypes of Persian walnut (*Juglans regia* L.) among seedling originated trees based on pomological characterizations. Scientia Horticulturae, 288, 110299.

Kazankaya A., Koyuncu M.A., Koyuncu F., Yarilgac T., Sen S.M. (2001). Some nut properties of walnuts (*Juglans regia* L.) of Edremit country. Acta Horticulturae 544:97–100.

Khadivi-Khub, A., Ebrahimi, A., Sheibani, F., Esmaeili, A. (2015). Phenological and pomological characterization of Persian walnut to select promising trees. Euphytica, 205(2), 557-567.

Luedeling E., Gassner, A. (2012). Partial least squares regression for analyzing walnut phenology in California. Agricultural and Forest Meteorology, 158, 43-52.

Mahmoodi R., Hassani D., Amiri M.E., Jaffaraghaei M. (2016). Phenological and pomological characteristics of five promised walnut genotypes in Karaj, Iran. Journal of Nuts 7(1):1-8.

Manthos I., Rouskas D. (2021). 'Ourania' walnut. HortScience, 56(4), 521-522.

Martínez M.L., Labuckas DO, Lamarque AL, Maestri D.M. (2010). Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products. Journal of the Science of Food and Agriculture, 90(12), 1959-1967.

McGranahan G.H., Leslie, C.A. (2009). Breeding walnuts (*Juglans regia*), Breeding Plantation Tree Crops: Temperate Species pp 249-273. https://doi.org/10.1007/978-0-387-71203-1

Sharma O.C., Sharma S.D. (2001). Genetic divergence in seedling trees of Persian walnut (*Juglans regia* L.) for various metric nut and kernel characters in Himachal Pradesh. Sci Hortic., 88:163–171.

Sharma S.D., Sharma O.C. (1998). Studies on the variability in nuts of seedling walnut (*Juglans regia* L.) in relation to the tree age. Fruit Var J., 52:20–23.

Solar A., Stampar F. (2003). Genotypic differences in branching pattern and fruiting habit in common walnut (*Juglans regia* L.). Ann Bot 92:317–325.

Soleimani A., Rabiei V., Hassani D., Mozaffari M.R. (2019). Phenological characteristics of walnut (*Juglans regia* L.) genotypes under environmental conditions of Karaj in Iran. Crop Breeding Journal, 9(2), 11-22.

Sutyemez M. (2016). New walnut cultivars: Maras 18, Sutyemez 1, and Kaman 1. HortScience, 51(10), 1301-1303.

Sutyemez M., Ozcan A., Bukucu S.B. (2018). Walnut cultivars through cross-breeding: 'Dirilis' and '15 Temmuz'. Journal of The American Pomological Society, Vol:72, No:3, Sayf. 173-180.

Sütyemez M., Bükücü Ş.B., Özcan A. (2019). Maraş 12: A walnut cultivar with cluster-bearing habit. HortScience, 54(8), 1437-1438.

Türemiş N.F., Kafkas S., Burğut A., Köymen, M.T. (2017). Bazı ceviz çeşitlerinin Adana koşullarına adaptasyonu. Bahçe, 46 (Özel Sayı 2), 41-46.

UPOV 2014. Union internationale pour le protection des obtentions vegetales. Draft guidelines for the conduct of tests for distinctness homogeneity and stability. Walnut (*Juglans regia* L.). UPOV, Geneva, Switzerland

Xioying M., Yufei H., Guogang C. (2014). Amino acid composition, molecular weight distribution and gel electrophoresis of walnut (*Juglans regia* L.) proteins and protein fractionations. International journal of molecular sciences, 15(2), 2003-2014.

Yarilgac T., Koyuncu F., Koyuncu M.A., Kazankaya A., Sen S.M. (2001). Some promising walnut selections (*Juglans regia* L.). Acta Horticulturae 544:93–100.

Yıldız E., Sümbül A. (2019). Mineral content in kernel of some local and foreign walnut cultivars and genotypes. Mustafa Kemal Universitesi Tarim Bilimleri Dergisi, 24(3), 174-180.

Zeneli G., Kola H., Dida M. (2005). Phenotypic variation in native walnut populations of Northern Albania. Sci Hortic 105:91–100.

EVALUATION OF THE EFFECT OF TESBI (*STYRAX OFFICINALIS* L.) SEED EXTRACTS ON SEEDLING DEVELOPMENT OF SOME PLANTS

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Abstract

Many plant species synthesize various biochemical substances from their various organs such as leaves, flowers, fruits, and roots. While some of these biochemical substances have a stimulating effect on the growth and development of other plants, some of them have an inhibitory effect. In this study, the effect of seed extracts of the tesbi (*Styrax officinalis* L.) bush, which spreads naturally in the Mediterranean and Aegean regions, on the seedling growth of sweet fennel (*Foeniculum vulgare* Mill. var. *dulce*), coriander (*Coriandrum sativum* L.), fenugreek (*Trigonella foenumgraecum* L.), camelina (*Camelina sativa* L.) and mustard (*Sinapis arvensis* L.) plants were investigated. In the study, the extracts prepared from the ground tesbi seeds with distilled water at 5% concentration were boiled in three different times (S0: control, S1:15 min, S2:30 min, and S3:45 min). To determine the effects of extracts on seedling growth, seedling height, root length, seedling fresh-dry weights, and true leaf numbers were determined. In S0, S1, S2 and S3 applications, seedling lengths were 14.8-34.5 cm, 9.3-30.4 cm, 12.3-33.5 cm and 10.2-28.0 cm, respectively; root length 5.2-27.0 cm, 4.1-20.1 cm, 6.4-25.6 cm, 4.4-19.5 cm; seedling fresh weight 0.05-0.549 g, 0.033-0.446 g, 0.046-0.136 g and 0.029-0.543 g; seedling dry weight was 0.008-0.123 g, 0.005-0.093 g, 0.007-0.075 g and 0.005-0.074 g and true leaf number was between 1.0-7.0, 2.0-5.0, 2.0-6.0 and 3.0-6.0, respectively. When the findings obtained from the research are evaluated in general, it was found that extracts exhibited an inhibitory effect on seedling growth.

Keywords: *Styrax officinalis, Seed extract, Seedling height, Root Length, Fresh weight.*

Introduction

Allelopathy is a biological phenomenon by which is one plant affecting another plant's germination, survival, and growth either positively or negatively (Kadıoğlu and Yusuf, 2004). German physiology scientist Albrecht Kossel divided metabolites are two categories named "primer and seconder metabolites". Primer metabolites (protein, fat, carbohydrate) are found in all cells and have various functions. On the other hand, seconder metabolites are not found in all cells or do not have important functions in the life of plants. Up until now, 100.000 seconder metabolites are discovered, and this is thought to be over 1 million (Mammadov, 2014). Seconder metabolites (anthocyanins, carotenoids or flavonols, etc.) are being synthesized in low quantities in plants' metabolism but a major role in the plant's fitness for survival (Thoma et al., 2020). However, important seconder metabolites such as phenolics, alkaloids, terpenoids, glucosinolates, etc. are known allelochemicals. Generally, these compounds act with concentration, low concentrations are promoting plant growth and increasing concentration shows allelopathy effect (Farooq et al., 2011).

Styrax officinalis L. is a deciduous shrub distributed in the east and southeastern Asia and the Mediterranean region (Cesur et al., 2019;). The plant seed constituents some biochemical substances such as eugenol, benzofuran, homoegonol, gonol-β-gentiotrioside, egonol-β-gentiobioside, and fatty acids (Jaradat, 2020; Cesur et al., 2019; Akgul and Anil, 2003).
A lot of research was done on the allelopathic effect of plant extracts to effects on germination. Especially medicinal plant extracts are very important due to their seconder metabolites content. Kadıoğlu and Yanar (2004) used 22 plant extracts for determining and their effect on nine weeds germination. The result of the study, researchers have determined that some extracts can be used as herbicides. Özbay (2017), investigated some medicinal plant extract for effect on pepper (*Capsicum annuum* L.) germination. The results showed that the germination of pepper was affected by the concentration of extracts. Teaca et al. (2008), applied three different medicinal plant extract on soybean, bean, rye, wheat, and triticale. Increasing the dose of extract inhibits seed germination. As these studies show, extracts obtained from different parts of plants can show different effects on plants by showing alleloopathic effects.
This study was conducted to investigate allelopathic effect of tesbi seed extracts on sweet fennel (*Foeniculum vulgare* Mill. var. *dulce*), coriander (*Coriandrum sativum* L.), fenugreek (*Trigonella foenumgraecum* L.), camelina (*Camelina sativa* L.), and mustard (*Sinapis arvensis* L.).

Material and Methods

Tesbi (*Styrax officinalis* L.) seeds were finely ground with a laboratory-type blender. Each 100 ml tap water 4 gram tesbi seed powder added and boiled until reach to 15, 30 and 45 min. The obtained solution is rest for two days and then filtered. The experiment design with small pots with 10 replicates. Each pot bearing three seeds. In the experiment sweet fennel, coriander, fenugreek, camelina, and mustard are used. Soil was used in pots and after sowing control is irrigated with tap water and others irrigation was carried out with the prepared extracts. Seedling grow approximately 15 cm long (56 days later, (after true leaves have appeared and new leaves are formed) experiment is ended and seedling growth, seedling height, root length, seedling fresh-dry weights, and true leaf numbers were determined. After seedling fresh weight was determined, plants were dried in the oven at 70 °C for 48 hours and then dry weight was found.

Statistical analysis

Data were collected and subjected to analysis of variance procedure. Means were separated for significance using LSD Test at $p \leq 0.05$. The statistical analysis was done using the Statistical Analysis System software v.9.0.

Result and Discussion

Effects of seed extract of *Styrax officinalis* on seedling development of *Foeniculum vulgare* Mill. var. *dulce*

The effects of *S. officinalis* at different times doses are observed on morphological traits of sweet fennel, which are given in Table 1. There was a significant ($p \leq 0.05$) difference between treatments (seedling length, root length, and leaf). Our results showed that the S0 and S2 applications of tesbi bush had a positive effect on the seedling length and root length of the fennel.

The shortest seedling length and root length were obtained from S1 and S3 applications, respectively. However, the shortest leaf number of sweet fennel was determined from the S0 application.

Table 1. Effects of *S. officinalis* extracts of different doses on sweet fennel

Dose	Seedling length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Leaf (number)
S0	22.507 a	13.087 a	0.124 ns	0.0140 ns	2.000 b
S1	14.820 b	6.327 b	0.084 ns	0.0099 ns	4.133 a
S2	21.507 a	12.660 a	0.153 ns	0.0127 ns	4.533 a
S3	14.620 b	6.640 b	0.081 ns	0.0103 ns	4.266 a
LSD	3.324	2.874	0.106	0.0042	0.776
CV	9.655	15.773	50.901	18.739	11.044

Means followed by the same letter within the column are not significantly different at P<0.05. ns, not significant

Effects of seed extract of *Styrax officinalis* on *Coriandrum sativum* L.

At high concentrations were significantly ($P \leq 0.05$) decreased to seedling lengths in coriander. The seedling length of the tesbi shrub applications was recorded as S0>S1>S2> S3. The results showed that different tesbi extracts negatively affected the root length, seedling fresh weight, and seedling dry weight of coriander compared to the control treatments (Table 2).

Table 2. Effects of *S. officinalis* extracts of different doses on coriander

Dose	Seedling length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Leaf number
S0	22.393 a	12.987 a	0.156 a	0.0245 a	2.266 b
S1	13.326 c	8.233 b	0.061 b	0.0079 c	4.200 a
S2	15.973 b	9.813 b	0.073 b	0.0130 b	4.800 a
S3	13.633 c	8.687 b	0.091 b	0.0091 c	4.666 a
LSD	2.094	2.673	0.050	0.002	0.643
CV	6.812	14.298	27.871	7.488	8.575

Means followed by the same letter within the column are not significantly different at P<0.05.

Effects of seed extract of *Styrax officinalis* on *Trigonella foenum-graecum* L.

The tesbi seed extracts (S1, S2, and S3) significantly ($p \leq 0.05$) reduced the seedling length and root length of fenugreek tested. It has been observed that the extracts affect the root development of the plant negatively. On the other hand, as the boiling time of the extracts increased, the fresh and dry weight, and leaf number of the seedlings increased. (Table 3).

Table 3. Effects of *S. officinalis* extracts of different doses on fenugreek

Dose	Seedling length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Leaf number
S0	24.967 a	16.613 a	0.292 b	0.039 c	2.200 c
S1	22.787 ab	13.827 ab	0.308 ab	0.041 bc	4.400 b
S2	20.827 b	12.460 b	0.394 a	0.055 a	5.133 a
S3	22.947 ab	13.180 b	0.398 a	0.049 ab	5.133 a
LSD	2.471	3.293	0.091	0.009	0.670
CV	5.736	12.474	13.806	9.788	8.440

Means followed by the same letter within the column are not significantly different at P<0.05.

Effects of seed extract of *Styrax officinalis* on *Sinapis arvensis* L.

The results on seedling growth of mustard are presented in Table 4. At high concentrations were significantly ($P \leq 0.05$) decreased to seedling lengths, root length, and seedling fresh weight in mustard. However, the S2 and S3 extracts increased the number of leaves (Table 4).

Table 4. Effects of *S. officinalis* extracts of different doses on mustard

Dose	Seedling length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Leaf number
S0	20.133 ab	11.040 ab	0.338 a	0.068 ns	2.733 c
S1	19.307 ab	10.387 ab	0.244 ab	0.043 ns	3.467 b
S2	20.740 a	12.540 a	0.218 b	0.036 ns	3.933 ab
S3	16.093 b	9.460 b	0.226 b	0.054 ns	4.533 a
LSD	4.286	3.009	0.100	0.048	0.634
CV	11.939	14.722	20.756	50.772	9.181

Means followed by the same letter within the column are not significantly different at $P<0.05$. ns, not significant

Although the camelina plant emerged in other applications except for the control application, there was no development in the seedlings. Therefore, it was excluded from the study.

In this study, the effects of extracts on seedling growth, seedling height, root length, seedling fresh-dry weights, and true leaf numbers were determined in different plants. When the control application and different applications of the test seed extract were compared, the highest values were obtained from the control, while some decreases were observed with increasing boiling time in different applications. Also, the responses of plant species were different. Allelopathy is one plant's directly affecting another plant's growth either positively or negatively, exuding chemical substances (Rice, 1984). Verma et al. (2012), reported that the inhibitory effect was exhibited by all the extracts with a maximum in leaf followed by root and seed extract. Karaaltin et al. (2004) reported that *Nerium oleander* extracts significantly reduced radicle length. Also, Baličević et. al. (2015), reported that seed allelopathic effect, both stimulatory and inhibitory, was reported in other species.

Conclusion

The results showed that extracts from tesbi bush negatively affected seedling length, root length, seedling fresh weight, and seedling dry weight of all tested crops compared to the control treatments. However, it was observed that it increased the number of leaves in all plants. The effect of the extracts differed depending on the boiling times and the plant species. We can say that the chemical composition of the extract changes depending on the boiling time. The findings obtained from this study are preliminary information and various phytochemical analyzes are needed to fully explain this effect.

References

Akgul Y. Y., Anil H. (2003). Benzofurans and another constituent from seeds of *Styrax officinalis*. Phytochemistry, Vol. 63, pp. 939-943.

Baličević R., Ravlić M., Ravlić I. (2015). Allelopathic effect of aromatic and medicinal plants on *Tripleurospermum inodorum* (L.) C.H. schultz. Herbologia Vol. 15, pp. 41-53.

Cesur C., Uskutoglu T., Coşge Şenkal B., Kiralan S. S., Kiralan M., Ramadan M. F. (2019). Infuence of harvest time on protein content and lipids profle of wild *Styrax ofcinalis* seeds from different locations in Turkey. Rendiconti Lincei. Scienze Fisiche e Naturali, Vol.30, pp. 689–698.

Farooq M., Jabran K., Cheema Z. A., Wahid A., Siddique K. H. (2011). The role of allelopathy in agricultural pest management. Pest management science, Vol. 67, pp. 493-506.

Jaradat N. (2020). Phytochemistry, traditional uses and biological effects of the desert plant *Styrax officinalis* L. Journal of Arid Environments, Vol. 182, pp. 104253.

Kadıoğlu İ., Yanar Y. (2004). Allelopathic effects of plant extracts against seed germination of some weeds. Asian Journal of Plant Sciences, Vol. 3, pp. 472-475.

Karaaltin S., Idikut L., Uslu O. S., Erol A. (2004). Effect of root, stem, leaf and bud extracts of oleander (*Nerium oleander*) on the germination of seed and seedling growth of bean and Wheat. KSU Journal of Science and Engineering, Vol. 7, pp. 111-115.

Mammadov, R. 2014. Tohumlu Bitkilerde Sekonder Metabolitler [Secondary Metabolites in Flowering Plants], Nobel Akademik Yayıncılık, Ankara.

Özbay N. (2018). Allelopathic effects of some herbs and medicinal plants' extracts on seed germination and seedling growth of pepper. Turkish Journal of Agricultural and Natural Sciences, Vol. 5, pp. 81-85.

Rice E. L. (1984). Allelopathy, Academic effect of the subterranea organs of johnsongrass press, New York.

Teaca C. A., Bodirlau R., Oprea A., Tanase C., Colceru S. (2008). Influence of plant extracts on germination and post-germination development of different species. Cellulose Chemistry & Technology, Vol. 42, pp. 47-53.

Thoma F., Somborn-Schulz A., Schlehuber D., Keuter V., Deerberg G. (2020). Effects of light on secondary metabolites in selected leafy greens: A review. Frontiers in plant science, Vol. 11, pp. 497.

Verma S. K., Kumar S., Pandey V., Verma R. K., Patra D. D. (2012). Phytotoxic effects of sweet basil (*Ocimum basilicum* L.) extracts on germination and seedling growth of commercial crop plants. European Journal of Experimental Biology, Vol. 6, pp. 2310-2316.

THE INFLUENCE OF NITROGEN ON THE GRAIN YIELD AND SOME AGRONOMIC CHARACTERISTICS OF SWEET SORGHUM (*Sorghum bicolor* var. *saccharatum*)

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Abstract

Sorghum species are widely grown in the sub-tropical and temperate regions of the world as animal feed, human food, or bioenergy feedstock. It is capable of overcoming adverse climatic conditions through variable growth alternatives and sustains grain and forage production. Sweet sorghum has also many advantages such as wide adaptability, drought and waterlogging tolerance, saline-alkali resistance, and fast growth. Nitrogen is a crucial component of plant nutrition, and its deficiency limits productivity of crops more than any other element. Nitrogen (N) levels can alter performance of sweet sorghum in the sustainable second crop production system. In this reason, a pot experiment was conducted to determine the effects of nitrogen levels on the grain yield and some agronomic traits of sweet sorghum (*Sorghum bicolor* var. *saccharatum*). The experiment was carried out at the Bornova experimental fields of Field Crops Department of Agriculture Faculty, Ege University, Turkey, during 2019 second-crop growing season with typical Mediterranean climate conditions. The experiment was laid out in randomized complete block with four replicates. Seven nitrogen levels (0-50-100-150-200-250-300 kg N ha^{-1}) were tested on sweet sorghum. Plant height, panicle length, biomass yield and grain yield were affected significantly in line with higher N rates except thousand grain weight. Application of the higher rates of nitrogen levels increased the grain yield and some agronomic yield characteristics compared to the control level. The highest grain yield was obtained with the application of 200 kg ha^{-1} N in sweet sorghum under second crop production system.

Keywords: *Sweet sorghum, N level, grain yield.*

Introduction

Sweet sorghum (*Sorghum bicolor* var. *saccharatum*) produces grain, which can be harvested for human consumption, like the grain sorghum, in tropical, temperate, semi-arid regions, and also poor quality soils of the world. It is quantitatively the world's fifth largest most important cereal grain, after wheat, maize, rice and barley. The crop is a C_4 species with greater strength to various agro-ecologies, limited water and fertilizer condition in additional to short lifecycle (Rao and Kumar, 2013). Basically, sweet sorghum provides like food, fodder and fuels all three requirements in one crop. For this reason it is called that 'smart' crop or 'angel' crop. And it is more profitable comparison to competing crops like maize and sugarcane under rainfed conditions (Mokariya and Malam, 2020).
To control nitrogen more succesfully in sweet sorghum production grown as second crop, it is critical to be aware of the N functioning of soil-sorghum ecosystems in more factor, for which knowledge about N uptake by sweet sorghum is needed (Harmsen, 2003). The application nitrogen results in enhancement in the grain yield and model plant growth of any crop until an

ideal rate is reached (Olugbemi, 2017). For food safety management and against the global warming potential, there is an inevitable need to reform nitrogen use planning in sustainable agricultural production system (Anas et al., 2020). The target of this study was to determine the effects of different nitrogen levels performance of grain productivity of sweet sorghum grown second crop production system.

Material and Methods

A pot study was conducted in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey during 2019 second crop growing season with typical Mediterranean climate conditions. Some meteorological data from the experimental area in Bornova-Izmir are presented in Table 1. The experiment soil was analyzed for pH: 5.83, soil texture: loamy, avaiable Ca:1300 ppm, avaiable P: 2.54 ppm, avaiable K: 40 ppm, $CaCO_3$: 0.82%, organic matter: 2.27%, total nitrogen: 0.092%, total salt: 0.03%, sand: 80.2%, clay: 1.8%, silt: 18.0%.

Table 1. Some meteorological data of research area in Bornova, Izmir, Turkey in 2019

	2019		Long Year Average	
	Temperature (ºC)	Precipitation (mm)	Temperature (ºC)	Precipitation (mm)
August	29.8	0.0	27.9	12.7
September	24.6	31.7	23.9	18.0
October	21.2	4.0	19.1	27.7
November	16.9	41.0	13.8	31.5
Total-Mean	23.1	76.7	21.2	89.9

"Sugar-drip" variety of sweet sorghum (*Sorghum bicolor* var. *saccharatum*) was used as tested crop material. The seeds were sown in a plastic pots filled with 17 kg experimental soil on 2th August 2019, at 1 cm depth. Seven rates of nitrogen (0, 50, 100, 150, 200, 250 and 300 kg N ha^{-1}) were distributed in a completely randomized block design with four replicates. Half a dose of nitrogen fertiliser (urea), 80 kg ha^{-1} P_2O_5 (triple superphosphate) and 100 kg ha^{-1} K_2O (potassium sulphate) were applied with sowing, and the rest of nitrogen ($(NH_4)_2SO_4$) was applied at beginning of stem elongation. During the experiment, all of the pots were irrigated by tap water. In possible rainy weather, the experiment was covered by nylon sheet. Weeds were manually removed into the pots; no herbicide was used to control weeds. Alphacypermethrin was applied at 6-7 leaf stage of crops to control European corn borer (*Ostrinia nubilalis*). Panicles in every pot were isolated by net for avoiding bird damage and obtaining grain yield.

The plants were harvested for grain at physiological maturity stage (~13% moisture) by hand. According to the different nitrogen levels applied, the date of grain harvest continued from 28th October to 9th November. Plant height (cm); the plant was measured from the soil surface to the top level of the plant before grain harvest (Anonymous, 2010). Panicle length (cm); the panicle of plant was measured from the origin of panicle to the tip part. Biological yield (g plant^{-1}); after the harvesting, total above-ground biomass of plant (with grains) was measured; Grain yield (g plant^{-1}); the grains of plant were weighted after harvesting (Anonymous, 2010). Sweet sorghum grains (100) were weighted and converted for calculation of 1000-seed weights. The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD test (0.01) was performed to separate them (Stell et al., 1997).

Results and Discussion

Plant height positively responded to increased N fertilization compared the control level (Table 2). The highest plant height was measured with N200 (179 cm), whereas the lowest plant height was observed with N0 (146 cm). And N200 and N250 were statistically in the same group. Nitrogen may influence plant growth through cell division and cell expansion which thus increase plant height (Stals and Inze, 2001). Some reports show that application of N increases plant height in sweet sorghum (Usofzadeh et al., 2013; Geren and Girgin, 2014). For example, Usofzadeh et al. (2013) studied nitrogen at 0 (166 cm), 100 (175.2 cm), 200 (185.4 cm) and 300 (186.5 cm) kg ha^{-1} on sweet sorghum and found that plant height was increased by nitrogen application compared to the control (0 kg N ha^{-1}). Present study totally matches the results of the researches mentioned above. ANOVA results exposed significant effect of N application at different levels on panicle length of sweet sorghum (Table 2). N200 gave the highest panicle length (19.8 cm) which was statistically the same group with N150 (17.8 cm) and N250 (17.9 cm). The lowest panicle length was observed from N untreated pots (N0:15.1 cm) which was statistically similar with N50 (15.6 cm). Gebremariam and Assefa (2015) applicated four various N levels (0, 50, 100 and 150 kg N ha^{-1}) on the sweet sorghum plants. Researchers stated that the increased panicle length at high level of N application in that study (N0:17, N50:18, N100:21 and N150:21 cm). On the other study, Kugbe et al. (2019) found that different N levels (0, 30, 60, 90, 120,150 kg N ha^{-1}) positively influenced panicle length of local sorghum varieties. Present findings are in parallel with those researcher's results.

Table 2. Effects of different N levels on the yield and some yield components of sweet sorghum

N levels	Plant height (cm)	Panicle length (cm)	Biological yield (g plant^{-1})	Grain yield (g plant^{-1})	1000 grain weight (g)
N0	146 d	15.1 d	38.8 g	13.7 e	16.8
N50	151 cd	15.6 cd	73.8 f	16.7 e	16.7
N100	155 c	16.9 bc	110.0 e	28.9 d	16.3
N150	169 b	17.8 b	123.8 d	31.2 d	16.6
N200	179 a	19.8 a	187.0 a	65.3 a	17.0
N250	173 ab	17.9 ab	162.3 b	49.9 b	16.0
N300	166 b	16.6 bc	137.5 c	38.2 c	15.7
Mean	162.6	17.1	119.0	34.8	16.4
LSD	**	**	**	**	ns

**: significant at $P<0.01$, ns: not significant

As shown in Table 3, response of biological yield per plant to different N rates was significant. Increasing N levels enhanced the biological yield up to N200 level, but after that, the biological yield noticeably reduced. The maximum biological yield was recorded at fertilizer level of N200 (187 g plant^{-1}) whereas the lowest biological yield was recorded from nitrogen untreated pots (N0:38.8 g plant^{-1}). Our result is in line with the findings of Melaku et al. (2018) who found higher biomass yield with increased levels of N in sweet sorghum. Their results determined that application of 23, 41, 64 and 87 kg N·ha^{-1} gave a yield increase of 40, 53, 62 and 69% over the control (0 kg N ha^{-1}), respectively. Nitrogen element in the plant displays a positive correlation with the plant biomass, as well as N absorption ability (Nguyen et al., 2003). There was a significant effect of N levels on grain yield of sweet sorghum (Table 2). The least grain yield (13.7 g plant^{-1}) was at N0, and the highest (65.3 g plant^{-1}) was at N200. The grain yield of sweet sorghum progressively increased with the increasing levels of nitrogen up to 200 kg N ha^{-1}, in the experiment. Nitrogen fertilizer occurred in maximum grain yields due to sufficient soil nutrient environment that advanced uptake and promoted efficient nutrient translocation from leaves and stems into the filling seed (Kugbe et al., 2019). Application of 81.25 kg ha^{-1} of nitrogen observed significantly higher values of grain yield on sweet sorghum (2.1 t/ha) than that of 65 kg ha^{-1} (2.0 t/ha) and 48.75 kg ha^{-1} (1.8 t/ha) (Hugar et al., 2010). Increasing nitrogen fertilizer increased grain yield of sorghum by many researchers around the world (Geren and Girgin, 2014; Ajeigbe et al., 2018; Arshad et al., 2020). 1000-grain weight of sweet sorghum was not affected by N levels in our study. Average 1000-grain weight was 16.4 g. Geren and Girgin (2014) stated that increasing N level (from 0 to 375 kg N ha^{-1}) had no effect on 1000-grain weight of sweet sorghum. Almodares et al. (2006) reported that grain yield in sweet sorghum increased by the application of 90 kg N+90 kg K per ha under Iran conditions, although increasing N+K levels did not effect 1000-grain weight. Amiri et al. (2014) emphasized that grain yield in sorghum increased with the increasing N rate, but there were no significant differences among 160 kg ha^{-1} or 240 kg ha^{-1} N doses, and effect of N rate was not significant on 1000-grain weight.

Conclusions

Our findings indicated that N levels had a significant effect on the all of components (except 1000-grain weight) of our tested crop material in this study. Based on the results to obtain highest grain yield, we suggested that sugar-drip cultivar of sweet sorghum may be preferred, which well adapted to experimental area, and 200 kg ha^{-1} N treatment should be applied for higher grain yields and related properties under second crop production system. However, this type of experiment should be repeated different locations and at least two years in order to give complete advice in the local farming system.

References

Ajeigbe, H.A., Akinseye, F.M., Ayuba, K., Jonah, J. (2018). Productivity and water use efficiency of sorghum (*Sorghum bicolor* (L.) Moench) grown under different nitrogen applications in Sudan Savanna Zone, Nigeria. International Journal of Agronomy. 2018:1-11.

Almodares. A., Taheri, R., Hadi, M.R., Fathi, M. (2006). The effect of nitrogen and potassium fertilizers on the growth parameters and the yield components of two sweet sorghum cultivars. Pakistan Journal of Biological Sciences. 9(12):2350-2353.

Amiri, M., Mojaddam, M., Shokouhfar, A. (2014). The effect of nitrogen management on the yield and yield components of grain sorghum (*Sorghum bicolor* L. Moench). International Journal of Biosciences 4(1):272-278.

Anas, M., Liao, F., Verma, K.K., Sarwar, M.A., Mahmood, A., Chen, Z.L., Li, Q., Zeng, X.P., Liu, Y., Li, Y.R. (2020). Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. 53(47):1-20.

Anonymous. (2010). Technical instruction for measuring agricultural values, T.R. Ministry of Agriculture and Rural Affair, The Center of Seed Registration and Certification, Sorghum (*Sorghum spp*.), 13p. Ankara, Turkey.

Arshad, M., Nawaz, R., Ahmad, S., Shah, G.A., Faiz, F., Ahmad, N., Irshad, M.A. Ranamukhaarachchi, S.L. (2020). Growth, yield and nutritional performance of sweet sorghum and legumes in sole and intercropping influenced by type of legume, nitrogen level and air quality. Polish Journal of Environmental Studies. 29(1):533-543.

Gebremariam, G., Assefa, D. (2015). Nitrogen fertilization effect on grain sorghum (*Sorghum bicolor* L. Moench) yield, yield components and witchweed (*Striga hermonthica* (Del.) Benth) infestation in Northern Ethiopia. International Journal of Agricultural Research, 10: 14-23.

Geren, H., Girgin, V.Ç. (2014). Effects of nitrogen levels on the grain yield and related characteristics of sweet sorghum (*Sorghum bicolor* (L.) Moench var. *saccharatum*) in a Mediterranean environment. 25th International Scientific-Experts Congress on Agriculture and Food Industry, Izmir, Turkey, 25 September 2014, 1:209-212.

Harmsen, K. (2003). A comparison of the isotope-dilution and the difference method for estimating fertilizer nitrogen recovery fractions in crops. I. Plant uptake and loss of nitrogen. NJAS Wageningen Journal of Life Sciences. 50:321-347.

Hugar, A.Y., Jayadeva, H.M., Rangaswamy, B.R., Shivanna, S., Chandrappa, H. (2010). Assessing the effect of nitrogen and harvesting stages on yield and yield attributes of sweet sorghum genotypes. Agricultural Science Digest. 30(2):139-141.

Kugbe, J.X., Mbawuni, A.M., Wisdom, A. (2019). Growth, yield and yield components response of local sorghum varieties to nitrogenous fertilizer rates in Northern Ghana. World Journal of Agriculture and Soil Science. 3(5):1-10.

Melaku, N.D., Bayu, W., Ziadat, F., Strohmeier, S., Zucca, C., (2018). Effect of nitrogen fertilizer rate and timing on sorghum productivity in Ethiopian highland. Journal of agronomy and Soil Science. 64(4): 480-491.

Mokariya, L.K. and Malam, K.V., (2020), Sweet Sorghum: A smart, multipurpose crop. Agriculture and Environment. 1(1): 61-68.

Nguyen, N.T., Nakabayashi, K., Mohapatra, P.K, Thompson, J., Fujita, K. (2003). Effect of nitrogen deficiency on biomass production, photosynthesis, carbon partitioning, and nitrogen nutrition status of Melaleuca and Eucalyptus species. Soil Science and Plant Nutrition. 49(1): 99-109.

Olugbemi, O. (2017). Sweet Sorghum and Nitrogen Fertilizer Application – A Review. MAYFEB Journal of Agricultural Science. 2:28-35.

Rao, S., Kumar, C.G. (2013). Characterization of Improved Sweet Sorghum Cultivars. Springer India. India.

SAS Institute (1998). INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Stals, H., Inze, D. (2001). When plant cells decide to divide. 6(8): 359-364.

Stell, R.G.D., Torrie, J.A., Dickey, D.A. (1997). Principles and Procedures of Statistics. A Biometrical Approach 3rd Edi, Mc Graw Hill Book. INC. NY.

Usofzadeh, M., Daneshvar, M., Almodares, A., Eisvand, H.R. (2013). 'Effects of nitrogen fertilizer and plant growth regulator on stalk yield and bioethanol in sweet sorghum stress'. Iranian Journal of Plant Physiology. 3(3):711-716.

POTENTIAL GROWTH AND HERBAGE PRODUCTIVITY OF COMMON BURNET (*Poterium sanguisorba*) AS AFFECTED BY NITROGEN FERTILIZATION

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Abstract

The objective of this study was to examine the effects of N fertilization on herbage yield and plant-yield components of common burnet. A pot study was carried out with six different nitrogen levels (0, 50, 100, 150, 200 and 250 kg N ha^{-1}) on common burnet (Bünyan 80 cv). The experiment was arranged as a completely randomized block design with four replications from October 2019 to November 2020 in the outdoor conditions at Izmir, Turkey. Results shown that rate of N has a significant effect on plant height, fresh and dry herbage yield, and crude protein (CP) yield of burnet. Based on the results of this study, application of nitrogen at the rate of 200 kg ha^{-1} can be recommended for obtaining the maximum yield in common burnet under Mediterranean climate conditions.

Keywords: *N level, Poterium sanguisorba, Dry herbage yield, CP yield.*

Introduction

Common burnet (*Poterium sanguisorba*) also known as sheep's burnet or salad burnet, or small burnet is herbaceous, perennial, evergreen forb in the *Rosaceae* family (Andrabi et al., 2012). Crop has a prominent taproot system, simple or branched stems, 20-60 cm tall, lower leaves adherent to the petiole and 10-15 cm long, each flower provides approximately 12 stamens and the four broad sepals (4-5 mm long, white to red or purple), but it has no petal (Cronquist, et al. 1997). It has been reported that a hay yield of 10-35 t/ha can be obtained according to growing conditions, and also 70-80 t/ha total fresh herbage yield can be obtained by cutting 5-11 times a year under irrigated conditions (Tansı and Anlarsal, 1991). Small burnet has well to excellent forage value for livestock and wildlife during all seasons (Arzani et al., 2006). It has drought, heavy frost (up to -12°C) and high summer temperatures (>30°C) tolerance (Wills et al., 1987; Stevens and Monsen, 2004). Burnet plants are also tolerant of intense grazing and satisfactory survival and production under low to moderate fertility conditions (Buckland et al., 1997).

Nitrogen is a paramount element for plants since it is a core component of many plant structures and for both their internal and external metabolic processes. One of the major facts of N is increasing chlorophyll accumulation; this process is done by creating bigger leaf structures with larger surface areas for the photosynthesizing pigment. Extravagant nitrogen fertilization has caused low N use efficiencies and critical ecological problems. For this reason, level N fertilization programs must be noted for achieving high plant yield and sustainable agriculture system (Xu et al., 2012; Zhu et al., 2016). Considering all these literatures, the objective of this study was to evaluate the influence of different nitrogen levels on the herbage yield and some yield components of burnet under Mediterranean climate agroecology conditions.

Material and Methods

A pot study was conducted in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey from October 2019 to November 2020 with typical Mediterranean climate conditions. Some meteorological data from the experimental area in Bornova-Izmir are presented in Table 1. The experiment soil was analyzed for pH: 5.83, soil texture: loamy, avaiable Ca:1300 ppm, avaiable P: 2.54 ppm, avaiable K: 40 ppm, $CaCO_3$: 0.82%, organic matter: 2.27%, total nitrogen: 0.092%, total salt: 0.03%, sand: 80.2%, clay: 1.8%, silt: 18.0%.

Table 1. Some meteorological data of research area in Bornova, Izmir, Turkey in 2019-2020

	Temperature (ºC)		**Precipation (mm)**	
Months	**2019-2020**	**Long Year Average**	**2019-2020**	**Long Year Average**
October	20.0	19.1	4.0	43.2
November	17.2	13.8	41.0	109.7
December	10.8	10.5	69.7	137.9
January	7.0	9.0	25.5	112.2
February	9.4	9.2	65.4	99.7
March	12.0	11.8	47.1	82.9
April	14.7	16.1	27.6	46.4
May	20.6	21.0	17.7	25.4
June	23.0	26.0	5.0	7.5
July	26.0	28.3	1.0	2.1
August	26.0	27.9	1.0	1.7
September	22.0	23.9	12.0	19.9
October	17.0	19.1	39.0	43.2
November	12.0	13.8	78.0	109.7
Total/Mean	17.0	17.8	434.0	841.5

Bünyan-80 variety of burnet (*Poterium sanguisorba*) was used as test material. The seeds were sown in a plastic pots filled with 17 kg experimental soil on 22th October 2019, at 1 cm depth. Six rates of nitrogen (0, 50, 100, 150, 200 and 250 kg N ha^{-1}) were distributed in a completely randomized block design with four replicates. 1/3 dose of nitrogen fertiliser (urea), 80 kg ha^{-1} P_2O_5 (triple superphosphate) and 100 kg ha^{-1} K_2O (potassium sulphate) were applied with sowing,

and 1/3 of the rest nitrogen dose was given in the first cutting, and the last 1/3 was given in the form of ammonium sulfate after the second cutting and sprinkled on the pot surface. Weed control was performed by hand. No evident crop diseases or pests were detected.

During the vegetation period, four harvests were made to determine the herbage yield of the burnet plant (21th April, 1th June, 12th August, and 3th November of 2020). It was expected that the plant would start to bloom in the harvests aimed at determining the herbage yield. The distance between above soil level and the tip point of the plant was measured before harvest with a ruler for the plant height (cm). The plant reaching the harvest period was cut by leaving 3 cm of stubble height from the soil level and the green part obtained was weighed for fresh herbage yield. The harvested crops were dried to a constant weight at 65ºC during 48 h and then were weighed (g plant^{-1}). The dried samples were ground in a mill passed through a 1 mm screen. CP contents of samples were determined using the Kjeldahl method (N%) with a conversion factor of 6.25. CP yield was calculated by multiplying the CP content with the dry yield. The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD test (0.01) was performed to separate them (Stell et al., 1997).

Results and Discussion

Results presented in Table 2 shows that nitrogen levels had a significant effect on the all of tested characteristics in this study.

Table 2. Effects of different N levels on the yield and some yield components of burnet

N levels	Plant height (cm)	Fresh herbage yield (g pot^{-1})	Dry herbage yield (g pot^{-1})	CP Content (%)	CP yield (g pot^{-1})
N0	34.2 d	255 e	71 e	10.7 e	7.6 e
N50	36.1 cd	312 d	96 d	12.3 d	11.7 d
N100	37.4 c	368 c	110 c	13.1 c	14.4 c
N150	43.1 a	434 b	133 b	13.6 b	18.0 b
N200	44.0 a	490 a	146 a	14.7 a	21.5 a
N250	39.8 b	446 b	129 b	14.7 a	18.9 b
Mean	39.1	384	114	13.2	15.0
LSD (1%)	2.04	17.62	10.99	0.29	1.53
CV (%)	3.69	3.24	6.81	1.6	7.05

ANOVA indicated that nitrogen levels had significant effect on plant height in this study (Table 2). The maximum plant height (44.0 cm) was observed from N200 while the lowest plant height (34.2 cm) was observed from N0. And also there were no significant differences between N150 and N200 levels. In our study, increasing N levels increased plant height of burnet compared to the control. Çavdar et al. (2021) applied 100 kg N ha^{-1} on burnet and they recorded 20.9 cm for the plant height with irrigated conditions at the indoor experiment. İpek and Sevimay (2002) stated that increased N levels increasing plant height (N0:66.6, N40:68.1, N80:70.0 cm) of burnet plants. N is known to be functional in the construction of amino acids and chlorophyll, which can influence directly plant height by affecting photosynthesis (Xu et al., 2012).

There were statistically significant differences in fresh and dry herbage yield among the different nitrogen levels (Table 2). The highest fresh (490 g pot^{-1}) and dry (146 g pot^{-1}) herbage yield was recorded in N200, whereas the lowest fresh (255 g pot^{-1}) and dry (71 g pot^{-1}) herbage yield was recorded in N0 in our study. And there were no statistical differences between N150 and N250 levels. The yields of fresh and dry herbage of burnet progressively increased with the increasing levels of nitrogen up to 200 kg N ha^{-1}, in the experiment. Doran (2020) applied 100 kg DAP ha^{-1} on different geneotypes of burnet plant and the fresh herbage yield varied between 0.4 and 1.9 t/ha, while the dry herbage yield varied between 0.17 and 0.591 t/ha in this research. Acar et al. (1999) found difference in the fresh and dry herbage yield of burnet during the two years, where 0, 40, 80 and 120 kg N ha^{-1} were applied. They stated that the maximum fresh (2.5 t/ha) and dry (0.9 t/ha) herbage yield were observed from 120 kg N ha^{-1} but the minumun fresh (1.1 t/ha) and dry (0.4 kg/ha) herbage yield were observed from 0 kg N ha^{-1} level (control). N significantly increases and enhances the yield by playing a vital role in biochemical and physiological functions of plant (Leghari et al., 2016). Our findings confirmed those researcher's results.

The CP content of burnet increased noticeably by increasing nitrogen application comparing to the control level (N0) (Table 2). N200 and N250 gave the highest CP content (14.7%) as a same group. The lowest CP content (10.7%) was obtained in N0 level. Researchers examined the effect of different maturity periods on CP content of burnet. CP contents were recorded of as periods of pre-flowering (20.7%), flowering (13.7%) and seed maturing (6.7%) stage, respectively (Kaplan et al., 2014). In another study on burnet crop, the variation range of CP contents is between 10.3% and 14.0% with the applied as constant 100 kg N ha^{-1} (Çavdar et al., 2021). The increase in CP content with increasing in N fertilizer levels perhaps the result of enhancement in amino acid formation due to fertilization (Patel et al., 1994). Our findings in the similar line those researcher's results for the CP content.

Statistical analysis indicated that nitrogen levels had positively significant effect on CP yield in the experiment (Table 2). The highest CP yield (21.5 g pot^{-1}) was determined in N200, but the minimum CP yield (7.6 g pot^{-1}) calculated in N0. There was no statistical difference between N150 and N250 levels. Acar et al. (1999) reported that CP yield (720, 1200, 1500 and 1800 kg·ha^{-1}) of burnet were increased by increasing N level (0, 40, 80 and 120 kg N ha^{-1}), respectively. In other study, 0, 40 and 80 kg ha^{-1} N fertilizers were given to burnet by İpek and Sevimay (2002). Researchers found that increased N levels enhanced the yield of CP as 480, 645 and 775 kg·ha^{-1}, respectively. Our findings in parallel those researcher's results with increase the CP yield by N fertilization.

Conclusions

The results revealed that increasing N levels were significantly improved crop yield and quality of common burnet compared to the control. Application of nitrogen at the rate of 200 kg ha^{-1} can be recommended for obtaining the maximum yield in common burnet under Mediterranean climate conditions. However, these results need to be confirmed in field trials conducted in different locations.

References

Acar, Z., Sancak, C., Ayan, İ. (1999). Determination of yield and some characteristics of lesser burnet (*Sanguisorba minor* Scop.) grown in different nitrogen dozes and row spacings. Journal of Agricultural Sciences. 5(2), 41-49.

Andrabi, S.M., Rehman, W., Reshi, Z.A., Naqshi, A.R., Ganie, A.H. (2012). *Sanguisorba minor* Scop. (*Rosaceae*), a new addition to the Indian flora. Taiwania. 57(4): 410-412.

Arzani, H., Basiri, M., Khatibi, F., Ghorbani, G. (2006). Nutritive value of some Zagros mountain rangeland species. Small Ruminant Research. 65:128-135.

Buckland, S.M., Grime, J.P., Hodgson, J.G., Thompson, K. (1997). A comparison of plant responses to the extreme drought of 1995 in northern England. Journal of Ecology. 85:875-883.

Cronquist, A., Holmgren, N., Holmgren, P. (1997). Intermountain Flora, Vascular Plants of the Intermountain West, U.S.A. Volume 3 part A Subclass Rosidae (except Fabales). The New York Botanical Garden. 446 p.

Çavdar, Ö.T., Tepecik, M., Geren, H. (2021). A preliminary study on the effect of deficit irrigation application on the yield and some yield characteristics of burnet (*Poterium sanguisorba*) in early growth stage. ISPEC Journal of Agricultural Sciences. 5(2): 362-371.

Doran, T. (2020). Determination of herbage yield and quality characteristics of different *Sanguisorba minor* Scop. genotypes. Erciyes University Institute of Natural and Applied Sciences, M.Sc. thesis, 41p.

İpek, A., Sevimay, C.S. (2002). Effects of nitrogenous fertilization on forage yield and yield components of garden burnet (*Sanguisorba minor* Scop.). Journal of Agricultural Sciences. 8(4): 274-279.

Kaplan, M., Kamalak, A., Kasra, A.A., Güven, İ. (2014). Effect of maturity stages on potential nutritive value, methane production and condensed tannin content of *Sanguisorba minor* hay. Kafkas University Journal of Veterinary Science, 20(3), 445-449.

Leghari, S.J., Wahocho, N.A., Laghari, G.M., Laghari, A.H., Bhabhan, G.M., Talpur, K.H., Bhutto, T.A., Wahocho, S.A., Lashari, A.A. (2016). Role of nitrogen for plant growth and development: a review. Advances in Environmental Biology. 10(9):209–218.

Patel, G.N., Patel, P.G., Patel, J.C. (1994). Effect of nitrogen and phosphorus on yield and quality of forage sorghum (*Sorghum bicolor*). Indian JournalAgronomy. 39: 123-125.

SAS Institute (1998). INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Stell, R.G.D., Torrie, J.A., Dickey, D.A. (1997). Principles and Procedures of Statistics. A Biometrical Approach 3rd Edi, Mc Graw Hill Book. INC. NY.

Stevens, R., Monsen, S.B. (2004). Forbs for seeding range and wildlife habitats. Chapter 19, USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

Tansı, V., Anlarsal, A.E. (1991). Determining the most suitable cutting interval in burnet (*Poterium sanguisorba* L.) in GAP region. National 2nd Grassland and Forage Crops Congress, Izmir, Turkey, p: 285-291.

Zhu, G., Peng, S., Huang, J., Cui, K., Nie, L., Wang, F. (2016). Genetic improvements in rice yield and concomitant increases in radiation and nitrogen use efficiency in middle reaches of Yangtze River. Scientific Reports. 6:210–249.

Xu, G., Fan, X., Miller, A.J. (2012). Plant nitrogen assimilation and use efficiency. Annual Review of Plant Biology. 63:153–182.

Wills, B.J., Sheppard, J.S., Begg, J.S.C. (1987). Evaluation of alternative dryland pasture plants and browse shrubs for soil conservation in drought-prone Otago grasslands. New Zealand Grassland Association. 48:115-118.

EFFECT OF LIGHT PHOTOPERIOD ON GROWTH AND PHOTOSYNTHETIC INDICES OF KALE SEEDLINGS

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Abstract

Fresh leafy greens are the most consumed vegetables worldwide because of their known potential to enrich human health with minerals and compound shaving antioxidant properties. Recently more greens have been produced in controlled environment agriculture (CEA) systems, where the plants are grown under artificial lighting such as light-emitting diodes (LEDs). The properly selected LED lighting parameters, including photoperiod, can ensure healthy plant growth and productivity. This study aimed to evaluate the influence of LED lighting photoperiod on the development and photosynthetic indices of kale seedlings (*Brassica oleracea* 'Red Russian'). Plants were grown for 17 days in a closed controlled climate chamber: day/night temperature 21/18±2 °C, relative humidity 55±5 %, white LED (5700 K, Heliospectra, Sweden) light at 12, 18 and 24 h photoperiod, and total photon flux density (PPFD) of 200 µmol m^{-2} s^{-1}. The results showed that seedling hypocotyls were shorter due to the prolonged 18 and 24 h photoperiod, but plants grew taller than under the 12 h photoperiod. Seedlings grew under 24 h photoperiod formed longer and wider first true leaves and thicker hypocotyls and had higher fresh and dry weight than those under 12 h or 18 h illumination. Although the light period did not affect the photosynthetic rate and stomatal conductivity, the intercellular CO_2 concentration and transpiration intensity of kale decreased due to the prolonged illumination time. The lower levels of chlorophylls and carotenoids were found in seedlings grown at 18 and 24 h for lighting compared to those exposed at 12 h. In summary, the photoperiod is an important factor determining the vital processes in the plant, so it is necessary to select the duration of lighting to upgrade the growth rates without disturbing the function of the photosynthetic system of kale seedlings.

Keywords: *artificial lighting, controlled environment agriculture, leafy greens, light-emitting diodes.*

Introduction

The climate change and unfavourable climatic conditions promote seedling growers to produce high-quality seedlings in controlled environment agriculture (CEA) systems ("vertical farms", "plant factories") (Gómez et al., 2019). Leafy vegetables are increasingly produced in CEA systems seeking year-round production. Moreover, CEA systems allow the development of short food supply chains related to supply from growers in urban areas and consumers. In high-tech CEA, light is the essential factor because it regulates plants' morphogenesis, photosynthesis, and metabolism through wavelength-specific photosynthetic (chlorophylls and carotenoids) and morphogenetic (UV8, cryptochromes, phototropins and phytochromes) light receptors.

Therefore, to maximise the productivity of plants, it is important to determine optimal conditions of light parameters. As an artificial light source, light-emitting diodes (LEDs) can be used to make the plants grow more quickly in CEA production systems (Xu et al., 2016). The technology of LEDs has many advantages over traditional light sources like high-intensity discharge lamps. The advantageous properties of LEDs include their monochromatic nature, high photon efficiency, long life. All these favourable characteristics make LED applications typical for the cultivation of leafy vegetables indoors. In the recent decade, much attention was paid to evaluating light spectrum and intensity effects on various horticultural plants (Izzo et al., 2020; Qian et al., 2020; Liu et al., 2021). The higher intensity of blue, red and far-red (BRFR) LEDs lighting led to a higher total carotenoid content in red pak choi and mustard microgreens (Brazaitytė et al., 2015); the blue light increased β-carotene concentration in lettuce (Li and Kubota, 2009), and total carotenoids in lettuce, spinach, kale, basil, and pepper (Naznin et al., 2019). Plant soluble sugars, as the main products of photosynthesis can profoundly affect plant growth, particularly cell division and expansion, storage, signalling, and stress acclimation (Eveland and Jackson, 2012). Zhou et al. (2012) showed that increasing the increasing intensity of LED lighting increased the contents of soluble sugars in butterhead lettuce. Sucrose concentrations increased in red- and green-leaf lettuce when the green or red light was added to RBFR LEDs (Viršilė et al., 2020).

Moreover, the light and dark period a plant is exposed to controls many developmental and metabolic responses in plants (Iqbal et al., 2021; Lopes-Oliveira et al., 2021; Lopez et al., 2021). However, the effects of light duration of LED lighting indoors on leafy greens are less studied. Therefore,this study investigated how the LED lighting photoperiod affects the growth, photosynthetic parameters, and accumulation of metabolites in kale 'Red Russian' seedlings.

Material and Methods

The experiments were performed at Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, on August-September, 2020. Kale (*Brassica oleracea*) 'Red Russian' seedlings (CN Seeds, UK) were germinated and grown in a walk-in controlled environment growth room (4 m × 6 m) where the day and night temperatures of 21/18±2 °C and relative air humidity of 55±5 % were maintained. Seeds were sown into 2.5 cm × 2.5 cm rockwool cubes, pre-soaked in deionised water with an adjusted pH of 4.4–4.5 using diluted (1:31) 95–98% sulfuric acid. Seeded cubes were placed in plastic trays and covered with a lightweight agro-textile until the seeds germinated. Seedlings were watered with a modified Hoagland solution to supply the following nutrients (in mg L^{-1}): N, 120; P, 20; K, 128; Ca, 72; Mg, 40; S, 53; Fe, 4; Mn, 0.08; Cu, 0.08; B, 0.16; Zn, 0.8. The pH was 5.5–6.5, and the electrical conductivity (EC) was 1.3–1.7 mS cm^{-1} (GroLine HI9814, Hanna Instruments, USA).

For lighting treatments, kale seedlings were grown at 12-, 18- and 24-hour photoperiod of white (5700K) light-emitting diode (LED) lighting (Heliospectra, Sweden), and the total photon flux density (PPFD) was 200 μmol m^{-2} s^{-1}. The daily light integrals (DLI) of 12-, 18- and 24-hour LED lighting were 8.6, 12.96, and 17.28 mol m^{-2} d^{-1}. The experiment was repeated two times.

After 17 d from seed sow, destructive measurements were conducted on ten kale seedlings from each lighting treatment and replication. Each plant was cut from the rock wool cube and shoot fresh weight (FW, g), and dry weight (DW, g) was measured using an analytical balance (Mettler Toledo AG64, Columbus, OH, USA). Leaf length (cm) and width (cm) of the second fully expanded leaf were measured. Shoots were dried in an oven (Venticell 222, MBT, Czech

Republic) at 105 ºC for 24 h before DW measurements. Water content was calculated as the fraction of the difference between shoot FW and DW in FW and used to re-calculate biochemical compound contents in the DW of plants. The stem diameter (SD, mm) was measured using a digital calliper.

The photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), intracellular CO_2 concentration (µmol mol^{-1}), and the ratio of intercellular to ambient CO_2 concentration (Ci/Ca) were measured on the second developed leaf, using a portable photosynthesis system (LI-COR 6400XT, USA). The leaf chamber conditions were set at 21 ºC, a CO_2 concentration of 400 µmol mol^{-1}, and 60% relative humidity. Artificial irradiation in the leaf chamber was supplied from 665 and 470 nm LED sources (665 and 470 nm), PPFD ~1000 µmol m^{-2} s^{-1}. Photosynthetic parameters were measured from 9 to 12 am. Water usage efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O) was also calculated.

The fructose and glucose contents were analysed in leaves of kale seedlings, according to the Ma et al. (2014) method with some modifications. First, 0.5 g of fresh plant tissue was ground and diluted with deionised water. Then, the extraction was carried out for 4 h at room temperature with mixing. Samples were centrifuged at 14.000 g for 15 min. According to Brons and Olieman's (1983) instructions, a clean-up step to remove soluble proteins was performed before the chromatographic analysis. The analyses were performed on a Shimadzu HPLC (Japan) instrument equipped with an evaporative light scattering detector (ELSD). The separation of carbohydrates was performed on a Shodex VG-50 4D HPLC column with deionised water and acetonitrile gradient at a flow rate of 0.8 mL min^{-1}.

Contents of carotenoids (neoxanthin, violaxanthin, lutein and zeaxanthin, α- and β-carotenes and chlorophylls were evaluated according to the modified method of Edelenbos et al. (2001). Carotenoids and chlorophylls were extracted using 80% acetone (0.5 g of sample grounded with liquid N and 5 mL^{-1} of solvent), centrifuged (5 min, 4000 g), and filtrated through a 0.22 µm nylon membrane syringe filter (BGB Analytik Vertrieb GmbH, Germany). The HPLC 10A system (Shimadzu, Japan) equipped with a diode array (SPD-M 10A VP) detector was used for analysis. The separation of carotenoids and chlorophylls was performed on a Chromegabond C30 (120Å 3µm, 2.1 x 150mm) column (ES Industries, Germany). Peaks were detected at 440 nm. The mobile phase consisted of 80% methanol and 20% water and 100% ethyl acetate gradient at a flow rate of 0.2 mL min^{-1}.

The Analysis of variance (ANOVA) and Tukey's honestly significant difference test ($\alpha = 0.05$) and principal component analysis (PCA) ($\alpha = 0.05$) were performed using Microsoft® Excel® for 'Microsoft 365' and Addinsoft XLSTAT 2020 statistical and data analysis solution (Long Island, NY, USA). The results presented in the PCA biplot indicate distinct effects of lighting photoperiod on plant growth, photosynthetic and biochemical parameters, and the Pearson's correlation matrix summarises relationships between measurements of kale seedlings under the lighting treatments.

Results and Discussion

The significantly longer hypocotyls were of kale seedlings under 12- compared to 18- and 24 h photoperiod. Also, kale under 12 h was the lower fresh (FW) and dry (DW) and formed narrower and shorter leaves and thinner stem than seedlings under 18- and 24 h photoperiods. The highest FW and DW were of kale seedlings under 24 h photoperiod (Fig. 1).

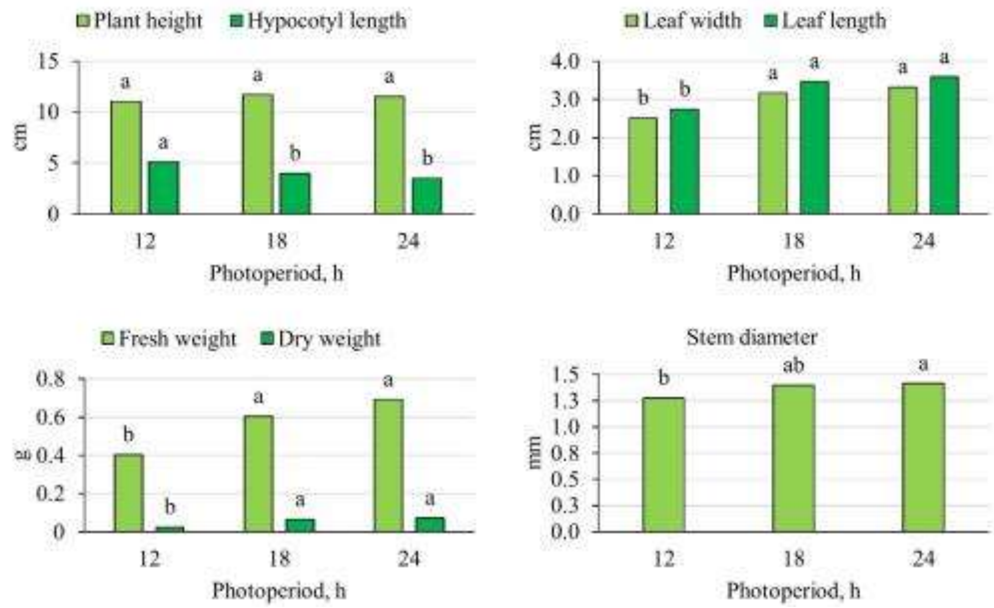

Figure 1. Growth parameters of kale seedlings grown under different LED lighting photoperiods. All values are expressed as mean. Means with different letters are significantly different at the $\alpha = 0.05$ (95%) level according to Tukey's HSD test.

The transpiration rate (Tr) of kale seedlings decreased with increasing LED lighting photoperiod. For example, the Tr of kale under 12 h was 1.3-fold higher than Tr under 24 h lighting. Contrary, the water usage efficiency (WUE) increased with increasing photoperiod determining the lowest WUE at 12- and the highest – at 24 h photoperiod. The photoperiod did not affect the photosynthetic rate, stomatal conductance, intercellular CO_2 concentration (Ci), as well as a ratio of intercellular to ambient CO_2 concentration (Ci/Ca) of kale seedlings (Fig. 2).
However, the changes in leaf pigments were determined (Fig. 3). The contents of chlorophyll *a* and *b* (Chl a, b) decreased with increasing photoperiod. About 2.0-fold lower contents of Chl a and b were determined under 24- compared to 12 h photoperiod. Moreover, the same tendency was determined on contents of neoxanthin, violaxanthin and lutein with zeaxanthin. The contents of α- and β-carotene were significantly 2.8- and 2.4-fold lower, respectively, in plants under 18- and 24 h than 12-h photoperiod.

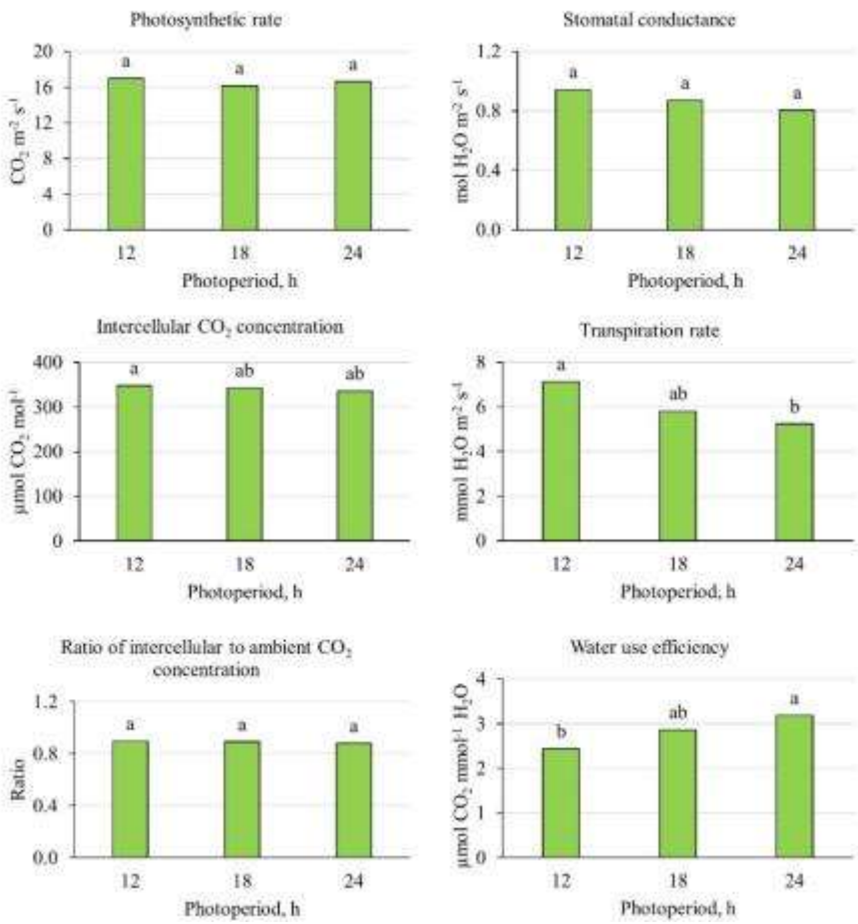

Figure 2. Photosynthetic parameters of kale seedlings grown under different LED lighting photoperiods. All values are expressed as mean. Means with different letters are significantly different at the $\alpha = 0.05$ (95%) level according to Tukey's HSD test.

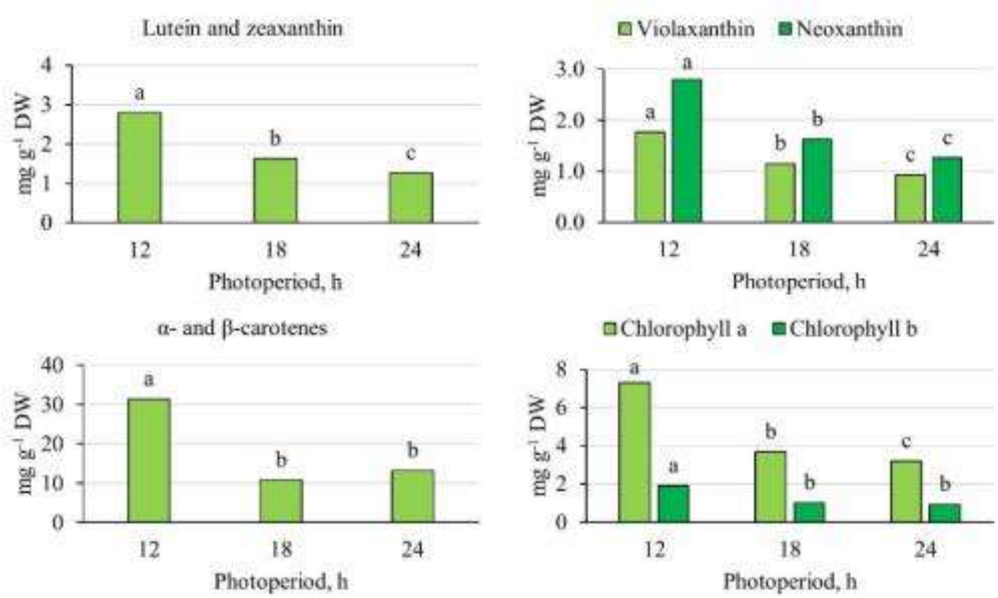

Figure 3. The contents of chlorophylls and carotenoids of kale seedlings grown under different LED lighting photoperiods. All values are expressed as mean. Means with different letters are significantly different at the α = 0.05 (95%) level according to Tukey's HSD test.

On the contrary, glucose and fructose contents increased with increasing photoperiod and were 7.1- and 5.4-fold higher in kale seedlings under 24 h photoperiod than plants grown under 12 h lighting (Fig. 4).

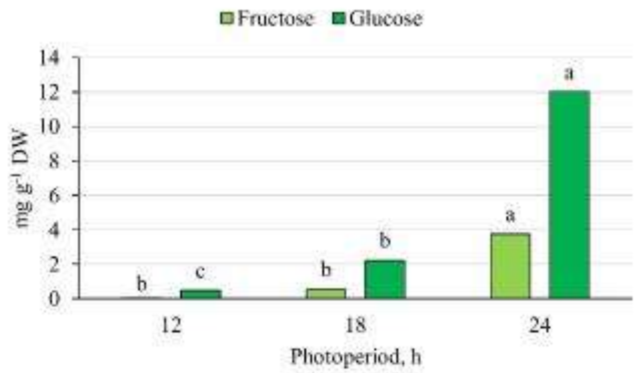

Figure 4. The contents of fructose and glucose of kale seedlings grown under different LED lighting photoperiods. All values are expressed as mean. Means with different letters are significantly different at the α = 0.05 (95%) level according to Tukey's HSD test.

The PCA biplots were analysed according to F1 and F2 factor loadings and scores to evaluate the associations between biometric, photosynthetic and biochemical measurements. It was found that 12-, 18- and 24 h photoperiods had distinct effects on parameters being assessed. The growth indices were associated with 18-, and leaf width and FW with 24 h photoperiod. The hypocotyl length and FW/DW ratio were not associated with any photoperiod, including 12 h. (Fig. 5, A). Tr and Pr were associated with 12- and WUE with 24 h photoperiod for photosynthetic parameters. The Ci, Ci/Ca, and gs were not associated with any photoperiod, including 18 h. (Fig. 5, B). The contents of sugars were linked to 24-, while all investigated carotenoids and Chl a and b were associated with the shortest 12 h photoperiod. The 18 h LED lighting was not associated with any investigated metabolite (Fig. 5, C).

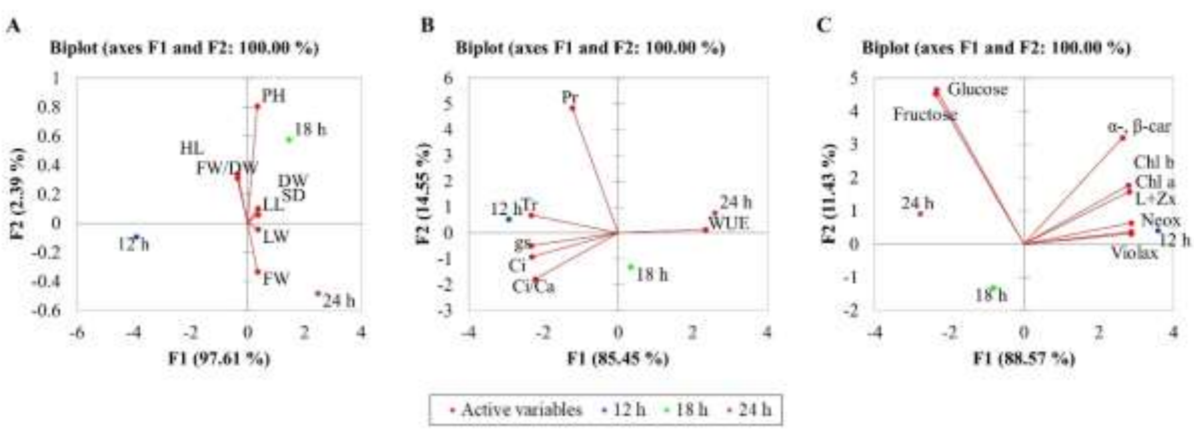

Figure 5. The PCA biplots, indicating distinct differences in growth (**A**) and photosynthetic parameters (**B**), and contents of chlorophylls and carotenoids (**C**) in kale seedlings grown under different LED lighting photoperiods. PH – plant height; HL – hypocotyl length, FW/DW – fresh and dry weight ratio, DW – dry weight; FW – fresh weight; SD – stem diameter; LW – leaf width; LL – leaf length; Pr – photosynthetic rate; Tr – transpiration rate; gs – stomatal conductance; Ci – intercellular CO_2 concentration; Ci/Ca- intercellular to ambient CO_2 concentration; WUE – water usage efficiency; α-, β-car – α- and β-carotene; Chl a and b – chlorophyll *a* and *b*; L+Zx – lutein and zeaxanthin; Neox – neoxanthin; Violax – violaxanthin.

Conclusions

In summary, the 18 h LED lighting photoperiod was the most appropriate for kale seedlings growth parameters. In addition, such lighting did not have a negatively impact on photosynthetic parameters. However, the 12- and 24 h photoperiods had a more pronounced effect on the accumulation of metabolites.

Acknowledgement

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References

Brazaitytė, A., Sakalauskienė, S., Samuolienė, G., Jankauskienė, J., Viršilė, A., Novičkovas, A., ... & Duchovskis, P. (2015). The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. *Food chemistry*, *173*, 600-606.

Brons, C., & Olieman, C. (1983). Study of the high-performance liquid chromatographic separation of reducing sugars, applied to the determination of lactose in milk. *Journal of Chromatography A*, *259*, 79-86.

Edelenbos, M., Christensen, L. P., & Grevsen, K. (2001). HPLC determination of chlorophyll and carotenoid pigments in processed green pea cultivars (Pisum sativum L.). *Journal of Agricultural and Food Chemistry*, *49*(10), 4768-4774.

Eveland, A. L., & Jackson, D. P. (2012). Sugars, signalling, and plant development. *Journal of Experimental Botany*, *63*(9), 3367-3377.

Gómez, C., Currey, C. J., Dickson, R. W., Kim, H. J., Hernández, R., Sabeh, N. C., ... & Burnett, S. E. (2019). Controlled environment food production for urban agriculture. *HortScience*, *54*(9), 1448-1458.

Iqbal, Z., Iqbal, M. S., Hashem, A., Abd_Allah, E. F., & Ansari, M. I. (2021). Plant defense responses to biotic stress and its interplay with fluctuating dark/light conditions. *Frontiers in Plant Science*, *12*, 297.

Izzo, L. G., Mele, B. H., Vitale, L., Vitale, E., & Arena, C. (2020). The role of monochromatic red and blue light in tomato early photomorphogenesis and photosynthetic traits. *Environmental and Experimental Botany*, *179*, 104195.

Li, Q., & Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*, *67*(1), 59-64.

Liu, J., & van Iersel, M. W. (2021). Photosynthetic Physiology of Blue, Green, and Red Light: Light Intensity Effects and Underlying Mechanisms. *Frontiers in plant science*, *12*, 328.

Lopes-Oliveira, P. J., Oliveira, H. C., Kolbert, Z., & Freschi, L. (2021). The light and dark sides of nitric oxide: multifaceted roles of nitric oxide in plant responses to light. *Journal of Experimental Botany*, *72*(3), 885-903.

Lopez, L., Fasano, C., Perrella, G., & Facella, P. (2021). Cryptochromes and the Circadian Clock: The Story of a Very Complex Relationship in a Spinning World. *Genes*, *12*(5), 672.

Ma, C., Sun, Z., Chen, C., Zhang, L., & Zhu, S. (2014). Simultaneous separation and determination of fructose, sorbitol, glucose and sucrose in fruits by HPLC–ELSD. *Food chemistry*, *145*, 784-788.

Naznin, M. T., Lefsrud, M., Gravel, V., & Azad, M. O. K. (2019). Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. *Plants*, *8*(4), 93.

Qian, M., Rosenqvist, E., Flygare, A. M., Kalbina, I., Teng, Y., Jansen, M. A., & Strid, Å. (2020). UV-A light induces a robust and dwarfed phenotype in cucumber plants (Cucumis sativus L.) without affecting fruit yield. *Scientia Horticulturae*, *263*, 109110.

Viršilė, A., Brazaitytė, A., Vaštakaitė-Kairienė, V., Miliauskienė, J., Jankauskienė, J., Novičkovas, A., ... & Samuolienė, G. (2020). The distinct impact of multi-color LED light on nitrate, amino acid, soluble sugar and organic acid contents in red and green leaf lettuce cultivated in controlled environment. *Food chemistry*, *310*, 125799.

Xu, Y., Chang, Y., Chen, G., & Lin, H. (2016). The research on LED supplementary lighting system for plants. *Optik*, *127*(18), 7193-7201.

Zhou, W. L., Liu, W. K., & Yang, Q. C. (2012). Quality changes in hydroponic lettuce grown under pre-harvest short-duration continuous light of different intensities. The *Journal of Horticultural Science and Biotechnology*, *87*(5), 429-434.

FEATURES OF THE REPRODUCTION AND PROPAGATION SYSTEM OF SOME SPECIES OF THE CAMPANULACEAE FAMILY

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Abstract

In this regard, it becomes necessary to study the processes that ensure the reproduction and restocking of higher plants. Knowledge of reproduction processes is extremely important for solving many problems of botany, including the problem of preserving and increasing the biological diversity of plants, identifying the features of their adaptations during introduction, clarifying the taxonomy, etc. Of no small importance are reproductive features also in the development of methods for rational use of natural resources and the introduction into culture of various economically valuable plants - medicinal, aromatic, ornamental. Since the reproductive biology of plants involves the study of a number of sequential and interconnected processes of the development of flower elements, the formation of generative structures, the peculiarities of flowering, pollination, seed formation and dissemination, knowledge of them makes it possible to establish the patterns of formation of reproductive elements, identify critical periods in reproduction, and develop methods for improving reproduction and propagation of rare plant species, etc. Comparative studies of reproductive biology and, in particular, plant embryology are widely carried out by researchers (Kamelina, 2009; Kamelina, Shevchenko, Kuzmina, Miroshnichenko, 2020; Klank et al., 2010; and others), but not all families have been studied in sufficient detail and deep. Many species of the Campanulaceae family, such as *Campanula sibirica* L., *C. talievii* Juz., *C. taurica* Juz., *C. alliariifolia* Willd., *Adenophora liliefolia* (L.) Ledeb. ex A. DC. (syn. *A. taurica* (Sukacz.) Juz., *Platycodon grandiflorus* (Jacq.) A. DC. deserve special attention. As a result of the studies of these species, common features of embryology and anthecology as well as individual features of their seed formation, were established.

Keywords: *family Campanulaceae, generative structures, anthecology, micro- and megasporangium, seeds formation.*

Introduction

According to the latest literature data (from October 2018), the Campanulaceae family takes 35th place in the system of flowering plants and is represented by 88 genera and 2385 species (https://ru.wikipedia.org/wiki/Flower_plant_families). The family includes 5 subfamilies, represented by different life forms (herbaceous perennials, tree-like forms, vines, etc.), which are found in different climatic zones and communities (Lammert, 2007). The literature contains extensive information on the study of all sorts of aspects: systematic affiliation, morphological features, growing conditions, etc. However, despite the fact that many representatives of the Campanulaceae family have been fairly well and comprehensively studied data on their embryology in the literature are insufficient. At the same time, the study of the reproductive structures, characteristics and possibilities of seed formation of these species will contribute to the expansion of the factual material on general embryology, and will also serve as additional information in solving controversial issues of the systematic affiliation of plants and the development of methods for optimizing their reproduction and propagation. In this regard, the aim of this work was to identify the features of the development of the reproductive structures of the some species, anthecological aspects of their reproduction, a comparative analysis of the possibilities of renewal in the conditions of their natural area or during introduction.

Materials and methods

The objects of these studies were 6 species of flowering plants from family Campanulaceae: *Campanula sibirica* L., *C. talievii* Juz., *C. taurica* Juz., *C. alliariifolia* Willd., *Adenophora liliefolia* (L.) Ledeb. ex A. DC. (syn. *A. taurica* (Sukacz.) Juz.) and *Platycodon grandiflorus* (Jacq.) A. DC. The question of the taxonomic affiliation of *Campanula sibirica* L., *C. talievii* Juz. and *C. taurica* Juz. in the literature it is debated: some authors define them as independent species (Yuzepchuk, 1951; Dremlyuga and Ziman, 2010), others (Viktorov, 1997) *C. talievii* and *C. taurica* refer to species *C. sibirica* as subspecies. Our observations allow us to join the opinion of the former.

C. sibirica (according to our observations and according to literature data) is a biennial herb. The stem is single, erect, slightly pubescent, up to 50 cm tall, with numerous flowers. The structure of the corolla corresponds to the characteristic features of the genus. Aerial shoots are in the form of leaf rosettes. Leaves are lanceolate, sessile. In the Crimea, it blooms from May to August, inclusive. The root of this species is tap-shaped, fusiform, branched, milky-white in color. The species is listed in the Red Books of the Udmurt Republic (2001), the Moscow (2006), the Voronezh (2018), the Rostov-na-Donu (2014), the Yaroslavl (2004) regions and the Republic of Belarus (2006). This species has been assigned the status of the 3rd rarity category.

C. taurica is a perennial plant up to 50 cm tall. Like *C. sibirica*, it blooms from May to August. Generative shoots pubescent, numerous, straight, the middle of which is usually higher than others. The flowers are numerous; the petals are serrated. Aerial shoots are in the form of rosettes of leaves, leaves are lanceolate. Underground shoots are creeping, slightly branched, light white in color.

C. talievii – it is a polycarpic, herbaceous, half-rooted plant up to 25-30 cm tall. According to V.N. Golubev (1996), this is the endemic of the Crimea. Generative shoots are pubescent, numerous. Flowers are also numerous. Above-ground shoots are represented by rosettes of lanceolate leaves. An underground shoot is branched, rod-shaped, fusiform-shaped, light-white in color. The species can be characterized as aeropedophyte and calfyte with a deep rod-root system.

C. alliariifolia (syn. *C. lamiifolia* var. *albotomentosa* Rupr.) is a perennial, densely pubescent plant up to 70 cm tall, stems are straight, ascending. Basal leaves are cordate, with long petioles, stem leaves are reduced, short-petiolate, the uppermost leaves are almost sessile. Blooms in August-September. The flowers are rather large (22-29 mm), fawn, on short pedicels, collected in a one-sided raceme, the teeth of the calyx are broadly lanceolate, ciliate along the edge, much shorter than the corolla. Grows in open limestone areas in the forest belt of mountains. We have noted the species on the bank of the Fiagdon river in the Kadargavan canyon of the Kurtatinsky ravine approximately at an altitude of 950 – 970 m above sea level.

Adenophora liliefolia is a herbaceous perennial with a height of 30-50 cm, with a thick fusiform or radish root and a cylindrical, longitudinally striped, glabrous or sparsely hairy, densely leafy stem. Stem leaves are alternate, elliptical, pointed, unevenly coarsely serrate-toothed at the edges, tiled overlapping each other in the middle part of the stem; upper leaves are lanceolate, sessile. Rosette basal leaves are round-cordate, long-petiolate, 4-10 cm long, drying quickly. *A. liliefolia* blooms in July-August, the flowering is acropetal, although sometimes there are shoots with basipetal blooming. The flowers are collected in a racemose inflorescence. Corolla bell-shaped, blue or light blue, shallowly divided into 5 lobes, up to 1.5 cm long, drooping flowers, spine-petal. The calyx is naked, its teeth are sharp, lanceolate-triangular, equal in length to the tube. The species is included in the European Red List (2011) and Red Books of various regions: Red Book of the Republic of the Crimea (2015), Red Books of Voronezh (2011) and Rostov (2014) regions.
Platycodon grandiflorus is a perennial herb. Stem with a large, single flower at the end and glaucous sessile leaves, ascending or straight from the base, strongly leafy, up to 50.0 cm high. When grown on the Southern Coast of the Crimea, *P. grandiflorus* blooms from late June to late July, bears fruit in August. The flowers are usually apical, the calyx is grayish, with straight, pointed teeth, the corolla is bright blue with pronounced dark veins. There are forms with white flowers and two circles of petals. The corolla is large, up to 7.0-8.0 cm in diameter, divided into ovoid lobes. The bright color of the *P. grandiflorus* flower and its size, which is much larger than the flowers of other representatives of the Campanulaceae family (many species of the genus *Campanula*, *Adenophora*), attract various insects. It should be noted that *P. grandiflorus* is very beautiful not only during the flowering period, but also during the budding period, since its buds are also colored and have an unusual shape, reminiscent of Chinese lanterns.
The material for embryological studies was fixed with a Carnoy solution (6: 3: 1) and Chamberlain fixative (ethyl alcohol 70% -90 parts: formalin 40% -5 parts: glacial acetic acid-5 parts). In the preparing of permanent preparations, conventional methods were used. Sections of 10-12 μm in thickness were obtained using a semi-automatic rotary microtome RMD-3000 (OOO "MedTekhnikaPoint", Russia). The preparations were stained with methylgrunpironin with an Alcyan blue tint and Heidenhain hematoxylin with Alcyan blue tint. The analysis of the preparations was carried out with the help of the AxioScope 1 microscope of Carl Zeiss (Jena), photographs were taken with a digital Axiocam 208 color. Anthecological observations were carried out under conditions of natural growth of the species under study.

Results and discussion

The flowering of three Crimean species of bellflower is rather long, due to which one can simultaneously observe buds, open and wilted flowers on one plant, and fruits are also added in August. The development of reproductive structures occurs in the year of flowering. Generative shoots in *C. sibirica* and *C. taurica* wither at the end of the growing season, and the death of aerial parts is observed after the completion of dissemination. In *C. talievii*, the stems dry out by the end of the growing season, but do not die off, as a result of which new rosettes of leaves are formed from dormant buds the next year. The flowers form loose inflorescences. As already mentioned, *C. alliariifolia* in its natural range blooms in July-September, the flowering is acropetal, the flowers are yellow, turning yellow over time, forming a loose racemose one-sided inflorescence.

It is known that one of the most important structural and functional parts of a flower is the androecium, which is a set of stamens, which largely determine the strategy of the species: how many anthers are in the flower, what is their position and developmental sequence, is there enough pollen for effective pollination. During evolution, the number of stamens can change, and this is due to the biology of pollination and pollinators, the presence or absence of nectaries.
Androecium in the species that we studied is represented by 5 stamens. The stamens are straight and equal, attached to the nectar disc, symmetrically arranged; in the bud they tightly cover the pistil. Filaments have widened bases, which, joining together, form a dome with an opening at the top (figure 1). This facilitates pollination by large insects, which can reach the nectar disc through the hole with their proboscis and extract nectar. As the flower opens, the anthers open up, the pistil grows, but its blades are still closed, that is, the phenomenon of protandria is observed.
The anther is 2-tec, 4-nested, there is a placentoid - a radial outgrowth of the connective tissue, which protrudes into the interior of each nest. The wall of the microsporangium develops centrifugally, the tapetum is a derivative of the primary parietal tissue. In the subepidermal layer, archesporium differentiates, the cell of which is distinguished by its size, a large nucleus. As a result of the division of the archesporial cell, parietal and sporogenic cells are formed, the latter of which forms sporogenic tissue, and a microsporangium wall develops from the parietal cell. After the cessation of divisions in the sporogenic tissue, its cells separate, callose is deposited around them, and microsporocytes are formed.

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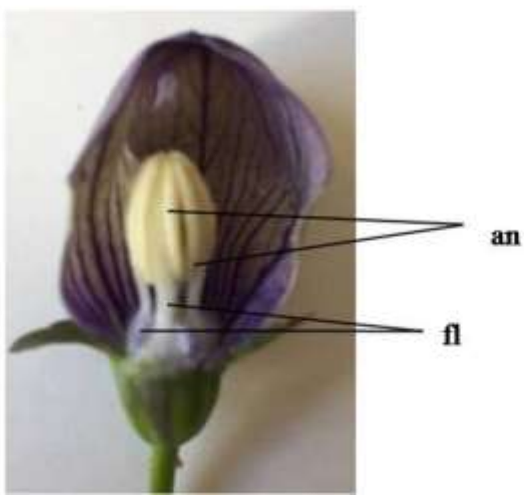

Figure 1. *Platycodon grandiflorus* bud with partially removed corolla (an - anthers, fl - filaments)

As a result of the meiosis passing through them, a tetrad of microspores is formed. The formation of a tetrad of microspores is simultaneous (cell septa are not formed after the first division of meiosis), tetrads of microspores are of isobilateral and tetrahedral types. The formed microsporangium wall consists of the epidermis, endothecium, one middle layer and secretory tapetum. In the young 2-cell pollen stage, individual tapetum cells are observed (figure 2). With the development of microsporocytes and the passage of meiosis in them, the cells of the middle layer gradually flatten and degenerate. Tapetum cells persist for a rather long time and are observed during the period of differentiating mitosis. At the stage of two-celled pollen grains, the microsporangium wall is represented by flattened cells of the epidermis, endothecium with fibrous thickenings, and remnants of tapetum cells. The wall of the mature anther consists of flattened cells of the epidermis, which is covered with cuticle, and the endothecium with fibrous thickenings. The endothecium is often two-row, from the side of the liaison it can be two- and three-row. Mature pollen grains are bicellular; spermatogenesis takes place in pollen grains on the stigma of the pistil. Most of the mature pollen grains are morphologically normal (presumably they are fertile); the amount of sterile pollen grains varies from 15 to 30%.

Female generative structures are the most stable and protected part of the flower, which is relatively resistant to external factors, and pathologies of its development usually indicate serious violations of the reproductive process of the species. In the species we studied, the gynoecium is coenocarpous (syncarpous variety), consists of 3 carpels, each of which contains many ovules that form the inferior ovary.

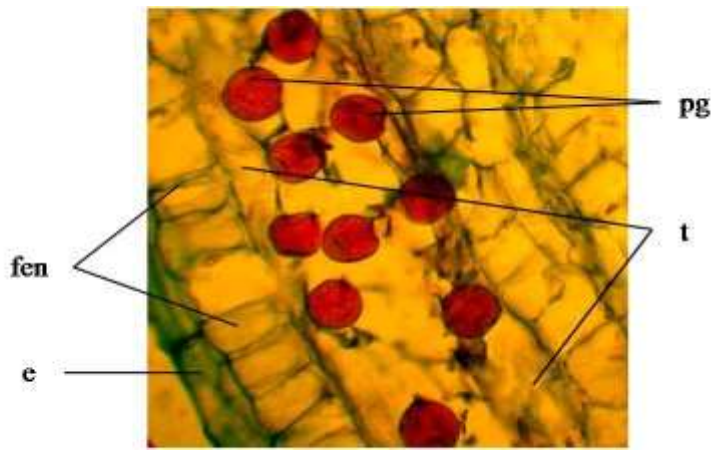

Figure 2. Fragment of the *Campanula taurica* microsporangium at the stage of young 2-cell pollen (pg - pollen grains, t - tapetum, fen - fibrous endothecium, e – epidermis)

The ovule is a structural element in which megasporogenesis occurs, the development of the female gametophyte, the fertilization process, as well as the subsequent processes leading to the formation of the seed. In the species we studied, the ovules are anatropic, medionucellular and unitegmal. The integument of epidermal origin is represented by 6-8 rows of cells. The micropile is simple, narrow, straight. The funiculus is short, weakly expressed, there is a funicular obturator. As a result of the congenital fusion of the funiculus and integument, rafe is formed. The vascular bundle reaches the chalaza. The outer epidermis of the ovule is represented by large elongated cells. Most of the cells of the inner epidermis differentiate into the integumental tapetum, which surrounds the embryo sac from the lateral sides, reaching the level of the egg apparatus.

The cells of the integumental tapetum differ in size; they are smaller at the micropylar and chalazal ends than in the central zone, where they are large, tabular, with rather well-defined nuclei and nucleoli. These data are consistent with the results of the study by I.I. Shamrov and N.A. Zhinkina *Azorina vidalii* (family Campanulaceae), in which a similar development and structure of the integumental tapetum was described. In the chalazal zone, the ovule forms a columnar postamento-podium, the cells of which differ from those around them in a thicker shell and adhere tightly to the embryo sac, and cup-shaped hypostasis.

The embryo sac is formed according to the Polygonum type. In the subepidermal layer of the meristematic tubercle, the archesporial cell differentiates, which differs from the surrounding cells by its larger size, a pronounced nucleus with a nucleolus. As a result of its division, parietal and sporogenous cells are formed, the latter of which is transformed into a megasporocyte. Like many other representatives of the Campanulaceae family, the embryo sac is monosporic and elongated (figure 3). The polar nuclei are first located in the center of the embryo sac, merge before fertilization to form the nucleus of the central cell of the embryo sac, which is then close to the egg apparatus.
The antipodes are located T-shaped in the chalazal zone of the embryo sac. They persist for a rather long time and degenerate after fertilization, in contrast to *Owsrowskia magnifica* and *Campanula latifolia*, in which antipodes are ephemeral and degenerated during fertilization.

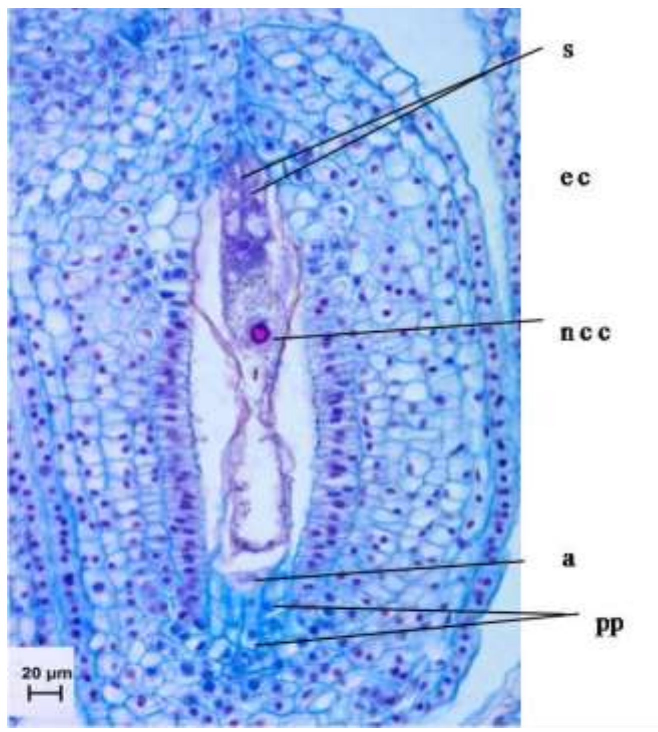

Figure 3. Fragment of the ovule and mature embryo sac *Adenophora liliefolia*
(s – synergids, e c – egg-cell, n c c – nucleus of central cell, a – antipods, pp – postamentopodium).

Thus, in the species of bluebells and adenophores we studied the mature embryo sac is 7-cell, with a well-differentiated egg apparatus, but its maturation occurs later than the male generative structures, and by the time the flower opens, the embryo sac is not yet differentiated and is not ready to accept male gametes. Its differentiation occurs later, which indicates the presence in these species of the phenomenon of protandria, which contributes to allogeny.
One of the most important stages in the process of reproduction of flowering plants is the period of their flowering, when the differentiation and maturation of the reproductive elements of the flower is completed and pollination is possible, which subsequently determines the processes of fertilization and seed formation. At the same time, it is necessary to note the importance of coordinated actions and processes of flower development and pollination agents, the effective

interactions of which ultimately determine the reproduction, dispersal and conservation of the species.

Depending on the method of pollination, its syndrome in various plant species is represented by various adaptations that maximally ensure the ingress of pollen on the stigma of the pistil. Members of the Campanulaceae family have a very specific pollination mechanism. In the species we studied, the flower is full, bisexual, actinomorphic; the limb of the flower, which attracts insects and serves as a landing site for them, is of great importance. Calyx toothed, wilting, non-falling. There are bent appendages equal to the length of the calyx, toothed petals. The corolla is bell-shaped, agglomerate, pubescent (the outer part of the corolla is not pubescent, and the inner part is weakly pubescent), 15-20 mm long, wilting, non-falling. At the closed-bud stage, the stamens completely encircle the pistil column. Filaments are short, widened at the base, in the lower part, joining together, form a kind of dome with small holes. The anthers break open at the stage of a loose bud; the stigma lobes are still closed at this time. The nectary is intra-flowered, in the form of a disk above the ovary. One pistil, central column, open, with a canal in the center, non-falling, covered with many unicellular hairs of epidermal origin. The stigma is apical, dissected, 3-lobed. The originality of pollination lies in the fact that the pollen from the flower is not carried out by the anthers, but by the columns covered with hairs. As the pistil grows, the hairs pry up the pollen grains from the open anthers and extract them, as a result of which the entire column is covered with pollen. In search of nectar, the pollinator insect flies up to the flower and goes to the nectar disk, which is covered with a dome of staminate filaments widened at the base. In the upper part of the dome there is a hole through which the pollinator reaches the nectar disc with its proboscis. As it moves to the nectar disc, the insect with its paws, belly, and sometimes wings, removes the pollen on the column. After visiting one flower and when flying to another, an open flower, the insect, moving to the nectar disk, touches the open lobes of the stigma of the pistil, leaving pollen on them, and thus carrying out pollination.

It should be noted that the studied species have the phenomenon of intussusception, or retraction, which is as follows. After the function of extracting pollen from the anthers and removing it from the column by insects, the hairs covering the column do not fall off, but are drawn into the surface tissue of the column, expanding the base of the hair until only its tip remains above the surface of the epidermis (figure 4). The retraction phenomenon encourages insects to reach the nectar disc and leave the flower, collecting pollen and transferring it to other flowers. After pollen enters the stigma of the pistil, spermatogenic division occurs, leading to the formation of two spermatozoa, the pollen tube grows along the canal in the column, reaches the embryo sac, passes through one of the synergids, bursts and pours out its contents. One of the sperm fuses with the nucleus of the central cell, and the second with the nucleus of the egg, that is, double fertilization occurs. In the species we studied, fertilization of the premitotic type, which is characterized by the union of the reproductive nuclei before the first mitotic division of the zygote. The zygote is in a dormant state for some time, then it stretches somewhat inside the embryo sac.

As a result of the fusion of sperm with the nucleus of the central cell, the primary endosperm nucleus is formed, the first division of which is transverse with the formation of chalazal and micropilar cells. The micropilar cell divides longitudinally, then these two cells divide transversely, resulting in the formation of a haustorium. The chalazal cell divides transversely and the endosperm itself is formed from the formed upper cell, and the lower cell divides longitudinally, forming a chalazal haustorium, which can be unicellular or bicellular. The micropilar haustorium begins to form even before the active development of the endosperm (figure 5). The endosperm observed in these species, according to the characteristics of the Campanulaceae family, is cellular. In accordance with the classification of O.P. Kamelina, it is tubifloral, characterized by the transverse division of the primary nucleus and the formation of micropilar and chalazal haustorium, which perform nutritional and secretory functions. According to I.I. Shamrova (2008), such endosperm formation can be defined as a micropilar-chalazal subtype of the cellular type with terminal haustoriums. The embryo in the species studied by us develops according to the Solanad-type. The zygote is at rest for a long time, increasing in size and growing inside the central cell. The first division of the zygote is transverse. Then both cells of the proembryo also divide transversely, forming a linear tetrad.

Further, cells derived from the apical cell divide longitudinally, and the basal cell divide transversely, forming a long suspensor and carrying the proembryo to the center of the embryo sac. Both the apical and the basal cells take part in the formation of the embryo itself - the main part of the embryo is formed from the apical cell and the derivatives of the basal cell form the suspensor and pituitary gland.

In accordance with the classification of Z.T. Artyushenko and A.A. Fedorov in the species Campanulaceae that we studied, the fruit is a lower, three-celled, multi-seeded, covered with stiff hairs, drooping, wilting, non-falling capsule. At the base of the capsule of the studied species, there are three pores, first covered with caps, which fold back during the drying of the capsule. An axicornus, a month-like outgrowth attached to an axial column, serves as a device for the formation of a pore and a cap. The resulting seeds are small, light brown, about 1 mm long, most of which are endosperm.

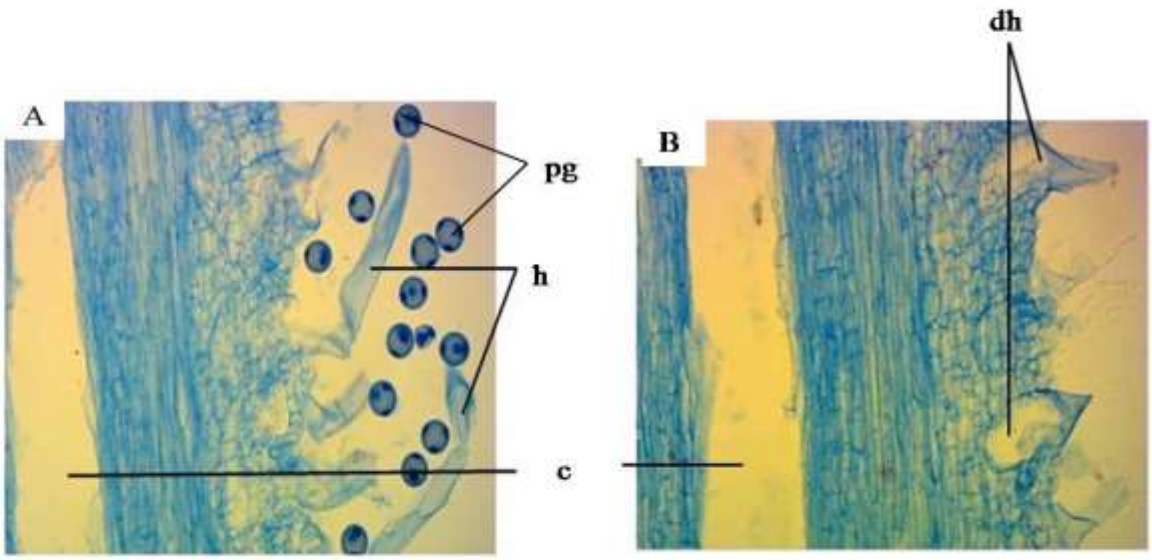

Figure 4. Fragments of style *Campanula sibirica* with epidermal hairs and pollen grains at the period pollination (A) and at the time of draw the hairs into style tissue (B) (h – hairs, pg – pollen grains, c – canal, dh – draw the hairs)

It should be emphasized that *Platycodon grandiflorus* stands out among the studied species. So, its flowers are large, more than 2 times the flowers of bells. Corolla bright blue with distinct veins. There are forms with white flowers and two circles of petals. *P. grandiflorus* is very decorative not only during the flowering period, but also during the budding period, since its buds are also colored and have an unusual shape, reminiscent of Chinese lanterns. In contrast to the bells studied by us, the sporogenic tissue in the microsporangium of *P. grandiflorus* is most often three-layered and only occasionally single-layered.

When *P. grandiflorus* is grown in the Peter the Great Botanical Garden V.L. Komarov Botanical Institute of Russian Academy of Sciences (St. Petersburg), microsporangium of fibrous thickenings is not formed in the endothecium, while when grown under conditions of the Southern Coast of the Crimea, endothecium with fibrous thickenings is formed, spermatogenesis in *P. grandiflorus* takes place in a pollen grain, and mature pollen grains in pollination period three-celled, 6-furrowed with a very small number of abnormal ones.

In contrast, N.A. Zhinkina and E.E. Evdokimova (2020) indicate that when this species is grown under the conditions of the Peter the Great Botanical Garden, 2-cell pollen with a significant number of anomalies is formed. The fruit of P. grandiflorus resulting from effective pollination and fertilization is a straight, ovoid, upwardly directed capsule with five holes at the top for dispersing seeds, in contrast to the three-nested bell capsule with three pores at the base.

Under the conditions of cultivation on the Southern Coast of the Crimea, a lot of seeds are formed, their viability after stratification in the refrigerator for a month is about 95.0%, in contrast to the Peter the Great Botanical Garden, where P. grandiflorus seeds are not set at all (Zhinkina, Evdokimova, 2020; Shevchenko and others, 2020).

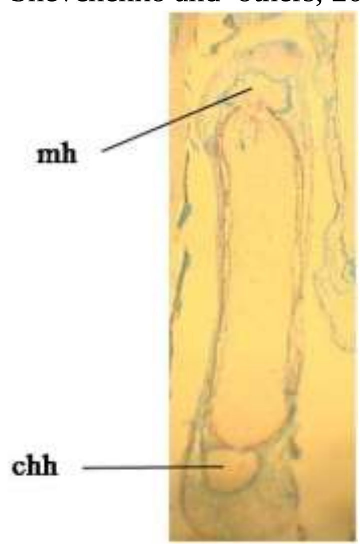

Figure 5. Fragment of the *Platycodon grandiflorus* seed with micropilar (mh) and chalasal (chh) haustoriums

Conclusions

Thus, the species studied by us are characterized by certain common features: dicotyledonous type of formation of the microsporangium wall, anatropic, medionucellular, unitegmal ovule, Polygonum-type of formation of the embryo sac, the presence of an integumental tapetum, tubifloral endosperm with micropylar and chalazal haustoriums. *P. grandiflorus* has some specific features that distinguish it from others: the size of the flower, the shape and color of the buds, a five-nested ovary and a stigma of a pistil with five lobes, unlike *Campanula* species with a three-nested ovary and a three-lobed stigma, large 3-celled six-furrowed pore pollen grains.

The insignificant amount of abnormal pollen grains in the total mass of pollen during the pollination period suggests a high viability and fertilizing ability of male generative structures of the studied species. The formation of a large proportion of high-grade seeds in their total mass indicates their high reproductive potential, the possibility of seed reproduction and use in landscaping parks on the Southern Coast of the Crimea.

It should be emphasized that the development of flower elements in the studied representatives of the Campanulaceae family, coordinated movements during the flowering process and the period of activity of pollinating insects, the pollination mechanism are closely interrelated, and their conjugation ensures the efficiency of pollination and subsequent seed formation processes, which indicates about high plasticity and reliability of their reproduction systems.

References

Golubev V.N. (1996). Biological flora of Crimea. Yalta. 126 p.

Dremlyuga N.G., Ziman S.M. (2010). Campanula talievii Juz. Rare endemic species in the flora of the Crimea // Ukr. Bot. Journal. T.67.No. 2. P. 225-230.

Kamelina O.P. (2009). Systematic embryology of flowering plants. Dicotyledons. Barnaul: Publishing house "Artika". 501 p.

Klank Ch., Andrea R. Pluess, Joaboury Chazoul. (2010). Effects of population size on plant reproduction and pollinator abundance in a specialized pollination system // J. Ecol. № 6. P.1389-1397.

Lammers T.G., Kubitzki K. (2007). Campanulaceae // The families and genera of vascular plants. – BerlinSpringer. V. 8. P. 26-56.

Shamrov I.I. (2008). The ovule of flowering plants: structure, function, origin / Ed. T.B. Batygina. Moscow: Partnership of scientific publications KMK. 350 p.

Shevchenko S.V. (2009). Reproductive biology of decorative and subtropical fruit plants of the Crimea. Kiev: Agrarian Science. 336 p.

Shevchenko S.V., Kuzmina T.N., Marko N.V., Yaroslavtseva A.D. (2010). Reproductive biology of some rare species of the Crimean flora. K.: Agrarian science. 392 p.

Shevchenko S.V. (2017). Reproduction and propagation of some rare species of the Crimean flora // Agticulture and Forestry. Vol.63. Issue 4, P. 99-106.

Shevchenko S.V., Kuzmina T.N., Miroshnichenko N.N. (2020). Structural and functional organization of the generative sphere of flowering plants / Simferopol: PH "ARIAL". 164 p.

Viktorov V.P. (2006). Taxonomy and variability of the genus Campanula (Campanulaceae) in Russia and neighboring countries: author. ... doct. biol. sciences. M.: MSU. 26 p.

Zhinkina N.A., Evdokimova E.E. (2020). Anther development in Platycodon frandiflorus (Campanulaceae) // Bot. zhurn. Vol. 105, No. 3. P. 300-309.

Yuzepchuk S.V. (1951). Campanula talievii Juz. sp. nova. // Bot. mater. herbarium of Bot. Institute of the USSR Academy of Sciences. XIV. P. 36.

INTERACTION OF FERTILIZATION AND SOYBEAN GENOTYPE ON NUMBER OF PODS, WEIGHT OF 1000 GRAINS AND GRAIN YIELD

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Abstract

In the last few decades, new soybean varieties with different characteristics, grain quality and purpose have been created, contributing to its expansion and increase in the cultivation area. Thanks to good agronomic characteristics, soybean has found its place in sustainable production systems. In order to increase the yield and quality of grain in sustainable soybean growing systems, different foliar treatments with different active substances have been increasingly applied. The aim of the study was to determine the impact of the application of EM Aktiv with effective microorganisms on the number of pods per plant, weight of 1000 grains and grain yield of different soybean cultivars grown in an integrated cultivation system. The research was conducted in the period 2016-2019 in the experimental field of the Institute of Field and Vegetable Crops Novi Sad. Varieties from three maturing groups Galina (0 group), Sava (I group) and Rubin (II group) were grown. Variants of fertilization application were: T1 control, T2 EM Aktiv was applied to the soil before sowing 20 $l.ha^{-1}$, and later in vegetation 6 $l.ha^{-1}$ (the first foliar treatment in the phase of three to four trefoils and the second before flowering in the budding phase), T3 NPK 8:15:15 300 $kg.ha^{-1}$ in basic treatment and T4 combination of T2 and T3 treatments. On average for all three years of the study were found statistically significant differences between the variables in all properties. The highest values were determined when applying T4 treatment. The number of pods was 55.92, the weight of 1000 grains was 163.61 g and the yield was 4.240 $kg.ha^{-1}$. The cultivar Rubin (II maturing group) showed the highest values for all examined variables on average after all treatments.

Key words: *soybean, fertilization, effective microorganisms, yield.*

Introduction

Soybean (*Glycine max* (L.) Merr) is one of the most important crops for the diet of the population due to its multiple purposes. Today, a large number of scientific and technological knowledge is constantly confirming its value and increasing its constant use. In addition to protein (40%) and oil (20-25%), soy contains various plant specialized (secondary) metabolites, such as isoflavones and saponins (Ahmad et al., 2014; Singh et al., 2017). Soybeans have a special genetic predisposition for a symbiotic relationship with bacteria that fix atmospheric nitrogen and arbuscular mycorrhizal fungi, which gives it an advantage for growing in a sustainable production system. Due to all that, the areas under soybean increase, however with greater oscillations in yields. For stable production of soybeans, it is necessary to know well the needs of the plant for nutrients, agroecological conditions for its production, to apply adequate technology as well as the appropriate assortment. The problem of soybean fertilization has been studied, but, in addition, many issues of nutrition of this plant have not yet been clarified, so there is no increase in yield under the influence of fertilization as in other plants. Soybean responds well to supplemental feeding foliar treatment of a variety of nutrients. Silberbush (2002) states that foliar nutrition of soybeans is widely used to correct plant deficiencies caused by improper root supply. Camberato et al. (2010) reports that if symptoms of nutrient deficiency occur during the growth phase, the most effective method to overcome deficiencies is to use a foliar diet. Mallarino (2005) concluded that foliar nutrition of soybeans in the early stages of growth increases grain yield by 10-30%. Oko et al. (2003) state that foliar treatment of soy with urea in phases R2 and R3 increases the yield by 6-68% compared to the control, while Haq and Mallarino (2005) pointed out that foliar application achieves an increase in protein and oil yield. However, with the development of methods in sustainable production systems, there is an increasing number of research related to the application of biophysics methods and various biofertigators. By applying low frequency electromagnetic waves with organic fertilizers, soybean yield can be increased (Cvijanović 2018) as well as the protein content in the grain (Đukić et al. 2017).

The use of microbiological multiple inocula with effective microorganisms (EM) is increasingly used in plant production. Different types of microorganisms and a large number of highly effective strains affect the stimulation of plant growth, protection against disease, increase resistance to abiotic stresses, improve the chemical properties of fruits (Filipović et al., 2020; Cvijanović et al., 2019) and increase yield. The use of effective microorganisms in plant production can increase the nutritional properties of fruits, according to the results of studies by Daiss et al., (2008), which found an increased content of phosphorus and magnesium in chard leaves. Yue et al., (2002) found that the application of EM can increase the intensity of photosynthesis in functional leaves during mid-vegetation. Using EM, the same authors found that there was a decrease in stomata openness, the activity of the enzyme nitrate reductase increased in the grain, while increasing the yield and quality of the grain (% protein and fat). The aim of the research was to determine the influence of EM Aktiv preparation with effective microorganisms on the number of pods per plant, weight of 1000 grains and grain yield of different soybean cultivars grown in an integrated cultivation system.

Materials and methods

The research was conducted in the period 2016-2019 in the experimental field of the Institute of Field and Vegetable Crops Roman trenches in Novi Sad. The land is of the chernozem type. The experimental field experiment in dry farming was set up according to the design of a split-plot experiment on an area of 2475 m^2. The experiment was set up in four replications, and the size of the basic plot was 15 m^2. Sowing was performed in the optimal agrotechnical period with three soybean genotypes of different FAO maturation groups: Galina (group 0), Sava (group I) and Rubin (group II). The fertilization was based on the principles of integrated production, where NPK fertilizer 8:15:15 was used for basic fertilization, the amount of 300 kg ha^{-1} during autumn tillage and the application of effective microorganisms in the preparation EM Aktiv (trade name) in the following variants:

T1-Control without fertilization; T2-Variant with the use of effective microorganisms in the preparation EM Aktiv (application initially on the ground before sowing in the amount of 20 liters per hectare and secondly foliar treatments in the development phase of plants from three to four trefoils and budding phase, ie buds on the soybean tree in the amount of 5 liters per hectare); T3-Variant with application of NPK fertilizer during basic tillage in autumn, fertilizer formulation 8:15:15, amount 300 kg ha^{-1}; T4-Variant where T2 + T3 treatments are combined.
In the phase of technological maturity of soybeans, 10 plants were taken from each basic plot for analysis. In this paper, the number of pods per plant, weight of 1000 grains and soybean grain yield were analyzed.

Results and discussion

The number of pods per plant represents the total number of pods from one soybean plant, from the main tree and branches. The average number of pods for the entire research period was 53.47. Observing the average values for the number of pods by individual varieties, it can be noticed that the highest value was recorded in the cultivar Rubin (59.91), which was statistically significantly higher than in the cultivar Galina (49.77). No statistically significant difference was found in relation to the Sava variety (55.06). According to the variants of fertilization, the number of pods was the highest when applying T4 treatment (55.92), which is statistically significantly ($p < 0.05$) higher number of pods compared to T3 treatment (52.60) and in relation to control (50.87) the significance was at the level of $p<0.01$. The number of pods in the variant with the preparation of EM Aktiv T2 (54.49) was statistically very significantly higher in relation to the control variant, while in relation to the T4 treatment no statistically significant difference in the number of pods per plant was found. The largest number of pods was found in the treatment of T4 with variety Rubin (62.94). According to Wiebold et al. (1981) by increasing the number of pods, soybean grain yield can be increased, which is one of the priority tasks in soybean cultivation and selection research. The obtained results can significantly improve soybean production.
The weight of 1000 grains is an important component of yield and an indicator of seed size. Grain mass can be affected by genotype, nitrogen diet, supplementation by various means. The average weight of 1000 grains for all three years of research was 162.28 g. Observing the average values for the weight of 1000 grains by individual varieties, it is noticed that the varieties Rubin (167.82 g) and Sava (162.94 g) had a statistically significant difference in the weight of 1000 grains in relation to the variety Galina (156.10 g). The difference in the mass of 1000 grains between the cultivar Rubin and Sava was not at the level of statistical significance. Observing the values for the weight of 1000 grains by fertilization variants, it is noticed that the highest value was recorded on the variant with application of T4 (163.61 g), and the lowest value on the control variant of the experiment (160.19 g), however these differences were not statistically significant. In the interaction of cultivars and treatments, the Rubin cultivar had the highest weight of 1000 grains at T4 treatment (169.73 g). According to the obtained results, it was determined that this trait is conditioned by a genetic factor, the amount of nitrogen (Haq and Mallarino, 2000), as well as foliar treatments. That soybean responds differently to foliar treatments has been shown by Dozet et al. (2016) in studies of foliar application with cobalt and molybdenum where there was a decrease in 1000 grain weight and yield height by 0.56%. Accordingly, the results of the application of a combined diet with mineral nitrogen and effective microorganisms are of great importance in the cultivation of soybeans of different genotypes.

Grain yield, as the ultimate goal of production, averaged 4034.81 kg ha^{-1}. The highest grain yield was in the variety Rubin 4240.22 kg ha^{-1}. In relation to the Sava variety, the increase was 300.06 kg ha^{-1} and in relation to Galina 494.10 kg ha^{-1}, which was a statistically significant increase in grain ($p < 0.01$). Regarding the treatment, the highest values were determined in the treatment T4 4240.22 kg ha^{-1}. The determined yield was higher by 583.17 kg ha^{-1} compared to the control (3657.05 kg ha^{-1}) ($p<0.01$). The other two treatments had statistically significant ($p<0.01$) differences (T2 for 486.16 kg ha^{-1} and T3 for 441.71 kg ha^{-1}) compared to the control. Between treatments T2 (4143.21 kg ha^{-1}) and T3 (4098.79 kg ha^{-1}) the difference of 44.42 kg ha^{-1} was not statistically significant.

Table 1. Influence of examined variables on number of pods, weight of 1000 grains and grain yield of soybean genotypes

Genotype (A)	Treatments (B) 1 control	2 EM Aktiv	3 NPK	4 NPK+EM Aktiv	$\bar{x}$ (A)
Number of pods per plant					
Galina	45.13	49.02	47.73	49.77	47.91
Sava	54.48	52.79	51.04	55.05	52.59
Rubin	56.02	61.67	59.03	62.94	59.91
$\bar{x}$ AB	50.87	54.49	52.60	55.92	
Average 2016-2019					**53.47**
LSD	A**		B**	AB**	
0.01	12.03		3.42	5.83	
0.05	8.88		2.30	4.30	
Weight of 1000 grains (g)					
Galina	153.50	155.59	157.18	158.14	156.14
Sava	160.69	163.05	165.05	162.96	162.94
Rubin	166.37	168.18	167.08	163.73	167.82
$\bar{x}$ AB	160.19	162.27	163.08	163.61	
Average 2016-2019					162.28
LSD	A**		B**	AB**	
0.01	11.31		5.05	4.14	
0.05	8.32		3.74	2.74	
Grain yield kg ha^{-1}					
Galina	3432.40	3853.07	3801.80	3909.21	3749.12
Sava	3622.23	3978.49	4011.19	4141.73	3940.16
Rubin	3909.40	4598.13	4483.37	4670.02	4415.23
$\bar{x}$ AB	3657.05	4143.21	4098.79	4240.22	
Average 2016-2019					**4034.81**
LSD	A**		B**	AB**	
0.01	166.12		153.81	151.07	
0.05	123,02		110.30	107.25	

The obtained results are compatible with the results of Xiaohou et al. (2001) who state that spraying with effective microorganisms can increase the yield and grain quality of different crops. The same authors state that by adding photosynthetic bacteria to the soil, the amount of nitrogen compounds that are the secretions of photosynthetic bacteria increases, which affects the increase of mycorrhizal fungi. These fungi can coexist with nitrogen-fixing bacteria, increasing nitrogen-fixing ability and grain yield. According to Javid (2006) foliar application of EM with the use of NPK fertilizers in the production of peas (*Pisum sativum* L.) increases grain yield by 126%. The same author found that applying EM in wheat production can increase grain yield by 27% compared to production where only chemical fertilizers were applied. By applying effective microorganisms, the yield of soybeans in two-year studies increased by an average of 10.84%, and by 6.86% and 14.81% per year (Dozet et al., 2014). Differences in yield were also conditioned by different agroecological factors.

Conclusion

The use of NPK fertilizers is justified in the production of soybeans, and the use of preparations with effective microorganisms has a positive effect on the tested properties. The application of effective microorganisms had better results compared to NPK treatment. The best results were obtained by applying a combination of NPK and effective microorganisms in all soybean genotypes. The Rubin variety, which belongs to 000 matures, had the best results.

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References

Ahmad, A., Hayat, I., Arif, S., Masud, T., Khalid, N., Ahmed, A. (2014). Mechanisms involved in the therapeutic effects of soybean (*Glycine max*) Int J Food Prop, 17 (6), 1332-1354

Camberato J., Wise K., Johnson B. (2010). Glyphosate - manganese interactions and impacts on crop production: the controversy. Purdue University Extension News and Notes [Online]. http://www.btny.purdue.edu/weedscience /2010/GlyphosateMn.pdf

Cvijanović, G., Đukić, V., Cvijanović M., Cvijanović, V., Dozet, G., Đurić, N., Stepić, V., (2019). Significance of soybean foliar treatments in different agroecological conditions on grain yield and oil content. Proceedings 60. Conference Production and processing of oilseeds, Jun 2019, Herceg Novi, 79-86.

Cvijanović, M. (2018). Iinnovation in application of biophysical methods in sustainable soybean production International scientific Conference Inovation as in initiatior of the development Conference 6th December, Belgrade 2018. Proceedings 16-26

Daiss N., Lobo, M.G., Socorro A.R., Bruckner, U., Heller, J., Gonzalez, M. (2008). The effect of three organic pre-harvest treatments on Swiss chard (*Beta vulgaris* L. var. *cycla* L.) quality. Eur Food Res Technol 226, 345–353

Dozet, G., Cvijanović, G., Djukić, V., Cvijanovic, D., Kostadinovic, Lj. (2014). Effect of microbial fertilizer on soybean yield in organic and conventional production. Turkish Journal of Agriculture and Natural Sciences, Special Issue 1, 2014, 1333-1339.

Dozet, G., Baleševoć-Tubić, S., Kostadinović, LJ., Đukić, V., Jakšić S., Cvijanović, M. (2016). The effect of proceding crops nitrogen fertilization and cobalt and molybdenum application on yeald amd quality od soybean grain, Romanian Agricultural Research, National Agricultural Research and Development Institute,DII 2067-5720 RAR, 51 (33), 1-11.

Đukić, V., Miladinov, Z., Dozet, G., Cvijanović, M., Tatić, M., Miladinović, J., Balešević-Tubić, S. (2017). Pulsed electromagnetic field – a cultivation practice used to increase soybean seed germination and yield, Zemdirbyste-Agriculture, vol. 104, No. 4, 345–352 doi: 10.13080/z-a.2017.104.044

Filipović, V., Cvijanović, G. Ugrenović, V., Aćimović, M., Popović, V., Radanović, D., Stanković, S. (2016). Use of Effective Micro-Organisms to Enhance the Productivity and Quality of Dry Biomass of the Basil Cultivar "Sitnolisni Aromatični". 7th International Scientific Agriculture Symposium "Agrosym 2016", Faculty of Agriculture, University of East Sarajevo, Bosnia and Herzegovina, Jahorina (B&H), 6-9 October 2016,. Proceedings, 1085 – 1091.

Haq, M.U., Mallarino, A.P. (2005). Response of soybean grain oil and protein concentrations to foliar and soil fertilization. Agronomy Journal, 97, 910-918

Haq, M.U., Mallarino, A.P. (2005). Soybean yield and nutritient composition as affected by early season for foliar fertilization. Agronomy Journal, 92 (1) 16.

Javaid, A. (2006). Foliar application of effective microorganisms on pea as an alternative fertilizer. Agron Sustain Dev 26, 257–262.

Mallarino, A.P. (2005). Foliar fertilization of soybean: Is it useful to supplement primary fertilization? In: Integrated Crop Manag, IC-494, 15, 125-126.

Oko, B.F.D., Eneji, A.E., BInang, W., Irshad, M., Yamamoto, S., Honna, T., Endo, T. (2003). Effect of foliar application of urea on reproductive abscission and grain yield of soybean. Journal of Plant Nutrition, 26, 1223-1234.

Silberbush, L.F. (2002). Response of maize to foliar vs. soil application of nitrogen-phosphorus-potassium fertilizers. Journal of Plant Nutrition, No 25, 2333- 2342.

Singh, B., Singh, J.P. Singh, N., Kaur, A. (2017). Saponins in pulses and their health promoting activities: a review Food Chem, No 233 540-549.

Yue, S., Wang, C., Xu, H., Dai, J. (2002). Effects of foliar application with effective microorganisms on leaf metabolism and seed yield in soybean. In: SANGKKARA, U. R. et. al. (eds.) Seventh International Conference on Kyusei Nature Farming. Christchurch Polytechnic, Christchurch, New Zealand, 62-65.

Xiaohou, S., Diyou, L., Liang, Z., Hu, W., Hui, W. (2001). Use of EM-tech-nology in agriculture and environmental management in China,Nat. Farm. Environ. 2, 9–18.

Wiebold, W.J., Ashley,, D.A., Boerma H.R. (1981). Reproductive abscission levels and patterns for eleven determinate soybean cutivars. Agronomy J. 73, 43-46.

EVALUATION OF PRESPRAUTING AND DIRECT COVERING TO ENHANCE OF EARLY TUBER YIELD OF POTATO CROPS (*Solanum tuberosum* L.)

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Abstract

The research focused on early potato (Solanum tuberosum L.) production in Gorenjska region, Slovenia. A field study was conducted during 2020 growing season, at the Experimental field of the Biotechnical Centre Naklo (an altitude: 420 m; φ = 46° 16′ 18″; λ = 14° 18′ 56″). The soil of the experimental plot was gravel clay with pH 6.7. The experimental layout was a split-split-plot design with four replications. Cultivar was the main plot ('Arrow', 'Esme' and 'Adora'), agro-textile covering (under polypropilene and non-covered) the subplot and presprauting the sup-subplot. The potato seeds of a size 28 to 35 mm were divided into two lots (presprouted and not presprouted tubers) over a four weeks period. Potato seeds planted mechanically within the rows of 30 and 75 cm between the rows. Planting date was 1 April and tubers were harvested at optimum maturity, 76 days after emergence. The crops were managed according good farming practices. Presprauting treatment gave on average 3.2 days earlier emergence compared with the control tubers. Cover crops enhanced speed of emergence to 3.9 days. In addition, presprouting seed produced significant more stems (3.4 stem/plant) than control-grown seed (2.7 stem/plant). Cover crops showed significant higer plants (36.8 cm), while no cover plants revealed shorter plants (32.1 cm). The leaf area index under cover crops was higher on average by 0.6 compared to the control crops. Covered treatments gave significant higer total (34.6 t/ha) and commercial (28.5 t/ha) yield of tubers compared to control (28.2 t/ha and 23.7 t/ha, respectively). Dry matter content in the tubers showed significantly higher level in presprouting and covering treatments compared with the control. The variation in total soluble sugar contentration in tubers depended mainly on the genetic potential of the cultivars.'Esme' contained more total soluble sugar than the other two cultivars (6.9% Brix). Potatoes grown by the different agricultural practices did not show any significant difference in the nitrate content.

Key words: *potato, cultivars, nonwoven polypropylene cover, presprouting, yield.*

Introduction

Potato (Solanum tuberosum L.) is the main crop among those producing tubers and one of the most popular vegetable crops grown all over the world in terms of human consumption and after rice, wheat and maize holds the 4th largest crop by global production volume (Marcomini et al., 2019). There are three main types of potato to grow, named according to when you plant and harvest them: first earlies, second earlies and main crop.

First early or 'new' potatoes are so-called because they are the earliest to crop, usually in May or early June. Early season potatoes reach maturity within 65 to 80 days. From a commercial standpoint, the term early potato may be applied to potatoes which aregenerally harvested before it is completely mature, marketed immediately after harvesting and whose skin can be easily removed by rubbing. Early potato is of high biological and nutritional value, a real delicacy

intended for garden-fresh home meals or placement through green markets or stores. Early potato tuber contains high levels of useful nutrients, it is easily digestible, high in energy and very low in harmful substances, primarily harmful nitrogen (Ilin et al., 2000).
Potato cultivation for early cropping involves considerable risk, due to the possibility of early frosts in the initial period of growth, which may cause significant damage to entire plantations. Growers can reduce some of the risks associated with early planting by adjusting management practices to fit the situation. For example, the soil surface covered by agrotextile (Wadas, 2016). Coverings not only provide protection against early frosts but also protects crops from high intensity rainfall (Benoit and Ceustermans, 1990) and the behaviour of insects that visit the plants (Qureshi et al., 2007). Coverings can also move up crop emergence by 3 to 8 days, and increase both total and commercial yields (Rębarz et al., 2015). In addition, covers reduce solar radiation (Benoit and Ceustermans, 1987), wind influences (Mermier et al., 1995) and evaporation rates (Choukr-Allah et al., 1994) while increasing air humidity (Hemphill, 1989) as well as the soil humidity (Wolfe et al., 1989). The most often used plants cover is made of spun bonded polypropylene (PP). Spun bonded cover is extremely lightweight (10–50 g/m2), transmits enough light (80–94%) and can be placed directly over crops without the use hoops for support (Wells and Loy, 1985).
Presprauting tubers is a further measure that can encourage the faster cultivation of potato. According to Struik (2007) presprouting is a way of affecting physiological age, which can be an agronomic tool to help the crop adjust to the ecological conditions of the growing season and perform optimally for its intended purpose.
In Slovenia, where the growing season is restricted, the early potatoes harvested in spring have a higher market value than later potatoes. This provides a good incentive for developing different methods to promote early tuber initiation, such as early cultivars, direct covering, presprouting etc.
Therefore, the present work was carried out aiming to evaluate the effect of nonwoven polypropylene (PP) covering and presprauting on the quantity and quality of early tuber yield of potato.

Material and Methods

Field experiment was conducted during the spring 2020 growing season at the Experimental field located at the Biotechnical Centre Naklo (46° 16′ 18″ N latitude; 14° 18′ 56″ E longitude, 420 m above see level) which is an area recently destined for early crop potato cultivation in Gorenjska region, Slovenia.
The soil samples from the upper horizon (0-25 cm depth) were collected before the experiment. Elemental analysis indicated that this soil was gravel clay with pH (water 1:1) 6.7, an organic matter content of 3.8%, and phosphorus and kalium contents (ammonium acetate extraction) of 24 mg and 32 mg per 100 g of dry soil, respectively. The preceding crop in the year before growing potato was annual ryegrass. Cattle manure was incorporated into the soil 20 days prior to planting seed potatoes. A basal dressing of 350 kg NPK (15-15-15)/ ha was applied prior to planting on to soil surface by hand and incorporated. Additional fertilizer other than N was supplied uniformly across the plot areas to correct any deficiencies indicated by soil test results.
Three cultivars: 'Arrow', 'Esme' and 'Adora' were evaluated. 'Arrow' is the most common variety grown in Gorenjska region, while 'Esme' and 'Adora' have been recently introduced at a local level by seed potato companies as promising cultivars for early potato production. These

cultivars are all considered to be suitable for the fresh market, with a comparable medium to medium-early maturing cycle.
The experimental layout was a split-split-plot design with four replications. Cultivar was the main plot, agro-textile covering (under PPand non-covered) the subplot and presprauting (presprouted and not presprouted tubers) the sup-subplot.The potato seeds of a size 28 to 35 mm were randomly divided into two lots and stored for three weeks at 70-80 %. The tubers of first lot were sprouted under fluorescente lights and the second (control) lot were left in bags until planting, when any sprouts present were removed.
Potato seeds planted mechanically within the rows of 30 and 75 cm between the rows. The crops were managed according good farming practices. Planting date was 1 April and tubers were harvested at early and optimum maturity by hand from 4 m of the middle two rows from each main plot, 76 days after emergence.
Analysis of variance was performed to assess the significance of treatment effects. Differences between treatment means were compared by using Tukey's multiple range test at the 0.05 probability level.

Results and Disscusion

The Voltcraft DL-121TH Multi-channel data logger was used to record the temperature. With the advance of the vegetation period, the values of daily mean temperatures under the cover and in open field were slightly increasing and consequently the differences between covered and no covered treatments were noticed. The mean temperatures during the growing season are presented in Figure 1. From the beginning of Aprile until the middle of June the mean temperature under the cover was 2.1 °C higher compared to outside temperatures.

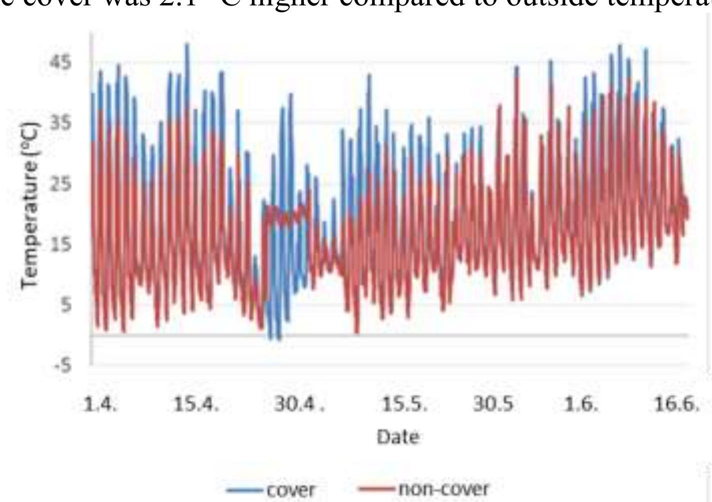

Figure 1. Temperature (°C) during the early potato growing period under PP cover and in open field

Some previous works showed that potato emergence can be affected by many factors, such as cultivar, seed-tuber age, cultural and enviromental conditions (Knowles and Knowles, 2006). As can be seen in Table 1 the percent of emerged plants was not significantly affected by the treatments; almost all planted tubers produced stems.
Presprauting treatment gave on average 3.2 days earlier emergence compared with the control tubers. The earlier emergence with presprouting were also reported by Möller and Reents (2007)

and Hagman (2012). The period from planting to emergence difference among cover and no cover treatments. Days to emergance was significant enhanced by 3.9 days. According Cholakov and Nacheva (2009) the application of PP covers enables earlier potato planting and forcing planting emergence. Wadas (2016) mentioned that PP covers shortened the period between planting and emergence by 2 to 8 days.

The number of main stem per plant was no significantly affected by cultivar and covering. But on the other hand this parameter consistently increased with presprouting. On average, presprouting seed produced significant more stems (3.4 stem/plant) than control-grown seed (2.7 stem/plant). These findings agree with the results of Caliskan et al. (2004). On the other hand, our results are not conformity with those of Allen and O'BRien (1986) who mentioned that presprouting have negligible effect on number of above ground stems. Dimante and Gaile (2014) assume that one of the main traits defining the physiological status of the seed tuber and potato plant growth vigour is number of stems per emerged plant. The number of stems is a crucial trait as it influences tuber number per plant.

Plant height from tubers of different cultivars was no significant varied. We also observed that presprouting had no effect on plant height. Nevertheless, differential responses of plant height performed by covering: on average, cover plants showed significant higer plants (36.8 cm), while no cover plants revealed shorter plants (32.1 cm). Our results are in accordance with previous findings of Cholakov and Nacheva (2009). The authors found out that potato growing under non-woven PP developed a higher stems compared to cultivation without covering.

A higher soil and air temperature under covers provides better conditions for plants immediately after planting and allows them to produce a higher leaf area index (LAI), which was confirmed in the present study. The LAI under PP fibre was significant higher on average by 0.6 compared to the plants cultivated without covers. According to Liu et al. (2012), a higher assimilation leaf area and LAI value could cause an increase in yield and improve its quality. No signifficant differences were recorded on LAI among cultivars and among presprauting tubers.

Table 1. The effects of presprauting and covering on percentage of emergence (%), on time to emergence (in days after planting-DAP), stem number (plant^{-1}), plant height (cm) and leaf area index of potatoes

Treatment	Emergence (%)	Emergence (DAP)	Stem number (plant^{-1})	Plant height (cm)	Leaf area index
Cultivar					
Arrow	99.8 a	26.5 a	3.1 a	32.4 a	3.2 a
Esme	99.5a	28.6a	2.8a	31.0 a	2.5a
Adora	100.0a	27.8 a	3.0a	31.3 a	2.6a
Cover					
Yes	99.4 a	24.5 a	2.8a	36.8 b	3.1 b
No	99.6 a	28.4 b	3.0 a	32.1 a	2.5 a
Presprouting					
Yes	99.7 a	25.2 a	3.4b	32.2 a	2.6 a
No	99.6 a	28.4 b	2.7 a	31.8 a	2.5 a

Mean values within treatment not followed by the same letter are significantly different at P = 0.05.

The cumulative total and commercial yield (tubers with a size of >45 mm) at the end of the experiment per unit area, was significantly enhanced by the all treatments.The different potato cultivars significantly affected potato yield. The highest total yielding cultivar were 'Arrow' at 32.4 t/ha and 'Adora' at 33.8 t/ha. The similar trend was observed in context from commercial yield. The 'Arrow' and 'Adora' cultivar were quite similar with 28.4 and 28.8 t/ha, respectively. 'Esme' provided the lowest average commercial yield (24.6 t/ha).
The application of PP fibre improved the growth and development conditions of plants and as a result increased the yield. Covered plants gave significant higer total (34.6 t/ha) and commercial (28.5 t/ha) yield of potato compared to uncovered plants (28.2 t/ha and 23.7 t/ha, respectively). Rębarz et al. (2015) reported a similar increase of total yield and share of commercial tuber yield of early potato. Presprauting had no effects on total yield but a significant variations in the commercial yield. On average, presprauting treatments gave higher yield (27.2 t/ha) compared with the control (23.4 t/ha). Our results are conformity with those of Caliskan et al. (2004).
The dry matter (DM) content were obtained after drying the samples in a forced air oven (65–70 °C) to constant weight. The obtained results proved that cultivar had a significant impact on the size of the percent of DM of tubers. 'Arrow' tubers showed the highest DM (22.1%) than those from the other two cultivars. Baranowska (2018) stated that genetic features of potato cultivars had a signifcant impact on the size of the yield of DM. The % of DM were higher in potato tubers cultivated under agro-textile (21.6%) than in potatoes cultivated without coverings (16.8%).This increase of DM in the treatment under thecover at early harvest is in relation with results obtained by Hamouz et al. (2005). Measurements of the DM content in the tubers showed significantly higher % of DM content in presprouting treatments (20.4%) compared with the control (17.2%). The higher DM content in the presprouted treatments indicated that these physiologically older seed tubers had reached a more mature stage at the time of harvest (Pandeyet el., 2017).
Total soluble sugar (TSS) concentration of the tuber extracted juice was determined using a hand-held Atago PR1 refractometer. TSS was mainly affected by cultivar and less by covering and presprouting. The maximum TSS (6.9% Brix) of tuber was recorded by 'Esme' and the minimum (4.6% Brix) was recorded by 'Adora'.
Furthermore, we noted that potato cultivar and type of the production system had no impact on the nitrate content in potato samples. Nitrate content was determined by HPLC according to Kmecl and Žnidarčič (2015). The nitrate content in all treatments was practically the same, the differences were within the experimental error.

Table 2. The effects of presprauting and covering on tuber yield (t/ha), dry matter (%), total soluble solids (%Brix) and nitrate content (mg/kg) in tubers

Treatment	Tuber yield (t/ha)		Dry matter (%)	TSS (%Brix)	Nitrate (mg/kg f.w.)
	Total	Commercial			
Cultivar					
Arrow	32.4b	28.4b	22.1 b	6.0b	65.2 a
Esme	28.7 a	24.6 a	18.3 a	6.5c	87.5 a
Adora	33.8b	28.8 b	17.6 a	5.2a	68.4 a
Cover					
Yes	34.6b	28.5 b	21.6b	5.8 a	73.1 b

No	28.2 a	23.7a	16.8 a	5.5 a	85.5 a
Presprouting					
Yes	30.9a	27.2 b	20.4 b	5.9 a	69.6 a
No	28.6 a	23.4 a	17.2 a	5.8 a	81.5 a

Mean values within treatment not followed by the same letter are significantly different at P = 0.05.

Conclusions

Production of early potato in Slovenia is importand because it provides this highly valued delicates food during the period from May to June, when is on the market low offer of other fresh vegetables. The greatest benefits of sprouting and direct covering of nonwoven PP cover were significantly influenced on commercial tuber yield. The efficacy of these agronomic treatments not only affected tuber yield quantity, but also contributed to improving of the tuber quality, especiallyby an increase in dry matter.

References

Allen, E.J., O'Brien, P.J. (1986). The practical significance of accumulated day-degrees as a measure of physiological age of seed potato tubers. Field Crops Res., 14:141–151.

Baranowska, A.J. (2018): Yield of dry matter and starch of edible potato tubers in conditions of application of growth biostimulators and herbicide. Acta Agrophys., 25: 397–407.

Benoit, F, Ceustermans, N. (1987). Advancing the harvest of bolt-sensitive endives by means of temporary single and double direct crop covering. Plasticulture, 7: 4–8.

Benoit, F., Ceustermans, N. (1990). Effect of the removal of the two direct cover sheetings on the development of carrots (*Daucus carota* L.). Acta Hortic., 267: 45–52.

Caliskan, M.E., Caliskan, S., Arioglu, H. (2004). Eeffects of presprouting and planting date on growth and yield of potato crop in a mediterranean type environment. Agronomy Section Meeting of the European Association for Potato Research, 23-27 June 2004, Mammaia, Romania, pp.189–196.

Cholakov, T.L., Nacheva, E.K. (2009). Results from using polypropylene cover in production of early potatoes. Acta Hort., 830: 603–608.

Choukr-Allah, R., Hafidi, B., Reyd, G., Hamdy, A. (1994). Influence of nonwovens on outdoor crops: Moroccan experience. Proceed. of 13th ICPA, Verona, Italy. 13 p.

Hagman, J. (2012). Pre-sprouting as a tool for early harvest in organic potato (*Solanum tuberosum* L.) cultivation. Potato Res., 55: 185–195.

Hamouz, K., Lachman, J., Dvořák, P., Pivec, V. (2005). The effect of ecological growing on the potatoes yield and quality. Plant Soil Environ., 51: 397–402.

Hemphill, D.D. (1989). Tomato, cucurbit, and sweet corn growth under agriplastics as a function of heat unit accumulation. Proceed. of 21st NAPC, Orlando, Florida, pp. 276-282.

Ilin, Ž., Đurovka, M., Marković, V., Sabadoš, V. (2000). Agrobiological basis for successful potato production. Arh. Poljopr. Nauke, 61(215): 101–114.

Kmecl, V., Žnidarčič, D. (2015). Accreditation of the analytical method used for nitrate determination in vegetables. Arch. Biol. Sci., Belgrade, 67(1), 295–302.

Knowles, N.R., Knowles, L.O. (2006). Manipulating stem number, tuber set, and yield relationships for northern and southern-grown potato seed lots. Crop Sci., 46: 284–296.

Liu, J., Pattey, E., Jégo, G. (2012). Assessment of vegetation indices for regional crop green LAI estimation from Landsat images over multiple growing seasons. Remote Sens. Environ., 123: 347–358.

Marcomini, G.R., Patino, M.T.O., Alam, M.J., de Amorim, M.R. (2019). Lessons from a cost's analysis in potato production. Emir. J. Food Agric., 31(11): 857–63.

Mermier, M., Reyd, G., Simon, J.C., Boulard, T. (1995). The microclimate under Agril P17 for growing lettuce. Plasticulture, 107: 4–12.

Möller, K., Reents, H.J. (2007). Impact of agronomic strategies (seed tuber pre-sprouting, cultivar choice) to control late blight (*Phytophthora infestans*) on tuber growth and yield in organic potato (*Solanum tuberosum* L.) crops. Potato Res., 50(1), 15–29.

Qureshi, M.S., Midmore, D.J., Syeda, S.S., Playford, C.L. (2007). Floating row covers and pyriproxyfen help control silverleaf whitefly *Bemisia tabaci* (Gennadius) Biotype B (Homoptera: Aleyrodidae) in zucchini. Aust. J. Entomol., 46(4): 313–319.

Pandey, V., Kumar, V.A., Brar, A. (2017). Biochemical behaviour of potato tubers during storage. Chem. Sci. Rev. Lett., 6(23): 1818–1822.

Rębarz, K., Borówczak, F., Gaj, R., Frieske, T. (2015). Effects of cover type and harvest date on yield, quality and cost-effectiveness of early potato cultivation. Am. J. Potato Res., 92: 359–366.

Struik, P.C. (2007). The Canon of potato science: 40. Physiological age of seed tubers. Potato Res., 50: 375–377.

Wadas, W. (2016). Using non-woven polypropylene covers in potato production: a review. J. Cent. Eur. Agric., 17(3): 734–748.

Wells, O.S., Loy, J.B. (1985). Row cover and high tunnels enhance crop production in the northeastern United States. HortTechnology, 3: 92–95.

Wolfe, D.W., Albright, L.D., Wyland, J. (1989). Modelling row cover effects on microclimate and yield. I. Growth response of tomato and cucumber. JASHS, 114(4): 562–568.

DETERMINATION OF THE PRODUCTIVITY AND DEVELOPMENT STATUS OF THE SECONDARY BUDS IN THE KARAERIK GRAPE VARIETY

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Abstract

This study was carried out to reveal the size of the secondary buds and the number of clusters they contain in the Karaerik grape variety. In addition, it was tried to determine whether there was a relationship between secondary bud size and cluster outline formation. In the study, winter buds in the first, second and third node of the grape variety Karaerik (*Vitis vinifera* L.), fertile in bottom buds, suitable for short pruning and have a very high table value in the region, were used as material. Secondary buds were separated from the primary and tertiary buds in winter buds with the help of a scalpel and were subjected to fixation, vacuuming, paraffin impregnation, paraffin embedding, freezing, sectioning and tissue staining, respectively. The stained samples were examined microscopically, their images were taken and these images were transferred to the computer, and the secondary bud size and cluster number were determined. In the study, it was revealed that the secondary buds in the second node were statistically different ($p<0.05$) from the secondary buds in the first and third nodes in terms of both cluster number and bud size. In addition, it was determined that there was a relationship between the bud size of the secondary buds in the second node of Karaerik grape variety and the number of clusters.

Keywords: *Secondary bud, bud size, cluster number.*

Introduction

Winter buds on the grapevine are quite complex due to their anatomical and morphological structure. Winter buds are of various shapes and appearances, but are generally large and angular. They contain leaf scales, shoots, leeches and cluster primordium and take their final shape in autumn. The bud core after the protective scales consists of three shoot beds. These shoot beds are expressed as primary bud, secondary bud and tertiary bud according to their growth capacities (Celik et al., 1998; Agaoglu, 1999). One of the shoot beds is in the middle and the other two are on the sides. The scales of the middle shoot bed are larger and the organs are better developed.

With the beginning of the vegetation period, the shoot bed in the middle develops and forms the summer shoot. In the shoot bed called the secondary bud, the organs are weaker than the middle shoot bed and in some case they may form clusters. The growth cone, which is called the tertiary bud and located at the top of the middle shoot bed, is more primitive than the other two shoot beds and does not contain clusters.

In addition, more than three shoot beds can be seen in a winter buds, although not very often. Although it is thought that the number of exile beds in the winter bud can be affected by genetic structure, the location of the bud on an old branch, cultural practices a year ago (Ilter, 1980; Agaoglu, 1993), this may occur under the influence of many factors. There is no clear explanation of the situation.

In terms of productivity of the grapevines, it is understood the productivity of the primary buds in the winter buds. The efficiency of secondary buds is important only in terms of compensating the losses that will occur due to the damage of the primary bud and ensuring the continuity of vegetative organs (Agaoglu, 1969).

In temperate climates, bud burst of grapevine is expected to occur with the continuation of the primary bud located in the middle of the winter buds, which have completed their development at the beginning of April. If this bud is damaged by late spring frosts, diseases and pests and mechanical impacts, the secondary bud comes into play, and if it is damaged, the tertiary bud becomes active. During this period, the dead primary bud can be observed among the other two developing buds (Dry and Coombe 1994).It is well known that primary buds in the winter buds are more efficient than secondary and tertiary buds, but they are more sensitive to frost damage than secondary and tertiary buds (Hemstead and Luby, 2000; Sivritepe et al., 2001) Smiley et al., 2008).Although the Üzümlü district of Erzincan province, where the study was conducted, has a microclimate feature, the low winter temperatures that occur in some years cause significant damage to the winter buds of the grapevine. Primary buds, which are primarily sensitive, are damaged in years when frost damage is observed. In this case, the secondary buds, which act as insurance, continue and the vegetative development of the grapevine continues. The continuation of secondary buds ensures the continuity of vegetative organs, and depending on the variety and year, sometimes products can be obtained at levels close to the normal product. Therefore, the productivity of the secondary bud in viticulture is also important (Guner, 2005). In different studies, it has been determined that secondary buds can yield varying levels according to the variety and year (Karaagaç, 2000; Aydın, 2001; Guner, 2005). However, in many national and international studies, all yield calculations were made on primary buds, ignoring the productivity of secondary buds in grapevine winter buds.

The main purpose of this study is to determine the size and productivity of the secondary buds, whose productivity is often neglected in winter buds, according to the node (position they are on an old branch). In this context, this study conducted on *Vitis vinifera* cv. Karaerik, secondary bud sizes and cluster primordia within the buds, depending on their positions, were determined by histological analysis. In addition, the relationship between the secondary bud size and the number of clusters in the Karaerik grape variety has been tried to be put on the line.

Material and Methods

Material

This study was carried out between 2019-2020 on a 30-year-old Karaerik vineyard established with the Baran system under the conditions of the Üzümlü district belongs to Erzincan province, which has microclimate characteristics in the Eastern Anatolia Region. The Karaerik grape variety, which is suitable for short pruning, is usually pruned over 2-4 buds, and taking into account the growth capacity of the vines, usually 30-40 buds are left on one vines (Kupe and Kose 2015). In the study, one of the old branches of the Karaerik grape variety, which is consumed for table consumption and has a very high market value in the region, was taken just before pruning (March) at the end of the winter rest period. Samples taken from the vineyard were delivered to the laboratory on the same day in polyethylene protection bags in order to avoid lost of moisture.

Methods

In the study, the other two buds (primary and tertiary buds) in winter buds were separated with the help of a scalpel in order to determine the productivity status of secondary buds. The remaining secondary buds are grouped according to their node (position) where the winter buds are on an old branch. For this purpose, the cluster primordia within the secondary buds inside the suspended winter buds with mixed bud structure were determined according to Odabas (1976). In the study, the first 3 nodes from the bottom, which are fertile for the Karaerik grape variety, were evaluated.

Each of the secondary buds grouped according to their nodes (the position they are on an old branch) were placed in separate bottles and placed in the desiccator by adding 2 ml of fixation liquid consisting of 5 ml formaldehyde, 5 ml glacial acetic acid, 90 ml 70% alcohol, and vacuuming process was carried out at regular intervals. Each fixed sample was passed through Johansen solutions (water + ethyl alcohol + tertiary butyl alcohol mixture) at concentrations of 50%, 70%, 85%, 95% and 100%, respectively, according to Johansen (1940).

The samples were kept in each solution for at least two hours. After the fixation process was completed, paraffin was added to completely cover the samples taken into the empty bottles, and paraffin impregnation was carried out by keeping them in the oven at 60 °C for 24 hours. Samples removed from the oven at the end of 24 hours (through liquid paraffin) were embedded in paraffin so that a longitudinal section could be taken. The paraffin blocks in which the samples are embedded were kept in the freezer at -20 ºC for 24 hours. Samples taken out of the freezer are stored at room temperature (22-24 ºC) for 5 minutes. After waiting for a period of time, longitudinal sections were taken with the help of a rotary microtome in thicknesses varying between 08-12 microns. The sections taken were laid in a gelatin-poured hot water bath (30 ºC). The samples adhered on the slide were heat treated in the oven at 60-70 ^{0}C for 1 hour and the paraffin was melted. In order to remove the melted paraffin from the tissue in the samples taken from the oven, it was kept in xylol (10 minutes) and 100% ethyl alcohol (5 minutes). After waiting for the water on the slide to dry, a drop of Toluidine blue was dropped on each section sample, after 5 minutes, the sections were washed with water and allowed to dry. The coverslips were adhered by dropping 1 drop-holding gel on each of the dried sections and the sections were made ready for examination under a light microscope. In histological examinations, images were transferred to the computer with the help of a camera (Moticam 480) integrated with the microscope.

With the help of the program installed on the computer (Motic images 2010), investigations were made on the photographs of the tissues. During the investigation, cluster outline numbers within the secondary buds were determined according to the positions of the buds. In addition, while determining the size of the buds according to their position, the width x height value measured in mm was taken into consideration as the bud size (mm^2) (Figure 1). In this study, some statistical analyzes have been made to determine the differences between the cluster plots that the secondary buds contain according to their location and years. T-test (One way Anova) was performed in SPSS program to determine how cluster outline numbers and bud sizes in secondary buds vary by position and year.

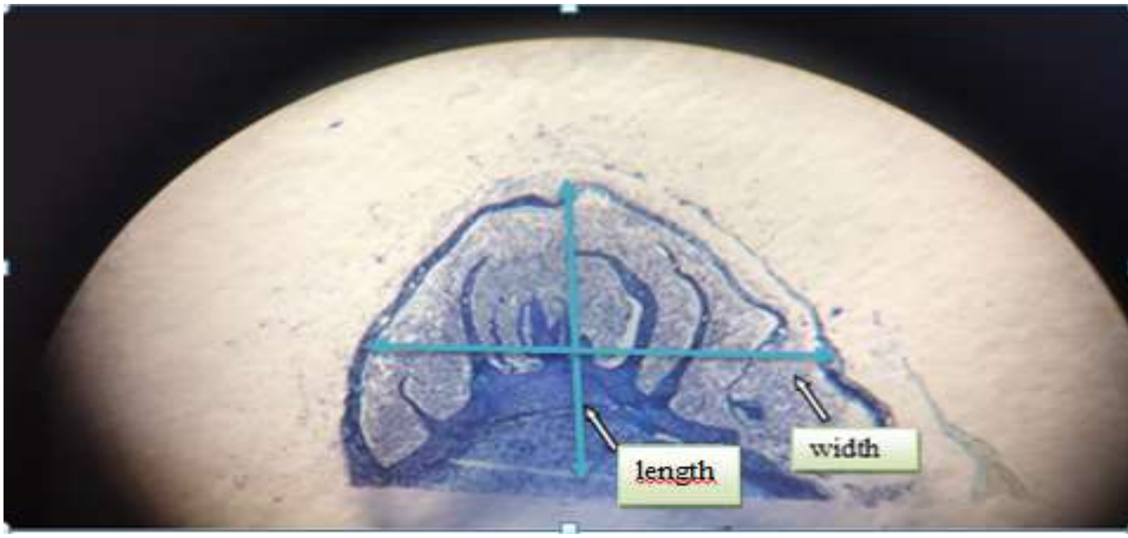

Figure 1. Determination of secondary bud size

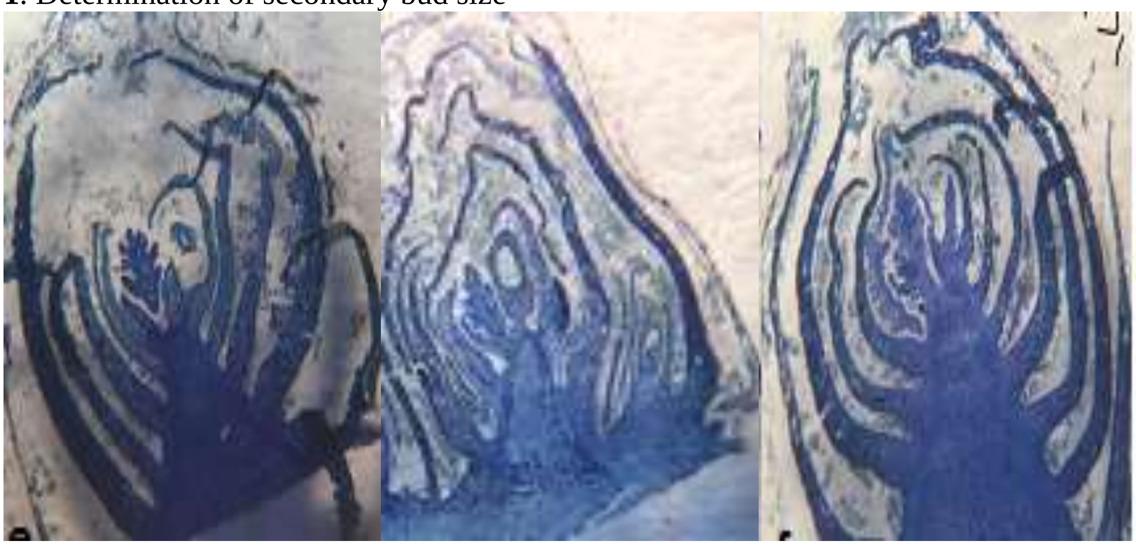

Figure 2. Samples of secondary buds containing clusters

Results and Discussion

The T Test (Oneway ANOVA), we made in order to reveal how the number of clusters contained in the secondary buds in the winter buds change according to the position is presented in Table 1. According to the results obtained in the study in which the number of clusters contained by the secondary buds were evaluated according to the first 3 nodes; It was determined that there was a statistically significant ($p < 0.05$) higher number of clusters between the 1st node and the 2nd node. Although it was determined that the number of clusters in 3rd nodes was higher than the 1st node and less than the 2nd node, the difference between these groups was statistically insignificant ($p > 0.05$). Similar results were obtained in both years of the study (Table 1).

Table 1. The number of clusters in secondary buds according to their position

Position	**Cluster numbers**			
	Year 1		**Year 2**	
	$\bar{X}$	S_x	$\bar{X}$	S_x
1. node	0.000^{b}	0.000	0.077^{b}	0.053
2. node	0.177^{a}	0.043	0.311^{a}	0.061
3. node	0.077^{ab}	0.053	0.111^{ab}	0.062
Mean	0.121	0.029	0.220	0.040
P	*		*	

a, b: There is no statistical difference between the values indicated with the same letter.
*: Significant difference at $p < 0.05$ level

In the first year of the study, no clusters were found in the secondary buds located in the first node. In the second node, it was determined that 17.7% of the secondary buds examined had 1 cluster. In the third node, it was determined that there was 1 cluster in 7.7% of the samples examined. In the second year of the study, it was determined that 7.7% of the secondary buds in the first node, 31.1% of the secondary buds in the second node, and 11.1% of the secondary buds in the third node. When the years and positions are evaluated together, it has been determined that 17% of the secondary buds have 1 cluster each (Table 1).
The T Test (Oneway ANOVA) we made in order to reveal how the size of the secondary buds in suspended winter buds changes according to the position is presented in Table 2. When the secondary bud sizes were examined in terms of their position in the study, the size of the secondary buds in the 2nd node was found to be statistically ($p < 0.05$) higher than the size of the 1st and 3rd buds in both years. In addition, no statistically significant difference ($p > 0.05$) was determined in terms of the size of the 1st and 3rd buds in both years of the study (Table 2).
According to the results of the study, it was determined that the largest secondary bud was in the second node in both years, and the secondary buds in this node had a size of 0.041mm^2 in the first year of the study and 0.048 mm^2 in the second year of the study. Although there was no statistically significant difference between the secondary buds in the first and third node in both years of the study, the secondary bud in the first node was larger than the secondary bud in the third node, and in the second year, the secondary bud in the third node was found to be larger than the secondary bud in the first node (Table 2).

Table 2. Secondary bud size according to their position

Position	**Bud Size**			
	Year 1		**Year 2**	
	$\bar{X}$	S_x	$\bar{X}$	S_x
1. node	0.031^{ab}	0.003	0.027^{b}	0.003
2. node	0.041^{a}	0.004	0.048^{a}	0.004
3. node	0.025^{b}	0.002	0.029^{b}	0.003
Mean	0.035	0.003	0.040	0.003
P	*		**	

a, b: There is no statistical difference between the values indicated with the same letter.
*: Significant difference at $p < 0.05$ level

In the test performed to determine the relationship between bud size and the number of clusters, the first year was significantly ($r = 0.199$, $p < 0.05$), and the second year was very significantly ($r = 0.359$, $p < 0.01$) between the number of clusters and the bud size in the study. In the correlation analysis, it was understood that there was a very significant relationship ($r = 0.250$, $p < 0.01$) between the number of clusters in the 2nd node and the size of the buds, but there was no significant relationship in the 1st and 3rd node.
Some important results were obtained in the study of determining the size and productivity of secondary buds on Karaerik grape variety (*V. vinifera* L.), which is widely grown in Erzincan (Üzümlü) province in Eastern Anatolia region of Turkey. It is known that primary buds in winter buds are more productive than secondary and tertiary buds, but they are more susceptible to frost damage than secondary and tertiary buds. Although this region where the study was conducted has a microclimate feature, the low winter temperatures that occur in some years cause significant damage to the winter buds of the grapevine. Primary buds, which are primarily sensitive, are damaged in years when frost damage is observed. In this case, secondary buds, which assume the role of insurance, continue the vegetative development of the grapevine. In this study, cluster numbers were examined in order to determine whether the secondary buds of the Karaerik grape variety had an effect on the yield of the grapevine. At the end of the study, it was revealed that Karaerik grape variety had 1 bud in 17% of the secondary buds.
When the number of cluster primordia contained by the buds according to their positions at the end of the study is evaluated, it is seen that the secondary buds in the 2nd node contain more cluster primordia than the secondary buds in the 1st and 3rd node. Since the yield of grape obtained from vineyards can vary depending on the position of the winter buds on the 1 year branch, it is of great importance to be able to determine the winter bud productivity in different positions (Karatas et al., 2010; Kupe and Kose 2015). Agaoglu and Kara, (1993), in their study, in which they determined the productivity of 37 grape varieties grown in Tokat region according to the positions of the first 10 internodes, reported that the maximum productivity varied between the 3rd and 10thbuds, and the first nodes were unproductive in some varieties. Agaoglu (1999) stated that the number of buds and clusters in anbud varies according to the position of the bud, and although it varies according to the varieties, generally the bottom and tip of the shoot contain fewer buds (shoot beds) than the middle part of the buds.
Although this situation is expressed for primary buds, it has been revealed that it has a similar relationship in secondary buds. In addition, the lowest number of cluster primordia was observed in the first node in both years of the study. We believe that this situation may cause very few cluster primordia or no cluster primordia to be seen in the secondary buds due to excessive lignification in the first node, where old branches begin to lignify from the bottom. Agaoglu (2002) and Celik (2008) stated that very strong vegetative developments delay the formation of cluster primordia in winter buds and decrease the number of clusters/bud ratio, and Celik (2008) stated that the growth power is one of the most important factors affecting the cluster and flower formation. Considering the fact that there is more wooding in the bottom buds as a part of vegetative development, it is seen that it supports our result. Similarly, Sartorius (1968) reported that very strong developments reduce bud productivity.
In the study, when the sizes of the secondary buds are compared according to their position, it is understood that the secondary buds in the second node have a larger structure than the secondary buds in the first and third node. Since we thought that there might be a relationship between bud size and the number of clusters in the study, we determined that there was a relationship between the size of the bud and the presence of a cluster outline in the statistical analysis we made to

determine this relationship. It has been demonstrated that this relation is strongest in the 2^{nd} node in the Karaerik grape variety, whose bottom buds are fertile. Lavee (2000) reported that shoots developed better due to the long vegetation in hot regions and there was a positive correlation between shoot development and bud development. However, Sartorius (1968) reported that very strong growth decrease bud productivity. Indeed, Huglin (1958), who stated that the growth power is one of the most important factors affecting the cluster and flower formation, reported that there is a positive relationship between the growth of the shoots and the number of clusters/bud, flower number/flower number/shoot ratio. In other words, the presence of clusters in the bud reinforces the possibility that the bud size may increase or the possibility of cluster outline formation in buds with large structure may be higher.

Conclusion

As a result of the study, it was observed that the secondary bud size for the Karaerik grape variety and the number of cluster outline in the bud may vary according to the position of the bud on an old branch and the bud size. Considering that the low winter temperatures that occurred in the region where the study was conducted for some years caused significant damage to the winter buds of the grapevine, it is very important to know how efficient these buds are, and the secondary buds that take on the role of insurance in the years of frost damage. If the secondary bud continues, it is ensured that the vegetative development of the grapevine will continue. In addition, by knowing the productivity levels of secondary buds, it will be possible to make a more accurate annual yield estimation for the variety in years of frost damage.

References

Agaoglu, Y.S., 1969. Comparative Studies on Bud Structures, Floral Growth Periods and Pruning Methods of Table Grape Varieties Hasandede, Kalecik Karası, Papaz Karası, Öküzgözü and Furmint. (PhD Thesis). Ankara University Faculty of Agriculture. Ankara.

Agaoglu, Y.S.ve Z. Kara, 1993. Studies on the Determination of Eye Productivity of Some Grape Varieties Cultivated in Tokat Region. Doğa Tr. J. of Agricultural and Forestry. 17 (1993), 451-458.

Agaoglu, Y.S., 1999. Scientific and Applied Viticulture (Vine Biology). Ankara University, Faculty of Agriculture. Volume I. Kavaklıdere Education Publications. No:1, 205 pp., Ankara.

Agaoglu, Y.S., 2002. Scientific and Applied Viticulture (Vine Physiology). Kavaklidere Education Publications No: 5, 445 pp., Ankara.

Aydın, I., 2001. Determination of productivity levels of secondary shoots formed as a result of damage to primary shoots in some wine grape varieties grown in Ankara conditions. (Master Thesis). Ankara university, Graduate School of Natural and Applied Sciences, Ankara.

Celik, H., Agaoglu, Y.S., Marasalı, B., Söylemezoglu, G., Fidan, Y., 1998. General Viticulture. Sun Fidan AS. Professional Books Series, No:1, 253pp., Ankara.

Dardeniz, D., Kısmalı, I., 2005. Studies on Determination of Winter Bud Productivity and Determination of Optimum Pruning Levels in Some Table Grape Varieties. Journal of Ege University Faculty of Agriculture, 42(2):1-10.

Dry, PR., Coombe, BG. (1994). Primary Bud-Axis Necrosis of Grapevines. I. Natural Incidence and Correlation with Vigor. Vitis, 33, 225-230.

Guner, N., 2005. The Relationship of Spreading Performance with Rootstock and Training-Pruning in Table and Wine Grape Varieties. (Master Thesis). Ankara Univ. Institute of Science, Ankara.

Hemstead, PR., Luby, JJ., 2000. Utilization of *Vitis riparia* for the Development of New Wine Varieties with Resistance to Disease and Extreme Cold. Acta Hort. 528:487-490.

Huglin, P., 1958. Recherches sur les Bourgeons de la Vigne. Initiation Florale et Développement Végétatif. Ann. Am él. Plantes 8:113-272.

Ilter, E., 1980. Investigations on the Effects of Some Chemical Substances Applied on Leaves on Winter Bud Productivity in Vines. E.U. Faculty of Agriculture Publications No: 372, 132pp.

Johansen, D. A., 1940. Plant Microtecnique. Mc. Graw-Hill Book Company, Newyork 523, 126-154.

Karaagac, E., 2000. Determination of productivity levels of secondary shoots formed as a result of damage to primary shoots in vines under mainland conditions. (Master Thesis). Ankara University Graduate School of Natural and Applied Sciences. Ankara.

Karatas, H., Değirmenci D., Agaoglu, Y., S. 2010. The Effects of Branch Directions on Grape Yield and Quality in Kalecik Karası Grape Varieties (*Vitis vinifera* L.). Journal of Uludag University Faculty of Agriculture. 24(1): 37-46.

Kupe, M, Kose, C. 2015. Determination of Appropriate Pruning Levels in Karaerik Grape Varieties Depending on Damage Level After Winter Cold. Journal of Atatürk University Faculty of Agriculture, 46 (1), 21-28.

Lavee S 2000. Grapevine (Vitis vinifera) growth and performance in warm climates. In: Temperate Fruit Crops in Warm Climates. (Ed. A Erez). Boston, London, pp. 343-366

Odabas, F., 1976. Determination of Productivity by the Investigation of Floral Developmental Stages of Some Important Grape Varieties Cultivated in Erzincan and Research on Fertilization Biology of these Varieties. Atatürk University Publications No:466, Research Series,No:141. 130pp., Erzurum.

Sartorius, O. 1968. Die Blütenknospen der Reben. Weinwissenschaft 23;309-338

Sivritepe, N., Burak, M., Yalçın, T., 2001. Determination of frost resistance of Ata Sarısı, Uslu and Yalova İncisi grape varieties. Journal of Uludag University Faculty of Agriculture 15: 25–38.

Smiley, LA., Domoto P., Nonnecke, G., Miller, WW., 2008. Cold Climate Cultivars. A Review of Cold Climate Grape Cultivars 'Baltica'. Iowa State University, Viticulture Home Page.

FATTY ACID DIVERSITY IN CULTIVATED AND WILD APRICOTS FROM EASTERN ANATOLIA IN TURKEY

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Abstract

Turkey is one of the richest countries in the world in terms of both horticulture crop production and diversity. Turkey is origin or second homeland of many fruit species. Diverse climate and soil conditions supports to growth a large number of fruit species, cultivars, genotypes, accessions even clones in the country. During the last two decades due to increasing interest to plant-derived natural products, apricots and its products has been gained more attention not only by consumers but also industries. Present study describes fatty acid profile of cv. Aprikoz and 4 wild apricot (Zerdali in Turkish) genotypes that has sweet kernels. A total nine fatty acids were found in cv. Aprikoz and four wild apricots and statistically significant differences are evident for major fatty acids among all searched cultivar and genotypes. Among the major fatty acids, oleic acid (18:2) were found to be the highest ratio for all analyzed apricot kernels which in range of 67.11% (wild apricot 4) and 70.44% (wild apricot 3), respectively. The other dominant fatty acids were linoleic acid and varied from 19.92% (wild apricot 4) 5) to 22.10% (wild apricot 3). All apricot samples had more monounsaturated fatty acids (MUFA) than saturated fatty acids (SFA) in seed oils. ΣPUFA/ΣSFA were in range of 3.22 and 3.75 among samples. Results indicated that fatty acid compositions of wild apricots are comparable with cv. Aprikoz.

Keywords: *apricot, wild, fatty acids, metabolic profile.*

Introduction

Turkish fresh fruit industry is not only the great diversity in the number of fruits grown but also the astonishing quantity of the total produce, about 20 million tons. The fresh produce grown in Turkey mainly consists of berries, pome fruits, stone fruits and citrus fruits. For apricots, the country leading both fresh and dried apricot production of the world for a long time. Turkey shares around 20% of world fresh apricot production and the country realizes more than 70 percent of the world dried apricot production. Uzbekistan, Italy, Iran, Algeria, Pakistan, Iran, Egypt, Ukraine, France and Spain were the other important apricot producer countries in the world (FAOSTAT 2020). Last two decades Turkey sharply increased apricot production from 315 thousand tons in 2002 at 750 thousand tons in 2018. During that period most of the main apricot producer countries either decreased their production or keep the same amount.
Approximately 35-40 percent of apricots produced in Turkey are consumed fresh and the remaining amount is dried or used in juice industry (Paydas et al. 1992; Ercisli 2009). In the production of dried apricots, the seeds are separated from the fruit flesh and the fruit flesh is dried and in general send to local markets or exported. Turkey is also an important apricot kernel producer in this sense. Apricot trees consisted in both cultivated and wild forms in Turkey, are

generally grown in semi-hot and arid regions, as well as in parts of the mountains that receive plenty of sun, and on the mountain slopes that do not hold water and are slightly calcareous in the south-facing parts of the mountains. These apricots are generally disease-free, delicious, sweet-tasting fruits with high dry matter content. Apricot trees are grown in Turkey mainly Malatya, Elazig, Mersin, Aras valley, Antalya, Kahramanmaras provinces, respectively (Asma et al., 2007; Polat et al., 2010; Caliskan et al., 2012; Polat et al., 2018). Aras valley, which has the one of the higher number of both cultivated and wild apricot trees, has traditional use of apricot kernels. In the Kagizman district, located in the Aras valley, apricot kernels are used especially in the production of pestil (a traditional product consisting of mulberry fruit, honey, milk and apricot kernels). In recent years, the kernels of apricot have been used as snacks as well and formed an important market segment (Gecer et al., 2020). In 2020, approximately 25 thousand tons of shelled kernels and 8 thousand tons of apricot kernels are obtained from dried apricots in Malatya city and average of 6-7 thousand tons of kernels exports per year. Italy is one of the most important buyers and approximately 2 thousand tons of apricot kernels exported to Italy. Malatya apricot kernel has also geographical indication (Anon. 2020). Consumers' orientation towards natural and unprocessed oils is increasing day by day. Oils that contain more phenolic components, high in oleic acid and rich in omega-3, can be offered for consumption in salads and sauces. In this sense, the fact that it is an alternative product to olive oil and rich in functionality reveals that it can have an important place in the market (Petrescu et al. 2020). The fatty acid composition of oil from kernels of apricot is highly favorable in human nutrition (Turan et al., 2007; Akinci et al., 2010). Apricot kernel oils had high oleic and linoleic oils and both had superior stability and nutritional importance. Linoleic acid, the main polyunsaturated fatty acid found in vegetable oil, nuts and seeds and lowers risk of coronary heart diseases (Farvid et al., 2014). Apricot kernels make up about 12% of the apricot fruit weight and consist of 15-20% protein, 4-5% cellulose, 52% oil. The oil content of the kernels varies between 27.7% and 66.7% (Femenia et al., 1995; Alpaslan and Hayta 2006). Apricot kernel oil is a raw material rich in unsaturated fatty acids, mostly consisting of oleic and linoleic fatty acids. Apricot kernel oil contains 58.3-73.4% of oleic acid and 18.8-31.7% of linoleic acid (Ozcelik, 2017). This content consists of 91.5-91.8% unsaturated fatty acids and 7.2-8.3% saturated fatty acids, as well as 95.7-95.2% neutral lipids, 1.3-1.8% glycolipids and 2.0% phospholipids. Seed oil contains 11.8 mg of campesterol, 9.8 mg of stigmasterol and 177.0 mg of sitosterol per 100 g (Alpaslan and Hayta, 2006). Apricot kernel oil contains high levels of γ-tocopherol, which can reach up to 475 mg/kg in oil. It also contains lesser amounts of α and β-tocopherol than γ-tocopherol (Ozcelik 2017).
The fact that there is not much research in fatty acid profiling of apricot kernel in particular on wild apricot kernel. Thus, the aim of this study is to determine fatty acid profile of 4 wild apricot kernels and cv. Aprikoz grown together in Kagizman district in Aras valley.

Material and Methods

Plant material

In present study cv. Aprikoz, and 4 wild grown apricots (kernels have sweet taste) used. All plants are grown in Kagizman district located in Aras valley in 2018. After harvest the apricot seeds separated from flesh and also removed of foreign substances.

Oil extraction

Automatic soxhlet device was used for total oil (lipid) extractions. Approximately one hundred fifty grams of dried grape seeds were used for oil extraction. Hexane used as solvent.

Determination of fatty acids

The fatty acids were analyzed by a GC (Perkin Elmer, Shelton, USA). Chromatographic separation was done using a (30 m×0.25 mm) column equipped with a flame ionization detector (FID). The oven temperature was 120 °C for 2 min, raised to 5 °C/min to 220 °C, which was held for 10 min, while the injector and the detector temperatures were set at 280 °C and 260 °C, respectively. The results were expressed in GC area % as a mean value and ± standard deviation.

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

Results and Discussion

The data of the fatty acid profiles of cv. Aprikoz and 4 wild apricot kernels are presented in Table 1. Statistically there were a great variation ($p<0.05$) in the palmitic acid, oleic acid and linoleic acid in seed oils of used cultivar and four wild apricot genotypes (Table 1).

Oleic acid, linoleic acid, palmitic acid and stearic acid were in range of 67.11-70.44%, 19.92-22.10, 4.56-5.46 and 0.97-1.23%, respectively (Table 1). For all apricot samples, oleic acid was predominant and the highest oleic acid content was obtained from wild apricot 3 genotypes (70.44%), and followed by wild apricot 1 (70.04%), cv. Aprikoz (68.10%), wild apricot 2 (67.33%) and wild apricot 4 (67.11%), respectively. However statistically there was no differences between wild apricot 1 and wild apricot 3 genotypes (Table 1). The kernel oil of the wild apricot 3 contained higher content of linoleic acid (22.10%) than those of the cv. Aprikoz (21.41%), wild apricot 1 (20.33%), wild apricot 2 (21.89%) and wild apricot 4 (19.92%) (Table 1) The wild apricot 2 genotypes had the highest palmitic acid (5.46%) in kernel than rest of the genotypes and cv. Aprikoz. The other three genotypes of wild apricots had lower palmitic acid content than cv. Aprikoz. The cultivar cv. Aprikoz and 4 wild apricot genotypes were differed each other in terms of saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA) and polyunsaturated fatty acids (ΣPUFA (Table 1). Apricot kernels were characterized by high ΣMUFA (67.86-71.24%) and followed by ΣPUFA (20.02-22.24%) and ΣSFA (5.82-6.86%), respectively (Table 1). Σ fatty acids were in range of 94.23-99.47% (Table 1). Considering general classification of fatty acid, sequence was MUFA>PUFA>SFA in seeds oils of cv. Aprikoz and wild apricots. We found PUFA/SFA ratio between 3.15 in wild 4 apricot and 3.75 for cv. Aprikoz, which shows good values for nutritional perspective as dietary fat by British Department of Health (Anon. 1994). Previous studies related to fatty acid profile of apricot kernels in apricot producing countries located in Asia (kernel is traditionally have been used and has special importance in Turkey and the other Asian countries) showed that the major fatty acids in apricot kernel oils was oleic acid and followed by linoleic and palmitic acid. The concentrations of oleic, linoleic and palmitic acid varied from 62.07-71.00%; 20.15-27.76%; 4.20-7.79% in India (Gupta et al., 2012; Yadav et al., 2018), 62.34-80.97%; 13.13-30.33%; 3.35-5.93% in Pakistan (Manzoor et al., 2012) and 70-29-71.25%; 22.31-23.00%; 4.57-4.87% in China (Zhou et al., 2016), respectively. In Turkey Juhaimi et al. (2018) found that apricot seed oils contain 65.98-71.86% oleic acid, 20.18-22.74% linoleic acid and 5.87-6.78% palmitic acid.

Table 1. Fatty acids in apricot seeds (% of Σ FAME)

	Aprikoz	Wild apricot 1	Wild apricot 2	Wild apricot 3	Wild apricot 4
Fatty acids					
Palmitic acid (C16:0)	5.33±0.01ab	4.56±0.01c	5.46±0.01a	4.77±0.01bc	5.03±0.01b
Margaric acid (C17:0)	0.10±0.01^{NS}	0.14±0.01	0.09±0.01	0.09±0.01	0.09±0.01
Stearic acid (C18:0)	1.23±0.01^{NS}	0.97±0.01	1.19±0.01	1.03±0.01	1.09±0.01
Arachidic acid (C20:0)	0.11±0.01^{NS}	0.15±0.01	0.12±0.01	0.10±0.01	0.14±0.01
Palmitoleic acid (C16:1)	0.74±0.01^{NS}	0.63±0.01	0.84±0.01	0.70±0.01	0.65±0.01
Oleic acid (C18:1)	68.10±0.07b	70.04±0.03a	67.33±0.04b	70.44±0.02a	67.11±0.02b
Gadoleic acid (C20:1)	0.09±0.01^{NS}	0.11±0.01	0.10±0.01	0.10±0.01	0.10±0.01
Linoleic acid (C18:2)	21.41±0.04b	20.33±0.01bc	21.89±0.01ab	22.10±0.01a	19.92±0.01c
α-Linolenic acid (C18:3)	0.13±0.01^{NS}	0.09±0.01	0.18±0.01	0.14±0.01	0.10±0.01
ΣSFA	5.96±0.03c	5.82±0.01d	6.86±0.01a	5.99±0.01c	6.35±0.01b
ΣMUFA	68.93±0.04c	70.67±0.01b	68.27±0.03d	71.24±0.02a	67.86±0.02e
ΣPUFA	21.54±0.06b	20.42±0.04c	22.07±0.04a	22.24±0.01a	20.02±0.01c
ΣPUFA/ΣSFA	3.75	3.51	3.22	3.71	3.15
Σ Fatty acids	96.43	96.91	97.2	99.47	94.23

Data ex expressed as the mean±standard deviation, n=3.*Different letters in the same row indicate significant difference (p<0.05). FAME, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. NS: Non significant

Sengun et al. (2021) used Hacihaliloglu apricot seed to characterize fatty acid composition and found that approximately 97% of kernel oil consists of oleic (63.5%), linoleic (28.1%) and palmitic acid (5.2%). Compared with the results of previous studies, it was determined that the oleic, linoleic and palmitic acid amounts of the apricot kernel oil examined in this study were in accordance with the literature. These studies revealed that the fatty acid components of apricot seed oil may vary depending on the genetic background, harvesting period, processing, storage conditions, geographical region in which apricot trees are grown etc. Candan (2019) examined fatty acid composition of apricot kernels obtained from supermarket and determined that it contained 63.87-69-53% of oleic acid, 23.35-26.01% of linoleic acid, and its α-linolenic acid content was below 1%. Gundogdu et al. (2011) used Sakit, Bebeco and Colomer apricot cultivars to determine fatty acid profile in eastern Turkey and they found quite variable fatty acid profile. They reported oleic acid, linoleic acid, palmitic acid and stearic acid were in range of 26.51-59.88%, 29.24-53.65%, 5.73-10.89%, 3.05-3.84%, respectively. Matthaus et al. (2016) used Hasanbey, Hacihaliloglu, Kabaasi and Soganci apricot kernels to determine fatty acid content. They found that fatty acid compositions of the oils extracted from apricot kernels collected at the different harvest times are variable. Oleic and linoleic acids were determined as major fatty acids in kernels. While oleic acid contents of apricot kernel oils increase depending on maturation at the harvest times, both palmitic and linoleic acid contents of apricot kernel oils decreased. Uluata (2016) used Hasanbey apricot cultivar kernels for fatty acid analysis by using solvent and listed fatty acids as oleic acid (72.10%), linoleic acid (19.90%), palmitic acid (6.40%), respectively. The total SFA, MUFA and PUFA values of Hasanbey apricot kernels were 7.50%, 72.60% and 19.90%, respectively. The same study indicated PUFA/SFA ratio 2.65 that was a little bit lower than our finding.

Conclusions

In literature, very limited studies have been concerned with fatty acid composition of oils obtained from wild apricot seeds. This highlights the importance of the present study. Results also indicate that cv. Aprikoz and wild apricots was outstanding with better PUFA/SFA ratio. Taking all these facts into account, apricot kernels can be recommended for human consumption due to comparable fatty acid profile. This study has its limitation, where the fatty acid profiles were only identified using limited number of apricot cultivar and wild apricot samples as well.

References

Akinci, F.Y., Yıldırım, A.N., Askin, M.A., Kankaya, A., 2010. Total oil, fatty acid composition and tocopherol content in kernels of several bitter and sweet apricot (*Prunus armeniaca* Batsch) cultivars from Turkey. Journal of Food, Agriculture and Environment. 8:196-201.

Alpaslan, M., Hayta, M., 2006. Apricot Kernel: Physical and Chemical Properties. Journal of the American Oil Chemists' Society. 83 (5): 469-471.

Anonymous. 1994. Nutritional Aspects of Cardiovascular Disease (report on health and social subjects No. 46), Hmso, U.K.

Anonymous. 2020. Crop report: Apricot. Ministry of Agriculture and Forestry of Turkey. Agricultural Economic and Policy Development Institute. 41 p.

Asma, B.M., Kan, T., Birhanli, O., 2007. Characterization of promising apricot (*Prunus armeniaca* L.) genetic resources in Malatya, Turkey. Genetic Resources and Crop Evolution. 54:205-212.

Caliskan, O., Bayazıt, S., Sumbul, A., 2012. Fruit quality and phytochemical attributes of some apricot *Prunus armeniaca* L.) cultivars as affected by genotypes and seasons. Notulae Botanicae Horti Agrobotanici Cluj Napoca. 40(2): 284-294.

Candan, A., 2019.Investigation of the properties of apricot seeds, flaxseed and grape seed oil extracted by different methods and pre-treatments. The Graduate School of Natural and Applied Science of Necmettin Erbakan Unıversity, Konya, Turkey. MSC Thesis, 37 p.

Ercisli, S., 2009. Apricot culture in Turkey. Scientific Research and Essays, 4 (8):715-719.

Farvid, M.S., Ding, M., Pan, A., Sun, Q., Chiuve, S.E., Steffen, L.M., Willett, W.C., Hu, F.B., 2014. Dietary linoleic acid and risk of coronary heart disease: A systematic review and meta-analysis of prospective cohort studies. Circulation, 130 (18):1568-1578.

Femenia, A., Rosello, C., Mulet, A., Canellas, J., 1995. Chemical composition of bitter and sweet apricot kernels. Journal of Agricultural and Food Chemistry. 43: 356-361.

FAOSTAT. Food and agricultural commodities production. Food and Agricultural organization Statistics. 2020 (accessed 13 June 2020).

Gecer, M.K., Kan, T., Gundogdu, M., Ercisli, S., Ilhan, G., Sagbas, H.I., 2020. Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. Genetic Resources and Crop Evolution. 67:935-945.

Gundogdu, M., Ozrenk, K., Kan, T., Gecer, M.K., 2011. Phenolic compounds, tocopherols and fatty acid contents of some apricot cultivars grown in Van vicinity. Iğdir University Journal of the Institute of Science & Technology. 1 (4): 31-36.

Gupta, A., Sharma, P.C., Tilakratne, B.M.K.S., Verma, A.K., 2012. Studies on physico-chemical characteristics and fatty acid composition of wild apricot (*Prunus armeniaca* Linn.) kernel oil. Indian Journal of Natural Products and Resources. 3(3):366–370.

Juhaimi, F.A., Ozcan, M.M., Ghafoor, K., Babiker, E.E., 2018. The effect of microwave roasting on bioactive compounds, antioxidant activity and fatty acid composition of apricot kernel and oils. Food Chemistry. 243: 414-419.

Manzoor, M., Anwar, F., Ashraf, M., Alkharfy, K.M., 2012. Physico-chemical characteristics of seed oils extracted from different apricot (*Prunus armeniaca* L.) varieties from Pakistan. Grasas y Aceites, 63:193–201

Matthaus. B., Ozcan, M.M., Al Juhaimi, F., 2016. Fatty acid composition and tocopherol content of the kernel oil from apricot varieties (Hasanbey, Hacihaliloglu, Kabaasi and Soganci) collected at different harvest times. European Food Research and Technology, 242:221–226

Ozcelik M.M., 2017, Obtaining apricot kernel oil by ultrasound and microwave assisted extraction methods and examining some parameters, Master Thesis, Süleyman Demirel University, Institute of Science and Technology, Isparta.

Paydaş, S., Kaşka, N., Polat, A.A., Gübbük, H., 1992. A study on adaptation of new apricot (*Prunus armeniaca* L.) cultivars in Adana ecological conditions. Proceedings of 1st National Horticultural Congress, Vol. 1 Fruits, p. 465-471

Petrescu, D.C., Vermeir, I., Petrescu-Mag, R.M., 2020. Consumer understanding of food quality, healthiness, and environmental impact: A cross-national perspective. International Journal of Environmental Research and Public Health. 17: 169.

Polat, A.A., 2010. Apricot production in the eastern Mediterranean region, Hatay, Turkey. Acta Horticulturae. 862: 343-350.

Polat, M., 2018. Yield and some pomological characteristics of organically grown "Alyanak" and "Hasanbey" apricots (*Prunus armeniaca* L.). Fresenius Environmental Bulletin. 27 (6): 4433-4439.

Turan, S., Topcu A., Karabulut, I., Vural, H., Hayaloglu, A.A., 2007. Fatty acid, triacylglycerol, phytosterol, and tocopherol variations in kernel oil of Malatya apricots from Turkey. Journal of Agricultural and Food Chemistry. 55: 10787-10794.

Sengun, I.Y., Yucel, E., Kilic, G., Ozturk, B., 2021. Determination of fatty acid composition and bioactive properties of pumpkin seed and apricot kernel oils. The Journal of Food, 46 (3): 608-620.

Uluata, S., 2016. Effect of extraction method on biochemical properties and oxidative stability of apricot seed oil. Academic Food, 14(4): 333-340.

Yadav, A.K., Pal, A., Dubey, A.M., 2018. Experimental studies on utilization of *Prunus armeniaca* L. (wild apricot) biodiesel as an alternative fuel for CI engine. Waste Biomass Valorization, 9(10): 1961-1969,

Zhou, B., Wang, Y., Kang, J., Zhong, H., Prenzler, P.D., 2016. The quality and volatile- profile changes of Longwangmo apricot (*Prunus armeniaca* L.) kernel oil prepared by different oil-producing processes. European Journal of Lipid Science and Technology. 118(2): 236-243.

THE INFLUENCE OF FERTILIZATION WITH PYROPHYLLITE ON VEGETATIVE GROWTH OF CABBAGE

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Abstract

In this study, research is focused on the application of pyrophyllite fertilization in combination with mineral fertilizer NPK (15:15:15) on the vegetative growth of cabbage hybrids Bravo F_1. Research was conducted during 2018 and 2019at the Institute of Agriculture of Federation of Bosnia and Herzegovina (FBiH).The aim of the study was to determine the extent to which the vegetative growth of cabbage depends on different doses of pyrophyllite combined with a standard amount of NPK mineral fertilizer. The influence of the following fertilization variants was investigated: var. 1 (control) - 800 kg/ha NPK 15:15:15, var.2 – 800 kg/ha NPK 15:15:15 + pyrophyllite 2.200 kg/ha, var.3 - 800 kg/ha NPK 15:15:15 + pyrophyllite 700 kg/ha, var.4 -800 kg/ha NPK 15:15:15 + pyrophyllite 1.200 kg/ha, var.5 800 kg/ha NPK 15:15:15 + pyrophyllite 700 kg/ha.The highest dose of applied pyrophyllite per unit area (2.200 kg/ha) had the greatest impact on the vegetative growth of cabbage.The results of the study indicate that the application of pyrophyllite can reduce the use of mineral fertilizer in the production of cabbage without adverse effects on the parameters of its vegetative growth.

Keywords: *fertilization, pyrophyllite, cabbage, vegetative growth.*

Introduction

Most agricultural land due to the continuous loss of nutrients in different ways, consequently lose their fertility and fertilizer efficiency and renewal of soil fertility remains insufficient. Precisely because of this, the use of pyrophyllite is taking on an increasing role in preserving and restoring soil fertility. Prospects for greater use of pyrophyllite in agriculture are based on the characteristics of its high adsorption capacity, high selectivity for some heavy metals and radionuclides (Hodžić,2016). According to Stoiljković (2002), pyrophyllite is one of the most promising minerals for use in agricultural production as a nutrient and biostabilizer. According to research of Churakov (2016) the pyrophyllite thanks to its physical and chemical properties has a positive effect on the fertility of the soil, improves the structure and the ability to retain moisture in the soil, regulates and distributes nutritional materiesand water to the plant when it needs most, at the same time for its crystal structure is tightly bound by heavy metals and polycyclic hydrocarbons. The results of Hasanbegović (2018) indicate that pyrophyllite is an important resource in the agricultural sector and other economic activities and its use in wider production practice can prevent serious environmental problems while obtaining quality agricultural products. In production practice, according to the research of Murtić et al. (2020) the application of pyrophyllite in agriculture is not significantly present due to poorer availability and insufficient knowledge of its potential for use.

Taking into account the previously mentioned facts, the aim of the study was to examine whether the application of pyrophyllite can reduce the use of mineral fertilizers in the production of white cabbage. Headed white cabbage was chosen as the subject of this experiment primarily because often in the production of cabbage with intentions for the highest possible yield there is an expensive irrational application of mineral fertilizers which inevitably causes harmful consequences not only on product quality but poses a potential risk of environmental pollution.

Material and methods

The research analyzed the pyrophyllite at the mine site Parsovići-Konjic, Bosnia and Herzegovina. In laboratory conditions of the Institute of Technology and Nuclear Physics in Belgrade and the Mining and Geology Institute of Tuzla were determined that certain physical and chemical parameters pyrophyllite. Physical traits of analyzed pyrophyllite were: specific weight of 2.7 g/cm^3, bulk density of 0.380 g/cm^3, pH value 8.5, the hardness (Mohs) 1. Chemical composition of a pyrophyllite are: SiO_2 62.35% Al_2O_3 15.00%, Fe_2O_3 2.03%, FeO 1.65%, MnO 0.08%, MgO 2.32%, CaO 5.11%, Na_2O 0.67%, K_2O 0.88%, CO_2 5.85%, SO_3 0.56%, H_2O 3.43%.
The head cabbage hybrid Bravo F_1 was used for the experiment. The hybrid Bravo F_1 is aDutch hybrid popular mainly for fresh consumption and it is also used for fermentation. Hybrid Bravo F_1characterized by tightly packed cabbage heads, good yield and uniform morphological characteristics. The average weight of the head is about 2-3 kg, the growing season is 90-95 days, the recommended harvest time is autumn.
The field experiment was set up on the experimental range of the Federal Institute of Agriculture in 2018 and 2019 by the block method with a random arrangement of plots in four replications. Before sowing, basic tillage was performed by plowing to a depth of 25 cm, followed by pre-sowing soil preparation by milling. Each experimental plot was 7 m^2(1.4 m wide x 5 m long). On the basic plot, 20 cabbage plants were planted per treatment in two-row strips at a distance of 70 cm between rows and 50 cm in a row. The distance between the plots was 100 cm. The total area of the experimental plot was 360 m^2. The following variants of pyrophyllite fertilization were used in the experiment:

Var. 1 - 800 kg/ha NPK 15:15:15 (control),
Var. 2 - 800 kg/ha NPK 15:15:15 + pyrophyllite 2,200 kg/ha,
Var. 3 - 800 kg/ha NPK 15:15:15 + pyrophyllite 700 kg/ha,
Var. 4 - 800 kg/ha NPK 15:15:15 + pyrophyllite 1200 kg/ha,
Var. 5 - 800 kg/ha NPK 15:15:15 + pyrophyllite 700 kg/ha.

During the development of cabbage, standard measures of cabbage care were taken.
The basic properties of the soil on which the experiment was set up were: pH in water 6.96, pH in KCL 5.90; N content 0.17%; $CaCO_3$ content 0.5%; humus content 2.26%; P_2O_5 7.65 mg/100g; K_2O 22.30 mg/100g. Soil analysis was performed in the Department of Agropedology of FBiH according to the standards: BAS ISO 10390: 2005, pH, $CaCO_3$, content N according to BAS ISO 11261: 2000, content P_2O_5 and K_2O Al - method.
The growth parameters were monitored: cabbage plant height (cm), cabbage rosette diameter (cm), cabbage head diameter (cm). The assessment of the significance of individual examined factors and their interrelation were tested on the basis of the LSD test for significance levels of 1% and 5%. The obtained results were processed by the method of analysis of variance of two - factorial experiment (ANOVA) using SPSS software.

Results and discussion

Fertilization with different doses pyrophyllite had a statistically significant effect on the plant height. Variant 2 exhibited statistical significance in comparison with all other variants, while variant 5 did not show statistical significance related to the control group. Similar results were obtained by Hasan and Solaiman (2012). The same authors state that the height of the plant depends exclusively on the type of fertilization. Olaniyi and Ojetayo (2011) mention that aplant grown with organic fertilizer obtains higher plants than plants grown with organomineral fertilizer and mineral fertilizer. A group of authors (Olaniyi et al., 2010; Olaniyi and Ojetayo, 2011) explain that organic fertilizer releases a significant amount of nutrients for plant use resulting in better vegetative growth.

Table1. Cabbage plant height (cm) depending on year and fertilization ± s. Deviation

Year	Date of measurement	Var. 1	Var. 2	Var. 3	Var. 4	Var. 5
2018	12.05 18.06	8.6 ± 0.11 14.4 ± 0.29	14.2 ± 0.39 19.8 ± 0.85	12.6 ± 0.21 17.1 ± 0.46	11.4 ± 0.46 15.9 ± 0.27	9.0 ± 0.32 14.8 ± 0.62
2019	12.05 23.06	8.2 ± 0.26 14.0 ± 0.40	13.4 ± 0.49 19.0 ± 0.28	12.0 ± 0.35 16.9 ± 0.34	11.0 ± 0.38 15.2 ± 0.54	8.4 ± 0.38 14.0 ± 0.26

Table 2. Variance analysis table for stable cabbage height

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	15.10	1	15.10	1.99	0,15965775	3.89
Columns	767,29	4	191.82	25.32 *	7,98544E-17	2.42
Interaction	1.26	4	0.32	0.04	0,996694004	2.42
Within	1439,26	190	7.58			
Total	2222,92	199				

Cabbage rosette diameter

The largest diameter of the rosette of cabbage plants was in variant 2 in both years of research. In variants 3, 4 and 5 in both years of the study, the diameter of the cabbage rosette decreased depending on the amount of pyrophyllite applied.

Table 3. Cabbage rosette diameter ($\bar{x}$ - cm/plant) depending on year and fertilization ± s.

Year	Date of measurement	Var. 1	Var. 2	Var. 3	Var. 4	Var. 5
2018	12.05 18.06	14.0 ± 0.34 28.2 ± 0.48	24.4 ± 0.45 44.0 ± 0.30	21.0 ± 0.78 33.2 ± 0.39	16.5 ± 0.33 30.0 ± 0.81	14.1 ± 0.36 28.2 ± 0.31
2019	12.05 21.06	13.4 ± 0.34 27.3 ± 0.29	23.0 ± 0.37 42.6 ± 0.40	19.6 ± 0.58 33.0 ± 0.53	15.2 ± 0.27 28.8 ± 0.41	17.0 ± 0.27 27.4 ± 0.36

Fertilization with different doses pyrophyllite had a statistically significant effect on the diameter of the rosette cabbage. The statistical significance was found in the Variant 2, in respect of all other variants, while Variant 5 with the least amount pyrophyllite did not have statistical significance compared to the control.

Table 4. Table of analysis of variance for the diameter of the cabbage rosette

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	17.58	1	17.58	0.30	0,581576556	3.89
Columns	4366,84	4	1091,71	18.92	4,11857E-13	2.42
Interaction	38.80	4	9.70	0.17	0,954396625	2.42
Within	10962.45	190	57.70			
Total	15385.68	199				

Cabbage head diameter

Based on the average value of the diameter of the cabbage head, it can be concluded that the plants in variant 2 with the largest amount of pyrophyllite applied had the largest average diameter of the cabbage head in both measurements. Our results are in parallel with the results of Murtić et al., (2020) who emphasize the positive influence of pyrophyllite on vegetative growth and lettuce yield.

Statistical difference was exhibited in the Variant 2, in respect of all other variants, while Variant 5 with the least amount pyrophyllite do not reach statistical significance compared to the control. Variants 3 and 4 in relation to the control also showed statistical significance. Similar to our results, Sarker et al. (2003) indicate that organomineral fertilizer has an effect on the diameter of the cabbage head.

Table 5. Cabbage head diameter ($\bar{x}$ - cm /plant) depending on year and fertilization ± s.

Year	Date measurements	Var.1	Var. 2	Var. 3	Var. 4	Var. 5
2018	01.08 10.08	13.0 ± 0.21 13.5 ± 0.44	17.0 ± 0.31 17.3 ± 0.39	16.0 ± 0.69 16.2 ± 0.30	15.0 ± 0.24 15.4 ± 0.32	13.1 ± 0.31 13.4 ± 0.40
2019	03.08 12.08	12.6 ± 0.30 13.0 ± 0.36	16.6 ± 0.60 16.8 ± 0.46	15.7 ± 0.50 16.0 ± 0.46	14.7 ± 0.52 15.2 ± 0.44	12.5 ± 0.51 13.0 ± 0.41

Table 6. Table of analysis of variance for cabbage head diameter

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	7.49	1	7.49	31.50 *	6,96572E-08	3.89
Columns	491,44	4	122.86	516.88 *	6,7755E-101	2.42
Interaction	2.03	4	0.51	2.13	0,078456396	2.42
Within	45.16	190	0.24			
Total	546,11	199				

Conclusion

Based on two years of research on the influence of fertilization with pyrophyllite on the vegetative growth of head cabbage of the Bravo F_1 hybrid and the obtained results, it can be concluded:

Conducted two-year research confirmed the effective fertilization with pyrophyllite on the parameters of vegetative growth of cabbage Bravo F_1,

- The most significant difference on the vegetative growth of cabbage was shown by variant 2 with the highest application of pyrophyllite (2.200 kg of pyrophyllite/ha) in relation to all other variants ,
- Variant 5 with the lowest applied amount of pyrophyllite (700 kg of pyrophyllite/ha) did not show statistical significance compared to the control,
- The results of the study indicate that the application of pyrophyllite can reduce the use of mineral fertilizer in the production of cabbage without adverse effects on the parameters of its vegetative growth.

References

Churakov S.V. (2006): Ab initio study of sorption on pyrophyllite: Structure and acidity of the edge sites. The Journal of Physical Chemistry B, 110 (9), 4135-4146.

Hasanbegović E. (2018): Determination of nitrate adsorption capacity from fertilizers and soil on pyrophyllite. Final master's thesis. University of Sarajevo-Faculty of Natural Sciences and Mathematics, Department of Chemistry.

Hasan M.R. and Solaiman AHM (2012): Efficacy of organic fertilizer on growth of Brassica oleracea L. (Cabbage). International Journal of Agriculture and Crop Sciences, Vol.4 (3), pp.128-138.

Hodžić A. (2016). Pyrophyllite is the future of ecology - international fair of energy, mining, environmental protection and related industries - Sarajevo, June 19-21, 2018.

Murtić S., Zahirović Ć., Karić L., Jurković J., Čivić H., Sijahović E. (2020): Use of Pyrophyllite as Soil Conditioner in Lettuice Production.Nature Environment and Pollution Technology. Vol.19. No.1.pp. 355-359.

Olaniyi J.O. and Ojetayo A.E. (2011): Effect of fertilizer types on the growth and yield of two cabbage varieties. Journal of Animal & Plant Sciences.Vol.12 (2), pp.1573-1582.

Olaniyi J.O., Akanbi W.B., Olaniran O.A., Ilupeju O.T. (2010): Effect of organic, inorganic and organominerals on growth, fruit yield and nutritional composition of okra *(Abelmoscus esculentus).* Journal of Animal and Plant Sciences. Vol.9 (1), pp.1135-1140.

Sarker M.Y., Begum F., Hasan MK, Raquibullah SM, Kader M.A. (2003): Effect of Different Sources of N utients and M ulching on G rowth and Y ield Contributing of Cabbage.Asian Journal of Plant Sciences, 2 (2), pp.175-179.

Stoiljković S. (2002): Application of some mineral raw materials in modern agriculture - Annual of scientific papers number 1, pages 86–92 UDC: 63: -032.

VARIABILITY OF SPIKE HARVEST INDEX IN WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Grain spike index indicate wheat productivity and can be used as criterion in wheat selection in the wheat breeding program. Aim of this investigation is study of variability of grain spike index in wheat cultivars grown under different environmental condition. Ten genetically divergent winter wheat cultivars were included in two year investigation in field experiment in randomized block design in three replications. The seeds of varieties were sown at the distance of 0.10 m in rows of 1.0 m length with the distance of 0.2 m. For analysis of grain spike index determined in proportion of grain mass spike^{-1}/mass of spike, 60 plants in full maturity stage (20 plants per replication) were used. On the base of analysis of variance by using MSTAT C (5.0 version) and computed F-test values, differences among cultivars according to value of grain mass spike^{-1}, mass of spike and also, spike harvest index were established. In the first year the highest spike harvest index was 0.82 (Fortuna) and the lowest 0.76 in Ljubičevka, with average for all varieties 0.79, while in the second year the highest spike index was 0.83 in Somborka, and the lowest 0.77 in Ljubičevka, with average value 0.80. The different values of spike harvest index indicate response of genotypes to environmental factors as well as interaction of genotype/environment.

Key words: *wheat, cultivar, variability, index of spike.*

Introduction

In wheat plant the large number of spike on the one hand and many grains spike^{-1} on the other hand is advantages in forming yield. There is differences in estimation of advantages number of spike per plant and mass of spike per plant based on negative correlation of size of spike and number of spike. The spike length has strong indirect influence on yield through number of spikelets spike^{-1} and further on number of grain and size and weight of grain (Zečević *et al.*, 2004). In breeding program in the aim of creating variety with high yield, the concept of plants contains more productive tillers, more spikes, spikelets spike^{-1}, grain spike^{-1}, mass of grain spike^{-1} (Knezevic et al., 2014**).** In many cases yield and biomass showed significant correlations with average yield and yield components example thousand grain weight. Height of plant have a weak negative association with yield. Absence of height reduction (*Rht*) gene alleles associated with high height of plant which is in negative association with harvest index (HI) (Rebetzke et al., 2011; Reynolds and Langridge, 2016). In production of total dry matter in wheat the biological yield is not main criterion for selection because grain yield is a main economic part of yield. The ratio of grain yield (economic) of plant and biological yield indicate on efficiency of genotype for formation of the economic yield or harvest index of plant (Knežević et al., 2015a). This indicate that model plant need have large spike and high mass (Knežević et al., 2011; 2019), especially high mass of grain spike^{-1} (Knežević et al., 2015b). The ratio of grain mass spike^{-1} and mass of spike variate due to fertilization of spikelets spike^{-1} or its number of fertilised florets as well number of grains spike^{-1} indicate efficiency of transport and translocation of dry matter through vessel bundles (phloem and xylem) to spike and grain of spike. Also, this indicate index of receptive capacity of grain spike^{-1} and post-anthesis photosynthetic capacity of stem. The large spike of wheat means that is a optimal number of florets per spike to ensure that their capacity of the spike does not represent a limit for fotosynthates translocation to grain. Size and number of spike per plant, number of spikelets spike^{-1} are genotype characters related to tillering (Moeller and Rebetzke, 2017) that are under genetic control and variate due to environmental condition as well as density of crops, seed rate, supply of nutrition and water in soil (Dodig et al., 2008). Aim of this investigation is study of variability of grain spike index in wheat vatieties grown under different environmental condition.

Materials and Methods

Ten genetically divergent winter wheat cultivars were used for investigation on experimental field in Kraljevo in two vegetative season 2015/16 and 2016/17. The experiment set up in randomized block design in three replications. The seeds of varieties were sown at the distance of 0.10 m in rows of 1.0 m length with the distance of 0.2 m. For analysis of grain spike index determined in proportion of grain mass spike^{-1}/mass of spike, 60 plants in full maturity stage (20 plants per replication) were used.

Based on the obtained average values of the mass of the seed and spike, the spike harvest index is calculated according to the formula:

$$Spike\ index\ (\%) = \frac{mass\ of\ seeds^{-spike}}{mass\ of\ spike}\ \mathrm{x}\ 100$$

Statistical data processing was done using the MSTAT C 5.0 version for analysis of variance by the mono factorial system for each year. 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 among cultivars according to grain index of spike were tested by means of test value of $LSD_{0.05}$ and $LSD_{0.01}$.

Weather conditions in the vegetation period

The total amount of precipitation and average temperature per month and per year were different in two year of experiment, during vegetative season (2015/16 and 2016/17) and differed in

relation to the long-term period (2000-2010). In the first year of the experiment, the average temperature was 9.9 °C and the total rainfall was 651.00 mm, which is significantly higher than in the second year and than in ten year period. In the second year the average temperature during the growing season was 8.7 °C and similar to ten year period while the total rainfall 523.1 mm was significant higher than in ten year period. During the grain filling phase of plants in the first year in April the average temperature was higher and in May the average precipitation was higher and favorable than in second year of experiment and than in ten year period (table 1)
.

Table 1. Average monthly temperatures and total monthly precipitation in Kraljevo

Parameter	Period	Oct	Nov	Dec	Jan	Feb	March	April	May	June	Xm	Total
Temperature ^{0}C	2015/16	11,6	7,3	3,3	-0,1	8,8	7,8	14,1	15,5	21,3	**9,96**	
Temperature ^{0}C	2016/17	10,6	6,8	0,0	-4,7	5,2	10,8	11,1	16,8	22,1	**8,74**	
Temperature ^{0}C	2000-2010	11,8	6,4	1,7	-0,1	2,6	5,9	11,6	16,4	20,4	**8,5**	
Precipitation (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**
Precipitation (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**
Precipitation (mm)	2000-2010	61,0	44,3	44,6	30,0	29,9	33,2	52,9	52,6	69,3	46,4	**417,8**

*source: Republic Hydrometeorological service of Serbia

Results and Discussion

The spike harvest index varied from 0.76 in Ljubičevka to 0.82 in Fortuna variety with average value of 0.79 in the first year of experimental vegetative season. The values of spike harvest index in second year of experiment varied from 0.77 in Ljubičevka to 0.83 in Somborka variety with average value 0.80 of all varieties (table 2). Wheat varieties with higher spike harvest index (SPHI) should be with developing more kernels spike^{-1} and a higher grain mass spike^{-1}, what depends on genotypes and their plasticity and response to environmental conditions.

In research on chernozem, similar results of spike harvest index 0.75 in winter wheat in sole crop and in intercrop (wheat/pea) 0.71 was found by Grčak et al. (2019a), while in spring wheat varieties spike harvest index was 0.72 in sole crop and 0.71 in intercrop wheat/pea (Grčak et al., 2019b). In earlier investigation of 22 divergent wheat genotypes presented that index of spike was in range from 0.68 to 0.91 Petrović et al. (2002).

Table 2. Analisys of variance for grain mass spike^{-1}, mass of spike and spike harvest index

Variety	Index of spike)		Average (g)	Grain mass spike^{-1} (g		Average (g)	Mass of spike (g)		Average
	I year	II year		I year	II year		I year	II year	
Fortuna	0.819ab	0.784bcdef	0.802A	2.910abcde	2.250g	2.580C	3.557def	2.860gh	3.208EF
Sasanka	0.781cdef	0.784bcdef	0.783ABC	3.123ab	2.753cdef	2.938AB	4.070a	3.310ef	3.690ABC
Danica	0.804abc	0.811abc	0.808A	3.223a	2.857bcde	3.040A	4.010abc	3.567def	3.788AB
Somborka	0.787bcdef	0.831a	0.809A	3.220a	3.033abc	3.127A	4.073a	3.643bcde	3.858A
Kremna	0.794abcde	0.822ab	0.808A	3.173ab	2.900abcde	3.037A	4.043ab	3.533def	3.788AB
Kosmajka	0.815abc	0.802abc	0.808A	2.847bcdef	2.233g	2.540C	3.530def	2.787h	3.158F
Šumadija	0.791bcde	0.794abcde	0.793AB	2.947abcd	2.583efg	2.765BC	3.737abcd	3.233efg	3.485CDE
Morava	0.798abcd	0.808abc	0.803A	2.660def	2.490fg	2.575C	3.330def	3.217fg	3.273DEF
KG-56S	0.7690ef	0.784bcdef	0.769BC	2.747cdef	2.623def	2.685BC	3.570def	3.333def	3.452CDEF
Ljubičevka	0.757f	0.763def	0.761C	2.743cdef	2.617def	2.680C	3.623cdef	3.417def	3.520BCD
Average	**0.790**	**0.799**	**0.794**	**2.96**	**2.63**	**2.79**	3.557def	2.860gh	**3.52**

LSD	Variety	Year	Variety x year	Variety	Year	Variety x year	Variety	Year	Variety x Year
(0,05)	0.268	0.076	0.379	0.255	0.071	0.360	0.2949	0.0829	0.4171
(0,01)	0.385	0.101	0.545	0.366	0.095	0.517	0.4237	0.1109	0.5992

Spike harvest index is value of ratio grain mass spike^{-1} and mass of spike. In this study the grain mass spike^{-1} varied in the range from 2.66 g in Morava to 3.22 g in Danica cultivar with average value 2.96 g in first year of experiment. The values of grain mass spike^{-1} in second year of experiment varied from 2.23 g in Kosmajka to 3.03 g in Somborka variety and average value was 2.63 g (table 2).

Generally, in all studied wheat varieties in first year expressed in average higher value of grain mass spike^{-1} than in second year. This indicates response of genotypes to environmental conditions. The obtained results showed significant differences in the average values of grain mass spike^{-1} and mass of spike per year, that indicating diversity of studied varieties (table 2).

In other studies the significantly lower of grain mass spike^{-1} were obtained, in a sole crop of 1.18 g while in intercrop (wheat/pea) 1.19 g in winter wheat grown on chernozem type soil (Grčak et al., 2019a) as well as in spring wheat grain mass spike^{-1} in a sole crop was 1.02 g and in intercrop (wheat/pea) 0.87 g (Grčak et al., 2019b).

The mass of spike varied in the range from 3.33 g in Morava to 4.07 g in Somborka variety with average value 3.75 g in first year of experiment. The values of mass of spike in second year of experimental vegetation season variate from 2.79 g in Kosmajka to 3.64 g in Somborka variety and average value was 3.29 g (table 2). Generally, the all tested varieties had higher mass of spike in the first year than in second year of experiment. The mass of spike were significantly different among the varieties and between the years of experiment. (table 2).

In other investigation of ten genotypes in two vegetative seasons were found significant differences in wheat varieties for the mass of spike which varied in range from 3.44 g and 4.12 g in first vegetation season and in the range from 3.28 g to 4.8 g in second year (Knežević et al. 2011). Similar values of mass of spike established in ratio 3.30 g – 4.49 g with average of 3.97 g in the first year and 3.20 g – 3.92 g with average 3.61g in the second year of investigation, which depending of variety and environment (Knežević et al., 2019). The significantly lower average values of mass of spike were obtained in research of winter wheat variety on the chernozem soil type, in a sole crop of 1.56 g while in intercrop (wheat/pea) 1.6 g (Grčak et al., 2019a). Also on chernozem in spring wheat varieties obtained in average similar values of mass of spike in sole crop 1.41 g an in intercrop (wheat/pea) 1.23 g (Grčak et al., 2019b). The obtained differences are due to differences of studied genotype (wheat variety) and environmental condition (soil, weather conditions, nutrition).

In these studies, it was found that some varieties, although they have a higher grain mass spike^{-1}, had a lower spike harvest index. For example in Danica found grain mass spike^{-1} 3.22 g, mass of spike 4.01 g and spike harvest index was 0.804 in the first year, on the other hand in Kosmajka grain mass spike^{-1} 2.85 g, mass of spike 3.53 g and spike harvest index was 0.806. In the second year, in Danica variety, the grain mass spike^{-1} was 2.86 g, the mass of spike was 3.57 g and the spike harvest index was 0.801, while in the Kosmajka variety the grain mass spike^{-1} was the lowest 2.23 g, the mass of spike was the lowest 2.79 and the value of the spike harvest index was 0.801, the same as in Danica variety. This indicates that the mass of chaff and rachis was higher in the variety Danica, i.e. that a larger amount of assimilate deposited in the chaff and rachis and was not fully translocated into grain.

Somborka variety in first year of experiment had the highest values of grain mass spike^{-1} (3.22g) and the highest mass of spike (4.07 g) but values of spike harvest index 0.79 it was not the largest, but it was smaller than in Morava, which in the first year had the lowest grain mass spike^{-1} of 2.66 g, the lowest mass of spike 3.33 g and the high value of the spike harvest index 0.799. Generally, in average all studied wheat varieties in first year expressed higher values of grain mass spike^{-1} as well mass of spike than in the second year. However, spike harvest index was higher in the first year in two variety (Fortuna 81.8% and Morava-79.9%) than in the second year (Fortuna 78.7% and Morava-78.5%).
These data indicate different efficiency of uptake, utilization of water and minerals and different efficiency of photosynthesis and translocation of photosynthesized organic matter into grain. Also, the obtained values of the spike harvest index indicate that they are not a reliable criterion for predicting higher values of grain mass spike^{-1} and grain yield, In early studies showed that correlation between spike HI, estimated on the basis 10 spike sample and plot harvest index, was low and statistically nonsignificant. Based on this study, one could not recommend using spike HI as a predictor of plot harvest index (Hucl and Graf, 1992).
For achieving high mass of grain spike^{-1} and grain yield of wheat the significant role has efficiency of photosynthesis related to efficiency of water absorption and plant nitrogen absorption, utilization and translocation into grain in highly favorable environmental conditions. Flag leaf area and green leaf duration have impact in grain filling and in relation to spike forming of grain number and potential size of grains significantly contribute to grain mass. Genetic and physiological characterization of wheat variety can help in creation new high yielding variety by crosses in terms of complementary traits and alleles (Reynolds and Langridge, 2016).

Table 3. Components of variance for spike harvest index, grain mass spike^{-1} and mass of spike

	Spike harvest index						Grain mas spike^{-1}					Mass of spike				
Source of variance	DF	SS	MS	F	Components of variance		SS	MS	F							
					σ^2	%				σ^2	%				σ^2	%
Repetitions	2	30.121	15.060	3.5698*	-	-	0.206	0.103	2.7149^{ns}	-	-	0.184	0.092	1.8222^{ns}	-	-
Variety	9	168.349	18.705	4.4337**	1,868	25,63	2.609	0.290	7.6285**	0,039	29,32	3.492	0.388	7.6722**	0,052	23,85
Year	1	10.753	10.753	2.5487^{ns}	0,109	1,50	1.588	1.588	41.7796**	0,051	38,35	3.234	3.234	63.9492**	0,106	48,63
Variety x year	9	67.464	7.496	1.7768^{ns}	1,092	14,98	0.490	0.054	1.4317^{ns}	0,005	3,76	0.699	0.078	1.5354^{ns}	0,009	4,13
Error	38	160.319	4.219	-	4,219	57,89	1.444	0.038	-	0,038	28,57	1.922	0.051	-	0,051	23,39
Total	59	437.006	-	-	7.288	100,0	6.337	-	-	0.133	100,0	9.531	-	-	0.218	100,0

The analysis of the components of variance showed that genotype had the greatest influence (25.63%) on expression of spike harvest index, while the share of environment was 1.50% and the share of interaction G/E was 14.98% (table 3). Different results reported Petrović et al. (2002) which found higher impact of environment. The highest impact on grain mass spike^{-1} had environmental condition (38.35%) and higher than genotype (29.32%), while the least impact had interaction G/E (3.76%). Analysis of component of variance showed that environment had the highest impact (48.63%) on expression of mass of spike and higher than impact of genotype (23.85%) while the impact of interaction G/E (4.13%) was the least (Table 3). The environmental factors as well temperature, 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).

Conclusion

In this study were established that the mass of spikes, grain mass and harvest index of spike are different between varieties and between years of experiment. The highest values of spike harvest index (83.1%) in Somborka variety expressed in the second experimental year, while the least (75.7%) in wheat Kosmajka in the first experimental year. The genotype had the greatest influence (25.63%) on expression of spike harvest index. The highest values of grain mass spike^{-1}, (3.22 g) in Danica and Somborka variety expressed in the first experimental year, while the least (2.23 g) in wheat Kosmajka in second experimental year. The highest values of mass of spike (4.07 g) in Somborka variety expressed in the first experimental year, while the least (2.79 g) in wheat Kosmajka in second experimental year.

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References

Dimitrijević M., Petrović S., Kraljević-Balalić M., Panković L. (2006): Genotype/external environment of grain mass per plant and spike index interaction in *Triticum sp*. Proceedings, 42: 227-236. Iinstitutes for field and vegetable crops, Novi Sad

Dodig, D., Zoric, M., Knezevic, D., King, S.R., Surlan-Momirovic, G. (2008): Genotype x environment interaction for wheat yield in different drought stress conditions and agronomic traits suitable for selection. *Australian J.of Agric.Res.*, 59, 536-545

Grčak, M., Grčak, D., Jevtić, R., Lalošević, M., Kondić, D., Živić, J., Knežević, D. (2019a): Variation of spike index of cereal and index pea pods in monocrops and intercrops system of cultivation. Genetika, 51 (3): 1185-1196.

Grčak, M., Grčak, D., Županski, V., Jevtić, R., Lalošević, M., Radosavac, A., Kondić, D., Živić, J., Paunović, A., Zećević, V., Mićanović, D., Knežević, D. (2019b): Effect of cereals + pea intercropping on spike index of spring wheat, triticale, oat and pods index of pea. Acta Agriculturae Serbica, XXIV:48):167-180.

Hucl, P. and Graf, R. J. (1992): Variation in spike harvest index among diverse genotypes of spring wheat and triticale. Can. J. Plant Sci. 72: 257 -261.

Knežević, D., Kovačević, V., Paunović, A., Madić, M., Đukić, N., Tanasković, S., Jelić, M. (2011): Variability of spike mass in wheat *Triticum aestivum* L. Proceedings 16(18):47-53. XVI Conference on Biotechnology with international participation, Čačak, 4-5. March 2011, University of Kragujevac, Faculty of Agriculture,

Knezevic, D., Zečević, V., Kondic, D., Markovic, S., Šekularac, A.. (2014): Genetic and phenotypic variability of grain mass per spike in wheat under different dose of nitrogen nutrition. Turkish journal of agricultural and natural sciences, vol., pp. 805-810

Knezevic, D., Kondic, D., Srdić, S., Paunović, A., Madić, M. (2015a): Harvest index and components of yield in winter wheat cultivars (*Triticum aestivum* L.). Proceeding of *Sixth International Scientific Symposium "Agrosym Jahorina 2015"*. (Editor in Chief: Dusan Kovacevic) October 15-18 2015, Jahorina Bosnia and Herzegovina), pp.368-372.

Knežević, D., Radosavac, A., Zelenika, M. (2015b): Variability of grain weight per spike of wheat grown in different ecological conditions. Acta Agriculturae Serbica, 39: 85-95.

Knežević, D., Paunović, A., Zečević, V., Urošević, D., Kondić, D., Mićanović, D., Živić, J., Madić, M., Djurović, V., Srdić, S., Kovačević, V. (2019): Variability of mass of spike in winter wheat (*Triticum aestivum* L.). Proceedings 24 (26): 37-44. XXIV Conference on Biotechnology with international participation, Čačak, 15-16. March 2019, University of Kragujevac, Faculty of Agriculture,

Marijanović, M., Markulj, A., Tkalec, M., Jozić, A., Kovačević, V. (2010): Impact of precipitation and temperature on wheat (*Triticum aestivum* L.) yields in Eastern Croatia. *Acta Agriculturae Serbica*, 30: 117-123.

Moeler, C., Rebetzke, G. (2017): Performance of spring wheat lines near isogenic for the reduced-tillering 'tin' trait across a wide range of water-stress environment-types. Field crops research, 200:98-113.

Petrović S., Dimitrijević M., Kraljević-Balalić M., Mladenov, L. (2002): Stability of spike index of divergent wheat genotypes. Proceedings, 36:251-261. Institutes for field and vegetable growing, Novi Sad

Petrović, S., Marić, S., Guberac V., Drezner G., Eđed A. (2008): Influence of environmental conditions and sowing rates on winter wheat yield. Cer.Res.Comm., 36:1307-1310

Reynolds, M,, Langridge, P. (2016): Physiological breeding. Curr Opin Plant Biol 31:162–171.

Rebetzke, G.J., Ellis, M.H., Bonnett, D.G., Condon, A.G., Falk, D., Richards, R.A.(2011): The Rht13 dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. Field Crop Res., 124: 323–331.

Zečević, V., Knežević, D., Kraljević-Balalić, M., Mićanović, D. (2004): Genetic and phenotypic variability of yield components in wheat (*Triticum aestivum* L.). Genetika, 36(2): 151-159

THE GRAIN YIELD STABILITY ANALYSIS OF THE ZP COMMERCIAL MAIZE HYBRIDS BASED ON MULTI-ENVIRONMENTAL TESTING

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Abstract

The Maize Research Institute, Zemun Polje (MRIZP) has been working intensively on maize breeding for 75 years and today occupies an important position in maize seed market in Serbia and region. Some of the main characteristics of the crop production in Serbia are many different environmental conditions (soil, weather) and farmers with very different crop management practices. Considering that genotype x environment interaction plays an important role in grain yield performance of maize, testing in a wide range of environments is necessary for precise evaluation of maize cultivars. Every year, the Marketing Department of the MRIZP conducts multi-environmental testing of the bestselling and the new promising commercial maize hybrids (so-called ZP hybrids) and uses these results for recommendations to farmers. The main goal of this research was to evaluate the grain yield and its stability of the ZP commercial maize hybrids and based on these results to determine which hybrid could accomplish the best its potential in which growing conditions. Seven maize hybrids (ZP 427, ZP 555, ZP 560, ZP 600, ZP 606, ZP 666 and ZP 707) of different maturity groups (FAO 400 – FAO 700) were grown at 99 locations in Serbia during three years (2018, 2019 and 2020). Based on the grain yield data, the stability analysis was performed using linear regression model proposed by Eberhart and Russel (1966). The mean grain yield ranged from 9.823 t/ha to 10.691 t/ha in the hybrids ZP 427 and ZP 606, respectively. The values of regression coefficient (b_i) differed between hybrids and ranged from 0.845 in the hybrid ZP 427 to 1.0951 in hybrid ZP 666. The results were compared, and the adaptation of the evaluated hybrids to different environments and growing conditions were discussed. Based on the results certain conclusions were made.

Key words: *maize hybrids, grain yield, genotype x environment interaction, multi-environmental testing, grain yield stability*.

Introduction

The Maize Research Institute, Zemun Polje (MRIZP) has been working intensively on maize breeding for 75 years and today occupies an important position in maize seed market in Serbia and region. Maize is the most important crop in Serbia where it is grown on approximately million hectares every year (FAOSTAT, 2021). The economic significance of the maize for Serbia is reflected in the fact that maize had a share of the gross national income of Serbia between 6 and 6.8% in the period from 2015 to 2017 (http://www.minpolj.gov.rs/), and that it regularly represents one of the most important export items of Serbia. According to the report of the Association for the Improvement of Production and Export of Oilseeds and Cereals from 2017, when it comes to the produced quantities of maize grain, Serbia is in the 6th place in Europe behind Ukraine, France, Russia, Romania and Hungary, while it is the 8th largest exporter of maize grain in the world (https://www.zitasrbije.rs/download/Izvestaj_Jun_2017-99.pdf).

Some of the main characteristics of the crop production in Serbia are many different environmental conditions (soil, weather) and farmers with very different crop management practices. It is well known that maize cultivars often very differ in their performance across environments (Kang et al., 1989; Crossa et al., 1999; Signor et al., 2001; Admassu et al., 2008; Kandus et al., 2010; Anley et al., 2013; Choukan, 2013; Ndhlela et al., 2014), for example genotypes that are the best in certain locations in some other locations are bad and vice versa. Considering that genotype x environment interaction plays an important role in grain yield performance of maize, testing in a wide range of environments is necessary for precise evaluation of maize cultivars. In order to establish the stability of the genotype performance on the basis of big data from many environments, stability analysis was designed. Many researchers proposed various different statistical methods for identification of the stable genotypes which are based on linear regression (Yates and Cochran, 1938; Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968).

Stability analysis aims to examine reaction of genotypes to different environments (Bernardo, 2020) which is very important in commercial breeding programs. It can provide information of the pattern of genotype performance in the different groups of environments in which farmers, breeders, seed producers and distributors are interested. For example, stability analysis aims to identify cultivars best suited to specific environment so that the production in that environment is maximized (Bernardo, 2020).

The main task of maize breeders is to develop cultivars with the best phenotype in the different growing conditions i.e. cultivars with good performance and stable. Both maize breeders and farmers want maize cultivars which express low genotype x environment interaction (Babić et al., 2010). For that reason, maize breeders and breeding companies are looking for genotypes, not only with high average grain yield, but with known yield stability. Farmers with very good crop practices show interest for genotypes which give the best results in the best growing conditions, and on the other hand farmers in poor environments who don't have a high level of crop practise want cultivars which give satisfactory results in bad or limited growing conditions. Based on all this, it is concluded that stability analysis of the maize cultivars can help all participants in the maize production, such as breeders, companies, seed producers and distributors, and what is the most important it can help farmers to achieve the best possible results with the offered assortment of maize cultivars at the market.

Every year, the Marketing Department of the MRIZP conducts multi-environmental testing of the bestselling and the new promising commercial maize hybrids (so-called ZP hybrids) and uses these results for recommendations to farmers. The main goal of this research was to evaluate the grain yield and the grain yield stability of the ZP commercial maize hybrids and based on these results to determine which hybrid can reach the best its potential in which growing conditions. Extensive stability analysis takes many locations and years and therefore the multi-environmental testing approach was selected.

Material and Methods

Seven commercial ZP maize hybrids (ZP 427, ZP 555, ZP 560, ZP 600, ZP 606, ZP 666 and ZP 707) of different maturity groups (FAO 400 – FAO 700) were grown at 99 locations in Serbia during three years (2018, 2019 and 2020). Plot area was 1000 m^2 and sowing density for each hybrid was the one generally recommended (Table 1). Standard crop practise of maize production was applied and harvesting was done mechanically. Based on the grain yield data, the stability analysis was performed using linear regression model proposed by Eberhart and Russel (1966). Data processing were done in Microsoft Excel and stability analysis were done in software GEA-R (Pacheco et al., 2015).

Table 1. Tested maize hybrids, their maturity groups and sowing densities

Maize hybrid	Maturity group	Sowing density (seeds/ha)
ZP 427	FAO 400	73500
ZP 555	FAO 500	68000
ZP 560	FAO 500	68000
ZP 600	FAO 600	63000
ZP 606	FAO 600	63000
ZP 666	FAO 600	63000
ZP 707	FAO 700	63000

The linear model proposed by Eberhart and Russel (1966) is:

$P_{ij}= (\mu + g_i) + b_i t_j + \delta_{ij} + e_{ij}$,

where P_{ij} is the mean of genotype I in environment j; $\mu + g_i$ is the mean of genotype i across all environments; b_i is the linear regresssion coefficient of P_{ij} on t_j; t_j, which serves as the environmental index, is the effect of environment j; δ_{ij} is the deviation of P_{ij} from the regression-fitted value of genotype i in environment j; and e_{ij}, is within-environment error, averaged across replications, associated with genotype i in environment j (Bernardo, 2020).

The b_i measures the change in the mean performance of a particular genotype per unit change in the mean of an environment (Bernardo, 2020). This measure of stability is estimated for each genotype as: $b_i = \Sigma_j P_{ij} t_j / \Sigma t_j^2$

The value of the regression coefficient (b_i) indicates genotype response to different environments and growing conditions as follows (in case that higher values of the trait are desired):

1. $b_i = 0$ indicates that the performance of a genotype is constant across environments;
2. $b_i = 1$ indicates that the response of a genotype to different environments is the same as the mean response of all other genotypes in the experiment;
3. $b_i > 1$ have genotypes which are well adapted to favorable environments and bad adapted to unfavourable environment (better than average response to good growing conditions and worse than average response to bad conditions);
4. $b_i < 1$ have genotypes which are adaptable to poor environments and bad adapted to good environments (better than average response to unfavorable environments and worse than average response to good environments).

Results and Discussion

The results showed that commercial ZP maize hybrids differed in the grain yield and the grain yield stability (Table 2). The highest average grain yield was recorded in the hybrid ZP 606 (10.691 t/ha), which is the best-selling hybrid of the Maize Research Institute, Zemun Polje. This hybrid also exhibited good response to favorable environments and to good growing conditions, according to the value of b_i above 1 (1.0782). Similar result for this hybrid was found by Crevar et al. (2011). The lowest average grain yield was recorded in the earliest hybrid (FAO 400) in this study, ZP 427 (9.823 t/ha). However, hybrid ZP 427 expressed a very good response to unfavorable environments considering very low value of b_i regression coefficient (0.8450), which is in accordance with results of Crevar et al. (2011) and Pavlov et al. (2011) who also found better adaptation of early maturity hybrids to poor environments.

Table 2. The average grain yield and b_i coefficient of tested maize hybrids

Maize hybrid	Average grain yield (t/ha)	b_i coefficient
ZP 427	9.823	0.8450
ZP 555	10.165	1.0033
ZP 560	10.250	0.9993
ZP 600	10.357	0.9577
ZP 606	10.691	1.0782
ZP 666	10.345	1.0951
ZP 707	10.567	1.0215

Hybrids with the most stable grain yield performance were ZP 555 and ZP 560 with regression coefficient values near 1 (1.0033 and 0.9993, respectively). The best adaptation to favorable environments was recorded in the hybrid ZP 666 (b_i = 1.0951), which is in accordance with results obtained by Crevar et al. (2011). Hybrids ZP 600 and ZP 707, third and second in grain yield in this research (10.357 and 10.567, respectively), expressed medium adaptation to poor environments (b_i = 0.9577) and good environments (b_i = 1.0215), respectively.

Conclusions

Based on the results of the grain yield and the grain yield stability obtained from multi-environmental testing, growing conditions in which certain maize hybrids can reach their potential were recognized. Hybrid ZP 606 as the most yielding and well adapted to favorable environments was recommended mostly for good growing conditions and good crop management practices. For the best results in the best growing conditions and the best environments hybrid ZP 666 is proposed. Hybrid ZP 427 is recommended only for poor growing conditions considering its best adaptation to unfavorable environments and the lowest average grain yield of all evaluated hybrids. Hybrids such as ZP 427 are not expected to disappoint farmers even in the worst environments and years. Hybrids ZP 555 and ZP 560, as the most stable hybrids in this research, represent hybrids which will meet expectations of farmers in any environment and year. Hybrids ZP 600 and ZP 707, as high yielding and relatively stable hybrids, are suitable for achieving high performance under different conditions.

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References

Admassu S., Nigussie M., Zelleke H. (2008). Genotype-environment interaction and stability analysis for grain yield of maize (Zea mays L.) in Ethiopia, Asian Journal of Plant Sciences, 7(2), (163-169)

Anley W., Zeleke H., Dessalegn Y. (2013). Genotype X environment interaction of maize (Zea mays L.) across North Western Ethiopia, Journal of Plant Breeding and Crop Science, 5(9), (171-181)

Babić V., Babić M., Ivanović M., Kraljević-Balalić M., Dimitrijević, M. (2010). Understanding and utilization of genotype-by-environment interaction in maize breeding, Genetika, 42(1), 79-90.

Bernardo R. (2019). Breeding for Quantitative Traits in Plants, MN: Stemma press, Woodbury.

Choukan R. (2011). Genotype, environment and genotype× environment interaction effects on the performance of maize (Zea mays L.) inbred lines, Crop Breeding Journal, 1(2), (97-103)

Crevar M., Vančetović J., Pavlov M., Stevanović M., Čamdžija Z., Božinović S., Prodanović S. (2011). Yield stability of new ZP maize hybrids, Selekcija i semenarstvo, 17(2), (49-55)

Crossa J., Vargas M., Van Eeuwijk F. A., Jiang C., Edmeades G. O., Hoisington D. (1999). Interpreting genotype× environment interaction in tropical maize using linked molecular markers and environmental covariables, Theoretical and applied genetics, 99(3), (611-625)

https://www.zitasrbije.rs/download/Izvestaj_Jun_2017-99.pdf Accessed on 06/06/2021

FAOSTAT (2021). Food and Agriculture Organization of the United Nations (*FAO*), *FAOSTAT* Database (website: http://www.fao.org/faostat/en/ Accessed on 06/06/2021)

Finlay K. W., Wilkinson G. N. (1963). The analysis of adaptation in a plant-breeding programme, Australian journal of agricultural research, 14(6), (742-754)

Eberhart S. T., Russell W. A. (1966). Stability parameters for comparing varieties 1, Crop science, 6(1), (36-40)

Kandus M., Almorza D., Boggio Ronceros R., Salerno J. C. (2010). Statistical models for evaluating the genotype-environment interaction in maize (Zea mays L.), Phyton-Revista Internacional de Botanica Experimental, 79, (39-46)

Kang M. S., Gorman D. P. (1989). Genotype× environment interaction in maize, Agronomy Journal, 81(4), (662-664)

Ndhlela, T., Herselman, L., Magorokosho, C., Setimela, P., Mutimaamba, C., & Labuschagne, M. (2014). Genotype× environment interaction of maize grain yield using AMMI biplots, Crop Science, 54(5), (1992-1999)

Pacheco A., Vargas M., Alvarado G., Rodríguez F., López M., Crossa J., Burgueño J. (2015). User's Manual GEA-R (Genotype by Environment Analysis with R), GEA-R *(*Genotype x Environment Analysis with R for Windows) Version, 4.

Pavlov J., Delić N., Stevanović M., Čamdžija Z., Grčić N., Crevar M. (2011). Grain yield of ZP maize hybrids in the maize growing areas in Serbia, In Proceedings - 46th Croatian and 6th International Symposium on Agriculture, Opatija, Croatia, (395-398).

Perkins J. M., Jinks J. L. (1968). Environmental and genotype-environmental components of variability, Heredity, 23(3), (339-356)

Signor C. E. L., Dousse S., Lorgeou J., Denis J. B., Bonhomme R., Carolo P., Charcosset A. (2001). Interpretation of genotype× environment interactions for early maize hybrids over 12 years, Crop Science, 41(3), (663-669)

Yates F., Cochran W. G. (1938). The analysis of groups of experiments, The Journal of Agricultural Science, 28(4), (556-580)

INFLUENCE OF THE SIZE OF VEGETATION SPACE ON THE QUALITY OF TOMATO SEEDLINGS

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable species, which is result of its high nutritional value and the area on which it is grown. Tomatoes are mainly produced from seedlings and the quality of seedlings is one of the basic elements of achieving high yields. The quality of seedlings largely depends on the vegetation space, ie the size of the cells in the containers in which they are produced. Thus, the aim of this study was to examine the influence of vegetation space size on the quality of tomato seedlings grown in containers with different cell volumes. The study used a cv. Big Beef F1 which seedlings were produced in containers with 24, 40, 60 and 104 cells. As indicators of growth and quality of tomato seedlings the following parameters were analyzed: height and diameter of hypocotyl, length and width of cotyledons, number of leaves, length and width of leaves, height and diameter of stem and mass of whole plant, aboveground part and roots. The dynamics of seedling growth was directly dependent on the size of the vegetation space. The highest quality seedlings were obtained in containers with 24 cells, in which 4 leaves were formed, the height of the plant was 36.117 cm, and the weight of the whole plant was 14.24 g.

Keywords: *Tomato, Vegetation space, Seedling quality, Growth dynamic*.

Introduction

Tomatoes are produced in the open field as an early, medium early and late culture and in all forms of greenhouses. Production can be directly from seeds (field, industrial) and from seedlings (field and in a greenhouse). The combination of both productions enables the supply of fresh tomatoes to the market throughout the year (Kumar and Singh, 2015).

Tomatoes in our country, are mainly produced from seedlings. Seedling is a young plant with 4-10 well-developed leaves and in the 4th or 5th phase of organogenesis, well-developed root system, firm, elastic and compact stem with high dry matter content, appropriate age depending on the time and method of production (Đurovka *et al*., 2006). The quality of seedlings according to Murtić *et al.*, 2018 is one of the most important items in tomato production. Several researchers state that yield, especially early yield and number of fruits per plant depends on seedling quality (Jankauskiene *et al*., 2013). Seedling quality is affected by a number of factors. The factors can significantly affects the morphological appearance even if they are minimall (what is visible to the eye - the development of the root system, height, thickness and elasticity of the stem, number and arrangement of leaves) and seedling quality (flow of individual stages of organogenesis of generative organs) (Todorović *et al*., 2015; Zeljković *et al*., 2017). Therefore, the management and maximum control of critical points in the production of seedlings ensures high quality and economy of seedling production, as well as vegetable production as a whole.

The purpose of vegetable production through seedlings is to shorten the time required for fruiting, save energy, better and more economical use of protected space (Todorović *et al*., 2019). In our country, seedlings are mostly produced in containers or pots. Container seedlings are mainly used for the production of seedlings in the open field. Very often, due to the economic effect and obtaining a larger number of plants per unit area, used containers do not provide enough space for quality growth of the tomato root system, which can be a limiting factor for obtaining quality seedlings (Lin *et al*., 2015). For these reasons, the aim of this study was to determine the influence of vegetation space size, ie the number of cells in the container, on seedling quality and tomato growth in the nursery period, where the length and width of cotyledons, height and width were measured as growth factors, as well as hypocotyl diameter, height and diameter of the stem, and the dynamics of leaf formation, number and size of leaves, as well as the mass of the seedling plant.

Material and Methods

The tests were performed on a tomato, a cv. Big Beef F1 (Monsanto Holland BV). Big Beef F1 is an early indeterminate beef-type tomato. The fruits are large, oval red in color, with excellent taste and medium firmness. Styrofoam containers with different number of cells were used: K1-24 cells (diameter 6.80 cm), K2-40 cells (diameter 4.30 cm), K3-60 cells (diameter 4.10 cm) and K4-104 cells (diameter 3.05cm). In each variant, 40 plants were analyzed. The experiment was set up in a greenhouse of 30 m^2 without additional heating with additional tunnels in the village of Jelicka, Prijedor municipality (N 44 ° 49′31.04 ″; E 16 ° 55′9.52 ″; altitude: 223 m) in Bosnia and Herzegovina. The research was conducted from April 2 to June 2, 2016. During the experiment, tomato seedlings were fed with water-soluble fertilizer Kristalon "Yara" formulation 13 + 40 + 13 + micro, as well as other care measures. The dynamics of growth and development of tomatoes was followed by the BBCH monograph (Meier, 2001). Eight days after emergence (BBCH_1: 10), the length and width of fully formed cotyledons and the height and diameter of the hypocotyl were measured in all four variants. Then, 9 days after complete formation of cotyledons, the length and width of the first leaf, diameter and height of the plant were measured in containers with 24 and 40 cells (BBCH_1: 11), and in containers with 60 and 104 cells, measurements were performed 12 days after complete formation of cotyledons. Measurement of the second leaf in a 24-cell container was performed 12 days after the appearance of the first leaf (BBCH_1: 12), as well as the height and diameter of the plant. Measurement of the second leaf in containers with 40 and 60 cells was performed 13 days after the formation of the first leaf, and in containers with 104 cells was performed 14 days after the formation of the first leaf. Measurement of plants in the third leaf phase (BBCH_1: 13) in a 24-cell container was performed 9 days after the formation of the second leaf, in a 40-cell container the measurement was performed after 10 days, and in a 60-cell container plants were measured after 12 days of forming another leaf. When measured in 104 cells containers the third leaf was not formed. The fourth leaf in a 24-cell container was measured eight days after the formation of the third leaf (BBCH_1: 14). At the time of transplanting, ie at the time of measurement, buds were formed in plants in a 24-cell container (BBCH_5: 51). Analysis during vegetation and at the end of the seedling production cycle included: length and width of cotyledons (cm), diameter and height of hypocotyl (cm), number of leaves, length and width of leaves (cm), stem diameter (cm), plant height (cm), the mass of the whole plant (g), the mass of the green part (g) and the mass of the root (g). Measurements of plant dimensions were done with a ruler and caliper MAUSER INOX, while the measurement of the mass of the whole plant, green part and roots was done on a digital scale AE ADL 600 in the laboratory at the Faculty of Agriculture, University of Banja Luka. Statistical analysis of morphometric data was done through analysis of variance and t-test.

Results and Discussion

Germination, ie emergence of cotyledons above the soil's surface (BBCH_0: 09) was determined in all four variants at the same time or 10 days after sowing. Measurement of cotyledons and hypocotyls was done in the phase of complete opening and occupying the vertical position of cotyledons (BBCH_1: 10).

Table 1. Characteristics of cotyledons

Variant	Cotyledons			
	Length (cm)		Diameter (cm)	
	$\bar{X} \pm S\bar{x}$	*Vk*	$\bar{X} \pm S\bar{x}$	*Vk*
K1-24	2.993±0.06	12.894	0.696±0.01	11.077
K2-40	2.764±0.05	12.907	0.641±0.01	11.292
K3-60	2.864±0.07	16.092	0.642±0.01	14.161
K4-104	2.850±0.05	11.596	0.685±0.01	9.475

Based on the results, it can be determined that there was no large variation in the length and width of the cotyledon depending on the vegetation space (Table 1). However, it should be noted that the largest cotyledons were in 24-cell containers, which averaged 2.993 cm long and 0.696 cm wide, while the smallest were in 40-cell containers, where they averaged 2.764 cm long and 0.641 cm wide.

Table 2. Characteristics of hypocotyls

Variant	Hypocotyl			
	Length (cm)		Diameter (cm)	
	$\bar{X} \pm S\bar{x}$	*Vk*	$\bar{X} \pm S\bar{x}$	*Vk*
K1-24	2.620±0.05	12.378	0.153±0.002	9.833
K2-40	2.402±0.03	9.996	0.134±0.002	11.757
K3-60	2.430±0.05	13.696	0.133±0.002	10.511
K4-104	2.461±0.04	11.215	0.124±0.002	12.169

The height and diameter of the hypocotyl in all variants were approximately the same, ie there were no significant differences. It must be emphasized that the plants from the container with the smallest number of cells (K1-24) had both the largest length (2.62 cm) and the diameter of the hypocotyl (0.153 mm).

Table 3. Plant height and seedling stem diameter (seedling age 60 days)

Variant	Plant height (cm)		*t-test*	Stem diameter (cm)		*t-test*
	$\bar{X} \pm S\bar{x}$	*Vk*		$\bar{X} \pm S\bar{x}$	*Vk*	
K1-24	36.117±0.374	6.483	K1:K2 20.925** K1:K3 30.228** K1:K4 59.529** K2:K3 11.324** K2:K4 39.066** K3:K4 17.455**	0.511±0.004	5.077	K1:K2 28.119** K1:K3 31.703** K1:K4 66.411** K2:K3 10.632** K2:K4 43.266** K3:K4 17.455**
K2-40	25.817±0.320	7.760		0.370±0.003	6.629	
K3-60	20.320±0.365	11.222		0.308±0.005	10.344	
K4-104	11.661±0.170	9.157		0.214±0.002	8.105	

The tallest and thickest seedlings were produced in 24-cell containers, with a plant height of 36.117 cm and a stem diameter of 0.511 cm. At the same time, the smallest seedlings were in 104-cell containers, with plant height of 11.661 cm and stem diameter of 0.214 cm. By testing the level of significance of differences in the height and diameter of the stem depending on the size of the vegetation space, ie the size and number of cells in the container, it was determined that there is a statistically highly significant difference between the examined variants.

Similar results were obtained by Liptay and Hoffmann (1988) who found that in containers with a larger cell volume (over 16 mm^3) seedlings of better quality were obtained and that its height did not vary significantly with a further increase in substrate volume. Also, the same law applies when it comes to the thickness of the seedling stem. As Weston and Zandstra (1986) found, the height of tomato seedlings varies from 10.1 to 26.8 cm depending on the size of the cells in the containers. Jankauskiene *et al*. (2013) came to the results that show that with increasing number of leaves, the height of the plant also increases.

Table 4. Dynamics of leaf formation

Variant	Days between the phases			
	BBCH_1:11	BBCH_1:12	BBCH_1:13	BBCH_1:14
K1-24	27	12	9	8
K2-40	27	13	10	
K3-60	30	13	12	
K4-104	30	14		

Based on the results of examining the influence of vegetation size on the quality of tomato seedlings, ie the number of leaves, it was determined that variant K1-24 showed the best where 4 leaves were formed, while variant K4-104 showed the worst where only 2 leaves were formed. In their research, Ilin *et al*. (2003) came to similar results, where they found that with increasing substrate volume in containers and pots, the formation of plants with a larger number of leaves occurs. Shopova and Haytova (2015) in their research found that with increasing size of vegetation space by reducing the number of cells in the container, the number of leaves increases.

Table 5. Leaf length and width (seedling age 60 days)

Variant	Leaf length (cm)		*t-test*	Leaf width (cm)		*t-test*
	$\bar{X} \pm S\bar{x}$	*Vk*		$\bar{X} \pm S\bar{x}$	*Vk*	
K1-24	13.492±0.145	6.731	K1:K2 20.650** K1:K3 25.890** K1:K4 44.847** K2:K3 8.854** K2:K4 23.757** K3:K4 8.256**	8.592±0.106	7.775	K1:K2 18.917** K1:K3 21.757** K1:K4 28.537** K2:K3 7.847** K2:K4 15.065** K3:K4 5.359**
K2-40	9.952±0.121	7.912		6.189±0.07	7.566	
K3-60	7.766±0.167	13.435		5.092±0.121	14.905	
K4-104	6.271±0.07	7.166		4.212±0.111	16.490	

Based on the results obtained during the monitoring of the increase in leaf length, differences in the examined variants are noticeable. In variant K4-104, the length of the first and second leaves was the same, and did not increase until the end of the nursery period. In other variants, an increase in leaf length was observed. In variants K2-40 and K3-60, the largest difference in leaf length was observed between the first and the second, ie a significantly larger length of the second leaf was observed in relation to the first, in both examined variants. The difference in leaf length between the second and third leaf was smaller. The intensity of growth of seedling leaf length in variant K1-24 was the highest in the period of development between the first and second leaf. The second and third leaves were almost the same length, while the fourth was significantly longer than the third leaf. In addition, it can be seen that the influence of vegetation space on leaf size is significant. Plants had the largest leaves in the variant with the largest vegetation space (K1-24), and the smallest with the smallest vegetation space (K4-104).
Similar tendencies of leaf width growth were observed as with its length. The smallest leaf width was in plants in variant K4-104, where there was no significant difference in width between the first and second leaf. In other tested variants, the largest width was in the second leaf, while the third leaf had a smaller width in relation to the second. In K1-24, in which the fourth leaf is formed, a smaller width is noticeable in relation to the third. It should be noted that the width of the leaves is directly dependent on the size of the vegetation space. Thus, the largest leaf width was in K1-24, while the smallest was in the container with the largest number of cells (K4-104).

Table 6. Plant weight (seedling age 60 days) (g)

Variant	Whole plant mass (g)		
	$\bar{X} \pm S\bar{x}$	*Vk*	*t-test*
K1-24	14.240±0.275	10.416	K1:K2 26.838** K1:K3 33.112** K1:K4 41.819** K2:K3 11.301** K2:K4 28.723** K3:K4 16.192**
K2-40	6.240±0.115	9.994	
K3-60	4.457±0.108	13.145	
K4-104	2.373±0.07	15.960	
	The above-ground part mass (g)		
K1-24	10.741±0.212	10.646	K1:K2 27.223** K1:K3 33.964** K1:K4 42.379** K2:K3 11.842** K2:K4 28.759** K3:K4 16.270**
K2-40	4.471±0.09	11.130	
K3-60	3.045±0.08	14.968	
K4-104	1.510±0.05	18.285	
	The root mass (g)		

K1-24	3.467±0.08	12.892	K1:K2 20.014** K1:K3 24.227** K1:K4 31.784** K2:K3 8.485** K2:K4 25.266** K3:K4 15.281**
K2-40	1.757±0.03	11.334	
K3-60	1.397±0.03	12.715	
K4-104	0.846±0.02	15.692	

The test results showed that the highest plant weight was in variant K1-24 (14.240 g), and the lowest in variant K4-104 (2.373 g). By testing the level of significance, a statistically highly significant difference was found between all examined variants. Ilin *et al*. (2003) came to similar results, they also determined the influence of vegetation space size on plant mass, whereby they obtained the plants of the largest mass in containers and pots that give the largest vegetation space. Jankauskienė *et al*. (2013) found that the most developed seedlings (formed 9–10 leaves) had the highest fresh weight. Oagile *et al*. (2016) found that as the cell size increases, so does the fresh mass of the plant. Fresh weight of 50-day-old seedlings ranged from 0.65 g to 3.6 g depending on the cell size of the container (Liptay and Hoffmann, 1988).
The largest mass of the green part of the plant had a container with 24 cells, which was 10.741 g, and the smallest container with 104 cells, which amounted to 1.510 g. By testing the level of significance, a statistically highly significant influence of the size of the vegetation space on the mass of the green (aboveground) part of the tomato seedling plant was determined.
The highest root mass was in variant K1-24 which was 3.467 g, and the lowest K4-104 which was 0.846 g. By testing the significance level, a statistically highly significant influence of the size of the vegetation space on the root mass of tomato seedling plants was determined. Some of researches came to similar results, who found that the largest mass of roots had seedlings produced in the largest pots and containers with a smaller number of cells, ie seedlings that had the largest volume of substrate for the development of the root system (Ilin *et al*., 2003).

Conclusions

Based on the research of the influence of the size of the vegetation space on the quality of tomato seedlings, it can be concluded that the volume of the container, ie the size of the cells in the container, significantly affects the quality of tomato seedlings. By measuring the length and width of the cotyledons and the height and diameter of the hypocotyl after tomato emergence, no significant differences were found between the examined variants. The variant with the smallest number of cells, ie the largest cell diameter, recorded the largest number of leaves, while the other variants recorded a smaller number of leaves. In all examined variants, it was determined that there is a statistically highly significant difference between them.

References

Đurovka M., Lazić B., Bajkin A., Potkonjak A., Marković V., Ilin Ž., Todorović V. (2006). Production of vegetables and flowers in protected area. Faculty of Agriculture in Novi Sad, Faculty of Agriculture in Banja Luka, p. 501.

Ilin Ž., Mišković A., Vujasinović V. (2003). NUTRITIONAL SUBSTRATE - the basis for successful and quality production of tomato seedlings. Contemporary vegetable garden, no.8.

Jankauskienė J., Aušra B., Česlovas B., Pavelas D. (2013). Effect of transplant growth stage on tomato productivity. Acta Scientiarum Polonorum: Hortorum Cultus 12, No. 2 str. 143-152.

Kumar R., Singh M. (2015). Production technology of Tomato. Improved production Technologies in Vegetable Crops. IIVR training manual, ICAR-Indian Institute of Vegetable Research, Varanasi, India (59), 81-88.

Lin L. J., Luther G. C., Hanson P. (2015). Raising healthy tomato seedlings. AVRDC–The World Vegetable Center publication, 15-795 (Google Scholar).

Liptay A., Hoffmann K. (1988). Extending upper cell density limits of processing tomato tray transplant production in greenhouses. In Symposium on High Technology in Protected Cultivation 230, str. 533-540.

Meier U. (2001). Growth stages of mono-and dicotyledonous plants, BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry.

Murtić S., Oljača R., Koleška I., Karić L., Todorović V. (2018). Response of cherry tomato seedlings to liquid fertilizers application under water stress. Horticultural Science (HortSci) 45 (1), 22-28.

Oagile O., Pitso G., Christinah M., Thembinkosi M. (2016). Effect of container size on the growth and development of tomato seedlings. Int. J. Curr. Microbiol. Appl. Sci 5, No. 4: 890-896.

Shopova N., Hayatova D. (2015). Effect of the Age and Planting Area of Tomato (*Solanum Lycopersicum* L.) Seedlings for Late Field Production on the Plants During the Growing Period, Scientific Papers. Series B, Horticulture. Vol. LIX, Agricultural University, Plovdiv, Bulgaria, str. 259-262.

Todorović V., Zeljković S., Koleška I., Bosančić B., Klokić I. (2015). Efficiency of biostimulants application in the regulation of tomato productivity. Proceedings of 2nd International Symposium for Agriculture and Food, Sedtion5: Vegetable, flower and decorative plants production, 7-9. october 2015., Ohrid, Republic of Macedonia, pp:427-432.

Weston L.A., Zandstra B.H. (1986). Effect of root container size and location of productio on growth and yield of tomato transplants. J.Amer. Soc. Hort. Sci. 111: 498-501.

Zeljković S., Šušak U., Parađiković N., Davidović Gidas J., Tkalec M., Todorović V. (2017). Application of zeolite, as a substrate conditioner, in the production of seedlings of surfinia (*Petunia hybrida* Juss.). Proceedings, 52nd Croatian and 12th International Symposium of Agronomists, Dubrovnik 12-17.02.2017, pp 290-294.

PLANT PROTECTION AND FOOD SAFETY

MICROBIOLOGICAL STATUS OF WATER IN FOOD INDUSTRY OF ANIMAL ORIGIN IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA) IN THE PERIOD 2018-2020 IN RELATION TO THE EXAMINED PARAMETERS

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Abstract

Zero-probability level of microbiological contamination of drinking water does not exist. The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard. The experiment used samples of water from food industry of animal origin from the territory of the Entity of Republic of Srpska (Bosnia & Herzegovina) sampled in the period 2018-2020. A total of 390 samples were examined. The aim of this study is to determine the microbiological status of water used in food industry of animal originin Republic of Srpska, in order to identify the risks to food safety. For microbiological testing of water methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1/A1 were used. Research shows that the microbiological status of water used in food industry of animal origin in Republic of Srpska in the period 2018-2020 differs significantly between individual regions. The analysis revealed a significantly higher number of unsatisfactory samples of well water with presence of intestinal enterococci, coliforms and *E. coli*. Water from water supply system has a better microbiological status, but pathogenic bacteria have also been detected in it, which is of concern because it can affect food contamination during the production process.

Keywords: *water, microbiology, food industry.*

Introduction

The production and distribution of biological stable drinking water can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution (Prest *et al*., 2016). Water supplies within food production premises should be subject to risk and hazard assessment to ensure that appropriate water quality is maintained throughout the production process (Dawson, 1998; Dawson, 2000). In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use (Sasakova *et al*., 2013; Fridrich *et al*., 2014). On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The presence of bacteria in drinking water *per se* is not an issue, as long as no pathogenic organisms are present: there are bacteria in drinking water, even in relatively high numbers (10^3 to 10^6 cells/mL), without consequences on human health (Hoefel *et al*., 2005; Hammes *et al*., 2008). Water temperature is an essential factor influencing bacterial growth kinetics and competition processes (Niquette et al., 2001). The temperature oscillates seasonally, even within a single drinking water distribution system. Elevated water temperatures have often been associated with increased bacterial abundance in drinking water distribution systems (Francisque et al., 2009; Liu et al., 2013). The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard (EU, 2020). *E. coli*, intestinal enterococci, coliform bacteria and colony count on 22°C shall be monitored in accordance with the monitoring frequencies. *E. coli* and intestinal enterococci are considered "core parameters". Minimum requirements for parametric values used to assess the quality of water intended for human consumption is 0/100 ml for *E. coli* and intestinal enterococci. In Republic of Srpska (B&H) the limit value for total count on 22°C (TC 22°C) is 100 CFU/ml and for total count on 37°C (TC 37°C) the limit is 20 CFU/ml. Also, coliform bacteria (CB), *Escherichia coli (E. coli)* and intestinal enterococci (EC) must not be detectable in 100 ml sample of water (Official Gazette RS, 2017). Indicator organisms are used to assess the microbiological quality of water. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated (Edberg, 2000). The use of indicator bacteria, in particular *Escherichia coli* (*E. coli*) and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen *et al*., 2000). The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* bacteria are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin (Edberg, 2000; WHO, 2008) and it can represent up to 95 % of the *Enterobacteriaceae* found in faeces. *E. coli* occurs in the faeces of all mammals, often in high numbers (up to 10^9 per gram of faeces) (Edberg, 2000; WHO, 2008). It is incorrect to state that drinking water distribution and delivery systems should be sterile. The active growth of microorganisms is considered indicative of failures in water processing units or distribution (Gottschal, 1992). Water is used in various ways in milk production and dairy industry, thereby becoming part of the food intentionally, inevitably or accidentally. The contamination of the food by water-borne microorganisms occurs directly, much more often, however, indirectly after multiplication of these microorganisms on the cleaned surfaces of the equipment used (Terplan, 1980). Some studies revealed that wash water can be source of bacterial contamination for milk and further compromise the quality and safety of milk or milk products (Kivaria *et al*., 2006; Perkins *et al*., 2009). The water used during handling and processing of milk products can be potential sources of microbial contamination with possible negative consequences on food safety. Especially, the water used in keeping the hygiene of milking and milk storage utensils is crucial to keep the quality and safety of the products. *E. coli* was isolated in 39.20% of samples of water (Amenu *et al*., 2016). In a study by Habes *et al*. (2015) it has been founded that all water samples used in the production of milk and milk products were microbiologically safe. Kalaba *et al*. (2020) found that in the period 2015-2017 of the 584 water samples, 26.20% were unsatisfactory. The aim of this study is to determine the microbiological status of water used in food industry of animal originin Republic of Srpska, in order to identify the risks to food safety.

Materials and methods

The experiment used samples of water from food industry of animal origin from the territory of the Entity of Republic of Srpska (Bosnia & Herzegovina) sampled in the period 2018-2020. A total of 390 samples were examined (122 in 2018, 127 in 2019 and 141 in 2020). Laboratory testing of samples was performed at the Public Institution Veterinary Institute of Republic of

Srpska "Dr Vaso Butozan" Banja Luka. Microbiological examination was carried out according to the Official Gazette RS (2017). This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C (TC 22°C) and 37°C (TC 37°C) according to BAS EN ISO 6222 (ISBIH, 2003a), coliform bacteria (CB) and *E. coli* according to BAS EN ISO 9308-1/A1 (ISBIH, 2018) and intestinal enterococci (EC) according to BAS EN ISO 7899-2 (ISBIH, 2003b). In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables and figures.

Results and discussion

The territorial and economic organization of the Republic of Srpska (B&H) is conditionally created at the level of six regions: Banja Luka, Prijedor, Doboj, Bijeljina, East Sarajevo and Trebinje. The distribution of water is such that it is not enough where it is most needed (in the northern, most developed part of Republic of Srpska), and flows are most scarce during periods of the year when needs are greatest and when water quality protection problems are most serious. Figures 1 to 4 show the average representation of the samples in relation to the region of origin and the category of samples.

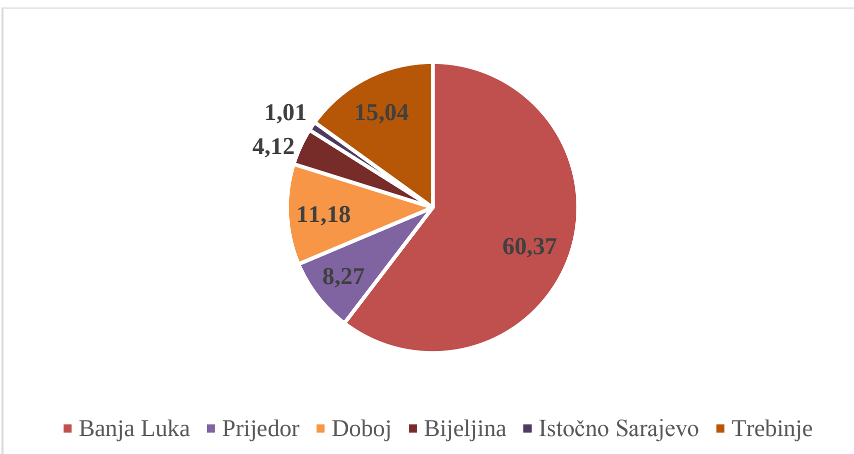

Figure 1. Representation of samples in relation to the region in % for the period 2018-2020

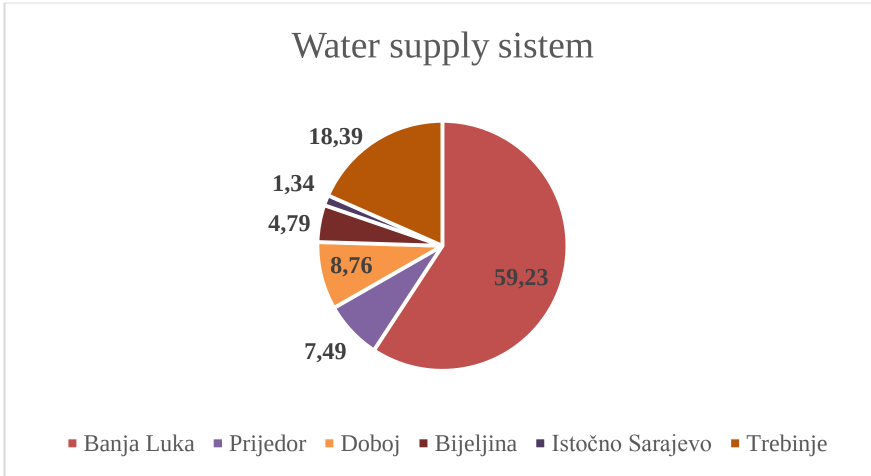

Figure 2. Average regional representation of samples originating from water supply system in % for the period 2018-2020

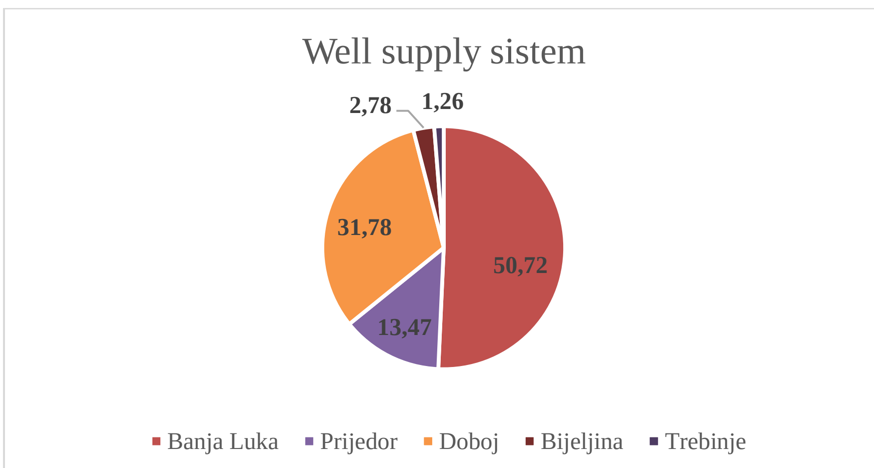

Figure 3. Average regional representation of samples originating from well supply system in % for the period 2018-2020 years.

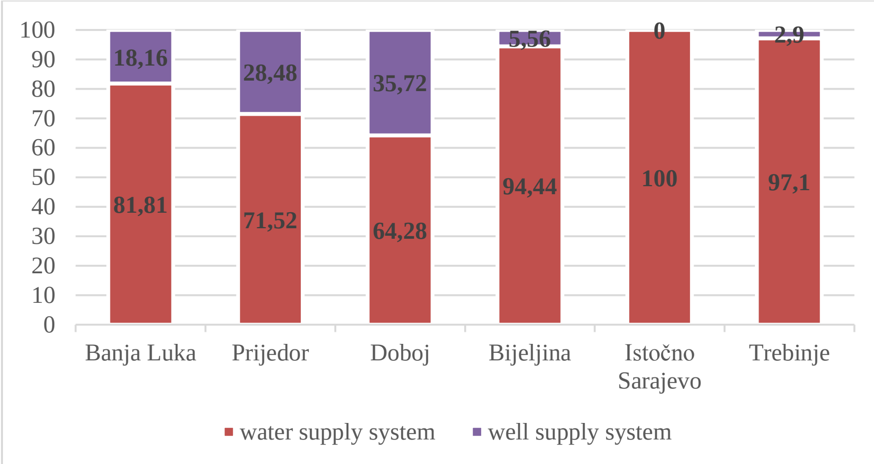

Figure 4. Representation of samples by regions in % in relation to the category for the period 2018-2020

Analysis of the representation of samples by region shows that the most regions predominantly use in the food industry water from water supply system. Most of the analyzed samples, as expected, come from region with the highest concentration of facilities in the food industry (Banja Luka). The analyzed number of samples originating from the region of East Sarajevo is negligibly small, so it was left out of the discussion.

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and may be subject to natural or manufactured sources of pollution that may result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination. Zero-probability level of microbiological contamination of drinking water does not exist (EU, 2020).

Table 1 shows the test results by region in relation to the total number of samples in % for the period 2018-2020 years, and Table 2 shows the results by region in % in relation to categories for the period 2018-2020 years.

Table 1. Test results by region in relation to the total number of samples in % for the period 2018-2020

Region	Satisfactory	Unsatisfactory
Banja Luka	90.26±7.41	9.74±7.41
Prijedor	86.16±6.26	13.84±6.26
Doboj	86.64±4.28	13.36±4.28
Bijeljina	100	0
Istočno Sarajevo	78.57±25.75	21.43±25.75
Trebinje	79.90±7.12	20.10±7.12

In most regions over four-fifths of the samples were satisfactory. The obtained results, in contrast to the results of Kalaba *et al*. (2020), indicate a much better microbiological status of water.

Table 2. Test results by region in % in relation to categories for the period 2018-2020.

Region	$\bar{x}$±δ			
	Water supply system		Well supply system	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
Banja Luka	91.86±6.54	8.14±6.54	64.10±24.43	35.90±24.43
Prijedor	86.51±17.55	13.49±17.55	83.33±28.87	16.67±28.87
Doboj	94.44±9.62	5.56±9.62	70.24±39.82	29.76±39.82

Bijeljina	93.33±11.55	6.67±11.55	33.33±57.74	0
Istočno Sarajevo	83.33±28.87	16.67±28.87	0	0
Trebinje	84.56±11.30	15.44±11.30	0	33.33±57.74

As expected, the microbiological status of water from the water supply system is significantly better than well water, in which one-third of the three regions have unsatisfactory samples.

According to the WHO (2008), *E. coli* are the only true indicator of faecal contamination; they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Faecal streptococci represent evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources.

Table 3 shows the average test results of the total number of samples by region in % in relation to the test parameter for the period 2018-2020.

Table 3. Test results of the total number of samples by region in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	9.30±6.68	8.02±6.69	1.12±1.95	2.14±0.79	2.57±0.31
Prijedor	13.84±6.26	13.84±6.26	8.59±8.35	5.56±9.62	5.56±9.62
Doboj	4.18±3.64	9.17±2.55	3.92±6.79	0	3.03±5.25
Bijeljina	0	6.67±11.55	0	0	0
Istočno Sarajevo	16.67±28.87	16.67±28.87	0	0	0
Trebinje	13.66±0.62	12.86±8.59	2.97±2.57	4.35±7.53	5.86±6.62

The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Official Gazette RS, 2017). A possible explanation for this is dilapidation and damage to water supply installations leading to water contamination.

Use of contaminated water in the handling and processing of milk products can cause a higher potential health risk than the risk through direct drinking. This is due to the fact that multiplication of pathogenic micro-organisms can occur in milk and milk products with amplification of the load of the pathogens (Amenu, 2013).

E. coli is a coliform bacteria and it is considered a primary indicator of fecal water contamination. Enterococci include a number of species that occur in the faeces of humans and warmblooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution (WHO, 2008).

Tables 4 and 5 show the results of testing water from water supply system and well water by region in % in relation to the test parameter for the period 2018-2020. years.

Table 4. Test results of water from water supply system by regions in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	7.68±5.76	6.05±5.88	1.33±2.31	1.65±1.50	6.34±6.72
Prijedor	13.49±17.55	13.49±17.55	0.56±0.96	0	0
Doboj	2.78±4.81	2.78±4.81	0	0	0
Bijeljina	0	6.67±11.55	0	0	0
Istočno Sarajevo	16.67±28.87	16.67±28.87	0	0	0
Trebinje	10.90±5.32	9.96±11.62	3.10±2.69	1.59±2.75	3.10±2.69

The obtained results are better than the results of the study of testing water from water supply system at milk collection points, were found 20.40% of unsatisfactory samples, of which 10.20% was detected *E. coli* and coliforms, and faecal streptococci in 12.24% (Denžić *et al*., 2013).

Table 5. Test results of well water by regions in % in relation to the test parameter for the period 2018-2020

Region	$\bar{x}\pm\delta$				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	30.34±21.31	13.67±17.45	0.85±1.48	0.85±1.48	5.56±9.62
Prijedor	16.67±28.87	16.67±28.87	16.67±28.87	16.67±28.87	16.67±28.87
Doboj	0	21.43±25.75	16.67±28.87	0	4.76±8.25
Bijeljina	0	0	0	0	0
Istočno Sarajevo	0	0	0	0	0
Trebinje	33.33±57.74	33.33±57.74	0	33.33±57.74	33.3±57.74

The obtained results indicate that the presence of bacteria, especially pathogenic ones, is significantly higher in well water than in water from water supply system. However, these results differ significantly from the results of analysis of water from milk collection points, originating from wells, where it was found 63.90% (Jaki *et al*., 2010) and 58% (Denžić *et al*., 2012) of unsatisfactory samples, including *E. coli* (20.45%), coliforms (32.55%) and enterococci (41.86%).

Conclusion

Research shows that the microbiological status of water used in food industry of animal origin in Republic of Srpska in the period 2018-2020 differs significantly between individual regions. The analysis revealed a significantly higher number of unsatisfactory samples of well water with presence of intestinal enterococci, coliforms and *E. coli*. Water from water supply system has a better microbiological status, but pathogenic bacteria have also been detected in it, which is of concern because it can affect food contamination during the production process.

References

Amenu K. (2013). Assessment of water sources and quality for livestock and farmers in the Rift Valley area of Ethiopia: implications for health and food safety Sierke Verlag Göttingen.

Amenu K., Shitu D., Abera M. (2016). Microbial contamination of water intended for milk container washing in smallholder dairy farming and milk retailing houses in southern Ethiopia. Springerplus 5(1):1195.

Dawson D. (1998). Water Quality for the Food Industry: An Introductory Manual CCRFA Guideline No. 21 Campden and Chorleywood Food Research Association Chipping Campden UK.

Dawson D. (2000). Water Quality for the Food Industry: Management and Microbiological Issues CCRFA Guideline No. 27, Campden and Chorleywood Food Research Association Chipping Campden UK.

Denžić M., Bojko I., Jaki Tkalec V., Majnarić D. (2012). Analysis of drinking water from milk tanks in the Bjelovar-Bilogora's district. Book of Abstracts, XVI Scientific-professional Conference Water and public water supply Trogir 203-204.

Denžić M., Sokolović J., Jaki-Tkalec V., Majnarić D., Pavliček D. (2013). Analysis of drinking water milk producers in the area of city Jastrebarsko. Book of Abstracts, VII Scientific-professional Conference Water and public water supply Lopar 139-40.

Edberg S. C., Rice E. W., Karlin R. J., Allen M. J. (2000). *Escherichia coli*: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology, 88, 106-16.

EU (2020). Directive (EU) 2020/2184 of the European Parliament and of the Council on the quality of water intended for human consumption (recast) Official Journal of Europian Union L435, 1-62.

Francisque A., Rodriguez M. J., Miranda-Moreno L. F., Sadiq R., Proulx F. (2009). Modeling of heterotrophic bacteria counts in a water distribution system. Water Res. 43, 1075-87.

Fridrich B., Krcmar D., Dalmacija B., Molnar J., Pesic V., Kragulj M., Varga N. (2014). Impact of wastewater from pig farm lagoons on the quality of local groundwater. Agricultural Water and Management 135, 40-53.

Gottschal J. C. (1992). Substrate capturing and growth in various ecosystems. J. Applied Bacteriol. Symp. Suppl. 73, 39-48.

Habes S., Hasanagic E., Aldzic A., Smjecanin E., Jukic H. (2015). Microbiological quality of processed water during processing of milk and dairy products. Balkan Journal of Health Science 3(2):34-38.

Hammes F., Berney M., Wang Y., Vital M., Koster O., Egli T. (2008). Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res. 42, 269-77.

Hijnen W. A. M., van Veenendaal D. A., van der Speld W. H. M., Visser A., Hoogenboezem W., van der Kooij D. (2000). Enumeration of faecal indicator bacteria in large volumes using in site membrane filtration to assess water treatment efficiency. Water Research 34, 1659-65.

Hoefel D., Monis P. T., Grooby W. L., Andrews S., Saint C. P. (2005). Profiling bacterial survival through a water treatment process and subsequent distribution system. J. Appl. Microbiol. 99, 175-86.

ISBIH (2003a). Water quality - Enumeration of culturable micro - organisms - Colony count by inoculation in a nutrient agar culture medium, BAS EN ISO 6222. Institute for standardization of Bosnia and Herzegovina, East Sarajevo, Bosnia & Herzegovina.

ISBIH (2003b) Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method, BAS EN ISO 7899-2. Institute for standardization of Bosnia and Herzegovina, East Sarajevo, Bosnia & Herzegovina.

ISBIH (2018). Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora, BAS EN ISO 9308-1/A1. Institute for standardization of Bosnia and Herzegovina, East Sarajevo, Bosnia & Herzegovina.

Jaki V., Majnarić D., Lukačić M. (2010). The analysis of drinking water from the milk collection point in the Križevci area. Book of Abstracts, 39th Croatian Symposium of Dairy Experts with International Participation Opatija 56-57.

Kalaba V., Golić B., Ilić T. (2020). Microbiological safety of water in primary production of food. Veterinary Journal of Republic of Srpska (Banja Luka) 20(1-2):66-80.

Kivaria F. M., Noordhuizen J. P. T. M., Kapaga A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. Trop Anim Health Prod. 38(3):185-94.

Liu G., Verberk J. Q., Van Dijk J. C. (2013). Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl. Microbiol. Biotechnol. 97 9265-76.

Niquette P., Servais P., Savoir R. (2001). Bacterial dynamics in the drinking water distribution system of Brussels. Water Res. 35, 675-82.

Official Gazette (2017). Rulebook on the health safety of water intended for human consumption Official Gazette of Republic of Srpska No 88/17.

Perkins N. R., Kelton D. F., Hand K. J., MacNaughton G., Berke O., Leslie K. E. (2009). An analysis of the relationship between bulk tank milk quality and wash water quality on dairy farms in Ontario, Canada. J Dairy Sci. 92, 3714-22.

Prest E. I., Hammes F., van Loosdrecht M. C. M., Vrouwenvelder J. S. (2016). Biological Stability of Drinking Water: Controlling Factors, Methods, and Challenges. Front. Microbiol. 7, 45.

Sasakova N., Veselitz-Lakticova K., Hromada R., Chvojka D., Koscco J., Ondrasovic M. (2013). Contamination of individual sources of drinking water located in environmentally polluted Central Spis Region (Slovakia). Journal of Microbiology, Biotechnology and Food Sciences 3. 262-65.

Terplan G. (1980). The role of water in the production and processing of milk. Zentralbl Bakteriol Mikrobiol Hyg B. 172(1-3):96-104.

WHO (2008). Guidelines for Drinking-water Quality, 3rd ed., Vol. 1. World Health Organisation.

RESISTANCE OF MAIZE HYBRIDS TO EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HUEBNER) UNDER THE CONDITIONS OF CENTRAL NORTHERN BULGARIA

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Abstract

Maize (*Zea mays* L.) is one of the oldest crops in the world and, as a C4 plant, it shows an incredible increase in biomass. In recent years, a significant number of varieties and hybrids of maize with different tolerance and resistance to diseases and pests have been introduced in Bulgaria. An important pest against which resistance has been created is the European corn borer (*Ostrinia nubilalis* Hb.). The aim of the present work is to monitor the incidence and development of the European corn borer in three maize hybrids in the region of Central Northern Bulgaria. The study was carried out on F1 maize hybrids: ISH714, ISH618, NOSTRANO AGROSUD. Flight dynamic was monitored daily from early May to late September using pheromone traps ZOEKON (ECB-1 capsules). The attack of each hybrid was detected by plant dissection. Damage above and below the cob and the number of larvae in each stem were reported. During the study period 2017-2019, it was found that the hybrids ISH 714 and ISH 618 were attacked equally by the first generation of the European corn borer and there were no proven differences between them. A higher number of larvae were reported in the NOSTRANO AGROSUD hybrid. The ISH 714 hybrid can be defined as the most resistant to the European corn borer, it had the lowest attack and degree of damage, as the differences with the other hybrids ISH 618 and NOSTRANO AGROSUD were statistically proven.

Keywords: *Ostrinia nubilalis, hybrids resistance.*

Introduction

Maize (*Zea mays* Linnaeus, 1753) is an economically important crop and, as a C4 plant, alongside wheat and rice, one of the most important sources of energy in the world. It is attacked by a very large number of insect pests. The European corn borer is a major pest of maize in many European countries, in the Mediterranean region of Africa and in limited areas in southwest Asia. It creates serious problems in maize production in Bulgaria and Europe (Pronko, 2011; Gagnon et al., 2019). In many regions with intensive maize cultivation, 50 to 80% and sometimes up to 100% of the plants are damaged, resulting in a yield loss of 30-40% (Lisowicz and Tekiela, 2004). In recent years, in addition to the use of insecticides, efforts have been made to create varieties and hybrids resistant to very dangerous pests such as cotton bollworm, European corn borer, corn aphids and others through breeding methods. The application of alternative methods to limit the use of plant protection chemicals is an important assignment to be solved by farmers and researchers. An increasing number of maize varieties and hybrids with different tolerance and resistance to diseases and pests are entering Bulgaria. However, it should be considered that insect pests adapt to crop resistance due to their natural evolutionary process, which is a serious problem for agriculture. Achieving higher yields and greater profitability implies systematic observations on the anticipatory adaptation of pests to the new and changing mechanisms of influence on them. Therefore, science is researching new ways and strategies to manage resistance. New plant protection programs must be accompanied by crop resistance monitoring to pests. In this aspect, the following of the density and dynamics of the European corn borer populations in resistant varieties and hybrids is of paramount importance. The aim of the present work is to monitor the occurrence and development of the European corn borer in three foreign maize hybrids in the region of Central-Northern Bulgaria.

Materials and Methods

Geographical location of the experimental areas

The results in the present work are based on carried out field investigations. The experimental area was located on the left bank of the Osam river valley about 30 km northeast of the city of Pleven, Bulgaria (43°50'441"N, 24°99'045" E).

Agrotechnical data for the experimental areas

The study was conducted in the period from 2017 to 2019 on an area of 0.5 ha. Sowing was carried out in the first week of April with hybrids ISH 618, ISH 714 and NOSTRANO AGROSUD at 72120 plants/ha and row spacing 19.8 cm. Maize was sown in this area according to the crop rotation scheme. The plant protection measures applied precluded the use of insecticides during the growing season. Control points were identified from which the necessary data were obtained to assess the resistance of maize hybrids. The values obtained from the three experimental years (2017-2019) were averaged.

Determination of the population density of European corn borer populations

The presence of the first and second generation was predicted based on the effective temperature sums from the date of permanent increase of the average daily temperatures above 10°C resp. 15 °C. Flight dynamics were monitored from early May to late September with Zoekon pheromone traps (ECB-1 capsules). They were mounted on woody vegetation 10 m from the maize crops, at a distance from each other (laterally) 75-100 m and at a height of 1 m above the ground surface. The wild herbaceous vegetation under and around the pheromones was not mowed.

As an auxiliary method to the monitoring with pheromone traps before the start of flight activities of *O. nubilalis*, the plants were observed for the presence of the first groups of eggs.

Monitoring of the flight would be more accurate if light traps were also used, but due to the difficulty of installing them in production experimental fields, the flight of moths was followed only with pheromone traps.

Determination of the infestation by the European corn borer

The infestation degree of each hybrid was assessed in 3 replicates by dissection of 15 plants (5 for each replicate). Damage above and below the cob and the number of larvae in each stem were counted. Due to the impossibility to compare the resistant hybrids with another non-resistant, the resistance was determined by a correlation between the number of the adults of European corn borer and damaged plants.

Characteristics of plant material

The study was carried out on three F1 maize hybrids: The ISH 714 hybrid is a late maturity maize hybrid (FAO 700). It is grown for the production of grain and silage. The hybrid has a good tolerance to *O. nubilalis*, very good to *Helminthosporium* spp. and medium to *Fusarium* spp. The ISH 618 hybrid is a medium-late maturity maize hybrid (FAO 600). Shows excellent yield under different conditions; suitable for silage in a second crop. Has a very good tolerance to *O. nubilalis*, very good to *Helminthosporium* spp. and *Fusarium* spp. The NOSTRANO AGROSUD hybrid is a medium early maturity maize hybrid (FAO 450). It is grown for grain production. Shows very good tolerance to *O. nubilalis*, good to *Helminthosporium* spp. and *Fusarium* spp.

Statistical analysis

The results were analysed using the software package Statistica 13.0 (TIBCO® Statistica™, CA, USA). One- and two-factor analysis of variance (ANOVA) was used to determine the influence of various factors on the studied features.

Results and discussion

Flight dynamics of the the European corn borer

It is known that in the observed area the European corn borer develops two generations per year and depending on climatic factors they are more or less differentiated.

The flight of first-generation was determined by pheromone traps and the presence of groups of eggs. The dynamics of flight for the region and the period 2017-2019 is presented graphically in Fig. 1. An increase in the *O. nubilalis* population was observed in the end of June. The peak of the population was registered in the end of June, when the plants were in the growth stage BBCH 55. The largest number of moths in the pheromone trap was observed between the end of June and the first ten days of July, followed by a clear decline in the population, and in the third ten days of July more moths were caught again.

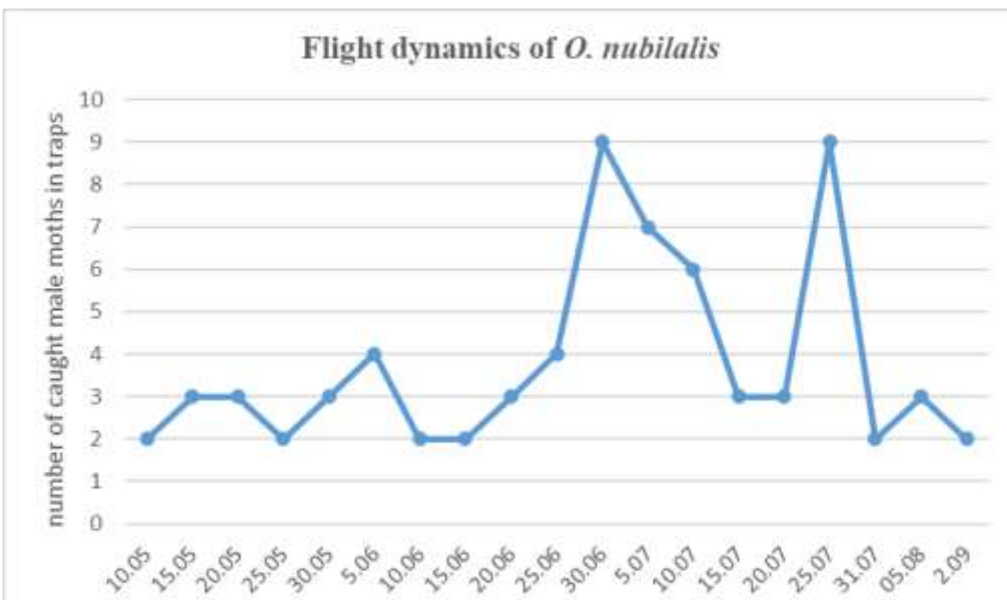
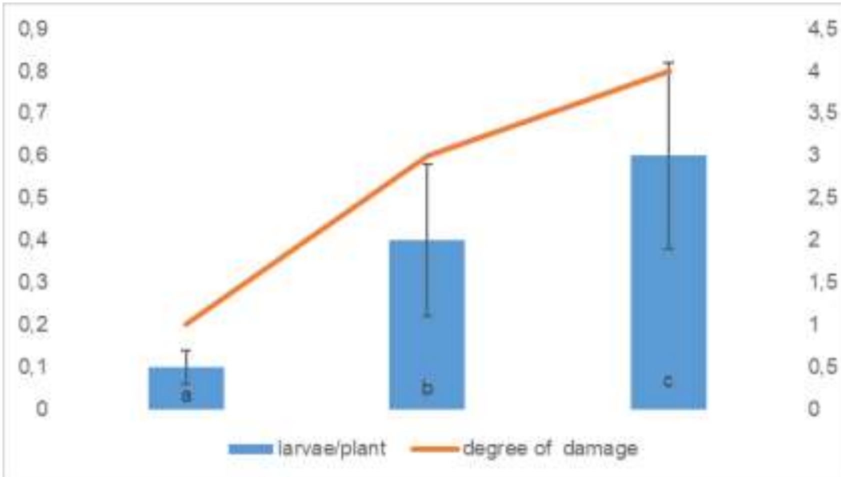


Figure 1. Flight dynamics of the European corn borer moths based on the catch with pheromone traps, 2017-2019, Pleven region (Bulgaria)

The monitoring carried out during 2017, 2018 and 2019 using pheromone traps showed that male *O. nubilalis* were present in the corn field between May and September. During the flight, two population peaks were observed: the first in the first ten days of June and the second in the second ten days of July. The second peak coincides with the peak of the second generation.

Degree of infestation and damage by European corn borer

The following results were obtained from the processing of European corn borer infestation data: a higher number of larvae (0.68 ± 0.15 larvae/plant) were registered in the studied plants in NOSTRANO AGROSUD hybrid than in the ISH 714 and ISH hybrids 618 (0.29 ± 0.15 larvae/plant resp. 0.38 ± 0.22 larvae/plant) (Fig. 2).



Mean ± SE; different letters = significant differences; P <0.05; -Tukey test

Figure 2. European corn borer infestation in hybrids ISH 714, ISH 618 and NOSTRANO AGROSUD

Despite the presence of larvae, significant damage was not found in all three hybrids. Data from the infestation and damage of second generation showed that the larvae were found mainly in the stems of maize plants. The proportion of larvae in the cobs was relatively low: in INOSTRANO AGROSUD 0.43 ± 0.22 larvae/stem and 0.25 ± 0.03 larvae/cob; in ISH 618 0.36 ± 0.17 larvae/stem as in 0.17 ± 0.01 larvae/cob, while in ISH 714 the number of larvae was 0.05 ± 0.04 larvae/stem, but no larvae were found in the cobs (Fig. 3).

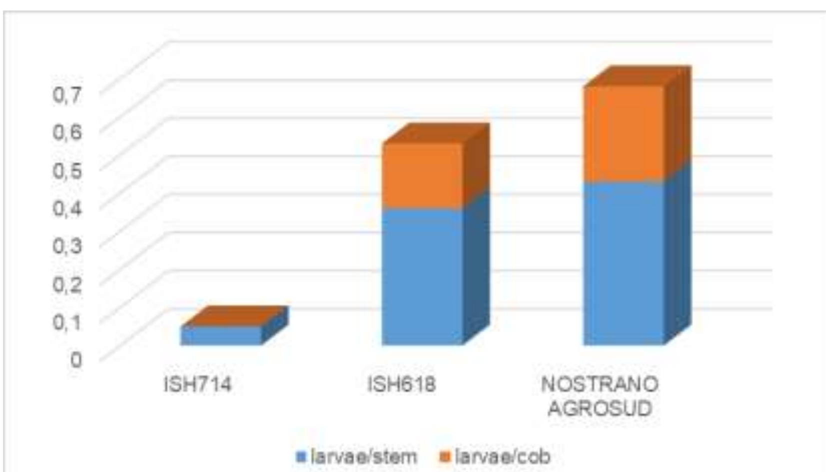
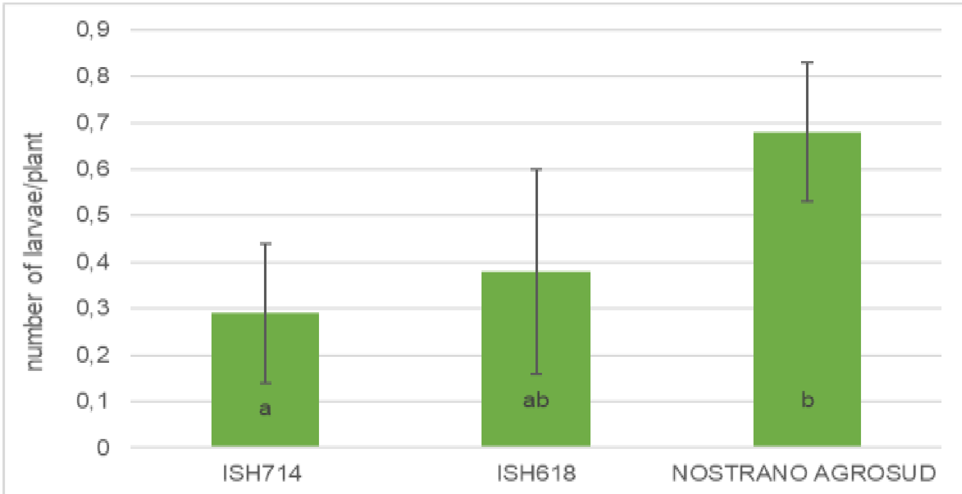


Figure 3. European corn borer infestation (second generation) in hybrids ISH 714, ISH 618 and NOSTRANO AGROSUD

The damage to the cobs was evaluated on a Hudon and Chaing (1991) scale from 1 to 9, with the highest degree of 9. A higher degree of damage was demonstrated for NOSTRANO AGROSUD (4) and ISH 618 (3) compared to ISH 714 (1) (Fig. 4).



Mean ± SE; different letters = significant differences; P <0.05; -Tukey test

Figure 4. Degree of damage by European corn borer in hybrids ISH714, ISH618 and NOSTRANO AGROSUD

Yield measurements were performed and the reported values were as follows: ISH 714: 13.700 t/ha; ISH 618: 14.000 t/ha; NOSTRANO AGROSU: 10.650 t/ha. The yields did not deviate significantly from those indicated in the characteristics of the hybrids. When comparing the number of caught moths with the number of larvae and the established damages, it can be seen that not always the larger number of moths is accompanied by more larvae and damages. For example, in July the average catch was 5.6 moths in a pheromone trap and the larval density was 0,1 ± 0,04 larvae/plant in ISH 714, 0,4 ± 0,18 larvae/ plant in ISH 618 and 0. 6 ± 0.22 larvae/plant in NOSTRANO AGROSUD. The number of reported moths also differs with the degree of damage. After correlation analysis of the data, it was demonstrated that the values of the correlation coefficients between the number of moths and larvae and the degree of damage for the period May-September were very insignificant (R = 0.29). This indicates that low values of recorded flight cannot be used as a criterion for predicting minimal damage or that infested hybrids are resistant to European corn borer. This finding is consistent with previous data of Nikolov and Stoyanova (2003). The authors look for a relation between the catch of European corn borer moths with light and pheromone traps and the damage from larvae on the pepper. They found that the values of the correlation coefficients between the number of moths and the density of the larvae, resp. the damage were insignificant. Similar data were obtained from Maini and Burgio (1999) in sweet corn. The authors found that there is a certain positive correlation (r = 0.75) between the number of caught females and the percentage of damaged cobs, while when reporting males such a correlation was not observed. It should be noted that many biotic and abiotic factors affect the fertility and sterility of adults, the hatching and parasitism of eggs during different years. However, the degree of infestation and damage by European corn borer, which we have observed in the three foreign maize hybrids, is important for more effective control of the pest and for obtaining information on possible pre-early adaptation of plants to new and changing mechanisms of influence.

Conclusions

Results showed that the hybrids ISH 714 and ISH 618 were infested equally by the first generation of the European corn borer and there were no proven differences between them. A higher number of larvae were reported in the NOSTRANO AGROSUD hybrid.
The hybrid ISH 714 can be determined as the most resistant in the conditions of central northern Bulgaria to the European corn borer. It had the lowest infestation and the lowest degree of damage (1), as the differences with the other hybrids ISH 618 and NOSTRANO AGROSUD were statistically proven. A relation between the catch of the European corn borer males with pheromone traps and the damage by larvae on hybrids was not established.

References

Gagnon, A. È., Bourgeois, G., Bourdages, L., Grenier, P., & Blondlot, A. (2019). Impact of climate change on Ostrinia nubilalis (Lepidoptera: Crambidae) phenology and its implications on pest management. Agricultural and Forest Entomology, Vol. 21, No. 3, pp. 253-264.

Hudon, M., Chaing, M. S. (1991). Evaluation of resistance of maize germplasm to the univoltine European corn borer *Ostrinia nubilalis* (Huebner) and relationship with maturity in Quebec [Canada]. Maydica (Italy), Vol. 36, No. 1, pp. 69-74.

Lisowicz F., Tekiela. A. (2004). Pests and diseases of mazie (in Polish). In: Dubas A. (eds) 3rd ed. Technology production of maize Wieś Jutra, Warszawa, 2004, pp. 52–64.

Maini, S., Burgio G. (1999). *Ostrinia nubilalis* (Hb) (Lep., Pyralidae) on sweet corn: relationship between adulrs caght in multibaited traps and ear damages. Journal of Applied Entomology, vol.123, No 3, pp. 179-185.

Nikolov, N., Stoyanova I. (2003). Dependence between the catch of butterflies by the corn borer (*Ostrinia nubalis* Hb) with light and pheromone traps and the damage by caterpillars on pepper (in Bulgarian). Plant Sciences, Vol. 40, pp.370-377.

Pronko, A. V. (2011). European corn borer (*Ostrinia nubilalis* Hbn.) incidence in European countries and Belarus. (in Russian). Plant Protection: manual of proceedings, Vol. 35, pp. 217-224.

SRAP MARKERS ASSOCIATED WITH RESISTANCE TO LOOSE SMUT IN SOME EGYPTA IN BARLEY GENOTYPES

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Abstract

Barley production has been constrained by various factors, of which loose smut (*Ustilago nuda*) is the main biotic factor. Molecular and agronomical screening analyses were evaluated to study the similarity levels and marker assisted selection associated with resistance to loose smut among eight barley genotypes using Sequence-Related Amplified Polymorphism (SRAP). Agronomical parameters during two consecutive 2020 and 2021 seasons were studied to identify their reaction to loose smut. The results exhibited significant differences among all studied genotypes for all studied characters, and the highest mean values for all studied traits were detected in Giza 136 and Giza 137. Six SRAP selected primer combinations were amplified and gave 56 total fragments, where primer combination me1+em3 gave the highest polymorphism (100 %) and the highest polymorphic information content of PIC was (0.96). The dendrogram of SRAP markers had clustered all studied genotypes into two main clusters, cluster I include all the resistance genotypes Giza 136, Giza 137, Giza 123, Giza 132, Giza 138 and Line 2. However, cluster II include only Line 1 and line 3 as a susceptible genotypes. Thus, SRAP marker could be efficiently used to assess genetic variation among barley genotypes and useful for barley germplasm management in terms of biodiversity protection and design of new crosses for loose smut breeding programs.

Keywords: *Hordeum vulgare, Loose smut (Ustilago nuda), Sequence-related amplified polymorphism (SRAP), UPGMA cluster analysis.*

Introduction

Barley (*Hordeum vulgare* L.) is a cereal crop that is grown throughout the world and is ranked fifth in world crop production. Barley can be grown in many different climatic regions due to its adaptability to diverse conditions. These climatic conditions include variable growing seasons, temperatures, and precipitation rates (FAOSTAT, 2020).

Barley, like most crops, is attacked by many disease-causing organisms. Some cause only minor damage while others can completely destroy the crops. One of the most devastating diseases is loose smut which is caused by a fungus (*Ustilago nuda*). This fungus can drastically reduce both the yield and quality of crops which has been considered as a common and world-wide seed-transmitted pathogen (Thomas, 2011). The mycelium, that is localized in the embryo, spreads systemically and asymptotically in the developing plant; during flowering, the inflorescence is largely replaced by sari containing the black teliospores of the fungus.

Seeds infected by loose smut produce normal-looking tillers up until the time of ear emergence. Thus, the affected plants compete for light, water and nutrients alongside healthy plants. (Zegeye et al., 2015).

Improvement of resistant cultivars is one of the most effective and economical means of controlling barley loose smut. Identification and incorporation of new and effective sources of resistance are key to the success of barley breeding programs. Molecular markers display an important role and considered as a tool in parallels with conventional breeding for barley improvement. The first step to design breeding program for useful trait is choosing parental genotypes based on its genetic dissimilarity (Mariey et al., 2021).

Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by (Li and Quiros, 2001*)*. It is simple, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing. SRAP markers have been successfully used to measuring the genetic diversity and relationships in barley (Yang et al., 2010; Mariey, 2021*)*. Nonetheless no much works for using SRAP as a marker for genetic diversity in barley for loose smut.

The objectives of the present study were to fingerprint and to determine the relationships among some barley genotypes based on the SRAP markers as the first work for for loose smut associated with loose smut resistance to use them in barley breeding programmers.

Materials and methods

Field Experiments and Plant Materials

Eight Egyptian barley genotypes were used in this study. Their names and pedigree are shown in Table (1). Barley genotypes were grown in the Experimental Research Station of Sakha (Egypt) during two growing seasons 2019/2020 and 2020/2021 with the aim to evaluate the yield and its related traits and loose smut reaction. Randomized complete block design with three replications was used. Plot size was 1.8 m^2 (6 rows x 0.2 m x 1.5 m). Studied characteristics determined in this study included plant height (cm), number of spike m^{-2}, number of grains $spike^{-1}$, grain yield ($t.ha^{-1}$)

Table 1. Names, type and pedigree for the studied barley genotypes

No.	Name	type	Pedigree
1	Giza 123	Hulled	Giza 117/FAO 86
2	Giza 132	Hulled	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
3	Giza 136	Hulless	Plaisant/7/cln-b/ligee640/3/s.p-b//gloriaar/ come b/5/falconbar/6/linocln-b/a/s.p-/lignee640/3/s.p-b//gloria-bar/come b/5/falconbar/6/lino
4	Giza 137	Hulled	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
5	Giza 138	Hulled	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")
6	Line1	Hulled	Giza 117/3/Alanda/Hamra//Alanda-01
7	Line2	Hulled	Giza 118/3/Alanda/Hamra//Alanda-01
8	Line3	Hulled	Giza 117/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01

Inoculation and disease evaluation

Smutted heads (SMT): (disease incidence): Assessment of 100 randomly selected plants from three central rows of each plot at the time of heading and calculated as percentage of plants showing symptoms**.**

Loose smut incidence (LSI) was assessed in each cultivar according to the method described by Menzies et al. (2009), and was calculated as follows:

$$LSI\ (\%) = \frac{\text{Number of smutted plants}}{\text{Total number of plant}} \times 100$$

Molecular markers assay

The molecular assay was carried out in the Genetics Laboratory of Genetics Department, Faculty of Agriculture, kaferelshikh University, Egypt. Genomic DNA was isolated using CTAB method from fresh leaves of the used eight genotypes of barley according to Doyle and Doyle (1990). The PCR reactions using nine SRAP combinations were used in this study as shown in Table (2). The reactions for SRAP was optimized and mixtures were prepared (in total volume of 25 µl). PCR cycling was carried out as the following program; initial denaturation at 94 °C for 4 min, followed by five cycles comprising 1-min denaturation at 94 °C, 1-min annealing at 35 °C, and 30 s of elongation at 72 °C. In the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 30 s were carried out, ending with an elongation step for 10 min at 72 °C. The amplified products were stored at 4 °C. The PCR products were separated by electrophoresis using 2% agarose gel in 1 x TBE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation.

Table 2. SRAP Primers name and sequences

No.	Name	Sequences SRAP 5'---- 3'	
1	me1+em1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT
2	me1+em2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC
3	me1+em3	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC
4	me2+em1	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT
5	me2+em2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC
6	me2+em3	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGAC

Data Analysis

***Agro-Morphological Traits Analysis*.**

Data collected from field experiment were statistically analyzed as a randomized complete block design (RCBD) using analysis of variance (ANOVA) as a combined analysis (Steel et al., 1997).

Molecular Marker Analysis

The amplified bands from SRAP were scored as a binary data under the heading of total scorable fragments, which was determined for each cultivar. The data were used to estimate the genetic similarity on the basis of number of shared amplification products *(*Nei and Li, 1979*)*. Polymorphism information content (PIC) values were done according to (Anderson et al., 1993). Cluster analysis was performed to produce a denderogram using PAST program adapted by Hammer et al. (2001).

Results and discussion

Agro-Morphological Traits Analysis

The results exhibited significant differences among the genotypes for all studied characteristics. This provides an evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied cultivars in Table 3. The results significantly varied in plant height clearly indicated that Giza 137 was the tallest cultivar (114.2 cm). However, Giza 132 was the shortest cultivar (85.0 cm). The mean performance of number of grains spike^{-1} indicted that Giza 137 gave the highest no. of grains spike^{-1} (63.5 grains spike^{-1}), however, the lowest no. of grains spike^{-1} was produced by Giza 123, which gave 61.5 grains spike^{-1}. Concerning number of spikes m^{-2}, the means of the cultivars showed that Giza 137 gave the highest number of spikes m^{-2} (513.2 spikes m^{-2}), while the lowest number of spikes m^{-2} was obtained by Giza 138 with value of 450.8 spikes m^{-2}. Regarding grain yield, Giza 137 had maximum value (3.7 t fed^{-1}). However, Giza 132 had the lowest grain yield (2.3 t fed^{-1}). The results were in close agreement with those obtained by (Mariey et 2021*)* who found high genetic variation for most of agro-morphological traits among barley genotypes under normal condition

High loose smut incidence (LSI) was assessed in line 1 and line 3

loose smut was common in all districts surveyed (Zegeye et al., 2015). Infected barleyseeds used for planting purposes in repeated cycles, has lead to the multiplication and distribution of the fungus across large areas of the region. line1 and line3 weren't acted as a potential activator of plant defense responses to biotic stresses. The genetic also helped the plant to become susceptible.

Table 3. Analysis of variances of combined mean values of eight Egyptian barley genotypes for agro-morphological traits and loose smut reaction

Cultivar	Plant height (cm)	No. grains spike^{-1}	No. of spikes m^{-2}	Grain yield (t h^{-1})	Loose smut Reaction (LST%)
Giza 123	**99.7**	**61.3**	**491.6**	**3.9**	**0**
Giza 132	**85.8**	**61.7**	**486**	**2.3**	**0**
Giza 136	**108.3**	**62.5**	**508**	**3.4**	**0**
Giza 137	**114.2**	**63.5**	**513.2**	**3.7**	**0**
Giza 138	**106.7**	**63.1**	**450.8**	**3.1**	**0**
Line 1	**99.0**	**61.5**	**465.2**	**2.5**	**100**
Line 2	**100.0**	**61.0**	**474**	**3.3**	**0**
Line 3	**101.8**	**60.5**	**497.2**	**3.4**	**80**
Average	**97.6**	**61.8**	**464.9**	**3.4**	**30.1**
LSD 0.05	**3.33**	**1.64**	**4.49**	**0.29**	**0.34**
F Test	**	*	*	**	*

Molecular markers analysis

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in the present paper shows that SRAP analysis is a powerful tool also for the characterization of barley genotypes. In our study, the SRAP markers were used for the first time for assessment the genetic diversity among Egyptian barley genotypes for loose smut resistance. The results obtained in this study showed that there were high levels of polymorphism in genotypes under study especially when the genotypes were compared for the loose smut reaction. Total of 56 fragments were amplified with six primer combinations, Results in Table 4 showed the average percentage of polymorphic loci for all primer combinations was 77.67 % and the average band number amplified from each pair of primers was 9.33% bands, of which included 7.5% polymorphic bands, which the maximum band number among the six primers combinations was 13 obtained by primer combination me1+em3 which gave the highest polymorphism (100 %) and highest Polymorphic information content (PIC) (0.96) which generated specific band associated to loose smut with size 550 bp Fig (1) found in the susceptible genotypes (line 1 and line 3), However, primer me1+em2 had the lowest polymorphism (57.4.0%) and lowest PIC value (0.58).

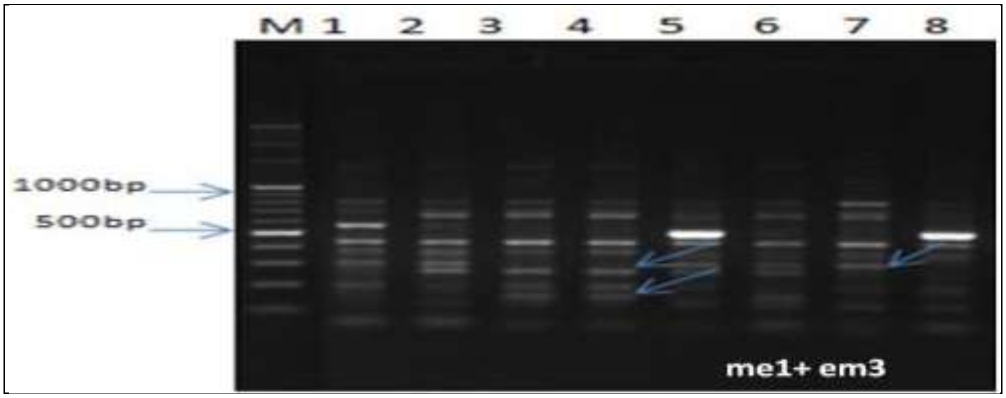

Fig (1) Agarose gel electrophoresis using SRAP primer combinations me1+em3 (A) and me2+em2 (B) in eight barley genotypes. (M) marker, (1) Giza 123, (2) Giza 132, (3) line 1, (4) line 2, (5) line 1, (6) Giza 136, (7) Giza 137 and (8) Line 3.

Table 4. Polymorphism number and rate for six SRAP primer pairs used to amplify genomic DNA templates from eight Egyptian barley genotypes

Primer combination	Number of Total fragments	Number of polymorphic fragments	Percentage of polymorphic fragments	polymorphic information content (PIC)
me1+em2	7	4	57.14	0.58
me1+em3	14	14	100.00	0.96
me2+em1	10	7	70.00	0.71
me2+em3	8	6	75.00	0.68
me1+em2	9	8	88.89	0.83
me1+em1	8	6	75.00	0.76
Average	9.33	7.5	77.67	
total	56	45		

A dendrogram (**Fig.2**) based on the genetic similarity coefficient was constructed using the six SRAP primers. In this dendrogram, all the eight barley genotypes divided in two main cluster, cluster I include all the resistance six barley genotypes Giza 136, Giza 137, Giza 123, Giza 132, Giza 138 and Line 2. However, cluster II include the genotypes Line 1 and line 3 were as a susceptible genotypes with genetics similarity (0.82).

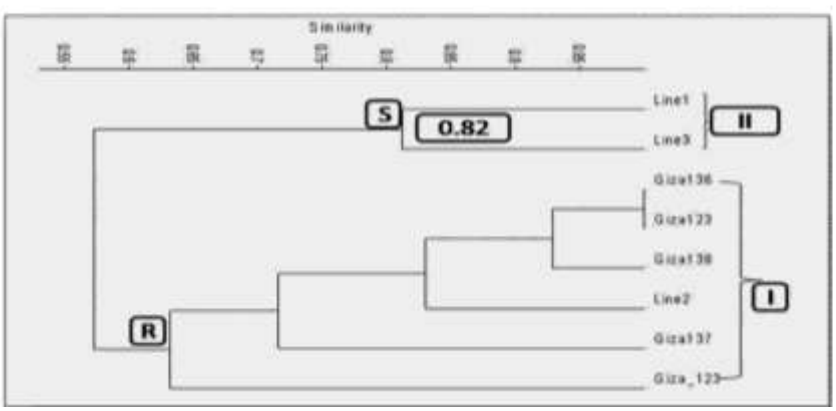

Fig (2): SRAP based dendogram of the eight barley genotypes constructed using Unweighted Pair–GroupMethod with Arithmetic (UPGMA).

In present study, genetic diversity level of Egyptian barley genotypes for loose smut is higher than other genetic diversity studies using different marker systems for loose smut such as such as RFLP (Eckstein et al.,1993), SSR (Cheng-Dao et al., 2001) and SCAR marker for loose smut in wheat (Draz et al., 2021) .SRAP markers mainly targets exons which are expected to be evenly distributed along all chromosomes with GC-rich regions and introns with AT-rich regions (Li and Quiros, 2001). Small and simple barley genome taken into consideration, many intron and exon regions may have influenced the number of excess polymorphic bands. Therefore, using SRAP marker to assessment the genetic diversity among barley genotypes for environment stress will be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for disease resistance to loose smut breeding programs . These results were in a good harmony with (Mariey et al., 2021). They reported that the SRAP marker will be efficiently used to assess genetic variation among barley genotypes and would be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for environment stress breeding program.

Conclusions

This is the first attempt to determine the genetic diversity resistance to loose smut in Egyptian barley genotypes using SRAP markers. The primer combination me1+em3 was (0.96) indicating that this primer combination is highly informative and might be useful tool to determine the genetic differences among barley cultivars for loose smut reaction.

References

Anderson, J. A., Churchill ,G. A., Autrique, J. E. Tanksley , S. D and Sorrells , M. E. (1993). Optimizing parental selection for genetic linkage maps. Genome. 36, 181–186

Cheng-Dao Li, Eckstein, P. E., Minyan Lu, Brain G. Rossnagel and Graham J. S. (2001). Targeted development of a microsatellite marker associated with a true loose smut resistance gene in barley (*Hordeum vulgare* L.). Molecular Breeding 8: 235–242.

Doyle, J.J. and J.L. Doyle (1990). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Focus, 12: 13-15.

Draz I.S., Darwish, A.K., Abou-Elseoud, M.S., Elassal, A.A. Komeil, D.A. (2021). Molecular discovery of new allele associated with loose smut resistance gene Ut-X in spring wheat. Agronomy Research 19(1) : 74–82.

Eckstein P.E., Duncan S.J.L., Scoles G.J. and Rossnagel B.G. (1993). An RFLP for true loose-smut resistance and for scald resistance. Proceedings of the 15th North American Barley Research Workshop (Guelph, Ontario, Canada).

FAOSTAT (2020). Food and Agriculture Organization of the United Nations Statistical Database. Available online: http://www.fao.org/faostat

Hammer Ø.; D.A.T. Harper; and P.D. Ryan (2001). Paleontological statistics software package for education and data analysis, Palaeontologia Electronica, 4: 1-9.

Li, G. and C.F., Quiros, (2001). Sequence-related amplified polymorphism (SRAP) a new marker system based on a simple PCR reaction, its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103, 455–461.

Menzies, J.G., Turkington, T.K. & Knox, R.E. 2009. Testing for resistance to smut diseases of barley, oats and wheat in Western Canada. Can. J. Plant Pathology 31: 265–279.

Mariey A. Samah, E. Mohamed , Z. Ghareeb and Abo Zaher E. (2021).Genetic Diversity of Egyptian Barley using Agro–physiological Traits, grain quality and molecular markers. Current Science International, 10 :58-71.

Nei, M. and Li W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76:5269–5273.

Steel, R.G.D., Torrie, J.H. and Deekey, D.T. (1997) Principles and procedures of statistics: "A Biometrical Approach" 3rd ed., McGraw Hill Book Co., Inc. New York

Thomas, P.L. (2011). Barley smuts in Manitoba and eastern Saskatchewan, 1972-74. Canadian Plant Disease Survey, 54, 124-128

Zegeye, W., Dejene, M. and Ayalew, D. (2015). Importance of loose smut [Ustilago nuda (Jensen) Rostrup] of barley (Hordeum vulgare L.) in Western Amhara Region, Ethiopia. East African Journal of Sciences, 9: 31-40.

EFFECT OF GELATIN BASED EDIBLE COATINGS ON MINIMALLY PROCESSED APPLE (MALUS DOMESTICA BORKH) CUBES

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Abstract

Apples are rich in bioactive compounds like antioxidants, polyphenols and other compounds having health promoting properties. The shift of modern-day consumer towards fresh, minimally processed, conveniently prepared food is making minimally processed apples more and more popular. Processing of fruits generates physiological stresses in the live tissues even if it is minimal processing. These stresses lead to quality deterioration and shorter shelf life as compared to fresh intact produces. Use of edible coatings is gaining popularity now a days for packaging of fruits and vegetables but not much information is available regarding the use of gelatin based edible coatings on minimally processed apple cubes. The present research work discusses the effect of edible coatings made of gelatin, citric acid and glycerin. Two different concentrations of gelatin, 8% and 10% were used and their effect was seen on carrots against the control samples (carrot samples having no coating). The concentration of glycerin was 2% and ascorbic acid was 1% in both the coating solutions. Apples were cubed, coating was applied using the dipping method. The control and coated samples were stored in ambient conditions. Total polyphenol content, Percent loss in weight, color values, textural analysis antioxidant capacity of apple cubes were recorded for 5 days. Coatings were proved to be good only for retention of total polyphenols and textural properties. It negatively influenced weight loss percent and browning.

Key words: *freshly cut vegetables, carrots, biodegradable, edible packaging, circular economy.*

Introduction

It has been proven by numerous scientific studies that a good lifestyle and eating habits promote health. Due to the omnipresence of the internet, consumers can access all the information including health-related in a flash of a second. Thus, modern day consumer is much more aware, conscious and demanding. Moreover, the busy lifestyles, new habits, and demand for ready to eat food has resulted in growth of minimally processed fruit and vegetable market [1]. Minimally processed fruits and vegetables are more convenient, have fresh characteristics, and health benefits. Minimal processing can be any of these unit operations- washing, sorting, peeling, coring, slicing, etc. [2]. But in the process, the integrity of fruits and vegetables is altered because of the wounding [3]. Apple is a very popular fruit, consumed all over the world. Apples always remain in the top three produced fruits around the world. The worldwide production of apples in 2020 was 63.9 million tons (World Data Atlas) and is projected to be about 75.9 million tons in 2021 (USDA).

Edible films and coatings are made up of edible biopolymers and food grade additives. The biopolymers can be proteins, polysaccharides, lipids, or their mixture. Edible films are free standing structures and are applied to food after they are made but edible coatings are applied directly to the food stuff [4]. The use of edible coatings for a wide range of food products, including fresh and minimally processed fruit, has received increased interest. Gelatin is natural water-soluble protein obtained by partial hydrolysis of collagen which is found in bones, skins, connective tissues and tendons of vertebrates and invertebrates [5]. Gelatin has very good gelling, thickening and water binding capacity[6].
Not much research has been done to see the effect of gelatin coatings on minimally processed apples. Considering the above, the objective of present work was to develop edible coating based on gelatin, apply it to minimally processed apple cubes and evaluate shelf life of the product based on color, texture, percentage loss in weight, total polyphenol content antioxidant capacity for a period of 5 days at ambient storage conditions.

Materials and methods

Materials

Materials used were gelatin, citric acid, glycerin, apples. Apples were brought from local supermarket in Budapest.

Sample Preparation and Coating

The experiments were performed in the laboratory of Department of Fruit and Vegetable Processing Technology located at Budapest in the month of June. 4 kilogram good quality apples were brought. They were peeled using a hand peeler and cut into cubes using Robert Bosch (Gmbh) FD8904 machine and knife. After cubing they were washed thoroughly in potable running water and then dipped in 0.1% Citric acid for a few seconds. Afterwards, the apple cubes were divided in three different batches. No coating was applied to the first batch, it was called control. The other two were treated with 2 different coatings. For the first coating 8% gelatin was heated with distilled water for 5 minutes. After that, glycerin (2%) and citric acid (1%) were added. The solution was brought to about 35-37℃ temperature. Same method was used to make the second coating, but the concentration of gelatin was 10%. Coatings were applied using dipping method. Each apple cube was coated three times and then observations were taken daily for 5 days. Control and coated apple cubes were stored in ambient conditions.

Analytical methods

Percentage weight loss

Weight of the samples were measured on each day of analysis using a laboratory scale high precision digital A & D company, FX3000i weighing balance with accuracy of 0.01g. Weight loss was then determined and expressed as percentage weight loss using the following formulae:
Weight loss (%) = $(W_i-W_f)/W_i * 100$ [W_i-the initial weight, W_f -measured weight (g) of sample on day of analysis]

Color values

Color measurements were performed by CIE Lab Color Measuring System with Konica Minolta CR 410 manual digital color meter.

Texture Analysis

The texture analysis was performed using Brookfield LFRA Texture Analyser-6514933. A stainless-steel needle probe was used having 1.0 mm diameter and 46 mm length. The penetration depth was 4mm and speed of the probe was 1mm/s.

Total Polyphenolic Content
Total Polyphenol Content (TPC) was determined using the method of (Singleton and Rossi). The spectrometric measurements were carried out with Hitachi U-2900 equipment (Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

Ferric Reducing Ability of Plasma (FRAP)
Antioxidant capacity was determined using Ferric Reducing Ability of Plasma (FRAP) assay using the method of [7] with slight modifications.

Statistical Analysis
All the experiments were done in triplicates/more than triplicates. There were two fixed factors, treatment (edible coating with different concentrations of gelatin) and storage time (number of days). Statistical evaluation was done using IBM SPSS V27 in 95% confidence interval.

Results and Discussion

Weight loss percentage
weight loss occurred in all the apple cubes. It is a natural phenomenon. When fruits and vegetables are exposed to environment, they lose weight [8]. The prime reason for weight loss could primarily be water evaporation facilitated by water vapor pressure gradient along with the loss of carbon (lost during formation of CO_2 during respiration) [9], [10]. Unexpectedly the weight loss % of control samples were less than the coated ones. The results for weight loss of apple cubes are assigned in Table 1.

Table 1. Effect of coating treatment and storage time (days) on weight loss% of the apple cubes. Values represent means of nine replicates and their corresponding standard deviations. Superscripts with small case letters indicate significant differences by time along the rows. Superscripts with the uppercase letters indicate significant differences by treatment along the columns. 8%G, and 10%G represent 8%, and 10% concentrations of the gelatin in the edible coatings. C represents the apple cubes without any coating or treatment.

weight loss% of apple cubes				
TREATMENT	Time (Days)			
	1	2	3	4
10%G	$59.51 \pm 3.32^{a,B}$	$82.63 \pm 9.04^{b,B}$	$86.64 \pm 0.55^{b,B}$	$86.66 \pm 0.59^{b,B}$
8%G	$59.94 \pm 1.83^{a.B}$	$84.92 \pm 0.97^{b,B}$	$85.99 \pm 0.76^{b,B}$	$86.04 \pm 0.74^{b,A,B}$
C	$37.59 \pm 3.21^{a.A}$	$69.72 \pm 2.58^{b,A}$	$83.41 \pm 1.60^{c,A}$	$85.14 \pm 1.50^{c,A}$

Color values
L*, a*, b* color values

Results for the L* color values are expressed in Table 2. Declining trend was seen throughout the storage. Control samples had highest L* values. Coated samples showed higher a* color values compared to control ones. The control samples showed comparatively higher values than the coated samples during the analysis period.

Table 2. Effect of coating treatment and storage time (days) on color values of the apple cubes.

	days	C	8%G	10%G
L*	0	77.73 ± 2.71 ^{b,B}	77.13 ± 4.52 ^{b,B}	72.03 ± 1.70^{b,A}
	1	79.73 ± 3.18 ^{b,B}	76.65 ± 4.82 ^{b,A,B}	73.89 ± 3.30 ^{b,A}
	2	75.47 ± 4.8 ^{a,B}	66.07 ± 4.41 ^{a,A}	62.08 ± 3.37 ^{a,A}
	3	70.83 ± 2.32 ^{a,B}	65.89 ± 4.48 ^{a,A}	63.70 ± 2.43 ^{a,A}
	4	68.60 ± 2.63 ^{a,B}	65.50 ± 3.94 ^{a,A,B}	62.66 ± 2.82 ^{a,A}
a*	0	1.29 ± 1.02 $^{a.A}$	2.02 ± 0.98 $^{a.A}$	2.14 ± 1.20^{a,A}
	1	1.27 ± 0.70 ^{a,A}	3.88 ± 2.92 ^{a,A}	5.70 ± 1.34 ^{b,B}
	2	4.45 ± 2.39 ^{b,A}	8.09 ± 3.32 ^{b,B}	10.25 ± 1.38 ^{c,B}
	3	6.85 ± 0.58 ^{c,A}	8.60 ± 3.46 ^{b,A,B}	9.65 ± 2.10 ^{c,B}
	4	8.21 ± 0.93 ^{c,A}	8.24 ± 3.42^{b,A}	9.91 ± 1.83 ^{c,A}
b*	0	24.18 ± 2.01 $^{a.A}$	22.29 ± 2..52 $^{a.A}$	24.27 ± 2.30^{a,A}
	1	25.20 ± 1.88 ^{a,A}	28.16 ± 2.55 ^{b,B}	29.67 ± 0.85 ^{b,B}
	2	28.63 ± 2.21 ^{b,B}	25.93 ± 1.67 ^{b,A}	25.15 ± 1.89 ^{a,A}
	3	26.34 ± 3.24^{a,b,A}	25.31 ± 2.34 ^{a,b,A}	26.32 ± 1.85 $^{a.A}$
	4	34.69 ± 2.23 ^{c,B}	25.39 ± 2.34^{a,b,A}	26.01 ± 2.95 ^{a,A}

Texture analysis

Hardness, Gumminess, Chewiness

The hardness of coated samples was found to be more than control samples. Treatment had no significant effect on gumminess and chewiness of the apple cubes during the storage period in our study.

Table 3. Effect of coating treatment and storage time (days) on hardness of the apple cubes.

hardness of apple cubes				
TREATMENT	Time (Days)			
	1	2	3	4
10%G	37.89 ± 9.33a,A	97.11 ± 26.27 b,B	114.61 ± 25.37 b,c,B	145.89 ± 59.82 b,A
8%G	43.06 ± 22.14 a.A	63 ± 13.63 a,b,A	89.89 ± 30.29 b,c,A,B	106.94 ± 30.41 c,A
C	45.22 ± 11.01 a.d,A	49.94 ± 11.29 a,b,A	64.89 ± 18.49 b,d,A	101.06 ± 16.67 c,A

Total Polyphenol Content (TPC)

The coated samples showed more TPC and it increased during the storage time. Same trend in results of carrots was reported by [11], [12].

Table 4. Effect of coating treatment and storage time (days) on TPC values of the apple cubes.

TPC values for apple cubes					
TREATMENT	Time (Days)				
	0	1	2	3	4
10%G	$22.75 \pm 0.72^{a,A}$	$22.45 \pm 1.26^{a,A}$	$71.98 \pm 2.31^{b,C}$	$119.70 \pm 4.98^{c,B}$	$113.60 \pm 1.27^{c,A}$
8%G	$20.23 \pm 4.04^{a,A}$	$23.66 \pm 3.11^{a,A}$	$42.42 \pm 1.47^{b,A}$	$73.85 \pm 5.06^{c,A}$	$107.80 \pm 3.26^{d,A}$
C	$21.59 \pm 0.49^{a,A}$	$18.36 \pm 3.11^{a,A}$	$56.40 \pm 2.05^{b,B}$	$103.31 \pm 10.09^{c,B}$	$107.50 \pm 2.63^{c,A}$

Ferric Reducing Ability of Plasma (FRAP)
Only time had significant effects on the FRAP values. The results can be seen in Table 5.

Table 5. Effect of coating treatment and storage time (days) on FRAP values of the apple cubes.

FRAP values for apple cubes					
TREATMENT	Time (Days)				
	0	1	2	3	4
10%G	4.04 ± 2.22a,b	6.57 ± 1.98 b	2.65 ± 1.22a	14.94 ± 0.38 c	19.56 ± 1.87d
8%G	4.06 ± 0.47 a,b	6.66 ± 5.32 b	0.89 ± 0.77 e	22.85 ± 0.70 c	37.76 ± 3.28d
C	4.17 ± 3.11 a	2.30 ± 0.92 a	4.72 ± 1.36a	15.28 ± 27.94a,b	30.05 ± 2.70b

Conclusion

Results show that the samples coated with new edible coatings performed better in terms of retention of Total Polyphenols. The positive effect of coatings was seen on texture analysis. Based on the results, it can be suggested that the application of coatings made of gelatin, citric acid and glycerin are not good for reducing the water loss percentage of apples. The coatings were not able to retard the browning. The coated samples had more browning than control ones. The FRAP values were affected only by time not by the effect of treatment.

References

C. Alegria, J. Pinheiro, E. M. Gonçalves, I. Fernandes, M. Moldão, and M. Abreu, "Evaluation of a pre-cut heat treatment as an alternative to chlorine in minimally processed shredded carrot," Innovative Food Science & Emerging Technologies, vol. 11, no. 1, Jan. 2010, doi: 10.1016/j.ifset.2009.10.008.

L. R. HOWARD and T. DEWI, "Minimal Processing and Edible Coating Effects on Composition and Sensory Quality of Mini-peeled Carrots," Journal of Food Science, vol. 61, no. 3, May 1996, doi: 10.1111/j.1365-2621.1996.tb13177.x.

N. M. Emmambux and A. Minnaar, "The effect of edible coatings and polymeric packaging films on the quality of minimally processed carrots," Journal of the Science of Food and Agriculture, vol. 83, no. 10, Aug. 2003, doi: 10.1002/jsfa.1509.

J. H. Han, "Edible Films and Coatings," in Innovations in Food Packaging, Elsevier, 2014. doi: 10.1016/B978-0-12-394601-0.00009-6.

S. Shankar, L. Jaiswal, and J.-W. Rhim, "Gelatin-Based Nanocomposite Films," in Antimicrobial Food Packaging, Elsevier, 2016. doi: 10.1016/B978-0-12-800723-5.00027-9.

B. Ramos, F. A. Miller, T. R. S. Brandão, P. Teixeira, and C. L. M. Silva, "Fresh fruits and vegetables—An overview on applied methodologies to improve its quality and safety," Innovative Food Science & Emerging Technologies, vol. 20, pp. 1–15, Oct. 2013, doi: 10.1016/J.IFSET.2013.07.002.

I. F. F. Benzie and J. J. Strain, "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': The FRAP Assay," Analytical Biochemistry, vol. 239, no. 1, Jul. 1996, doi: 10.1006/abio.1996.0292.

G. I. Olivas and G. v. Barbosa-Cánovas, "Edible Coatings for Fresh-Cut Fruits," Critical Reviews in Food Science and Nutrition, vol. 45, no. 7–8, Oct. 2005, doi: 10.1080/10408690490911837.

M. Mohebbi, E. Ansarifar, N. Hasanpour, and M. R. Amiryousefi, "Suitability of Aloe Vera and Gum Tragacanth as Edible Coatings for Extending the Shelf Life of Button Mushroom," Food and Bioprocess Technology, vol. 5, no. 8, Nov. 2012, doi: 10.1007/s11947-011-0709-1.

K. M. Kim, J. A. Ko, J. S. Lee, H. J. Park, and M. A. Hanna, "Effect of modified atmosphere packaging on the shelf-life of coated, whole and sliced mushrooms," LWT - Food Science and Technology, vol. 39, no. 4, May 2006, doi: 10.1016/j.lwt.2005.02.015.

R. G. Klaiber, S. Baur, A. Koblo, and R. Carle, "Influence of Washing Treatment and Storage Atmosphere on Phenylalanine Ammonia-Lyase Activity and Phenolic Acid Content of Minimally Processed Carrot Sticks," Journal of Agricultural and Food Chemistry, vol. 53, no. 4, Feb. 2005, doi: 10.1021/jf049084b.

L. R. HOWARD and L. E. GRIFFIN, "Lignin Formation and Surface Discoloration of Minimally Processed Carrot Sticks," Journal of Food Science, vol. 58, no. 5, Sep. 1993, doi: 10.1111/j.1365-2621.1993.tb06113.x.

https://apps.fas.usda.gov/psdonline/circulars/fruit.pdf accessed on 19th July 2021.

https://knoema.com/atlas/topics/Agriculture/Crops-Production-Quantity-tonnes/Apples-production accessed on 19th July 2021.

THE EFFECT OF EDIBLE COATING BASED ON THE RICE BRAN OIL AND ACETYLATED POTATO STARCH ON QUALITY CHARACTERISTICS OF GRAPE

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Abstract

The use of agricultural products and their wastes as in the production of edible coatings and films has been developed in recent years. In this study, optimization of the edible coating production was carried out using response surface methodology (RSM) and optimal custom design with 20 treatments. Different levels of acetylated potato starch (APS) (3, 5, and 7 gr), rice bran oil (RBO) (0, 0.5, 1, 1.5, and 2 gr), and ultra-thorax rate (homogenization) (0.35×10^{-3}, 1.53×10^{-3} and 3.51×10^{-3} g) were used. Factors such as weight loss, stability, and *aw*, as the responses of experimental design were considered for investigation. Emulsion gel consisting of 6.57 gr of the acetylated potato starch and 2 gr of the rice bran oil produced at 0.35×10^{-3} g homogenization rate was selected as the best combination of edible coating. The effect of the emulsion gel based on acetylated potato starch, rice bran oil was studied on some of the grape's chemicals (TA, TSS, pH, weight loss, and spoilage percentage) and sensory properties. Consisted Edible coating of the rice bran oil and acetylated potato starch increased acidity and brightness, but reduced pH and TSS during storage.

Keywords: *Edible coating, Rice bran oil, Acetylated potato starch, Grape*

Introduction

Fruits are suitable environments for microbial growth due to the high content of sugars and other nutrients as well as high humidity (Tournas and Katsoudas, 2005). "Grape" (it was scientifically named *Vitis vinifera*) has been considered as an important fruit among its producing countries. Its preservation is difficult due to its perishable nature. Post-harvest fungal contamination causes a lot of waste of the grape. Therefore, manufacturers seek alternative approaches to maintain the quality of the grape. In the past, to increasing the shelf life of this product was being mainly used sulfur dioxide (Smilanick et al., 1990; Mustonen, 1992; Karabulut et al., 2003).
The edible coating is a thin layer of materials (mostly Biopolymers) that extend the shelf life of foods by protecting them against the transport of gases, water vapor, soluble solids, and mechanical damages (Baldwin, 2006). Various materials including hydrocolloids (polysaccharides and proteins), lipids (fatty acids, glycerides, and waxes) have been used to produce the edible coatings (Mariniello et al., 2003). Starch is one of the most important polysaccharides that has made good films. The starch-based films often are transparent or semi-transparent without odor, taste, and color. Potato starch produces gels with high viscosity due to its high phosphorus content (Rajabian, 2018). "Acetylated starch" reduces gelatinization temperature and increases transparency, viscosity, and freezing stability of the gel (Luallen, 1985; Rogals, 1986; Luallen, 1988; Schierbaum and Kettliz, 1994). Previous studies indicated the effectiveness of starch-based coatings on storage life and control the decay of the red grape and strawberry fruits (Maria et al., 1998; Farayde et al., 2015). Adding lipids to hydrocolloid compounds has improved their moisture-barrier properties due to the hydrophobic properties of lipids (Debeaufort et al., 2002). "Bran" is the by-product of rice processing (Grist, 1985). Its oil

increases the shelf life of fruits by reducing weight loss, transpiration, and respiration which are major agents of their spoilage in the refrigerator (Sala et al., 2009).
In this study, optimization was performed on the production of edible coating based on rice bran oil (RBO) and acetylated potato starch (APS) at different homogenization rates. Then, the effects of the produced coating on some quality characteristics of grape were investigated.

Materials and Methods

Grape (Asgari cultivar) was obtained from Kashmar (Khorasan Razavi) with characteristics such as much sweet taste and high storage capacity. The berries were uniform in size and color (light-green). Clusters were washed with brine and dried at ambient temperature for 2 hours just before the experiments. Then, the clusters which were free from any disease, insect, injury, or machine damage were selected for further study.
Acetylated potato starch- produced under the protection of Denmark- was purchased from Arsha Pouyeh Company (Tehran-Iran). Edible refined rice bran oil was prepared by Saman Oil Company (Khorasan Razavi-Mashhad) with a purity of 99.99 %.
Response surface method and optimal custom design were used for the optimization of 20 treatments and two repetitions under three independent variables including acetylated potato starch at three levels (3, 5, and 7 gr), rice bran oil at five Levels (0, 0.5, 1, 1.5 and 2 gr) and homogenization rate at three levels (0.35×10^{-3}, 1.53×10^{-3} and 3.51×10^{-3} g) (g is relative centrifugal force or RCF). Weight loss of coated grapes (percentage), the stability (percentage), and a_w of emulsion gels (percentage) parameters were considered as test responses to investigate the effect of different levels of treatments on them. The weight loss of 20 treatments belonging to the optimization stage was also calculated as a percentage with three repetitions every two weeks. The edible coating was prepared by continuous mixing a certain amount of acetylated potato starch in 100 ml of distilled water through gelatinization of starch into a hot water bath at 90 °C for 5 min (Riku, 2007). After cooling (for 30 min), the resulting gel was homogenized by adding a certain amount of rice bran oil (gr) and 2 % Tween 80 as emulsifier under a certain rate of Ultraturex (rpm) for 5 min (T25 digital, Made in Germany) (Shaw, 2002).
To measure the stability of emulsion gels, a certain amount of every sample was poured into a falcon and centrifuged for 30 min under 1999.52 g at ambient temperature (D72- Made in Germany). The separated phase was removed from the container by reversing the falcon on filter paper. The stability was calculated as a percentage based on the ratio of the secondary weight of the falcon containing emulsion gel to its initial weight (Baliga & Madaiah, 1970).
To determine a_w of gels was used a water activity meter (HYGROLAB-3, Made in Swiss). Thus, a certain weight of every coating sample was poured into a device (at the temperature of 20 °C). After establishing equilibrium, the amount of water activity was calculated based on the ratio of equilibrium respective humidity to 100 (Iranian National standard, 1992).
Coated treatments were weighed by digital Scale with the accuracy of 0.001 ± 1 gr (Ouauz GT 2100, Made in the USA) before and after storage in the refrigerator. Their weight loss was reported as a percentage through the ratio of the difference between the initial weight of the cluster and its initial weight (Parvaneh, 1986). To determine the total soluble solid (TSS) of the samples, a Refractometer (PAL-3, Made in the USA) was used at three repetitions (Hassani et al., 2010). The titratable acidity (TA) was measured using 5 ml of grape juice filtered in 95 ml of distilled water, 0.1 N NaOH, and Plenolphthalein reagent to an end-point of pH 8.1 at three repetitions (Shirin & Asgar, 2014). Grape extracted juice pH value was measured by a pH meter

at three repetitions (CP-744, Made in England) (Abdollahi, 2008). The CIE Laboratory color scale was used to determine the parameters L* (Black to White), a* (Red to Green), and b* (Yellow to Blue). All measurements were performed at three repetitions (Ebrahimi et al., 2018). The coated grapes were judged by 7 panelists to evaluate the sensory characteristics and general acceptance (in the terms of odor, color, flavor, appearance, texture, and overall acceptability) every two weeks. To study the mentioned parameters, the 5-point Hedonic ranking method was used. The scores 5 and 1 were considered for a very good and bad attributes, respectively.
Design-Expert software (10.0.3.0-x64-Softcozar.com.msi) in the form of optimal custom design with 20 treatments, 3 measured variables, and 3 responses were used to evaluate the results and determine the optimal conditions for the production of edible coating based on acetylated potato starch and rice bran oil. On the other hand, SPSS version22 software was used for statistical analysis. Duncan's multiple range tests (DMRT) with the maximum acceptable error of 5% were used to measure significant differences between samples, analysis of variance (ANOVA).

Results and Discussions

The Results of Optimization

As it can be seen from figure 1, weight loss decreased and then increased with increasing the concentration of APS. This difference in result can be due to the inherent moisture loss of the fruit during storage, its weight was reduced. In the following, the environment slowed down the process of weight loss of grape by keeping fruit moisture; it even increased the weight of samples. Also, an increase in the concentration of RBO had a significantly effect on the weight loss so that the lowest percentage of weight loss was observed in the high concentrations of both the independent variables. Therefore, rice bran oil has been able to prohibit moisture loss of grape during storage by preventing the transmission of its.

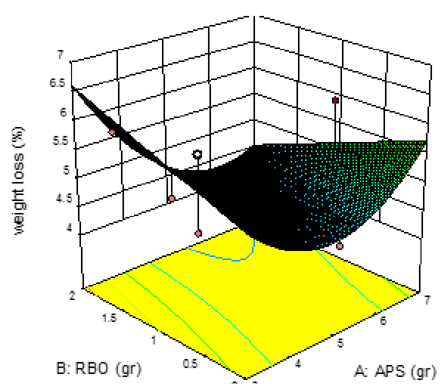

Fig.1. The effect of different levels of Acetylated Potato Starch and Rice Bran Oil on the weight loss of the clusters.

According to figure 2, the stability went through an upward trend with increasing the concentration of APS. The highest stability obtained at the highest level of acetylated potato starch. But none of the values of RBO and homogenization rate had any effect on the stability of emulsion gel. The reason for this case could be the more and better strength of gel due to filling the maximum of empty spaces between two continuous and diffused phases. Also, uniform and more mixing the components of the coating increased its stability the gelling agent (acetylated potato starch) was the effective parameter of this flow.

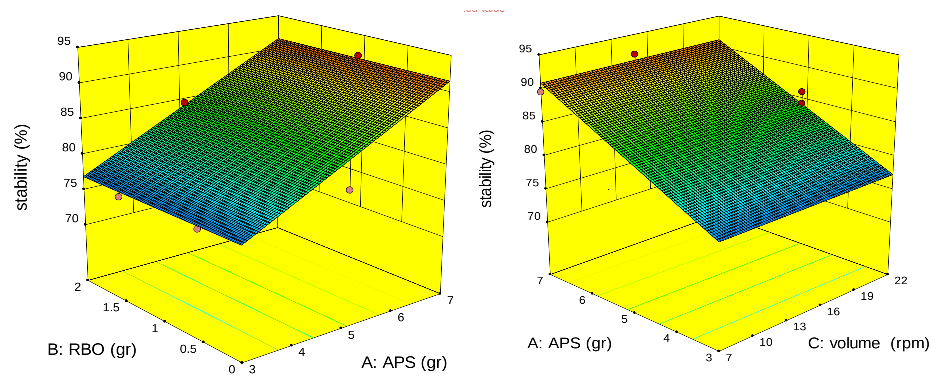

Fig. 2. The effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and the rate of homogenization on the stability of the emulsion gel.

Figure 3 shows clearly the effect of different levels of acetylated potato starch, rice bran oil, and stirring speed on water activity. Increasing the concentration of both acetylated potato starch and rice bran oil had a very significant effect on the changes in water activity parameter. The effect was impressive to some extent that the highest and lowest a_w were obtained at the minimum (0 gr) and maximum (3 gr) of RBO, respectively. But in the case of APS, results were significantly opposite obtained; thus, the water activity of emulsion gel increased with the use of the high levels of starch. Also, the different levels of X_3 did not affect the water activity of the gel. Consequently, it can be concluded that a desirable water content (less than 0.8 %) is going to create for the emulsion gel by increasing the level of consumption of rice bran oil as a plasticizer in the composition of the edible coating.

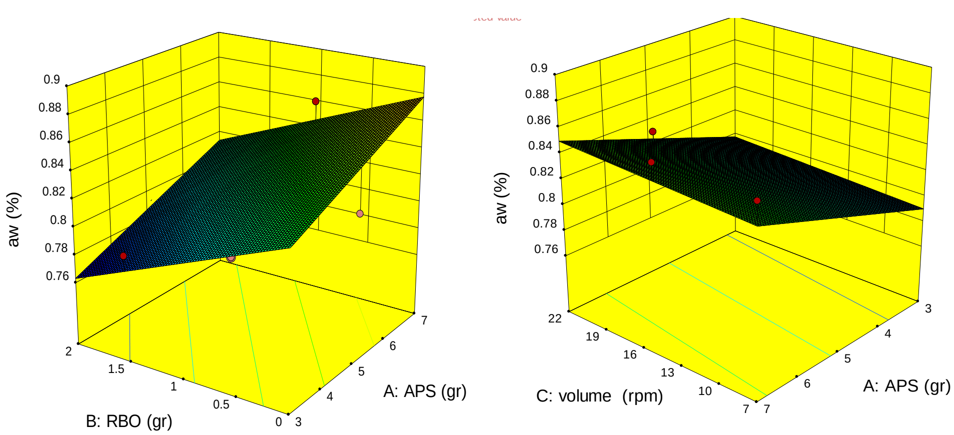

Fig. 3. The effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and the rate of homogenization on the amount of aw emulsion gel.

The results obtained from the effect of emulsion gels were produced (20 samples of edible coating) on the design responses (weight loss, stability, and a_w) is inserted in Table 1.

Observations	APS (gr)	RBO (gr)	Homogenize speed (g)	Weight loss %	Stability %	a_w %
1	7	1	10^{-3} 1.53 ×	5.44	88.6	**0.8**
2	7	2	10^{-3} 3.51×	4.94	93.6	**0.81**
3	7	0	10^{-3} 3.51×	18.21	76.75	**0.84**
4	7	1	10^{-3} 1.53 ×	5.9	91.94	**0.86**
5	7	0	10^{-3} 0.35 ×	6.17	91.46	**0.9**
6	7	2	10^{-3} 0.35 ×	6.83	66.52	**0.77**
7	7	0	10^{-3} 3.51×	5.67	74.29	**0.83**
8	7	0.5	10^{-3} 0.35 ×	5.11	72.25	**0.81**
9	3	0	10^{-3} 0.35 ×	5.17	80.26	**0.82**
10	3	2	10^{-3} 3.51×	2.47	78.73	**0.78**
11	7	0	10^{-3} 3.51×	5.11	91.85	**0.89**
12	7	1	10^{-3} 0.35 ×	4.5	89.61	**0.86**
13	3	1.5	10^{-3} 1.53 ×	6.1	76.26	**0.79**
14	3	0.5	10^{-3} 1.53 ×	6.3	69.52	**0.81**
15	5	1	10^{-3} 3.51×	4.3	86.15	**0.83**
16	3	0.5	10^{-3} 1.53 ×	5.046	76.56	**0.81**
17	5	2	10^{-3} 1.53 ×	4.054	84.54	**0.78**
18	5	0	10^{-3} 1.53 ×	4.52	80.21	**0.82**
19	7	2	10^{-3} 0.35 ×	2.44	90.69	**0.79**
20	5	1	10^{-3} 3.51×	4.2	84.34	**0.80**

Therefore, it can be predicted that using 2 gr rice bran oil, 6.57 gr acetylated potato starch and 0.35×10^{-3} g homogenization rate led to the formation of optimal emulsion gel with the most stability (90.075%), the least a_w (0.80%), and weight loss (4.403%) (Table1)

The results of Chemical parameters

A significant percentage of soluble solids in fruits contains sugars and a small portion of them, amino acids, organic acids, vitamins, and minerals. The amount of soluble solids increases with fruit ripening (Hassani et al., 2010).

Figure 4 shows (effect of different levels of APS, RBO, and Volume on the TSS parameter in the second week of storage), on the second week of storage, the highest TSS was obtained at the maximum oil concentration, 2 gr. Also, with increasing homogenization rate (Volume) and APS, this parameter decreased with a slight slope. Its maximum value was observed in 3 gr of starch. An increase in the soluble solids during storage occurs due to decrease product moisture and the decomposition of compound sugars into simple sugars. At the end of storage, by increasing starch levels, TSS decreased with a large slope; but, the effect of the RBO parameter did not change. During this period, the homogenization rate factor showed an inverse effect so that the maximum soluble solid was obtained at 3.51×10^{-3} g (Figure 5). These results were consistent with reports of kiwi fruit, Ghezel Ozum grape, and fresh strawberries (Hassani et al., 2010; Asghari & Ahadi, 2012; Emamifar, 2014). This difference in Brix changes in the two storage periods can be attributed to the moisture changes in grape.

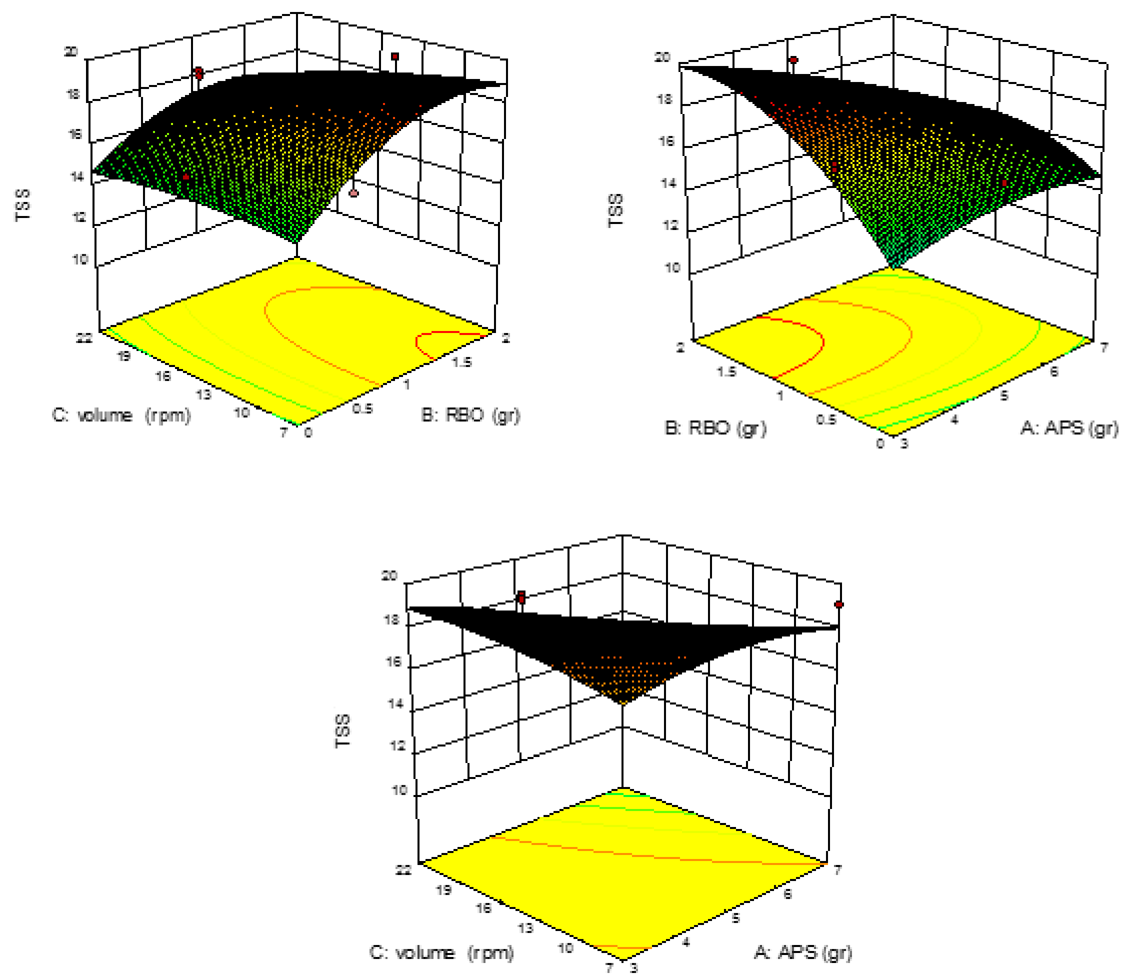

Fig. 4. The effect of different levels of APS, RBO, and Volume on the TSS parameter in the second week of preservation.

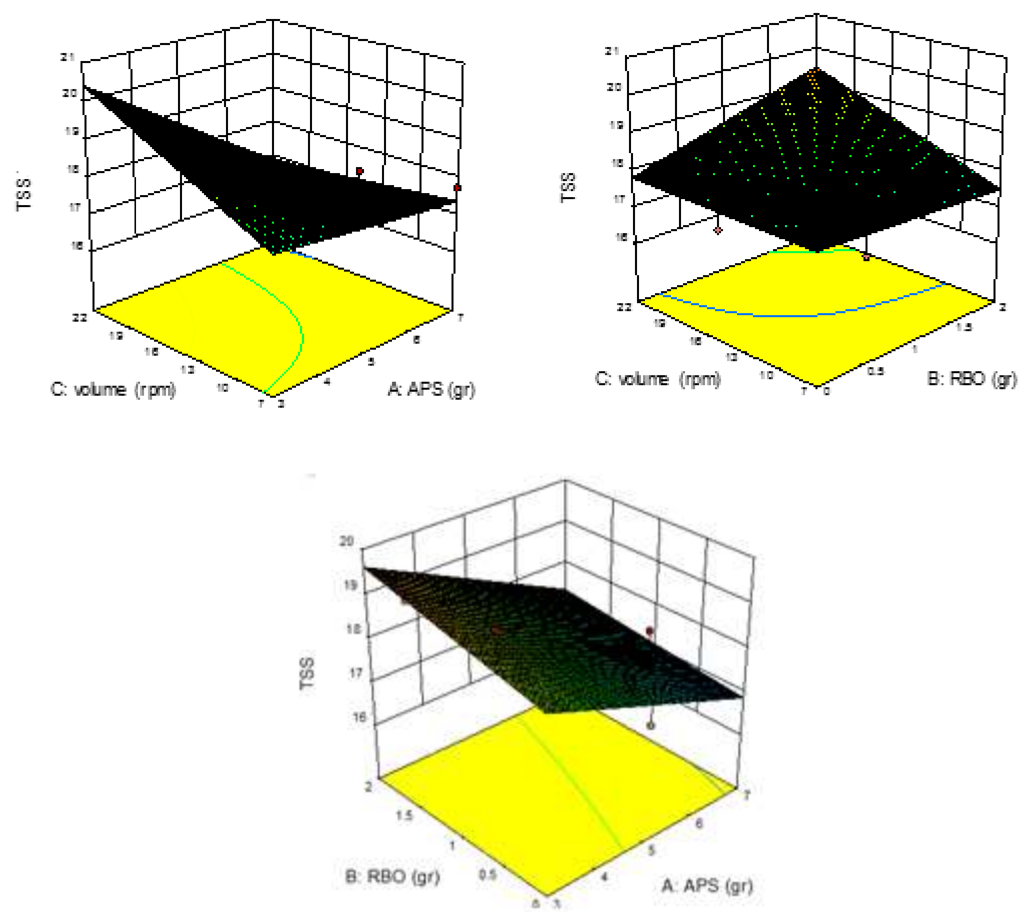

Fig. 5. The effect of different levels of APS, RBO, and Volume on the TSS parameter in the Fourth week of preservation.

The amount of titratable acids (TA) depends on the ripeness of the fruit, so that when ripe, the amount of organic acids, and the sour taste in the product decrease. The amount of these acids in the harvest period depends on the content of soluble solids and the rate of decomposition of acids (Hassani et al., 2010).

Figure 6 shows that with increasing starch concentration and rate of homogenization, acidity decreased and the highest amount of TA obtained at the lowest levels of these two variables, 3 gr and 0.35×10^{-3} g. But in the fourth week of storage, different results obtained so that with increasing APS and volume, there was an upward trend in acidity (Figure 7).

A decrease in the acidity occurs due to the decomposition of organic acids during the refrigeration period following a drop in humidity and increases the soluble sugar content of the fruit. This reason justifies the reduction in acidity in the first period of storage. In the last two weeks, due to rehydration of the sample from the environment, fruit TSS decreased during this period and the acidity of the product increased. These results are consistent with the reports of researchers to increase the shelf life of fruits such as kiwi, peach, and apricot. They stated that organic acids decrease as they mature and increase metabolic activity within the crop; however, edible coatings maintain the more and better titratable acidity by reducing the rate of fruit respiration, (Hassani et al., 2010; Galvis-Sanchez et al., 2003; Gerossi et al., 2009).

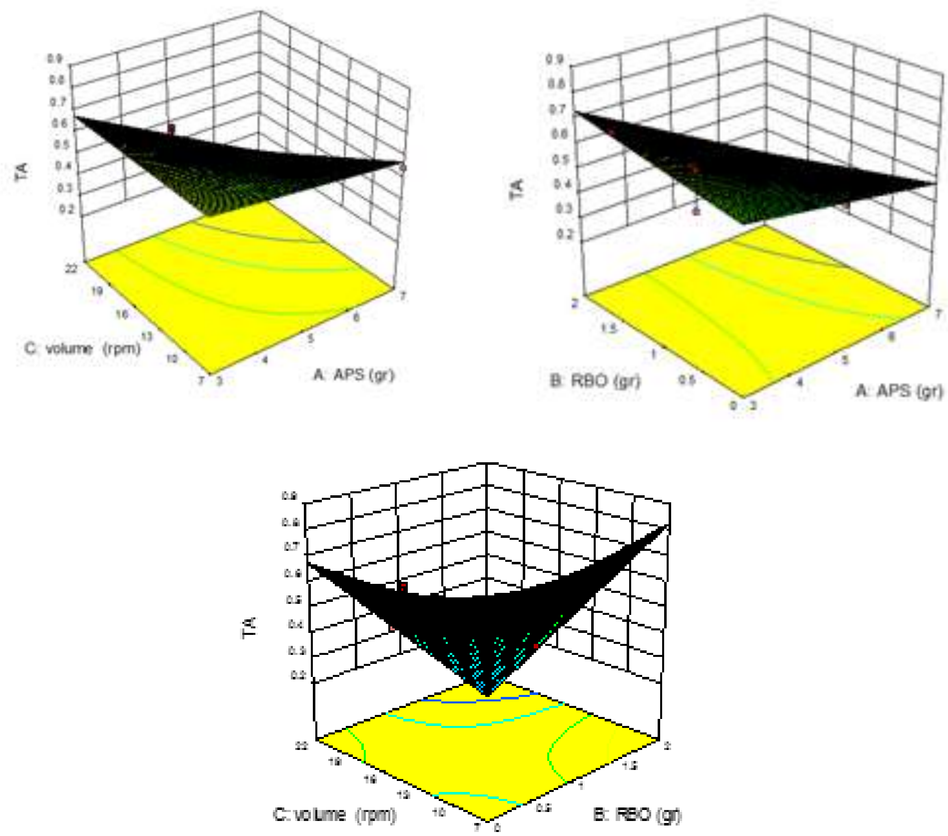

Fig. 6. The effect of different levels of APS, RBO, and Volume on the TA parameter in the Second week of preservation.

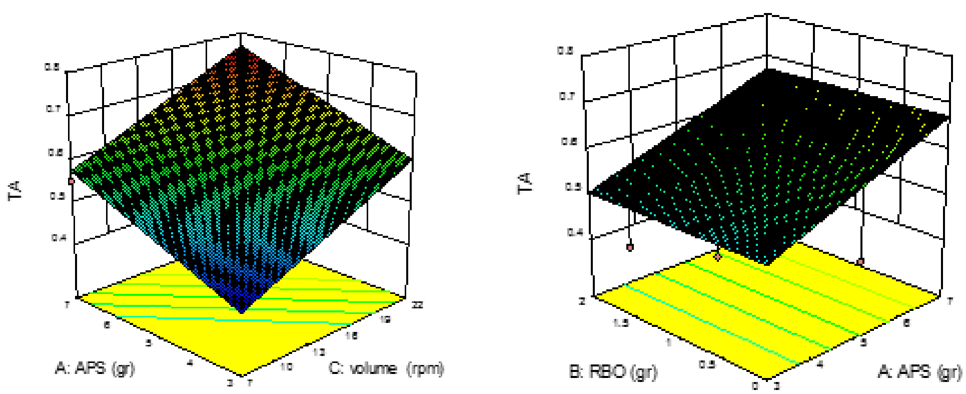

Fig. 7. The effect of different levels of APS, RBO, and Volume on the TA parameter in the Fourth week of preservation.

The pH increased and decreased by increasing APS and RBO, respectively. But the rate of homogenization did not affect this parameter (Figure 8). This increase in pH is due to the increase in sugar content and decrease in grape acidity. In the fourth week of storage of samples, the highest pH level was evaluated at the highest level of bran oil consumption, 2 gr; because it preserves as much moisture as possible and consequently prevents the decrease of acidity and increase of fruit sugar. In contrast, the maximum amount of this parameter was calculated in 3 gr of starch (minimum concentration) (Figure 9). The above discussion is consistent with the results obtained from studies on the use of edible coatings for peach and grape products (Ochoa-Velasco et al., 2014; Asghari & Ahadi, 2012).

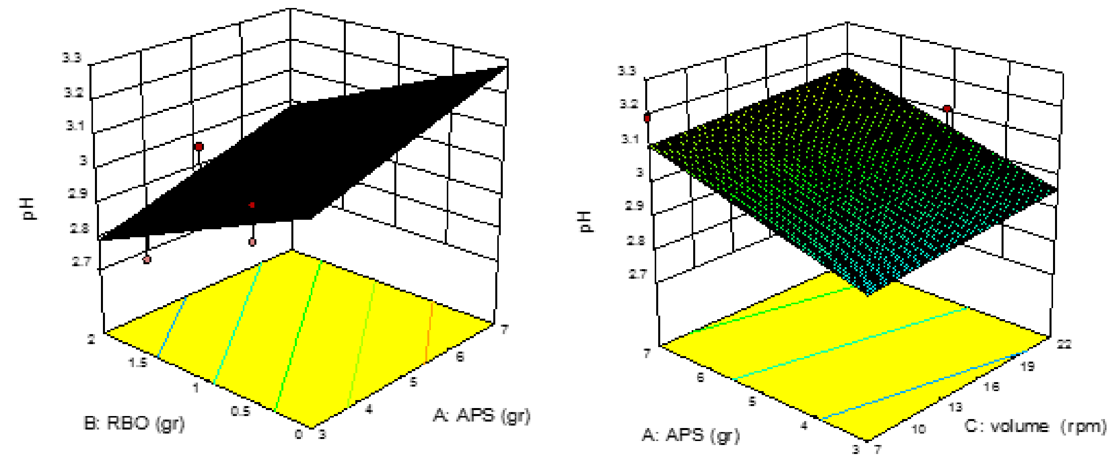

Fig. 8. The effect of different levels of APS, RBO, and Volume on the pH parameter in the Second week of preservation.

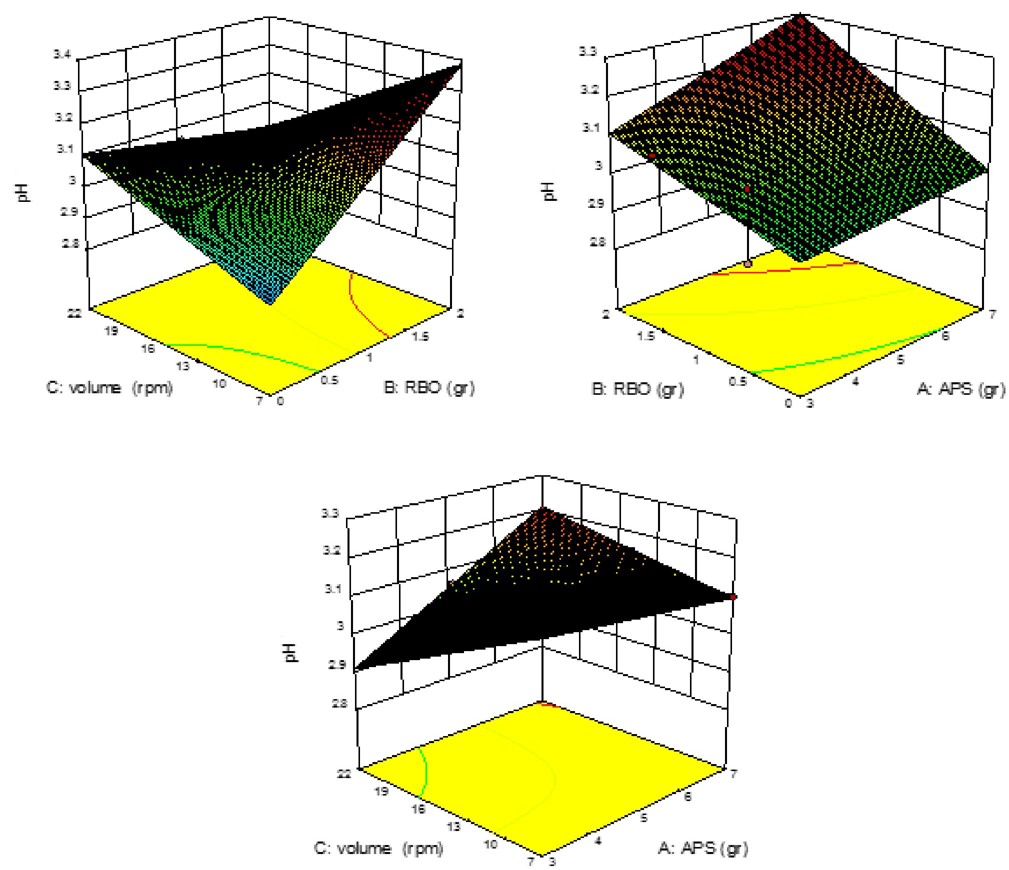

Fig. 9. The effect of different levels of APS, RBO, and Volume on the pH parameter in the Fourth week of preservation.

According to Figure 10, with increasing starch level, the amount of green color increased and with increasing oil concentration, the parameter a* of gel decreased. On the other hand, the X1X2 interaction reduced the vegetables in the edible coating.
With increasing X1, X2, and X1X2, the amount of blue decreased and yellowing increased, but with increasing the rate of homogenization, the color of the gel tended to blue (Figure 11).
From Figure 12 it can be concluded that the coatings containing the highest level of starch and oil consumption provided the most transparency. The luminosity of the coated clusters depends on the phosphate monoesters present in the potato starch structure, the amount of which is very high in this plant source. High results are according to Basiak et al. (2013) and Semigen et al (2018).

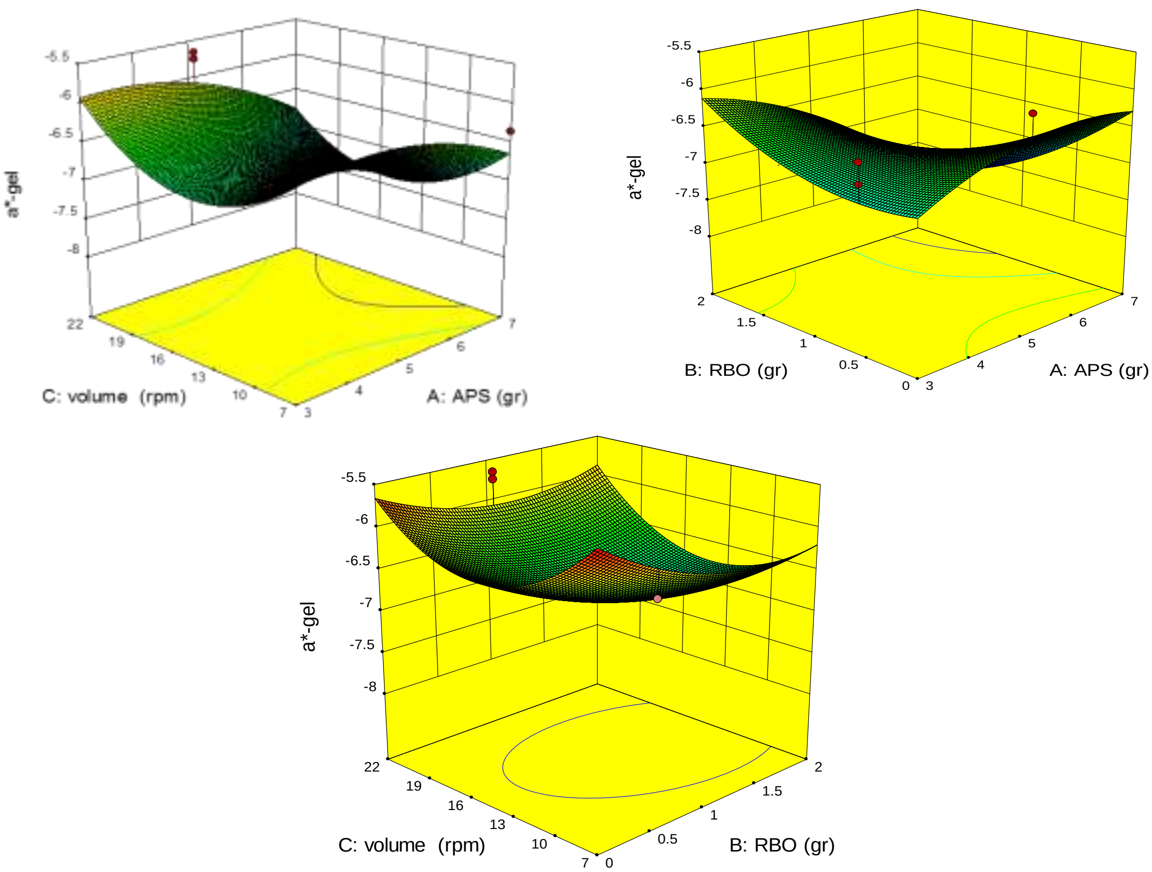

Fig. 10. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter a* of Gel.

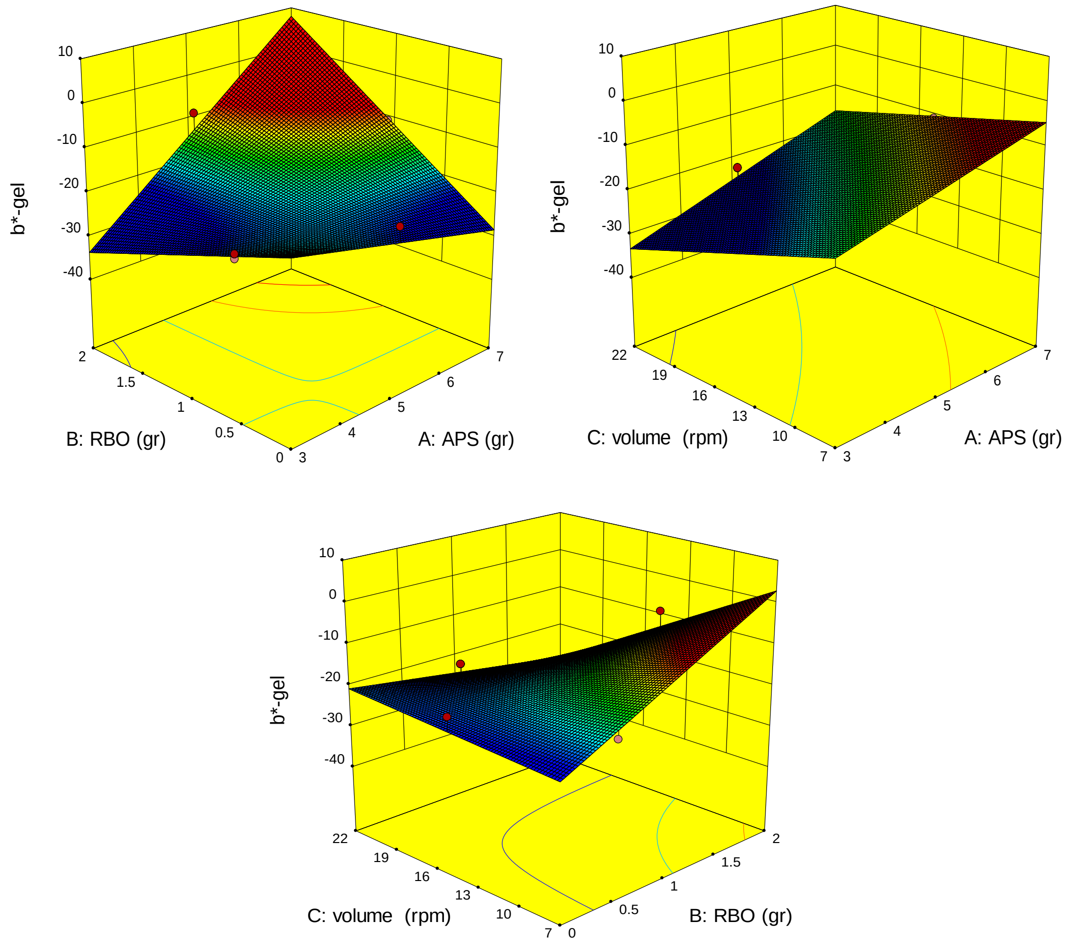

Fig. 11. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter b* of Gel.

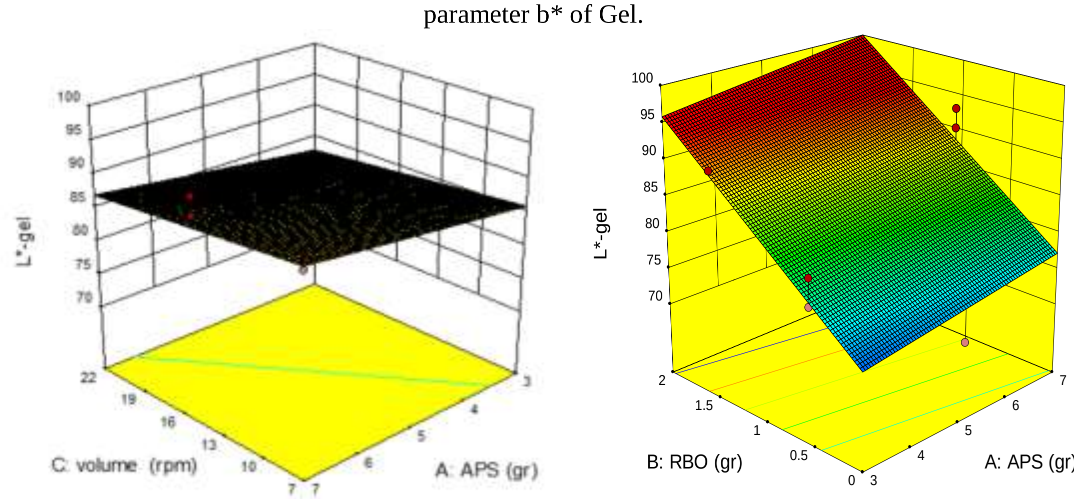

Fig. 12. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter L* of Gel.

The enzymatic browning is one of the effective factors in changing the color of fruits and vegetables during storage. On the other hand, edible coatings, by reducing the contact of oxygen with the tissue of products, prevent or delay the progression of this reaction (Akbarian, 2012).
Potato starch concentration factor had the greatest effect on the amount of a*. As you can see, with increasing the concentration of starch in the edible coating, the amount of grape' a* decreases with a sharp slope (Figure 13).
According to Figure 14, as the amount of variables X1 and X3 increased, the yellowness of the grape clusters underwent a decreasing trend, which was steeper for starch. As the amount of starch consumed and the rate of homogenization in the edible coating increased, the luminosity of the coated samples during 28 days of storage followed an increasing path (Figure 15).

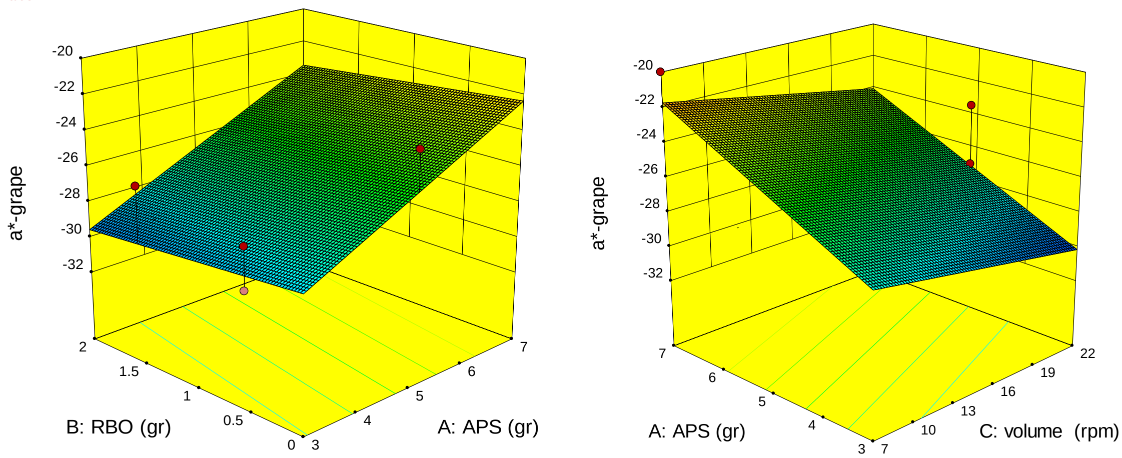

Fig. 13. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter a* of grape.

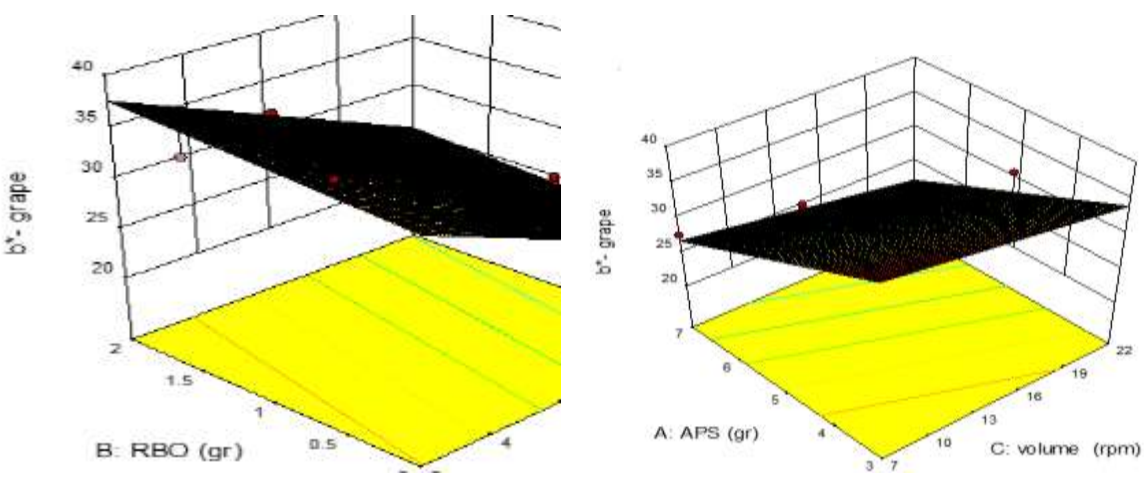

Fig. 14. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter b* of grape.

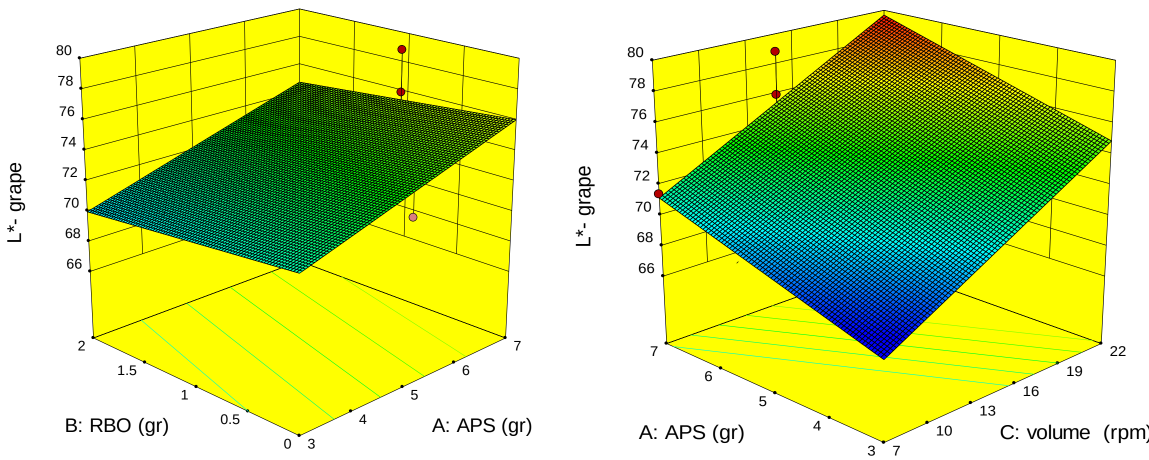

Fig. 15. Effect of different levels of Acetylated Potato Starch, Rice Bran Oil, and Ultratorex speed on the color parameter L* of grape.

Results of Sensory Test

In the first two weeks, no significant difference in flavor was observed between the control and coated samples. In the last two weeks of storage, there was a significant difference between all treatments, and the control cluster had an unfavorable taste along with samples 18 and 5. Also, the best taste belonged to the clusters with a small amount of starch and oil.

In terms of appearance, in the first 14 days of storage, there was no significant difference between the coated and uncoated clusters. Low homogenization rate due to the production of porosity and the prevention of bubbles that make the appearance of the gel unpleasant, as well as emulsion gel with a low amount of starch, which causes less thickness on the grape clusters and faster absorption by the shell.

At the end of refrigeration period, a very significant difference was seen between all treatments in terms of appearance, and samples 16 and 4 were selected by the evaluators as the most desirable and worst clusters in terms of shape, respectively.

The best fruit texture was attributed to samples 13 and 16 (lower starch content and medium oil) and the worst to samples 2 and 5 (highest starch concentration). But no significant difference was observed between control and coated clusters in the first two weeks of maintenance.

But in the last two weeks, there was a very significant difference between the control sample tissue and the coated samples. In terms of texture, the samples of 16 and 13 were determined as the best and the worst treatments, respectively. The results indicate that the use of starch with lower concentration and oil with a medium level in the composition of the coating reduces the moisture loss and consequently the optimal preservation of fruit texture during storage.

There was no significant difference between control and coated clusters in terms of overall acceptance in the first two weeks of storage. Sample 7 had the highest and treatments 2 and 12 had the lowest overall acceptance percentage from the evaluators' point of view. After 28 days of storage, samples containing less starch and oil with moderate concentration were more desirable than the control cluster and other coating compounds for the evaluators.

All the sensory parameters in question, through the edible coating, were able to induce a favorable feeling to the consumer when eating fruit. As for the flavor parameter, the evaluators felt better taste than other treatments only when consuming samples containing less oil and starch.

Table 2. The effect of edible coating on the sensory parameters of grape clusters.

Sensory parameters	Color		Odor		Flavor		Appearance		Sensory Texture		Overall Acceptance	
	Time (day)		Time (day)		Time (day)		Time (day)		Time (day)		Time (day)	
Sample	28	14	28	14	28	14	28	14	28	14	28	14
Control	3.70^{bcdef}	3.70^{bc}	3.20^{ab}	3.30^{ab}	2.80^{bcdef}	2.80^{abc}	3.50^{fg}	3.60^{b}	3.50^{def}	3.40^{a}	3.10^{bcdef}	3.10^{abcde}
1	3.20^{abcd}	3.60^{bc}	3.00^{ab}	3.60^{b}	1.90^{ab}	2.50^{abcd}	2.50^{abcd}	3.50^{ab}	3.00^{abcd}	3.20^{a}	2.70^{abc}	3.00^{abcde}
2	3.50^{bcdef}	2.80^{ab}	3.90^{cd}	2.50^{a}	2.60^{abcde}	2.00^{a}	2.50^{abcd}	2.60^{ab}	3.00^{abcd}	2.60^{a}	2.90^{abcd}	2.30^{a}
3	3.30^{abcd}	3.40^{bc}	3.50^{abc}	3.40^{ab}	2.60^{abcde}	3.30^{bcd}	2.50^{abcd}	3.00^{ab}	3.10^{abcde}	3.00^{a}	2.80^{abcd}	3.10^{abcde}
4	3.10^{abc}	3.40^{bc}	3.60^{bcd}	3.30^{ab}	2.20^{abc}	2.40^{abc}	2.10^{a}	3.40^{ab}	3.30^{cdef}	3.00^{a}	3.00^{bcde}	2.90^{abcde}
5	3.10^{abc}	3.00^{bc}	3.40^{abc}	3.10^{ab}	1.70^{a}	2.50^{abcd}	2.20^{a}	2.40^{a}	3.40^{def}	2.60^{a}	2.80^{abcd}	2.50^{abc}
6	3.00^{ab}	3.80^{c}	3.30^{abc}	2.90^{ab}	2.80^{bcdef}	2.90^{abcd}	2.60^{abcde}	3.30^{ab}	3.50^{def}	3.30^{a}	3.00^{bcde}	3.00^{abcde}
7	3.60^{bcdef}	3.60^{bc}	3.40^{abc}	3.50^{ab}	3.50^{ef}	3.60^{d}	3.00^{cdef}	3.50^{ab}	3.00^{abcd}	3.10^{a}	3.40^{defg}	3.80^{e}
8	3.60^{bcdef}	3.40^{bc}	3.60^{bcd}	3.10^{ab}	2.40^{abcd}	2.80^{abcd}	3.20^{defg}	3.20^{ab}	3.30^{cdef}	2.90^{a}	3.10^{bcdef}	2.80^{abcd}
9	3.20^{abcd}	3.40^{bc}	3.70^{bcd}	3.60^{b}	3.60^{f}	3.50^{cd}	3.30^{efg}	3.70^{b}	3.60^{def}	3.40^{a}	3.90^{g}	3.60^{de}
10	3.80^{cdef}	3.60^{bc}	3.60^{bcd}	3.40^{ab}	3.30^{def}	2.60^{abcd}	3.50^{fg}	3.50^{ab}	3.70^{ef}	3.20^{a}	3.60^{efg}	2.70^{abcd}
11	3.50^{bcdef}	2.90^{abc}	2.80^{a}	3.00^{ab}	2.30^{abc}	2.70^{abcd}	2.60^{abcde}	2.60^{ab}	2.60^{ab}	3.10^{a}	2.30^{a}	2.40^{ab}
12	3.40^{bcde}	3.20^{bc}	3.50^{abc}	2.80^{ab}	2.10^{abc}	2.30^{ab}	2.50^{abcd}	2.70^{ab}	2.70^{abc}	2.90^{a}	2.80^{abcd}	3.20^{a}
13	3.60^{bcdef}	3.40^{bc}	3.30^{abc}	3.20^{ab}	2.80^{bcdef}	2.90^{abcd}	2.90^{bcdef}	3.50^{ab}	2.50^{a}	3.70^{a}	3.20^{cdef}	3.10^{abcde}
14	3.90^{def}	3.20^{bc}	3.70^{bcd}	3.20^{ab}	2.80^{bcdef}	3.10^{abcd}	3.30^{efg}	3.40^{ab}	3.10^{abcde}	3.00^{a}	3.40^{defg}	2.90^{abcde}
15	3.40^{bcde}	3.30^{bc}	3.50^{abc}	2.90^{ab}	2.10^{abc}	2.90^{abcd}	2.60^{abcde}	3.60^{b}	2.60^{ab}	3.50^{a}	2.90^{abcd}	3.20^{abcde}
16	4.10^{ef}	3.70^{bc}	4.30^{d}	3.60^{b}	2.90^{cdef}	3.30^{bcd}	3.80^{g}	3.10^{ab}	3.80^{def}	3.60^{b}	3.70^{fg}	3.30^{bcde}
17	4.20^{f}	3.20^{bc}	3.50^{abc}	3.30^{ab}	2.80^{bcdef}	2.70^{abcd}	2.80^{acdef}	2.90^{ab}	3.60^{def}	3.70^{a}	3.20^{cdef}	3.00^{abcde}
18	2.60^{a}	2.10^{a}	3.30^{abc}	3.30^{ab}	1.80^{a}	3.30^{bcd}	2.70^{abcde}	2.40^{a}	3.10^{abcde}	3.20^{a}	2.50^{ab}	3.10^{abcde}
19	3.60^{bcdef}	3.30^{bc}	3.10^{ab}	3.10^{ab}	2.40^{abcd}	2.20^{ab}	2.30^{abc}	3.30^{ab}	3.10^{ab}	3.60^{a}	2.90^{abcd}	3.40^{cde}
20	3.40^{bcde}	3.40^{bc}	3.60^{bcd}	3.50^{ab}	2.90^{cfef}	2.60^{abcd}	2.40^{abc}	2.60^{ab}	3.40^{def}	3.40^{a}	3.10^{bcdef}	2.90^{abcde}
Standard Error	0.053	0.063	0.052	0.065	0.068	0.077	0.056	0.072	0.048	0.073	0.047	0.065

Conclusion

Edible coatings due to the use of biodegradable, recyclable, and edible in their production have become a useful alternative to synthetic films and coatings. Using the combination of 2 gr rice bran oil, 6.57 gr acetylated potato starch, and stirring speed of 0.35×10^{-3} g was selected as the best treatment because it was led to the coating with the highest stability and the least a_w and weight loss. Moreover, it improved the chemical properties of grapes by reducing oxygen transfer and controlling moisture diffusion. Also, it increased the shelf life (more than 2 weeks) and the marketability of grape samples. It was also showed that the employment of the edible coating based on the APS and RBO increased the brightness as well as the acidity of the grape cluster while their pH and TSS were decreased during four-week storage. Moreover, the coated Samples with higher levels of Oil and less Starch scored higher in terms of all sensory parameters except taste and were more desirable from the point of view of evaluators.

References

Abdollahi, A., 2008. Effect of three herbal senses on the control of fungal diseases after harvest of two grape varieties. Master Thesis. Uremia University.

Akbarian, M., 2012. Optimization of formulations of edible coatings and osmotic solutions in osmotic dehydration and drying of fruit "by" by response surface methodology. Master Thesis, University of Tabriz - Aras International Campus [In Persian].

Asghari, M., Ahadi, L., 2012. The effect of application post-harvest Salicylic acid and Aloe-vera gel on the qualitative characteristics and antioxidant activity of Uzum red Grape cultivar. HorticSci, Agric Sci and Ind., 3, 342-349. [In Persian]

Baldwin, E. A., 2006. Use of edible coating to preserve pecans at room temperature. J. Food Research International. 41, 188-192.

Baliga, B., Madaiah, N., 1970. Quality of Sausage emulsion prepared from mutton. J. Food Sci. 35, 383-385.

Basiak, E., Lenart, A., Debeaufort, F., 2017. Effect of starch type on the Physico-chemical properties of edible films. Inter J. Biol Macro.

Debeaufort, F., Peroval, C., Despre, D., Courthaudon, J. T., Voilley, A., 2002. Arabinoxylan – Lipid-based edible films and coatings. Influence of drying temperature on film structure and functional properties. J. Agric and Food Chem. 50, 2423-2428.

Ebrahimi, B., Mohammadi, R., Rouhi, M., Mortazavian, A.M., Shojaee-Aliabad, S., Koushki, M.R., 2018. Survival of probiotic bacteria in carboxymethyl cellulose-based edible film and assessment of quality parameters. J. Food Sci and Technol. 87, 54-60.

Emamifar, A., 2014. Evaluation of the effect of Aloe-vera gel as an edible coating on the microbial, physicochemical and sensory characteristics of fresh Strawberries during storage. Food Sci and Technol., 2nd Year, 6, pp. 15-29. [In Persian]

Farayde M., F., Silvia Maria, M., Thiago, C., José Ignacio, V., Lucia Helena, I.M., 2015. Edible films and coatings based on starch/gelatin: film properties and effect of coatings on quality of refrigerated red crimson grapes. J. Postharvest Biol and Technol. 109, 57-64.

Galvis-Sanchez, A. C., Fonseca, S. C., Morais, A. M., Malcata, F. X., 2003. Physicochemical and sensory evaluation of, Rocha" Pear following controlled atmosphere storage. Food Sci., 68, 318-327.

Gerossi, F., Javanmard, M., Hassani, F., 2009. Application of edible coating based on whey protein and Gellan gum for Apricot fruit. Islamic Azad University, Department of Food Ind. Food Sci and TechnoL., 8, 29. [In Persian]

Grist, D.H., 1985. Rice, 5th edn (Longman, London, UK).

Hassani, F., Javanmard, M., Grossiy, F., 2010. Evaluation of shelf-life of Kiwi fruit coated with whey protein concentrate and rice bran oil. Food Sci and Technol Res., 167-158. [In Persian]

Iranian National standard. 1992. Fruit and vegetable products- determination of acidity- Test method. Iranian Institute of Standards and Industrial Research (First ed), pp. 373. [In Persion]

Karabulut, O.A., Smilanick, J.K., Franka, M., Mansour, M., Droby, S., 2003. Near-harvest applications of Metschnikowia fruit cold, ethanol and sodium bicarbonate to control postharvest diseases of grape in Central California. J. Plant Disease. 87, 1384-1389.

Luallen, T. E., 1985. Starch is a functional ingredient. J. Food Technol. 39, 59-63.

Luallen, T. E., 1988. Structure, characteristics, and uses of some typical carbohydrate food ingredients. J. Cereal Foods World. 33, 924-927.

Marıa, A., Garcı´a, M. N., Martino and Noemı´, E. Zaritzky., 1998. Plasticized Starch-Based Coatings to Improve Strawberry (Fragaria _ Ananassa) Quality and Stability. J. Agric Food Chem. 46, 3758-3767.

Mariniello, L., Di Pierro, P., Esposito, C., Sorrention, A., Masi, P., Porta, R., 2003. Preparation and mechanical properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. J. Biotechnol. 102, 191-198.

Mustonen, H.M., 1992. The efficacy of a range of sulfur dioxide generating pads against Botrytis cinerea infection and on out-turn quality of Calmeria table grapes. J. Experimental Agric. 32, 389-393.

Ochoa-Velasco, C. E., Guerrero-Beltran, J. A., 2014. Post-harvest quality of peeled prickly Pear fruit treated with Acetic acid and Chitosan. Post-harvest Biol and Technol., 92, 139-145.

Parvaneh. v., 1986. Food quality control and chemical testing. Tehran University Press.Rajabian, M., 2018. Study of the effect of edible coating of potato starch containing mountain coconut essential oil and thyme on the microbial and chemical properties of chicken breast kept at refrigerator temperature. Ph.D. Thesis in Food Health, Shahrekord University.

Rajabian, M., 2018. Study of the effect of edible coating of potato starch containing mountain coconut essential oil and thyme on the microbial and chemical properties of chicken breast kept at refrigerator temperature. Ph.D. Thesis in Food Health, Shahrekord University. [in Persion]

Riku, A., Talja, H., Hele´n, Y.H., Roos and Jouppila, K., 2007. Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. J. Carbohydr Polym. 67, 288-295.

Sala, G., T. Van Vliet, M. A., Cohen Stuart, G. A., Van Aken, F. van de Velde., 2009. Deformation and fracture of emulsion-filled gels. Effect of oil content and deformation speed. J. Food Hydrocoll. 23, 1381-1393.

Semeijn, C., Pieter L. Buwalda., 2018. Potato Starch. Chapter 9.

Schierbaum, F., Kettlitz, B., 1994. Studies on rye starch properties and modification. Part III. Viscograph pasting characteristics of rye starches. Starch/Stärke. 46, 2-8.

Shaw, N. B., Monahan, F. J., Ó Riordam, E. D., Ó Sullivan, M., 2002. Effect of soya oil and glycerol on physical properties of composite WPI film. J. Food Eng. 51(4), 299-304.

Shirin, S., Asghar, R., 2014. Effect of natural aloe vera gel coating combined with calcium chloride and citric acid treatments on grape (Vitis vinifera L. Cv. Askari) quality during storage. J. Food Sci and Technol. Vol. 2, No. 1, 1-5.

Smilanick, J.L., Harvey, J.M., Hartsell, P.L., Henson, D.J., Harris, C.M., Fouse, D.C., Assemi, M., 1990. Factors influencing sulfite residues in table grapes after sulfur dioxide fumigation. J. Enol and Vitic. 41, 131-136.

Tournas, V.H., Katsoudas, E., 2005. Mold and Yeast flora in fresh berries, grapes, and citrus fruits. Inter. J. Food Microbio. 105, 11-17.

RADIATION LEVELS IN SAMPLES OF DICALCIUM PHOSPHATE (DCP) WITH A GAMMA SPECTROMETRY METHOD

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Abstract

Radioactive contamination of living organisms and body tissues primarily depends on the level of contamination of the food they consume, and to a lesser extent on drinking water and inhalation. The aim of this study was to determine the level of radioactivity present in dicalcium phosphate (DCP) samples used as a feed additive. DCP is used as a major source of phosphorus and calcium because it strengthens the skeleton and accelerates the growth of the animal. It is therefore important to familiarize yourself with these levels as part of this radioactivity is likely to be transmitted to humans through the food chain. Measurements were made in order to determine the risks that those quantities may bring. Radioactivity levels are measured using a standard spectroscopic system with a high-resolution HPGe detector. The mean values for the measured activities in the DCP samples were 5.27Bq.kg-1 for 226Ra, 2.87 Bq.kg-1 for 228Th. The mean value measured for 40K was 22.26 Bq.kg-1, respectively. From the results obtained for DCP, it can be seen that the activity of 226Ra is significantly lower than the activity of 228Th, while the value measured for 40K in the samples does not pose a risk to human health, even to animals, because potassium is an essential mineral for living organisms. Considering that the radioactive contamination of the tissues of the animal body originates primarily from the level of contamination of the used animal feed, as well as from the water used for irrigation of the animals, a preventive measure is to control the radioactivity of the feed in their use, i.e. the concentration of radioactive isotopes should be as low as possible, ensuring that they do not pose a threat to the animal organism.

Key words: *DCP, gamma spectrometry, feeds.*

Introduction

Minerals are unavoidable elements in daily animal nutrition, which should be offered in quantities by following animal's requirements. In the same time, the fact that minerals e.g. phosphorus play an important part in environmental contamination should be taken into consideration. Therefore, animal nutritionist should balance animal's daily diet by using high quality phosphorus and in the same time by limiting the excess amount of phosphorus into the environment.
There is a significant need for radiological analysis in the environment, i.e. the soil, food for humans and animal feed. For radioecology, the study of the migration of radionuclides in the soil as a basic resource in agricultural production is of particular interest. In most cases the radionuclides in the soil are tightly bound, however the root of the plant can still absorb them.

Since the first discovery of radioactivity, there has been concern about the damage it can cause to humans, living things and the environment. Many studies have been devoted to monitoring the amount of activity of radionuclides in food and dietary supplements, especially those related to human nutrition, in order to determine the risks that those amounts may bring (Tchokossa et. Al., 2013; Zagato et al., 2007).
The process of artificial fertilizer production distributes radionuclides into the environment and in the same time incorporates them into final products and intermediate products (Saueia et al., 2005). The phosphate rock that is usually used for phosphate fertilizer production, contains natural radionuclides from U and Th series (Mazzilli et al., 2000). Phosphoric acid is a starter material for production of: triple superphophate, simple mono superphosphate, monoamonia phosphate and diamonia phosphate, Nitrogen-Phosphorus-Potassium fertilizer and dicalcium phosphate.
Phosphate-based products, such as dicalcium phosphate (DCP) and monocalcium phosphate (MCP) contain approximately 20% phosphorus, and for this reason they are important in the modern production of live raw materials. These compounds are most commonly used in the diet of cattle, pigs and poultry (Arruda et al., 1997), as well as as a dietary supplement in humans. Normally, animal feed is developed on an organic basis and is intended to provide the most complete nutrition possible. In order to increase the nutritional value, elements that can actually increase the amount of radionuclide activity in animal feed are increasingly added, which was discussed by Arruda et al., (1997). It should be noted that phosphorus is one of the elements often added to food, as it is one of the most important minerals for living organisms, including humans. It is an essential element for all living cells and a key element for the cellular energy transportation (Roessler, 1990). It affects several important biological processes, such as osmotic pressure and all metabolic reactions (Casacuberta et al., 2007).
In this study, radioactivity was measured in a representative number of DCP samples, wherewith specific activities of certain natural radionuclides were calculated, compared with other studies and calculated doses.

Materials and Methods

Sampling
The samples were placed in 0.5 l Marinelli beakers which were fully filled, sealed and stored in order to establish a balance between ^{226}Ra and ^{222}Rn before the measurements are performed. (6) Eighteen samples of DCP were analyzed, which were being used as a feed additive. The measurements were performed at intervals and the background spectrum of the acquisition system is checked on a weekly basis. The process of homogenization of these samples was performed according to the international standard protocol to ensure homogeneity. All studied samples of animal feed were from different farms in the Republic of Macedonia.

Instrument
The samples were measured on an instrument-gamma spectrometer (Canbera Packard) with a high purity germanium detector. The measurement was carried out in beakers that were hermetically sealed so that ^{222}Rn produced by the decomposition of ^{226}Ra would not result in gas leakage. After ensuring a time balance between the successors of the ^{238}U and ^{232}Th series, these sealed samples were prepared for analysis. GENIE 2000 software was used for data acquisition and analysis. The specific activity of ^{226}Ra is calculated for the energy line of 186.1 (keV) and

^{232}Th through its decay descendant ^{228}Ac (second in the decay chain), i.e. through its three gamma decay energy lines which occur at 338.4; 911.07 and 968.9 (keV).
The activities of ^{40}K were determined from its γ-line of 1460 keV, while the activities of ^{137}Cs were determined by means of an estimation of the γ-line at 661.66 (keV). The time interval for calculation (counting) was 108000 seconds.

Activity calculation
The specific activity (A) is determined according the equation

$$A = \frac{\frac{N}{t} - \frac{N_0}{t_0}}{\varepsilon \cdot \gamma \cdot m} \quad (\mathrm{Bq \cdot kg^{-1}})$$

Where, N is clean surface of peak accumulated from a specific radionuclide in analysis of a specific sample (number of readings), N_0 is clean surface of peak accumulated from the spot of a specific radionuclide without an analysis of sample (number of readings), t is live time of accumulation of the sample spectrum (s), t_0 is live time of accumulation of the phone spectrum (s), ε is detector efficiency for a given energy (for a specific peak), γ is intensity of gamma transition in radioactive decay for a respective radionuclide (%), and m is sample mass (kg) (Garcia-Talaver, 2007).

Radium equivalent activity Ra_{eq}
The calculation of the radium equivalent activity (Raeq) is a value for comparing the specific activities of the samples with different contents of ^{226} Ra, ^{232}Th and ^{40}K. The uniformity with respect to radiation exposure was defined in terms of the radium equivalent activity (Raeq) in Bq/kg in order to compare the specific activity of the materials containing different amounts of ^{226}Ra, ^{232}Th and ^{40}K. It is assumed that 370 Bq/kg of ^{226}Ra, 259 Bq/kg of ^{232}Th and 4810 Bq/kg of ^{40}K produce the same gamma-ray dose rate. It is calculated by using the following ratio (Beretka and Methew, 1985).

Ra_{eq}(Bq/kg)= $A_{Ra}+1.43A_{Th}+0.07A_k$

A_{Ra}, $A_{Th,}$ A_k - specific activiites (Bq/kg) of ^{226}Ra, ^{232}Th and ^{40}K, respectively. The value of the radium equivalent activity of 370 Bq/kg corresponds to the maximum allowed dose for a population of 1 mSv.

External and internal hazard index
In order to assess the equivalent average of the annual effective dose imposed to the residents of each area, the external hazard index for the soil samples was calculated.
$H_{eks} = A_{Ra}/370 + A_{Th}/259 + A_k/4810 \leq 1$
A_{Ra}, $A_{Th,}$ $A_{k\,-}$ specific activities (Bq/kg), ^{226}Ra, ^{232}Th and ^{40}K, respectively (Kurnaz et al., 2007)

Results and discussion

The different radionuclide activities in DCP samples could be derived from two causes: the geological characteristics of the phosphate rock; and the industrial manufacturing processes used for its production. According to the obtained results in regard to DCP, it is observable that the activity of ^{226}Ra is higher than the activity of the thorium series. The mean value for ^{226}Ra was

(5.27) Bq.kg-1, while the value for ^{232}Th reached (2.87) Bq.kg-1. According to Silva et al., (2001), ^{226}Ra is the biggest source of radioactivity in phosphogypsum and can enter living organisms because it follows the same biological pathway of calcium. However, the activity for ^{40}K was (22.26) Bq.kg -1, i.e. with the highest value in all samples of DCP, since K is an essential element for living organisms, therefore the radioactivity of ^{40}K cannot be avoided (Abbady et al., 2005). Thus, the results regarding the activity for this radionuclide in this research and for the DCP samples comply with the expectations and do not create any additional concern.

Table 1. Radionuclide concentration of ^{232}Th, ^{226} Ra, ^{40}K in DCP (Bq/kg)

^{40}K ($\bar{x} \pm S_{\bar{x}}$)	**^{226}Ra ($\bar{x} \pm S_{\bar{x}}$)**	**^{232}Th ($\bar{x} \pm S_{\bar{x}}$)**
31.3±2.0	1.70±0.3	7.63±2.0
<7.80	0.30±0.1	<4.5
45.24±1.0	6.23±0.1	5.22±2.0
51.1±0.4.	6.82±0.2	6.13±2.0
21.5±2.0	2.04±0.2	<7.1
23.0±1.0	7.73±0,3	3.03±1.5
26.31±2.2	2.55±0.2	4.54 ±1.0
33.37±1.0	1.42±0.1	6.00±1.0
<9	<1.3	<3.1
<5.4	<2.0	<4.20
11.54±2.0	1.66±0.2	2.97±2.1
<6.20	<1.00	<1.0
31.32±2.0	2.13±0.4	4.13±0.2
32.56±1.0	1.65±0.1	4.22±0.2
15.71±1.0	<1.5	<4.20
21.33±2.2	1.12±0.2	<4.5
34.15±2.0	31.3±0.5	4.17±1.0
22.40±2.0	31.3±0.3	3.66±2.0

Table 2. Comparison of the DCP activities in the research literature

Reference	^{40}K	^{226} Ra	^{232}Th
Present work	22.26	5.27	2.87
Casacuberta (2007)	28	/	<148
Saueia (2005)	148	<2	10
Arruda Neto (1997)	/	/	/

The mean value of the radiation risk index H_{eks} is minimum and it amounts to 0.03, and its value is lower than the maximum allowed value which is <1 for H_{eks}. The value of the radium equivalent activity Ra_{eq} is below the maximum recommended limit, i.e. 370 Bq kg^{-1} and it amounts to 10.93 Bq.kg^{-1}.

The specific activity values and the calculated radiation risk index (H_{eks}) and radium equivalent (Ra_{eq}) obtained in this study also did not exceed the safety limits, noting the negligible radiation hazard arising from naturally occurring terrestrial radionuclides.

Conclusions

The activity concentrations of natural radionuclides, ^{40}K, ^{226}Ra and ^{232}Th, were assessed in this study, in different samples of DCM used in the Republic of Macedonia. The results were also compared with other similar studies in other parts of the world. However, the values of the concentrations of the tested radionuclides are within the permissible values, i.e. the transmission of such levels to animal feed and, ultimately, to humans, through the radionuclide pathway, will not pose a threat when humans eventually consume poultry meat, poultry products and eggs from poultry fed with this food. However, it would be necessary to establish radionuclide monitoring systems in the main food in order to reduce human exposure to radiation through the consumption of animal products. Prevention would be the best manner to reduce radioactive contamination in animals and further in consumers as well. Monitoring the level of natural and artificial radionuclides in animal feed in order to mitigate the amount of radioactive substances that can reach the human body through the trophic chain is the basis for radiological contamination.

References

Abbady A., Uosif M., El-TaherA., (2005). Natural radioactivity and dose assessment for phosphate rocks from Wadi El-Mashash and El-Mahamid Mines, Egypt. Journal of Environmental Radioactivity 84, 65-78.

Arruda-Neto J.D.T, Tavers M.V., Filadelfo M. (1997). Concentrations of uranium in animal feed supplements: Measurements and dose estimate. Journal of Radioanalytical and Nuclear Chemistry, volume 221, pages 97–104.

Beretka, J. Methew, P.J. (1985). Natural radioactivity of Australian building materials, industrial wastes and by-products, Health Physics 48, 87-95

C.E. Roessler C.E. (1990) Control of radium in phosphate mining, beneficiation and chemi-cal processing, The Environmental Behaviour of Radium, vol.2, Technical Report Series, No. 310, IAEA Vienna, pp. 269–279.

Casacuberta, N., Masque, P., Garcia-Orellana, J., Bruach-Menché, J.M., Sanchez-Cabeza, J.A., Villa, M., Hurtado, S., Manjón, G., Garcia-Tenorio, R. (2007). Dicalcium Phosphate: Contents of Uranium Decay Series. NORM V, March 19-22, Seville, Spain.

Garcia-Talaver,. M. (2003). Evaluation of the Suitability of Various γ Lines for the γ Spectrometric Determination of 238U in Environmental Samples. Applied Radiation & Isotopes. 59: 165-173.

Kurnaz A, Küçükömeroğlu B, Keser R, Okumusoglu NT, Korkmaz F, Karahan G, Çevik U. (2007). Determination of radioactivity levels and hazards of soil and sediment samples in Fırtına Valley (Rize, Turkey). Applied Radiation and Isotopes. 65: 1281–1289.

Mazzilli B., Palmiro V., Saucia C., Nisti M.B. (2000). Radiochemical Characterization of Brazilian Phosphogypsum." Journal of Environmental Radioactivity, Vol. 49, No. 1, pp. 113-122.

Saueia C.H., Mazzilli B. P., Favaro D. I. T. (2005). Natural Radioactivity in Phosphate Rock, Phosphogypsum and Phosphate Fertilizers in Brazil. Journal of Radioanalytical and Nuclear Chemistry, Vol. 264, No. 2, 2005, pp. 445-448.

Silva, N.C., Fernandes, E.A.N., Cipriani, M., Taddei, M.H.T. (2001). The natural radioactivity of Brazilian phosphogypsum. Journal of Radioanalytical and Nuclear Chemistry 249, 251-25.

Tchokossa P., Olomo J.B., balogun F.A. and C.A. adesanmi C.A. (2013). Assessment of Radioactivity Contents of Food in the Oil and Gas Producing Areas in Delta State, Nigeria. International Journal of Science and Technology Vol 3, No. 4, pp 245 - 250.

Tufail, M., Iqbal, M., Mirza,. S. M. (2000). Radiation Doses Due to Natural Radioactivity in Pakistan Marble. Radioprotection, Vol. 35, No. 3, pp. 299-310.

Zagato, V. A. B. Medina, N. H.; Okuno, E.; Umisedo, N.K. (2007). Natural Radiactivity in Bananas. 8th International Symposium (NRE VIII) AIP Conf. Proc. 1034, pp. 264-268.

BACILLUS AMYLOLIQUEFACIENS I3: AN EFFECTIVE BIOCONTROL AGENT IN THE INDUCTION OF SYSTEMIC RESISTANCE IN *ARABIDOPSIS THALIANA*

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Abstract

The bacteria *Bacillus* species were described as inducers of plant systemic resistance in relation to their antagonistic activity. The objective of this study was to evaluate the effect of selected strain of *Bacillus amyloliquefaciens* (I3) on inducing systemic resistance in *Arabidopsis thaliana* as a model for plant molecular genetics. The microorganisms were identified and confirmed for their antagonistic potential *in vitro* and *in vivo* in previous studies. In order to explore this mechanism, two mutants of *A. thaliana* carrying a *PR1* promoter (a conventional marker of salicylic acid (SA) pathway) and *LOX2* promoter (a marker triggering jasmonic acid (JA) pathway activation) were analyzed after inoculating antagonists. Transgenic reporter line analysis demonstrated that *B. amyloliquefaciens* I3 induced *A. thaliana* defense pathways by activating SA and JA at a high level compared to lines treated with chemical elicitors of reference~~s~~ [acibenzolar-*S*-methyl; Bion 50 WG, SA, and methyl jasmonate]. The efficacy of *B. amyloliquefaciens* I3 in inducing the defense mechanism in *A. thaliana* was demonstrated in this study.

Keywords: *seed treatment, Bacillus amyloliquefaciens I3, salicylic acid, jasmonic acid, β-glucuronidase.*

Introduction

Among the biological control alternatives to the extensive use of chemical pesticides, the application of non-pathogenic bacteria living in association with plant is promising. Treatment with these microorganisms was in many cases associated to the reduction of plant diseases severity. As bacterial treatment at the root level confers protection against foliar or systemic pathogens, has been termed induced systemic resistance (ISR) (Kloepper *et al.*, 1992). ISR is effective against a broad range of diseases, the potential to activate ISR is assigned to antagonistic microorganisms (van Loon *et al.*, 2006) or to their secondary metabolites (Ongena *et al.*, 2007). The plant defense mechanism is typically related to ISR activation (Mejri *et al.*, 2017; van Wees *et al.*, 2008), the signaling pathways of which depend on cultivars, pathogen, and microorganism inducer (Leeman *et al.*, 1995; van Wees *et al.*, 1997). *Bacillus amyloliquefaciens* I3 isolated from wheat leaves in Morocco demonstrated high antagonistic potential against *Zymoseptoria tritici in vitro* and in greenhouse conditions (Barakat *et al.*, 2018). Also, it was demonstrated that surfactine extracted from *B. amyloliquefaciens* strain S499 triggers ISR in wheat against *Z. tritici* (Le Mire *et al.*, 2018).

In this study, the objective was to evaluate the potential of *B. amyloliquefaciens* I3 to induce and activate defense pathways in *Arabidopsis thaliana* by seed treatment. This property was described *in vitro* and *in vivo* using mutant lines of *A. thaliana* which report the activity of gene promoters *PR1* and *LOX2*, markers of salicylic acid (SA) and jasmonic acid (JA) defense pathways, respectively.

Materials and Methods

Biological Material

Mutant lines pPR1::GUS and pLOX2::GUS (N6357 and N57953, respectively) were used as reporter lines for SA and JA activity, respectively. These lines were provided by Nottingham *Arabidopsis* Stock Center.

In-plant analysis of β-glucuronidase (GUS) activity in *Arabidopsis* lines, genetically transformed by transcriptional fusions of the *UIDA* gene with promoters of the *PR1* (PR1 line) and *LOX2* (LOX2 line) genes allows the activation of these promoters already described as markers of induction of the SA and JA pathways, respectively (Millet *et al.*, 2010; Jensen *et al.*, 2002).

Preparation of Antagonist Inoculum

B. amyloliquefaciens I3 inoculum was prepared in Luria Bertani (LB) Agar (Luria et Burrous, 1957) and incubated 72 h at 27 °C in the dark.

Arabidopsis Seedling Preparation

Ten milligrams of seeds (6000 *Arabidopsis* seeds) were stirred in 1 mL of sterilizing solution (ethanol 70%, sodium hypochlorite 10%) for 10 min, subsequently rinsed three times with ethanol 70%, and dried up using Whatman papers disinfected with ethanol.

Arabidopsis was cultivated *in vitro* and *in vivo* for studying its different early growth stages (germination, cotyledons, leaf production, and rosette production); also, on late growth stages (flowering and silique ripening). *In vitro* seedlings were grown in Murashige and Skoog basal salt mixture agar media in petri plates of 90 mm in diameter. *In vivo* seedlings were sown in pots of 7 × 7 × 8 cm, with three seeds in each pot.

Seeds were stratified at 4 °C in the dark before adding the antagonist suspension. Afterwards, 10 µL of the antagonist suspension (10^9 colony-forming units (cfu)/mL) were added on the top of each seed in *in vitro* conditions. In the *in vivo* conditions, the substrate was inoculated with 8 mL of the antagonist suspension (10^9 cfu/mL). Both plates and pots were transferred to a growth room kept at long-day conditions.

In this study, four controls were made: a negative control in which seeds were treated with sterile milliQ water, and positive controls where seeds were treated with acibenzolar-*S*-methyl at 925 µM (Bion 50 WG), SA at 140 µg/mL which induced *PR1* activity, and methyl jasmonate (MeJa) at 60 µM which induced *LOX2* activity in reporter cell lines. All plants in controls were treated using foliar spray 24 h before being stained with GUS solution.

Histochemical Staining

Ten plants were used to study every growth stage modality cultivated *in vitro*, and three plants were used to study in vivo growth modality in pots. Plants were dipped in GUS mixture for 12 h and incubated in the dark at 37 °C while stirring for 150 rotations per minute. Tissues were then washed with ethanol 70%. Stained tissues were studied with a binocular loupe, and the ratio of surface area stained over total surface area of each plant was estimated using ImageJ software. This assay was repeated three times.

Statistical Analysis
SPSS 21 statistic software was applied for histochemical data analysis. Statistical analyses were established at two-factor level; the first corresponded to treatments (*B. amyloliquefaciens* I3, Bion 50 WG, SA, MeJa, and negative control) and the second factor indicated the growth stages (germination, cotyledons, leaf production, rosette, and flowering). ANOVA analysis was applied for analysis of mean data variation, while the Duncan test was used for the comparison of means at p = 0.05.

Results and discussion

The antagonist inoculation in transgenic *Arabidopsis* (*PR1* and *LOX2* reporter lines) demonstrated the induction of SA (*PR1* line) and JA (*LOX2* line) pathways. Results of β-glucuronidase (GUS) staining in both reporter lines (Figures 1 and 2) whose seeds were treated by *B. amyloliquefaciens* I3 revealed the effective activation of both *LOX2* and *PR1* promoters and, thus, the induction of SA and JA pathways. Similar GUS staining results were shown in lines treated with chemical elicitors (Bion 50 WG, MeJa, and SA) at different growth stages (germination, cotyledons, leaf production, rosette, and flowering).

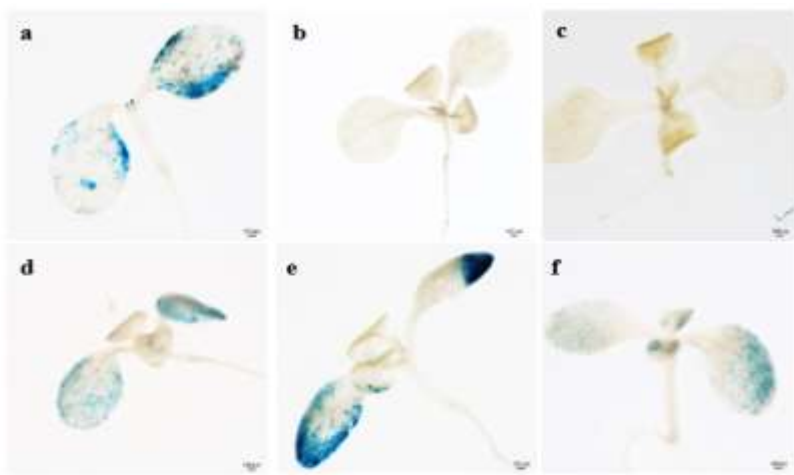

Figure 1. β-Glucuronidase (GUS) activity in pPR1::GUS (**a, d, e**) and pLOX2::GUS (**b, c, i**) lines of *A. thaliana* at cotyledon stage: (**a, c**) seeds treated with *B. amyloliquefaciens* I3; (**d**) treatment by Bion 50 WG; (**e**) treatment by SA; (**b**) treatment by milliQ water; (**f**) treatment by MeJa.

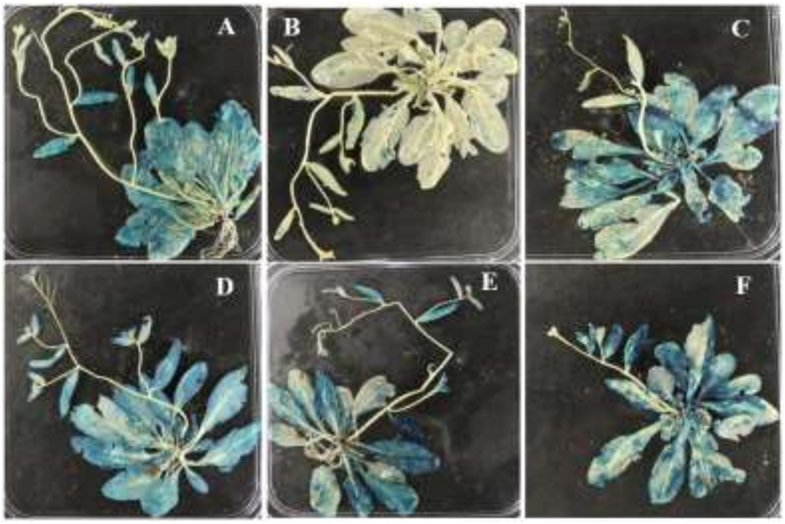

Figure 2. β-Glucuronidase activity in pPR1::GUS (**A, D, E**) and pLOX2::GUS (**B, C, F**) lines of *A. thaliana* at flowering stage: (**A**,**C**) seeds treated with *B. amyloliquefaciens* I3; (**D**) treatment by Bion 50 WG; (**E**) treatment by SA; (**B**) treatment by milliQ water; (**F**) treatment by MeJa.

GUS staining pattern in the germination stage of transgenic *Arabidopsis* lines carrying pPR1::GUS was observed in treated plants with the two positive controls, Bion 50 WG and SA. Plants inoculated with *B. amyloliquefaciens* strain I3 developed an intense histochemical staining which was dependent on the developmental stage. The former ranged from 51% in the cotyledon stage to 91% in the flowering stage. *Arabidopsis* lines carrying pPR1::GUS and treated with *B. amyloliquefaciens* I3 showed a very high GUS stain pattern compared to those observed in plants treated with water at different growth stages. GUS staining covered at the most 3% of the plants in the flowering stage treated with water (Figure 3).

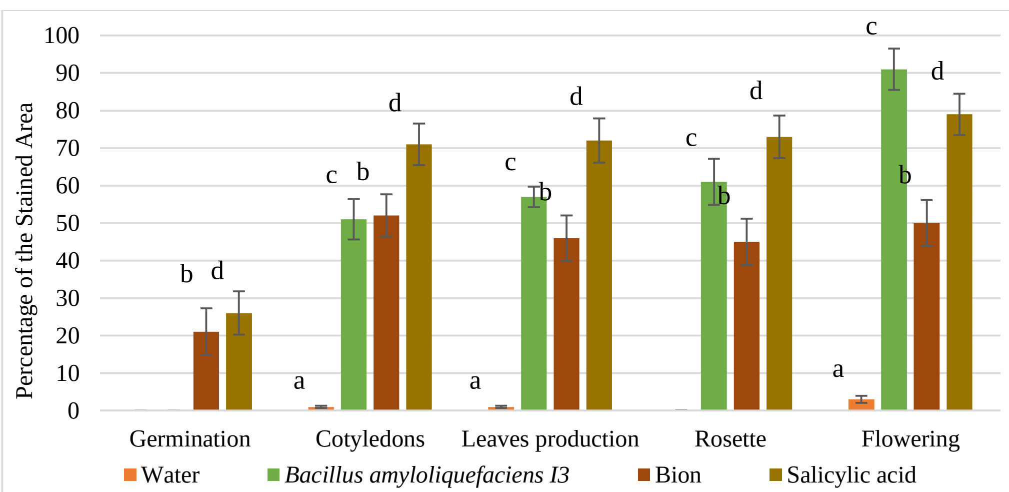

Figure 3. GUS stain pattern surface for each treatment and growth stage in the pPR1::GUS line. Bars correspond to standard errors of means. Letters denote homogeneous groups in the Duncan test at p = 0.05.

The transgenic *Arabidopsis* line carrying pLOX2::GUS, planted *in vitro,* did not show any GUS stain pattern when treated with both antagonists during all the studied developmental stages (germination, cotyledon, leaf production, rosette). On the other hand, when this line was treated with methyl-jasmonate, the GUS stain patterns ranged from 79% in germination to 81% in the rosette stage (Figure 4). Plants presented highly significant GUS stain differences in all the treatments in the flowering stage. The GUS stain patterns covered 84% of the leaf surfaces when plants were treated with *B. amyloliquefaciens* I3. The GUS stain patterns covered 82% of the leaf surfaces when treated with MeJa. On the other hand, the negative controls were stained on less than 2% of the total surface (Figure 4).

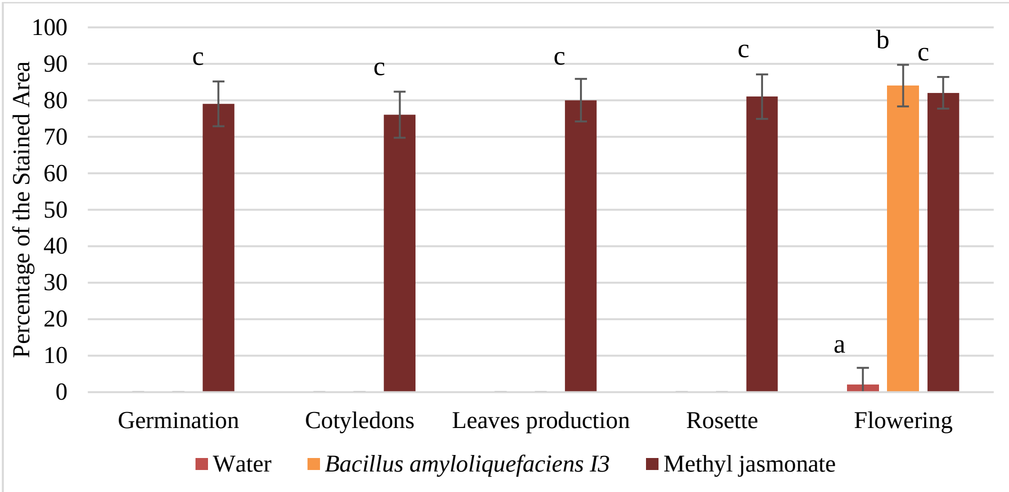

Figure 4. GUS stain pattern surface for each treatment and growth stage in the pLOX2::GUS line. Bars correspond to standard errors of means. Letters denote homogeneous groups in the Duncan test at $p = 0.05$.

In the present study, the induction of SA and JA pathways by *B. amyloliquefaciens* I3 from the cotyledons to the flowering stage was demonstrated using GUS staining patterns (Figures 1 and 2). The GUS staining pattern in the plant surface was significantly higher ($p = 0.05$) when plants were treated with antagonist at the flowering stage compared to the percentage of GUS dyes in plants treated with the commonly tested chemical references, Bion 50 WG and SA (Figure 3). There was no visible GUS staining in pLOX2::GUS lines observed in in vitro cultivated plants; however, in the flowering stage, the GUS staining was observed. On the other hand, the GUS staining pattern was very high in plant leaves treated with *B. amyloliquefaciens* I3, which demonstrate the potential of strains I3 in inducing the JA pathway (Figure 4). It is important to note that both transgenic plants exhibited a positive GUS staining and were also induced in the flowering and the silique ripening stages, while the antagonist inoculation was established in the substrate during the early developmental stages (Figure 2).

Our results are in agreement with the study conducted by Niu *et al.* (2011), who demonstrated that *B. cereus* AR156 induces ISR in *A. thaliana*, by simultaneously activating SA and JA pathways. Similarly, van Wees et al. (1997) proved that strain WCS417r of *Pseudomonas fluorescens* induces ISR in tomato and *A. thaliana* against *Pseudomonas syringae* pv. *tomato* and *Fusarium oxysporum* f. sp. *raphanin*, respectively. Ryu *et al.* (2004) also demonstrated that different plant growth-promoting rhizobacteria (PGPR) (*Serratia marcescens* 90–166, *Bacillus pumilus* SE34, *P. fluorescens* 89B61, *P. fluorescens* 89B27, *B. amyloliquefaciens* IN937, and *B. subtilis* IN937) induce SA and JA pathways in cucumber. However, it is important to note that surfactines and fengycines, which are lipo-peptides produced by the genus of *Bacillus*, may interact with plant membrane cells and disturb them (provoking stress), leading to a similar defense reaction in the plant that may be confused with ISR induction (Ongena *et al.*, 2007).

Conclusion

Our results demonstrated the potential of *B. amyloliquefaciens* to systemically induce SA and JA pathways in *A. thaliana.* According to the biocide effect, the antagonists may offer more sustainable plant protection in field conditions using the treated seeds with *B. amyloliquefaciens* I3.

Acknowledgments

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References

Barakat, I.; Chtaina, N.; El Guilli, M.; Ezzahiri, B. (2018). Evaluation du potentiel antagoniste de quelques isolats de bactéries et de *Trichoderma spp.* dans le contrôle de *Z. tritici* agent causal de la septoriose du blé. *Rev. Maroc. Sci. Agron. Vét*, *6*, 345–354.

Barakat, I.; Chtaina, N.; El Guilli, M.; Ezzahiri, B. (2019). Efficacy of *Bacillus amyloliquefaciens* and *Trichoderma harzianum* as biocontrol agent of septoria of wheat by seed coating. Revue Maroc. Prot. Plantes, 13, 9–18.

Jensen, A.B.; Raventos, D.; Mundy, J. (2002). Fusion genetic analysis of jasmonate-signalling mutants in Arabidopsis. *Plant J*. *29*, 595–606.

Kloepper JW, Tuzun S, Kùc JA. (1992). Proposed definitions related to induced disease resistance. Biocontrol Sci Technol 2:349–351

Le Mire, G.; Siah, A.; Brisset, M.N.; Gaucher, M.; Deleu, M.; Jijakli, M.H. (2018). Surfactin Protects Wheat against *Zymoseptoria tritici* and Activates Both Salicylic Acid-and Jasmonic Acid-Dependent Defense Responses. *Agriculture*, *8*, 11.

Leeman, M.; Van Pelt, J.A.; Den Ouden, F.M.; Heinsbroek, M.; Bakker, P.A.H.M.; Schippers, B. (1995). Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology*, *85*, 1021–1027.

Luria, S.E.; Burrous, J.W. (1957). Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol*. *74*, 461–476.

Mejri, S.; Siah, A.; Coutte, F.; Magnin-Robert, M.; Randoux, B.; Tisserant, B.; Krier, F.; Jacques, P.; Reignault, P.; Halama, P. (2017). Biocontrol of the wheat pathogen *Z. tritici* using cyclic lipopeptides from *B. subtilis*. *Environ. Sci. Pollut. Res.* 1–12.

Millet, Y.A.; Danna, C.H.; Clay, N.K.; Songnuan, W.; Simon, M.D.; Werck-Reichhart, D.; Ausubela, F.M. (2010). Innate Immune Responses Activated in Arabidopsis Roots by Microbe-Associated Molecular Patterns. *Plant Cell*, *22*, 973–990. doi:10.1105/tpc.109.069658.

Niu, D.D.; Liu, H.X.; Jiang, C.H.; Wang, Y.P.; Wang, Q.Y.; Jin, H.L.; Guo, J.H. (2011). The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate-and jasmonate/ethylene-dependent signaling pathways. *Mol. Plant-Microbe Interact*. *24*, 533–542.

Ongena, M.; Jourdan, E.; Adam, A.; Paquot, M.; Brans, A.; Joris, B.; Arpigny, J.L.; Thonart, P. (2007). Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol*. *9*, 1084–1090.

Ryu, C.M.; Murphy, J.F.; Mysore, K.S.; Kloepper, J.W. (2004). Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid dependent signaling pathway. *Plant J*. *39*, 381–392. doi:10.1111/j.1365-313X.2004.02142.x.

Van Loon, L.C.; Geraats, B.P.J.; Linthorst, H.J.M. (2006). Ethylene as a modulator of disease resistance in plants. *Trends Plant Sci. 11*, 184–191. doi:10.1016/j.tplants.2006.02.005.

Van Wees, S.C.M.; Pieterse, C.M.J.; Trijssenaar, A.; Van't Westende, Y.A.M.; Hartog, F.; Van Loon, L.C. (1997). Differential Induction of Systemic Resistance in Arabidopsis by Biocontrol Bacteria. *Mol. Plant-Microbe Interact. 10*, 716–724.

Van Wees, S.C.M.; Van Der, S.; Pieterse, C.M.J. (2008). Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant Biol*. *11*, 443–448. doi:10.1016/j.pbi.2008.05.005.

INTERFERENCE COMPETITION BETWEEN LADYBIRD BEETLE ADULTS (COLEOPTERA: COCCINELLIDAE) ON PRICKLY PEAR CACTI PEST *DACTYLOPIUS OPUNTIAE* (HEMIPTERA: DACTYLOPIIDAE)

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Abstract

The outbreaks of mealybug, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) have created problems to Prickly Pear crops in Morocco in recent years. The native ladybird *Hyperaspis campestris* (Herbst 1783) (Coleoptera: Coccinellidae) and introduced ladybird *Cryptolaemus montrouzieri* (Mulsant, 1853) (Coleoptera: Coccinellidae) were used to control this menace. Petri dishes were used to determine the minimum number of *D. opuntiae* females that females of the introduced ladybird beetle *C. montrouzieri* require per day to achieve maximum fecundity and to assess the effects of interference competition from conspecific and heterospecific [*Hyperaspis campestris* (Herbst 1783)] adult ladybirds on its growth and reproduction. The number of *D. opuntiae* females at which females of *C. montrouzieri* ate most, and achieved maximum reproduction was 30. The presence of *H. campestris* does not significantly affect the reproductive numerical response of *C. montrouzieri* females. The presence of a conspecific or heterospecific (*H. campestris*) did not significantly affect the mean number of clutches laid by *C. montrouzieri* females. Also, mean clutch size and number values were significantly increased as prey density increased from 3 to 25 and does not significantly change from 25 to 30 density. The mean number of clutches laid by *C. montrouzieri* females was independent of the presence or sex of another lady beetle. Based on these results we suggest that the population abundance of *C. montrouzieri* will not be affected by the native ladybird *H.campestris.*

Keywords Coccinellidae, *Dactylopius opuntiae*, *Hyperaspis campestris*, Interference competition.

Introduction

The prickly pear cactus is a weed in some contexts but is also exploited for its fruit and cladodes as a vegetable source for humans, fodder for livestock, and as a barrier hedge. In Morocco, cactus was introduced in 1770 and it is now well represented throughout the national landscape. As a result of drought, the cactus area has evolved significantly in the past two decades: from 50 000 ha in 1998 to > 150 000 ha in 2014 (Paolo *et al.*, 2017). The modernization of Moroccan agriculture (Green Morocco Plan) encourages cactus plantation as an alternative crop in less favorable regions. Each year, over 4 000 ha are planted in the center and south of the country (Ait Hamou, 2007). Since 2016 *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) a possibly introduced mealybug has been causing severe damage to prickly pear crops in Morocco. *Dactylopius opuntiae* establish and spread more easily than many other insect species due to: a waxy coating on their dorsal side, which protects them from insecticides, a high reproductive rate, and the propensity to spread quickly through natural carriers such as plant products, wind,

water, rain, birds, human beings, and farm animals (khan *et al.*, 2012). Mealybugs suck sap through phloem tissues causing chlorosis and premature dropping of cladodes and fruits. Severe infestations (>75 % of the cladode surface) can result in death of the plant (Mann, 1969; Vanegas-Rico *et al.*, 2010, 2015). They also seem to be able to become dormant on inert material for considerable periods of time under unfavorable conditions (Khan *et al.*, 2012). Furthermore, problems with insecticide resistance and non-target effects on natural enemies make chemical control a less desirable control option to combat *D. opuntiae*. Therefore, to reduce insecticide use, there is a need to establish an integrated pest management program (Van den Bosch *et al.*, 1982) for *D. opuntiae* by introduction of some natural enemies. Arthropod natural enemies associated with *D. opuntiae* and other Dactylopiidae include only predators (Mann, 1969; Zimmermann *et al.*, 1979; VanegasRico *et al.*, 2010) of which the Coccinellidae *Chilocorus cacti L*. and *Hyperaspis trifurcata* Schaeffer are the most abundant in Mexico and the USA (Mann, 1969; Gilreath and Smith, 1988; Badii and Flores, 2001; Rodríguez-Leyva *et al.*, 2010; Vanegas-Rico *et al.*, 2010, 2015). Besides *C. cacti L*. and *H. trifurcata* Schaeffer, *Cryptolaemus montrouzieri* is also known as a generic predator of mealybugs and scales, and it has been used in 50 countries for control of several mealybug species (Srinivasan and Babu, 1989; Olivero *et al.*, 2003; Al-Khateeb and Asslan, 2009; Rosas-Garcia *et al.*, 2009). *Cryptolaemus montrouzieri* is not a common predator in Morocco, so morocco has had to import the beetle from different countries where its use as a biological control agent of mealybugs. In addition July 2017, *Hyperaspis campestris* (Herbst 1783) was also observed in cactus crop feeding on *D. opuntiae* in the state of Zemamra locality (32°37'48" N, 8°42'0" W) in the Sidi Bennour region (120 km north-west of Marrakech), Morocco. The combination of predators produced varying degrees of success, both in fields and greenhouses. Failures may be the result of unfavorable environmental factors, incompatible pest management practices, and antagonistic interactions among the biological control agents (khan *et al.*, 2012). *Cryptolaemus montrouzieri* has the potential of interfering with the biological control of the mealybug (Sengonca and Yanuwiadi, 1994).
This study aimed to determine (1) the minimum number of *D. opuntiae* females that females of *C. montrouzieri* need per day to maximize their fecundity; (2) the effect of the presence of the adults of conspecifics and the heterospecific *Hyperaspis campestris* on the growth and reproductive capacity of *C. montrouzieri* females.

Materials and methods

Insects

Adult of *Hyperaspis campestris* were collected from prickly pear plantations from Zemamra (32°37'48" N, 8°42'0" W) and immediately identified. The ladybird was maintained on Cladodes infested with *D. opuntiae* nymphs in the laboratory at 27°C. The *C. montrouzieri* colony was established from adults imported by the laboratory of entomology at INRA (National Institute of Agricultural Research). Adults were placed in entomological cages (80-80-80 cm) comprised of a wooden frame covered by a mesh fabric to allow ventilation. Access to water was provided via a cotton wick inserted into a 25 ml glass vial of water. Cladodes infested with *D. opuntiae* were introduced into the cages to provide food and substrates for *C. montrouzieri* oviposition. Infested cladodes were withdrawn weekly and *C. montrouzieri* larvae transferred to another cage with the same characteristics to complete their development.

Effect of *D. opuntiae* females density on biological features of *C. montrouzieri* females

Cryptolaemus montrouzieri adult females were (15 days old) were placed individually in Petri dishes (14 cm in diameter) and provided by different densities of *D. opuntiae* females. 20 replicates randomly distributed were used for each combination of predator and prey density. Each of the 20 replicates contained seven treatments, defined by the density of the prey they would subsequently be offered. These densities were, 3, 5, 10, 15, 20, 25, and 30. All experiments were carried out at 27°C. *Dactylopius opuntiae* females were replaced daily for 3 days. Each set of *D. opuntiae* females was weighed before being presented to the predators. The females' voracity and reproductive capacity (measured as the number of eggs laid per female per day, number of eggs laid per clutch per female per day, and number of clutches laid per female per day) were recorded over the final 24 h of the 3-day experimental period.

Daily voracity (V0) was estimated using the equation of Soares et al. (2003): $V0 = (A- a_{24})* ra_{24}$. where V0 is the number of prey consumed, A is the number of prey offered, a_{24} is the number of prey alive after 24 h, and ra_{24} is the proportion of prey alive in the absence of a predator after 24 h. Another measure of voracity is the biomass of prey ingested (mg) per unit of time, the so-called relative consumption rate (RCR). RCR was evaluated according to Soares et al. (2004): $RCR_{(mg)} = (W_i/N)*V_0*PUC$. Where RCR (mg) = relative consumption rate, Wi = total weight of insects provided, N = number of insects provided, V0 = number of insects eaten and PUC = prey utilization coefficient. In a separate experiment, the PUC of *C. montrouzieri* females was estimated to be 61.44 ± 9.35% using the following model (Schanderl, 1987) $PUC=[(W_0-W_1-(W'_0-W'_1)/W_0]*100$. where W_0 = body weight of one *D. opuntiae* female provided, W_1 = *D. opuntiae* female carcass weight, W'_0= initial body weight, and W'_1= final bodyweight of one *D. opuntiae* female in the control treatment.

Effect of the presence of conspecific and heterospecific adults on the growth and reproductive capacity of *C. montrouzieri* females

In this study, *C. montrouzieri* adult females (15 days old) were placed individually in Petri dishes (14 cm in diameter) and provided with 15 *D. opuntiae* gravid females (egg production stage). At the end of the second day, one conspecific (treatment 1) or one heterospecific (treatment 2) beetle was also released in the experimental setup. All experiments were carried out in the same conditions 27°C, and all adults used in this experiment were reared under the same conditions. On the third day, the reproductive capacity of *C. montrouzieri* females was recorded. In the replicates with two females of *C. montrouzieri*, results for reproductive capacity were compared and presented per female. 20 replicates randomly distributed (ten females and ten males) were used for each treatment.

Statistical analysis

The data of voracity, relative consumption rate, reproductive capacity, the mean number of eggs laid, the mean number of eggs laid per clutch per female and mean number of clutches laid per female kept alone, with a conspecific or a heterospecific were analyses using ANOVA followed by a multiple comparison test (LSD test) with the software package SPSS ver. 18.0 (SPSS, 2012).

Results and discussion

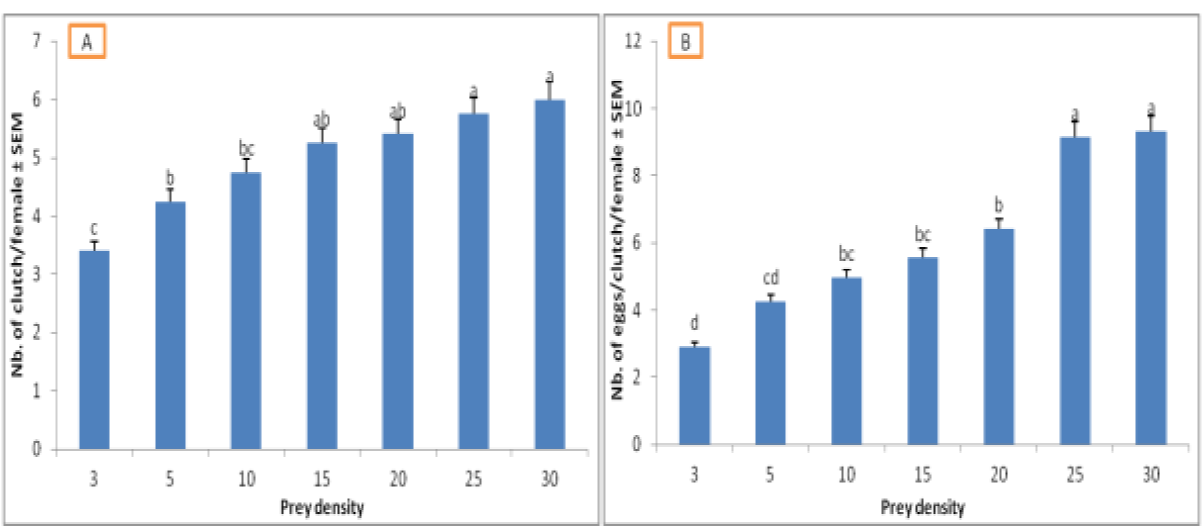
Effect of *D. opuntiae* females density on biological features of *C. montrouzieri* females

Daily voracity of *C. montrouzieri* females against *D. opuntiae* females was significantly increased (ANOVA, F = 178.854, df = 6, P < 0.0001) and reached maximum values when 30 mealybugs were provided. Biomass ingested was also significantly increased (ANOVA, F = 156.658, df = 6, $P \leq 0.0001$) to reach maximum values when 20 mealybugs were provided (Table 1). Fecundity varied significantly with treatment (ANOVA, F = 41.868, df = 6 , P < 0.0001). Number of eggs laid by *C. montrouzieri* females significantly increased as prey density increased from 3 to 20 and does not significantly change from 20 to 30 density (Fig. 1A). Mean clutch size differed significantly with treatment (ANOVA, F = 42.407, df = 6, P < 0.0001). It significantly increased as prey density increased from 3 to 25 and does not significantly change from 25 to 30 density (Fig. 1B). The number of clutches differed significantly with treatment (ANOVA, F = 24.840, df = 6, P < 0.0001). Clutch number increased significantly as prey density increased from 3 to 25 and does not significantly change from 25 to 30 density (Fig. 1C).

Table 1. Mean voracity (mean number of *Dactylopius opuntiae* females eaten ± SE and relative consumption rate (milligrams of biomass ingested ± SE) of *C. montrouzieri* female, provided with different numbers of *D.opuntiae* females

Number of *Dactylopius opuntiae* females provided	Daily Voracity	Relative consumption rate
3	2.17±0.49 e	27.76±6.32 d
5	3.13±0.50 d	40.72±6.83 c
10	4.20±0.78 c	55.54±10.25 b
15	4.53±0.91 c	60.43±12.06 b
20	4.95±0.75 b	66.11±9.97 a
25	5.35±0.71 a	65.90±9.05 a
30	5.55±0.75 a	66.08±8.97 a

Bars with different letters are significantly different according to Tukey's protected LSD test (alpha = 0.05).



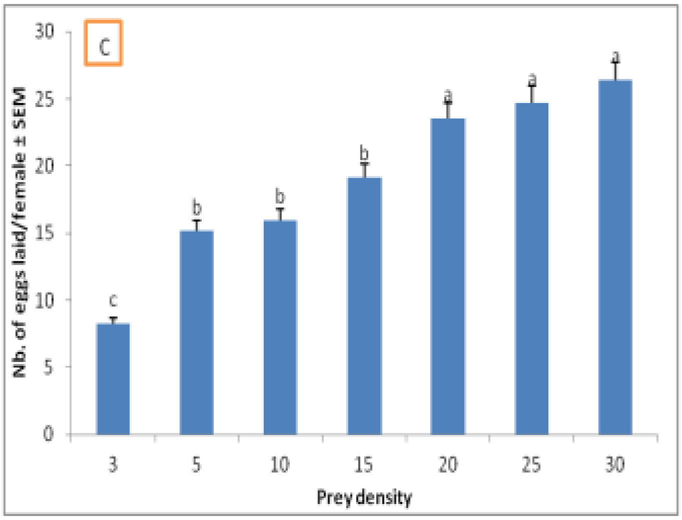


Fig. 1. Reproductive capacity [A clutch number (mean number of clutches per day per female ± SE), B clutch size (mean number of eggs per day per clutch ± SE) and C fecundity (mean number of eggs per day per female ± SE)] of *C. montrouzieri* females in 24 h on seven different prey densities. Bars with different letters are significantly different according to Tukey's protected LSD test (alpha = 0.05).

Effect of the presence of conspecific and heterospecific adults on the growth and reproductive capacity of *C. montrouzieri* females

Fecundity of *C. montrouzieri* females was not significantly reduced in both treatments. In the presence of conspecifics (ANOVA, F = 0.549, df = 3, P = 0.652), and also in the presence of heterospecifics ladybird (ANOVA, F = 1.52, df =3, P = 0.225). The mean number of eggs laid by *C. montrouzieri* female was not significantly affected by the presence of another lady beetle species independent of its sex (Fig. 2). The presence of heterospecifics ladybird did not significantly affect the clutch size of *C. montrouzieri* (ANOVA, F = 1.510, df = 3, P = 0.228), but clutch size was significantly lower in the presence of male conspecifics (ANOVA, F = 3.458, df = 3, P = 0.026). Mean clutch size was not significantly affected by the sex of the heterospecifics (Fig. 2). The presence of a conspecific or heterospecific did not significantly affect the mean number of clutches laid by *C. montrouzieri* females (ANOVA, F = 2.475 , df = 3 , P = 0.077; ANOVA, F = 2.376, df = 3, P = 0.086, respectively). The mean number of clutches laid by *C. montrouzieri* females was independent of the presence or sex of another lady beetle (Fig. 2).

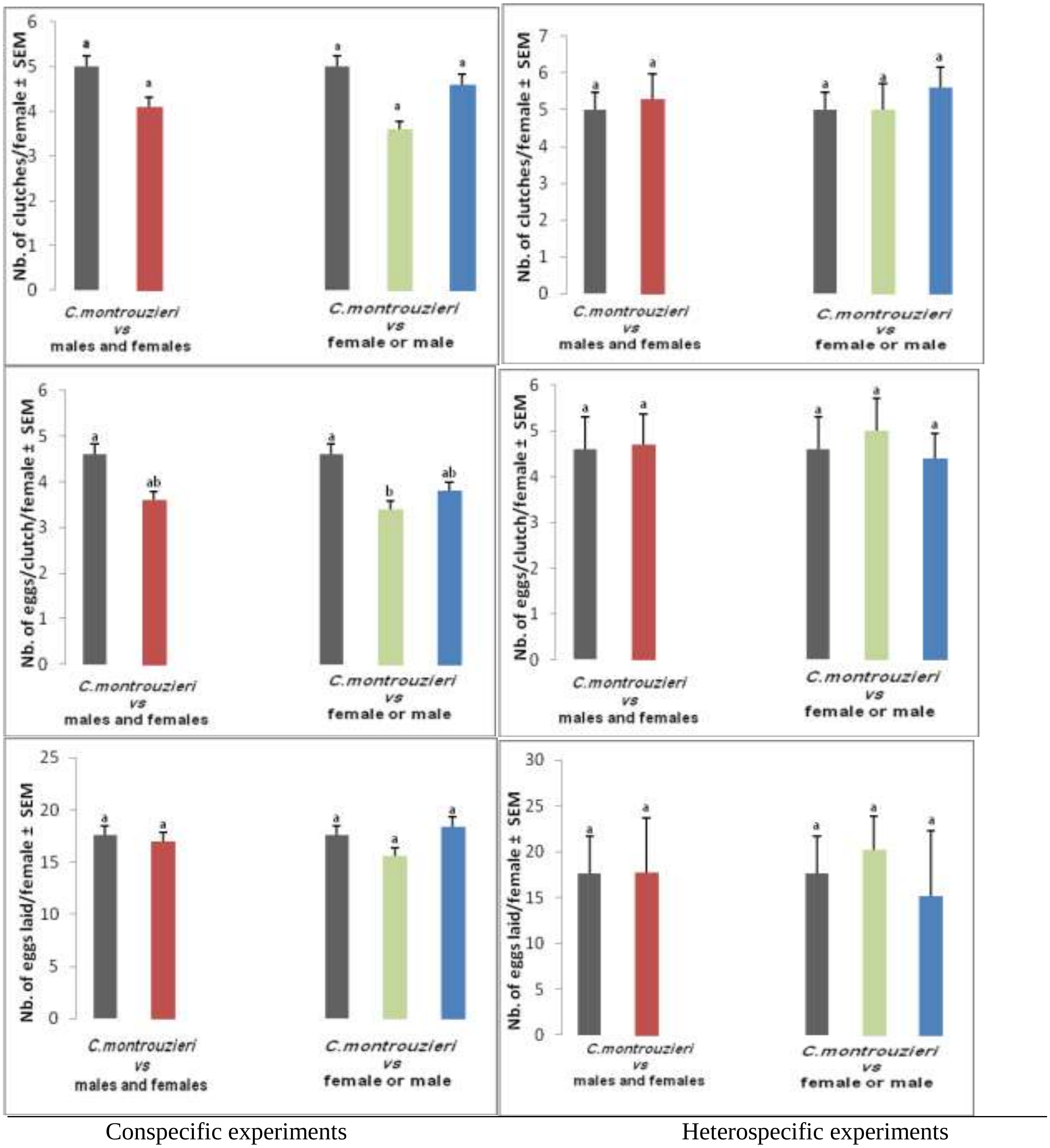


Fig. 2. Mean changes in mean number of eggs laid, number of eggs per clutch per female, and mean number of clutches per female of *C. montrouzieri* females in the presence of ladybird beetles *H. campestris.* Histograms within the same subgroup of experiments having the same letter are no significantly different (P < 0.05; Tukey's protected LSD test). Black bars *C. montrouzieri* females kept alone; brown bars *C. montrouzieri* females in the presence of conspecifics or heterospecifics; green bars *C. montrouzieri* females in the presence of a male conspecific or heterospecific; and bleu bars *C. montrouzieri* females in the presence of a female conspecific or heterospecific.

The results of this study showed that there was a significant difference among the number of prey consumed and reproductive capacity of *C. montrouzieri* females at different prey densities. The maximum consumption and reproductive capacity were recorded at high prey densities, and they were significantly higher than those obtained at the lowest densities. The voracity and reproductive capacity of coccinellids depend on prey density. The increase in prey density results in the increase of number of prey eaten and its reproductive numerical response (Hodek and Honêk, 1996). Over the range of 3–30 *D. opuntiae* females provided per day, the mean number of eggs per clutch increased significantly. These results suggest that *C. montrouzieri* females maximize their fitness by laying large clutches. Our results reported that The presence of heterospecifics ladybird did not significantly affect the reproductive capacity of *C. montrouzieri* females. *Cryptolaemus* has the potential of interfering with the biological control of mealybugs (Sengonca and Yanuwiadi, 1994). Since clutch size was significantly lower in the presence of male conspecifics. The mechanism for this lower clutch size is not clear. However, one of the possible explanations is that *C. montrouzieri* females expended some time and/or matter and/or energy on competition or avoiding competition, and then there was less of these resources available for larging theirs clutches. The combination of predators produced varying degrees of success, both in the fields and greenhouses. Failures may be the result of unfavorable environmental factors, incompatible pest management practices, and antagonistic interactions among the biological control agents (Khan *et al.*, 2012). Our results suggest also that both predators show strong predatory potential against *D.opuntiae*, with *C. montrouzeiri* being the most ravenous feeder. From a biological control point of view, this study shows that *H. campestris* could not endanger the population abundance and/or induce competitive displacement of *C. montrouzieri*, Thus the combination of the two predators can reduce more significantly *D. opuntiae* population.

Conclusion

The laboratory results presented here suggest that the local polyphagous ladybug (*H. campestris*) is not able to negatively impact the reproductive parameters of the introduced beneficial ladybug *C. montrouzieri*; however, an extrapolation of these results to the field will not be entirely accurate. Therefore, a direct field assessment of the impact that this local ladybug species (*H. campestris*) may have on introduced beneficial ladybugs such as *C. montrouzieri* is needed.

References

Ait Hamou A. (2007). An overview on medicinal and cosmetic uses of cactus in Morocco. Cactusnet Newsletter, Vol. 11, pp. 51-56.

Al-Khateeb N., Asslan L. (2009). Preliminary data on settlement and adaptation of the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) in colonies of mealybugs on citrus trees in the Syrian coast. Egyptian Journal of Biological Pest Control, Vol. 19, pp. 191-192.

Badii MH., Flores AE. (2001). Prickly pear cacti pests and their control in Mexico. Florida Entomologist, vol 84, pp. 503-505.

Gilreath ME., Smith JWJr. (1988). Natural enemies of *Dactylopius confusus* (Homoptera: Dactylopiidae): exclusion and subsequent impact on *Opuntia* (Cactaceae). Environmental Entomology, Vol. 17, pp. 730-738.

Hodek I., Honêk A. (1996). Ecology of Cocinellidae. Kluwer Academic Publishers, Dordrecht.

Khan HAA., Sayyed AH., Akram W., Raza S., Ali M. (2012). Predatory potential of *Chrysoperla carnea* and *Cryptolaemus montrouzieri* larvae on different stages of the mealybug, *Phenacoccus solenopsis*: A threat to cotton in South Asia. Journal of Insect Science, Vol.12, pp. 147.

Mann J. (1969). Cactus-feeding insects and mites. United States National Museum Bulletin, Vol. 256, pp.1-158.

Olivero J., Garcia E., Wong E., Marquez AL., Garcia S. (2003). Defining a method to determine the release dose of *Cryptolaemus montrouzieri* Muls. based on the incidence of *Planococcus citri* Risso in citrus orchards. Bulletin OILB/SROP, Vol. 26(6), pp. 163-168.

Paolo, I., Candelario M., Juriquilla Q., Ali N., Carmen. (2017). Crop Ecology, Cultivation and Uses of Cactus Pear.

Rodríguez-Leyva E., Lomeli-Flores JR., Vanegas-Rico JM. (2010). Enemigos naturales de la grana cochinilla del nopal *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae). In: Portillo L, Vigueras AL (eds) Conocimiento y aprovechamiento de la grana cochinilla. Colegio de Postgraduados. Estado de México, México, pp. 101-112.

Rosas-Garcia NM., Duran-Martinez EP., de Luna-Santillana JE., Villegas-Mendoza JM. (2009). Predatory potential of *Cryptolaemus montrouzieri* Mulsant towards *Planococcus citri* Risso. Southwestern Entomologist, Vol. 34, pp. 179-188.

Schanderl H., Ferran A., Garcia V. (1988). L'e´levage de deux coccinelles *Harmonia axyridis* et *Semiadalia undecimnotata* a` l'aide d' oeufs *d'Anagasta kuehniella* tue´s aux rayons ultraviolets. Entomologia Experimentalis Applicata, Vol. 49, pp. 417-421.

Sengonca C., Yanuwiadi B. (1994). Frassverhalten des schmierlausraubers *Cryptolaemus montrouzieri* Mulsant beidurch *Leptomastix dactylopii* (Howard) parasitierten *Placococcus citri* (Risso). Mitteilungen der Deutchen Gesellschaft fur Allgemeine und Angewandte Entomologie, Vol. 9, pp. 121-124.

Srinivasan, TR., Babu PCS. (1989). Field evaluation of *Cryptolaemus montrouzieri* Mulsant, the coccinellid against grapevine mealybug *Maconellicoccus hirsutum* Green. South Indian Horticulture, Vol. 37, pp. 50-51.

Soares AO., Coderre D., Schanderl H. (2003). Effect of temperature and intraspecific allometry on predation by two phenotypes of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Envernmental Entomology, Vol. 32, pp. 939-944.

Soares AO., Coderre D., Schanderl H (2004). Dietary selfselection behaviour by the adults of the aphidophagous ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae). Journal of Animal Ecology, Vol. 73, pp. 478-486.

SPSS (2012). SPSS for Windows, release. version 21.0. SPSS, Chicago.

Van den Bosch R., Messenger PS., Gutierrez AP. (1982). An introduction to biological control. Plenum Press.

Vanegas-Rico JM., Lomeli-Flores JR., Rodríguez-Leyva E., Mora-Aguilera G., Valdez JM. (2010). Natural enemies of *Dactylopius opuntiae* (Cockerell) on *Opuntia ficus-indica* (L.) Miller in Central Mexico. Acta Zoológica Mexicana (ns), Vol. 26, pp. 415-433.

Vanegas-Rico JM., Lomeli-Flores JR., Rodríguez-Leyva E., Perez-Panduro A., Hernandez-Gonzalez H., Marin-Jar-amillo A. (2015) *Hyperaspis trifurcata* (Coleoptera: Coccinellidae) y susparasitoides en el centro de Mexico. The Revista Colombiana de Entomología, Vol. 41, pp. 194-199.

Zimmermann HG., Erb HE., McFayden RE. (1979). Annotated list of some cactus-feeding insects of South America. Acta Zoológica Lilloana, Vol. 33, pp.101-112.

THE INFLUENCE OF POLYOLS USED AS OSMOTIC AGENTS IN OSMOTIC DEHYDRATION PROCESS

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Abstract

Osmotic dehydration (OD) is a technique used in food technology for the partial removal of water from plant tissues that often precedes different preservation treatments, especially drying. The OD process can improve the food product quality and reduce energy consumption in the next processes allowing to create new products. It positively affects retention of flavour, colour and nutrients and causes a reduction of both water content and water activity of a dehydrated material. Many factors have an impact on the efficiency of OD (e.g., temperature, time, solution type and concentration, the ratio of the mass of the dehydrated product to the mass of the solution, the sample geometry, the material structure). For the OD process, different types of agents might be applied. One of the most common osmotic agents is sucrose solution. However, because of the worldwide epidemic of obesity, there is an increasing demand for its less-caloric alternatives. In recent years, the use of polyols as the osmotic agents is widely investigated due to their low energy value and a comparable sweetness to sucrose. The aim of this study is to discuss the applicability of chosen substances from the polyol group as the osmotic agents. The sugar profiles, kinetics parameters and sensory properties of dehydrated food products with the use of polyols are analysed. Literature review points out that some substances from the polyol group can successfully be alternatives to sucrose in the OD process. Erythritol and xylitol is the most studied and promising osmotic agents of sugar alcohols.

Keywords: *osmotic dehydration, polyols, sugar alcohols, plant tissue.*

Introduction

Food preservation involves delaying the growth of microorganisms and inhibiting biochemical reactions. Its aim is to prevent foods from spoiling and therefore to minimize wastage (Sridhar *et al.*, 2020; Yadav and Singh, 2014). Although the concept of food preservation was introduced in ancient times, it is constantly evolving. With increasing demands of quality and safety of foods, preservation techniques have been improved and modernized (Amit *et al.*, 2017; Sridhar *et al.*, 2020). One of the techniques that enables to obtain a high nutritional and organoleptic quality product is osmotic dehydration (OD).

OD can be used as a technique for obtaining minimally processed food products (Tappi *et al.*, 2017) or as a pre-treatment before other processes, e.g., freezing (Dadan *et al.*, 2020) or drying Assis *et al.* (2017). In the latter case, it causes energy saving as well as increases retention of nutritional value, colour, flavour and texture of dried fruits and vegetables (Yadav and Singh, 2014). OD is a process used for partial dewatering of plant tissues such as fruits and vegetables by immersing in hypertonic solution. During OD, a counter-current transfer of mass occurs through the transfer of water from the food tissue to the osmotic solution, and at the same time the osmotic substance goes into the dehydrated material. Additionally, the food's solutes, i.e., vitamins, minerals, organic acids, saccharides, or colourants are leached out of the tissue. This transfer is quantitatively negligible compared with the first two types but it also affects the change in the chemical composition of the product. The driving force for mass flow is the chemical potential difference across a semi-permeable membrane between the material and the solution (Ahmed *et al.*, 2016; Akbarian *et al.*, 2014; Nowacka *et al.*, 2021). The efficiency of the OD process is influenced by several factors such as type and concentration of the osmotic solute, food structure, maturity level, shape, and size of the raw material, ratio of the osmotic solution to the product, as well as the process parameters (time, temperature, pressure, agitation) (Ahmed *et al.*, 2016; Ciurzyńska *et al.*, 2016; Yadav and Singh, 2014). The osmotic dehydration process is also affected by solubility, molecular weight, physicochemical properties, and the ionic state of the solute. A low molecular weight osmotic agent can more easily penetrate the cells of plant tissues as compared to a high molecular weight osmotic agent (Gribova *et al.*, 2021). Furthermore, the solute cost, harmlessness, organoleptic compatibility with the food product and additional preservation effect by the solute should be considered when choosing osmotic agents (Akbarian *et al.*, 2014). The most used osmotic agent for fruits is sucrose (Chandra and Kumari, 2014). However, its high consumption might cause negative consequences on human health (Codella *et al.*, 2016). Therefore, it is important to find alternative osmotic agents to sucrose. Various substances that enable to achieve products with desirable nutritional, functional, and organoleptic properties were recently proposed, for example fruit juice concentrates (Lech *et al.*,2017; Samborska *et al.*, 2019), steviol glycosides (Nowacka *et al.*, 2018) and polyols (Cichowska *et al.*, 2018; Mendonça *et al.*, 2017).

Polyols (polyhydric alcohols or sugar alcohols) are low digestible carbohydrates, which are obtained by substituting an aldehyde group with a hydroxyl one. They are naturally present in certain kinds of fruits, vegetables, or mushrooms. Sugar alcohols have lower caloric value (from 0.2 to 2.7 kcal/g) compared with sucrose (4 kcal/g) and relative sweetness of around 30-100 % compared with sucrose (Grembecka, 2015). The use of sugar alcohols during pre-treatment can reduce sugar content in the product, resulting in the reduction of calories (Cichowska *et al.*, 2019a).

This study aims to analyse the possibility of use of selected polyols as the osmotic agents. Certain parameters, including water activity, mass transfer kinetics, sugar profiles, colour changes, sensory properties depending on the type of sugar alcohol are described.

Material and Methods

This paper is based on a literature review of journal articles and book chapters. The presented conclusions were drawn from the analysis and synthesis of current information on the research topic. The information was gathered from the main online scientific databases including Science Direct, Elsevier, and Google Scholar.

Results and Discussion

The present review focuses on the effect of the use of the chosen sugar alcohols such as maltitol, xylitol erythritol and sorbitol as osmotic agents. Those substances were chosen as their applicability in the OD is the most studied substances among all polyols so far.

Maltitol is a disaccharide polyol that consists of glucose and sorbitol in equal parts. Although it is characterized by a pleasant sweet taste, the insulin response associated with its ingestion is significantly reduced due to its slow absorption. Maltitol characterizes with properties the most resembling the ones of sucrose among sugar alcohols. Its sweetness amounts up to 90% of the one attributed to sucrose, but its caloric value is lower (2.1-2.4 kcal/g). Maltitol causes laxative effect when consumed in amounts exceeding 25-30 g/kg body weight per day (Grembecka, 2015). The molecular weight of maltitol amounts 344.31 g/mol and it is similar to the molecular weight of sucrose (342.30 g/mol). Kowalska *et al.* (2019) stated that maltitol caused comparable mass exchange rate to sucrose when both substances were used in the same concentration. However, the mass transfer kinetics of the OD of yacon with maltitol was less effective in relation to the other polyols as xylitol, erythritol, sorbitol. This result can be attributed to its significantly higher molecular weight (Mendonca et al., 2016). The changes of water activity in the OD are related to water content of dehydrated product. Therefore, the influence of maltitol on decrease of water activity is not significant compared to sucrose (Cichowska *et al.*, 2018). The OD in maltitol solution may significantly increase the content of this substance in the dehydrated tissue. It was observed by Cichowska *et al.* (2019b), where significant increase of colour intensification was noted but the total colour difference after the OD was quite small. Furthermore, the OD in polyols solutions as a pre-treatment before convective drying was studied by Bialik *et al.* (2020). It was demonstrated that the utilization of maltitol at 30°C improved the retention of the carotenoids. Moreover, the use of maltitol at 30°C shortened the drying time compared to pre-treatment in sucrose or without pre-treatment.

Xylitol is a 5-carbon polyol. It characterizes with the same sweetness as sucrose (with one-third fewer calories), so it is the sweetest among all polyols. Human tolerance of xylitol amounts to 100 g per day (Grembecka, 2015). Kowalska *et al.* (2019) demonstrated that the OD process in xylitol solution is more efficient than in sucrose solution. It is caused by the low molecular weight of xylitol (152.15 g/mol). For the same reason, the difference between water activity of fresh sample and dehydrated in xylitol is bigger than the difference between the fresh sample and the sample dehydrated in sucrose. Furthermore, the study showed that colour changes of apples dehydrated in xylitol and sucrose solutions were similar. Additionally, the contents of sucrose, fructose and glucose in the samples decreased and the content of xylitol increased during the whole OD process, compared to the fresh material. It is worth mentioning that the absolute changes in sugar profiles were stronger when xylitol was used compared to sucrose and maltitol due to the higher osmotic pressure which is the driving force of sugars' diffusion (Kowalska *et al.*, 2019). The efficiency of mass transfer kinetics and decrease of water activity when xylitol is the osmotic agent were confirmed also for kiwiberries (Bialik *et al.*, 2020), apples (Cichowska *et al.*, 2019a; Cichowska *et al.*, 2018) and yacon (Mendonça *et al.*, 2017).

In the experiment performed by Cichowska-Bogusz *et al.* (2020), an impact of OD with sugar alcohols on drying was analysed. It was found that the OD with xylitol shortens the drying time in both convective and vacuum-microwave drying compared to drying without the OD. Moreover, convective drying was shorter when xylitol was used as osmotic agent compared to sucrose and erythritol. According to this study, no negative effect on taste was observed when xylitol was used in the production of dried apples. In addition, it enabled to obtain a noticeable cooling/refreshing effect felt in the mouth. The use of xylitol enabled the production of dried snacks with lower calorific value, compared to dried apples obtained by the same method without initial OD. Xylitol residues in the dried product were at a level that guaranteed consumer safety, understood as not exceeding the dose resulting in gastric problems. The safety of the level of xylitol residues in dried apples was confirmed by Kowalska *et al.* (2019). However, it was highlighted that the dried fruit should be considered as a snack and consumed in the amount of 20-60 g a day, i.e., in portions that are available on the market for other dry snacks.

Erythritol (1,2,3,4-butanetetrol) is a stable compound that does not decompose in either acid or alkaline environments. Due to its small molecular size, erythritol has a high level of digestive tolerance in comparison to other polyols. As the molecular weight of erythritol (122.12 g/mol) is almost three times lower than sucrose's molecular weight (342.20 g/mol), it could be an alternative osmotic agent to sucrose (Cichowska *et al.*, 2019a). During OD of apples in 30% (w/w) solutions (a solution/fruit mass ratio was 4:1) at 40°C Kowalska *et al.* (2019) showed that the use of erythritol causes stronger mass exchange effect than the use of sucrose, xylitol and maltitol as well as it decreases the value of water activity the most. Moreover, erythritol caused stronger diffusion of sugars from the tissue to the solution than other substances due to the higher osmotic pressure. Additionally, Cichowska *et al.* (2019b) stated that during OD of apples in erythritol solution, there is an increase of brightness which is considered as a positive. The brightness could be higher due to sugar coating, which appeared as a result after a process of OD. This substance has a high crystallization capacity. Cichowska-Bogusz *et al.* (2020) also observed that the microwave-vacuum drying time was shorter when apples were dehydrated previously in erythritol or sucrose solution (their efficiency was the same). However, the OD in erythritol as pre-treatment was not effective in case of convective drying. The use of erythritol similarly to the use of xylitol in production of dried apples causes a noticeable cooling/refreshing effect felt in the mouth and it is good in taste. It might be a safe and healthy snack with reduced calorie, when is consumed in recommended amount. Moreover, dried apples that were previously osmo-dehydrated in erythritol characterized by the best hygroscopic properties among tested polyols. This results in the best storage stability of the dried product, due to the low ability to absorb water from the environment.

Sorbitol is a 6-carbon sugar alcohol and that is the polyol mostly found in nature. Sorbitol has low calorie (2.4 kcal/g) and the sweetening power up to 70 % of the sweetness of sucrose (Brochier *et al.*, 2015). A molecular weight that equals 182.17 g/mol. A lower molecular weight of the solute results in a greater driving force for the process (Mendonça *et al.*, 2017). Therefore, sorbitol is a good alternative to replace the sucrose as the osmotic agent in OD which has been shown in studies conducted by Assis *et al.* (2017) and Hui *et al.* (2017). The low molecular weight of sorbitol resulted also in the reduction of water activity of pineapple osmo-dehydrated in sorbitol in comparison to the OD in sucrose, which is important in extension the shelf life of the product (Hui *et al.*, 2011).

Assis *et al.* (2017) studied the effect of the OD with sorbitol on convective drying. The authors found that the use of sorbitol as osmotic agent did not affect the water loss rate in the hot air-drying process, in comparison to sucrose. However, sorbitol was more effective in reducing the air-drying time and water activity of dried products. It may be explained by the fact that osmotically dehydrated samples with sorbitol solutions presented already lower values of water activity before the hot air drying than the samples osmotically dehydrated with sucrose solutions.

The sensory analysis conducted by Sritongtae *et al.* (2011) showed that partial replacement the sucrose solution with sorbitol at 10 or 15% (w/v) did not cause a significant difference in sample's colour and get worse the quality of the obtained dried products. However, in the study of Vilela *et al.* (2016), in which sensory profiles of three types of candied fruits (pineapple, orange peel and citron) were analysed, the use of sorbitol in the previously conducted OD resulted in significant differences of the textural and sensorial properties of the final products when compared to the traditional process. The sensory evaluation was ambiguous as some characteristics were considered as positive but others can be depreciated by consumers, so consumer tests should be conducted.

Conclusions

The solutions of polyols as maltitol, sorbitol, xylitol and erythritol could be alternatives to the solution of sucrose in the OD process. The mass transfer rates are satisfactory for all the substances when the same solution concentrations are compared. The molecular weight is an important parameter that determines the efficiency of mass transfer kinetics as well as the reduction of water activity and the sugar diffusion to the plant tissue. The molecular weights of erythritol, xylitol, sorbitol and maltitol amount 122.10, 152.15, 182.17, and 344.31 g/mol, respectively. Therefore, erythritol was the most efficient osmotic agent, then xylitol, sorbitol and maltitol, respectively. The use of polyols in the OD process before drying might positively affect the drying time or retention of bioactive compounds in the plant tissue. The replacement of sucrose with erythritol or xylitol results in a noticeable cooling/refreshing effect felt in the mouth, the lower calorific value, and the safe level of polyols in the final products.

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References

Ahmed I., Qazi I. M., Jamal S. (2016). Developments in osmotic dehydration technique for the preservation of fruits and vegetables. Innovative Food Science and Emerging Technologies, Vol. 34, pp. 29-43.

Akbarian M., Ghasemkhani N., Moayedi F. (2014). Osmotic dehydration of fruits in food industrial: A review. International Journal of Biosciences, Vol. 4 (No. 1), pp. 42-57.

Amit S.K., Uddin M.M., Rahman R., Islam S. M. R., Khan M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. Agriculture & Food Security Vol. 6 (No. 51), pp. 1-22.

Assis F. R., Morais R. M. S. C., Morais A. M. M. B. (2016). Mathematical modelling of osmotic dehydration kinetics of apple cubes. Journal of Food Processing and Preservation, Vol. 41 (No. 3), pp. 1-16.

Assis F. R., Morais R. M. S. C., Morais A. M. M. B. (2017). Osmotic dehydration with sorbitol combined with hot air convective drying of apple cubes. Journal of Food Science and Technology, Vol. 54 (No. 10), pp. 3152-3160.

Bialik M., Wiktor A., Witrowa-Rajchert D., Gondek E. (2020). The Influence of Osmotic Dehydration Conditions on Drying Kinetics and Total Carotenoid Content of Kiwiberry (Actinidia Arguta). International Journal of Food Engineering, Vol. 16 (No. 1-2), pp. 1-8.

Brochier B., Marczak L. D. F., Noreña C. P. Z. (2014). Osmotic Dehydration of Yacon Using Glycerol and Sorbitol as Solutes: Water Effective Diffusivity Evaluation. Food and Bioprocess Technology, Vol. 8 (No. 3), pp. 623-636.

Chandra S., Kumari D. (2014). Recent Development in Osmotic Dehydration of Fruit and Vegetables: A Review. Critical Reviews in Food Science and Nutrition, Vol. 55 (No. 4), pp. 552–561.

Cichowska J., Figiel A., Stasiak-Różańska L., Witrowa-Rajchert D. (2019a). Modelling of Osmotic Dehydration of Apples in Sugar Alcohols and Dihydroxyacetone (DHA) Solutions. Foods, Vol. 8 (No. 1), pp. 1-17.

Cichowska J., Woźniak Ł., Figiel A., Witrowa-Rajchert D. (2019b). The Influence of Osmotic Dehydration in Polyols Solutions on Sugar Profiles and Color Changes of Apple Tissue, Periodica Polytechnica Chemical Engineering, Vol. 64 (No. 4), pp. 530-538.

Cichowska J., Żubernik J., Czyżewski J., Kowalska H., Witrowa-Rajchert D. (2018). Efficiency of Osmotic Dehydration of Apples in Polyols Solutions. Molecules, Vol. 23 (No. 2), pp. 446-465.

Cichowska-Bogusz J., Figiel A., Carbonell-Barrachina A.A., Pasławska M., Witrowa-Rajchert D. (2020). Physicochemical Properties of Dried Apple Slices: Impact of Osmo-Dehydration, Sonication, and Drying Methods. Molecules, Vol. 25 (No. 5), pp. 1-20.

Ciurzyńska A., Kowalska H., Czajkowska K., Lenart A. (2016). Osmotic dehydration in production of sustainable and healthy food. Trends in Food Science and Technology, Vol. 50, pp. 186-192.

Codella R., Terruzzi I., Luzi L. (2017). Sugars, exercise and health. Journal of Affective Disorders, Vol. 224, pp. 76-86.

Dadan M., Nowacka M., Czyzewski J., Witrowa-Rajchert D. (2020). Modification of food structure and improvement of freezing processes by pulsed electric field treatment. Pulsed Electric Fields to Obtain Healthier and Sustainable Food for Tomorrow, Academic Press (1st edition), Oxford, UK, Chapter 9 pp. 203-226.

Grembecka M. (2015). Sugar alcohols their role in the modern world of sweeteners: a review, European Food Research and Technology, Vol. 241 (No.1), pp. 1-14.

Gribova N.A., Perov V.I., Eliseeva L.G., Berketova L.V., Nikolayeva M.A., Soltaeva N.L. (2021). Innovative technology of processing berries by osmotic dehydration. IOP Conference Series: Earth and Environmental Science 2021, Vol. 624, pp. 1-8.

Hui L. C., Sulaiman R., Shamsudin R., Rukayadi Y. (2017). Effect of Storage on Physicochemical Properties and Microbiological Stability of Osmodehydrated Pineapple (Ananas comosus) Treated with Sucrose-Sorbitol Mixtures. Research Journal of Pharmaceutical, Biological and Chemical Sciences, Vol. 2, pp. 296-307.

Kowalska H., Woźniak Ł., Masiarz E., Stelmach A., Salamon A., Kowalska J., Piotrowski D. Marzec A. (2019). The impact of using polyols as osmotic agents on mass exchange during osmotic dehydration and their content in osmo-dehydrated and dried apples. Drying Technology, Vol. 38 (No.12), pp. 1620-1631.

Mendonça K., Correa J., Junqueira J., Angelis-Pereira M., Cirillo M. (2017). Mass transfer kinetics of the osmotic dehydration of yacon slices with polyols. Journal of Food Processing and Preservation, Vol. 41, pp. 1-8.

Nowacka M., Dadan M., Tylewicz U. (2021). Current Applications of Ultrasound in Fruit and Vegetables Osmotic Dehydration Processes. Applied Sciences, Vol. 11 (No. 3), pp. 1-22.

Nowacka M., Tylewicz U., Tappi S., Siroli L., Lanciotti R., Romani S., Witrowa-Rajchert D. (2018). Ultrasound assisted osmotic dehydration of organic cranberries (Vaccinium oxycoccus): Study on quality parameters evolution during storage. Food Control, Vol. 93, pp. 40-47.

Samborska K., Eliasson L., Marzec A., Kowalska J., Piotrowski D., Lenart A., Kowalska H. (2019). The effect of adding berry fruit juice concentrates and by-product extract to sugar solution on osmotic dehydration and sensory properties of apples. Journal of Food Science and Technology, Vol. 56, pp. 1927–1938.

Sridhar A., Ponnuchamy M., Kumar P.S., Kapoor A. (2020). Food preservation techniques and nanotechnology for increased shelf life of fruits, vegetables, beverages and spices: a review. Environmental Chemistry Letters, Vol. 19, pp. 1715-1735.

Sritongtae B., Mahawanich T., Duangmal K. (2011). Drying of Osmosed Cantaloupe: Effect of Polyols on Drying and Water Mobility. Drying Technology, Vol. 29 (No. 5), pp. 527-535.

Tappi S., Mauro M. A., Tylewicz U., Dellarosa N., Dalla Rosa M., Rocculi P. (2017). Effects of calcium lactate and ascorbic acid on osmotic dehydration kinetics and metabolic profile of apples. Food and Bioproducts Processing, Vol. 103, pp. 1-9.

Vilela A., Sobreira C., Abraão A.S., Lemos A.M., Nunes F.M. (2016). Texture Quality of Candied Fruits as Influenced by Osmotic Dehydration Agents. Journal of Texture Studies, Vol. 47, pp. 239–252.

Yadav A.K., Singh S.V. (2014). Osmotic dehydration of fruits and vegetables: A review. Journal of Food Science and Technology, Vol. 51 (No. 9), pp. 1654-1673.

EFFECT OF DRYING TEMPERATURE ON ANTIOXIDANT ACTIVITY OF WHITE AND RED MAIZE (*ZEA MAYS* L.) SEEDS

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Abstract

Maize is the most commonly consumed cereal in the world. Its various compounds are associated with nutraceutical properties and health-promoting benefits. Therefore, maize seeds are considered to be functional food with high antioxidant activities. The drying conditions have a huge impact on maintaining the seed quality. This study aimed to evaluate the effect of temperature on the antioxidant activities of white and red maize (*Zea mays* L.) cultivars. A drying time of 1 h at a temperature of 45 °C was tested. The antioxidant activity expressed in percentages was evaluated as radical scavenging activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. The results revealed that the antioxidant activity of the dried white seeds (74.08 %) was not significantly different ($p>0.005$) with respect to the un-dried seeds used as control (72.6 %). Contrary, drying at 45 °C caused a significant ($p<0.005$) rise of antioxidant activity in the red-coloured seeds' being 85.95 % and 92.25 % before and after the treatment, respectively. The obtained results show that the antioxidant activity may be a reliable indicator for the estimation of the cereal seeds' quality, which is useful in the food industry and agriculture.

Keywords: *maize seeds, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, oven-drying, quality.*

Introduction

Maize (*Zea Mays* L.) is a principal cereal crop cultivated worldwide and one of the major food sources. High diversity exhibited in cultivated maize is recognized phenotypically by different seed colours, shapes, and sizes, as well as differences in the cob and plant characteristics (Martínez-Martínez et al., 2019). The quality of pigmented grain could be correlated to the antioxidant activity (AA%). Secondary metabolites, phenolics, and carotenoids found in pigmented maize are attributed to the high antioxidant properties of their seeds. The most commonly present phenolic compounds in the seeds are flavonoids, which are responsible for the different coloration of plants, and phenolic acids (Žilić et al., 2012). The color of purple corn is due to anthocyanin which has been using for coloring beverages, jellies, candies (Vilcacundo et al., 2020).

Health promotion benefits of edible plants are correlated to biologically active compounds, called antioxidants, that protect the cells from the harmful effect caused by active oxygen species and prevent the development of various diseases (Harakotr et al., 2014; Zargoosh et al., 2019). Beneficial health effects of nutrients that feature high antioxidant capacity are considered to be functional food ingredients.

Drying cereal seeds is a method of preservation common in the food industry used also to produce food with desired characteristics. During this process, retained moisture is evaporating up to a certain level, while the storage period of dried products is increased (Hihat et al., 2017).

Effects of drying of seeds were investigated experimentally in several separate research studies and the obtained conclusion was that increasing drying temperature reduces nutraceutical properties. Other studies reported the effects of different drying techniques on the antioxidative activity of seeds (López-Vidaña et al., 2017).

In the present work, the effect of drying temperature on antioxidant activity of different coloured maize seeds (white and red) was investigated. The impact of drying was evaluated using DPPH assay. The difference in antioxidant activity between treated (dried at 45 °C) and untreated (control) seeds of two different coloured maize variants was detected.

Material and Methods

Sample preparation

The seed material was supplied by the Maize Research Institute (Zemun Polje, Serbia). Experimetns were carried out at the Institute for Multidisciplinary Research, University of Belgrade. Half of the maize seeds (both white and red coloured) were dried for 1 h at 45 °C in an oven (Daihan Scientific, South Korea) while the other half was stored at room temperature (25 °C) to be used as control. After the heat treatment, maize seeds were homogenized. The seeds were grinded in a mill, followed by further powdering with liquid nitrogen in a mortar with pestle, and used for detection of antioxidant activity with DPPH assay.

DPPH Assay

Antioxidant activity (AA) of different coloured maize seeds, before and after drying, was measured by employing a 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA) assay. DPPH was dissolved in 96 % ethanol to a final concentration of 0.4 mM. Freshly prepared DPPH solution was stored in the dark at 4 °C until use. The samples of powdered maize seeds were immersed in 3 ml of ethanol (70 % v/v) and shaken on a vortex mixer for a few seconds. After extraction, 1 ml of 0.4 mM DPPH solution was added to each sample to a final concentration of 0.1 mM. The reaction was carried out while the mixture was constantly shaken and incubated for 30 minutes in the dark. Absorbance was measured at 517 nm on a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Pure deionized water was considered as a blank while the control consisted of 0.1 mM DPPH in 96 % ethanol solution. Antioxidant activity was calculated as a percentage of depleted reagent and the results were presented as a mean value of four replicates (n=4) and the standard error. The Mann-Whitney U nonparametric test has been used for tested the means values.

Results and Discussion

Obtained results of the antioxidant activity of the two analyzed maize seeds dried at 45 °C compared to the control (untreated) seeds are presented in Figure 1. Drying treatment (1h at 45 °C) of red coloured seeds significantly (P<0.05) increased their AA to 92 % as compared to AA of 86 % recorded in control samples. Contrary to the red pigmented seeds, there is no significant difference (P>0.05) in AA between treated (74.08 %) and control (73 %) seeds of white coloured maize seeds.

Red pigment of the maize seed is attributed to a high content of a class of flavonoids called anthocyanins, with high antioxidant and antiradical activities, located in aleurone layer or pericarp (Žilić et al., 2012). These compounds may contribute to an increase in antioxidant activity in red pigmented seed after exposure to temperature treatment at 45 °C. These red pigmented seeds might be used for producing of the sprouts which have attracted the interest of the food industry for the nutritional value of their components and their biological properties such as the antioxidant activity.

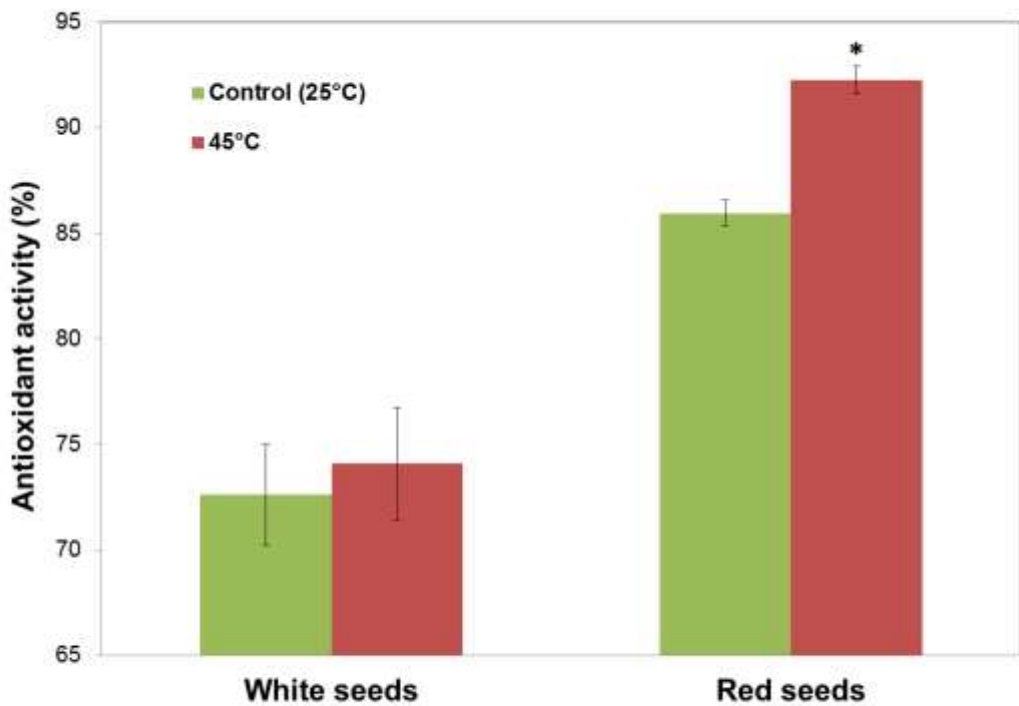

Figure 1. Antioxidant activity of white and red maize seeds after 1 h drying at 45 °C compared to the control (seeds stored at room temperature, 25 °C). Values are given as the mean percentage ± SD (n=4). Asterisk (*) indicates a significant difference at $p<0.05$.

Conclusions

Our results indicate that the heat treatment of the maize seeds (1 h-drying at 45 °C) increased antioxidant activity compared to the control samples. The difference is statistically significant ($p<0.005$) only for red pigmented maize. The two different coloured maize types have different phytochemical profiles, therefore corresponding antioxidant activity differs significantly between the two seed types.

Acknowledgement

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References

Harakotr B, Suriharn B, Tangwongchai R, Scott MP, Lertrat K (2014). Anthocyanins and Antioxidant Activity in Coloured Waxy Corn at Different Maturation Stages, Journal of Functional Foods, 9: 109–118.

Hihat S, Remini H, and Madani K (2017). Effect of Oven and Microwave Drying on Phenolic Compounds and Antioxidant Capacity of Coriander Leaves, International Food Research Journal, 24(2): 503–509.

López-Vidaña EC, Figueroa IP, Cortés FB, Rojano BA, Ocaña AN (2017). Effect of Temperature on Antioxidant Capacity during Drying Process of Mortiño (Vaccinium Meridionale Swartz), International Journal of Food Properties, 20(2): 294–305.

Martínez-Martínez R, Vera-Guzmán AM, Chávez-Servia JL, Aquino-Bolaños EN, Carrillo-Rodríguez JC, Pérez-Herrera A (2019). Bioactive Compounds and Antioxidant Activities in Pigmented Maize Landraces, Interciencia, 44(9): 549–556.

Vilcacundo E , García A , Vilcacundo M, Morán R, Samaniego I, Carrillo W (2020). Antioxidant purple corn protein concentrate from germinated Andean Purple corn seeds. Agronomy, 10: 1382.

Zargoosh Z, Mansureh G, Bacchetta G, Tavili A (2019). Effects of Ecological Factors on the Antioxidant Potential and Total Phenol Content of Scrophularia Striata Boiss, Scientific Reports, 9(1): 1–15.

Žilić S, Serpen A, Akıllıoğlu G, Gökmen V, Vančetović J (2012). Phenolic Compounds, Carotenoids, Anthocyanins, and Antioxidant Capacity of Colored Maize (Zea Mays L.) Kernels, Journal of Agricultural and Food Chemistry, 60(5): 1224–1231.

DISSIPATION DYNAMICS OF ANTHRANILIC DIAMIDE INSECTICIDES IN SOME FRUITS

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Abstract

Nowadays for the control of a wide range of insects pests, different chemical groups of insecticides are available on the market, but in spite of that inappropriate agricultural practice often leads to pest resistance development, as well as various harmful effects on human health or the environment. To overcome these, a few relatively new class of insecticides, as spinosines and anthranilic diamides, are developed. However, even if they are applied in accordance with good agricultural practices, the pesticides may leave residues, and cause side effects on human health. Due to this, it is very important to evaluate the persistance of pesticides in the agroecological conditions where they are applied mostly. In this paper behavior and the fate of the anthranilic diamides, chlorantraniliprole and cyantraniliprole, in peach, sweet cherry and pear fruits were evaluated. Field trials were conducted at different localities in Vojvodina, Serbia. Products based on cyantraniliprole and chlorantraniliprole were foliar applied at the recommended rate, for the control of main peach, sweet cherry, and pear pests. The extraction procedure of insecticides involved a widely used QuEChERS-based method, while residues were analyzed by HPLC-DAD. Methods were validated according to SANTE criteria. The half-life (DT_{50}) of cyantraniliprole in peach and sweet cherry fruits obtained in this study was 2.5 and 3.0 days, respectively. Furthermore, chlorantraniliprole dissipated rapidly in peach and pear fruits, with half-lives of 3.15 and 2.76 days. This study suggests that chlorantraniliprole and cyantraniliprole could be safely used in peach, sweet cherry and pear orchards, with an important role in the control of insecticide resistance.

Keywords: *anthranilic diamide, dissipation dynamic, peach, sweet cherry, pear.*

Intoduction

Contemporary agricultural production, with high, satisfactory yields is almost impossible without the use of plant protection products (PPP) and suitable agrotechnical measures. On the market, currently are available different chemical groups of insecticides, but in spite of that, unsuitable agricultural practice often leads to pest resistance development, as well as various harmful effects on human health or the environment. Nowadays, a few relatively new class of insecticides, as spinosines and anthranilic diamides, with the potential to replace insecticides such as neonicotinoids and pyrethroids in pest management are developed (Schmidt-Jeffris and Nault, 2016). Anthranilic diamides, with a novel mode of action, are highly effective in pest control, which is especially important for reducing pest resistance. The ryanodine receptor is the target site of action of these compounds. They intensely activate this receptor, by releasing stored calcium from the sarcoendoplasmic reticulum, which causes a disorder in the regulation of muscle contraction (Cordova et al., 2006). Active ingredients chlorantraniliprole,

cyantraniliprole, flubendiamide, cyclaniliprole and tetraniliprole belong to this class. Chlorantraniliprole and cyantraniliprole, second-generation anthranilic diamide insecticides, are two active ingredients that are currently registered for use in many crops. The systematic activity and residual control make them ideal for controlling a wide range of pests in many different crops. Anthranilic diamides are also considered to be fairly selective, with low toxicity to many non-target and beneficial insects (Schmidt-Jeffris and Nault, 2016). These two insecticides are used in very low application rates for controlling rangde of pests belonging to Lepidoptera order and some other insects belonging to different orders. They act contact and digestive, with ovicidal, larvicidal activity on all larval stages, as well as adulticidal activity. In Serbia, cyantraniliprole and chlorantraniliprole based insecticides are registered for the control of *Grapholita molesta*, *Anthonomus rubi*, *Rhagoletis cerasi*, *Cacopsylla pyri*, *Lobesia botrana*, *Clysia ambiguella*, *Cydia pomonella*, *Lithocolletis blancardella*, *Delia antique*, *Pieris rapae*, Thysanoptera order, *Helicoverpa armigera*, *Trialeurodes vaporariorum*, *Tuta absoluta*, *Leptinotarsa decemlineata* in peach, raspberry, sweet cherry, pear, grape, apple orchards and, onion, cabbage, cucumber, paprika, tomato, and potato crops (Plant Doctor, 2021).
However, even if they are applied in accordance with good agricultural practices, the pesticides may leave residues (Lazić et al., 2012). This is especially important for the fruits and vegetables consumed mostly fresh. Factors such as climate, soil, application rates, etc., strongly influence pesticide fate in the environment and agricultural products, thus it is important to evaluate persistence under agro-ecological conditions where they are applied. In this paper, authors were evaluated behavior and the fate of the anthranilic diamides, chlorantraniliprole and cyantraniliprole, in peach, sweet cherry and pear fruits.

Material and methods

Field experiment in peach orchards. Field trials were set up at the localities Čerević and Mala Remeta, Serbia, during 2017. The experiments were conducted according to the standard EPPO methods for the experimental design, data analysis, insecticide efficiency, and phytotoxicity (2014, 2015). Products based on cyantraniliprole (100 g a. i./l, SE) and chlorantraniliprole (200 g a. i./l, SC) were foliar applied at the rate of 0.6 and 0.2 l/ha, respectively with water consumption of 1000 l/ha. The orchards were 5 years old with the peach cultivar Royal gem. The treatment was conducted for the control of the first generation of the *G. molesta*. At the time of the treatment, the peaches were in the BBCH 74 phenophase. The experiment was set up in four replications, with the layout of the basic plots according to a randomized block system. To evaluate the cyantraniliprole and chlorantraniliprole residues in the peach fruits, fruit samples were randomly picked from each repetition and delivered to the laboratory. For the determination of cyantraniliprole residues, the fruit samples were taken 1 h after the pesticide application and, during the next 7 days starting from the first, while for the determination of chlorantraniliprole residues, samples were taken 1 h after the application and after 1, 3, 5, 7, 9, 11, 13 and 15 days.

Field experiment in sweet cherry orchard. For the dissipation dynamic analysis of insecticide cyantraniliprole in sweet cherry fruits, the experiment was set up in 2018 at the locality Čerević in Vojvodina Province, Serbia. The trial was created according to EPPO methods, due to the fruit phenophase and biology of treated pests. Insecticide based on cyantraniliprole (100 g/l, SE) was applied at the recommended rate of 0.75 l/ha, with 1000 l water/ha, for the control of *R. cerasi* in sweet cherries. Randomly picked fruit samples were taken right after the drying deposit and every next day, for seven days.

Field experiment in pear orchard. To determine the residues and dissipation dynamics of the insecticide chlorantraniliprole in pear fruits, the experiment was set up according to standard EPPO methods, at the locality of Kula (Vojvodina, Serbia) in 2019. The treatment was performed foliar with water consumption of 1000 l/ha when the pears were in the phenophase BBCH 74. The plot was under the pear cultivar Williams and treated with PPP (45 g/l chlorantraniliprole, 18 g/l abamectin), at a concentration rate of 0.1%. Sampling was performed one hour after the treatment, and after 1, 2, 4, 6, 8, 10, 12, and 14 days.

In all experiments, 500 g of fruit samples were collected and transported to the laboratory, and stored at a temperature of -18 ºC until the analysis. Also, the untreated samples were collected and used as a control for the validation of the methods.

Extraction and determination. The extraction procedure of insecticides involved a widely used QuEChERS-based method (EN 15662) (Anastassiades et al. 2003), while residues were analyzed by high-performance liquid chromatography (HPLC) with a diode-array detector (DAD) (Figure 1). Methods were validated according to SANTE/1183/2017 and SANTE/12682/2019 criteria.

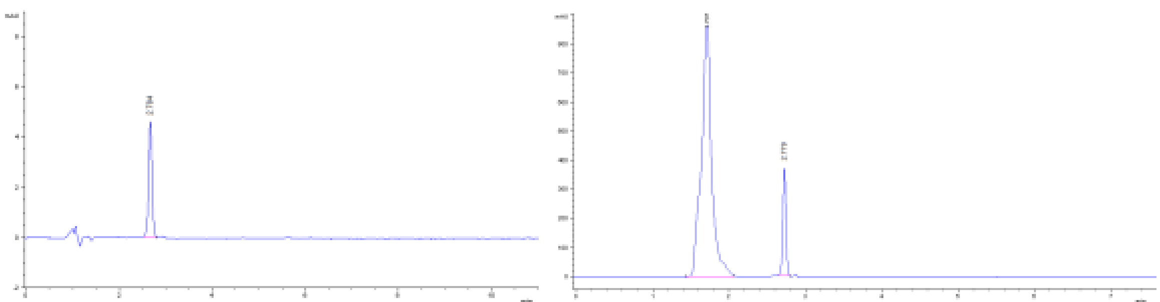

Figure 1. Chromatogram of cyantraniliprole standard solutions in acetonitrile and sweet cherry sample

Results and Discussion

For the analysis of the cyantraniliprole and chlorantraniliprole residues in the fruits, the methods validated in accordance with the requirements of the SANTE/1183/2017 and SANTE/12682/2019 standard were applied.

Table 1. Half-life of the cyantraniliprole and chlorantraniliprole in peach fruits

Insecticide	**Regression equation**	**Correlation coefficient**	**Degradation constant**	**Half life, DT_{50} (days)**
Cyantraniliprole	$y=1.46e^{-0.28x}$	0.28	0.965	2.5
Chlorantraniliprole	$y=2.32e^{-0.22x}$	0.22	0.961	3.15

The concentration of cyantraniliprole in the peach fruits was at the EU MRL (Commission Regulation EU 2018/832) of 1.5 mg/kg, immediately after the drying of the deposit (1.46 mg/kg), while at the end of pre-harvest interval (PHI), cyantraniliprole concentration was 0.19 mg/kg. Using these results, the calculated half-life (DT_{50}) of cyantraniliprole in peach fruits was 2.5 days (Table 1).
After the application of chlorantraniliprole, the maximum level of the residues in the peach fruits was determined after drying the deposit and it was 2.32 mg/kg. The study established that the level of chlorantraniliprole was below the MRL of 1 mg/kg seven days after the application. During the research, it was found that the chlorantraniliprole content in the samples was progressively decreasing. After the PHI of 14 days, the level of chlorantraniliprole was 0.12 mg/kg, which is significantly below the allowed level. The half-life of chlorantraniliprole, calculated based on the residue content, with the use of chlorantraniliprole in the amount of 0.2 l/ha, was 3.15 days.
Over the past few years, a number of studies were conducted with the aim of assessing the behavior of cyantraniliprole and chlorantraniliprole in crops, but none of them were conducted in peach fruits. Hong-Mei et al. (2014) established that the half-life of cyantraniliprole in paprika ranged from 9.2–11.2 days. Also, Sun et al. (2012) reported that the half-life of cyantraniliprole ranged from 2.9–6.4 days in the pak choi. In tomatoes, the half-life of cyantraniliprole was 2.6 days, with a PHI of 3 days, after treatment at the recommended dose (Malhat et al. 2018). According to Hu et al. (2013), the DT50 values of cyantraniliprole were 1.1 and 2.7 days in watermelons. The cyantraniliprole half-lives determined in rice straw at three localities were 3.2, 4.4 and 6.3 days (Zhang et al. 2013). According to the available literature, this was one of the first research of cyantraniliprole and chlorantraniliprole dissipation dynamics in peach fruits. Based on the obtained results, it can be concluded that cyantraniliprole and chlorantraniliprole in the peach fruits decrease rapidly and have low half-life values. Therefore, it is recommended to use cyantraniliprole and chlorantraniliprole-based insecticides for control the of *G. molesta* in peach orchards.

Table 2. Half-life of the cyantraniliprole in sweet cherry fruits

Insecticide	**Regression equation**	**Correlation coefficient**	**Degradation constant**	**Half life, DT_{50} (days)**
Cyantraniliprole	$y = 4.57e^{-0.23x}$	0.23	0.960	3.0

After drying the deposit, the amount of cyantraniliprole residues in sweet cherry fruits was 4.75 mg/kg (Table 2). In sweet cherry fruits, the initial concentration of cyantraniliprole residues was below the EU MRL of 6 mg/kg (Commission Regulation EU 2018/832). Analysis has shown a decrease in the amount of residues, with 0.80 mg/kg of cyantraniliprole in the fruit sample collected last day of the sampling period. The performed study proved that insecticide cyantraniliprole half-life in sweet cherries, was 3 days. The PHI of 7 days was correctly prescribed, however very fast cyantraniliprole degradation allows the use of this product, if needed, even shortly before harvesting, without the risk of the high level of residue content.
Lee et al. (2019) conducted an experiment about the dissipation of cyantraniliprole and its metabolite IN-J9Z38 in proso millet during cultivation. There was a 91.1 and 89.1% decrease in cyantraniliprole residues in grain and straw, respectively, from the initial residues (14-7 days) to

the final plot (40-30 days before harvest). The calculated half-life of cyantraniliprole was 11.3 and 9.4 days for grain and straw, respectively.

Table 3. Half-life of the chlorantraniliprole in pear fruits

Insecticide	Regression equation	Correlation coefficient	Degradation constant	Half life, DT_{50} (days)
Chlorantraniliprole	$y = 4.95e^{-0.25x}$	0.25	0.988	2.76

Since the use of chlorantraniliprole is also allowed in control of pear orchards against *C. pyri*, the method for the determination of its residues in pear fruits was validated. The maximum level of chlorantraniliprole residues in pear fruits was detected after drying the deposit and it was 4.95 mg/kg (Table 3). By analyzing samples collected during the 14 days, a gradual decrease in residue concentration was observed. In Serbia and EU prescribed PHI for chlorantraniliprole in pear orchards is 14 days, while the MRL in pear fruits is 0.5 mg/kg. During this experiment it was determined that the level of this insecticide was below MRL after six days from the day of application. At the end of the PHI the level of chlorantraniliprole in pear fruits was 0.16 mg/kg, which is significantly below the MRL. At the end of the research, the half-life for chlorantraniliprole in pear fruits was calculated, and it was 2.76 days.
Vijayasree et al. (2013) conducted research about chlorantraniliprole residues in cowpea fruits. It was concluded that the concentration of chlorantraniliprole dissipates rapidly in this crop. The calculated half-life was 1.31 days. Kar et al. (2013) conducted an experiment with three applications of chlorantraniliprole at the recommended dose and with a doubled recommended dose. The deposits were found to be less than MRL, which is 2 mg/kg. Residue concentration dissipated below the limit of quantification (0.1 mg/kg) after 3 and 5 days at recommended and double doses respectively. The calculated half-life was 1.36 days following application at the recommended dose while the prescribed waiting period was 1 day. A study about the dissipation of chlorantraniliprole, chlorpyrifos-methyl and indoxacarb in apples was conducted by Szpyrka et al. (2017). The half-lives of chlorantraniliprole, chlorpyrifos-methyl and indoxacarb were 16–17, 4–6 and 20–24 days, respectively. The initial residue levels declined gradually and reached the level of 0.01 mg/kg in 1 month for chlorpyrifos-methyl, 2 months for chlorantraniliprole and 2.5 months for indoxacarb, while MRL was 0.01, 0.5 and 0.5, respectively. By monitoring the degradation dynamics of chlorantraniliprole in grapes in Egypt, a half-life of degradation of 2.7 days was determined (Malhat, 2012).
Based on the available literature, there are no data related to the half-life of degradation of chlorantraniliprole in pear fruits, as well as its degradation dynamics in this fruit species.

Conclusions

This study indicates that anthranilic diamide insecticides, chlorantraniliprole and cyantraniliprole, could be safely used in peach, sweet cherry and pear orchards in the recommended dose in our agroecological conditions. Chlorantraniliprole and cyantraniliprole insecticides dissipated rapidly, with half-lives between 2.5 and 3.15 days. This study suggests that anthranilic diamide insecticides, chlorantraniliprole, cyantraniliprole could be safely used in peach, sweet cherry and pear, with an important role in the control of insecticide resistance.

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References

Anastassiades M.S., Lehotay J., Štajnbaher D., Schenck F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International, 86: 412–431.

Bagi F. (Ed.) (2021). Plant Doctor - Plant Protection Products on the Serbian Market, 49, 3-4/2021. Department for Environmental and Plant Protection, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia.

Commission Regulation EU 2018/832, Official Journal of the European Union.

Cordova D., Benner E., Sacher M.D., Tao Y., (2006). Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. Pesticide Biochemistry and Physiology 84, (3), 196-214.

EPPO Standards (2014). Phytotoxicity assessment, PP 1/135(4). European and Mediterranean Plant Protection Organization, 44: 265–273.

EPPO/OEPP Bulletin (2015). Efficacy evaluation of insecticides, PP 1/288 (1) *Grapholita molesta*. European and Mediterranean Plant Protection Organization, 45: 347–350.

Hong-Mei H., Chun-Rong Z., Ya-Hong Z., Chang-Peng Z., Li-Feng P., Hua Z., Min W. Tao T., Xiao-Ming C., Zhen L. (2014). Residue and degradation of Cyantraniliprole and its main metabolite in pepper and soil. Chinese Journal of Analytical Chemistry, 42: 1177–1182.

Hu X., Zhang C., Zhu Y., Wu M., Cai X., Ping L., Li Z. (2013). Determination of residues of cyantraniliprole and its metabolite j9z38 in watermelon and soil using ultra-performance liquid chromatography/mass spectrometry. Journal of AOAC International, 96: 1448–1452.

Kar A., Mandal K., Singh B. (2013). Environmental fate of chlorantraniliprole residues on cauliflower using QuEChERS technique. *Environmental Monitoring and Assessment*, vol. 185, pages 1255–1263.

Lazić S., Šunjka D., Grahovac N., Guzsvany V., Bagi F., Budakov D. (2012). Application of liquid chromatography with diode-array detector for determination of acetamiprid and 6-chloronicotinic acid residues in sweet cherry samples. Pesticides & Phytomedicine, 27: 321–329.

Lee J., Woo Jung M., Lee J., Lee J., Shin Y., Kim J. (2019). Dissipation of the Insecticide Cyantraniliprole and Its Metabolite IN-J9Z38 in Proso Millet during Cultivation. Scientific Reports, vol. 9, Article number: 11648

Malhat F., Kasiotis K.M., Shalaby S. (2018). Magnitude of cyantraniliprole residues in tomato following open field application: pre-harvest interval determination and risk assessment. Environmental Monitoring and Assessment Journal, 190: 115–116.

Malhat, F.M. (2012). Determination of chlorantraniliprole residues in grape by high-performance liquid chromatography. Food Analytical Methods, 5: 1492–1496.

SANTE/11813/2017 (2017). Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. European Commission, Directorate General For Health and Food Safety.

SANTE/12682/2019 (2020). Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. European Commission, Directorate General For Health and Food Safety.

Schmidt-Jeffris R.A., Nault B.A. (2016). Anthranilic Diamides Can Potentially Replace Neonicotinoid Seed Treatments in Vegetable Crops. Entomology Today.

Šovljanski R., Lazić S. (2007). Basics of phytopharmacy. University of Novi Sad, Faculty of agriculture, Novi Sad, Serbia.

Sun J., Feng N., Tang C., Qin D. (2012). Determination of cyantraniliprole and its major metabolite residues in pakchoi and soil using ultra-performance liquid chromatography-tandem mass spectrometry. Bulletin of Environmental Contamination and Toxicology, 89: 845–852.

Szpyrka E., Matyaszek A., Słowik-Borowiec M. (2017). Dissipation of chlorantraniliprole, chlorpyrifos-methyl and indoxacarb—insecticides used to control codling moth (*Cydia Pomonella* L.) and leafrollers (*Tortricidae*) in apples for production of baby food. Environ Sci Pollut Res Int., 24(13): 12128–12135.

Vijayasree V., Bai H., Beevi S.N., Mathew T.B., Kumar V., George T., Xavier G. (2013). Persistence and Effects of Processing on Reduction of Chlorantraniliprole Residues on Cowpea Fruits. Bull Environ Contam Toxicol, 90:494–498.

Zhang C., Hu X., Zhao H., Wu M., He H., Zhang C., Tang T., Ping L., Li Z. (2013). Residues of cyantraniliprole and its metabolite J9Z38 in rice field ecosystem, Chemosphere, 93: 190–195.

MICROBIOLOGICAL QUALITY OF SURFACE WATER AND SAFE VEGETABLE PRODUCTION

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Abstract

Good microbiological quality of irrigation water has a great importance for health-safety agricultural production. In order to avoid microbiological contamination of crops, the microbiological quality of the water supplying the crops should be monitored. The aim of this paper is investigation of the microbiological quality of the water from 5 channels and one pool from the municipality of Surcin which can potentially be used for irrigation, as well as determination the microbiological quality of the vegetables which are irrigated from the pool. Microbiological parameters of water and vegetables quality are determined by the standard methods prescribed by the Rulebook on parameters of surface waters (MPN method) and Rulebook of food hygiene of production of Republic of Serbia. The values of the tested parameters in one of the five investigated channels, Surcin channel water, were higher in relation to the limit values of the Rulebook. The increased levels of total and fecal coliforms, *Escherichia coli*, *Salmonella,* and *Shigella* were found. The Surcin channel water requires appropriate treatment before its use for crop irrigation. The values of other tested waters did not go beyond the limit values prescribed by the Rulebook, but for the safer agricultural production their treatment is recommended. Microbiological analyzes of vegetables were also done. The total and fecal coliform bacteria, as well as *E. coli*, *Salmonella* sp. and *Shigella* sp., were not detected in the tested vegetable samples.

Key words: *microbiological quality of irrigation water, coliform bacteria, E. coli, Salmonella sp.*

Introduction

Consumption of fresh fruits and vegetables is essential for healthy nutrition and prevention of various diseases The World Health Organization (WHO) underline necessary for consumption at least 400 g of raw fruits and vegetables daily. However, these products could be microbiologically contaminated and pose a risk for human health. Based on that, it is necessary to take care about microbiological safety of fresh vegetables and fruits. It implies the absence of human pathogens, because consuming raw vegetables that are microbiologicaly contaminated can cause many health problems and sometimes can even lead to death. Human pathogens could be associated with gastrointestinal diseases and cause many health diseases such as dysentery, hepatitis, hemolytic uraemic syndrome, typhus (Halablab et al., 2011).

Microbiological contamination of fresh vegetables can occur at any point of production chain and the sources of contamination could be numerous, but one of the most common is contaminated irrigation water or faecal contamination from humans or animals (Halablab et al., 2011). The surface waters are used for irrigation but they are very often polluted (Uyttendaele et

al., 2015) and that contamination comes from runoff, animal faeces, sewage (Gemmell and Schmidt, 2013).
Irrigation water is the good environment for many human pathogens such as viruses (adenovirus, norovirus, enterovirus, rotavirus), human pathogen bacteria (*Campylobacter sp*., *E. coli*, *E.coli* O157: H7, *Bacillus cereus*, *Salmonella sp*., *Shigela sp*.) (Ahmed et al., 2014). These microorganisms are able to colonize vegetable plant tissue through irrigation water and contaminate edible parts of plants because plant root niches are a good environment for their survival. Thus, irrigation water from two rivers in Norway had higher level of *E. coli* compared to national regulatory standard (Paruch et al., 2015). The research of Mcheik et al., (2018) showed huge microbiological contamination in the 33 well water samples taken from 5 sites. All water samples were contaminated with total coliforms and most of them with fecal coliforms, *E. coli* and *S. aureus*. These waters were used for irrigation which caused negative effect on microbiological safety of vegetables. The high microbial loads of coliforms and *S. aureus* were noticed at lettuce, mint, dandelion, spinach, coriander and radish. Such a large number of bacteria on mentioned leafy species of vegetables is a consequence of the larger surface area exposed to contaminated irrigation water. Also, the reason for this is entrance and penetration human pathogen bacteria in plant inner tissues, root and leaves (Kljujev et al., 2018a; Kljujev et al., 2018b). Also, there is evidence that surface of leafy vegetables could be a host for potential human pathogens (Dees et al., 2015). The enteric-bacterial-contaminated irrigation water has impact on the microbiological safety of vegetables. The large number of coliforms and *E. coli* in channel irrigation water leads to contamination vegetables. The high number of coliforms and *E. coli* was at spring onion, cabbage, carrot and tomato (Douti et al., 2021).
It is very important to take care about microbiological quality of irrigation water (Jeong et al., 2016; De Pascale et al., 2011), especially those which is used for irrigation of vegetables that are eaten in raw. It is necessary to regularly control these waters in order to enable safe vegetable production.

Materials and methods

Sampling of water and vegetables for microbiological analysis was done on the territory of the Surcin, municipality near Belgrade in Serbia. The microbiological analysis included 6 water samples and 3 vegetable samples. Out of 6 water samples, 5 samples were originated from channel water and another one was from pool used for irrigation. The locations for water samples were: Channel ”Surcin”, Channel ”Donje polje”, Channel between P.S ”Donje polje” and P.S Petrac”, Channel ”Petrac”, Channel ”Galovca”, Pool ”I.S.C.”. The lettuce sample was taken from open field but hot peppers and cucumbers samples were taken from greenhouse. Analyzed vegetables are irrigated with water from Poll ”I.S.C.”.
The channel "Galovica" covers an area of about 70 000 ha and its capacity is 24 m^3 of water per second. It flows into the river "Sava" near the settlement of New Belgrade and it brings water to the "Galovica" pumping station. The channel "Petrac" covers an area of 8 000 ha, it has capacity 8 m^3 water/sec. and its length is 24 496 m. Both channels ("Galovica" and "Petrac") run in parallel at a distance of 65 m from each other and they are interconnected by a connecting side channel for water inlet and outlet. The water in these channels represents an excess of water and has a important role in irrigation especially in summer. There are two big companies (PKB Corporation A.D. Belgrade and Farm "July 7"-Jakovo) which use waters of Channel "Donje polje'' and Channel between P.S ''Donje polje'' and P.S ''Petrac'' for irrigation their fields with

corn, sunflower and other mainly industrial plants during the summer. Also, the water sample was taken from the pool of the agricultural farm "Iceberg Salat Center". This company uses water from well of 25 meters depth for irrigation.

The microbiological quality of lettuce (*Lactuca sativa*), hot pepper (*Capsicum annuum*) and cucumber (*Cucumis sativus*) was examined. Vegetable samples were taken from the agricultural areas of the company "Iceberg Salad Center". Lettuce was grown in the open field and hot peppers and cucumbers were grown in greenhouses.

Microbiological analysis included determination of: aerobic heterotrophs (psychrophilic and mesophilic), total and fecal coliforms, *Escherichia coli*, *Salmonella* sp., *Shigella* sp. Determination of indicator microorganisms (their presence and number) was done inoculated appropriate nutrient media by direct and diluted samples. The number of heterotrophs was determined on Nutrient Agar (Torlak, Serbia). The water samples were diluted in Peptone Water (Torlak, Serbia) and after medium inoculation, incubation was done at 26°C, 5-7 days (mesophiles), 22°C, 5-7 days (psychrophiles). After incubation, colony counts were done.

The detection of total, fecal coliforms and *E.coli* was done using MPN method. A series of test tubes with MacConkey Broth (Torlak, Serbia) and Durham tubes were inoculated with 1 ml of samples diluted in peptone water, and it was done in triplicate. Incubation was done at 37°C for 48 hours. The tubes with changed medium color and bubbles in Durhams were taken as positive. Based on the number of positive tubes, the most probable number of coliform bacteria in 100 ml of water is determined using the MPN table. The confirmation test using EMB agar (Biomerieux, France) was done. The detection of *Salmonella* sp. and *Shigella* sp. was done using Selenite Broth (Biomerieux, France) for enrichment and SS Agar (Biomerieux, France) for confirmation. Incubation was done at 37°C for 24^{h}. The appearance of typical black colonies is confirmed as *Salmonella sp*. and uncolored and colonies were estimated as *Shigella sp*. The vegetable samples were tested to presence of aerobic heterotrophs, total and fecal coliform bacteria, *E. coli*, *Salmonella sp*. and *Shigella sp*. The 20g of vegetable pieces was homogenizated with Peptone Water on a shaker 250 rpm for 30 min. The 1 ml of dilution was used for inoculation of Nutrient Agar and selective media MacConkey Broth, Selenite Broth, SS Agar, EMB Agar. Incubation was done at 22°C, 37°C and 44°C for $24 – 48^{h}$. The number of bacteria is represented as cfu/ml and MPN/100 ml for water samples and cfu/g DM and MPN/gDM (DM- Dry Matter) for vegetable samples. The obtained results were interpreted according to Rulebook on parameters of ecological and chemical composition of surface waters and parameters of chemical and quantitative status of groundwater ("Official Gazette of RS", no. 74/2011) and Rulebook on general and special conditions of food hygiene at any stages of production, processing and trade ("Official Gazette of RS", No. 72/10).

Results and discussion

Aerobic heterotrophic bacteria are indicators of water status and quality. Their presence in large numbers indicates that the water is loaded with biodegradable organic pollutants. Our results showed that the number of total aerobic heterotrophs depends on the sampling location and the highest number was detected in Channel "Surcin".

Table 1 . The number of aerobic heterotrophs in water samples

Location	Unit	Temperature (t^o)	
		22^{o}C	37^{o}C
Channel "Surcin"	cfu/ml	551	368
Channel "Donje polje"	cfu/ml	14	14
Channel between P.S "Donje polje" and P.S "Petrac"	cfu/ml	338	99,5
Channel ''Petrac''	cfu/ml	24	36
Channel ''Galovca''	cfu/ml	12	9,5
Pool ''I.S.C.''	cfu/ml	24,5	18.5

The number of psychrophilic bacteria was 551 cfu/ml, and mesophilic was 368 cfu/ml which indicate that water is loaded with organic matter. The smallest number of heterotrophic bacteria was noticed in Channel "Galovica" (12 cfu/ml and 9.5 cfu/ml.) (Table 1). The results of the tested vegetable samples showed the high number of aerobic heterotrophs at lettuce (103 cfu/g DM and 54 cfu/g DM) (Table 2).

Table 2. The number of aerobic heterotrophs on vegetable samples

Vegetable sample	Unit	Temperature (t^o)	
		22^{o}C	37^{o}C
Lettuce	cfu/g DM	103	54
Hot pepper	cfu/g DM	14,5	0
Cucumber	cfu/g DM	9	5

Determination of total coliforms (TC), fecal coliforms (FC) and *E.coli* is important for microbiological quality of irrigation waters and vegetables. The coliform bacteria indicate to fecal contamination. The highest number of coliform bacteria (total and fecal) was detected in water of Channel "Surcin", more that 1 100 000 (MPN/100 ml) and according to the Rulebook on parameters of ecological and chemical composition of surface waters and parameters of chemical and quantitative status of groundwater ("Official Gazette of RS", no. 74/2011) this water belongs to IV-V class.

Based on our results, the water of Channel "Surcin'' should not be recommended for irrigation crops without appropriate treatment. Channel "Surcin" is located about 200 m from the municipality center near markets, feed factory, livestock farm and many other facilities. It is assumed that all waste, generated from the mentioned facilities, arrive into the channal, as well as the local population often leave their waste into the Channel "Surcin". Also, there is an inflow of sewage water into the Channel "Surcin" under the bridge near downtown which indicates that the water is much polluted.

The water from Channel "Donje polje'', Channel between P.S ''Donje polje'' and P.S ''Petrac'', Channel ''Petrac'' and Pool ''I.S.C.'' belong to II and III class according to the above-mentioned Rulebook. These waters could be used for irrigation according to the Regulation on Water Classification ("Official Gazette of RS", No. 5/68). The smallest number of coliforms was

noticed in Channel ‘’Galovca’’ water 430 MPN/100 ml which means that this water belongs to I-II class and it is safe for irrigation crops (Table 3).
Also, the number of fecal coliforms as well as *E. coli* was the highest in Channel “Surcin’ water. The *E. coli* was also detected in waters of Channel “Donje polje’’ and Channel between P.S‘’Donje polje’’ and P.S‘’Petrac’’ but its number was much smaller. Other investigated waters were free for *E. coli*. Our results are in agreement with other authors which detected total coliforms in channel water ranged between 3.74 and 3.92 (logCFU/100ml) while the fecal coliforms results ranged from 3.39 and 3.82 (logCFU/100ml), respectively. The *E. coli* ranged from 3.17 to 3.54 (logCFU/100ml), while *Salmonella* sp. was not detected (Douti et al., 2021).
According to above-mentioned Rulebook (‟Official Gazette of RS‟, No. 74/2011) for presence of fecal coliforms in groundwaters, Channel ‘’Surcin” water belongs III-IV class. The large number of *E. coli* in this water indicates its huge contamination with fecal matter and it will be a big risk if this water should be used for irrigation, esspecially vegetables which are eaten in raw. The presence of *Salmonella sp.* and *Shigella sp.* was confirmed in waters of Channel ”Surcin’’ and Channel “Donje polje’’ (Table 3). The study of Mcheik et al.~~,~~ (2018) showed that fecal contamination was in the range from 92.4% to 17.5% out of 33 total well water samples and *S. aureus* was in the range from 78.7% to 14.5%.
The number of total coliform bacteria in vegetable samples (lettuce, hot pepper, cucumber) was insignificant, les then 30 MPN/gDM each sample. The pathogens, *E coli*, *Salmonella sp*. and *Shigella sp*. were also not detected and which indicates no fecal contamination and vegetables are safe for consumption. Unlike our results, Mcheik et al. (2018) noticed high level of faecal coliforms and *E.coli* at vegetables which were irrigated with contaminated water and the highest level of bacteria was at lettuce (6.0 and 5.0 $\log_{10}$CFU.g^{-1} fresh weight). Also, other authors found out high level of *E. coli* in vegetable samples irrigated with polluted water. The average number of *E. coli* for tomato samples were 3.27 logCFU/10g, for carrot samples 3.33 logCFU/10g and for spring onion and cabbage were 3.50 and 3.34 respectively (Douti et al., 2021).

Table 3. The number of coliforms (MPN/100 ml) and presence of pathogens in water samples

Water sample	Channel ”Surcin”	Channel “Donje polje”	Channel between P.S”Donje polje”and P.S”Petrac”	Channel ‘’Petrac’’	Channel ‘’Galovca’’	Pool ‘’I.S.C.’’
TC	>1.100.000	7.300	2.400	2.400	430	930
FC	70	< 30	< 30	< 30	< 30	< 30
E. coli	1100	200	36	0	0	0
Salmonella	+	+	-	-	-	-
Shigella	+	+	-	-	-	-

According to the German standard for irrigation waters (DIN 19650), the water of the Channel ”Surcin” should not be used for irrigation crops which are consumed in raw. Also, according to mentioned German standard, waters from Channel ”Donje polje’’ and Channel between P.S ”Donje polje’’ and P.S. ”Petrac’’ would not be used for irrigation, but our Regulation on water classification ("Official Gazette of RS", No. 5/68) allows using these water for irrigation (II-III class).

The presence of *Salmonella* sp. and *Shigella* sp. in waters of Channel "Surcin'' and Channel "Donje polje" indicate for fecal contamination and their presence in the groundwater is not specified by our law for waters which could be potentially used for irrigation. Considering worldwide epidemics caused by consumption of *Salmonella*-contaminated fresh products, it could not recommend these waters for irrigation. According to the Rulebook on general and special conditions of food hygiene at any stages of production, processing and trade ("Official Gazette of RS", No. 72/10) it is not allowed presence of Salmonlla *sp*. in food. *Salmonella sp.* and *Shigella sp.* were not detected in tested vegetable samples which indicate good microbiological quality of investigated vegetables.

Conclusion

Based on the microbiological analyzes of water, it could be concluded that investigated water have medium to good microbiological quality. The Surcin channel water has very poor microbiological quality and this water is a good environment for many human pathogens. It is not usable for irrigation, especially for irrigation vegetables which are eaten in raw. This water should undergo certain purification processes in order to get microbiologically safety water for irrigation.

The other tested waters could potentially be used for irrigation but appropriate water treatment should be recommended in order to improve microbiological quality of water and achieving health safety agricultural production.

The water from the well of the company "Iceberg Salat Center", which is otherwise used for irrigation, has satisfactory microbiological quality as well as vegetables which are healthy safe for consumption.

References

Ahmed, W., Brandes H., Gyawali P., Sidhu J. P. S., Toze S. (2014) Opportunistic pathogens in roof-captured rainwater samples, determined using quantitative PCR. Water Res. 2014, 5, 361-369

De Pascale, S., Costa, D L., Vallone, S., Barbieri, G., Maggio, A. (2011) Increasing Water Use Efficiency in Vegetable Crop Production: From Plant to Irrigation Systems Efficiency. HortTechnology, Volume 21: Issue 3, 301–308, Jun 2011.

Dees M. W., Lysøe E., Nordskog B., Brurberg M.B. (2015) Bacterial communities associated with surfaces of leafy greens: Shift in composition and decrease in richness over time. Appl. Environ. Microbiol. 2015, 81, 1530-1539.

Douti N. B., Amuah E. E. Y., Abanyie S. K., Amanin-Ennin P. (2021): Irrigation water quality and its impact on the physicochemical and microbiological contamination of vegetables produced from market gardening: a case of theVea Irrigation Dam, U.E.R., Ghana. J Water Health (2021) 19 (2): 203–215.

Gemmell M. E., Schmidt S. (2013) Is the microbiological quality of the Msunduzi River (KwaZulu-Natal, South Africa) suitable for domestic, recreational, and agricultural purposes? Environmental Science and Pollution Research 2013, 20, 6551-6562.

Halablab M. A., Sheet I. H., Holail H. M. (2011) Microbiological Quality of Raw Vegetables Grown in Bekaa Valley, Lebanon, "American Journal of Food Technology, Vol. 6, No. 2, 2011, pp. 129-139.

Jeong, H., Kim, H., Jang, T. (2016) Irrigation Water Quality Standards for Indirect Wastewater Reuse in Agriculture: A Contribution toward Sustainable Wastewater Reuse in South Korea. *Water* 2016, *8*(4), 169;

Kljujev I., Raicevic V., Vujovic B., Rothballer M., Schmid M. (2018) Salmonella as an endophytic colonizer of plants - A risk for health safety vegetable production. Microbial Pathogenesis, Volume 115, 199-207. (a)

Kljujev I., Raicevic V., Jovicic-Petrovic J., Vujovic B., Mirkovic M., Rothballer M. (2018) Listeria monocytogenes–Danger for health safety vegetable production. Microbial Pathogenesis, Volume 120,23-31. (b)

Mcheik A., Awad A., Fadel A., Mounzer C., Nasreddine S. (2018): Effect of Irrigation Water Quality on the Microbial Contamination of Fresh Vegetables in the Bekaa Valley, Lebanon. American Journal of Agriculture and Forestry 2018; 6(6): 191-197.

Paruch A. M., Mæhlum T., Robertson L. (2015) Changes in microbial quality of irrigation water under different weather conditions in southeast Norway. Environ. Proces. 2015, 2, 115-124.

Uyttendaele M., Jaykus L., Amoah P., Chiodini A., Cunliffe D., Jacxsens L., Holvoet K., Korsten L., Lau M., McClure P., Medema G., Sampers I., Jasti P. R. (2015) Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production. Compr. Rev.in Food Sci. Food Saf. 2015, 14, 336-356.

THE PROGNOSIS OF *CYDIA POMONELLA* L.THE MOST SIGNIFICANT PESTS OF APPLES

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Abstract

The prognosis of *Cydia pomonella* L. appearance in apple plantations enables proper undertaking of protective measures without harmful effects on man and useful organisms. The indication pest extermination deadlines improve the efficacy of protection and diminishe the appearance of toxic substances in apple fruit. The aim of this work is to point out the significance of methods for the prognosis of pest appearance as the basis for pest suppression and the rational application of pesticides. The paper is accompanied by a flight of *Cydia pomonella* L. at two sites in Rasina district.The pest presence determination visually, by the usage of feromone traps, by calculation of efficient temperatures, enables the proper application of chemical protective measures, as well as the production of healthy organic food achieving the preservation of the environment. Among the areas with extremely suitable ecological conditions for intensive production of apples is the Rasina district. The modern growth of apples as a high intensive growing of fruit species iwass threatened by many economically important pests and pathogens. In order to suppress them effectively and economically, it is necessary to monitor the upgrowth of apple pests, to make the prognosis of their appearance and signalize the terms of treatment.

Keywords: *prognosis, indication, codling moth, apple.*

Introduction

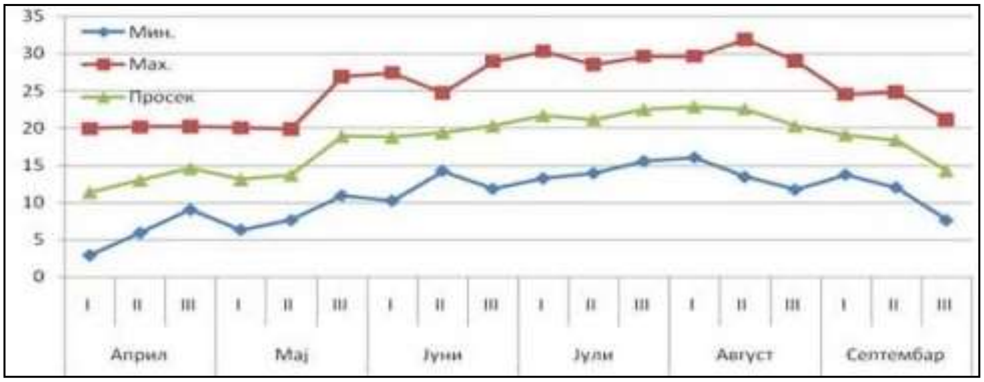
Fruit growing has a very significant place in the modern agricultural production. The special intention is paid to the spreading and organizing the fruit production in orchards. Among the areas with extremely suitable ecological conditions for intensive production of apples is Rasina district. The modern growing of apples as high intensive growing of fruit species is threatened by a large number of economically important pests and pathogens (Milenković and Stamenković, 2003). In order to suppress them effectively and economically, it is necessary to monitor the upgrowth of apple pests, to make the prognosis of their appearance and signalize the terms of treatment (Stamenković and Stamenković, 2000). Economically the most significant apple pest is codling moth – *Cydia pomonella* L., against which is necessary to make the measures of protection since it is regularly present each year in plantations in all the regions of apple production (Nikolić and Stamenković, 2003). The damage made by grubs of apple fryer can be direct (vermiculation, deformity and the earlier falling of fruit and indirect (rotting of fruit) and in some years the apple production and profitability can be threatened. The aim of the research is to point at the significance of the methods of prognosis of pest appearance as the base for their effective repression. Signalization of the terms of repression provides the rational use of pesticides and reduces the presence of toxic substances in apple fruit.

Material and methods

During the examination the appearance and prognosis of apple fryer in apple plantations were monitoring, at the territory of Rasina district (Serbia), in 2018. The main objects of research were the already existing apple plantations at two localities: Mala Drenova (between Kruševac and Trstenik) and Donji Stupanj (between Kruševac and Aleksandrovac). In Donji Stupanj flying of the butterfly of apple fryer was monitored at the apple area of 1,8 ha where the following species are: Golden delicious, Klon reinders, Idared, Granny smith and Red chief. The space between planting is 3,8 x 1,2 m. The cultivated shape is the modified pyramid and is in good health for now. In planting the chemical treatment was done against weed by Zoomer 390 SC (glyphosate 360 g/l + oxyfluorfen 30 g/l). Butterfly flying was monitoring from 20 April to 31 August 2018. In the area of Mala Drenova, the surface of planting was 1,5 ha, Idared, spindle shaped bushes. Planting space was 3,5 x 1m. Treatment against weed was done by Glyphosate 5 l/h, and mechanical inter row treatment. Butterfly flying was monitoring from 18 April to 30 September 2018. The gathering of data about meteorological conditions was done in the Weather station in Kruševac. Orientational prognosis was done at the basis of monitoring the sum of average daily temperatures. Appearance and butterfly flying were monitored by pheromonal batches which were set at the edge of orchard at the height of 1,5-2 m. Applying the everyday check the calculating and recording of caught butterflies were done. By visual method plant organs were examined and the first appearance of butterflies was determined, the beginning of oviposition, the beginning of grub drilling in the fruits and monitoring of their upgrowth. The sums of effective temperatures were calculated as the total of average daily temperatures which were diminished for 10^{o} C. The prognosis of flying of apple fryer can be done by monitoring the sum of daily temperatures. The sum of daily temperatures starts to be counted from so called biofix. Biofix stands for the first stable fly of butterfly and usually takes place around blooming, or after blooming. When biofix is determined from the day after the degree days (DD) are counted, that is, the sum of effective temperatures which is counted by subtracting 10^{o} C from the average value of temperature. On the basis of such monitoring, eggs are appearing at the sums of temperatures from 69 to 111, and cocoons from 200 to 345 .

Results and discussion

The climate in Kruševac and its surrounding is moderate continental with distinct seasons where autumn is slightly warmer than spring. When the data of perennial average value and average temperatures were analyzed in 2018 (Graph 1.), it can be seen that winter in 2017 was milder than perennial average value, while the other months were of approximate values.



Graph 1. Minimal, middle and maximal average temperature per decade in Kruševac in 2018.

During the research the presence of apple fryer was determined in all the localities from the period of vegetation to the end of the apple vintage. The activity of butterflies depends to a large amount on meteorological factors, while the greatest influences have the air temperature and rainfalls. The beginning of flying of butterflies in spring is one of the most important moments in the biology of pests since the period from laying eggs to grub drilling in fruits is the most convenient for the efficacy of chemical agents in the fight against apple fryer.

3.1. Locality Mala Drenova – The results of dynamics of the number of *C. pomonella* are shown in table 1. Looking at the results it can be seen that the first butterflies were noticed on 19 April 2018 and that they were presented during the whole vegetation period. The increase of number was particularly noticeable at the end of the first decade of May in the first generation and at the beginning of July in the second generation. The constant presence of adult butterflies during August indicates to the presence of the third generation of *C. pomonella*. In none of the generations the higher peaks are not expressive, but the mild increase in number in each generation of growth is clearly noticed. It is clear that climate changes, above all variable temperatures and increased number of rainy days in the year had the influence to not appearing of the regularity of the number of butterflies. The variability of upgrowth *of C. pomonella* depending on climate factors was pointed out by many researchers in their research (Rafoss and Sæthre, 2003). Climate factors (temperature, light, rainfalls, air flow, etc.) influence together and their influence is not the simple sum of separate factors . However, in the conditions of moderate climate the crucial role in the upgrowth of apple fryer has the temperature

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Table 1. Dynamics and number of *C. pomonella* in Mala Drenova in 2018.

Number of *C.pomonella*	**April**	**May**	**June**	**July**	**August**	**September**
1		1	0	3	2	2
2		0	0	4	1	1
3		1	1	5	3	1
4		0	0	8	2	1
5		0	1	3	1	0
6		3	0	1	1	0
7		5	3	0	1	1
8		10	3	1	1	0

9		5	3	1	2	0
10		4	2	1	2	0
11		2	3	1	3	1
12		4	3	0	4	0
13		1	1	0	4	1
14		0	26	0	1	0
15		0	3	0	1	0
16		0	4	0	3	1
17		0	1	1	3	0
18	0	1	2	3	4	1
19	2	1	1	3	3	1
20	0	0	1	2	3	0
21	1	4	3	0	1	0
22	0	7	1	2	3	0
23	1	1	0	6	4	0
24	1	1	1	1	5	0
25	1	0	2	3	1	0
26	0	3	8	3	2	0
27	0	3	1	0	3	0
28	0	0	1	4	2	0
29	1	1	1	3	1	0
30	0	0	1	0	1	0
31		1		0	1	
	0.54	1.90	2.57	1.90	2.23	0.37

3.2. Locality Donji Stupanj – At the locality Donji Stupanj the greater number of adult butterflies of *C. pomonella* was notified during the whole vegetation period in accordance to the locality Mala Drenova. The beginning of flying was notified on 21 April 2018 and the number was increasing up to 9 May, when the maximal flying of 28 imagoes was notified. The increasing of the number of butterflies and maximal flying are in accordance with temperatures at the beginning of May. The higher number of butterflies of *C. pomonella* in comparison to the locality in Mala Drenova is the surrounding of the monitored orchard by fewer apple trees nearby whose protection is deficient and movement of butterflies to the examined plantation. Apple fryer was earlier considered as "sedentary" species , but during the research it has been determined that particular individuals of this species can move even to few kilometers which significantly influences the spreading of this pest (Keil *et al.*, 2001) . This is particularly important since in that way the resistant types of *C. pomonella* are spreading. The significant role in that has also man. It is familiar that on the wooden boxes which are used for transporting apples grubs make their cocoons and in this way the pest spreads to further distances.

Table 2. Dynamics and number of *C. pomonella* in Donji Stupanj in 2018.

Number of *C.pomonella*	April	May	June	July	August
1		4	0	20	1
2		5	0	6	1
3		3	1	4	0
4		3	0	3	0
5		3	1	3	1
6		4	2	1	1
7		4	2	0	0
8		12	3	1	1
9		28	3	1	2
10		11	2	0	0
11		5	3	1	1
12		1	2	1	0
13		4	6	0	1
14		2	2	1	1
15		1	3	0	1
16		0	2	0	2
17		0	1	1	3
18	0	2	5	2	2
19	0	1	3	3	3
20	0	0	4	0	2
21	1	4	2	1	1
22	0	3	7	2	3
23	1	2	5	5	0
24	0	5	6	2	0
25	0	0	8	3	1
26	0	1	12	5	2
27	2	1	6	6	2
28	1	0	8	5	2
29	3	1	10	5	1
30	2	0	11	7	1
31		1		10	2
	0.77	3.58	4.00	3.19	1.23

Theoretical model for the prognosis of appearance of *C. pomonella* butterflies is based on the sum of effective temperatures which is monitored from 1 March. On the base of the sum of effective temperatures the appearance of first butterflies should be expected in the interval from 22 to 30 April, and larvae from 22 May and later if it is considered that according to the sum needed for the upgrowth of a butterfly is from 69-111, and eggs 200-345. The average temperature above 10^{o} C was notified on 17 March and it was 11 days to the end of the month with temperatures above 10^{o} C. The sum of effective temperatures in March was 22,8; in April 93,4; in May 170; in June 296,1; in July 367,5; in August 369,5; in September 219.Wildbolz stresses that for the upgrowth of butterflies in spring the temperature should be 100 ^{o}C, grub for

eggs 90^{o} C, and only for one generation (from the phase of eggs to grown up butterflies) the needed sum of effective temperatures 610^{o} C.
According to that calculation the appearance of the second generation should be expected in the middle of July. According to our data the second generation appeared was a bit earlier, at the beginning of July. Literature is familiar with such a notion and it is connected to the effect of insecticide by whose use the resistance to certain chemical substances appears, on one hand, and the shortage of developing cycles, on the other hand.

Conclusions

On the base of shown results of appearance and dynamism of flying of *C. pomonella* it can be concluded the following: at both localities the appearance of three generations of *C. pomonella* was determined. The first generation appeared at the end of the first decade of May, the second at the beginning of July and third in the middle of August 2018. The first butterflies at both localities were noticed at the beginning of the last decade of April and is caused by variable meteorological conditions in that period.
The use of pheromone batches was a useful method for monitoring the flight of *C. pomonella* butterflies and the protection of plantation on the base of that.
Prognosticating the presence of apple fryer by calculating the sum of effective temperatures and monitoring meteorological factors provides the timely determination of treatment terms for its suppression. In that way the effective protection is reached, rational use of pesticides and protection of the environment.

References

Milenković S., Stamenković S. (2003). Uloga feromona u sistemu integralne zaštite. Padinska Skela: XVII savetovanje agronoma, veterinara i tehnologa. Zbornik naučnih radova, 9 Str. 207-212.

Stamenković T., Stamenković S. (2000). Atlas štetočina i bolesti jabuke sa programom mera zaštite. Čačak: Centar za voćarstvo i vinogradarstvo. Institut za primenu nauke u poljoprivredi, Beograd. Str. 28-32.

Nikolić K., Stamenković S., (2003). Rasprostranjenost *Cydia pomonella* L. (*Lepidoptera, Tortricidae)* u Jablaničkom okrugu. VI Savetovanje o zaštiti bilja, Zlatibor. Str. 24-28.

Rafoss T., Sæthre M. G. (2003). Spatial and temporal distribution of bioclimatic potential for the Codling moth and the Colorado potato beetle in Norway: model predictions versus climate and field data from the 1990s. *Agricultural and Forest Entomology*. Vol.5, pp. 75–86.

Keil S., Gu H., Dorn S. (2001). Response of *Cydia pomonella* to selection on mobility: laboratory evaluation and field verification. *Ecological Entomol*ogy. Vol. 26, pp. 495-501.

THE RESISTANCE OF AUTOCHTHONOUS VARIETIES OF APPLES AND PEARS ON *ERWINIA AMYLOVORA*

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Abstract

Erwinia amylovora, a bacterial blight of apple fruit, is one of the most important diseases of apples and pears. It is very difficult to suppress the disease and sometimes the infection is 100% and practically the fruits are not for use. Bacterial blight caused by E. amylovora belongs to the group of the economically most significant diseases of apple fruits. Since E. amylovora parasitizes all the organs of the plant (flower, fruit, skeletal branches, and trunk) and causes necrosis and tissue damaging on them, this bacterium is considered as one of the most destructive pathogens. With further significant spread of the infection in the Republic of Serbia, due to insufficient, superficial, or non implementation of measures to protect sensitive host plants, even more significant economic losses from the quarantine bacteria E. amylovora, should be expected in the near future. One of the measures that could ensure the normal yield of apples and pears in the coming period is the cultivation of autochthonous varieties. According to the data from the literature, autochthonous varieties are resistant to plant diseases. Apple ("Sarunka", "Sumatovka", "Kolacara", "Budimka") and pear varieties ("Vodenac", "Kaludjerka", "Lubenicarka", "Jecmenka", "Letnji kajzer" and "Karamanka") were used in the research. By using specific laboratory tests (ELISA test, IF test, Biologist test) as well as checking pathogenicity, inoculation of young apples and pears, the presence of Erwinia amylovora at autochthonous varieties will be proved. "Gloucester" apples and "William" pears were used as controls. The autochthonous varieties of apple and pear used in this study are resistant to Erwinia amylovora, although the occurrence of bacterial exudate by artificial inoculation was recorded.

Keywords: *Erwinia amylovora, apple, pear, autochthonous varieties.*

Introduction

Erwinia amylovora is the causative agent of bacterial blight of plants of the *Rosaceae* family. The most important hosts of this bacteria are pome fruit species (pear, apple, medlar and quince), *Sorbus spp*., *Cotoneaster spp*., *Crategus spp*. and *Pyracantha spp*. Hosts are also species of the genera *Chaenomeless* and *Photinia* (Panić and Arsenijević, 1996; Gavrilović and Arsenijević, 1998; Balaž *et al*., 2004; Gavrilović *et al*., 2008). Bacterial blight caused by *E. amylovora* belongs to the group of the economically most significant diseases of apple fruits. Since *E. amylovora* parasitizes all the organs of the plant (flower, fruit, skeletal branches, trunk) and causes necrosis and tissue damaging on them, these bacteria are considered as one of the most destructive pathogens. With further significant spread of the infection in the Republic of Serbia, due to insufficient, superficial, or non implementation of measures to protect sensitive host plants, even more significant economic losses from the quarantine bacteria *E. amylovora*, should be expected in the near future. One of the protection measures in the future could be the use of antibiotics, which are already used and give good results. It is also necessary to introduce bactericides into the market and use them, which we currently do not have on the Serbian market. However, not all apple varieties are equally sensitive to *E. amylovora*. Very sensitive are: Fuji, Gala and Jonathan, sensitive: Granny Smith, Golden Delicious and Jonagold, and resistant: Red Delicious, Prima and Priscilla (Fisher and Richter, 1998; Mohan *et al*., 2001; Boyd and Jacobi, 2005). Therefore, intensive work is being done to study this pathogen, the symptoms caused by it, its bacteriological characteristics (Meyer, 1973; Paulin, 1983) and to predict the occurrence of the disease. Adequate and effective measures to suppress pathogens include: cultivation of resistant plant genotypes, as well as the application of physical, biological, chemical and mechanical measures to suppress *E. amylovora* (Garrett, 1990; Beer, 1991), emphasizing that it must be carried out with mandatory disinfection of work equipment (Panić and Arsenijević, 1996). Intensive work is being done to find and create resistant genotypes to *E. amylovora* (van der Zwet and Beer, 1995). One of the measures that could ensure the normal yield of apples and pears in the coming period is the cultivation of autochthonous varieties. According to the data from the literature, autochthonous varieties are resistant to plant diseases (Gavrilović *et al*., 2014).

Material and methods

Artificial inoculation of immature fruits of autochthonous varieties of apple and pear was performed by applying drops of bacterial suspension, originating from the culture, by pricking a needle in three places of each fruit. Apple fruits of the varieties: "Sarunka", "Sumatovka", "Kolacara" and "Budimka" were inoculated, as well as pear fruits of the varieties: "Vodenac", "Kaludjerka", "Lubenicarka", "Jecmenka", "Letnji kajzer" and "Karamanka" (Figures 1-6). Inoculated fruits were stored in a humid chamber for 1-2 days, moistened by spraying, using hand sprayer, and then covered with moistened polyvinyl (Kudela, 1988; Arsenijević, 1988). Plant material was collected in the municipality of Nis, Serbia. 10 fruits were inoculated from each variety. Firstly they were sterilized with 70% ethyl alcohol on the surface, and then washed under a stream of distilled water, after which they were placed on filter paper for drying. The dried fruits were placed in Petri boxes, which were placed in plastic containers lined with moist filter paper, in order to create the optimal humidity necessary for the infection process and the formation of exudates. The control were the fruits of the "Gloucester" apple and the "William" pear.

Figure 1. Autochthonous variety "Lubenicarka"

Figure 2. Autochthonous variety "Vodenac"

Figure 3. Autochthonous variety "Kaludjerka"

Figure 4. Autochthonous variety "Karamanka"

Figure 5. Autochthonous variety "Jecmenka"

Figure 6. Autochthonous variety "Letnji kajzer"

Results and discussion

By inoculation of autochthonous pear varieties, the appearance of bacterial exudate was recorded on pear fruits, varieties: "Lubenicarka", "Letnji kajzer", "Kaludjerka", "Vodenac" and "Karamanka", while there was no change on variety "Jecmenka" (Table 1, Figure 7). The control strain of pear, the "William" variety, reacted positively and expressed its reaction with the appearance of bacterial exudate, which gives us confirmation of the correctness of the experiment.

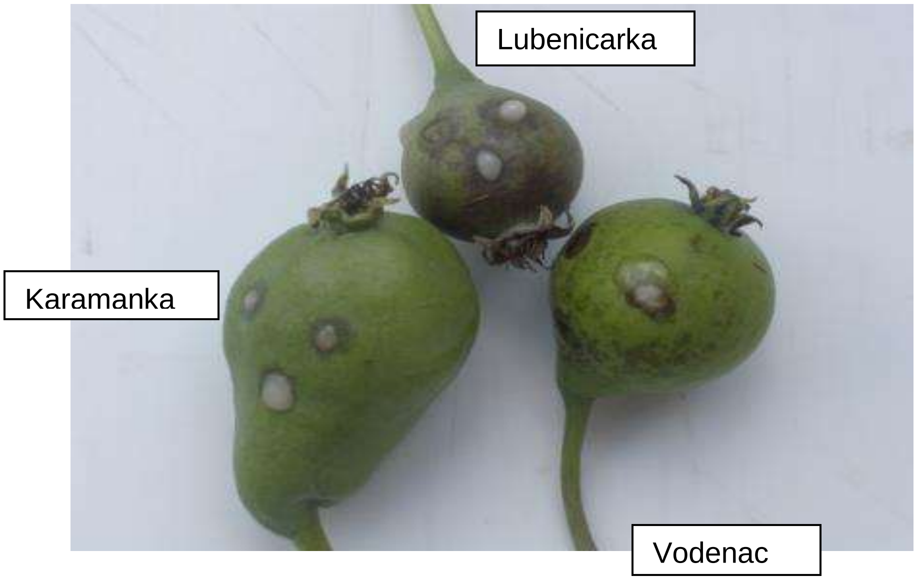

Figure 7. *E. amylovora*: Appearance of exudates on autochthonous pear varieties

By inoculation of autochthonous apple varieties, the appearance of bacterial exudate was not recorded on the fruits of the varieties: "Sarunka", "Sumatovka", "Kolacara" and "Budimka" (Table 1). The control strain of apple, the "Gloucester" variety, reacted positively and expressed its reaction with the appearance of bacterial exudate, which gives us confirmation of the correctness of the experiment.

Table 1: Appearance of exudates on autochthonous pear and apple varieties

Plant species	**Variety**	**Appearance of bacterial exudate**
Pear	Vodenac	+
Pear	Kaludjerka	+
Pear	Lubenicarka	+
Pear	Jecmenka	-
Pear	Letnji kajzer	+
Pear	Karamanka	+
Apple	Sarunka	-
Apple	Sumatovka	-
Apple	Kolacara	-
Apple	Budimka	-
Apple - control	Gloucester	+
Pear - control	William	+

The studied samples of fruits, leaves and branches, of apples and pears originating from autochthonous varieties taken from nature, show a negative reaction to the ELISA test, if test and express test (Table 2), which proves that autochthonous varieties of apples and pears in the natural environment do not suffer from bacterial blight of apple fruit.

Table 2. Specific tests for the identification of *E. amylovora* on autochthonous apple and pear varieties from samples taken from nature

Plant species	Isolate code	ELISA test	IF test	EXPRESS test
Pear	Vodenac	-	-	-
Pear	Kaludjerka	-	-	-
Pear	Lubenicarka	-	-	-
Pear	Jecmenka	-	-	-
Pear	Letnji kajzer	-	-	-
Pear	Karamanka	-	-	-
Apple	Sarunka	-	-	-
Apple	Sumatovka	-	-	-
Apple	Kolacara	-	-	-
Apple	Budimka	-	-	-
Apple - control	Gloucester	+	+	+
Pear - control	William	+	+	+

Control of *E. amylovora* is hampered by the lack of appropriate bactericides to prevent its spread. Copper-based pesticides show some efficacy, but may be phytotoxic in phenophases when the risk of infection is greatest (Arsenijević and Gavrilović, 2007). Antibiotics (streptomycin and oxytetracycline) are highly effective, but their long-term use leads to the development of resistance, which was recorded in the 1970s in the USA (Van der Zwet and Keil, 1979). In addition, antibiotics are not registered for use in plant protection in many European countries, but also in Serbia. Due to all this, intensive research has been conducted in many countries in recent decades with the aim of selecting resistant varieties of pear and apple to *Erwinia amylovora*. One of the possible solutions could be the cultivation of autochthonous varieties of apple and pear resistant to *Erwinia amylovora*.

Conclusion

Autochthonous varieties of apples and pears in nature do not suffer from bacterial blight of apple fruit caused by the bacteria *Erwinia amylovora*. This is confirmed by specific pathogen identification tests (ELISA test, IF test, Biologist test). By artificially infecting young apple fruits varieties: "Sarunka", "Sumatovka", "Kolacara" and "Budimka", and pear varieties: "Lubenicarka", "Letnji kajzer", "Kaludjerka", "Vodenac", "Karamanka" and "Jecmenka", the presence of bacterial exudate was determined on pears varieties: "Lubenicarka", "Letnji kajzer", "Kaluđerka", "Vodenac" and "Karamanka", while the variety "Jecmenka" did not show any changes. Artificially infected apple varieties did not show the appearance of bacterial exudate (Vojinović, 2010). The appearance of bacterial exudate proves the presence of *Erwinia amylovora*.With this research we wanted to draw attention to the fact that in this way the presence and spread of *Erwinia amylovora* can be controlled. This experiment was done on a small sample and errors are possible. The final confirmation of whether the autochthonous varieties of apple and pear are resistant or tolerant to *Erwinia amylovora* can be determined by analysis at a higher level using the latest technology. In addition to resistance to plant diseases, these varieties have other qualities that recommend them for cultivation.

References

Arsenijević, M. (1988): Bacteriosis of plants. II edition, p. 464. Scientific book

Arsenijević, M., Gavrilović, V. (2007): A practical handbook on bacterial blight of apple fruit trees. Institute for Plant Protection and Environment, Belgrade, pp 1-80.

Balaz, J. Knezevic, T., Smiljanic, A., Stojsin, B. (2004**)**: Chaenomeles japonica and Cotoneaster horizontalis, new hosts of *Erwinia amylovora* in Serbia, 10th international Workshop on Fire Blight Bologna, Italy 5-9 juli 2004. Book of abstracts 22.

Beer, S. V. (1991): Fire blight, pp 61-63. In: Compendium of Apple and Pear Diseases, by A.L. Jones and H.S. Aldwinckle. APS Pres, St. Paul.

Boyd, J. E. and Jacobi W. R. (2005): Fike blight, Colorado Gardener's Calendar

Ficher, C., Richter, K. (1998): Results of fire blight resistance within the Pillnity apple breeding program. 8th International Workshop on Fire blight. Kuşadasi, Turkey, 12-15 October, 1998. Book of Abstract pp. 56.

Garret, C. M. E. (1990): Control of fire blight of Pomoideae.Applied research in Europe (1997-88), pp. 54-78. Comision of the European Comunities, Luxembourg.

Gavrilović, V., Arsenijević, M. (1998): Fire thorn - new host of *Erwinia amylovora* for our country. Herbalist 1: 52-55, Novi Sad.

Gavrilović, V., Živković, S., Ivanović, Ž., Vojinović, M. (2008): Sorbus domestica and Sorbus torminalis new hosts of *Erwinia amylovora* in Serbia. "Plant Protection" Vol 58 (1-4) No.263-266, 69-79. Belgrade

Gavrilović, V.,Stanisavljević, R., Stošić S., Stevanović m., Aleksić G., Stajić M., Dolovac N. (2014): Examination of resistance of pear varietes to Erwinia amylovora by inoculation of immature fruits. "Plant Protection" Vol 65 No.289,117-123. Belgrade

Küdela, V. (1988): *Erwinia amylovora* - Causal Agent of Fire Blight Rosaceous Plants in Chechoslovakia. Ochr. Rostl. 24 (3): 173-182

Meyer, J. (1973): Feuerbrand-*Erwinia amylovora* Winslow et al. an der Westkuste Schleswinghilsteins. Nachrichtenblatt F. den Deutschen Pflanzenschutzdienstes 25:153-157.

Mohan, S., Fallahi, E., Bijman, V. P, (2001): Evaluation of apple varietes for susceptibility to *Erwinia amylovora* by artificial inoculation under field conditions. 9th international Workshop on Fire Blight. Hawke Bay, New Zealand, 8-12 October 2001. Book of abstrakt P52.

Panić, M., Arsenijević, M. (1996): Bacterial blight of fruit trees and ornamental plants - *Erwinia amylovara.* Monographic study. Community for fruits and vegetables, Belgrade D.D. and Faculty of Agriculture, Novi Sad, p.413.

Paulin, J. P. (1983): Bacterial blight: I disease, II Bacterial blight control methods in fruit and ornamental arboriculture, pp. 9-34.Ed. Inra-stifl, Paris.

van der Zwet, T., Keil, H.L. (1979): Fire Blight – A Bacterial Disease of Rosaceous plants. U.S. Department of Agriculture, Agriculturae Handbook 510, Washington, D.C., pp., 200.

van der Zwet, T., Beer, S. V. (1995): Fire blight - Its Nature, Prevention and Control. A Practical Guide to Integrated Disease Menagment U.S. Department of Agriculture. Agricultural Information Bulletin No.631, 97 pp.

Vojinović, M., (2010): Comparative characteristics of *Erwinia amylovora* strains obtained from diferent hosts, areas and altitudes in Serbia, Ph thesis, Faculty of Agriculture in Kosovska Mitrovica, 2010

EFFECT OF 1-METHYLCYCLOPROPENE ON STORAGE OF PEAR 'WILLIAMS'

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Abstract

The paper presents the influence of the use of 1-methylcyclopropene (1-MCP) on the shelf-life quality of pear fruits of the cultivar 'Williams' with two different cultivation sites in the vicinity of Čačak (Serbia). The fruits were stored separately under the same cooling conditions in a controlled atmosphere (CA) at -0.5-0.5°C after treatment. The tests were performed before the treatment of fruits, and then 1 day, 30 days and 90 days after treatment and shelf life of 7 days of aging at room temperature (20°C). The application of 1-MCP affected the preservation of fruit weight and strength at different measurement intervals. The soluble solid content and pH increase, and total acids content decreases with storage length, with smaller variations in treated compared to untreated fruits. Based on the examined parameters, the optimal storage period of fruits treated with 1-MCP from both sites was 30 days and shelf life of 7 days. The storage period of 90 days affected the biochemical parameter of the fruits and their use immediately after taking them out of the cooler. The length of fruit storage depends on the results of the initial examined parameters during the harvest, the content of macronutrients and their relationship, climatic conditions, the length of the period between harvest and treatment with 1-MCP, etc.

Key words: *1-Methylcyclopropene, Shelf-Life Quality, Pear, 'Williams'.*

Introduction

Pear is one of the most widely grown fruit species across the world, but of all cultivars, the best and most appreciated is for sure 'Williams' or 'Bartlett' as better known in the West. Storage of 'Williams' pears is typically limited to 2–3 months in open air, even under ideal storage conditions at -1°C. In most cases, the end of storage life is due to the onset of physiological disorders, particularly superficial scald and core breakdown. Ethylene produced by the fruit can exacerbate these disorders, as well as cause premature yellowing and softening during storage (Ekman et al., 2004). Late harvested fruit are particularly susceptible to these disorders, which may begin to appear within a month of harvest (Ju et al., 2001).

Pears are climacteric fruits, in which the major negative changes are the results of maturation. During ripening, some changes are observed in firmness, color, acidity, sugar content and development of aroma (Alpalhao et al., 2006). After harvest, pears are conditioned with ethylene and marketed immediately or stored at low temperatures until ready to be marketed (Villalobos-Acun˜a and Mitcham, 2008). Controlled atmosphere related disorders in fruit can be minimized by delaying the exposure to CA storage after harvest or by pre-storage conditioning fruit under ambient air at higher temperatures than the final storage temperature (Flaherty et al., 2018). Besides storing fruits at lower temperatures, it is necessary to control fruit ripening using blockers at the site of ethylene binding. In recent years, increasing applications of 1-methycyclopropene (1-MCP) in the preservation of freshness for harvested fruits have been reported. As a result, treatment with 1-MCP reduces the production of ethylene and other

volatiles, respiration, peel color development from green to yellow and the rate of softening (Watkins, 2006; Chiriboga et al., 2013a, 2013b; Hendges et al., 2018). The ethylene perception inhibitor 1-methylcyclopropene (1-MCP) is known to delay softening and peel yellowing in pears following their transfer from low temperature and CA storage to shelf-life conditions (Villalobos-Acuna and Mitcham 2008; DeEll and Ehsani-Moghaddam 2011; Gago et al., 2015).
The aim of our research is to determine the influence of 1-MCP on the storage potential of 'Williams' pears in a controlled atmosphere (CA) from different production plantations and at different storage temperatures.

Materials and methods

Plant material
Pear fruits were collected from the plantations pear 'Williams' established in 2013, grafted on rootstock quince BA 29, with interstems 'Kaluđerka' in production plantations of an agricultural producer in the village Trnava and Viljuša, Cacak (Serbia), under conditions of temperate continental climate. The beginning of the harvest in the first plantation was 14.08-20.08.2020, and in the second orchard 16.08-22.8.2020. Fruits were harvested at optimal maturity stage and size for tested cultivar.

1-methylcyclopropene (1-MCP) treatment
Pear fruits of uniform shape and without visible mechanical damage were selected for the needs of the experiment, in order to include 30 fruits in three replications from each production plant, while an equal number of samples was left in the cold storage to serve as a control variant. After selection of samples for analysis, the fruits were placed in the cold storage with a control atmosphere (CA) for a period of 4-7 days (at a temperature of 1°C). The fruits selected for treatment were arranged in crates and placed in a chamber with a volume of 500 m^3 in the first cold storage with an occupancy capacity of 50% (CS 1) from the first production plant in Trnava and with a volume of 250 m^3 in the second cold storage with an occupancy capacity of 90% (CS 2) from another production plantation in Viljuša. Treating the fruits with the agent acting as an ethylene blocker was performed by dissolving tablets of 1-MCP (SmartFresh^{TM}) with activator tablets (blue activator tablets) in citric acid. After diluting 1-MCP (SmartFresh^{TM} protab), the chamber was sealed airtight for the next 24 hours, until the gas concentration of 750 ppb was achieved. Control fruits were stored in wooden crates and placed in an adjacent empty chamber, under the same cooling conditions, The temperature during treatment in the first cold storage was 1°C and in the second cold storage 0°C.

Fruit storage and sampling
All of the replications of the fruits selected for treatment, as well as the ones that were not subjected to the 1-MCP treatment, were placed in wooden crates and kept in vertical tiers. The fruits were kept in a cold atmosphere: 0-0.5°C in the first cold storage and -0.5 to 0°C in the second cold storage, 90 ± 5% relative humidity (20.9kPa 0_2+<0.5kPa CO_2), in the period of 90 days. Sampling of fruits for analyses was carried out before fruit treatment (Ø), and then in three phases: 1 day (1), 30 days (2) and 90 (3) days after treatment. After taking the samples from the cold storage, the fruits were left in conditions of normal atmosphere: 20 ± 1°C and 60 ± 5% RH, for 7 days (shelf life), followed by the displayed measurement and chemical analyses of fruits (1-control; 2-treatment). Fruit weight (FW) was determined on a Mettler 0.01 g precision scale (Switzerland). The fruit firmness (FF) measuring was performed using the penetrometer Fruit

Pressure Tester FT 327 (Winopal Forschungsbedarf GmbH, Germany) with an 8 mm probe. Two measurements were made in six fruits per each replication on both sides expressed in Newton (N/cm^2) = fruit firmness value (kg/cm^2) × 9.81.

Assessments

The soluble solid content (SSC) of the fruit was determined on a manual refractometer (3828, Carl Zeiss, Germany). Actual acidity (pH value) was measured by a pH Meter Iskra MA 5707, Slovenia. Titratable acidity (TA) was determined by neutralization of fruit extract with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator. Acidity was expressed as mg malic acid/100 g fresh weight.

Content of macro and microelements in the fruits of pear

The content of macro and microelements was determined before the experiment setup, by sampling 1 kg of average samples in three replications, for each cultivar. The content of macro (K, Ca, Mg, Na) and microelements (Cu, Zn, Mn, Fe, Co, Ni, Pb) was anlaysed using modified method (Moraisa et al. 2017). Readings were performed on AAS, Perkin-Elmer, PinAAcle 500 (USA 2018), and values were expressed in mg kg^{-1} of fresh weight of the sample, whereas K+Mg/Ca, K/Ca ratio was determined using calculation.

Statistical analysis

The data was subjected to analysis of variance (*ANOVA*) using statistical package MSTAT-C (Michigan State University, USA). The least significance difference (LSD) was used to compare treatment means and treatments declared different at $p = 0.05$ level of significance.

Results and discussion

Prolonging the period of fruit storage and delaying the ripening of fruits requires a better understanding of the physiological and biochemical role, especially the ratio of Ca, K, Mg, K+Mg/Ca, K/Ca, and the content of microelements is also important. In tab. 1, results of macroelement content in fruits of pear are shown. The content of K and Ca had higher values in samples number 2, and the lower content of Mg affected the lower ratio of K + Mg/Ca and K/Ca (17,57-17,10), which is a good predisposition for longer storage of fruit. In fruits of apple, if the K/Ca ratio is higher than 30, and in certain cultivars higher than 25, even 20, there comes to physiological diseases and lower potential of fruit keeping, accordingly.

Table 1. Content of macroelements and their relation in pear fruits

Samples	K	Ca	Mg	Na	K+Mg/Ca	K/Ca
	mg·kg^{-1}				computational ratio	
plantation 1	969,4±35,57a	45,20±9,62a	58,20±5,52a	22,00±2,36a	22,70±2,43a	21,50±2,89a
plantation 2	1096,2±76,85a	64,20±6,78a	32,00±3,23b	3,00±0,90b	17,57±2,08a	17,10±1,82a
ANOVA	ns	ns	*	*	ns	ns

The Mn content shows significantly higher values in sample 2 (0.66 mg·kg^{-1}), and the Fe content in sample 1 (4.95 mg·kg^{-1}). The other elements did not differ significantly in their content between the pear samples (tab. 2).

Table 2. Content of microelements in pear fruits

Samples	Cu	Zn	Mn	Fe	Co	Ni	Pb
	mg·kg^{-1}						
plantation 1	3,02±0,60a	3,10±0,28a	0,06±0,03b	4,95±0,72a	0,03±0,01a	0,25±0,04a	0,13±0,03a
plantation 2	3,10±0,21a	2,36±0,20a	0,66±0,16a	1,86±0,26b	0,05±0,02a	0,20±0,09a	0,06±0,02a
ANOVA	ns	ns	*	*	ns	ns	ns

Rapid deterioration of eating quality and high rates of disorders occurring in over-mature pears would diminish repeat consumer purchases (Gallardo et al., 2011). Postharvest 1-MCP application effectively suppressed ethylene receptors, maintained FF and green color retention, and reduced physiological disorder development in pears. 1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene receptors, thus retarding ethylene-dependent responses such as ripening, senescence and physiological disorders (Watkins, 2006).

Taken as a whole, pear fruits in the first cold storage had greater weight, RSM and TA. With the length of storage, measurements show a decrease in fruit weight and firmness. SSC and TA increase up to 30 days of fruit storage then decrease, and the pH varied without special regularities. The application of 1-MCP significantly affected the preservation of fruit hardness, and the SSC and pH were increased in relation to untreated fruits. The initial hardness of 'Williams' fruits in the first cold storage was 54.39N, and in the second 68.02N, representing slightly lower values of hardness before treatment compared to the research of other authors (tab. 3). 'Bartlett' pear is harvested at 80 to 89N when the fruit is still green and firm but physiologically mature (Villalobos-Acun˜a et al., 2010). Recommended harvest maturity for 'Bartlett' pear is indicated by flesh firmness (FF) at 84 N; maturity is completed at 76 N, over the course of 14 days (Wang et al.,2016; Zhi and Dong, 2018). However, labor short working season and high temperatures in certain periods of vegetation, narrow the harvest period and as a result, a significant number of pears are picked at an over-mature stage. Over-mature pears are more prone to reduced FF, yellowing, physiological disorders (i.e. decay and scald) and rapid softening after long-term storage or long-distance shipping (Villalobos-Acu~na et al., 2011; Calvo et al., 2015; Zh and Dong, 2018).

Table 3. Effects of 1-MCP application on fruit weight, FF, SSC, pH and TA

Treatments			FW	FF (N)	SSC	pH	TA
Cold Storage (A)		1	184,38±3,78a	27,06±2,39b	12,52±0,16a	3,81±0,03b	0,27±0,005a
		2	172,57±3,57b	28,98±3,01a	11,34±0,13b	4,01±0,02a	0,24±0,006b
Measurements (B)		1	190,26±4,38a	61,20±1,61a	11,04±0,24c	3,92±0,05b	0,23±0,007c
		2	186,04±6,57ab	28,04±2,55b	12,43±0,22a	4,03±0,04a	0,30±0,007a
		3	174,31±5,36bc	14,63±1,65c	12,56±0,21a	3,78±0,03c	0,26±0,007b
		4	163,29±3,18c	8,21±0,77d	11,71±0,18b	3,91±0,04b	0,24±0,006bc
Treatments (C)		1	176,80±3,81a	22,02±2,81b	11,66±0,14b	3,86±0,03b	0,26±0,006a
		2	180,15±3,67a	34,02±2,43a	12,21±0,18a	3,96±0,03a	0,25±0,006a
Cold Storage x Measurements (AXB)	1	1	196,20±4,69a	54,39±1,39b	11,92±0,35b	3,70±0,07b	0,26±0,008bc
		2	195,24±9,61ab	30,30±3,71c	13,27±0,28a	3,98±0,05cd	0,30±0,007a
		3	184,34±7,76ab	14,17±2,28e	13,05±0,28a	3,71±0,04b	0,28±0,008ac
		4	161,74±4,37c	9,38±1,48f	11,86±0,27b	3,85±0,05c	0,24±0,008d
	2	1	184,32±7,26ab	68,02±1,79a	10,17±0,15c	4,13±0,02a	0,20±0,006c
		2	176,83±8,68bc	25,78±3,51d	11,58±0,19b	4,08±0,05ac	0,30±0,011a
		3	164,29±6,80c	15,10±2,45e	12,06±0,28b	3,86±0,05c	0,23±0,009d

		4	164,84±4,71c	7,03±0,34f	11,57±0,23b	3,97±0,04cd	0,25±0,008db
Cold Storage x Treatments (AXC)	1	1	179,93±5,63ab	20,82±3,49b	12,19±0,19a	3,77±0,04c	0,27±0,006a
		2	188,83±5,02a	33,30±2,98a	12,86±0,26a	3,85±0,04c	0,27±0,008a
	2	1	173,67±5,17b	23,22±4,46b	11,13±0,16c	3,95±0,04b	0,25±0,009b
		2	171,48±5,00b	34,74±3,88a	11,56±0,22b	4,07±0,03a	0,24±0,008b
A			*	*	*	*	*
B			*	*	*	*	*
C			ns	*	*	*	ns
AXB			ns	*	*	*	*
AXC			ns	*	*	*	ns

The firmness and color of the fruit is characteristic of the cultivar, and the emission of ethylene is an indicator of the ripeness of the fruit. The content of SSC and TA during harvest had significantly lower values in sample number 2 (10.17 and 0.20, respectively) and a higher pH value, compared to the sample from the first cold store. Certain differences in initial parameters during fruit harvest in different orchards and storage in two different cold storages contributed to differences between measurement and treatment, but both treated and untreated fruits required market placement after 30 days of storage. Yingli L. et al., in 2021 were harvesting 'Williams' fruit for storage, the hardness of the pear was 84.6 N, the SSC was 11.5%, and the TA was 0.33%. Our research results show the variation of fruit hardness, SSC, pH and TA during fruit harvest in the year of the experiment.

In tab. 4, the correlation between the analyzed parameters is shown. Pear samples from different coolers had a medium degree of negative correlation to SSC content and a low degree to FW and TA, and a positive correlation was expressed to pH value. Measurement intervals show a high degree of negative correlation on pear FF (-0.840), a low degree on FW and a low positive correlation on SSC. Different treatments had a low positive effect on N, SSC and pH. Fruit hardness at SSC and TA and a positive effect on pH had a low degree of negative correlation. SSC has a low positive correlation with TA and a negative one at pH.

Table 4. Correlation of examined parameters

Parameters	CS	Meas.	Treatm.	FW	FF	SSC	pH	TA
CS	1	0,00	-0,00	-0,187*	0,042	-0,422*	0,398*	-0,277*
Meas.		1	0,00	-0,328*	-0,840*	0,171*	-0,117	-0,030
Treatm.			1	0,053	0,262*	0,197*	0,206*	-0,040
FW				1	0,252*	0,135	-0,007	0,037
FF					1	-0,204*	0,214*	-0,203*
SSC						1	-0,271*	0,322*
pH							1	-0,046
TA								1

Many studies have reported that 'Williams' pears generally ripen within 7 days at 20°C after purchase. Usually, this information is based on experiments that were performed in a controlled environment. In reality, at the point of sale, storage time and conditions are not known, neither commercial handling procedures implemented to extend the shelf life after storage (Huč et al., 2020). Measurements of these parameters in our studies were performed after 7 days of shelf life at room temperature, which further affected the results obtained.

Conclusion

Based on the results of the study of the influence of 1-MCP on the storage length of different fruits of pear `Williams', it was found that the initial parameters for longer storage of fruits were more favorable in pear samples from plantation 2, observing the ratio of K + Mg/Ca and K/Ca (17.57-17.10) and FF 68.02N. The pear fruits in the first cold storages had a higher FW, RSM and TA. With the length of storage, measurements show a decrease in fruit weight and firmness, and SSC and TA increase until the 30^{th} day of fruit storage, then decrease, while the pH varied without special regularities. The application of 1-MCP significantly affected the preservation of fruit hardness, and the SSC and pH were increased in relation to untreated fruits. Certain differences in initial parameters during fruit harvest in different orchards and storage in two different cold storages contributed to differences between measurement and treatment, but both treated and untreated fruits required market placement after 30 days of storage. The shelf life at room temperature of 7 days significantly affected the quality of the fruit and it is necessary to consider reducing the shelf life, especially in pears compared to apples, which are more resistant to longer storage.

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Reference

Alpalhão, A., Neto, C., Goulão, L., Clemente, J., Henriques, J., Lourenço, I., Oliveira, C. M. (2006). Efeito do 1-metilciclopropeno em pêra 'Rocha' em diferentes estados de maturação armazenadas em atmosfera normal. In: I Simpósio Nacional de Fruticultura Alcobaça, 12 e 13 de Outubro de 2006. Actas Portuguesas de Horticultura n.º 11, 232-238.

Calvo, G., Candan, A. P., Civello, M., Giné-Bordonaba, J., Larrigaudière, C. (2015). An insight into the role of fruit maturity at harvest on superficial scald development in 'Beurré D'Anjou' pear. Scientia Horticulturae, 192, 173–179.

Chiriboga, M-A., Saladi ́e, M., Gin ́e Bordonaba, J., Recasens, I., Garcia-Mas, J., Larrigaudi`ere, C. (2013a). Effect of cold storage and 1-MCP treatment on ethylene perception, signalling and synthesis: influence on the development of the evergreen behaviour in 'Conference' pears. Postharvest Biology and Technology, 86, 212–220.

Chiriboga, M-A., Schotsmans, W.C., Larrigaudi`ere, C., Dupille, E., Recasens, I. (2013b). Responsiveness of 'Conference' pears to 1-methylcyclopropene: the role of harvest date, orchard location and year. Journal of the Science of Food and Agriculture, 93, 619–625.

DeEll, J.R., Ehsani-Moghaddam, B. (2011). Timing of postharvest 1-methylcyclopropene treatment affects Bartlett pear quality after storage. Canadian Journal of Plant Science, 91, 853–858.

Ekman, J.H., Clayton, M., Biasi, W.V., Mitcham, E.J. (2004). Interactions between 1-MCP concentration, treatmentinterval and storage time for 'Bartlett' pears. Postharvest Biology and Technology 31, 127–136.

Flaherty, E.J., DeEll, J.R., Shelp, B.J., Bozzo, G.G. (2018). 1 Methylcyclopropene affects the shelf-life quality of controlled atmosphere stored 'Cold Snap™' pears. Canadian Journal of Plant Science 98, 1365–1375.

Gago, C.M.L., Miguel, M.G., Cavaco, A.M., Almeida, D.P.F., Antunes, M.D.C. (2015). Combined effect of temperature and controlled atmosphere on storage and shelf-life of 'Rocha' pear treated with 1-methylcyclopropene. Food Science and Technology International, 21, 94–103.

Gallardo, R. K., Kupferman, E., Colonna, A. (2011). Willingness-to-Pay for Optimal "Anjou Pear Quality. HortScience 46, 452-456.

Huč, A., Vidrih, R., Trebar, M. (2020). Determination of Pears Ripening Stages Based on Electrochemical Ethylene Sensor. IEEE Sensors Journal, 20 (23), 13976 – 13983.

Ju, Z., Curry, A. E., Duan, Y., Guo, A. (2011). Plant Oil Emulsions Prevent Senescent Scald and Core Breakdown and Reduce Fungal Decay in `Bartlett' Pears. Horticultural Science, 126 (3), 358-363.

Li, Y., Zhang, S., Dong, Y. (2021). Improving storability, physiological disorders, and antioxidantproperties of 'Bartlett' and 'd'Anjou' pears (Pyrus communis L.) bypre-harvest 1-methylcyclopropene spraying. International Journal of Food Science and Technology, 56, 115–125.

Morais D. R., Rotta M. E., Sargi C. S., Bonafe E. G., Suzuki R. M., Souza N. E., Matsushita M., Visentainer V. J. (2017). Proximate Composition, Mineral Contents and Fatty Acid Composition of the Different Parts and Dried Peels of Tropical Fruits Cultivated in Brazil. Journal of the Brazilian Chemical Society. 28 (2), 308-318.

Villalobos-Acuña, M., Mitcham, E.J. (2008). Ripening of European pears: the chilling dilemma. Postharvest Biology and Technology, 49, 187–200.

Villalobos-Acun˜a, M. G., Biasi, V.W., Flores, S., Mitcham, J. E., Elkins, B. R., Willits, H.N. (2010). Preharvest Application of 1-Methylcyclopropene Influences Fruit Drop and Storage Potential of 'Bartlett' Pears. Hortscience, 45 (4), 610–616.

Watkins, C.B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389–409.

Villalobos-Acuna, M.G., Biasi, W.V., Flores, S., Jiang, C.-Z., Reid, M.S., Willits, N.H., ˜ Mitcham, E.J. (2011). Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. Postharvest Biology and Technology, 59, 1–9.

Wang, Y. (2016). Storage Temperature, Controlled Atmosphere, and 1-Methylcyclopropene Effects on α-Farnesene, Conjugated Trienols, and Peroxidation in Relation with Superficial Scald, Pithy Brown Core, and Fruit Quality of 'd'Anjou' Pears during Long-term Storage. Journal of the American Society for Horticultural Science, 141 (2), 177–185.

Watkins, C.B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389–409.

INFLUENCE OF REFINING PROCESS ON MYCOTOXIN CONTENT IN VEGETABLE OILS AND FATS

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Abstract

Oilseeds and crude oils may contain various contaminants that accumulate in the oil during vegetation, processing and storage of raw materials. All contaminants and their metabolites are harmful to human health. The refining process, in different stages, leads to the removal of these contaminants and they should not be found in refined oils. In addition to removing unwanted ingredients by refining, there are components of vegetable oils that need to be preserved due to their positive effect. As a result of the refining process, by-products appear to under go further processing. A special place among the contaminants is occupied by fungi (molds) that have the ability to produce mycotoxins, and the most important molds are *Aspergillus*, *Fusarium* spp., *Penicillium*, *Alternaria*. Some of the most common mycotoxins are aflatoxin, ochratoxin, fumonisins, trichothecenes, zearalenone, and patulin. The aim of this paper is to review the latest scientific knowledge, which relates to the influence of individual stages of the refining process on the content of mycotoxins in vegetable oils and fats, as well as the possibility of removing these contaminants from oils and fats during the refining process. In the production of vegetable oils and fats, the refining process is effective for reducing the content or removal of certain mycotoxins, in order to obtain quality, health-safe edible refined oil or fat. Since there are two basic refining processes, research has shown that chemical refining is, in that sense, more suitable than physical. The stages of refining during which most mycotoxins are removed are neutralization, bleaching and deodorization/dezoneutralization.

Keywords: *Edible oil, Contaminants, Mycotoxins, Removal.*

Introduction

Fungi (molds) contamination usually occurs during harvest or during storage of seeds and/or fruits. Inadequate storage conditions and other eco-physiological factors, especially those prevailing in tropical and subtropical conditions, such as high temperature and humidity, greatly contribute to the rapid growth of fungi. Fungis manage to produce mycotoxins even at low water activity (Bhat and Reddy, 2017). Food and feed fungi contamination is very widespread, and the most important fungi that contaminate food and feed are *Aspergillus*, *Penicilium* and *Fusarium*. They produce a group of various secondary metabolites - mycotoxins, showing a wide range of toxic effects on humans and animals (Gelderblom et al., 2014; Ji et al., 2015; Romanić et al., 2020). Several hundred different mycotoxins with high structural diversity have been discovered, differing in chemical and physicochemical characteristics. Most abundant mycotoxins occurrence are: aflatoxin and ochratoxin (produced by *Aspergillus* spp.), fumonisins, trichothecenes, zearelenone (produced by *Fusarium* spp.), patulin (*Penicillium* spp.). These compounds are strong toxins and have a wide range of effects on animals and humans because of their cytotoxic, neurotoxic, nephrotoxic, carcinogenic, mutagenic, immunosuppressive, teratogenic effect. In addition, most mycotoxins are causing dermatitis in humans and animals (Krska et al., 2009). Human exposure to mycotoxins mainly occurs through the food chain by consuming contaminated agricultural products, such as cereals, nuts, oilseeds and food products obtained from these sources (Lacoste, 2013; Gelderblom et al., 2014; Romanić et al., 2020; Abdolmaleki et al., 2021).

Good agricultural practice, plant disease management and adequate storage conditions of raw materials reduce the level of mycotoxins in the food chain, but mycotoxins are not completely removed from it in this way. Food processing can further reduce the content of mycotoxins by physical removal and chemical or enzymatic transformation of mycotoxins into less toxic products (Mariod and Idris, 2015; Karlovsky et al., 2016; Pinton et al., 2019). In edible vegetable oils and fats, the mentioned changes occur in the refining process. Refining, as a technological process, involves operations aimed at reducing the content or completely removing certain undesirable ingredients from crude oil, in order to obtain high quality edible oil. The refining process aims to remove all the ingredients from crude oil that reduce the sensory properties, quality and sustainability of refined oil. Undesirable ingredients in crude oil are of various origins and can be ingredients specific to a particular oil (protein residues, phospholipids, pigments, sulfur compounds, etc), degradation products formed during seed or during storage of seeds (free fatty acids, peroxides, aldehydes, ketones, non-hydrated phospholipids, etc) and converted into oil, residues of chemicals added during plant growth, degradation products and derivatives of these chemicals-pesticides, herbicides, desiccants, phosphoric acid-impurities from equipment or other oils (solvent, metals, soaps, etc) and pollution from soil, water, air or during seed drying (PAHs, dioxins, 3-MCP-diol, etc.) *(van Dujin, 2016; Chang et al., 2020; Zio et al., 2020; Abdolmaleki et al., 2021).

Two refining processes classical chemical (alkaline) refining and physical refining are represented (Ji et al., 2015; Gupta, 2017). Physical refining is suitable for oils with a high content of free fatty acids and a low content of non-hydrate phospholipids. Therefore, this refining process is suitable for crude palm and coconut oil. In case of the chemical refining of these oils, a large amount of soap would form, leading to a significant loss of neutral oil. Physical refining is also suitable for rapeseed oil with previously removed phospholipids, and in the case of sunflower and soybean oil it is possible only in the case of complete or enzymatic degumming in order to remove phospholipids and reduce phosphorus content to less than 10 ppm. Chemical refining is often used in the vegetable oils production, and mostly for oils of various oilseeds. In this process, the oil is pre-treated with an acid (e.g. phosphoric, etc) and then neutralized with a base solution (e.g. sodium hydroxide). In this phase, soaps are formed which are separated by centrifugation (separation), but also by water rinsing (Ji et al., 2015; Gupta, 2017; Flora et al., 2018; Abdolmaleki et al., 2021).

Influence of the refining process on mycotoxins as contaminants of vegetable oils and fats

Mycotoxins can be determined in vegetable oils and fats using chromatographic methods. Mycotoxins can be removed during different stages of refining process depending on their type, but they are all removed to the level of detection, ie below 1 μg/kg (Abdolmaleki et al., 2021). Table 1 shows the effect of physical and chemical refining on different contaminants in oils.

Table 1. Vegetable oils contaminants content and their removal in refining process stages (van Dujin, 2014; Pinton et al., 2019)

Contaminants	Mineral origin hydrocarbons (mg/kg)	PAH (polycyclic aromatic hydrocarbons) (μg/kg)	Pesticides (mg/kg)	Aflatoxin B1 (μg/kg)	Zearalenone (μg/kg)
Allowed amounts in oils	0.1-1	2		2	400
Degumming					
Neutralization			**	*	*
Bleaching	*	*	**	***	***
Deodorization	*	*	*		

* - chemical refining
** - chemical and physical refining
*** - physical refining

Aflatoxins are mostly removed during neutralization and bleaching. During bleaching, adsorption with bleaching earth is responsible for their removal. During deodorization, aflatoxins are removed in smaller quantities, because they can withstand high temperatures. For the removal of some mycotoxins, chemical refining of oil is better compared to the physical process, so zearalenone is removed more efficiently by chemical (80-98%) compared to physical treatment (70-80%) (van Dujin, 2014; Mariod and Idris, 2015). Short information about change in mycotoxin content during seed oil extraction and refining are available. In general, unrefined oils are excluded from the application of heat during the pressing process and are extracted mainly by mechanical process. This type of oil is washed, filtered or centrifuged without additional treatments such as alkali neutralization, bleaching or deodorization, while the reduction of mycotoxin content in refined oils can be attributed to the different stages of the refining process applied (Mariod and Idris, 2015; Bhat and Reddy, 2017; Abdolmaleki et al., 2021).

Degumming

The degumming process reduces the phospholipid content in the crude oil to a very low level. Phospholipids are structural components of plant cells and are extracted from seeds with oil. They have a great influence on the flavor, color, hydrolytic and oxidative stability of refined, bleached and deodorized oil, as well as on lower yields or higher losses of refined oil (Gupta, 2017). Degumming process is applied for removal of components precipitating from oil (during transport and storage), lecithin production (a very valuable by-product), removing ingredients that act as emulsifiers (phospholipids, carbohydrates) and physical refining, from the aspect of adequate oil "preparation".

Neutralization

Directly after harvest, lipolytic processes begin in the oilseeds, which can cause enzymatic or microbiological hydrolysis of oils and fats. The chemical and oxidative processes that occur cause the formation of free fatty acids. These fatty acids must be removed because they limit the use of oils and fats. Standard crude oils contain 1-3% free fatty acids (FFA). Up to 20% of FFA can be found in palm, olive and fish oil. Well-refined oils have about 0.1% FFA (Bockisch, 1993). Oil neutralization is the refining phase with the highest oil losses during refining. Two ways of neutralization, physical and chemical, are represented. Chemical neutralization by alkali is the most abundant.

Effect of the neutralization process on mycotoxins

The neutralization process includes heating the oil to 60°C, adding 13% sodium hydroxide, stirring for 10 minutes, centrifugation at 2000 rpm for 5 minutes. Thereafter, adding of water to the separated oil and rinsing with water until pH 7 is reached.

After alkaline treatment, the residual content of aflatoxin B1 is about 23%, aflatoxin B2 about 27%, and aflatoxin G1 and G2 below 0.1% in relation to the initial amounts, as seen in Figure 1 (Kamimura et al., 1986).

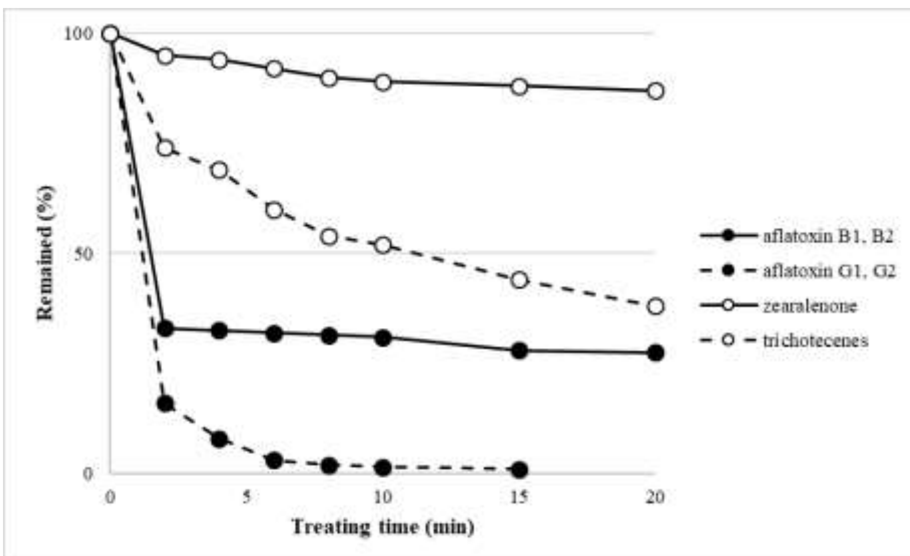

Figure 1. Stability of mycotoxins during neutralization (Kamimura et al., 1986)

Table 2 shows the mycotoxins content in oil and water used for oil washing after neutralization. After centrifugation, aflatoxin B1 and B2 were found in traces (0.01%), as well as deoxynivalenol and nivalenol. Aflatoxin G1 and G2 and zearalenone were not detected.

Table 2. Mycotoxin content in oil and water used for oil washing after neutralization (Kamimura et al., 1986)

Mycotoxins	Centrifugation (separation)		Washing	
	Oil (%)	Sludge (%)	Oil (%)	Water (%)
Aflatoxin B1	<0.01	48	nd	82
Aflatoxin B2	<0.01	46	nd	87
Aflatoxin G1	nd	in traces	nd	37
Aflatoxin G2	nd	in traces	nd	53
Zearalenone	nd	96	nd	98
Deoxynivalenol	<0.01	84	<0.01	91
Nivalenol	<0.01	78	nd	88

*nd - not detected

Bleaching

Bleaching is performed to remove color components from the oil. These components affect the appearance of the oil and also can negatively affect the taste and oxidative stability (Bockisch, 1993).

The pigments in the oil are in dissolved or colloidal form. In addition to the fact that bleaching is a physical process, ie. adsorption, so is the chemical process because there is an interaction between the pigments and the chemically active centers of the bleaching earth. In both cases the process takes place on the surface of the bleaching earth. Stained particles are bound adsorptively (hemisorption or physical adsorption) and are removed by filtration together with bleaching earth (Bockisch, 2004).

Effect of the bleaching process on mycotoxins

The bleaching process aims at adsorption and removal of pigments and trace components in the oil. Kamimura et al. (1986) investigated the influence of bleaching process on mycotoxind content. Active earth was used as an adsrobent, and the aim of the study was to determine whether mycotoxins are removed together with active soil or not. Bleaching earth was added in 2% to the oil and after 15 minutes it was separated from the oil, using a Bihner funnel. The part of the mycotoxin retained in the oil as shown in the Figure 2. After 15 minutes, zearalenone remained in the amount of 82%, and aflatoxins, deoxynivalenone and nivalenone were lagging behind in quantities below 0.5% (Kamimura et al., 1986).

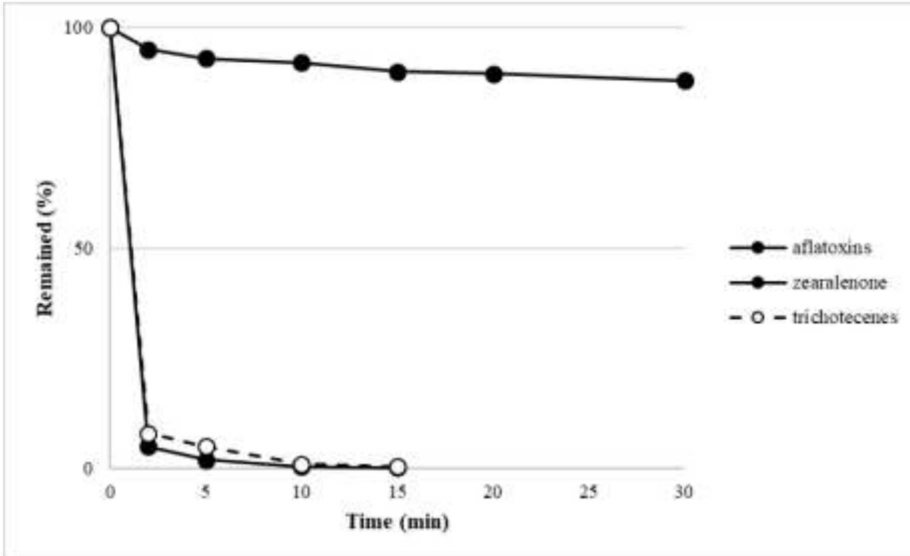

Figure 2. Effect of bleaching process on the mycotoxins content in oil (Kamimura et al., 1986)

Winterization

Winterization is a simplified form of fractionation, because it is not separated into two fractions, but a part of the oil components is removed. These components are triacylglycerols and high melting point waxes. If the oils are stored at low temperatures (in the refrigerator), the listed components will be separated. This adversely affects the appearance of the oil and consumers get the impression that the oil is spoiled. Also, under unfavorable conditions, the high content of waxes in the oil can affect the decomposition of oil emulsions, margarine, due to crystallization and separation of waxes. Thus, the goal of winterization is to reduce oil turbidity that occurs at temperatures from 0 to 8°C, by removing turbid substances (solid triacylglycerols, waxes, and oil polymerization products) (Bockisch, 2004).

Deodorization/dezoneutralization

Deodorization is the final step in the processing of vegetable oils and fats, by refining. This is followed by storage, packaging and distribution of the product, which means that the oil leaving the deodorizer must be ready for placing on the market (Gupta, 2017). Oils and fats contain undesirable components that give the oil an unpleasant taste and/or flavor and have to be removed during deodorization. Undesirable components include aldehydes, ketones, alcohols, hydrocarbons and other compounds formed during the oxidation and thermal degradation of peroxides, pigments and other natural constituents of oils, as well as free fatty acids. Deodorization is a physico-chemical process and represents the most complex operation in the refining process of vegetable oils and fats. This process represents the distillation of all undesirable volatile components at elevated temperatures in vacuum with the addition of direct superheated water steam.

Effect of the deodorization/dezoneutralization process on mycotoxins

Kamimura et al. (1986) investigated the thermal stability of mycotoxins at a temperature of 240°C and a pressure of 2-5 mmHg during the deodorization process. After 120 minutes of the process, aflatoxin B1 showed a tendency to decrease to 86% of the remaining aflatoxin B1, aflatoxin B2 to 80%, aflatoxin G1 to 72% and aflatoxin G2 to 70%, zearalenone and deoxyvalenol to 45%, while the level of nivalenol decreased to 7%, Figure 3 (Kamimura et al., 1986)

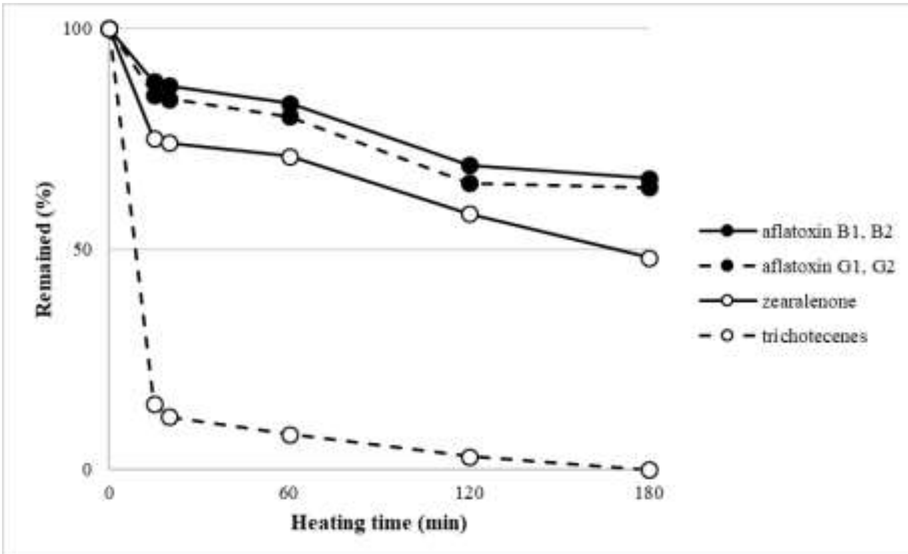

Figure 3. Stability of mycotoxins during heat treatment at reduced pressure (5 mmHg) (Kamimura et al., 1986)

Table 3 shows the content of mycotoxins in the oil before and after the refining process.

Table 3. Mycotoxins content in the oil before and after the refining process (Kamimura et al., 1986)

Mycotoxins	Aflatoxin				Zearale-none	Deoxyni-valenol	Nivale-nol
	B1	B2	G1	G2			
Initial oil (ppm)	1.0	0.8	1.0	0.8	10	8.0	8.0
Refined oil (ppm)	<0.02	<0.01	<0.02	<0.01	<1.0	<1.0	<1.0

Conclusions

Fats and oils, due to their high energy value and as important source of essential fatty acids and liposoluble vitamins, occupy an important place in human nutrition. On the other hand, raw materials for oil production are often contaminated caused by the production (cultivation) conditions and storage. These conditions can lead to the fungi growth and the formation of mycotoxins. Whether the oil is obtained by pressing or extraction, research has shown that

mycotoxins pass from the raw material into the oil. The maximum allowed amounts of mycotoxins in food around the world are regulated by appropriate regulations. Good agricultural practice, adequate plant disease management and adequate storage conditions limit the level of mycotoxins in the food chain, but do not completely eliminate their presence.
Various studies show that in the vegetable oils and fats production, the refining process is effective in reducing or removing certain mycotoxins, in order to obtain quality, health-safe edible refined oils or fats. Since there are two basic refining processes, research has shown that chemical refining is, in that sense, more suitable than physical. Within chemical refining, the best refining phases for mycotoxins removal are neutralization, bleaching and deodorization.

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References

Abdolmaleki K., Khedri S., Alizadeh L., Javanmardi F., Oliveira C.A.F., Khaneghah A.M. (2021). The mycotoxins in edible oils: An overview of prevalence, concentration, toxicity, detection and decontamination techniques. Trends in Food Science & Technology, 115, 500-511.

Bhat R., Reddy K.R.N. (2017). Challenges and issues concerning mycotoxins contamination in oil seeds and their edible oils: Updates from last decade, Food Chemistry, 215, 425-437.

Bockisch M. (1993). Nahrungsfette und -öle. In Nahrungsfette und -öle, pp. 436-483.

Bockisch M. (2004). Fats and Oils Handbook, Academic Press and AOCS Press, pp. 613-808.

Chang X., Liu H., Sun J., Wang J., Zhao C., Zhang W., Zhang J., Sun C. (2020). Zearalenone removal from corn oil by an enzymatic strategy. ***Toxins,*** 12: 2, 117, 1-14.

Flora O., Oluyemisi O.E., Olateju K.S., Mobolaji O.A., Adelodun K.L. (2018). Extent of microbial contamination of refined and unrefined vegetable oils sold in south-west Nigeria, Turkish Journal of Agriculture - Food Science and Technology, 6: 4, 396-400.

Gelderblom W.C.A., Shephard G.S., Rheeder J.P., Sathe S.K. (2014). Edible nuts, oilseeds and legumes (Ch. 12). In: Food Safety Management, Academic Press is an imprint of Elsevier, pp. 301-324.

Gupta M.K. (2017). Practical Guide to Vegetable Oil Processing, Academic Press/Elsevier and AOCS Press, pp. 27-260.

Ji N., Diao E., Li X., Zhang Z., Dong H. (2016). Detoxification and safety evaluation of aflatoxin B1 in peanut oil using alkali refining. ***Journal of the Science of Food and Agriculture***, 96: 12, 4009-4014.

Kamimura H., Nishijima M., Tabata S., Yasuda K., Ushiyama H., Nishima T. (1986). Survey of Mycotoxins Contamination in Edible Oil and fate od mycotoxins during oil refining processes, Journal od the Food Hygienic Society of Japan, 27, 59-63.

Karlovsky P., Suman M., Berthiller F., De Meester J., Eisenbrand G., Perrin I., Oswald I., Speijers G., Chiodini A., Recker T., Dussort P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination, Mycotoxin Research, 32, 179-205.

Krska R., Schubert-Ullrich P., Molinelli A., Sulyok M., MacDonald S., Crews C. (2009). Mycotoxin analysis: An update, Food Additives & Contaminants: Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 25: 2, 152-163.

Lacoste F. (2014). Undesirable substances in vegetable oils: anything to declare?, OCL Oilseeds & fats Crops and Lipids, 21: 1, A103, 1-9.

Mariod A.A., Idris Y.M.A. (2015). Aflatoxin B1 levels in groundnut and sunflower oils in different Sudanese states, Food Additives & Contaminants: Part B, 8: 4, 266-270.

Pinton P., Suman, M., Buck, N., Dellafiora, L., De Meester J., Stadler, D., Rito E. (2019). Practical guidance to mitigation of mycotoxins during food processing. Report, Commissioned by the ILSI Europe Process-Related Compounds and Natural Toxins Task Force, ILSI Europe - International Life Sciences Institute, ILSI Europe a.i.s.b.l., Brussels, Belgium, pp. 30-31.

Romanić R., Lužaić T., Roganović M. (2020). Contaminants in vegetable oils. Proceedings of the XI International Scientific Agricultural Symposium „Agrosym 2020", October 08-09, 2020, Jahorina, Bosnia and Herzegovina, pp. 444-454.

van Dujin G. (2016). Fate of contaminants during the refining process of vegetable oils and fats: A calculation model. European Journal of Lipid Science and Technology - Special Issue: Contaminants in fats and oils, 118: 3, 353-360.

van Dujin, G. (2014). Oils and Fats (Ch. 13). In: Food Safety Management - A Practical guide for the Food Industry, Academic Press is an imprint of Elsevier, pp. 325-345.

Zio S., Cisse H., Zongo O., Guira F., Tapsoba F., Somda N.S., Hama-Ba F., Songre-Ouattara L.T., Zongo C., Traore Y., Savadogo A. (2020). The oils refining process and contaminants in edible oils: A review. Journal of Food Technology Research, 7: 1, 9-47.

COLOR CHARACTERISTIC OF NON-REFINED OILS OBTAINED BY COLD PRESSING OF THE SEEDS OILS OBTAINED FROM CONFECTIONARY SUNFLOWER HYBRIDS

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Abstract

Most vegetable oils obtained by cold pressing are yellow or yellowish-brown, with red or green tone. The color of the oil is a consequence of the presence of colored macromolecules (pigments). The most common natural pigments in the oils are carotenoids (yellow) and chlorophylls, green color pigments. The color is also affected by the content of various non-glyceride ingredients present in the oils, but also by various products formed during the inadequate management of the technological process. The aim of this study was to examine the content of the most abundant pigments (total carotenoids and total chlorophylls) in sunflower oil obtained by cold pressing of three confectionary (non-oily) sunflower hybrids (NS-H-6318, NS-H-6307 and NS-H-6308), as well as to determine their influence on instrumentally determined color parameters (CIE $L^*a^*b^*$ system and transparency). Negative a^* values noticed in examined samples indicate presence of green pigments in examined oils. Total chlorophyll content found in samples amounted from 0.41 to 0.70 mg/kg. Very strong negative correlation (R = -0.99) was determined between a^* color parameter and total chlorophyll content. On the other hand, strong correlation (R = 0.74) was noticed between yellow balance (b^*) and total carotenoids content. These compounds content amounted from 6.31 to 10.24 mg/kg. Also, very strong correlation (R = 0.90) was found between L^* color parameter indicating lightness and transparency obtained spectrophotometric..

Keywords: *Cold pressed oil, Sunflower, Pigments, CIE L*a*b*, Transparency.*

Introduction

Carotenoids and chlorophylls are the most abundant pigments in sunflower oil. Carotenoids are tetraterpenoids, made up of eight isoprene molecules and containing 40 carbon atoms. They are classified into two groups: xanthophylls, which contain oxygen, and carotenes, which are pure oxygen-free hydrocarbons. Xanthophylls, together with dihydroxy carotenoids (mainly lutein), are the most abundant carotenoids in sunflower oil and make up 76 to 81% of total carotenoids (Rade et al., 2004). Carotenoids have a significant biological and protective role in the body, acting as provitamins and in protection against UV rays (De Leonardis et al., 2001; Tuberoso et al., 2007; Dimakou and Oreopoulou, 2012). In unrefined oils, carotenoids not only contribute to the color of the oil as an important parameter of sensory quality, but also have a strong antioxidant effect, such as tocopherols (Choe and Min, 2006; Dimakou and Oreopoulou, 2012).

The addition of β-carotene to sunflower oil increases oxidative stability at room temperature and under daylight due to the synergistic effect with tocopherols (Yanishlieva et al., 2001). Franke et al. (2010) found carotenoids in cold-pressed sunflower oil, but not in refined. The high proportion of carotenoids removed during refining is attributed to their thermolability (Ouyang et al., 1980). The content of total carotenoids in cold-pressed sunflower oil ranges from 2 to 4 mg/ml (Tuberoso et al., 2007), while Dimić et al. (2018) found a significantly higher content of total carotenoids (from 4.80 to 14.43 mg/kg). Other cold-pressed oils contain lower carotenoids content: walnut oil contains 0.93 ± 0.05 mg/kg (Martínez et al., 2013), grape seed oil 0.2 mg/kg (Lutterodt et al., 2011), blueberry oil 19 mg/kg (Parry et al., 2005).
Chlorophylls are green pigments, affect color, and are an important factor in sensory evaluation of oil (Matthäus and Brühl, 2004). Chlorophyll and chlorophyll derivatives are the most active promoters of photooxidation of oil in the presence of light and largely make oils susceptible to oxidative processes. Recent research also indicates the antioxidant properties of chlorophyll present in the oil (Tynek et al., 2012). Chlorophyll derivatives, pheophytin A, have shown a mild antioxidant effect in the dark, probably donating hydrogen to free radicals and thus interrupting the chain oxidation reaction (Dobarganes and Velasco, 2002). Crude sunflower oil has a low content of chlorophyll, which is removed during bleaching process. The chlorophyll content in cold-pressed sunflower oil ranges from 2.3 mg/kg (Tuberoso et al., 2007) to as much as 5.15 mg/kg in highoileic sunflower oil (Dimić et al., 2011). Dimić et al. (2018) examined the chlorophyll content in cold-pressed sunflower oils obtained from seeds with different proportions of organic impurities and hull and obtained a significantly lower content (from 0.00 to 1.21 ± 0.01 mg / kg). In refined oils, chlorophylls are found in amounts up to 0.6 mg/kg (Warner et al., 1989). In other cold-pressed oils, chlorophylls are found in higher concentrations, and in virgin olive oil the chlorophyll content can be higher than 31.97 mg/kg (Giuffrida et al., 2007), in cold-pressed rapeseed oil the content of total chlorophylls ranges from 22 to 118 mg/kg (Matthäus and Brühl, 2004), while cold-pressed flaxseed oil contains 0.8 to 5.76 mg/kg (Choo et al., 2007).

Material and Methods

Material
The latest confectionary (non-oily) sunflower hybrids (NS-H-6318, NS-H-6307, NS-H-6308) were used in the experimental research. Sunflower hybrids were grown in the experimental field of the Institute of Field and Vegetable Crops (45°15'6.01"N, 19°50'12.98"E), Novi Sad, Serbia from the 2017 growing season. Sunflower is grown under standard growing conditions without irrigation. All hybrids were sown in three blocks, randomly distributed. Up to 10 kg of seeds of an individual hybrid were collected, depending on the amount obtained in the experimental field. A representative amount of seeds was stored for 6 months at 20°C until the time of oil production (pressing). The seeds were pressed on a screw press. Before the beginning of the pressing process, the press was heated to the operating temperature (80 - 100°C) by a heater. The obtained cold-pressed oils were after precipitation for 48 h filtered through filter paper into 500 mL PET bottles, filled without empty space, closed and stored in a refrigerator at 6 ± 1°C until analysis.

Methods

Total carotenoids content, expressed as β-carotene, was determined by the standard spectrophotometric method (British standard, 1977) by measuring sunflower oil dissolved in cyclohexane (Lach-Ner, Czech Republic), at a wavelength of 452 nm, relative to pure cyclohexane as a blank, as described Dauqan et al. (2011).

Total chlorophyll content, expressed as pheophytin a, was determined by spectrophotometric method, by measuring the absorbance of pure oil, at a wavelength of 667 nm, relative to an empty cell as a blank, as described by Pokorny et al. (1985).

Oil color was determined by measuring instrumental color indicators in the CIE *L*a*b** system. The measurement was performed using a Minolta Chroma Meter CR-400 (Konica Minolta, Japan), using accessories for liquid materials, according to the manufacturer's instructions. To determine the color parameters, a colorimeter with an opening on the measuring head 8 mm and a standard measuring nozzle CR-A33b (Konica Minolta, Japan) was used. Measurements were performed in D-65 illumination with a standard shelter angle of 2°. Before each measurement series, the instrument was calibrated using a white calibration plate CR-A43 (Konica Minolta, Japan), according to the manufacturer's standard procedure. Oil samples were heated to room temperature (25°C) for 2 h before the color measurement.

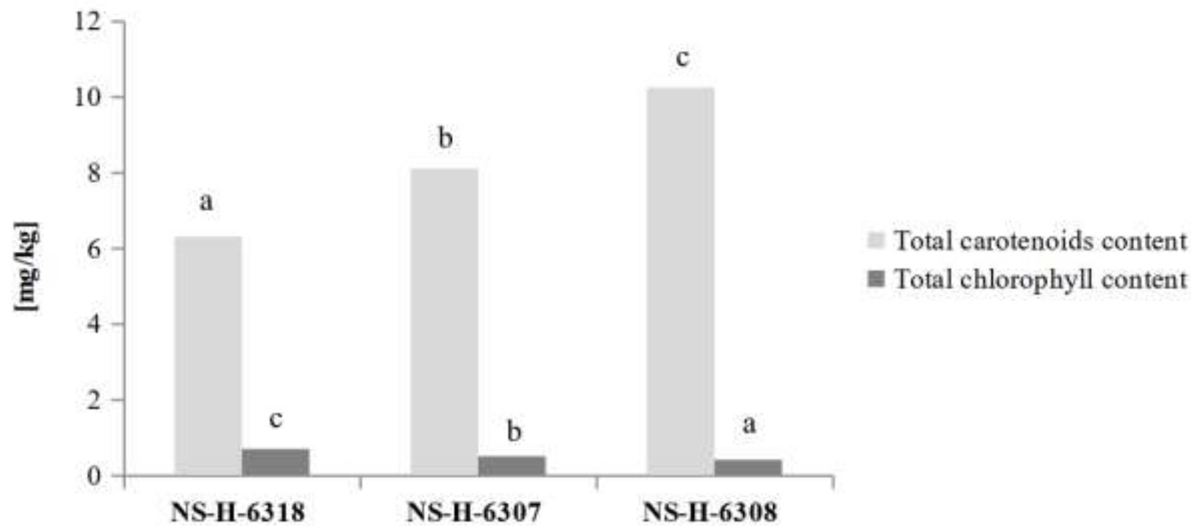
The oil color was also determined by spectrophotometric method, by measuring the transparency (% T) of pure oil, at 455 nm in relation to the empty cell as a blank (Dimić and Turkulov, 2000).

All spectrophotometric measurements were performed in a 1 cm - glass cells using a UV-VIS spectrophotometer, model T80 + (PG Instruments, UK).

Results are presented as mean ± standard deviation (n = 3). One-way analysis of variance (ANOVA) using the Tukey HSD test was used to determine significant differences between data at a significance level of $p < 0.05$. The degree of linear relationship between the two variables was measured using the Pearson correlation coefficient. For statistical data processing, the programs Statistica version 13.5.0.17 (StatSoft, Tulsa, Oklahoma, USA) and Microsoft Excel 2013 were used.

Results and Discussion

Carotenoids, yellow pigments, and chlorophylls, green pigments, in addition to affecting the sensory quality of unrefined oils and color formation (Parker et al., 2003; Matthäus and Brühl, 2004), also affect other aspects of quality, such as oil stability and shelf life (Pokorny et al., 1993; Karabagias et al., 2013). Carotenoids present in unrefined oils show an antioxidant activity, similar to tocopherols (Choe and Min, 2006; Rodriguez-Amaya, 2010; Dimakou and Oreopoulou, 2012). Some studies have also confirmed the antioxidant activity of chlorophyll (Lanfer-Marquez et al., 2005). The total carotenoids content (expressed as β-carotene) in the tested oil samples ranged from 6.31 ± 0.17 to 10.24 ± 0.15 mg/kg (Figure 1). The total chlorophyll content (expressed as pheophytin a) in the tested oil samples was determined in significantly lower quantities in relation to the total carotenoids content, up to 0.70 mg/kg (Figure 1), which is also characteristic for unrefined sunflower oils.



*different letter are significantly different based on Tukey HSD, at $p < 0.05$

Figure 1. Total carotenoids and total chlorophyll content of unrefined oil samples

Instrumental color parameters: *L** (lightness), *a** (+ red value / - green value), *b** (+ yellow value / - blue value) and transparency (% T) are summarized in Table 1. Oil color is a consequence of the presence of the pigments in cold-pressed sunflower oil. The lightness and the transparency are directly conditioned by the pigment content, with the increase of the pigment content the mentioned values decrease. The values of lightness ranged from 47.50 ± 0.02 to 48.93 ± 0.00, while the obtained values of transparency were in the range of 40.23 ± 0.05 to 55.93 ± 0.05%. However, a significant correlation of 0.90 ($p = 0.29$) was obtained between the values of lightness and transparency, shown in Table 2.

Table 1. Instrumental color parameters unrefined oil samples

Sample	Lightness *L**	+ red value / - green value *a**	+ yellow value / - blue value *b**	Transparency [% T]
NS-H-6318	47.96 ± 0.03^{b}	-4.76 ± 0.04^{a}	36.77 ± 0.04^{b}	51.67 ± 0.05^{b}
NS-H-6307	48.93 ± 0.00^{c}	-4.19 ± 0.01^{b}	35.31 ± 0.01^{a}	55.93 ± 0.05^{c}
NS-H-6308	47.50 ± 0.02^{a}	-3.87 ± 0.03^{c}	40.63 ± 0.05^{c}	40.23 ± 0.05^{a}

*different letter are significantly different based on Tukey HSD, at $p < 0.05$

The color values expressed through the *a** parameter ranged from -3.87 ± 0.03 to -4.76 ± 0.04. The obtained negative values indicate the presence of green pigments (chlorophyll) in the tested oils, but in small quantities (Figure 1). On the other hand, the color values expressed through the *b** parameter were in the range from 35.31 ± 0.01 to 40.63 ± 0.05, which indicates the presence of yellow pigments (carotenoids), in larger quantities compared to green pigments content (Figure 1). The obtained correlations between the *a** values and total chlorophyll content (R = -

0.99; p = 0.03) and between *b** values and total carotenoid content (R = 0.74; p = 0.47) confirm these statements (Table 2).

Table 2. Correlations between investigated parameters in unrefined oil samples

Correlation between	Pearson correlation coefficient R	Probability p
*L** and % T	0.90	0.29
*a** values and total chlorophyll content	-0.99	0.03
*b** values and total carotenoids content	0.74	0.47

Conclusions

Obtained negative *a** values indicate the presence of green pigments in the oils. Significantly higher *b** values indicate a significantly higher total carotenoids content in relation to total chlorophyll, which was confirmed in this paper.
The sunflower oil sample obtained by cold pressing of seeds of the NS-H-6307 hybrid had the highest lightness (48.93 ± 0.00) and transparency (55.93 ± 0.05) values. The lowest total pigments (carotenoids and chlorophyll) content was not found in the mentioned sample, which indicates that the oils color is affected by various non-glyceride components present in oils, as well as various products formed during the production process.
In this paper, the lightness and transparency of unrefined oils obtained by cold pressing of confectionary sunflower hybrids were also examined. Confectionary hybrids achieve a higher temperature during pressing, which can lead to the creation of products affecting the mentioned color parameters. Therefore, the obtained lightness and transparency results can also serve as a parameter of production process control. Also, the seeds of confectionary sunflower hybrids have a large hull content that can be of different colors, which affects the examined color parameters of unrefined oils obtained by cold pressing.

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References

British standard (1977). Methods of analysis of fats and fatty oils. Other methods. Determination of carotene in vegetable oils. British Standards Illustrations, London, (BS 684-2.20).

Choe, E., Min, D. B. (2006). Mechanisms and factors for edible oil oxidation, Comprehensive Reviews in Food Science and Food Safety, 5(4), 169-186.

Choo, W. S., Birch, J., Dufour, J. P. (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils, Journal of Food Composition and Analysis, 20(3–4), 202–211.

Dauqan, E., Sani, H. A., Abdullah, A., Muhamad, H., Gapor Md Top, A. B. (2011). Vitamin E and beta carotene composition in four different vegetable oils, American Journal of Applied Sciences, 8(5), 407–412.

De Leonardis, A., Mcciola, V., De Felice, M. (2001). Chemical and commercial characteristics of cold pressed sunflower oils, Italian food and beverage technology, 25, 46-52.

Dimakou, C., Oreopoulou, V. (2012). Antioxidant activity of carotenoids against the oxidative destabilization of sunflower oil-in-water emulsions, LWT - Food Science and Technology, 46(2), 393–400.

Dimić, E., Premović, T., Radočaj, O., Vujasinović, V., Takači, A. (2018). Influence of seed quality and storage time on the characteristics of cold-pressed sunflower oil: Impact on bioactive compounds and colour, Rivista Italiana Delle Sostanze Grasse, 95(1), 23–36.

Dobarganes, M. C., Velasco, J. (2002). Analysis of lipid hydroperoxides, European Journal of Lipid Science and Technology, 104(7), 420–428.

Franke, S., Frölich, K., Werner, S., Böhm, V., Schöne, F. (2010). Analysis of Carotenoids and Vitamin E in Selected Oilseeds, Press Cakes and Oils, European Journal of Lipid Science and Technology, 112(10), 1122–1129.

Giuffrida, D., Salvo, F., Salvo, A., Pera, L. L., Dugo, G. (2007). Pigments composition in monovarietal virgin olive oils from various sicilian olive varieties, Food Chemistry, 101(2), 833–837.

Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I., Kontominas, M. G. (2013). Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses, Food Research International, 54(2), 1950–1958.

Lanfer-Marquez, U. M., Barros, R. M. C., Sinnecker, P. (2005). Antioxidant activity of chlorophylls and their derivatives, Food Research International, 38: 885-891. https://doi.org/10.1016/j.foodres.2005.02.012

Lutterodt, H., Slavin, M., Whent, M., Turner, E., Yu, L. (2011). Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours, Food Chemistry, 128(2), 391–399.

Martínez, M. L., Penci, M. C., Ixtaina, V., Ribotta, P. D., Maestri, D. (2013). Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions, LWT - Food Science and Technology, 51(1), 44–50.

Matthäus, B., Brühl, L. (2004). Cold-pressed edible rapeseed oil production in Germany, INFORM - International News on Fats, Oils and Related Materials, 15(4), 266–268.

Microsoft Excel 2013, Microsoft. Microsoft Excel. Computer Software. Redmond, Washington, USA, 2013. (www.microsoft.com).

Ouyang, J. M., Daun, H., Chang, S. S., Ho, C. T. (1980). Formation of Carbonyl Compounds from β-Carotene during Palm Oil Deodorization, Journal of Food Science, 45(5), 1214–1217.

Parker, T. D., Adams, D. A., Zhou, K., Harris, M., Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils, Journal of Food Science, 68(4), 1240–1243.

Parry, J., Su, L., Luther, M., Zhou, K., Peter Yurawecz, M., Whittaker, P., Yu, L. (2005). Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils, Journal of Agricultural and Food Chemistry, 53(3), 566–573.

Pokorny, J., Dobiasova, S., Davidek, J. (1985). Repeatability of the determination of oxidative stability of vegetable oils using the Schaal oven test, Scientific papers of the Prague Insitute of Chemical Technology, 58, 163–173.

Rade, D., Mokrovcak, Z., Struceli, D., Skevin, D., Nederal, S. (2004). The Effect of Processing Conditions on the Nontriacylglycerol Constituents of Sunflower Oil, Acta Alimentaria, 33(1), 7–18.

Rodriguez-Amaya, D. B. (2010). Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids-A review. Journal of Food Composition and Analysis, 23(7), 726-740.

StatSoft, Inc. (2010). STATISTICA (data analysis software system), version 10.0. Available from: http://www.statsoft.com/.

Tuberoso, C. I. G., Kowalczyk, A., Sarritzu, E., Cabras, P. (2007). Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use, Food Chemistry, 103(4), 1494–1501.

Tynek, M., Pawłowicz, R., Gromadzka, J., Tylingo, R., Wardencki, W., Karlovits, G. (2012). Virgin rapeseed oils obtained from different rape varieties by cold pressed method - their characteristics, properties, and differences, European Journal of Lipid Science and Technology, 114(3), 357–366.

Warner, K., Frankel, E. N., Mounts, T. L. (1989). Flavor and oxidative stability of soybean, sunflower and low erucic acid rapeseed oils, JAOCS, Journal of the American Oil Chemists' Society, 66(4), 558–564.

Yanishlieva, N. V., Raneva, V. G., Marinova, E. M. (2001). β-Carotene in Sunflower Oil Oxidation, Grasas y Aceites, 52(1), 10–16.

EFFICACY OF FUNGICIDES IN CONTROL OF CHERRY PATHOGEN *MONILINIA LAXA* (ADER. and RUHL.)

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Abstract

Cherry is a deficient fruit species in the world and in Serbia, where it has become the most economically viable fruit species. There has been a growing interest among agricultural producers in raising orchards and growing cherries, in recent years. During flowering, in spring, climatic conditions are convinient for the development of the pathogen *Monilinia laxa*, which causes drying of flowers, twigs, branches and fruits. In order to produce healthy food and protect the environment, it is necessary to choose the right fungicides, as well as their timely and proper application, which is not cared about a lot in the area of southeastern Serbia. The research was conducted during 2020 and 2021 on the registered agricultural farm of the municipality of Lebane in Serbia, KM (cadastral municipality) Bošnjace. The experiments were performed according to a block system with three variants. The fungicide boscalid+pyraclostrobin (Signum) was applied in the first variant and in the second - cyprodinil (Ciprodex). The third variant was control, without the application of chemical protection measures. Evaluation of the effectiveness of the tested fungicides was performed by counting infected (dried) and healthy flowers on the tested trees. A thousand flowers were counted in a variant, and samples were taken from four different sides of the tree. The effectiveness of the tested fungicides was calculated according to the Abbott formula. The efficiency of chemical measures was high in both years of research and ranged from 94.56% for the fungicide Ciprodex in 2020, to 97.69% for the fungicide Signum in 2021.

Key words: *Cherry Burlat, Monilinia laxa, efficiency, fungicides.*

Introduction

Cherry is a deficient fruit species in the world and in Serbia, where it has become the most economically viable fruit species. There has been a growing interest among agricultural producers in raising orchards and growing cherries, in recent years. The pathogen *Monilinia laxa* can be a limiting factor in cherry cultivation due to its harmfulness. It is the most destructive stone fruit pathogen in USA, Europe, Australia and New Zeland (Archer, 1988). Byrde and Willets (1997) state that *M. laxa* causes large losses on stone fruit species with amount of about 50%. In California, *M. laxa* often causes damage to all types of stone fruit, and the greatest damage is caused on cherries in the area of large lakes, because there are ideal climatic conditions for the rapid development of this pathogen - humid weather and temperatures from 24 to 25^{o}C (Christensen 2003). It causes significant damage in years when the thermo-hygrometric factor is very favorable for the pathogen, i.e. when the flowering period and fruit ripening are accompanied by rainy weather and when the flowering period is extended (Ivanović, 1992; Perić, *et al*., 1996). If flowering is prolonged and time promotes the development of infection, may be necessary to perform interfacial treatment from the popcorn stage (Gubler et al., 2006). Perennial diseases of stone fruits lead to their exhaustion, general weakening and drying (Gudžić and Stojanović, 2011). There are many authors which pointed out this pathogen destructiveness on almost all stone fruit species (Kišpatić, 1985; Radman, 1979; Marić, 1991; Ivanović, 1992). The pathogen overwinters in the form of mycelium in cancer wounds in the bark of infected branches and twigs and in mummified fruits. Monilinia species form conidia on mummified fruits and diseased branches already at a temperature of 5^{o}C under favorable humidity conditions (Ogawa et al., 1995). The perfect, teleomorphic stage of the fungus (formation of apothecia) is a rare occurrence in Europe, so ascospores are not of significant importance in the epidemiology of this fungus (Balaž, 2000). Vojvodić (1979) states that the number of treatments against moiniliosis depends on the distribution of precipitation and that the number of treatments increases if it often rains or the humidity increases during flowering. In order to produce healthy food and protect the environment, it is necessary to choose the right fungicides, as well as their timely and proper application, which is not cared about a lot in the area of Lebane municipality in Serbia.

Material and Methods

The research was conducted during 2020 and 2021 in orchard on the registered agricultural farm of the municipality of Lebane in Serbia, on cadastral parcel 1243/3, KM (cadastral municipality) Bošnjace (Fig. 1). The fruit variety in orchard is Cherry Burlat, six years old. The cultivation system is a canopy in the shape of a vase, the planting density is 6 x 4 m and the exposure is southeast. The experiments were performed according to a block system with three variants, each variant in four repetitions and each repetition with five trees, so the study covered a total of 60 trees. The treatments were performed in the white balloon phase and in full bloom in both trial years. The fungicide boscalid+pyraclostrobin (Signum) was applied in the first variant and in the second - cyprodinil (Ciprodex), which are registered in Serbia for this purpose. The third variant was the control one, without the application of chemical protection measures. Evaluation of the effectiveness of the tested fungicides was performed by counting infected (dried) and healthy flowers on the tested trees. A thousand flowers were counted in a variant and samples were taken from four different sides of the tree. The effectiveness of the tested fungicides was calculated according to the Abbott formula.

Figure 1. Experimental field in the locality Bošnjace - Lebane

According to our observations, the cultivar Burlat is of medium early flowering. Cherry blossoms in the area of the municipality of Lebane took place in the third decade of March in both years of testing (Table 1). The onset, length and end of flowering are directly correlated with temperature as well as infection with the pathogen *M. laxa*.

Table 1. Dynamics of cherry blossoms and flowering length in the municipality of Lebane

Year	Phenophase of flower development			
	The start of Flowering (BBCH 61)	Full flowering (BBCH 65)	Overblown (BBCH 67)	Flowering length
2020	21.03.	27.03.	4.04.	14
2021	22.03.	29.03.	4.04.	13

The year 2020 was very rainy, with special reference to March, which was on average the warmest and rainiest, in the most part of southeastern Serbia. There were medium air temperatures, but also quite dry weather during April. Weather conditions with increased humidity favored the spread of the pathogen *M. laxa.* during the flowering period in 2021. It was rainy in this part of Serbia in terms of the amount of precipitation during the spring. The number of treatments directly dependent on the weather conditions and the length of cherry flowering (Perić i Perić, 2000; Todorović i Perić, 1996).

Results and Discussion

M. laxa, the causative agent of drying flowers, twigs and brown fruit rot is one of the most economically significant pathogens of stone fruits. The thermohygrometric factor was favorable to the pathogen *M. laxa* during the two-year study in 2020 and 2021 in the area of the municipality of Lebane. The rainy season in the flowering phase in both years affected the realization of the cherry blossom infection. Drying of flower bouquets was observed on control trees in both years. They turned brown, dried and mostly fell off, or remained stuck on the branch. It was noticed in further observation, that the pathogen from the diseased flower parts moved into the branches and caused their drying, and as a consequence of their drying, the leaves on them also became extinct (Fig. 2).

Figure 2. *Monilinia laxa* – drying of cherry flowers and twigs

Two treatments were performed during the two-year trial. The effectiveness of the tested fungicides was calculated according to the Abbott formula. The efficiency of applied fungicides during 2020 was high, for Signum 96.69%, and Ciprodex 94.56%. In 2021, higher efficiency was achieved in both variants, the preparation Signum, 97.69%, and Ciprodex 95.38%. The percentage of infected flowers in the control was different and ranged from 51.5% in 2020, and 47.7% in 2021 (Tables 2 and 3).

Table 2. Efficacy of chemical measures in the control of *M. laxa* on cherries in 2020 in the municipality of Lebane

Number of variants	Plant protection product	Concentration (%)	Average infection (%)	Efficiency (%)
V_1	Signum	0.075	1.7	96.69
V_2	Ciprodex	0.05	2.8	94.56
V_3	Control	-	51.5	-

Table 3. Efficiency of chemical measures in the control of *M. laxa* on cherries in 2021 in the municipality of Lebane

Number of variants	Plant protection product	Concentration (%)	Average infection (%)	Efficiency (%)
V_1	Signum	0.075	1.1	97.69
V_2	Ciprodex	0.05	2.2	95.38
V_3	Control	-	47.7	-

Differences in the efficiency of the tested preparation and the number of infected flowers are reflected in the different distribution of precipitation during flowering. During the flowering in 2020, all the conditions for the realization of the infection were fulfilled, the flowering lasted longer, so that a stronger infection was recorded, and a slightly lower efficiency of the applied preparations in relation to 2021.

Conclusions

There has been a growing interest among agricultural producers in raising orchards and modern cherries growing, in recent years. The pathogen *M. laxa* occurs almost every season, so it is necessary to prevent economic losses with as few treatments as possible. Persistence is important for the suppression of this pathogen, because depending on the year, the application of fungicides can be reduced to 1-4 treatments. Thermohygrometric factor in the years of research favored the development of the pathogen *M. laxa* on cherry. Treatment tests were performed at the beginning of flowering (20-30% of open flowers) and in full bloom (70-80% of open flowers) in both years. The second treatment should be done even in the absence of precipitation in order to reduce the infectious potential and avoid the possibility of infection, which would later cause fruit rot. The fungicide boscalid+pyraclostrobin (Signum) was applied in the first variant and in the second - cyprodinil (Ciprodex). Our two-year (2020-2021) studies show that the effectiveness of chemical measures was high in both years, ranging from 94.56%, for the fungicide Ciprodex in 2020, to 97.69%, for the fungicide Signum in 2021, as well as the fact that with two timely treatments, excellent results are achieved in the control of the very dangerous cherry pathogen *M. laxa*. The research is important, not only from the aspect of increasing productivity, i.e. economy, but also because of obtaining ecologically correct food and preserving the natural ecosystem.

References

Archer, S. A. (1988). Monilinia frictigena (Aderhold & Ruhl.) Honey ex Whetzel. European Handbook of Plant Diseases, pp. 439-440, (eds. I. M. Smith et al.), Backwell Scientific Publication, London.

Balaž, J. (2000). Monilia spp. kao parazit voćaka (Monilia spp. as a fruit parasite). Biljni lekar br. 2-3, pp. 155-162, Novi Sad.

Byrde, R. J. W., Willets, H. J. (1997): The Brown Rot Fungi of Fruit: their Biology and Control. Perg. Press, Oxford.

Christensen, J. (2003). Brown Rot of Stone Fruits. Department of Plant Pathology. University of Nebraska. Lincoln.

Gubler, W. D., Adaskaveg, J. E., Pickel, C., Niederholzer, F. J. A., Teviotdale, B. L. (2006). Brown Rot Blossom and Twig Blight. Statewidw IPM Program, Agriculture and Natural Resources, Universitiy of California.

Gudžić, S., Stojanović, S. (2011). Bolesti voćaka i vinove loze (Diseases of fruit trees and vines). Poljoprivredni fakultet, Kosovska Mitrovica-Lešak.

Ivanović, M. (1992). Mikoze biljaka (Plant mycoses). Nauka, Beograd.

Kišpatić, J. (1985). Opšta fitopatologija (General phytopathology), Zagreb.

Marić, A. (1991). Opšta fitopatologija (General phytopathology), Novi Sad.

Ogawa, J. M., Zehr, E. I., Biggs, A. R. (1995). Brown rot: in Compendinum of Stone Fruit Diseases (ed. Ogawa et al.), APS Press.

Perić, R., Todorović, D., Blagojević, R. (1996). Prilog proučavanju uzroka propadanja cvetova i grana Oblačinske višnje u Toplici i mogućnosti suzbijanja uzročnika (Contribution to the study of Cherry Oblacinska flowers and branches decay causes in Toplica and the possibilities of controlling the causative agents). Zbornik naučnih radova. Prvi naučni skup, Viša poljoprivredno-prehrambena škola u Prokuplju, pp. 219-224, Prokuplje.

Perić, R., Perić, S. (2000). Najopasniji parazit višnje Monilinia laxa u Toplici i mogućnosti njihovog suzbijanja (The most dangerous cherry parasite Monilinia laxa in Toplica and possibilities of its suppression). Savetovanje «Dani višnje» saopšteni rezultati. Prokuplje.

Radman, LJ. (1979). Fitopatologija, bolesti voćaka i vinove loze (Phytopathology, diseases of fruit trees and vines), Zadrugar, Sarajevo.

Todorović, D., Perić, R. (1996). Mesto i značaj Oblačinske višnje u voćarstvu juga Srbije i problemi zaštite (The place and significance of cherry Oblacinska in Southern Serbia fruit growing and protection problems). Zbornik radova I naučni skup Više poljoprivredno-prehrambene škole, Prokuplje.

Vojvodić, Đ. (1979). Prilog proučavanju suzbijanja Monilinia laxa (Enhr.) Sacc. na višnji (Contribution to the study of Monilinia laxa (Enhr.) Sacc. suppression on cherry). Zaštita bilja (147), pp. 71-76, Beograd.

MORPHOLOGICAL CHARACTERISTICS OF *EUTYPA LATA* ISOLATES FROM GRAPEVINE IN SERBIA

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Abstract

In an *in vitro* experiment, the morphological characteristics of three *Eutypa lata* isolates (EL129, EL153 and EL199), previously determined at the molecular level, and two reference isolates BX1.10 and 8F obtained from the Institute National de la Recherche Agronomique, INRA, France were observed. The following morphological features of the studied isolates were studied on the nutrient medium of potato dextrose agar (PDA): macroscopic (appearance, color and zoning of the front and back of the culture) and microscopic (vegetative organs of mycelium, reproductive organs of anamorphs - conidia, pycnidia, conidiophores and reproductive organs of teleomorphs - perithecium, ascus with ascospore). On potato dextrose medium (PDA) 24 h after placing, the studied *E. lata* isolates formed at the beginning a white mycelium. After 10 days, the mycelium had a cottony appearance, white color with a weak air growth. The entire colony developed evenly and had a fine diffuse edge. After 30 days, the face of the colony was white with a thick cotton aerial mycelium and a pale yellow conidial mass, which was excreted from the pycnidia. The studied *E. lata* isolates formed pycnidia, which were formed in culture. The pycnidia of the studied *E. lata* isolates were spherical or irregular in shape, from dark brown to black in color. The dimensions of the pycnidia were from 0.5 to 1 mm. Conidial mass was a cream to pale orange color on a PDA base. All studied isolates formed conidia in culture. None of the studied *E. lata* isolates, originating from the stem and branches of the grapevine from Serbia, as well as the control isolates, formed perithecia in culture.

Key words: *Isolates, breeding traits, substrates, Eutypa lata.*

Introduction

The grapevine dieback occurs in almost all countries of the world where vines are grown commercially. This is understandable given the wide range of host plants that *E. lata* fungus attacks. In addition to the wide distribution, grapevine dieback as an economically important grapevine disease is more significant in areas where annual rainfall is above 600 mm. On the other hand, it rarely occurs in areas where the average annual rainfall is less than 250 mm (Carter, 1994). In Serbia, this disease was first detected by Živković *et al*. (2012a, b).
E. lata is a pathogen of a large number of plants, from different genera of stone fruits, apples, berries and other fruits and causes great damage. The fungus is also isolated from a large number of ornamental, wild or woody plants. According to Carter *et al.* (1985) the grapevine (*Vitis* sp.) is the universal host for the distribution of *E. lata* to other species growing nearby.
Trouillas and Gubler (2010) studied the host circle of *E. lata* in the vineyard regions of California. The authors monitored the presence of *E. lata* perithecia on vine stems, in orchards

on apricot, almond, cherry, sweet cherry, apple and pear trees, as well as on natural hosts of large-leaved maple, willow and oleander. By phylogenetic analysis of the DNA sequences of the ITS region and the β-tubulin gene, the DNA-dependent RNA polymer gene (RPB2) was confirmed and identified by *E. lata* isolated from different plant hosts. The interspecific phylogenetic diversity according to Trouillas and Gubler (2010) does not match the geographical origin of the isolates, and the interspecific groups after different DNA phylogenies do not match. Significant phenotypic variations of *E. lata* isolates were detected on the vine, in terms of the length of ascospores and conidia as well as the levels of aggressiveness of isolates. The aim of this study was to describe the morphological (macroscopic and microscopic) properties of three isolates of *Eutypa lata* (EL129, EL153 and EL199) on a nutrient medium PDA.

Material and methods

The tested isolates were obtained from grapevine plants with grapevine dieback symptoms collected in the period 2015-2019. Sampling was performed in the main vine production areas on the territory of the Republic of Serbia from 14 sites: Dobričevo, Drenovac (Pomoravlje district), Praskovče, Lipovac (Nišavski district), Kobilje, Bela Voda, Krvavica, Suvaja, Trnavci, Tulež (Rasina district), Gudurica (South Banat district), Karbulovo (Bor district), Strezovac (Pčinja district), Sremski Karlovci (South Banat district). A total of 50 samples were taken and analyzed. After being brought to the laboratory, the samples were first washed with running water, and then fungal isolation was performed using standard phytopathological methods. Pathogen isolation was performed from grapevine stems and cordons. In order to remove surface impurities, parts of the stem and cordon were washed with running water for 2 h, and then cut into fragments 1 cm long. Fragments of the stem and cordon were cut at the junction of necrotic and healthy tissue, surface disinfected for 5 minutes in 5% sodium hypochlorite solution (NaOCl) (14% NaOCl, Superlab, Belgrade) and washed 3 times for 5 minutes in sterile distilled water. The fragments were transferred to sterile filter paper to remove excess fluid and then placed on a nutrient medium. Potato dextrose agar (PDA) with the addition of 300 μl / l gentamicin sulfate was used to isolate the pathogen. This medium was prepared from 200 g of potatoes, 20 g of dextrose (Torlak, Institute of Immunology and Virology, Belgrade), 20 g of agar (Torlak, Institute of Immunology and Virology, Belgrade) and 1 l of distilled water (Dhingra and Sinclair, 1995). Petri plate with fragments were incubated in a thermostat at a temperature of 24 ± 2˚C, in the dark until the development of fungal colonies around the fragments, and incubated at 24 ° C in the 24 h UV light for 30 days. Individual conidia were selected and transferred directly to the PDA plate according to the procedures described by (Dhingra and Sinclair, 1995), and stored on PDA in tubes at 4 ° C. In this study, the macroscopic and microscopic morphological characteristics of 3 isolates (EL129, EL153, EL199) isolated from Serbia and 2 reference isolates BX1.10 and 8F obtained by the Institute National de la Recherche Agronomique, INRA, France, were studied. Of the macroscopic characteristics, the traits of colonies are described, such as the appearance, color and zoning of the face and back of the culture on the tenth day after transferring (Muntanola-Cvetković, 1987). Identification was done by pathogenicity test (Peros and Berger, 1994), morphology (Glave and Rogers, 1982; Glave *et al.,* 1982) and PCR methods (Lecomte *et al*., 2000).
Considering the microscopic morphological characteristics, the appearance of mycelium, the presence of asexual (conidia, pycnidia, conidiophores) and sexual reproduction (perithecia, asci, ascospores) were studied.

Macroscopic and microscopic morphological characteristics of 3 *Eutypa lata* isolates and 2 *E. lata* reference isolates were studied on a PDA medium, according to the method of Glawe *et al.* (1982). The appearance, color and zoning of the face and back of the culture were observed, followed by the appearance, structure, color and growth of the mycelium. Inoculation of the studied isolates on medium was performed by aseptic application of round fragments of colonies of pure cultures of the studied isolates with a diameter of 10 mm, in the center of Petri plate, which were then grown at 25 ° C, and under 24 h UV light for 30 days. In all 3 isolates of *E. lata* and 2 reference isolates of *E. lata*, the shape and dimensions of conidia were studied. The study of conidia was performed by observing 100 randomly selected conidia of the studied isolate, using a microscope at a magnification of 400 times (Olympus BX51 / BX52, Japan) and a digital camera (Olympus DP71, Japan). The average dimensions of the conidia were determined by measuring the length and width of 100 randomly selected conidia of the studied fungal isolates, grown on a PDA medium using a light microscope and a digital camera.
To monitor the formation of the teleomorphic stage, 3 studied *E. lata* isolates and 2 reference *E. lata* isolates were grown on PDA medium, cultures were grown in a thicker layer (40 ml of medium per Petri dish with a diameter of 100 mm), at a temperature of 25 ° C. Readings of the presence of teleomorphic formations were performed after 30 days, 6 and 12 months. Monitoring of the appearance of teleomorphs was also performed in nature. After removing the bark from the dead wood of naturally infected grapevine plants, fragments of dead wood were cut and the presence of a stroma with perithecia was observed under binocular microscope (Olympus SYX7, DFPLAPO 1 x 4).
Statistical analysis performed in order to determine the relationship between *E. lata* isolate and two reference isolates 8F and BX1.10. Data were analyzed by variance analysis (ANOVA) using the computerized software (PROC GLM, SAS, System, version 8.1; SAS Institute, Cary, NC). To satisfy the assumptions of the ANOVA, the arcsine transformation of the proportion was used ($Y=2x \text{ arcsine } \sqrt{p}$). Homogeneity of groups was assessed using Duncan's test with $p = 0.05$.

Results and discussion

Examination of vineyards in several sites of grapevine cultivation in our country in the period from 2015 to 2019, revealed symptoms of grapevine dieback. Symptoms on the leaves of diseased vines are manifested in the form of small, chlorotic spots, distributed along the edge of the leaves, while the central part of the leaf blade had a wrinkled appearance. The edges of the leaves were worn and bent downwards, and in severe infections the surface of the leaves is mostly covered with necrotic spots. The shoots are light green in color, have a shortened appearance and the so-called zigzag rise of internodes.
On potato dextrose medium (PDA) 24 h after transferring, the studied *E. lata* isolates form the beginning of white mycelium. After 10 days, the mycelium was a cottony appearance, white color with a weak air growth. The entire colony develops evenly and were a fine diffuse edge. The back of the colony is cream in color, without fruiting structures (Figure 1). After 15 days, a dark pigment forms in the substrate. After 30 days, the face of the colony is white with a thick cotton aerial mycelium and a pale yellow conidial mass, which was excreted from the pycnidia. Macroscopic examination of colonies of *E. lata* isolates showed no differences in the morphological characteristics of the mycelium. Glawe *et al.* (1982) and McKemy *et al.* (1993) described cotton-white colonies of *E. lata* isolates on a PDA medium whose color changes to cream over time, which is in agreement with our results.

The studied *E. lata* isolates form pycnidia, which are formed in culture (Figure 1). The pycnidia of the studied *E. lata* isolates are spherical or irregular in shape, from dark brown to black in color. Conidial mass is a cream to pale orange color on a PDA base. The dimensions of the pycnidia were from 0.5 to 1 mm. Namely, under the influence of a change of 12 h of light - 12 h of darkness and at alternating temperatures of 19 ° C and 15 ° C, sporulation occurs after 30 days, which agrees with the statements of Carter (1994) and Munkvold (2001). The same authors state that in some cultures a gray pigment is formed after 15 days and the back of the colony turns black. Three to four weeks after placing, small black pycnidia appear in the culture under the influence of constant UV light. Conidia were excreted from the pycnidia in the form of a cream to an orange gelatinous mass (Rolshusen *et al.*, 2006; Trouillas and Gubler, 2010).

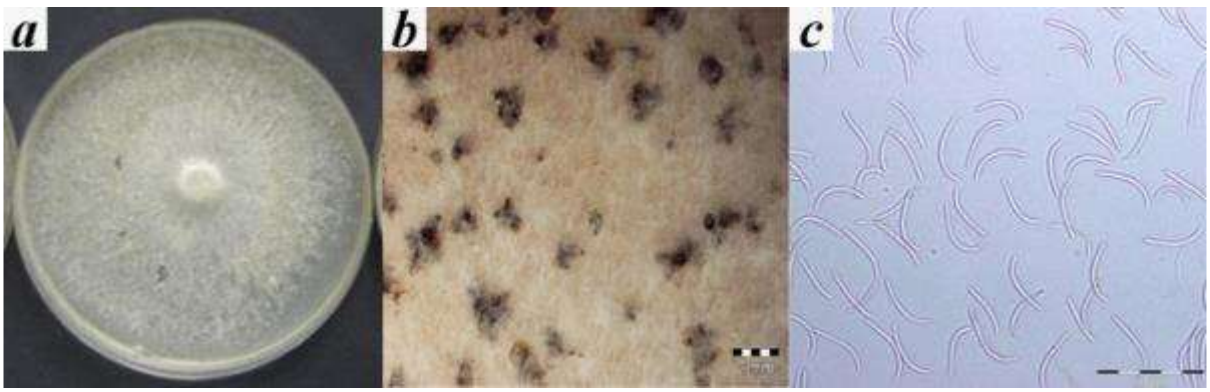

Figure 1. *Eutypa lata* EL129 isolate on PDA medium: a) Colony of isolates on PDA medium after 10 days of development; b) Stromatic formations of isolates in cultures 30 days old; c) Conidia EL153 in 30-day-old cultures.

In the studied *E. lata* isolates there are no morphological differences related to the appearance of conidia. All conidia were unicellular, unseptated, hyaline, elongated, and moderately curved with a flattened base (Figure 1). Conidia length and width were parameters that were individually statistically analyzed in order to determine suitability as a criterion for distinguishing between the tested *E. lata* isolates (Table 1). Based on data from the literature, the morphological characteristics of the anamorphic stage of the genus *Eutypa* were not sufficiently reliable traits for the identification of isolates up to the species level, although they were of great importance in the classification of the *Diatrypaceae* family (Trouillas *et al.*, 2010; Rolshausen *et al.*, 2014).
The genus *Eutypa* exhibits a very high similarity in the appearance of conidia, the ontogenesis of conidia, the characteristics of cultures, as well as the similarity of the teleomorphic stage with other genera within the fam. *Diatrypaceae*, such as the genus *Eutypella*, *Diatrype*, and *Diatrypella*. This makes it difficult to separate and identify the genus *Eutypa* (Glawe and Rogers, 1982; Rolshausen *et al*., 2006; 2014). On the contrary, the morphological characteristics of the teleomorphic stage of these fungi, due to their high uniformity, are very important for the determination of pathogens up to the genus level. However, during the tour of the terrain in the vineyards, no teleomorph was found in any locality where the symptoms of the dieback of the grapevine were observed. Therefore, the characteristics of teleomorphs were not used in the determination of this pathogen. Investigations of the morphological characteristics of anamorphs of the fungus served to identify the cause of the grapevine dieback to the level of genus. The

morphological and biometric values of the anamorphs were in agreement and fully corresponded to the reference values from the literature considering the pathogen *E. lata*. Considering the morphological characteristics of anamorphs, unicellular, conical, hyaline and slightly bent conidia with a flattened base, 19.50- (24.69) -33.34 μm long and 1.20- (1.69) -2.74 μm wide, were observed on PDA medium (Table 1), which is characteristic of *E. lata* species. These results were in agreement with the results presented by other authors. Glawe *et al.* (1982) describe the conidia of *E. lata* as unicellular, conical, slightly bent and hyaline, measuring 25-74 μm × 1–2 μm. Carter (1994) stated that conidia are 18-45 μm × 0.8–1.5 μm in size. Munkvold (2001) described the conidia of *E. lata* isolates as conical, straight or curved, very numerous, measuring 20-45 μm × 0.8–1.5 μm. Rolshausen *et al.* (2006, 2014) state that the dimensions of conidia of *E. lata* isolates from different hosts were 23.6-35.00 μm × 1.8-4.7 μm. In all tested isolates, conidia were formed in pycnidia, which is in agreement with the literature (Glawe and Rogers, 1982; Munkvold, 2001).

Table 1. Biometric values of conidia dimensions of the studied *Eutypa lata* isolates on PDA substrate

Isolate	Conidia length (μm)			Conidia width (μm)		
	Max	Min	Average*	Max	Min	Average*
EL 129	33.34	23.24	26.75	2.74	1.28	1.81
EL 153	23.10	19.50	20.94	1.81	1.20	1.54
EL 199	33.25	23.06	27.80	1.50	1.39	1.45
8F	26.25	22.54	24.33	2.19	1.58	1.94
BX1.10	24.99	20.04	23.62	2.16	1.28	1.71

*Average value of 100 repetitions

None of the studied *E. lata* isolates, originating from the stem and branches of the vine from Serbia, as well as the control isolates of *E. lata*, formed perithecia in culture, which agrees with the statements of Munkvold *et al.* (1993) and Munkvold (2001). Despite the very long storage of cultures until their complete exhaustion and observation on several occasions, after 30 days, 6 and 12 months, there was no formation of the teleomorphic stage. Also, the teleomorphic stage did not appear on grapevine plants inoculated with *E. lata* isolates, even after 27 months. Despite many years of monitoring this disease in vineyards, no teleomorphic stage has been found in nature. Glawe and Rogers (1982) and Carter (1994) stated that it is prevalent in vineyards older than 10 years in areas with frequent rainfall, and in vineyards where irrigation systems exist Munkvold *et al.,* (1993).

Conclusion

Macroscopic examination of cultures on a PDA medium showed that the studied *E. lata* isolates developed uniform colonies. In all studied isolates, conidia are formed in pycnidia from which a mucous mass (matrix) is secreted, which is a cream to a pale orange color. Isolates identified as *E. lata* had unicellular, smooth, hyaline, slightly bent, at the end narrowed, stringy conidia, 19.50- (24.69) -33.34 μm in length and width of 1.20- (1.69) -2.74 μm.

References

Carter, M.V. (1994). Wood and root diseases caused by fungi. *Eutypa dieback.* In R. C. Pearson, and A.C. Goheen (ed.), Compendium of grape diseases, 3rd ed. APS Press. St. Paul, Minnesota: 32-34.

Carter, M.V., Bolay, A., English, H. and Rumbos, I. (1985). Variation in the Pathogenicity of *Eutypa lata* (= *E. armeniacae*). Aust. J. Bot., 33: 361-366.

DeScenzo, R. A., Engel, S.R., Gomez, G., Jackson, E.L., Munkvod, G.P., Weller, J. and. Dhingra, O.D., J.B. Sinclair, J.B. (1995). Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida, (F).

Dhingra, O.D., J.B. Sinclair, J.B. (1995). *Basic Plant Pathology Methods*. CRC Press, Boca Raton, Florida, (F).

Glawe, D.A., Skotland, C.B., Moller, W.J. (1982). Isolation and identification of *Eutypa armeniacae* from diseased grapevines in Washington state. Mycotaxon 16: 123-132.

Glawe, D.A., Rogers, J.D. (1982). Observations on the anamorphs of six species of *Eutyp*a and *Eutypella*. Mycotaxon 14: 334-346.

Lecomte, P., Peros, J.P., Blancard, D., Bastien, N., Delye, C. (2000). PCR Assays That Identify the Grapevine Dieback Fungus *Eutypa lata.* Applied and Environmental Microbiology 66 (10): 4475-4480.

McKemy, J.M., Glawe, D.A., Munkvold, G.P. (1993). A hyphomycetous synanamorph of *Eutypa armeniacae* in artificial culture. Mycologia, 85(6): 941-944.

Munkvold, G.P. (2001). Eutypa dieback of grapevine and apricot. Online. Plant Health Progress doi: 10.1094/PHP-2001-0219-01-DG. Accessed 20.12.2020.

Munkvold, G.P., Duthie, J.A., and Marois, J.J. (1993). Spatial patterns of grapevines with Eutypa dieback in vineyards with or without perithecia. Phytopathology 83: 1440-1448.

Muntañola-Cvetković, M. (1987). Opšta mikologija (General mycology). NIRO "Književne novine". Beograde.

Peros, J.P., Berger, G. (1994). A rapid method to assess the aggressiveness of *Eutypa lata* isolates and the susceptibility of grapevines cultivares to *Eutypa* dieback. Agronomie 14: 515-523.

Rolshausen, P.E., Mahoney, N.E., Molyneux, R.J., Gubler, W.D. (2006). A Reassessment of the Species Concept in *Eutypa lata*, the Causal Agemt of Eutypa Dieback of Grapevine. Phytopathology 96: 369-377.

Rolshausen, P.E., Baumgartner, K., Travadon, R., Pouzoulet, J. (2014). Identification of *Eutypa* spp. causing Eutypa dieback of grapevine in Eastern North America. Plant Dis. 98: 483-491.

Trouillas, F.P., and Gubler, W.D. (2010). Host range, biological variation, and phylogenetic diversity of *Eutypa lata* in California. Phytopathology 100 (10): 1048-1056.

Trouillas, F.P., Úrbez-Torres, J.R., and Gubler, W.D. (2010). Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102 (2): 319-336.

Živković, S., Vasić, T., Anđelković, S., Jevremović, D., Trkulja, V. (2012a). Identification and Characterization of *Eutypa lata* on Grapevine in Serbia. Plant Disease 96 (6): 913.

Živković, S., Vasić, T., Trkulja, V., Krnjaja, V., and Marković, J**.** (2012b). Pathogenicity on grapevine and sporulation of *Eutypa lata* isolates originating from Serbia. Romanian Biotechnological Letters. No 17(3): 7379-7388.

IDENTIFICATION OF MYCOPOPULATION ON AMERICAN HIGHBUSH BLUEBERRY IN SERBIA

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Abstract

In recent decades, the American highbush blueberry (*Vaccinium corymbosum* L.) has gained an increasingly important place in the fruit production of several countries around the world, especially in the highly developed ones. Also, there is a growing interest of berry fruit producers from Serbia in growing highbush blueberry plantations. Currently, in Serbia, highbush blueberries are grown on an area of about 2,500 ha with a tendency to increase production. Blueberries are attacked by numerous diseases that can cause greater economic damage, and in exceptional cases lead to the decline of entire orchards. There have been no systematic studies of blueberry mycopopulation in Serbia so far. In this study, we present the results of preliminary research on the mycopopulation of blueberries originating from Serbia. Samples were collected in the period June-August 2020. A total of 18 samples from five sites were analyzed (Ivanjica 3, Čačak 3, Sopot 3, Bačka Topola 6 and Zlatibor 3). The developed mycelium was placed on a new PDA medium and, after the initial growth, the top part of the mycelium was placed again on PDA. Microscopic examination was performed using an Olympus CX31 microscope. Morphological identification of fungi by genus was performed using standard keys. A total of 350 plant parts were examined from which 8 genera of fungi were isolated: *Fusarium, Phomopsis* (*Diaporthe*), *Alternaria, Epicoccum, Penicillium, Ulocladium, Trichoderma* and *Pestalotiopsis*. Macroscopic symptoms of infection were clearly visible on the plants from which the fungi were isolated. Given the growing importance of blueberries as a berry fruit species, the aim of this study is to determine fungi, potential pathogens of blueberries, in order to better understand the problems (plant dieback, yield reduction, etc.) that arise as a result of fungal presence on blueberries.

Keywords: *blueberry, fungal diseases, isolation, Serbia.*

Introduction

In recent decades, highbush blueberries have gained an increasingly important place in the fruit production of many, especially in highly developed, countries. The economic importance of blueberries, identical to other types of berries, is determined by: a) the use value of the fruit, b) profitability and b) high marketability of production (Leposavić and Jevremović, 2020). Greater interest in growing highbush blueberry plantations in Serbia arose in the 1990's, as a result of reduced interest in raspberries and the increase in demand for blueberries on the world market. During that period, plantations were established at several sites: Lučani, Viča, Osečina, Pecka and others (Leposavić, 2006). Highbush blueberry is the host of numerous pathogens and pests that can cause minor or major economic damage, and sometimes even lead to crop failure. Economically, the most important mycotic (fungal) causes of blueberry disease are *Botrytis*

cinerea (gray rot), *Colletotrichum gleosporioides* and *C. acutatum* (anthracnose) (Wharton and Schilde, 2003), *Monillinia vaccinii-corymbosi* (monilinia), *Phomopsis vaccinii* (blight), *Microsphaera vaccinii* (powdery mildew), *Fusarium acuminatum* (Fusarium branch blight) (Wright *et al*., 2014), *Septorioa albopunctata* (leaf and shoot spot), *Thekospora minima* (blueberry rust), *Valdenisinia heterodoxa* (leaf spot), *Godronia cassandrae* (blueberry shoot cancer), *Botryosphaeria dothidea* (blueberry trunk cancer) (Cardinaals *et al.*, 2018; Phillips *et al*., 2006), *Alternaria tenuissima* (alternaria blueberry leaf spot) and *Phytophthora cinnamomi* (blueberry root rot) (Wright et al., 1998; Michalecka *et al.,* 2017; Tennakoon *et al.,* 2018; Petrović *et al.,* 2020). While the most economically significant bacterioses on blueberries are *Pseudomonas syringae* pv. *syringae* (bacterial blueberry blight) and *Agrobacterium tumefaciens* (blueberry root cancer) (Leposavić and Jevremović, 2020). The American highbush blueberry is a host to numerous viruses and virus-like organisms. The presence of two viruses has been confirmed in Serbia: the blueberry red ringspot virus (BRRV) and the blueberry mosaic associated ophiovirus (BIMaV). Also, eight blueberry viruses have been detected, worldwide, that cause significant economic losses: blueberry scorch virus (BIScV), blueberry shock virus (BIScV), blueberry leaf virus (BISSV), Blueberry leaf mottle virus (BLMoV), Peach rosette mosaic virus (PRMV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV) and one phytoplasma blueberry stunt phytoplasma (Jevremović *et al.,* 2015; Jevremović *et al*., 2016a, b; Jevremović *et al*., 2019).
The aim of this research was to identify fungi on blueberries in Serbia, as possible causes of disease, in order to better understand the problems (plant dieback, reduced yields, etc.) that arise as a result of the presence of fungi on blueberries.

Material and methods

For the study of mycopopulation, samples of different varieties ('Duke', 'Draper', 'Cantichleer', 'Patriot', 'Berkly') of highbush blueberry (*Vaccinium corymbosum* L.) originating from Serbia were collected. Samples were collected in the period June-August 2020. A total of 18 samples from five sites were analyzed (Ivanjica 3, Čačak 3, Sopot 3, Bačka Topla 6 and Zlatibor 3). The plant parts of the branches and roots were carefully washed under running water. After rinsing, parts of the branches and roots were cut into pieces measuring 0.5-1 cm. Samples prepared in this way were disinfected with 96% ethanol for 10 seconds, 1% sodium hypochlorite (NaOCl) for 1 min. and 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 plant parts were placed in each Petri plate in five repetitions. They were kept in a thermostat at 24 °C. The examination was performed every 3 days, and on most samples the mycelium developed by the 14th day. The developed mycelium was then transferred again on a new PDA medium and, after the initial growth, the top part of the mycelium was placed again on PDA.
Microscopic examination was performed using an Olympus BX61 microscope. Morphological identification of fungi by genus was performed using standard keys. The frequency of isolation in % was calculated using the formula (Vrandečić *et al*., 2011):

$$\textit{Frequency of isolation} = \frac{\textit{Number of parts colonised with fungi}}{\textit{Total number of analysed plant parts}} \times 100$$

Results and discussion

In these studies of the mycopopulation of blueberry plants, a total of 350 plant parts were examined (Table 1). Symptoms on the stem, in the form of necrotic spots and lesions, were clearly expressed on all plants from which the fungi were isolated. Fungi from the genera *Phomopsis* (*Diaporthe*), *Alternaria* and *Pestalotiopsis* were isolated from these plants. Also, in a large number of plants, light to dark brown changes (necrosis) were observed on the cross section of the roots, and fungi from the genera *Fusarium* and *Epicoccum* were isolated from these plants. In a large number of plants, black fruiting bodies (pycnidia) were observed on the leaves and stem. It was determined that they belong to the genera *Phomopsis* (*Diaporthe*) and *Pestalotiopsis*. A difference in the frequency of fungi isolation was noticed between the sites Ivanjica, Čačak, Sopot, Bačka Topola and Zlatibor. Namely, fungi from the genus *Pestalothiopsis* were isolated from samples from all studied sites (Ivanjica) in different percentages and the frequency of isolation ranged from 20% (Ivanjica 2) to 80% (Čačak 2) (Table 1). It was determined that the frequency of isolation includes fungi of the genus *Alternaria*, which were isolated in most samples in a large percentage of 30 to 80%. Regarding fungi of the genus *Phomopsis*, a difference in the frequency of isolation by sites was observed. In the samples originating from the sites Zlatibor 1, 2, 3 and Sopot 1, 2, 3, there were no isolates of fungi from this genus. In all other samples, the frequency of isolation of fungi from the genus *Phomopsis* ranged from 10 to 40%. Also, it was observed that fungi of the genus *Fusarium* were isolated from samples from all studied localities and the frequency of isolation ranged from 30 to 80%. The obtained results indicate that highbush blueberry is susceptible to attack by a large number of fungi, which can significantly affect and reduce its yield and quality.

Table 1. Frequency of fungal isolation on highbush blueberries in Serbia.

Region	***Number of samples***		***Fungi genus-branch***	***freq. of isol. (%)***	***Fungi genus-root***	***freq. of isol. (%)***
	Branch	***Root***				
Ivanjica 1	10	10	*Phomopsis* sp. *Alternaria* sp.	40 60	*Epicoccum* sp. *Fusarium* sp.	20 80
Ivanjica 2	10	10	*Alternaria* sp. *Pestalotiopsis* sp.	80 20	*Fusarium* sp. *Trichoderma* sp.	30 70
Ivanjica 3	10	10	*Alternaria* sp. *Phomopsis* sp.	30 40	*Trichoderma* sp.	70
Čačak 1	10	5	*Phomopsis* sp. *Pestalotiopsis* sp. *Penicillium* sp.	20 40 40	*Trichoderma* sp. *Fusarium* sp.	20 40
Čačak 2	10	10	*Pestalotiopsis* sp.	*80*	*Fusarium* sp. *Penicillium* sp.	60 30
Čačak 3	10	10	*Alternaria* sp. *Penicillium* sp.	70 30	*Epicoccum* sp. *Fusarium* sp.	60 40
Sopot 1	10	10	*Alternaria* sp. *Pestalotiopsis* sp.	60 30	*Fusarium* sp. *Trichoderma* sp.	30 50

Sopot 2	10	10	*Alternaria* sp. *Ulocladium* sp.	30 20	*Epicoccum* sp. *Fusarium* sp.	10 40
Sopot 3	10	10	*Alternaria* sp.	50	*Fusarium sp.* *Trichoderma* sp.	20 40
Bačka Topola 1	10	10	*Alternaria* sp. *Phomopsis* sp. *Pestalotiopsis* sp.	20 10 50	*Fusarium* sp. *Penicillium* sp.	20 40
Bačka Topola 2	10	10	*Alternaria* sp. *Phomopsis* sp. *Pestalotiopsis* sp.	40 10 50	*Fusarium* sp. *Trichoderma* sp.	40 10
Bačka Topola 3	10	10	*Alternaria* sp. *Phomopsis* sp.	50 20	*Fusarium* sp.	30
Bačka Topola 4	10	10	*Alternaria* sp. *Pestalotiopsis* sp.	30 60	*Fusarium* sp. *Penicillium* sp.	20 30
Bačka Topola 5	10	10	*Alternaria* sp. *Phomopsis* sp. *Pestalotiopsos* sp.	30 10 50	*Fusarium* sp.	20
Bačka Topola 6	10	10	*Alternaria* sp. *Phomopsis* sp. *Pestalotiopsis* sp.	50 10 20	*Fusarium* sp.	20
Zlatibor 1	10	5	*Ulocladium* sp. *Pestalotiopsis* sp.	50 30	*Epicoccum* sp. *Fusarium* sp. *Trichoderma* sp.	10 30 10
Zlatibor 2	10	10	*Alternaria* sp. *Pestalotiopsis* sp.	30 60	*Penicillium* sp. *Trichoderma* sp. *Epicoccum* sp.	10 10 20
Zlatibor 3	10	10	*Pestalotiopsis* sp. *Ulocladium* sp.	60 20	*Fusarium* sp.	40

Clearly visible disease symptoms were present on all plants from which the isolations were made. A difference in the frequencies of isolation of individual genera of phytoparthogenic fungi was observed in samples from different localities in these studies. It was determined that in samples originating from Bačka Topola, Čačak and Ivanjica, isolated fungi from the genus *Pestalotiopsis* were more frequent. Also, the genus *Phomopsis* is more often isolated in samples originating from Čačak. Regarding the remaining six genera of isolated phytopathogenic fungi, they were present in all studied sites.

Phomopsis (*Diaporthe*) species are known to cause several blueberry diseases, including powdery mildew, branch cancer, and fruit rot (Lombard *et al.*, 2014). The same authors describe three species from the genera *Diaporth*, *D. asheicola*, *D. baccae*, and *D. sterilis*, based on phylogenetic analyzes, morphological, and molecular traits. *Diaporthe baccae* and *D. sterilis* originate from Europe and *D. asheicola* originates from Chile. *Diaperher baccae* and *D. sterilis* have been found to be sterile and therefore described on the basis of molecular characteristics. *Diaporthe vaccinii* causes cancer of twigs and rot of blueberry fruits and is currently listed as a quarantine disease in the European Union. In the Netherlands, two species from the same genus *Diaporthe*, *D. eres* and *D. rudis*, were detected, which were isolated from infected blueberry twigs (Cardinaals *et al.*, 2018). The same authors state that based on the results of the experiment in the greenhouse, the species *D. vaccini* is more aggressive than the species *D. eres.* In the field, it was shown experimentally that the development of lesions caused by *D. eres* did not differ significantly from those caused by *D. rudis*. Cardinaals *et al.* (2018) stated that fungi isolated in Europe, *D. eres* and *D rudis*, do not cause significant damage to blueberries. Although several *Diaporthe* species have been observed on blueberries, in various countries of the world, *D. vaccinii* remains a major problem in Europe. Wright *et al.* (2014) reported the existence of a new parasite on the blueberry in Argentina - fungus *F. acuminatum*. In their research, they came to the conclusion that fungi from the genus *Fusarium* can cause damage to blueberry plants, especially in plants that are weakened by frost and cold weather. Symptoms of the fungus *F. acuminatum* were observed during cold springs. The species *A. tenuissima* and *A. alternata* are considered to be the cause of leaf spot and rot of blueberry fruits around the world. However, most *Alternaria* species show significant morphological similarities including *A. tenuissima* and *A. alternata* species (Zhu and Xiao, 2015). According to research by Zhu and Xiao (2015) in California, only 5% of isolates of *A. tenuissima*, 61.5% of *A. alternata* and 32.9% of *A. arborescens* caused damage to blueberries. In their research, the same authors, in addition to the species *A. tenuissima, A. alternata, A. arborescens*, also detected the species *A. infectoria* and *A. rosae*. *A. tenuissima* has been identified as a cause of leaf spot and fruit rot on blueberries in Argentina, China, the United States and New Zealand (Fernández *et al*., 2015). *Pestalotiopsis guepinii* has been found to cause damage to blueberries in China, Turkey, Argentina (Wright *et al*., 1998) and Chile (Zhang *et al*., 2003; Fernández *et al*., 2015). In Chile, in addition to *Pestalotiopsis guepinii*, the species *Pestalotiopsis clavispora* (syn. *Pestalotia clavispora* GF Atk.), *P. neglecta* (syn. *Pestalotia ignocta* Thüm.) and *Truncatella angustata* (syn. *Pestalotia angustata* (Pers.) Arx) (Espinoza *et al*., 2008) have been also detected as causes of branch cancer and the dieback of blueberry plants.

Conclusions

This paper presents the preliminary results of mycopopulation of 18 samples of highbush blueberry from five sites. So far, there has been no significant research in this direction in Serbia, so future research related to highbush blueberries will go in the direction of studying fungal pathogens as well as finding ways of controlling them.

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References

Cardinaals J., Wenneker M., Voogd J. G. B. and van Leeuwen G. C. M. (2018). Pathogenicity of *Diaporthe* spp. on two blueberry cultivars (*Vaccinium corymbosum*). Bulletin OEPP/EPPO Bulletin (2018) 0 (0), 1–7 ISSN 0250. ISSN 0250-8052. DOI: 10.1111/epp.12451. Accessed 20.12.2020.

Espinoza J. G., Briceño E. X., Keith L. M., and Latorre B. A. (2008). Canker and twig dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. Plant Disease, 92: 1407- 1414.

Fernández R.L., Rivera M. C., Varsallona B., Wright E.R. (2015). Disease Prevalence and Symptoms Caused by *Alternaria tenuissima* and *Pestalotiopsis guepinii* on Blueberry in Entre Ríos and Buenos Aires, Argentina. American Journal of Plant Sciences, 6: 3082-3090

Jevremović D., Leposavić A., Paunović S. (2019). Genetic diversity of *Raspberry leaf blotch emaravirus* in red raspberries from Serbia. Spanish Journal of Agricultural Research, 17(1), e1004.

Jevremović D., Leposavić A. and Paunović S. (2016a). Incidence of viruses in highbush blueberry (*Vaccinium corymbosum* L.) in Serbia. Pesticidi i fitomedicina, 31(1-2): 45-50.

Jevremović D, Leposavić A. and Paunović S. (2016b). *Raspberry leaf blotch virus* – a common raspberry pathogen in Serbia. Journal of Mountain Agriculture on the Balkans 19: 147-156.

Jevremović D., Leposavić A., Paunović S. (2015). First report of *Blueberry mosaic-associated virus* in highbush blueberry in Serbia. Journal of Plant Pathology, 97, 3: 541.

Leposavić A., Jevremović D. (2020). Borovnica-tehnologija gajenja, zaštite i prerade (Blueberry-technology of cultivation, protection and processing). Naučno-voćarsko društvo Srbije, Čačak.

Leposavić A. (2006). Tehnologija gajenja borovnice (Blueberry cultivation technology). Opšta zemljoradnička zadruga Ari Nova, Arilje, Institut za istraživanja u poljoprivredi SRBIJA, Beograd.

Lombard L., van Leeuwen G.C.M., Guarnaccia V., Polizzi G., van Rijswick P.C.J., Rossendahl K.C.H.M., Gabler J. and Crous P.W. (2014). *Diaporthe* species associated with *Vaccinium*, with specific reference to Europe. Phytopathologia Mediterranea, 53, 2: 85−97.

Michalecka M., Bryk H., Seliga P. (2017). Identification and characterization of *Diaporthe vaccinii* Shear causing upright dieback and viscid rot of cranberry in Poland. Eur J Plant Pathol,148:595–605. DOI 10.1007/s10658-016-1114-4. Accessed 20.12.2020.

Petrović S., Milošević T., Jevremović D., Glišić I., Milošević, N. (2020). Jagodasto voće - Tehnologija gajenja, zaštite i prerade (Strawberry - growing, protection and processing technology). Beograd-Zemun: Agronomski fakultet, Čačak & Institut za voćarstvo, Čačak.

Phillips A.J.L., Oudemans P.V., Correia A. and Alves A. (2006). Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. Fungal Diversity 21: 141-155.

Tennakoon, K. M. S., Ridgeway, H. J. Jaspers, M. V. And Jones E. E. (2018). Factors Affecting *Neofuscicoccum ribis* Infection and Disease Progression in Blueberry. European journal of plant pathology 151: 87–99.

Vrandečić K., Ćosić J., Jurković D. i Poštić J. (2011). Mikopopulacija ljekovitog bilja u Hrvatskoj (Mycopopulation of medicinal plants in Croatia). Poljoprivreda, 17 (2): 18-21.

Wharton P., Schilder A. (2003). Blueberry Fruit Rot Identification Guide. Extension Bulletin E-2847. MICHIGAN STATE U N I V E R S I T Y. http://www.blueberryfacts.org.

Wright E.R., Rivera M.C., Flynn M.J. (1998). First report of *Pestalotiopsis guepini* and *Glomerella cingulata* on blueberry in Buenos Aires (Argentina). Bull. OEPP 28: 219-220.

Wright E. R., Rivera M. C., Campanella E. R., Farinon O. M., Berretta M. F. and Perez B. A. (2014). Fusarium branch blight on highbush blueberry in Argentina. African Journal of Biotechnology. Vol. 13(51), pp4628-4634, 17. DOI: 10.5897/ AJB2014.14156

Zhang, J.X., Xu, T., Ge, Q.X. (2003). Notes on *Pestalotiopsis* from Southern China. Mycotaxon, 85, 91-92.

Zhu X.Q., Xiao C.L. (2015). Phylogenetic, Morphological, and Pathogenic Characterization of *Alternaria* Species Associated with Fruit Rot of Blueberry in California. Phytopathology, 105: 1555-1567.

OCCURRENCE OF POWDERY MILDEW AND SEPTORIA LEAF BLOTCH IN WHEAT AFFECTED BY NITROGEN DOSE AND FOLIAR FERTILIZATION

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Abstract

Modern wheat production increasingly relies on its additional nutrition through leaves, and numerous biostimulators and water-soluble fertilizers are used for foliar feeding. This kind of nutrition regime makes plants more resistant to pathogens. During its growth wheat is attacked by many pathogenic fungi. Septoria leaf blotch (*Mycosphaerella graminicola*) and powdery mildew (*Blumeria graminis*) are the diseases occurring every year. This investigation was carried out with three wheat cultivars (Sosthene, Simonida and Evropa), during growing season 2018/2019, in the area of Gračanica municipality (K&M), on vertisol soil type. The trial was set in RCBD with four replications. The treatments were the following: 1) Control; 2) $N_{50}P_{90}K_{90}$; 3) $N_{100}P_{90}K_{90}$; 4) $N_{150}P_{90}K_{90}$; 5) $N_{100}P_{90}K_{90}$+ Agrostemin; 6) $N_{100}P_{90}K_{90}$ + Murtonik; 7) $N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik. Occurrence and development of both diseases were observed, and attack intensity was graded during the first weeks of June, according to the standard scales for septoria leaf blotch and powdery mildew. Fertilization regime significantly affected occurrence and intensity of septoria leaf blotch. In cultivar Sosthene foliarly applied Agrostemin and Murtonik reduced the disease development. The treatments with those two supplements applied ($N_{100}P_{90}K_{90}$ + Agrostemin and $N_{100}P_{90}K_{90}$ + Murtonik) had the lowest infection intensity (5.0%). Powdery mildew was registered in all three studied cultivars, but not in all the treatments. Unlike septoria leaf blotch, powdery mildew occurred sporadically, and with low intensity.

Keywords: *Wheat, Septoria leaf blotch, Powdery mildew, Fertilization.*

Introduction

Wheat is one of the field crops making a group of strategic products within the total agricultural industry. Along with soybean, wheat is a "top-selling merchandise", because its price on global stock market strongly affects international trade relations (Cvijanović *et al.*, 2017). Yield and quality of wheat grain is largely influenced by balanced mineral nutrition, weather conditions (Đekić *et al.*, 2017), use of non-certified seed (Protić *et al.*, 2005) and occurrence of diseases, such as septoria leaf blotch (causal agent *Mycosphaerella graminicola*) and powdery mildew (causal agent *Blumeria graminis*).
Demand for nutritive elements is dependent on the amount of planned grain yield. Blanchet *et al.* (2016) points out the application of fertilizers as a necessary measure to keep sustainable productivity of agricultural ecosystems. Among the major elements of mineral nutrition, nitrogen plays the main role in grain yield increase (Đekić *et al.*, 2014; Jelić *et al.*, 2015). Inadequate nutrition can weaken plant vitality so they lose resistance to pathogens. Modern wheat production increasingly relies on its additional nutrition through leaves, and numerous biostimulators and water-soluble fertilizers, containing both macro and micro elements, are used

for foliar fertilization. Absorption of nutritive elements from these formulations is several times greater than the absorption through the root pathway (Bolcu and Andrei, 2003). This kind of nutrition regime, besides grain yield and quality improvement, makes plants healthy and more resistant to pathogens.

Septoria leaf blotch is the disease present in the all regions where wheat is grown and, according to Stammler *et al.* (2008), it is one of the most important wheat diseases in northern Europe. During humid years the disease can massively attack susceptible genotypes, and in Serbia it occurs on a regular basis with a high percent of infection (Jevtić *et al.,* 2008). Powdery mildew is a common disease in the all wheat production areas, including the territory of Serbia. It occurs every year with greater or lower intensity. The causal agent is considered as a high-risk pathogen because of its significant adaptability potential and change of virulence in populations of the pathogen (Lalošević *et al.*, 2016).

Small grain diseases cannot be completely eradicated from our production fields. The basic goal is to make them less damaging, which can be achieved by applying various measures (creating and growing resistant cultivars and applying proper agrotechnique practice). If plants are developed under the optimal nutrition regime, they will be able to express their natural resistance to pathogens. This investigation aimed to establish the effect of increasing nitrogen doses, as well as application of water-soluble fertilizers and biostimulators, on occurrence and intensity of septoria leaf blotch and powdery mildew of wheat, in the conditions of natural infection.

Material and Methods

The investigation, dealing with the effect of increasing nitrogen doses and application of water-soluble fertilizers and biostimulators on occurrence and intensity of septoria leaf blotch and powdery mildew of wheat, has been carried out with three wheat cultivars (Sosthene, Simonida and Evropa), during the growing season 2018/2019, in the area of Gračanica municipality (K&M), on vertisol soil type. Local coordinates of the location were the following: latitude 42º 36', longitude 21° 11', altitude 588 m. The trial was set in a randomized complete block design with four replications. Elementary plot area was 6 m^2 (3x2). The experimental treatments were the following:

1. Control (unfertilized)
2. $N_{50}P_{90}K_{90}$
3. $N_{100}P_{90}K_{90}$
4. $N_{150}P_{90}K_{90}$
5. $N_{100}P_{90}K_{90}$ + Agrostemin
6. $N_{100}P_{90}K_{90}$ + Murtonik
7. $N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik.

Nitrogen was applied in three doses, namely 50, 100 and 150 kg ha^{-1}, while the dose of phosphorus and potassium was 90 kg ha^{-1} for each element. The whole amount of phosphorus and potassium was applied pre-sowing, along with a part of nitrogen. The rest of nitrogen, following predetermined combinations, was administered in dates during plant growth, by the fertilizer LAN (limestone ammonium nitrate, 27% of N). Foliar application of biostimulator Agrostemin was at the dose of 25 g ha^{-1}, while water-soluble fertilizer Murtonik (19:9:27 + microelements) was administered at the dose of 3 kg h^{-1}. During the period of maximal development of septoria leaf blotch and powdery mildew (the first ten days of June), occurrence and development of both diseases were observed. Intensity of attack by septoria leaf blotch was estimated according to the scale of Gešele (1978), grading severity of infection from 0 to 100%. For powdery mildew, reaction mode of the investigated cultivars was graded by determining infection type from 0-4 and severity of infection from 0-100% (Peterson *et al.*, 1948). On the basis of determined infection type and severity of infection we calculated coefficient of infection.

Results and Discussion

Septoria leaf blotch was registered in the all three investigated wheat cultivars and in all the treatments (table 1).

Table 1. Attack intensity of *Mycosphaerela graminicola* during growing season 2018/19

Treatments	Cultivars		
	Sosthene	Simonida	Evropa
	I (%)	I (%)	I (%)
Ø (unfertilized control)	18	20	20
$N_{50}P_{90}$ K_{90}	7	11	12
$N_{100}P_{90}$ K_{90}	7	5	10
$N_{150}P_{90}K_{90}$	7	7	10
$N_{100}P_{90}K_{90}$ + Agrostemin	5	10	18
$N_{100}P_{90}K_{90}$ + Murtonik	5	9	18
$N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik	8	10	18
Average	8.14	10.28	15.14

Comparing the studied wheat cultivars, the highest average intensity of the disease caused by *M. graminicola* was observed in the cultivar Evropa (15.14%), then in the cultivar Simonida (10.28%), and the lowest one was observed in the cultivar Sosthene (8.14%). Such result indicate that in this study the cultivar Sosthene showed the greatest resistance to the causal agent of septoria leaf blotch. An elaborate comparison can clearly reveal that the difference in the average attack intensity between the cultivars Evropa and Simonida was 4.86%, while between Evropa and Sosthene it was 7%. Similar reaction of the cultivar Evropa to the causal agent of septoria leaf blotch in the conditions of natural infection at Kosovo and Metohia province, during 2015 (10%) and 2016 (20%), has been reported previously by Gudžić *et al.* (2018). Various nutrition regimes affected significantly occurrence of the disease and severity of infection. The disease occured in the all three cultivars with the highest attack intensity at unfertilized plots of the trial (control). At this variant, the cultivar Sosthene was attacked with intensity of 18%, while in the cultivars Simonida and Evropa attack intensity was slightly higher and amounted 20% in each. There was a clearly visible trend in the cultivar Evropa that attack intensity of the variants with foliar fertilization applied ($N_{100}P_{90}K_{90}$ + Agrostemin, $N_{100}P_{90}K_{90}$ + Murtonik and $N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik) was slightly lower than in unfertilized control. Attack intensity of those variants was 18%. In this cultivar the lowest disease intensity was observed in the variants $N_{100}P_{90}$ K_{90} and $N_{150}P_{90}K_{90}$, and in the variant $N_{50}P_{90}K_{90}$ was insignificantly higher and amounted 12%. The effect of fertilization on increasing resistance of wheat to the causal agent of

septoria leaf blotch was visible better in the cultivar Simonida, and especially in the cultivar Sosthene. Sosthene expressed a positive influence of foliarly applied Agrostemin and Murtonik on the disease occurrence. In the variants where one of these two preparations was applied ($N_{100}P_{90}K_{90}$ + Agrostemin and $N_{100}P_{90}K_{90}$ + Murtonik) the lowest attack intensity was observed (5%), which was not the case in the variant where they were applied together ($N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik). *M. graminicola* is a pathogen causing problems for wheat production. Growing susceptible cultivars, suitable weather and sufficient amount of inoculum present at the crop are necessary conditions for this disease to occur (Kalentić *et al.*, 2006).
Along with septoria leaf blotch, occurrence of powdery mildew was also observed in the investigated wheat cultivars (table 2).

Table 2. Attack intensity of *Blumeria graminis* during vegetation 2018/19

Variants	Cultivars								
	Sosthene			Simonida			Evropa		
	t	I	IC	t	I	IC	t	I	IC
Ø (unfertilized control)									
$N_{50}P_{90}K_{90}$				2	10	4			
$N_{100}P_{90}K_{90}$	1	10	2						
$N_{150}P_{90}K_{90}$							3	20	16
$N_{100}P_{90}K_{90}$ + Agrostemin									
$N_{100}P_{90}K_{90}$ + Murtonik									
$N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik	2	10	4						
Average		2.86			1.43			2.86	

t – Infection type; I – Severity of infection (%); IC – Infection coefficient

The obtained results showed that powdery mildew occurred sporadically, and in a low intensity when it did. In the cultivar Sosthene severity of infection of 10% was observed in the variants $N_{100}P_{90}K_{90}$ and $N_{100}P_{90}K_{90}$ + Agrostemin + Murtonik. In the other two cultivars powdery mildew was observed in only one variant each. So in the cultivar Simonida severity of infection of 10% was found in the variant $N_{50}P_{90}K_{90}$, while in the cultivar Evropa the disease was observed only in the variant where the highest amount of nitrogen was applied ($N_{150}P_{90}K_{90}$), and severity of infection was 20%. Previous reports by Stojanović *et al.* (2004) and Gudžić *et al.* (2008) pointed out to specific reaction of various wheat cultivars to the causal agent of powdery mildew in the conditions of natural infection. Lower severity of wheat infection by the causal agent of powdery mildew was the result of a prolonged latent period and less successful infection (Jerković and Jevtić, 2000).

Conclusions

Septoria leaf blotch was registered in the all three investigated wheat cultivars and in all nutrition treatments. Occurrence of the disease was found highest for the all three cultivars in the absence of any mineral fertilization of wheat. The highest average attack intensity was observed in the cultivar Evropa (15.14%), and the lowest one in cultivar Sosthene (8.14%). Application of the biostimulator and foliar fertilizer, used both alone and combined, showed the best effect on septoria leaf blotch in the cultivar Sosthene. Powdery mildew occurred sporadically, and in fertilization combinations where it did, severity of infection was low.

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References

Bolcu, C., Andrei, A. (2003): Preliminary research in obtaining foliar concentrated semiecological fertilizers. ISIRR 2003, Hunedoara, Romania, 29-34.

Blanchet, G., Gavazov, K., Bragazza, L., Sinaj, S. (2016) Responses of soil properties and crop yields to different inorganic and organic amendments in a Swiss conventional farming system. Agriculture, Ecosystems & Environment. 230: 116–126.

Cvijanović, G., Roljević, S., Đurić, N., Dozet, G., Đukić, V., Cvijanović V. (2017): Uticaj različitih varijanti đubriva na kvantitet i kvalitet zrna pšenice [Effect of fertilization on different variants quantity and quality of grain of wheat]. 22nd Meeting on Biotechnology, Proceedings, book 1, 22, (24): 45-52, Čačak, Serbia.

Đekić, V., Milovanović, M., Popović, V., Milivojević, J., Staletić, M., Jelić, M., Perišić, V. (2014): Effects of fertilization on yield and grain quality in winter triticale. Romanian Journal of Agricultural Research, 31: 175-183.

Đekić, V., Milojević, J., Staletić, M., Jelić, M., Popović, V., Branković, S., Terzić, D. (2017): Uticaj mineralne ishrane na prinos ozime pšenice (*Triticum aestivum* L.) [The influence of mineral nutrition on winter wheat yield (*Triticum aestivum* L.)]. 22nd Meeting on Biotechnology, Proceedings, book 1, 22, (24): 207-212, Čačak, Serbia.

Gešele, E. E. (1978). Osnovi fitopatologičeskoj ocenki v selekciji rastenij [The basics of phytopathological grading in plant breeding]. Kolos, Moscow, USSR.

Gudžić, S., Deletić, N., Gudžić, N., Aksić, M. (2008): Otpornost nekih sorti prema prouzrokovaču pepelnice pšenice [Resistance of some wheat cultivars to the causal agent of powdery mildew]. Agroznanje, 1, 67-73.

Gudžić, S., Deletić, N., Nikolić, K., Aksić, M., Gudžić, N., Grčak, M., Grčak, D. (2018): Occurrence of the Septoria leaf blotch causal agent in some winter wheat cultivars. Book of Proceedings, IX International Scientific Agricultural Symposium "Agrosym 2018", 1040-1044, Jahorina, Republic of Srpska.

Jerković, Z., Jevtić, R. (2000): Rezultati oplemenjivanja pšenice na otpornost prema prouzrokovačima bolesti [Results of wheat breeding for resistance to disease causal agents]. Book of Abstracts of the XI Yugoslav Symposium on Plant Protection and Meeting on Pesticide Application, 45, Zlatibor, Serbia.

Jevtić, R., Telečki, M., Malešević, M. (2008). Opravdanost primene fungicida u usevima strnih žita [Rationale for fungicide application in small grain crops]. Book of Abstracts of the IX Meeting on Plant Protection, 13-15, Zlatibor, Serbia.

Jelić, M., Milivojević, J, Nikolić, O., Djekić, V., Stamenković, S. (2015): Effect of long-term fertilization and soil amendments on yield, grain quality and nutrition optimization in winter wheat on an acidic pseudogley. Romanian Journal of Agricultural Research, 32: 165-174.

Kalentić M., Jevtić. R., Telečki M. (2006). Intenzitet napada *Septoria tritici* na genotipovima pšenice u 2006. godini [Intensity of *Septoria tritici* attack on wheat genotypes in 2006]. Book of Abstracts of the VIII Meeting on Plant Protection. 32-33, Zlatibor, Serbia.

Lalošević, M., Jevtić, R., Župunski, V., Maširević, S. (2016): Pepelnica: aristokratska bolest pšenice [Powdery mildew: an aristocratic disease of wheat]. Biljni lekar, 44, (5-6): 426-433.

Peterson, R.F. Campbell, A.B., Hannah, A.E. (1948): A diagramic scale for estimating rust intensity on leaves of cereals. Can. J. Res., 26:496-500.

Protić, R., Janković, S., Poštić, D., Protić, N. (2005): Stanje useva i mere nege pšenice u proizvodnoj 2005/2006. godini u Republici Srbiji [Condition of wheat crops and the applied agotechnique of the production season 2005/2006 im Sebia]. Poljoprivredne aktuelnosti, 3-4, 17-27.

Stojanović S., Staletić M., Milovanović M., Pešić V., Gudžić S. (2006): Otpornost nekih genotipova pšenice prema prouzrokovačima pepelnice, lisne rđe i sive pegavosti lišća [Resistance of some wheat genotypes to causal agents of powdery mildew, leaf rust and Septoria leaf blotch]. 11th Meeting on Biotechnology, Proceedings, 11(11-12), 325-330, Čačak, Serbia.

Stammler, G., Kem, L., Semar, M., Galaettli, A., and Schoefl, U., (2008). Sensitivity of *Mycosphaerella graminicola* to DMI fungicides related to mutations in the target gene cyp 51 (14 a-demethylase). In: 15 th International Reinhardsbrunn Symposium, Modern Fungicides and Antifungal Compouds, at Freiedrichroda, Germany, pp. 137-142.

SWEET CORN AS FUNCTIONAL FOOD

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Abstract

Sweet corn is a well-established product in the market, and popular food all around the world. It is a source of phytochemicals which, due to their antioxidant activities, demonstrate significant beneficial contribution in reducing the risk of many diseases, and therefore sweet corn could be considered as a functional food. The aim of this study was to examine phytochemicals content in four sweet corn hybrids. Carotenoids, tocopherols and phenolic acid content was determined by HPLC-DAD/FLD and functional oligosaccharide by HPAEC-PAD. The highest content of lutein + zeaxanthin was found in hybrid ZP515su (37,53 μg/g), while hybrid ZP553su had the highest content of β-carotene (1,01 μg/g). Sweet corn grains are a considerable source of phenolic acids such as ferulic, caffeic and p-coumaric acids which have a wide scope of effects against human diseases including cancer, cardiovascular and neurodegenerative diseases. The highest content of total phenolic acids was found in hybrid ZP553su (73,94 μg/g) and ferulic acid in hybrid ZP515su (26,67 μg/g). Functional oligosaccharides could bring benefit to human health and are consider as dietary fiber and prebiotics. Hybrid ZP504su had the highest content of trehalose and turanose, hybrid ZP355su maltose and panose, as well as hybrid ZP515su raffinose, isomaltose and maltotriose. As an important component of a healthy diet, sweet corn provides high nutrition value in addition to the enjoyment in taste. Therefore, developing of sweet corn genotypes with high phytochemicals content is challenge for breeders.

Key words: *sweet corn, functional sugar, phytochemicals, health benefits.*

Introduction

Sweet corn is used around the world in the human diet in the milk (technological) phase of maturity when the grain is tender, sweet, and juicy and is therefore considered a vegetable. In Europe, the largest producers, according to the sown areas, are Hungary (39,000 ha) and France (26,000 ha). In the world, however, the largest producer and exporter of sweet corn is the United States. According to statistics, in the last decade, the total world export volume of frozen and canned products based on sweet corn has increased by about 49% and 33% respectively (FAOSTAT, 2016). This data indicates an increased demand and consumption of sweet corn in the world and at the same time indicates a greater need for its production. Functional foods contain substances that promote human health. Many studies have been published and a positive correlation has been shown between consuming functional foods and reducing various diseases. Carotenoids, tocopherols, and phenolic compounds that exhibit antioxidant properties are receiving increasing attention. Carotenoid consumption is associated with a number of human health benefits, such as prevention of heart and vascular diseases (Agarwal *et al.*, 2012), prevention of cataracts and macular degeneration of the eye, (Basu *et al.,* 2001), cancer chemoprotection (Tanaka *et al*., 2012), as well as neurodegenerative diseases (Obulesu *et al*., 2011). Vitamin E is known as a powerful antioxidant and has anticancer (Saini and Keum, 2016), antihypertensive (Kizhakekuttu and Widlansky, 2010), and neuroprotective activity (Rashid *et al.*, 2015). One of the most studied phenolics metabolites is ferulic acid, which is an outstanding antioxidant agent very common in vegetables. Chaudhary *et al*. (2019) reported that ferulic acids have a wide scope of effects against human diseases including cancer, diabetes, and cardiovascular and neurodegenerative diseases. Also, recent research shows that functional oligosaccharides exhibit antioxidant activity and provide positive effects on human health. The beneficial roles of functional oligosaccharides are primarily prebiotic, but they also increase mineral absorption; improve HDL/LDL cholesterol ratio; reduce serum lipids as well as blood cholesterol; they eliminate toxins from the body and have a low glycemic index (Cheng *et al*., 2017). Sweet corn kernel in addition to sugar contains 2.1 - 4.5% protein, 3 - 20% starch, 1.1 - 2.7% fat, 0.9 - 1.9% cellulose, 9-12 mg of vitamin C, small amounts of vitamins A, B1, B2 and mineral components. Also, sweet corn has a wide range of phytochemicals exhibiting health benefits of lowering risk of chronic diseases. The health benefits of sweet corn are mainly attributed to the high contents of carotenoids and phenols. Health benefiting properties of carotenoids and phenolic compounds are related to high anti-oxidant and radical scavenging activities and also to anti-inflammatory, anticancer and neuroprotective properties (Balasubashini *et al*, 2003; Tokuji *et al*, 2009). The aim of this study was to examine phytochemicals content (carotenoids, tocopherols, phenolic acid and functional oligosaccharide) in four sweet corn hybrids.

Material and methods

In this research phytochemicals profile (carotenoids, tocopherols, phenolic acids and sugars) was determined in kernel of four sweet corn hybrids ZP504*su*, ZP355*su*, ZP553*su* and ZP515*su*, planted 2019 in Maize Research Institute trail field. Carotenoids and tocopherols extraction and quantification by HPLC-DAD/FLD (Dionex UltiMate 3000 Thermo Scientific, Germany) was the same as it was proposed by Mesarović *et al*., 2019. The detection of tocopherols and carotenoids was conducted by fluorescence (λ_{ex} 290 nm; λ_{em} 325 nm) and photodiode array (at 450 nm and 470 nm) detector, respectively. The extraction of free phenolic acids was achieved by using (2 × 5 mL) 80% methanol (Mesarović *et al*., 2017). After homogenization in the ultrasound bath (30 min at 25 ºC) for all analyses, the extracts were centrifuged, filtered (0.45 µm nylon syringe filter) and directly injected into the HPLC system (Dionex UltiMate 3000 Thermo Scientific, Germany). The free phenolic acids were detected on 278, 260, 290 and 320 nm. The sugars extraction from approximately 5g of fresh kernel was accomplished by using ultraviolet ultrapure water (50ml), (Schmitzer *et al*., 2011), and sugar quantification was accomplished by high performance anion-exchange chromatography – HPAEC (Thermo Scientific) with pulsed amperometric detection-PAD. Concentrations of the analyzed phytochemicals are expressed as µg per g of dry matter (DM), except for sugars which were expressed as mg/g DM and reported as the mean value of three independent injections.

Results and discussion

Sweet corn contains phytochemicals such as carotenoids, tocopherols and phenolic acids (Ibrahim and Juvik, 2009; Das and Singh, 2016). The content of those phytochemicals is highly influenced by the genotype and also by applied agrotechnical practice (Suri and Tanumihardjo, 2016, Mesarović *et al.*, 2019). Since, humans cannot synthesize carotenoids they have to obtain them from the diet to meet minimal nutritional requirements and maintain optimal health (Jerome-Morais, *et al*, 2011; Sen and Chakraborty, 2011). Sweet corn contains significant amount of lutein, zeaxanthin and other carotenoids (Junpatiw *et al*., 2013) and has been widely consumed as a healthy food. The genetic variation for carotenoids among maize hybrids showed zeaxanthin and lutein were the most predominant. Lutein and zeaxanthin are the primary pigment compounds found in the retina where they protect eye tissue from phototoxic damage by absorbing 90% of harmful high-energy blue light (Roberts and Dennison, 2015).

Content of lutein + zeaxanthin ranged from 17,88–37,53 μg/g, and *β*-carotene content vary from 0,52–1,01 μg/g, Table 1. The higest content of L+Z had hybrid ZP515*su* (37,53 μg/g), and the highest β-carotene content had hybrid ZP553*su* (1,01 μg/g). Regarding total carotenoid content of all four hybrids, ZP515*su* hybrid achieves the highest value, namely 38,04 μg/g, followed by hybrid ZP553*su*, which recorded values of 25,66 μg/g. The content of lutein+zeaxanthin and *β*-carotene in kernel of four sweet corn hybrids was in accordance with results of Kurilich *et al* (1999) and Ibrahim *et al* (2009). In our previously research (Drinic *et al*., 2019) carotenoids were determined in the set of maize inbred lines with different kernel type (dent, flint, sweet corn, popcorn) and kernel color (yellow and orange). The highest average lutein+zeaxanhtin and β – carotene contents had lines with orange kernels, folowed by yelow inbreds, sweet corn and popcorn.

Table 1. The content of carotenoids and tocopherols in sweet corn hybrids (μg/g)

	ZP504*su*	ZP355*su*	ZP553*su*	ZP515*su*
Lutein+zeaxantin	24,25±0,75	17,88±0,17	24,75±0,97	37,53±0,63
β-carotene	0,92±0,04	0,53±0,01	1,01±0,04	0,52±0,06
Σ carotenoids	24,78±0,78	18,40±0,18	25,66±0,98	38,04±0,69
δ- tocopherol	0,15±0,04	0,75±0,03	0,83±0,02	0,81±0,02
β+*γ*- tocopherol	8,23±0,07	20,06±0,09	10,00±0,05	12,66±0,07
α-tocopherol	2,06±0,09	9,48±0,07	3,89±0,01	2,48±0,04

The highest value of *β*+*γ* -tocopherols has hybrid ZP355*su* (20,06 μg/g) as well as *α*-tocopherols (9,48 μg/g), Table 1. The hybrid ZP553su has the highest value of *δ* –tocopherol (0,83 μg/g). Tocopherols content detected in grain of four sweet corn hybrids was in accordinance with results of Kurilich *et al.* (1999), Ibrahim *et al.* (2009), Xie *et a.l* (2017), and Baseggio *et al.* (2018).

Our previous study of tocopherols content in set of different inbred lines showed that sweet corn inbreds had higher β+γ tocopherols content than inbred lines with standard kernel type but α tocopherol content was lower than in inbreds with standard kernel type (Drinic *et al.* 2017, 2019).

Table 2.The content of phenolic acids in sweet corn hybrids (μg/g)

	ZP504*su*	ZP355*su*	ZP553*su*	ZP515*su*
sinapic acid	8,07±0,08	9,90±0,03	22,71±0,00	12,31±0,01
cinnamic acid	2,18±0,05	2,31±0,06	1,87±0,04	3,72±0,02
protocatechuic acid	20,42±0,50	22,30±0,61	29,73±0,71	17,82±0,54
caffeic acid	1,11±0,03	1,46±0,04	1,05±0,02	1,08±0,03

p-coumaric acids	4,92±0,02	7,59±0,03	8,26±0,03	9,21±0,06
ferulic acid	11,80±0,01	11,89±0,05	10,31±0,01	26,67±0,38
Σ phenolic acids	48,50±0,69	55,45±0,83	73,94±0,90	70,81±1,05

The highest total content of phenolic acids was recorded in hybrid ZP553*su* (73,94 μg/g), Table 2. The highest content of ferulic acid was shown by the hybrid ZP515*su* (26,67 μg/g) which also has high content of total phenolic acid (70,81μg/g), and the highest content of *p*-coumaric acids and cinnamic acid (9,21μg/g and 3,72 μg/g), respectively. The hybrid ZP553*su* has the highest value of protocatechuic acid and sinapic acid but the lowest value of ferulic acid. The hybrids ZP355*su* has the highest caffeic acid content (1,46 μg/g). However, the content of phenolic acids obtained in our study was lower in comparison with the results obtained by Das and Singh (2016). Zhang *et al.* (2017) showed significant genotypic differences for phenolic acids exist in the tested sweet corn varieties. Mesarović *et al.* (2018) reported a significant effect of growing season on total phenols in the grain of three sweet corn hybrids. Total phenol content was lower in drier growing season for all hybrids.

Due to diverse health benefits of ferulic acid, including antioxidant, anti-inflammatory, and anticancer activities, it is regarded as one of the most important phenolic acids and receiving increasingly more attention (Kroon and Williamson, 1999). Also p-coumaric acid is of great interest due to the antioxidant and cardiovascular protective properties (Jyoti and Stanely, 2013).

Recently, functional oligosaccharides (isomaltose, panose, trehalose, maltotriose, raffinose, etc.) are receiving increasing attention according their functional proprieties. They are indigestible and provide positive effects on human health, and therefore considered dietary fiber and prebiotics (Cheng *et al*., 2017). Primarily the beneficial roles are prebiotic that enhance beneficial bacteria in the colon, but they also increase mineral absorption, improve HDL/LDL cholesterol ratio, reduce blood cholesterol, eliminate toxins from the body and have a low glycemic index (Ibrahim *et al*., 2018).

Table 3. Content of functional oligosaccharides in sweet corn hybrids (mg/g)

	ZP504*su*	ZP355*su*	ZP553*su*	ZP515*su*
trehalose	2,00±0,02	1,98±0,08	0,56±0,03	1,26±0,02
turanose	11,56±0,09	8,56±0,07	4,10±0,02	4,42±0,00
maltose	11,10±0,09	27,61±0,04	14,17±0,01	10,53±0,02
panose	1,09±0,09	2,84±0,04	0,40±0,02	0,25±0,04
raffinose	18,33±0,15	18,41±0,25	14,93±0,09	72,50±0,93
isomaltose	4,09±0,03	3,90±0,03	6,35±0,05	18,40±0,30
maltotriose	0,89±0,07	1,30±0,02	0,44±0,03	3,33±0,06

In the case of hybrids ZP504*su*, the highest content of trehalose (2,00 mg/g), and turanose (11,56 mg/g) were recorded, Table 3. The ZP355*su* hybrid had the highest content of maltose (27,61 mg/g) and panose (2,84 mg/g). The highest contents of isomaltose (18,40mg /g) and maltotriose (3,33 mg/g) were found in grain of hybrid ZP515*su*.

Conclusion

The hybrid ZP515*su* had a high level of lutein+zeaxanthin, total carotenoids, cinnamic acid, *p*-coumaric acids, ferulic acid, raffinose as well as moderate content of tocopherols. Hybrid

ZP355*su* had the favorable content of tocopherols and maltose, panose and caffeic acid while hybrid ZP553*su* has the highest contents of total phenolic acids, protocatechuic acid, sinapic acid, β-carotene, and δ-tocopherol. The hybrid ZP504*su* has the highest contents of trehalose and turanose, and the lowest one of *p*-coumaric acids, sinapic acid and tocopherols. The genetic background undoubtedly influences chemical quality and hybrids with high content of particular phytochemicals could be considered as functional food. Consumption of sweet corn would afford health benefits to consumers so it useful for functional food industry.

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Reference

Agarwal, M., Parameswari, R., Vasanthi, H., Das, D. (2012). Dynamic action of carotenoids in cardioprotection and maintenance of cardiac health. Molecules, 17(4), 4755-4769.

Balasubashini, M.S., Rukkumani, R., Menon, V.P. (2003). Protective effects of ferulic acid on hyperlipidemic diabetic rats. Acta Diabet 40(3): 118–224

Baseggio M., Murray M., Magallanes-Lundback M., Kaczmar N., Chamness J., Buckler E.S., Smith M.E., DellaPenna D., Tracy W.F., Gore M.A. (2018). Genome-wide association and genomic prediction models of tocochromanols in fresh sweet corn kernels, Plant Genome 11 180038.

Basu, H., Del Vecchio, A., Flider, F., Orthoefer, F. (2001). Nutritional and potential disease prevention properties of carotenoids. Journal of the American Oil Chemists' Society, 78(7), 665-675.

Chaudhary, A., Jaswal, V.S., Choudhary, S., Sharma, A., Beniwal, V., Tuli, H.S., Sharma, S. (2019). Ferulic Acid: A Promising Therapeutic Phytochemical and Recent Patents Advances. Recent Pat. Inflamm. Allergy Drug Discov., 13, 115–123.

Cheng, W., Lu, J., Li, B., Lin, W., Zhang, Z., Wei, X., Sun, C., Chi, M., Bi, W., Yang, B., Jiang, A., Yuan, J. (2017). Effect of functional oligosaccharides and ordinary dietary fiber on intestinal microbiota diversity. Frontiers in Microbiology, 8.

Das, A.K., Singh, V. (2016). Antioxidative Free and Bound Phenolic Constituents in Botanical Fractions of Indian Specialty Maize (Zea mays L.) Genotypes. Food chemistry, 201, 298–306.

Drinic Mladenovic S., Mesarević J., Stevanović M., Srdić J., Kravić N., Filipović M., Anđelković V. (2017). Variability of tochopherols content in maize inbred lines. Book of proceeding VIII International Scientific Agriculture Symposium "AGROSYM 2017", pp 618-622.

Drinić Mladenović S., Mesarović, J., Kravić, N., Srdić, J., Stevanović, M., Filipović, M., Anđelković, V. (2019). Micronutritient variability in maize inbred lines. AGROFOR International Journal, Vol. 4, Issue No. 2, 43-50.

FAOSTAT (2016). Food and Agriculture Organization of the United Nations, Statistics Division. http://www.fao.org/faostat/en/#data

Ibrahim, K., Juvik, J. (2009). Feasibility for improving phytonutrient content in vegetable crops using conventional breeding strategies: case study with carotenoids and tocopherols in sweet corn and broccoli. Journal of Agricultural and Food Chemistry, 57, 4636–4644.

Ibrahim, O. (2018). Functional oligosaccharide: chemicals structure, manufacturing, health benefits, applications and regulations. Journal of Food Chemistry & Nanotechnology, 4(4).

Jerome-Morais, A., Diamond, A. M., Wright, M. E. (2011). Dietary supplements and human health: For better or for worse? Molecular Nutrition & Food Research, 55, 122–135.

Junpatiw, A., Lertrat, K., Lomthaisong, K. and Tangwongchai, R. (2013). Effects of steaming, boiling and frozen storage on carotenoid contents of various sweet corn cultivars. Internat. Food Res. J., 20 (5): 2219-2225.

Jyoti, R.A., Stanely, M.P. (2013). Preventive Effects of p-coumaric acid on Lysosomal Dysfunction and Myocardial Infarct Size in Experimentally Induced Myocardial Infarction. European Journal of Pharmacology, 699, 33–39

Kizhakekuttu T., Widlansky M. (2010). Natural Antioxidants and Hypertension: Promise and Challenges. Cardiovascular Therapeutics 28, e20–e32.

Kroon, P.A., Williamson, G. (1999). Hydroxycinnamates in Plants and Food: Current and Future Perspectives. Journal of the Science of Food and Agriculture, 79, 355–361.

Kurilich AC, Juvik JA, (1999). Quantification of carotenoid and tocopherol antioxidants in Zea mays. J Agric Food Chem 47: 1948-55Mesarović, J., Srdić, J., Mladenović-Drinić, S., Dragičević, V., Simic, M., Brankov, M., Milojković-Opsenica, D. (2018). Antioxidant status of the different sweet maize hybrids under herbicide and foliarfertilizer application. Genetika, 50 (3), 1023-1033.

Mesarović, J., Dragičević, V., Mladenović Drinić, S., Ristić, D., Kravić, N. (2017). Determination of free phenolic acids from leaves within different colored maize, Journal of the Serbian Chemical Society, Vol 82 (1): 63–72.

Mesarović J., Mladenovic Drinic, S., Dragicevic, V., Srdic, J., Simic, M., Brankov, M. (2017). Variability of tocopherols in sweet maize grain induced by herbicides. Journal of International Scientific Publications, Agriculture & Food, 5, 42- 49.

Mesarović, J., Srdic, J., Mladenovic Drinic, S., Dragicevic, V., Simic, M., Brankov, M., Milojkovic-Opsenica, D. (2019). Evaluation of the nutritional profile of sweet maize after herbicide and foliar fertilizer application. J. Cereal Sci., 87, 132–137.

Obulesu, M., Dowlathabad, M., Bramhachari, P. (2011). Carotenoids and Alzheimer's Disease: An insight into therapeutic role of retinoids in animal models. Neurochemistry International, 59(5), 535-541.

Rashid, K., M., Ahsan, H., Siddiqui, S., Siddiqui, W. (2015). Tocotrienols have a nephroprotective action against lipid-induced chronic renal dysfunction in rats. *Renal Failure*, 37(1), 136-143.

Roberts, J.E., Dennison J. (2015). The photobiology of lutein and zeaxanthin in the eye, J. Ophthalmol. 687173.

Saini, R., Keum, Y. (2016). Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection. Food Research International, 82, 59-70.

Schmitzer, V., Slatnar, A., Mikulic-Petkovsek, M., Veberic, R., Krska, B., Stampar, F. (2011). Comparative study of primary and secondary metabolites in apricot (Prunus armeniaca L,) cultivars. Journal of the Science of Food and Agriculture, 91, 860–866.

Sen, S., Chakraborty, R. (2011). The role of antioxidants in human health. ACS Symposium Series, 1083, 1–37.

Suri, D., Tanumihardjo, S. (2016). Effects of different processing methods on the micronutrient and phytochemical contents of maize: From A to Z. Comprehensive Reviews in Food Science and Food Safety, 15(5), 912-926.

Tanaka, T., Shnimizu, M., Moriwaki, H. (2012). Cancer Chemoprevention by Carotenoids. Molecules, 17, 3202-3242.

Tokuji, Y., Akiyama K., Yunoki K., Kinoshita M., Sasaki K., Kobayashi H., Wada M., Ohnishi M. (2009). Screening for Beneficial Effects of Oral Intake of Sweet Corn by DNAMicroarray Analysis. Journal of Food Sci 74: 197-20

Xie L., Yu Y., Mao J., Liu H, Hu J.G., Li T., Guo X., Liu R.H.(2017). Evaluation of biosynthesis, accumulation and antioxidant activity of vitamin E in sweetcorn (*Zea mays* L.) during kernel development,Int.J.Mol.Sci.18, 2780.

Zhang R., Huang L., Deng Y., Chi J., Zhang Y., Wei Z., Zhang M. (2017) .Phenolic content and antioxidant activity of eight representative sweet corn varieties grown in South China, International Journal of Food Properties, 20:12, 3043-3055.

EFFECT OF SOIL MANAGEMENT SYSTEMS ON THE GENERATIVE POTENTIAL AND FRUIT QUALITY OF BLACK CHOKEBERRY

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Abstract

This experiment evaluated the effect of soil management systems (bare soil and black foil mulch) on the generative potential (number of clusters per inflorescence, number of flowers per inflorescence, number of berries per cluster, berry set percentage, cluster weight, berry weight, yield per bush, yield per unit area) and fruit quality (soluble solids content, total sugars, total acids, pH value and protein content) in black chokeberry [*Aronia melanocarpa* (Michx.) Elliott] of cultivar 'Nero'. Significant differences in generative potential were detected among soil management systems. The experimental results showed that black foil mulch, due to its ability to increase temperature and moisture in the soil directly led to an increase in the number of clusters per inflorescence, number of flowers per inflorescence and number of berries per cluster. In contrast, bare soil, with lower soil water content and temperature stimulated an increase in the cluster weight and berry weight, as well as yields per bush and per unit area. Also, soil management systems had a significant effect on the chemical composition of the fruit. Black foil mulch promoted the synthesis of soluble solids, sugars and protein, while bare soil favoured the accumulation of acids in chokeberries. Generally, the results suggested that black chokeberry grow well under varied soil management systems, and indicated that soil management systems had a significant effect on the generative potential and fruit quality.

Keywords: *Aronia melanocarpa, bare soil, black foil mulch, generative potential, chemical fruit traits*

Introduction

Black chokeberry [*Aronia melanocarpa* (Michx.) Elliott] is a deciduous shrub native to North America, botanically classified as belonging to the family *Rosaceae*. Shrub of chokeberry grows 1.2 to 2.4 m producing beautiful white flowers and navy-blue berries of tart-sweet taste and aromatic flavour (Jeppsson, 2000; Brand, 2010). Chokeberry plantation can be established in any climate condition. It is a cold-hardy plant tolerant of very low temperatures even below –35°C and it is not sensitive to the spring frosts (Bussières *et al*., 2008). Chokeberries are noted for their modest requirements and adaptability to different soil types (sandy, acid and humid soils) and soil management systems. They grow well under bare soil or continuous tillage as the most common system tested, but mulching with foil has increasingly been used in recent years. Numerous studies have highlighted the importance of mulch as an effective method for weed control and for maintaining a favourable soil structure and regulating the water and temperature regimes of the soil. According to Kawecki and Tomaszewska (2006), mulching the soil with foil promotes the vegetative and generative potential of black chokeberry.

In recent years, chokeberry has become more popular among small berries for their nutritional quality and potential health benefits. Berries have high levels of primary metabolites, especially

sugars, acids, tannins, pectins and pigments (Wu *et al*., 2004; Oszmianski and Wojdylo, 2005). Many epidemiological studies have showed that consumption of berries on regular basis offers potential health benefits to a number of diseases, including cardiovascular diseases, cancer, aging, neurological diseases, inflammation, diabetes and gastritis (Kokotkiewicz *et al*., 2010; Denev *et al*., 2014; Jurikova *et al*., 2017). The primary metabolites of berries may be affected by various factors, such as genotype, harvesting time, growth conditions and cultivation method (Jeppsson and Johansson, 2000).
Considering that there are not many scientific studies on the soil management systems in black chokeberry growing, the objective of this research was a comparative analysis of the effect of soil management systems in a black chokeberry orchard on the generative potential and fruit quality.

Material and method

The research was conducted at the Fruit Research Institute, Čačak (Serbia), during 2015-2017. The experiment was including chokeberry grown on the bare soil and black foil mulch in cultivar 'Nero'. The black chokeberry orchard was established in the spring of 2013. It was grown at a spacing of 3 m between rows and 1.5 m in the row. The experiment was set up at a randomized block design with three replications, giving a total of 60 black chokeberry bushes. During the trial, standard cultural, training and pruning practices were used, including drip irrigation.
Generative potential parameters (number of cluster per inflorescence, number of flowers per inflorescence, number of berries per cluster) were observed by counting. Cluster weight and berry weight were determined on a Mettler precision scale with an accuracy of 0.01 g. Yield per bush was determined by weighing harvested fruit on an ACS system electronic scale, whereas yield per unit area was the result of multiplying yield per bush by number of bushes per hectare.
Chemical analysis of the fruit included following: 1. Soluble solids content was determined by a digital refractometer (Kruss); 2. Total sugars were determined using the Loof-Schoorl method (Egan *et al*., 1981); 3. Total acids were measured using a burette containing 0.1 N NaOH; 4. pH value was measured by potentiometer (pH Meter MA 5707, Iskra, Slovenia); 5. Protein content was determined by Kjeldahl's method (Helrich, 1990).
During the experimental period, the average air temperature from May (fruit set) to August (fruit maturation) was 21.9°C in 2015, 21.3°C in 2016 and 20.7°C in 2017. Rainfall totals from May to August were 192.6 mm in 2015, 299.4 mm in 2016, and 383.5 mm in 2017, respectively.
The experimental data obtained during the three-year research period were subjected to statistical analysis using Fisher's two-factor analysis of variance - ANOVA. The significance of differences between the mean values of the tested factors and and their interactions was determined by LSD test at $P \leq 0.01$ and $P \leq 0.05$ significance levels.

Results and discussion

The analysis of the data showed significant differences in generative potential among soil management systems (Tables 1a and 1b). Mulching with foil directly led to an increase in the number of clusters per inflorescence (4.01), number of flowers per inflorescence (28.21) and number of berries per cluster (22.71). It can be assumed that black foil due to its ability to absorb much sunlight, directly increases soil temperature throughout the growing season and conserves more soil moisture, which had a positive effect on the some tested parameters of generative potential compared to bare soil. On the other hand, bare soil increased the cluster weight (13.93 g) and berry weight (0.83 g), as well as yields per bush (1.94 kg) and per unit area (4.265,1 kg/ha). Bare soil and foil mulch treatments did not differ in the berry set percentage. The results of the present study are comparable with those of Kawecki and Tomaszewska (2006), who found higher number of blossoms per cluster (15.2-18.3) and number of berries per cluster (9.4-17.0), and lower berry weight (0.78-0.85 g) in bushes mulched with black plastic compared with the other mulch treatments. In contrast, Paunović *et al*. (2016a) reported that bare soil was effective in increasing generative potential in black currants, while the lowest values were obtained under foil mulch. Namely, Kasirajan and Ngouajio (2012) recorded that plastic mulch prevents soil moisture loss and weed occurrence, which is good to balance vegetative and reproductive growth, and does not reduce the flower bud formation in chokeberry.

Table 1a. Generative potential of chokeberry cultivar Nero

Treatment/Year		Number of cluster per inflorescence	Number of flowers per inflorescence	Number of berries per cluster	Berry set (%)
Treatment (A)	bare soil	3.64±0.12 b	24.75±0.89 b	20.87±1.46 b	83.67±3.30
	foil mulch	4.01±0.15 a	28.21±1.27 a	22.71±1.34 a	81.16±4.41
Year (B)	2015	3.56±0.14 c	24.22±1.17 c	20.17±1.06 b	83.23±1.76 b
	2016	4.08±0.15 a	28.80±1.12 a	26.83±0.81 a	93.38±1.81 a
	2017	3.83±0.19 b	26.43±1.68 b	18.37±0.42 b	70.62±3.91 c
ANOVA					
Treatment (A)		**	**	ns	ns
Year (B)		**	**	**	**
A x B		ns	ns	ns	ns

Means followed by different letters within the treatment and year columns are significantly different at P≤0.01 and P≤0.05 according to LSD test and ANOVA (F-test) results.

In general, Kurtović *et al*. (2016) recorded similar number of clusters per inflorescence (3.03-4.08), number of flowers per inflorescence (14.02-25.74), number of berries per cluster (13.53-25.54) and berry weight (0.52-0.71 g), while Skender *et al*. (2017) reported a lower number of berries per cluster (13.0-14.6), but higher berry weight (1.02-1.20 g). Also, berry weight is in agreement with the results of Jeppsson (2000), who reported that berry weight varies from 0.61 to 0.85 g, which may be considered large fruits. In contrast, the present results are significantly lower than in the study of Strik *et al*. (2003), who obtained values for berry weight from 1.03 to 2.80 g.

Table 1b. Generative potential of chokeberry cultivar Nero

Treatment/Year		Cluster weight (g)	Berry weight (g)	Yield per bush (kg)	Yield per unit area (kg/ha)
Treatment (A)	bare soil	13.93±0.70 a	0.83±0.04 a	1.94±0.32 a	4.265,1±98.8 a
	foil mulch	9.50±0.73 b	0.65±0.06 b	1.53±0.39 b	3.153,0±85.2 b
Year (B)	2015	11.09±1.46 b	0.62±0.06 b	0.425±0.06 c	606.1±27.2 c
	2016	10.41±1.22 b	0.71±0.07 b	2.08±0.16 b	4.567,2±71.9 b
	2017	13.65±0.77 a	0.89±0.04 a	2.71±0.20 a	5.953,9±95.1 a
ANOVA					
Treatment (A)		**	**	**	**
Year (B)		**	**	**	**
A x B		ns	ns	ns	ns

Means followed by different letters within the treatment and year columns are significantly different at P≤0.01 and P≤0.05 according to LSD test and ANOVA (F-test) results.

As regards yield, the results of the present study are comparable with the findings of Kawecki and Tomaszewska (2006), who reported that the five-year yield in chokeberry was highest in bushes mulched with conifer bark, and lowest in bushes mulched with black plastic. Namely, McKay (2001) reported that plants of chokeberry responded with high yield when mulched properly. The author found that chokeberry in the first years gives small yield, because plants have weak branches that fall over onto the ground, while in the fourth year can be expected yield about 7-8 tons per hectare. Additionally, Kivijarvi *et al*. (2005) found that mulching orchard soil with foil contributes to increased yields compared to unmulched soil. In contrast, according to Bakshi *et al*. (2014) and Pandey *et al*. (2015), strawberry mulched with black polyethylene produced higher yield by 24.20% and 17.80% compared to control. Generally, Kurtović *et al*. (2016) observed that yield per plant ranged from 0.64 to 0.79 kg and from 1.926,3 to 2.370,6 kg per unit area, in the first years after planting. Radanović *et al*. (2012) found yield per plant of 340 g already in the second year and 4200 g of fruits per plant in the third year, while Jeppsson (2000) harvested an average from 440 to 1.050 g per plant for 3- to 5-year-old plantings. However, all of these reported yields were low compared with the findings of Strik *et al*. (2003) in Oregon, which obtained 4.4 to 24.1 kg per plant for 3- to 5-year-old plantings of various selected cultivars of black chokeberry.
Soil management systems during the experiment had an effect on synthesis and accumulation of the chemical ompounds in berries (Table 2). Black foil mulch had a significant positive effect on the synthesis of soluble solids (23.57%), total sugars (10.75%) and protein (1.18%), while bare soil had the effect on the higher accumulation of acids (2.31%) and pH value (3.45). The present results of primary metabolites are within the range determined by Skender *et al*. (2017). Also, Jeppsson and Johansson (2000) and Ochmian *et al*. (2012) reported that aronia berries are high in sugar (12 to 20% soluble solids), and have a pH of 3.3 to 3.7, and 0.7 to 1.2% titratable acidity.

Table 2. Fruit quality in black chokeberry cultivar Nero

Treatment/Year		Soluble solids content (%)	Total sugars (%)	Total acids (%)	pH	Protein (%)
Treatment (A)	bare soil	20.74±0.46 b	10.36±0.21 b	2.31±0.26 a	3.45±0.11 a	1.09±0.07 b
	foil mulch	23.57±0.34 a	10.75±0.25 a	2.23±0.24 b	3.38±0.09 b	1.18±0.09 a
Year (B)	2015	21.37±0.77 c	9.98±0.08 c	2.97±0.02 a	3.83±0.03 a	1.33±0.06 a
	2016	21.83±0.83 b	10.26±0.11 b	1.28±0.01 c	3.21±0.02 b	1.25±0.04 b
	2017	23.27±0.56 a	11.43±0.15 a	2.56±0.03 b	3.19±0.01 b	0.83±0.01 c
ANOVA						
Treatment(A)		**	**	**	**	**
Year (B)		*	**	**	**	**
A x B		ns	ns	ns	ns	ns

Means followed by different letters within the treatment and year columns are significantly different at $P \leq 0.01$ and $P \leq 0.05$ according to LSD test and ANOVA (F-test) results.

In terms of soil management systems, the present results are comparable to those obtained by Bakshi *et al*. (2014), who reported that black polyethylene mulch affected the increase of soluble solids and sugars, and a decrease of acids in strawberry. Also, Melgarejo *et al*. (2012) observed the higher contents of soluble solids and sugars, and lower acids content in plum fruits from trees grown under plastic mulching compared to control. The highest values of primary metabolites, as observed under black polythene mulch, indicate a greater role of elevated soil temperature as a catalyst for root activities, which is ultimately reflected in higher yield and better quality of the fruit (Pandey *et al*., 2016). According to Albert *et al*. (2010), plastic mulch in blueberries resulted in the lowest content of soluble solids, while Vool *et al*. (2007) recorded that the highest soluble solids content was obtained under plastic mulch compared to non-mulch in blackberries and raspberries. Actually, Paunović *et al*. (2016b) reported that bare soil had a significant positive effect on the synthesis of total sugars, while foil mulch had the effect on the accumulation of total acids.

During berry formation and ripening, air temperature was higher and rainfall amount was lower in 2015 than in 2016 and 2017, which had a stimulating effect on the synthesis of acids and protein. Conversely, moderate air temperatures and precipitation amounts in 2016 promoted the number of clusters per inflorescence, number of flowers per inflorescence, number of berries per cluster and berry set in comparison to 2015 and 2017. Heavier precipitation and lower air temperature in 2017 increased cluster weight and berry weight, as well as yields per bush and per unit area, and had a positive effect on the synthesis and accumulation of soluble solids and sugars compared to the other two experimental years. Kawecki and Tomaszewska (2006) reported that the number of blossoms per cluster, the number of berries per cluster, and berry weight of chokeberry varied considerably from year to year. Also, Asănică *et al*. (2017) reported that the average number of flowers inside the inflorescences did not vary too much between years, while Jeppsson (2000) recorded lower mean berry weight and a remarkable loss in yield in higher temperature and precipitation. In terms of chemical composition of fruits, Paunović *et al*. (2016c) recorded that higher air temperature and lower rainfall had a positive effect on the synthesis of soluble solids, sugars and proteins, and negative on the synthesis of total acids and fruit pH value. Rubinskiene *et al*. (2006) observed a positive correlation between air temperature and the content of soluble solids and sugars, and a negative correlation between rainfall and these parameters. Similarly, as determined by Kaldmae *et al*. (2013), temperature is positively correlated with the contents of soluble solids and sugars, as opposed to precipitation. The difference in chemical compounds contents can be explained by strong variations in their synthesis and accumulation under different climates.

Conclusion

Soil management systems have a significant effect on the generative potential, and promote the synthesis and accumulation of chemical compounds in berries, which should be considered when establishing commercial black chokeberry orchards. Also, climatic factors (air temperature and precipitation) have an important effect on the generative potential and the biosynthesis of primary metabolites in the fruit, thus affecting the quality and commercial value of the fruit. In general, chokeberry is an exceptionally rich source of primary metabolites, suggesting that berries can be used as natural agents in the pharmaceutical and food industries.

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References

Albert T., Karp K., Starast M., Paal T. (2010). The effect of mulching and pruning on the vegetative growth and yield of the half-high blueberry. Agronomy Research, 8(1): 759–769.

Asănică A., Stănică F., Lacob A. (2017). New vertical training systems for *Aronia melanocarpa* L. Fruit Growing Research, 33: 41–46.

Brand, M.H. (2010). *Aronia*: Native shrubs with untapped potential. Arnoldia, 67(3):14–25.

Bakshi P., Bhat D.J., Wali V.K., Sharma A., Iqbal M. (2014). Growth, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.) cv. Chandler as influenced by various mulching materials. African Journal of Agricultural Research, 9(7):701–706.

Bussieres J., Boudreau S., Clement–Mathieu G., Dansereau B., Rochefort L. (2008). Growing black chokeberry (*Aronia melanocarpa*) in cut-over peatlands. HortScience, 43(2): 494–499.

Denev P., Kratchanova M., Ciz M., Lojek A., Vasicek O., Nedelcheva P., Blazheva D., Toshkova R., Gardeva E., Yossifova L., Hyrsl P., Vojtek L. (2014). Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. Food Chemistry, 157: 37–44.

Egan H., Kirk R., Sawyer R. (Eds) (1981). The Luff Schoorl method. Sugars and preserves. In Pearson's chemical analysis of foods. 8thedn. Harlow. UK: Longman Scientific and Technical, pp. 151–153.

Helrich K. (1990). Official methods of analysis of the association of official analytical chemists Arlington: Association of Official Analytical Chemists Inc., p. 807.

Jeppsson N. (2000). The effects of fertilizer rate on vegetative growth, yield and fruit quality, with special respect to pigments, in black chokeberry (*Aronia melanocarpa*) cv. `Viking'. Scientia Horticulturae, 83: 127–137.

Jeppsson N, Johansson R (2000). Changes in fruit quality in black chokeberry (*Aronia melanocarpa*) during maturation. The Journal of Horticultural Science and Biotechnology, 75: 340–350.

Jurikova T., Mlcek J., Skrovankova S., Sumczynski D., Sochor J., Hlavacova I., Snopek L., Orsavova J. (2017). Fruits of black chokeberry *Aronia melanocarpa* in the prevention of chronic diseases. Molecules., 22: 944.

Kaldmae H., Kikas A., Arus L., Libek A. (2013). Genotype and microclimate conditions influence ripening pattern and quality of blackcurrant (*Ribes nigrum* L.) fruit. Zemdirbyste-Agric, 100(2): 164–174.

Kasirajan, S. Ngouajio, M. (2012). Polyethylene and biodegradable mulches for agricultural applications : A review. Agronomy for Sustainable Development, 32: 501–29.

Kawecki Z., Tomaszewska Z. (2006). The effect of various soil management techniques on growth and yield in the black chokeberry (*Aronia melanocarpa* Elliot). Journal of Fruit and Ornamental Plant Research, 14: 67-73.

Kivijarvi P., Tuovinen T., Kemppainen R.(2005). Mulches and pheromones - plant protection tools for organic black currant production. In: NJF Report, Nordic Association of Agricultural Scientists, 1: 87–90.

Kokotkiewicz A., Jaremicz Z., Luczkiewicz M. (2010). Aronia plants: A review of traditional use, biological activities, and perspectives for modern medicine. Journal of Medicinal Food, 13: 255–269.

Kurtović M., Smajlović H., Grbo L., Grahić J. (2016). Morphological characteristics and production effects of chokeberry (*Aronia melanocarpa* L.) cultivation in the area of Nisicka uplands. Proceedings of Symposium „Improvement of agriculture, forestry and water management in the region the hilly and mountainous areas – sustainable use and protection", Sarajevo (Bosnia and Herzegovina), pp. 85–96.

McKay S.A. (2001). Demand increasing for aronia and elderberry in North America. NY Fruit Quarterly, 9: 2–3.

Melgarejo P., Calín-Sánchezb A., Hernándeza F., Szumnyc A., Martíneza J.J., Leguaa P., Martíneza R., Carbonell-Barrachina A.A. (2012). Chemical, functional and quality properties of Japanese plum (*Prunus salicina* Lindl.) as affected by mulching. Scientia Horticulturae, 134: 114–120.

Ochmian I., Grajkowski J., Smolik M. (2012). Comparison of some morphological features, quality and chemical content of four cultivars of chokeberry fruits (*Aronia melanocarpa*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40: 253–260.

Oszmiański, J., Wojdylo, A. (2005). *Aronia melanocarpa* phenolics and their antioxidant activity. European Food Research and Technology, 221: 809–813.

Pandey S., Singh J., Maurya I.B. (2015). Effect of black polythene mulch on growth and yield of Winter Dawn strawberry (*Fragaria × ananassa*) by improving root zone temperature. Indian Journal of Agricultural Science, 85(9): 1219–1222.

Pandey S., Tewari S.G., Singh J., Rajpurohit D., Kumar G. (2016). Efficacy of mulches on soil modifications, growth, production and quality of strawberry (*Fragaria* x *ananassa* Duch.). International Journal of Science and Nature, 7(4): 813–820.

Paunović S.M., Nikolić M., Miletić R., Milivojević J., Veličković M. (2016a). Effect of soil management systems on the generative potential of black currant (*Ribes nigrum* L.) cultivars. Journal of Pomology, 50(193–194): 25–30.

Paunović S.M., Nikolić M., Miletić R., Popović B., Mitrović O., Kandić M. (2016b). Effect of soil management systems on the content of primary metabolites and sensory attributes of black currant (*Ribes nigrum* L.) fruit. Journal of Mountain Agriculture on the Balkans, 19(2): 233–246.

Paunović S.M., Nikolić M., Miletić R., Milinković M., Karaklajić-Stajić Ž., Tomić J. (2016c). Effect of climatic factors on fruit quality of black currant (*Ribes nigrum* L.) cultivars. Book of Proceedings of VII International Scientific Agriculture Symposium "Agrosym 2016", Jahorina (Bosnia and Herzegovina), pp. 834–838.

Radanović S.D., Antić-Mladenović B.S., Sekulić Đ.P., Ninkov M.J., Radanović D.B. (2012). Initial growth and yield of the black chokeberry (*Aronia Melanocarpa*) grown on the dystric cambisol and calcareous chernozem soils and mineral composition of its fruits. Proceedings of the 7th CMAPSEEC, pp. 284–290.

Rubinskiene M., Viskelis P., Jasutiene I., Duchovskis P., Bobinas C. (2006). Changes in biologically active constituents during ripening in black currants. Journal of Fruit and Ornamental Plant Research, 14(l2): 237–246.

Strik B., Finn C., Wrolstad R. (2003). Performance of chokeberry (*Aronia melanocarpa*) in Oregon, USA. Acta Horticulturae, 626: 447–451.

Skender A., Joldić S., Kurtović M., Alibabić V., Hadžiabulić S. (2017). Effects of fertilization on vegetative growth, yield and fruit quality of black chokeberry (*Aronia melanocarpa* Elliot). Agro-Knowledge Journal, 18(2): 85–94.

Vool E., Karp K., Moor U., Starast M. (2007). Yield quality in some taxona of the genus *Rubus* depending on the cultivation technology. European Journal of Horticultural Science, 72: 32–38.

Wu X., Liwei G., Prior R.L., McKay S. (2004). Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. Journal of Agricultural and Food Chemistry, 52: 7846–7856.

COLOUR STABILITY OF CYANIDIN IN SLIGHTLY ACIDIC SOLUTION

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Abstract

Anthocyanins are the more important plant pigments visible to the human eye. They make a significant contribution to the colour, and hence acceptability, of many fruits, some vegetables and associated products, including beverages and preserves. The study of natural colourants is extensive and active area of investigation due to the growing interest of substituting synthetic colourants with toxic effects in humans with anthocyanins which possess an array of health-promoting benefits. Considering the beneficial effect for the health of these molecules, their incorporation in food and beverages industries represents an important value. Similar to other anthocyanins, cyanidin can take on various structural forms in aqueous environments, which depend on pH. To determine their stability in detail, we stored (4320 min) cyanidin in aqueous solution at pH 6.0 at room temperature (25.0 °C) and tracked the colour stability by UV–Vis spectrometry. The aim of this study was to investigate colour stability of the cyanidin in slightly acidic solutions during storage at room temperature in order to obtain more precise information to evaluate the possibility of its use as a food colour. Cyanidin colour stability in the first three hours after dissolution was high. However, after three days of storage, colour stability was decreased to half and the colour of the solution changed. The use of anthocyanins like cyanidin as food colourants in slightly acidic products should therefore be considered at least in products with limited storage.

Keywords: *cyanidin, anthocyanins, colour stability, UV–Vis spectrometry.*

Introduction

Anthocyanins (in Greek *anthos* means flower, and *kyanos* means blue) are the more important plant pigments visible to the human eye. They belong to the widespread class of phenolic compounds collectively named flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Kong, 2003). These pigments are responsible of the shiny orange, pink, red, violet and blue colours in the flowers and fruits of some plants (Castañeda-Ovando et al., 2009). Up to now there are reports of more than 500 different anthocyanins and 23 anthocyanidins of which only six are the most common in vascular plants, pelargonidin, peonidin, cyanidin, malvidin, petunidin and delphinidin (Castañeda-Ovando et al., 2009). The glycoside derivatives of the three non-methylated anthocyanidins (cyanidin, delphinidin and pelargonidin) are the most common in nature, being

found in 80% of pigmented leaves, 69% in fruits and 50% in flowers (Castañeda-Ovando et al., 2009; Kong, 2003).
The isolated anthocyanins are highly instable and very susceptible to degradation. Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Castañeda-Ovando et al., 2009). Anthocyanins are unique among flavonoids as their structures reversibly undergo pH-dependent transformation in aqueous solution (He & Giusti, 2010). Anthocyanins can be found in different chemical forms which depend on the pH of the solution (Figure 1) (Rakić et al., 2019). At pH 1, the flavylium cation (red colour) is the predominant species and contributes to purple and red colours. At pH values between 2 and 4, the quinoidal blue species are predominant. At pH values between 4 and 6, four structural forms of the anthocyanins coexist: flavylium cation, anhydrous quinoidal base, colourless carbinol base and the pale yellow chalcone. At pH values higher than 7, the anthocyanins are degraded depending on their substituent groups (Castañeda-Ovando et al., 2009). In general, anthocyanins show their highest colour intensity in the flavylium ion form (Harborne & Williams, 2001; Rakić et al., 2015; Rakić & Poklar Ulrih, 2021).

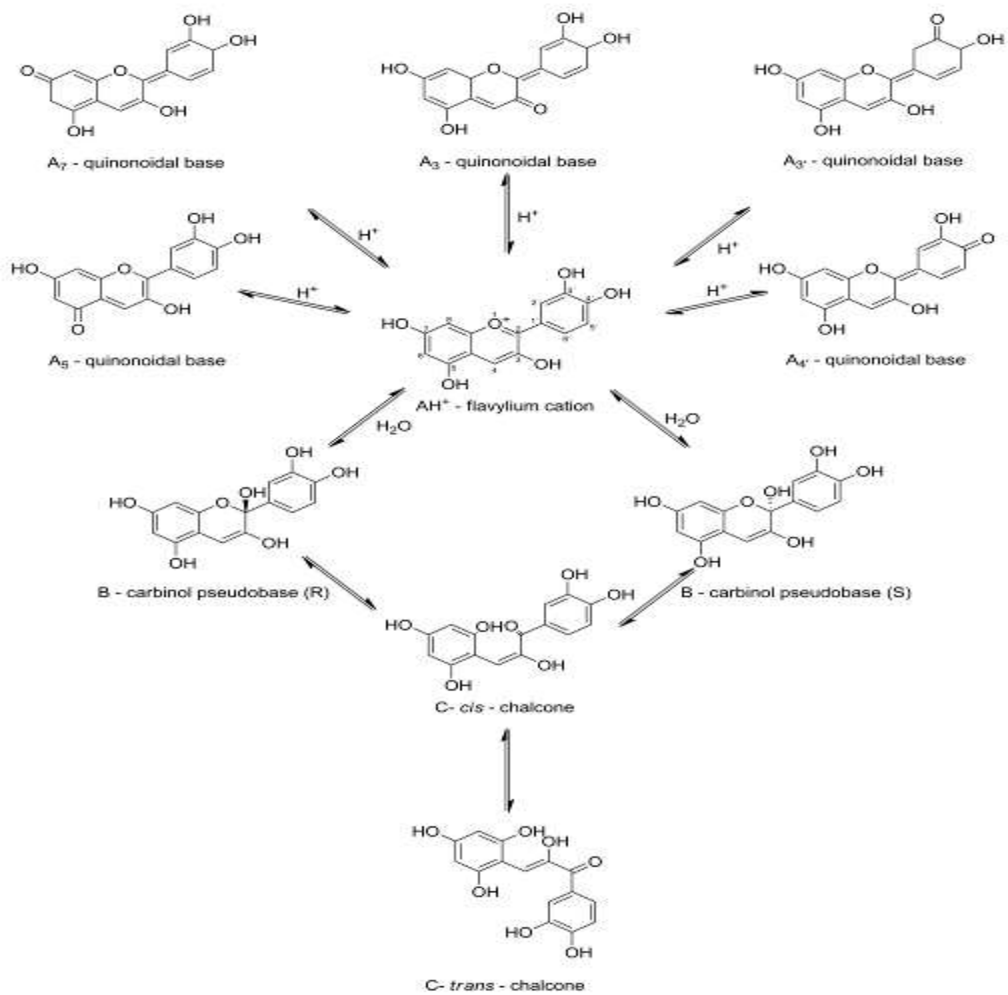

Figure 1. Transformations of cyanidin in aqueous solutions at different pHs (Rakić et al., 2019).

The anthocyanin content of fruit and vegetables, and their corresponding antioxidant activity, contribute to protective effects against degenerative and chronic diseases, thus also giving them further nephroprotective and anti-inflammatory effects, among others (Castañeda-Ovando et al., 2009; Popović, Kocić, Katić, Jović, et al., 2019; Popović, Kocić, Katić, Zarubica, et al., 2019; Veljković et al., 2017).
Anthocyanins make a significant contribution to the colour, and hence acceptability, of many fruits, some vegetables and associated products, including beverages and preserves. It was recognized that anthocyanin-rich extracts might have potential as food additives (Clifford, 2000). Anthocyanins are used as food colourants primarily in the beverage industry. As public concern about synthetic food dyes has increased recently, consumers and food manufacturers desire colourants from natural sources. Synthetic dyes commonly used in the food industry have been suspected to cause adverse behavioral and neurological effects. Consumers are willing to pay more for products that are perceived more natural, healthier, and with potential disease prevention benefits in addition to their nutritional value. This, in turn, is stimulating the food industry toward the incorporation of more natural ingredients into foods, including the use of anthocyanin-based colourants as an alternative to the use of synthetic dyes. Use of anthocyanin based colourants presents a number of challenges, including stability for processing and storage. Application of anthocyanin-based colourants in fruit yogurt and many types of fruit-flavored dry mixes is becoming more popular. Indeed, synthetic dyes are not allowed in the rapidly growing natural foods market, where anthocyanins are becoming increasingly important (He & Giusti, 2010). Considering the beneficial effect for the health of these molecules, their incorporation in food and beverages industries represent an important value (Castañeda-Ovando et al., 2009). To determine their stability in detail, we storage the cyanidin in aqueous solution at pH 6.0 at room temperature (25.0 °C) and tracked the colour stability by UV–Vis spectrometry. The aim of this study was to investigate colour stability of the cyanidin in slightly acidic solutions (pH 6.0) during storage at room temperature (25.0 °C) in order to obtain more precise information to evaluate the possibility of its use as a food colour.

Materials and methods

Chemicals and reagents

The chloride salt of cyanidin was from Polyphenols Laboratories AS (Sandnes, Norway). Sodium hydroxide was from Merck (Darmstadt, Germany). The aqueous solution was prepared from Milli-Q water (resistivity >18 MΩ cm; Millipore, Bedford, MA, USA).

Measurements of stability

The colour stability of the cyanidin in aqueous solution was determined at 25.0 °C. The chloride salt of cyanidin was dissolved in Milli-Q water to 0.2 mmol/L. The pH of cyanidin solution was adjusted to pH 6.0 using NaOH (concentration 9.8×10^{-3} mol/L). The sample tube was sealed with parafilm, in a water bath at 25.0 °C (room temperature) and the cyanidin was stored under air atmosphere. The pHs was measured using a pH meter (Seven Easy; Mettler Toledo, Schwerzenbach, Switzerlend) equipped with a micro electrode (InLab; Mettler Toledo, Schwerzenbach, Switzerlend). UV–Vis measurements were made immediately after dissolution and then after specified time intervals, during 4320 min (3 days). The absorption spectra (190–900 nm) were recorded at 25.0 ± 0.1 °C, using a UV– Vis spectrophotometer (Cary 100 Bio; Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-pathlength quartz cell, with

Milli-Q water as the reference, and a resolution of 1 nm. Colour stability was expressed as the percentage of absorbance remaining after a certain time interval, measured at visible absorption maxima ($\lambda_{vis-max}$) at pH 6.0.

Results and discussion

The colour stability in the 0.2 mmol/L cyanidin aqueous solution was studied at pH 6.0 during 4320 min (3 days) of storage at room temperature (25.0 °C). According to Cabrita et al. (Cabrita et al., 2000) and Fossen et al. (Fossen et al., 1998) colour stability was expressed as the percentage of the absorbance remaining after a certain time interval, measured at absorption maxima.

The shape of the absorption band in the visible spectra of cyanidin at pH 6.0, immediately after dissolution, was broad with visible absorption maximum at 580 nm (Figure 2). Broad visible absorption band suggest presence of mixture of various equilibrium forms (Castañeda-Ovando et al., 2009; Fossen et al., 1998). The shape of the absorption band did not change during first 150 minutes. Accordingly, the position of absorption maximum did not change during this period. This indicates high and almost constant colour stability of cyanidin in first 150 minutes of storage. The colour stability of cyanidin was above 98.0% in the first 150 min (Figure 3).

The absorption band changed its shape and decreased in intensity after 4320 min. Also, the position of the absorption maximum was changed. Consequently, the colour of the solution has changed. This was probably a consequence of the change in ratio of various equilibrium forms and cyanidin degradation. After 4320 min (3 days) of storage colour stability decreased to 51.1%.

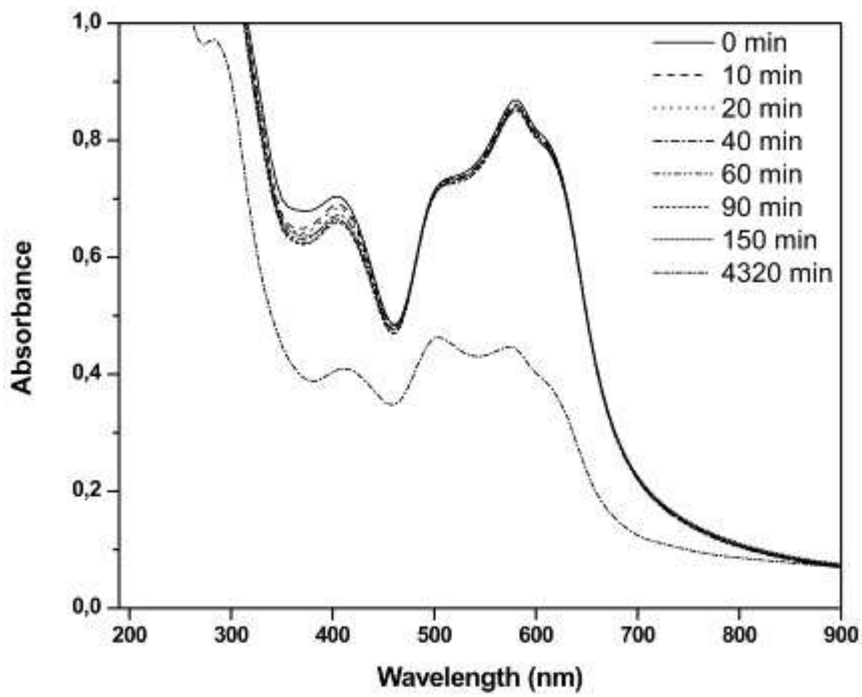

Figure 2. Changes in the UV–Vis spectra of 0.2 mmol/L cyanidin solution during storage (up to 4320 min) at pH 6.0, and 25.0 °C.

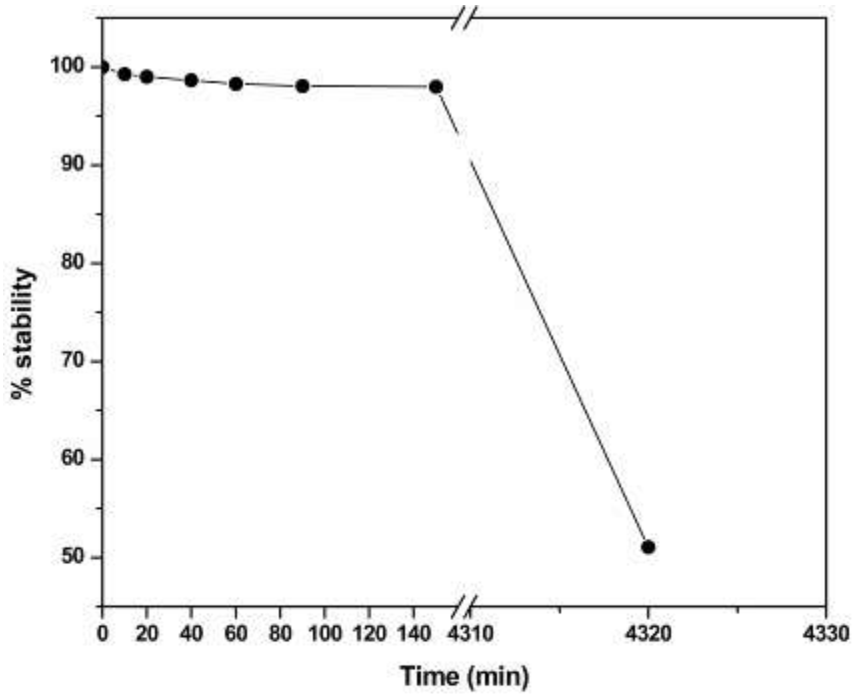

Figure 3. Colour stability of 0.2 mmol/L cyanidin solution during storage (up to 4320 min) at pH 6.0, and 25.0 °C.

Conclusions

Cyanidin colour stability in the first three hours after the dissolution was high. However, after three days of storage, colour stability was decreased to half and colour of the solution has changed. The use of anthocyanins like cyanidin as food colourants in slightly acidic products should therefore be considered at least in products with limited time of storage.

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References

Cabrita, L., Fossen, T., & Andersen, Ø. M. (2000). Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. *Food Chemistry, 68*(1), 101–107. https://doi.org/10.1016/S0308-8146(99)00170-3

Castañeda-Ovando, A., Pacheco-Hernández, M. de L., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113*(4), 859–871. https://doi.org/10.1016/j.foodchem.2008.09.001

Clifford, M. N. (2000). Anthocyanins – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 80*(7), 1063–1072.

Fossen, T., Cabrita, L., & Andersen, M. (1998). Colour and stability of pure anthocyanins inuenced by pH including the alkaline region. *Food Chemistry*, *63*(4), 435–440.

Harborne, J. B., & Williams, C. a. (2001). Anthocyanins and other flavonoids. *Natural Product Reports*, *18*(3), 310–333. http://www.ncbi.nlm.nih.gov/pubmed/11476484

He, J., & Giusti, M. M. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annual Review of Food Science and Technology*, *1*(1), 163–187. https://doi.org/10.1146/annurev.food.080708.100754

Kong, J. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, *64*(5), 923–933. https://doi.org/10.1016/S0031-9422(03)00438-2

Popović, D., Kocić, G., Katić, V., Jović, Z., Zarubica, A., Janković Veličković, L., Nikolić, V., Jović, A., Kundalić, B., Rakić, V., Ulrih, N. P., Skrt, M., Sokolović, D., Dinić, L., Stojanović, M., Milosavljević, A., Veličković, F., & Sokolović, D. (2019). Protective effects of anthocyanins from bilberry extract in rats exposed to nephrotoxic effects of carbon tetrachloride. *Chemico-Biological Interactions*, *304*(December 2018), 61–72. https://doi.org/10.1016/j.cbi.2019.02.022

Popović, D., Kocić, G., Katić, V., Zarubica, A., Veličković, L. J., Ničković, V. P., Jović, A., Veljković, A., Petrović, V., Rakić, V., Jović, Z., Ulrih, N. P., Sokolović, D., Stojanović, M., Stanković, M., Radenković, G., Nikolić, G. R., Lukač, A., Milosavljević, A., & Sokolović, D. (2019). Anthocyanins protect hepatocytes against CCL4-induced acute liver injury in rats by inhibiting pro-inflammatory mediators, polyamine catabolism, lipocalin-2, and excessive proliferation of kupffer cells. *Antioxidants*, *8*(10). https://doi.org/10.3390/antiox8100451

Rakić, V., & Poklar Ulrih, N. (2021). Influence of pH on color variation and stability of cyanidin and cyanidin 3-O-β-glucopyranoside in aqueous solution. *CYTA - Journal of Food*, *19*(1), 174–182. https://doi.org/10.1080/19476337.2021.1874539

Rakić, V., Rinnan, Å., Polak, T., Skrt, M., Miljković, M., & Ulrih, N. P. (2019). pH-induced structural forms of cyanidin and cyanidin 3-O-β-glucopyranoside. *Dyes and Pigments*, *165*(June 2018), 71–80. https://doi.org/10.1016/j.dyepig.2019.02.012

Rakić, V., Skrt, M., Miljković, M., Kostić, D., Sokolović, D., & Poklar-Ulrih, N. (2015). Effects of pH on the stability of cyanidin and cyanidin 3-O-β-glucopyranoside in aqueous solution. *Hemijska Industrija*, *69*(5), 511–522. https://doi.org/10.2298/HEMIND140711072R

Veljković, M., Pavlović, D. R., Stojiljković, N., Ilić, S., Jovanović, I., Poklar Ulrih, N., Rakić, V., Veličković, L., & Sokolović, D. (2017). Bilberry: Chemical Profiling, in Vitro and in Vivo Antioxidant Activity and Nephroprotective Effect against Gentamicin Toxicity in Rats. *Phytotherapy Research*, *31*(1), 115–123. https://doi.org/10.1002/ptr.5738

INFLUENCE OF DIFFERENT DENSITIES DURING SOWING ON BUCKWHEAT (*FAGOPIRUM ESCULENTUM* L.) YIELD AS A SUBSEQUENT IN AGRICULTURE

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Abstract

Buckwheat is a very old culture native to Asia. It does not show great demand for the soil, it can be grown on slightly acidic soil (up to pH 5.5), but it requires a more humid climate (with more precipitation) because it is quite sensitive to drought and high temperatures, especially during flowering and pouring grain. Sowing is done when the soil is heated to about 15 °C. Sowing can start from 01.05. to 15.05. when there is no danger of frost. The density of the assembly is about 200-250 germinating seeds per m^2. An experiment with 12 different buckwheat populations was set up on the PSS "Sombor" experimental field. Three experiments were set up with the same row spacing of 25 cm, while the row spacing on the first variant was 5.0 cm, on the second 3.75 cm and on the third variant 2.5 cm. During the experiment, the condition of the crops was monitored, as well as the yields after the harvest, which clearly showed that when using buckwheat as a side crop, we had to go for a denser sowing (2.5 cm). Due to relatively low yields (about 2 t/ha), the production of buckwheat as the main crop in Vojvodina is less profitable. Buckwheat is a very competitive plant with weeds due to its rapid germination and strong initial growth, so it quickly over shadows the surface, and thusthus sprouted weeds. That is why it is good to sow it as a functional food, and if necessary for green manure.

Keywords: *agriculture, subsequent, research, experiment, population.*

Introduction

Buckwheat is a culture native to Asia and has been grown in Europe since the 16th century (Bystricka et al, 2014). Significant species of the genus Fagopyrum are also *F.giganteum* Krotov, which was formed by crossing the tetraploid form *F. tataricum* and F.cymosum (Stoletova, 1940; Krotov, 1963.). Buckwheat is grown for its grain, which is well digested and also serves as a dietary food. Due to the similarity in the chemical composition of the grain as well as the manner of use, it is classified as a cereal, although according to its botanical affiliation it belongs to the family Polygonacea. Hulled grain contains about 80% starch, 10-15% protein, 1-2% crude fiber, 2-3% fat and 1-2% minerals, iron, phosphorus, iodine and increased content of vitamins B1 and B2. Buckwheat seeds are equated in nutritional value with straw of spring cereals, but cattle should not be given in larger quantities because they contain the alkaloid phagopyrin. Buckwheat is pollinated by bees because it blooms for a long time and has a flower that bees easily come to, but only in the morning. Under favorable conditions, 80-100 kilograms of honey can be obtained from one hectare. From 100 kilograms of buckwheat grains, 60-70 kilograms of dark flour can be obtained. Green leaves and buckwheat flowers can be used as a source of rutin, a glycoside used in medicine as a remedy for diseases such as hypertension (high blood pressure), rheumatism and diabetes, while buckwheat tea helps in recovery from radiation and chemotherapy. It has a well-developed spindle root of good absorbing strength. It penetrates the soil up to 120 centimeters. The stem is branched, hollow, about 150 centimeters high, and when ripe it acquires a reddish color. The leaves are heart-shaped and the grain is triangular in shape. The leaves are reddish in color and 5 to 10 cm long (Clayton, 1997). The weight of 1000 grains is 20-30 grams, and the hectoliter weight is 50-65 kilograms. White flowers are gathered on flower twigs that grow from the bud in the axils of the leaves. Flowering lasts a long time, about 30 days.

Photo 1. Buckwheat in bloom (photo by Vladmir Sabadoš)

Buckwheat is a crop that has low heat needs but is sensitive to both high and low temperatures (Björkman, 2000). The minimum soil temperature required for sowing and sprouting buckwheat is 4-9 °C (Varga, 1966). The optimal temperature for the growth and development of buckwheat is between 18 and 23 °C, and the optimal temperature for flowering is 17-19 °C. Inhibition of flowering occurs below 15 °C (delayed flower germination and reduction in the number of flowers). If the temperature drops below minus 1 °C, then the plants die, also below 10 °C assimilation (drying of flowers) stops, and above 25 °C fertilization stops (Guan and Adachi, 1992). The sum of the temperature required for buckwheat vegetation is from 1000 to 1200 °C. During flowering, temperature influences yield formation directly through influencing flower development and fertility, (Björkman, 2000). Buckwheat stalk is erect, hollow and branched into 5 to 10 branches, but can be without side branches, 3 to 15 mm in diameter, (Gondola and Papp, 2010). There are no big demands on the land. Due to relatively low yields (about 2 t / ha), the production of buckwheat as the main crop in the area of Vojvodina is less profitable, so in our country it is grown almost exclusively as a side crop after removing barley and winter fodder plants.

Material and Methods

For the area of Vojvodina Autonomous Province in Serbia, post-sowing of buckwheat is recommended, where solid yields are achieved. An experiment with 12 different buckwheat populations was set up on the Agriculture Extension Service "Sombor" experimental field. Three experiments were set up with the same row spacing of 25 cm, while the row spacing on the first variant was 5.0 cm, on the second 3.75 cm and on the third variant 2.5 cm. About 80 kg / ha of seeds are needed for sowing buckwheat in the earlier sowing period, while 80-100 kg / ha of seeds are needed for late sowing. They are sown with planters in narrow (spacing between rows about 10 cm) or wide rows (spacing between rows 45 - 50 cm) to a depth of 4 - 5 cm. After sowing, the soil is rolled.

If a crust forms after sowing, and before germination, it should be destroyed occasionally by harrowing. The spacing within a row depends on the density of the assembly. Buckwheat is a very competitive plant with weeds due to its rapid germination and strong initial growth, so it quickly overshadows the surface, and thus sprouted weeds. The experiment was sown with a single-row Wintersteiger seed drill, which is used for sowing smaller experiments. In order to have good germination when measuring the seeds, we added 20% more seeds.
During the experiment, the condition of the crops was monitored, as well as the yields after the harvest, which clearly showed that when using buckwheat as a side crop, we had to go for a denser sowing (2.5 cm). Due to relatively low yields (about 2 t/ha), the production of buckwheat as the main crop in Vojvodina is less profitable.

Results and Discussion

The results of the research are presented in Table 1. The tables list all the varieties that were tested and also the row spacing with sowing densities. Since the sowing was on May 11, the harvest went successively when most of the plantations began to ripen, when two thirds of the grain took on a dark brown color, with the use of grain harvesters, with a sieve that rotates a little slower, so as not to happen yield loss. It is very important to start just then, in order to avoid seed shedding.

Table 1. Results of varieties of buckwheat in post-sowing on PSS Sombor plots

	Variety	sowing 25 x 5 cm		sowing 25 x 3.75 cm		sowing 25 x 2.5 cm	
		yield t/ha	mass 1000 grains (gr)	yield t/ha	mass 1000 grains (gr)	yield t/ha	mass 1000 grains (gr)
1	Oberon	1.30	30	1.58	30	1.90	30
2	B. Petrovac	2.58	26	2.80	26	2.45	25
3	Darja	3.25	26	2.80	26	1.92	26
4	Populacija B.T.	2.37	27	2.87	28	2.15	28
5	Novosadska plus	2.52	25	3.20	26	3.10	26
6	Češka	2.65	28	2.57	28	2.77	29
7	Bamby	1.75	26	1.55	26	1.70	25
8	Novosadska	1.32	27	1.95	25	2.10	24
9	B.Petrovac exp.2	1.95	26	1.60	25	2.20	24
10	B.Petrovac exp.3	1.65	28	1.40	27	1.87	25
11	K - 11	1.65	24	1.35	24	1.15	26
12	Bily	1.75	28	1.80	26	1.92	25
	Average	2.06	27	2.12	26	2.10	26

Statistically significant were two varieties where we can see that the variety Darija gave the highest yield of 3.25 t/ha in the variant 25 x 5 cm, while the variety Novosadska plus gave the highest yield in the variant 25 x 3.75, as well as in variants 25 x 2.5 cm at 3.10 t/ha.
Based on these data, we can conclude that different populations have different sowing densities. Some varieties, if they have more room for growth, develop a higher plant height and the number of internodes, while in other varieties the opposite is true, some varieties with increasing density

give opposite results. As for the mass of 1000 grains, we could clearly see that the sowing density in the variety Oberon has no effect on the mass of 1000 grains, while in other varieties it is different. In some varieties we have an increase in the weight of 1000 grains with increasing density, while in others we have a decrease in the amount of grain weight with decreasing density. If we generally look at the results on average, we can see that the best variant was sowing at a row spacing of 25 cm and a density of 3.75 cm. With this agrotechnics we got an average yield of 2.12 t / ha.

Conclusions

Buckwheat (*Fagopyrum esculentum*) it is a very useful plant that is used inhuman nutrition, for animal feed, as bee pasture, and its honey is of exceptional quality. Buckwheat grain yields in post-sowing are from 1.5 to 2 t/ha. Based on the results, we can conclude that buckwheat, depending on the variety, gives different yields depending on the method and spacing of sowing in a row. Some varieties prefer denser sowing, while some rarer. Sustainability of agricultural production and biodiversity of agroecosystems are significantly increasing by introducing stubble crops into the crop rotation which should be its indispensable component. The use of arable crops leads to the improvement of agricultural production due to numerous benefits they bring to plant production systems. It is very much in the system of side farming it self important proper selection of plant species that will be used as such for various purposes. As a satisfactory solution when choosing side crops is buckwheat because of its characteristics that effectively lead to numerous benefits for the agroecosystem as a siding crop. Buckwheat quickly and efficiently covers the ground thus fulfilling the first and basic function of each post-cover crop, which is soil cover. This protects the soil from the negative effects of water and wind, direct solar insolation and excessive water loss which is otherwise pronounced due to evaporation on "bare" ground. Because it has a rapid initial growth, a large amount of biomass has a positive effect on reducing weeds through direct competition with weeds plants. It enriches the soil with nutrients and is very important as grazing by bees due to the long period flowering. It also attracts numerous entomofauna and increases biodiversity.

References

Amann, C., Amberger, A. (1989.): Phosphorus efficiency of buckwheat (*Fagopyrum esculentum*). Journal of Plant Nutrition and Soil Science, 181-189.

Bavec, F., Bavec, M. (2006.): Organic production and use of alternative crops. Taylor & Francis Group, Boca Raton, London, New York. 66-78.

Shail, J. (2008.): Buckwheat cover crop handbook. Cornell University.

Björkman, T. (2009.): Information for buckwheat growers. N.Y. State Agricultural Experiment Station, Cornell Univ.USA

Björkman, T. (2010.): Buckwheat Production: Harvesting. Cornell University, Ithaca, NY.

Björkman, T., Shail., J. W. (2010.): Cover Crop Fact Sheet Series: Buckwheat. Cornell University Cooperative Extension, Ithaca, NY.

Bystricka, J., Musilova, J., Tomas, J., Vollmannova, A., Lachman, J., Kavalcova, P. (2014.): Changes of Polyphenolic Substances in the Anatomical Parts of Buckwheat, 558-568.

Zhang, Q. H., Li, S. (2001.): „Advances in tha development of functional foods from buckwheat". Food Science and Nutrition, 451-464.

THE BLACK PARLATORIA SCALE *PARLATORIA ZIZIPHI* (LUCAS, 1853) (HEMIPTERA: COCCOMPORHA: DIASPIDIDAE): BIOLOGY, CONTROL OPTIONS AND PEST MANAGEMENT APPROACH

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Abstract

The Black Parlatoria Scale (BPS) *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae) is an invasive notorious scale insect species that affects citrus crops, notably in the southern Mediterranean basin. No approach to an Integrated Pest Management (IPM) strategy has currently been adopted for BPS control in the newly invaded citrus regions (Algeria, Egypt, and Tunisia). Our research aims to elucidate the pest management rules of the BPS in citrus orchards through a comprehensive study on the pest, exploring the bio-ecological environment and the life cycle stages in relation to the available control tools. The distinct life history dynamic of the BPS and the control measures, as reported in several citrus-growing countries, were critically reviewed. Eventually, interactions between the application of agro-chemicals and the life cycle stages were also revealed. Control timing is highly crucial for the BPS, since treatment methods vary significantly for each stage (feeding, moulting, dormant, reproducing, protected, producing wax) and its biological discreteness (generation overlapping, protected forms, high survival). Appropriate treatment must be timed to coincide with BPS population peaks, targeting the first crawler generation at their settlements and precisely before migration to fruits. A life cycle stage-based control model for the BPS is established to outline the future research directions of an integrated control program in the newly invaded citrus regions. Complementary studies on ecological and biological aspects of the insect population are required in the invaded citrus areas.

Key words: *P. ziziphi*, citrus, overview, chemical control, management approach.

Introduction

The Black Parlatoria Scale (BPS) *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae) is one of the most successful invader diaspids of citrus areas and a scale insect which is difficult to control (EPPO, 2015; Garcia Morales *et al*., 2016). The species has a very limited host range, restricted to the Rutaceae species from Citrus, Murraya, Poncirus, and Severinia. *Citrus* spp. are the predominant host plants to the BPS with a preference to lemon, orange and mandarin trees, and to a lesser extent on other species often used in the ornamental trade (Balachowsky, 1953; CABI, 1964, 2007; Pellizzari and Germain, 2010). The BPS is probably native to Asia in Southern China, and has been spread around the world where citrus is grown, in more than 90 countries across four continents (Longo *et al*., 1995; Miller and Davidson, 2005; Foldi and Germain, 2018; Jendoubi and Suma, 2021). The BPS has on many occasions been the major threat to citrus crops (Citrus spp.: Rutaceae) in different citrus growing areas with an important economic impact on fruit production (Talhouk, 1975; Kosztarab, 1990;

Miller and Davidson, 2005). Nowadays, severe outbreaks of the pest are reported in the southern Mediterranean countries (e.g. Algeria, Egypt, Tunisia), reaching severe pest status and recording complicated control situations (Jendoubi and Suma, 2021).
Despite the worldwide pest position of the BPS, a few detailed and advanced studies have been published. Old and recent experiments are focused on the bio-ecological behavior of this diaspid and have provided almost inconclusive results. Until recently, the BPS population has remained misidentified and has been confused with particles on citrus fruits and *Parlatoria* species during inspections and monitoring of citrus trees (Jendoubi *et al*., 2021; Jendoubi, 2018). No Integrated Pest Management (IPM) concept has been adopted for the management of the BPS in citrus orchards. The control of the BPS population has previously been almost entirely based on insecticides applications which, in many cases, were not very efficient. Different chemical actives have been evaluated by researchers, but very few alternatives to chemicals have been studied (Ipraloran, 1971; Grafton *et al.,* 1996; Morse *et al.,* 1996; Harrathi, 2008; Mangoud, 2008).
Recently, a detailed field description of all the life cycle stages for both male and female lineage of BPS was carried out recently, as one of the primary crucial steps of an IPM program that should be completed for this invasive pest (Jendoubi *et al*., 2021). Then, the actual pest status and the spread history of the scale insect were reviewed. The spread and establishment of BPS populations in new geographical zones appears to be rapid, mainly in the Mediterranean basin. This study was considered as a starting point of references for pest management decision-makers (Jendoubi and Suma, 2021). Therefore, it was necessary to better know the fundamental biological features of the BPS, examine the relations between chemical control and developmental stages, finally establishing the impact of all parameters on the management of the BPS in the different invaded citrus areas.
In the present research, a chemical control model, including life cycle stages, is proposed for controlling populations of this diaspid within an IPM program. Basic information regarding the BPS biology/life cycle and control methods, being followed presently in the different citrus growing countries from the Mediterranean basin and South Asia were reviewed. This information was used as support to discuss the rules of a chemical control scheme adapted to the BPS.

Extreme variability in generation number between regions

In Mediterranean countries, the BPS completes several overlapping generations each year, numbering from two to five. Conversely, in the Asiatic area, the generation number is relatively higher and the overlapping generations are less accentuated (table 1). Variations in cycle duration were observed too in different countries, even if they share similar climates. In Egypt, on mandarin trees, the first spring generation starts from early March recording peaks in mid-April early May (43.17 individuals), and ends in early June or mid-July, lasting 3.5-4 months. The second autumn generation starts in early July, peaks in mid-October (104.83 individuals) and continues until December (El-Bolok *et al*., 1985; Salama *et al*., 1985; Tawfeek and Abu-Shall, 2010; ElSayed-Darwish, 2016). In southern Italy (i.e. Sicily), the complete life cycle is much shorter than in Egypt, taking 1-1.5 months under favorable conditions (Monastero, 1962). In Tunisia, which has comparable climate conditions to Sicily, Bénassy and Soria (1964) noted that a generation requires more time than in Sicily to be accomplished, specifically 75-80 days during warm periods and up to 160 days during cool periods. A generation in Taiwan requires approximately 42 days to develop from June to August and around 93 days during cooler weather (Huang *et al*., 1988; Miller and Davidson, 2005). These differences may be related to the difficulty to 3 factors 1. The segregate generations during sampling due to the permanent presence of all instars along the year, 2. The mean temperature differences and 3. The host plant susceptibilities.

All BPS stages are affected in their vertical distribution by the different microclimates resulting from the tree architecture. Sessile populations tend to form three separable groups along the heights of trees, showing an important accumulation in the central core. The highest populations densities (50.54% of first instar, 48.15% of first molt stage, 46.69% of female stages and 43.7% of male nymphs) colonize the middle stratum (Sigwalt, 1971; El-Bolok *et al*., 1985; Tawfeek, 2012; ElSayed-Darwish, 2016; HJ personal communication, 2016).

Actually, the distribution pattern of the BPS in citrus orchards was correlated with environmental conditions such as sun inclination, wind direction, and hot temperatures in the same citrus orchard. The species chooses in fact shady zones more than the sunny exposed zones of trees, looking for the most suitable sites for its protection upon settlement, development and oviposition. In fall and spring, populations were accumulated on leaves in the western parts of trees, while summer populations settled in the southern parts (Salama *et al*., 1985; Harrathi, 2008; Medjoub, 2014).

Table 1. Number of generations of *P. ziziphi*

Location	**Citrus spp.**	**Generations**	**References**
Spain	Orange	3-5	Gomez-Clemente, 1943
Egypt	Mandarin trees	2	Sweilem *et al*., 1985
Egypt	Sour orange Grapefruits Navel orange	3	El-Bolok *et al*., 1985; Salama *et al*., 1985; Tawfeek and Abu-Shall, 2010; ElSayed-Darwish, 2016
Italy (Sicily)	Orange	5	Monastero, 1962
Tunisia (Cap Bon)	Orange	3	Bénassy and Soria, 1964
Algeria	Orange	3	Boukhobza, 2015
Caucasus	Orange	2.5	Garcia Morales *et al*., 2016
Taiwan	Orange	7	Huang *et al*., 1988; Miller and Davidson, 2005

Control options limited to agro-chemicals

Currently, no approach to IPM strategy has been adopted for the BPS. The main control options available are limited to some preventive practices, and mainly to agro-chemicals (Ipraloran, 1971; Grafton *et al*., 1996; Mangoud, 2008; Jendoubi, 2018). Chemical group of organophosphates (chlorpyrifos, malathion, parathion, quinalphos, methidathion, omethoate) and pyrethroids (lambda-cyhalothrine, fenvalerate, cypermethrine) are the main insecticides applied to manage BPS infestations. In China, it is efficiently controlled with total eradication, using different agro-chemical actives as parathion, quinalphos, methidathion, omethoate or lambda-cyhalothrine and fenvalerate (Dekle, 1976; Huang *et al*., 1988). In Tunisia, the regular application of chlorpyrifos and its dimethyl homologue, chlorpyrifos-methyl, and methidathion actives, has led to an important decrease in the populations of the BPS in some citrus orchards. The methidathion efficacy could reach 91% (Jendoubi *et al*., 2008; Harrathi, 2008).

In California and different Mediterranean countries (such as Algeria, Egypt and Tunisia), many experiments have been conducted with mineral oils (Citrole, Misrona, Volk-Ete, Triona) to

reduce crawler populations during the growth season. Dekle (1976) recommended combining oils and insecticides, precisely Malathion 0.05% plus oil 0.01% in citrus orchards from Florida. In Tunisia, Harrathi (2008) demonstrated that the mineral oil Citrole was as effective as methidathion in the control of the BPS on citrus in the northeastern area. Coll and Abd-Rabou (1998) have reported Triona oil was more effective than Shecrona oil and reduced BPS populations by up to 99%. The two spray oils used did not significantly affect the activity of the parasitoid *Encarsia citrina* (Craw), the primary parasitoid of the BPS. Mangoud (2008) demonstrated that Misrona oil and the insect growth regulator buprofezin had a long-term insecticidal effect on the BPS population occurring on citrus in Egypt. Therefore, the application of paraffin oils (Triona 81%, Misrona 95%) applied with buprofezin treatments should be employed as an alternative to other synthetic insecticides used for controlling the BPS populations in citrus orchards (Mangoud, 2008). Oils must be used as a contact insecticide, which requires thorough complete coverage and repetitive applications. In Algeria, three essential oil concentrations (0.4ml, 0.2ml, 0.1ml) extracted from grapefruits leaves showed a high significant insecticide effect against the BPS crawlers on citrus trees (89-90.2% larvae mortality at 0.2ml, 0.1ml; 94.4% mortality at 0.4ml) (Belguendouz *et al*., 2014).
Accordingly, pesticides play a key role in pest management programs for the BPS and may be the only control method available in cases of large invasion or chronic infestation. The use of oil spray could be one of the most common and effective scale control options used in out-season to control BPS crawlers. Nowadays, the market has increased the legislative restrictions on the presence of insecticidal residues on fruits, making the management of this pest increasingly more complicated, especially because alternatives to chemical control are limited and natural enemies associated with the BPS are not well investigated.

Potential role of natural enemies

A number of parasites have been recorded in literature as effective in controlling the BPS (table 2) (Miller and Davidson, 2005). In Tunisia, the aphelinid wasp *E. citrina* have reduced the BPS population at different periods of the year, causing two important declines at the beginning of July and at the end of August and attacking 20-40% of individuals (Sigwalt, 1971; Jerraya, 2003). Moustafa (2012) have demonstrated a strong correlation between both parasitoids *E. citrina* and *Aphytis lingnanensis* Compere and BPS pest population in citrus trees in Egypt (r = 0.39, 0.85 in 2011; 0.45, 0.81 in 2010, respectively). Augmentative release of the aphelinid wasp *A. melinus* was used for BPS control in Egypt in five citrus growing locations where the parasitism level (91%) resulted in a significant decrease of BPS populations. This parasitoid *A. melinus* could constitute a promising biocontrol program of BPS in citrus orchards as the case of the California red scale, *Aonidiella aurantii* (Maskell) (Coccoidea: Diaspididae) in different Mediterranean countries (Abd-Rabou, 2009). As predators (table 2), Stathas et al. (2008) demonstrated that the predation of the coccidophage *C. bipustulatus* reached 34% under laboratory conditions in Greece. As acari, studies carried out on the predatory activity of the phytoseiid mite *Euseius scutalis* (Athias-Henriot) under laboratory conditions, showed a 100% survival rate and a consistent average number of consumed prey eggs by adults, 16.11 and 10.30 eggs per individual (El-Sharabasy *et al*., 2017).
Additionally, the fungus *Aschersonia placenta* (Berkeley, 1873) is recognized as an important biological control agent able to causing epizootic disease in whiteflies and scale insects in the tropics and subtropics (table 2) (Zhu *et al*., 2008). Recently, Homrahud et al. (2016) demonstrated that the pest cumulative mortality of BPS population is higher when increasing the

conidial concentration of the fungus *A. placenta*. Conidial suspension at 1 × 109 conidia/ml caused 23.73% and 27.42% mortality of BPS population 14 and 21 days post inoculation, respectively. In Cuba, El-Choubassi et al. (2001) observed up to 49% infection of the BPS by two fungi belonging to the same genus, namely *A. aleyrodis* (Webber, 1897) and *A. goldiana* (Sacc. & Ellis, 1899). Entomopathogenic fungi belonging to *Aschersonia* genus can play an important role in the mortality of the BPS and eventually be successful biocontrol agents. The natural enemies' potential for the biological control of BPS appeared to be promising in the field.

Table 2. List of auxiliaries reported on *P. ziziphi*

	BPS instars affected	Country	References
Parasitoids			
Aphytis proclia	Nymphs, adults		
Encarsia citrina (Craw.)	Nymphs, adults	Algeria, China, Egypt, Morocco, China and Hawaii	Garcia Morales *et al*., 2016
Habrolepis aspidioti Compere & Annecke	Nymphs, adults	Egypt	Abd-Rabou, 1999 Sthatas *et al*., 2008
Encarsia lounsburyi (Berlese & Paoli)	Nymphs, adults	China	Garcia Morales *et al*., 2016
Predators			
Chilocorus kuwanae	Nymphs, adults	China	Garcia Morales *et al*., 2016
Chilocorus nigrita	Nymphs, adults	USA	Garcia Morales *et al*., 2016
Chilocorus bipustulatus (Linnaeus, 1758) *C. kuwanae* (Silvestri, 1909)	Larvae	China	Huang *et al*., 1988; Cruz and Segarra, 1991
Cybocephalus nipponicus	Nymphs, adults	China	Garcia Morales *et al*., 2016
Halmus chalybeus	Nymphs, adults	USA	Garcia Morales *et al*., 2016
Leptodiplosis aonididiellae	Nymphs, adults	Morocco	Garcia Morales *et al*., 2016
Rhyzobius lophanthae	Nymphs, adults	USA	Garcia Morales *et al*., 2016
Telsimia emarginata	Nymphs, adults	China	Garcia Morales *et al*., 2016
Episyrphus balteatus (De Geer, 1776)	BPS colonies	Algeria	Biche, 2012
Pathogens			
Nectria aurantiicola	Nymphs, adults	Taiwan	Garcia Morales *et al*., 2016
Nectria diploa	Nymphs, adults	Taiwan	Garcia Morales *et al*.,

			2016
Nectria flammea	Nymphs, adults	Java	Garcia Morales *et al*., 2016
Podonectria aurantii	Nymphs, adults	Brazil, Taiwan	Garcia Morales *et al*., 2016
Podonectria cocciola (Ellis & Everhart.) Petch,	Nymphs, adults	Japan, Taiwan	Abd-Rabou, 1999 Yen and Tsai, 1969
Pseudomicrocera henningsii (Koord.) Petch *Sphaerostilbe aurantiicola* (B. & Br.) Petch	Nymphs, adults	Taiwan	Yen and Tsai, 1969
Fusarium coccophilum (Desm.) Wr. & Rg., *Nectria flammea* (Tul.) Dingley (teleomorph of *F. coccophilum*), *Tetracrium coccicolum* Hohnell, *Podonectria coccicola* (Ellis & Everhart) Petch *Myriangium duriaei* Mont. & Berk	No data	Brazil Taiuva	Andréia *et al*., (1999)
A. aleyrodis (Webber, 1897) *A. goldiana* (Sacc. & Ellis, 1899)	No data	Cuba	El-Choubassi *et al*., (2001)

Control rules in relation to BPS biological features

Actually, the BPS has developed more adaptation capacities over time to survive different conditions, from tropical to temperate regions, becoming lately a key pest of citrus in the southern Mediterranean countries (Jendoubi and Suma, 2021). In these regions, the BPS marks three important periods of activity during the growth seasons of citrus trees in autumn, spring and occasionally in summer. The lowest population levels on foliage have been recorded usually during the coldest or/and hottest months, when the BPS infests mainly leaves and moves to fruits (Miller and Davidson, 2005; Tawfeek, 2012; Bouazza and Guenaoui, 2015; Taibi *et al*., 2016; Jendoubi, 2018). Then, the BPS time control must be maintained in mid-April (Algeria, Egypt, and Tunisia) and in mid-October (Egypt, Tunisia) or in November (Algeria).

Crawlers are the most difficult instars to be detected and identified in the field due to their small size and high mobility. The crawler emergence varies depending mainly on seasonal weather conditions and location. The crawling time is usually short, lasting only a few days. Chemical treatment must be applied when the first crawlers start emerging and approximately 10 days later to control those still emerging during all the period of laying eggs. It should be applied with sufficient volume and pressure to thoroughly cover all the woody portions of the tree, because the BPS individuals are usually concealed inside the canopies of trees, which makes them

invisible to farmers and allows them to escape the chemical treatments (Salama *et al*., 1985; Tawfeek, 2012; Medjoub, 2014; EPPO, 2015; HJ personal communication, 2016).
A regular plant inspection (visual inspection, sampling) is highly recommended for BPS life cycle monitoring and control. Catching males using chromatic traps should be introduced in monitoring programs of BPS populations in the field (Jendoubi *et al*., 2016). This tool gave consistent information on male abundance and flight patterns, allowing for pest injury detection before the symptoms appeared.
BPS populations must usually be kept in check by the natural limiting factors (e.g. temperature, cultural practices, and soil fertilizers) that should be considered in control program and used in combination with insecticides. Population growth, abundance and survival of BPS are limited in fact by the extensive summer heat, drought and cold. The natural mortality can even exceed 74-80% of the population for the BPS citrus orchards from Tunisia. During the tropical summer, the global mortality of this species presents 3 peaks around the year with the summer rate being the highest one [November (62%), February (66%) and August (75%)]. In some countries with extremes climatic conditions (e.g. Egypt), an important proportion of the spring population is overwintering as dormant stages during August (Bénassy, 1975; Salama *et al*., 1985; Harrathi, 2008; Tawfeek and Abu-Shall 2010). BPS control is so needless in summer and should be delayed to the autumn or spring seasons.

Chemical control approach for the BPS: life cycle stages in making control decision

A good IPM strategy for the BPS has three components: identifying the pest, monitoring infestation and selecting the best management tactics depending on the situation.
Identification of the pest life cycle stages is critical for the BPS, since appropriate management methods may vary dramatically for each stage. Recently, Jendoubi *et al*. (2021) have provided a field guide of the life cycle male and female, so as to achieve the correct and rapid recognition of the pest at immature and adult stages. Accordingly, two sorts of life cycle stages were indeed distinguished for the BPS, depending on their susceptibility to insecticides. The feeding stages group is the most vulnerable to agro-chemicals, while the second group contains protected instars which are the dormant, moulting and reproducing stages (Jendoubi *et al*., 2021).
The newly emerged first-instar nymph is highly mobile and the main dispersal agent either passively or actively for the species. **The BPS crawler stage** is the only instar to be controlled efficiently. These instars are vulnerable to agro-chemicals due to the absence of protective scales. Systemic agro-chemicals are unnecessary to be used at this stage. Chemical spray with contact insecticides should be applied at a specific time when the crawlers are the most dominant instar of the population, appearing as white circular masses on plant surfaces. Treatment, at this stage, should be applied exactly at the white cap stage with the settlement of crawlers on the host plant and before the beginning of the moulting process. Later, once crawlers start to cement wax and the dorsum becomes heavily sclerotized, systemic insecticides are recommended and insecticides of contact are only slightly efficient. The systemic ones can provide excellent options for the BPS control, due to their chemical properties which could fight the overlapping aspect and the protective scales of the diaspid.
When young males and females are developed, the use of systemic insecticides as a foliar spray to control this stage during the growing season is obligatory. Treatment, at this stage, should also be applied with care while scales are feeding (secretion of new wax around previous armor) not moulting (blackness of armor) at the appearance of black scale armors on leaves and fruits of citrus trees.

The adult stage should be used in the monitoring system as indicators of crawler emergences. Adult female is armored and does not feed and it is mated and producing eggs. The treatment of this stage is ineffective with any agro-chemicals. The photo illustrations provided by Jendoubi *et al*. (2021) can allow growers to recognize the developmental phases of each BPS stage and should be used as decision tool for its management. As in example, is looking at the exuviae rejected on plant surfaces by males. The presence of the third exuvium indicates that the emergence of males will occur in a short time as well as the mating process. Also the presence of the yellowish-brown wax fringe at the posterior end of the female body indicates as the production and lying of eggs is starting. When, the fringe becomes dark brown the emergence of crawlers start (Jendoubi *et al*., 2021). So, with these indications, it will be easy to limit the egg hatching duration and predict the emergence time of crawlers and then to fix the proper timing of crawler sprays, considering obviously the climate conditions differences between seasons and regions.

The dormant stages should be never treated. The diapause and the moulting are two dormant stages which do not need to be treated or sprayed by insecticides, as the insects are protected and not feeding**.** Hence, during the blackness of the female scales (moult stage), until the appearing of the orange ring moult, the treatment is a misapplication and a waste. Accurately,
oil sprays are used to control dormant stages in winter, while oil penetration may not be completed as the stages are protected by their armor. A descriptive model for the control treatment rules of the BPS is set out in figure 1.

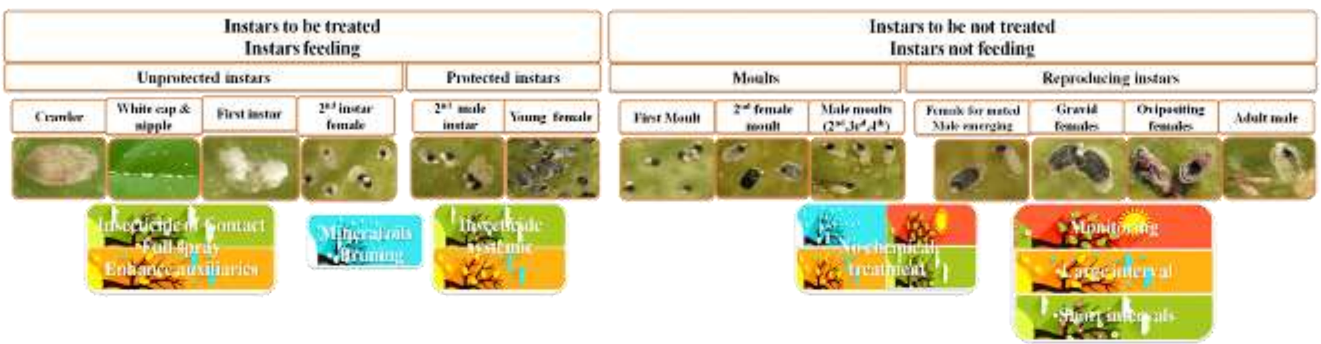

Figure 1. Control scheme of the BPS in citrus orchards

Conclusion

The BPS is a highly specialized plant-parasitic insect, to which the citrus is the main and quasi only host plant. Nonetheless, the BPS is a historic pest of citrus with a legacy of persistence to control methods and high adaptation potential to different climates. Inconsistent knowledge about this pest still exist in literature and no comprehensive approaches have yet been developed to allow for efficient monitoring and management of the BPS in many citrus-growing countries, mainly in the newly invaded areas from the southern Mediterranean basin. This paper attempts to provide valuable background information on the BPS, while paying particular attention to the potential use of all parameters together as baselines in view of a sustainable management solution for the expanding challenge that the BPS poses to the Mediterranean citrus production.

A compilation of an information management vision for the BPS is actually achieved as a primary step of an effective management program for the pest. A chemical control concept based on life cycle stages, in conjunction with other IPM components and early regular monitoring, is given for the sustainable control of the BPS in Mediterranean and African countries. Insecticides play a key role in pest management programs for the BPS. The feeding stages are the most vulnerable to pesticides, while the dormant, moult and protected stages shall not be treated as they do not feed and are armored. The reproducing females should be better used during monitoring as indicators of crawler emergence and to subsequently control timing. Some biological control agents (*A. melinus, A. placenta, E. citrina, E. scutalis* and *C. bipustulatus*) are promising in controlling BPS populations in regions where they are abundant, despite their efficacy is unclear under field conditions.

Perspectives

Currently, future research areas should be investigated, using the currently-existing management information option, to prevent further expansion of the pest in citrus-growing countries. Studies about pest interactions with citrus trees, the environmental factors affecting population growth and the selection of performing auxiliaries to enhance biological control, should be deeply investigated. For BPS bio-control, the challenge lies in finding successful combinations of entomopathogens, predators and parasitoids along with other interventions to maintain populations of the BPS below the economic threshold level.
We expect in the future to develop advanced approaches, such as bioinformatics solutions for the BPS to forecast the sessile population dynamics on citrus trees based on weather data and pest biological behavior.

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References

Abd-Rabou S. (1999). An annotated list of the Hymenopterous parasitoids of the Diaspididae (Hemiptera: coccoidea) in Egypt, with new records. Entomologia, 33, 173-177.

Abd-Rabou S. (2009). Evaluation of *Aphytis melinus* De Bach (Hymenoptera: Chalcidoidea: Aphelinidae) in citrus orchards as a biocontrol agent of black scale, *Parlatoria ziziphi* (Lucas) (Hemiptera: Coccoidea: Diaspididae) in Egypt. Journal of Biological Control, 23 (1), 37-41.

Balachowsky A. S. (1953). Les cochenilles de France d'Europe, du Nord de l'Afrique, et du bassin Méditerranéen VII-Monographie des Coccoidea; Diaspidinae IV, *Odonaspidini*, *Parlatorini*. Actualités Scientifiques et Industrielles Entomologie Appliquée, 1202, 725-929.

Belguendouz R., Harchouche C., Biche M., Houmani Z. (2014). Evaluation de l'effet insecticide de l'huile essentielle de *Citrus aurantium* (Rutacées) sur la dynamique des populations de *Parlatoria ziziphi* Lucas. (Homoptera, Diaspididae). Revue of Arid Regions, 35 (3), 1981-1991.

Bénassy C. (1975). Les cochenilles des agrumes dans le bassin méditerranéen. Annals of the Agronomic National Institute of Algeria, 4, 118-137.

Bénassy C., Soria F. (1964). Observations écologiques sur les cochenilles diaspines nuisibles aux agrumes en Tunisie. Annals of INRAT, 37, 193-222.

Biche M. (2012). Les principaux insectes ravageurs des agrumes en Algérie et leurs ennemis naturels. National Institut of crop protection of Alger and Regional Integrated Pest Management Program in the Near East-FAO, Alger, Algeria.

Bouazza I., Guenaoui Y. (2015). Density Infestations of *Parlatoria ziziphii* (Lucas) (Hemiptera: Diaspididae) on Citrus in the Northwestern Algeria. Proceedings of VI International Scientific Agricultural Symposium Agrosym, Dusan K., 15-18 October 2015, Jahorina, Bosnia, pp. 793-798.

Boukhobza L. (2015). L'effet des sels minéraux du sol sur l'écologie de *Parlatoria ziziphi* (Homoptera: Diaspididae) dans un verger d'oranger à Rouiba. Master thesis in Sciences of Nature and Life, High National institute of Agronomy El-Harrach, Algeria, 159 pp.

CABI Center for Agriculture and Bioscience International (1964). Distribution Maps of Plant Pests *Parlatoria ziziphus* (Lucas), Map 186. CAB International Publisher, Wallingford, United Kingdom.

CABI Center for Agriculture and Bioscience International (2007). Crop Protection Compendium. *Parlatoria ziziphi* (Lucas, 1853), black parlatoria scale. CAB International Publisher, Wallingford, United Kingdom.

Coll M., Abd-Rabou S.H. (1998). Effect of oil emulsion sprays on parasitoids of the black parlatoria, *Parlatoria ziziphi*, in grapefruit. Biocontrol, 43, 29–37.

Cruz C., Segarra A. (1991). Recent biological control experiences in Puerto Rico. Caribbean Meetings on Biological Control, Guadeloupe, France.

Dekle G. W. (1976). Black parlatoria scale *Parlatoria ziziphi* (Lucas) (Homoptera: Diaspididae). Entomology circular, 171, 1-2.

Elsayed-Darwish A. A. F. (2016). Some ecological and behavioral aspects of the black parlatoria, *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae) and its parasites on mandarin trees. International Journal of Entomology Research, 1 (4), 33-38.

El-Bolok M. M., Sweilem S. M., Abdel-Aleem R. Y. (1985). Seasonal variation in the population of *Parlatoria zizyphus* (Lucas) at Giza region. Entomological Society Bulletin of Egypt, 65: 281-288.

El-Choubassi, W., Iparraguirre-Cru, M.A., Sisne-Lui, M.L., Grillo-Ravelo H. (2001). Incidencia del genero Aschersonia sobre la población de *Parlatoria ziziphi* (Lucas) (Homoptera: Diaspididae) en naranjo Valencia (Citrus sinensis) de la provincia de Ciego de Avila Centro Agricola, 28, 42-45.

El-Sharabasy H.M., Sholla S.M.E., Helmy S.M.Y. (2017). A study on the Biology of Predatory Mite, *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) Feeding on Black Scale Insect, *Parlatoria ziziphi* (Lucas) (Homoptera: Diaspididae). Egyptian Academic Journal of Biological Sciences, 10 (1), 71– 75.

EPPO European and Mediterranean Plant Protection Organization (2015). PQR database, www.eppo.int/.

Foldi I., Germain J. F. (2018). Checklist of the scale insects of France (Hemiptera, Coccomorpha). Entomological Society Bulletin of France, 123 (1), 7-18.

Garcia Morales M., Denno B. D., Miller D. R., Miller G. L., Ben-Dov Y., Hardy N. B. (2016). A literature-based model of scale insect biology and systematic. ScaleNet database, http://scalenet.info/catalogue/parlatoria%20ziziphi/.

Gomez-Clemente F. (1943). Cochinillas que atacan a los agrios en la región de Levante. Boletín de Patología Vegetal y Entomología Agrícola, 12, 299-328.
Grafton-Cardwell E.E., Striggow R.A., Ouyang Y. (1996). Pesticide resistance in armored scale influences citrus IPM in California. Proceeding International Society, 1, 553-555.
Harrathi A. (2008). Le pou noir de l'oranger *Parlatoria ziziphi* Lucas (Hemiptera: Diaspididae): bioécologie et essai de lutte chimique. Ingineer thesis in Agronomic Sciences-Phytiatry, National Agronomic Institute of Tunisia, University of Carthage, Tunisia, 150 pp.
Homrahud D., Sopon U., Tipvadee A. (2016). Cultivation of *Aschersonia placenta* Berkeley and Broom and its efficacy for controlling *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae). Agriculture and Natural Resources, 50, 179-185. doi.https://doi.org/10.1016/j.anres.
Huang L. L., Wang D. W., Zhang Q. B., Lei H. D., Yue B. S. (1988). Study of bionomics and control of *Parlatoria zizyphus*. Acta Phytophylactica Sinica, 15 (1), 15-21.
Ipraloran J. C. (1971). Les agrumes: techniques agricoles et productions tropicale.- Maisonneuve and the Rose, Paris, France.
Jendoubi H. (2018). The scale insect fauna of citrus in Tunisia: A critical overview. International Journal of Fauna and Biological Studies, 5 (3), 169-178.
Jendoubi H., Grissa K. L., Suma P., Russo A. (2008). Scale insect fauna (Hemiptera, Coccoidea) of citrus in Cap Bon region (Tunisia). Bulletin IOBC/wprs, 38, 87-93.
Jendoubi H., Suma P. (2021). Pest status of the black parlatoria scale *parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae). Proceedings of XII International Scientific Agriculture Symposium-Agrosym, 07-10 October 2016, Jahorina, Bosnia and Herzegovina, pp. 5 (in press).
Jendoubi H., Suma P., Russo A. (2016). Response of the parlatoria citrus scale insects (Hemiptera, coccoidea) to coloured sticky traps. Proceedings of VII International Scientific Agriculture Symposium-Agrosym, 06-09 October 2016, Lisbon, Portugal, pp. 1529-1535.
Jendoubi H., Suma P., Russo A. (2021). The Black Parlatoria Scale, *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae): a guide to field identification. Bulletin of Entomological Research, 111 (1), 39-48.
Jerraya, A. (2003) Principaux nuisibles des plantes cultivées et des denrées stockées en Afrique du Nord : Leur biologie, leurs ennemis naturels, leurs dégâts et leur contrôle. Climat Pub, Tunis, Tunisia.
Kosztarab M. (1990). Economic importance, armored scale insects as pests. In: Rosen D. (ed.) Armored scale insects: their biology, natural enemies and control, Word Crop Pests, 4B. Elsevier Science, Netherland, Amsterdam, pp. 307-311.
Longo S., Marotta S., Pellizzari G., Russo A., Tranfaglia A. (1995). An annotated list of the scale insects (Homoptera: Coccoidea) of Italy. Israel Journal of Entomology, 29, 113-130.
Lucas H. (1853). *Coccus ziziphi*.Annales de la Société Entomologique de France, 1, 28–29.
Mangoud A. A. H. (2008). Insecticidal effect of some chemical and natural control agents against the black parlatoria scale, *Parlatoria ziziphi* (Lucas) and associated parasitoids on citrus trees. Egyptian Journal of Agricultural Research, 86, 2157–2167.
Medjoub Y. (2014). Bio-écologie de la cochenille noire *Parlatoria ziziphi* (Homoptera, Diaspididae) sur les agrumes dans la station d'El Fhoul à Tlemcen. Master thesis in Agronomy, University of Abou Bekrbelkaid Tlemcen, Algeria, 73 pp.
Miller D.R., Davidson J.A. (2005). Armored Scale Insect Pests of Trees and Shrubs. Cornell University Press, New York, United States America.

Monastero S. (1962). Le cocciniglie degli agrumi in Sicilia (Mytilococcus beckii New, *Parlatoria zizyphus* Lucas, *Coccus hesperidum* L., *Pseudococcus adonidum* L., *Coccus oleae* Bern., *Ceroplastes rusci* L.). Bollettino dell'Istituto di Entomologia Agraria dell'Università Palmero, 4, 65-151.

Morse J. G., Luck R. F., Gump D. J. (1996). Citrus pest problems and their control in the Near East. Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy.

Moustafa M. (2012). Scale insects (Coccoidae: Hemiptera) infested citrus trees and their natural enemies, with a key of these pests in Egypt. Egyptian Academic Journal of biological Sciences, 5 (1), 1- 23.

Pellizzari G., Germain J. F. (2010). Scales (Hemiptera, Superfamily Coccoidea). BioRisk, 4 (1), 475-510.

Salama H. S., Abdel Salam A. L., Donia A., Megahed M. I. (1985). Studies on the population and distribution pattern of *Parlatoria ziziphus* (Lucas) in citrus orchards in Egypt. Insect Science and its Application, 6, 43-47.

Sigwalt B. (1971). Les études de démographie chez les cochenilles diaspines à l'Oranger en Tunisie. Cas particulier d'une espèce à générations chevauchantes : *Parlatoria ziziphi* Lucas, applications à trois espèces nuisibles. Annals of Zoological and Ecological Animalia, 8 (1), 5-15.

Stathas G. J., Eliopoulos P. A., Japoshvili G. (2008). A study on the biology of the diaspidid scale *Parlatoria ziziphi* (Lucas) (Hemiptera: Coccoidea: Diaspididae) in Greece. Proceedings of the XI International Symposium on Scale Insect Studies, 24-27 September 2007, Lisbon, Portugal, pp. 95-101.

Sweilem S. M., El-Bolok M. M., Abdel Aleem R. Y. (1985). Biological studies on *Parlatoria zizyphus* Lucas (Homoptera: Diaspididae). Bulletin de la Societé Entomologique d'Egypte, 65, 301-318.

Taibi A., Gacemi A., Medjdoub Y., Medjdoub I. (2016). Citrus Infestation by the Black Scale *Parlatoria ziziphi* Lucas (Homoptera: Diaspididae) in Tlemcen, Algeria. American Eurasian Journal of Agriciculture and Environmental Science, 16 (5), 928-933.

Talhouk A. M. S. (1975). Citrus pests throughout the world. Technical Monograph, 4, 1-21.

Tawfeek M. E. (2012). Distributions of armoured scale insects infesting citrus trees in different localities in Egypt. Journal of Entomology, 9, 429-434.

Tawfeek M. E., Abu-Shall A. M. H. (2010). Seasonal population dynamics of *Parlatoria ziziphus* (Lucas) (Homoptera: Diaspididae) in Menoufia Gavernorate. Alexandaria Science Exchange Journal, 31, 331-338.

Yen D. F., Tsai Y. T. (1969). Entomogenous fungi of citrus Homoptera in Taiwan. Plant Protection Bulletin, 11 (1), 1-10.

Zhu Y. P., Guan X., Pan J. R., Qiu J. Z. (2008). Optimization of nutritional requirements for mycelial growth and sporulation of entomogenous fungus Aschersonia aleyrodis Webber. Brazilian Journal of Microbiology, 39, 770-775.

PEST STATUS OF THE BLACK PARLATORIA SCALE *PARLATORIA ZIZIPHI* (LUCAS, 1853) (HEMIPTERA: COCCOMPORHA: DIASPIDIDAE)

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Abstract

The Black Parlatoria Scale (BPS) *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae) is a serious citrus pest, and has the potential for establishment and spread, and for economic consequences in the citrus growing areas. Pest status dynamic and spread history of the scale insect are reviewed. The incidence of *P. ziziphi* is usually associated with host plant, climate and control programs adopted by national authorities. BPS was not a key insect pest of citrus in many countries until the 1970s. The pest has been established on citrus as primary host in Asiatic areas (China, Iran and Taiwan) and then has spread within the crop expansion to different areas through the trade movement of citrus plant materials, showing a high adaptation to different climates mainly to tropical and Mediterranean regions. The pest is established where citrus is grown, in more than 90 countries across four continents. Nowadays, severe outbreaks of the pest are reported in the southern Mediterranean countries (Algeria, Egypt, France, Italy, Spain, and Tunisia), reaching major pest status. Pest impact is more noticeable in North Africa which has recorded complicated control status. Potential future research lines should be delineated for BPS control in the newly invaded citrus areas. An integrated management approach should be developed and accurately applied in citrus orchards, focusing on monitoring techniques and biological control. Bio-control, in conjunction with other IPM components and regular monitoring, will provide sustainable control of the BPS in the newly infested citrus regions.

Key words: *P. ziziphi, citrus, distribution, Mediterranean countries.*

Introduction

The Black Parlatoria Scale (BPS) *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomorpha: Diaspididae) is referred to a worldwide pest of citrus crop (Miller and Davidson, 2005). BPS was originally described in 1853 by Dr. Lucas Pierre Hippolyte as *Coccus ziziphi* based on female specimens collected on *Ziziphus pinnachristi* (Lam) from France. However, the original description of Lucas is so brief and indefinite. There is legitimate question to the correctness of the association of the host plant name and the common black citrus-infesting *Parlatoria*. Later, the species was described under the genus *Parlatoria* Targioni Tozzetti (1868) as *P. lucassi*. Ever since, it has received different synonyms (*Parlatoria lucasii* TargioniTozzetti (1868), *Parlatoria zizyphi*, Signoret (1869), *Parlatoria zizyphus* (Lucas) Cockerell (1900)) and is recognized today under the first name, *P. ziziphi* (Lucas, 1853; Balachowsky, 1953; Mckenzie, 1956; Bénassy, 1975; Garcia Morales *et al*., 2016). The BPS is well distinguished from other *Parlatoria* species at adult stage. The female body is membranous, elongate oval with an ear-like lateral lobe on each side of the head at the level of anterior spiracles (Balachowsky, 1953). It

is the only *Parlatoria* species having earlike lobes, which make its identification under microscope evident from other species. On citrus trees, the habit pattern is mostly aggregative, appearing as black compact colonies encrusted on leaves and fruits. The female scale is a sub-rectangular black cover (1.25-1.4 mm long; 0.6-0.75 mm wide) made of two previous instars exuviae, and ending with a narrow fringe of white wax on the posterior margin. Adult males are completely different from females. The scale cover is white, elongated, and flat, with large black terminal exuviae, and is approximately 1/3 the size of the female (Mckenzie, 1945 & 1956; Balachowsky, 1953; Miller and Davidson, 2005). The BPS is considered by different coccidologists as the only truly monophagous scale species of citrus crop (Miller and Davidson, 2005). Commonly known as the Mediterranean scale or the citrus parlatoria, BPS is a sucking insect feeding on plant cells by piercing and sucking the plant phloem sap through a set of highly adapted mouthparts. Feeding behavior affects the young shoots, the leaves and the fruits of citrus trees, leading to reduced vigor of the host plant, and resulted in development of yellow discoloration patches on leaves and fruits (Dekle, 1976; Miller and Davidson, 2005).
Pest status was described in almost citrus growing areas of the world through different periods since it was first recorded in the thirty years (Dekle, 1976; Garcia Morales *et al*., 2016). Mckenzie (1945) has noted BPS to be a very serious pest of citrus in various parts of the world. Beardsley and González (1975) considered BPS to be one of 43 major armored scale pests and one of five *Parlatoria* species known to damage citrus crop. Talhouk (1975) reported BPS has long been treated as a pest of serious economic importance on citrus crop, mainly in citrus growing countries (Algeria, Brazil, China, Egypt, Iran, Italy, France, Libya, Morocco, Nigeria, Puerto Rico, Spain, Taiwan, and Tunisia). Later Miller and Davidson (2005), in their book '*Armored Scale Insect Pests of Trees and Shrubs'*, defined this species a serious world pest of citrus crop.
The species has received several studies during the seventies mostly carried out in Asiatic and Mediterranean countries, treating chemical control options and some bio-ecological aspects of the pest (Garcia Morales *et al*., 2016). Few details were available on the economic losses caused by this insect, though it is listed in several countries as organism that may cause damages. In the last decade, an increase of studies is noted again particularly in the southern Mediterranean countries where about thirty documents were published. These include susceptibility of the citrus varieties to BPS, infestation spread and some chemical trials of new agro-chemical actives (Jendoubi *et al*., 2008; Berrabah, 2012; Tawfeek, 2012; Medjdoub, 2014; EPPO, 2015; Taibi *et al*., 2016; Ramos Portilla and Caballero, 2017; Jendoubi, 2018). Currently, large occurrences is reported for the species in Mediterranean countries such as Algeria, Egypt, Libya, and Tunisia where it commonly reaches the highest densities compared with other pest species occurring in the same citrus regions (Tena and Garcia Mari, 2011; Bouazza and Guenaoui, 2015; Taibi *et al*., 2016). The recent outbreak and the high worldwide spread of BPS should be studied in the different citrus areas.
The paper aims to revise the current distribution of BPS and incidence within time and regions to provide the actual pest status in the main citrus growing countries. A detailed revision of most published papers on the BPS is reported to clear up the pest status dynamic and difficult control situations recorded in the invaded countries.

BPS incidence on citrus fruit production

The BPS has many characteristics that make it particularly more economically damaging than other citrus diaspid species. The important longevity and reproductive rate, the accentuated overlapping of generations (two to seven generations per year); the high ability to survive and propagate in new environments, and the high adaptation potential to different climates (cool, hot and dry) make this species harmful for the host plants. The presence of the strong body armor, the immobility and its cryptic behavior provides an excellent protection from insecticides, auxiliaries and adverse climatic conditions (Bénassy and Soria, 1964; Salama *et al*., 1985; Quilici, 2003; ElSayed-Darwish, 2016). Furthermore, the most characteristic damage of BPS is the virtually irremovable masses of black scales on fruits during cleaning and packinghouse procedures. The visual aspect of citrus is affected, which cause rejection in most fresh fruit markets and may render them unusable and unfit for human consumption (Henderson, 2011; Tawfeek, 2012; EPPO, 2015).

Feeding damages

The main injury caused by the BPS to citrus plants occurs as a result of feeding activities and involves two processes: the penetration of plant tissues by the stylets and the removal of resources needed for plant growth. Depletion of plant sap leads to the reduction of plant vigor, premature shedding of the leaves and fruits. While penetrating the plant tissues with their piercing stylets, they also inject saliva, which appears to be toxic to the plant. The saliva often produces greenish discoloration and spotting on leaves and fruits. Heavy infestations of BPS on citrus cause premature senescence and leaf drop reducing yield. Fruit quality (poor taste and color) and production is decreased (Dekle, 1976; Miller and Davidson, 2005; Henderson, 2011). Nevertheless, documentation of BPS economic importance on citrus production in literature is rather poor. It is only stated that this organism may seriously damage citrus, which is not supported with any quantitative data on yield reduction or loss of income. BPS has shown particular preference to feeding on leaves, mainly on the upper face near the middle nerve; but fruits and branches are also attacked in case of high infestation. In Algeria and Tunisia, fruit infestation caused by the BPS varied from 33% to 64% and 87.9% on Thomson variety, 79% for the Portuguese variety and can reach 100% in case of high attack. On leaves, the species infests 84% and 96% of the canopy (Harrathi, 2008; Medjoub, 2014; Taibi *et al*., 2016).

Spread dynamic history

The BPS is probably native to Asia in Southern China, and has been transported around the world on nursery citrus plants. Nowadays, the scale insect occurs in Africa (22 countries), Asia (48 countries), Central and South America, Europe (14 countries), Oceania in Austria, and the West Indies. Otherwise, the pest has limited distribution in several countries as Lebanon, Saudi Arabia, Malaysia, Singapore, Guam, Peru and Venezuela or locally spread to some counties within a country. In the Western Hemisphere in Northern Mariana Islands, Argentina and Bermuda, it has not been recorded since 1938 (CABI, 1964; Bénassy, 1975; Talhouk, 1975; Longo *et al*., 1995; Claps *et al*., 2001; Jendoubi *et al*., 2008; Pellizzari, 2010; Tena and Garcia Mari, 2011; EPPO, 2015; Garcia Morales *et al*., 2016**;** Foldi and Germain, 2018). Recently, Ramos Portilla and Caballero (2017) reported a new introduction of the BPS on *Citrus* spp. to Colombia, since Blackburn and Miller (1984) mentioned its occurrence in America without indicating the host plant.

The BPS has become the major threat of citrus crop at different times in several countries. In 1930s, BPS had been recorded on citrus mainly in Asia, (Malaya, Philippines, Burma, India, and Thailand), Europe and other tropical and sub-tropical countries (Clausen, 1933; Ferris, 1937).
In the last seventy years, the BPS has further spread to many citrus growing countries mainly to those from Asia (China). BPS was not previously reported as a pest of major importance anywhere in the world, except in china where regular control was required. An important economic impact on citrus fruit production was observed in this country, despite all efforts conducted to limit the BPS population growth control. It was qualified as the most difficult species to control (Talhouk, 1975; Huang *et al*., 1988). Otherwise, the BPS was considered as a new pest infesting citrus trees or as a secondary citrus pest. Talhouk (1975) in his chart of the geographic distribution and economic importance of mites and insects gave more precisions about the pest status of BPS in different countries during this period. In Algeria, Tunisia, Morocco and south east of Asia, the species was present at low densities that occasionally require control measures. In Tunisia, BPS was recorded to be one of the main diaspid attacking citrus, with two other species *Chrysomphalus dictyospermi* (Morgan, 1889), and *Lepidosaphes beckii* (Newman, 1869) (Sigwalt, 1971). No studies were probably reported in Algeria or Morocco during this period. Nevertheless, in Spain, Italy, Greece, Israel, Egypt, India, and South Africa, the species was present but of little economic importance (Ipraloran, 1971; Talhouk, 1975; Dekle, 1976; Amin and Salem, 1978).
In the ninety years, after being established in Asiatic citrus areas, the BPS became a serious world pest reaching the highest densities in several countries from America, Africa, to Europe. At that time, serious measures of eradication were successfully taken in Europe and America (Florida, France, Italy, Palestine, and Spain) and in some zones in China. BPS was controlled efficiently in China, using many pesticides such as chlorpyrifos, methidathion, quinalphos, lambda-cyhalothrine, fenvalerate or cypermethrine (Huang *et al*., 1988). In Florida, the use of oil sprays combined with agro-chemicals (malathion, dimethoate or parathion), was highly recommended as a competent control solution, having reduced BPS populations under economic level (Dekle, 1976).
In 2010, the pest moved to the Mediterranean basin and it was called since that time a Mediterranean citrus pest. The infestation has been rapidly spreading northward and eastward and has remained limited to this area until now. In the recent years, the pest has persisted in the south Mediterranean basin in some African countries such as Egypt, Tunisia, and Algeria.

Current pest status in the southern Mediterranean countries

In Egypt, the insect was observed for the first time in 1937 on mandarin trees in Alexandria and near Cairo and since, the insect is spreading in citrus regions, reaching more and more high densities in citrus orchards (Hosny, 1943; Amin and Salem, 1978). In the 1980s, many authors carried out intensive studies on the bio-ecology of the pest as being a major pest of citrus orchards and causing serious damages in the country. The BPS widespread in Egypt has coincided with the citrus cultivation expansion period. Furthermore, the favorable climatic conditions have greatly contributed to the insect progress and its establishment. A regular chemical control was applied on BPS populations during the eighties in order to reduce population level below the economic threshold (El-Bolok *et al*., 1985; Salama *et al*., 1985; Sweilem *et al*., 1985). In the years since 1998, BPS populations emerged again in Egypt in commercial citrus orchards. Studies on BPS increased in the 2010s, treating the seasonal population dynamic, behavior of the pest on the host plant, and associated parasitoids in order to develop a control strategy for BPS in citrus orchards. BPS presents now localized distribution, becoming the most dominant species on citrus in Shebin El-Kom, Menoufia, and Qalubia. Infestation level varies between high to moderate (Tawfeek and Abu-Shall, 2010; Tawfeek, 2012; ElSayed-Darwish, 2016). All authors are actually increasing research on the necessity to apply integrated pest management strategies against BPS to limit the spread of this pest in citrus areas.

In Tunisia, the pest has persisted since 1938 in citrus orchards and is surviving the environmental conditions of the country. The scale insects injuring citrus trees have historically been overlooked by farmers and authorities in the country unless they become destructive pests, and difficult to control (Pagliano, 1938). In 2007, faunistic observations carried out on scale insects in the most infested citrus groves of Tunisia revealed the occurrence of BPS at high densities, having invaded a large part of northern citrus areas. Additionally, it was reported that the Tunisian citrus groves host a large community of exotic scale insects and BPS is classified in the group of notorious citrus pests (Harrathi, 2008; Jendoubi *et al*., 2008). Since ten years ago, farmers have been applying intensive chemical control programs using systemic agrochemicals but many foci of BPS and other species are still increasing. According to observations made with farmers during recent surveys, chemical treatment is frequently applied by field men late in summer in citrus groves at the appearance of black armors on leaves or fruits. Some other farmers prefer to not control this group of pests and are just cleaning fruits after harvest (Jendoubi, 2018). The chemical treatments applied in Tunisia have failed due to two main causes. The pesticide has not been applied at the correct time or the pest may have been in a life cycle stage or in a location where it was not susceptible to pesticides. Hence, populations that are present in concealed areas may be part of new infestations that developed long after the chemical was applied; that abundance and the high occurrences of BPS occurred in several commercial citrus regions of Tunisia.

In Algeria, the situation is noticeable. The BPS was ignored for a long time by farmers until they caused considerable economic damage. No studies have been reported during the eighties or in recent times. Indeed, the number of researches has increased during the last ten years, studying the ecological features of the pest, infestation characteristics, interaction with the host plant (Telemcen, Blida, Mitidja, Rouiba), but not yet the control programs (Medjoub, 2014; Belaribi and Halladj, 2015; Boukhobza, 2015; Taibi *et al*., 2016). BPS is one of the main *Parlatoria* species occurring in large number in the northern citrus Algerian regions. The scale insect commonly reaches the highest densities compared with other species occurring in the same orchard and trees. The highest infestation level on fruits reaches nearly 88% on Thomson and 79% on Portuguese citrus varieties, and can reach 100% in cases of high attack. This species is abundant in the upper surface of leaves, affecting the southern and central part of trees mostly (Medjoub, 2014; Belaribi and Halladj, 2015; Boukhobza, 2015; Taibi *et al*., 2016). These results are imprecise and incomplete to achieve an integrated control program for BPS. Further surveys and applied studies on BPS and other scale insect species present in the citrus area from Algeria are needed.

BPS establishment in the invaded citrus areas and actual management tactics

Actually, the spread of BPS native to Asia throughout the world is highly correlated primarily to the host plant distribution and the climatic conditions are secondary. Four factors could be included in the large spread of BPS in different citrus areas: the misidentification of the pest in the field with similar species at nymphal stages, the poor quarantine measures, and the limited control tools available for the BPS and their inaccurate use, and its associated distinctive bio-morphological features**.**

The probability of entry in new geographical zones and the probability of establishment for BPS populations in all invaded countries (previously discussed) are both moderate to high. However, the spread after establishment appears to be rapid, mainly in the Mediterranean basin. The BPS outbreak at high occurrences and large numbers is noticeable in Egypt, Tunisia, and Algeria. Attacks of this pest in Tunisia and Algeria are more notable than in Egypt. The particular geographic position of Tunisia and Algeria, encompassing high mountains and connection to the Mediterranean Sea has allowed the BPS populations growth and numerous exotic scale insect species in citrus orchards.

Control measures are needed to maintain existing pest populations of BPS at acceptable levels in northern African countries, to avoid the potential spread in local areas that were not previously infested, and to limit serious crop damage that have been noted previously in Asiatic or American states. Descriptions of control measures are outside the scope of this paper but they are commented on briefly. Most of control programs designed to combat the BPS have historically relied heavily on the use of broad-spectrum insecticides that have been ineffective in some cases (Rosen, 1990; Jendoubi *et al*., 2021). Nonetheless, the market nowadays has increased the legislative restrictions on the presence of insecticidal residues on fruits, making the management of this pest increasingly more complicated, especially because alternatives to chemical control are limited and associated natural enemies to the BPS are rare (Garcia Morales *et al*., 2016; HJ personnel observation, 2016).

Actually, BPS can be effectively controlled by a range of insecticides (Organophosphates, Pyrethroids, Ketoenols (spirotetramat)) combined with oil sprays and a few number of biological control agents (the parasitoids *Encarsia citrina* (Craw) and *Aphytis melinus* De Bach and the fungus *Aschersonia placenta* (Berkeley, 1873)) that have been identified in the current area of distribution. Augmentative releases of the aphelinid wasp *A. melinus* used for BPS control in Egypt resulted in a significant decrease of BPS populations; the parasitism level was 91%. The aphelinid wasp *E. citrina* marks a parasitism rate of 20-40% causing two declines of the population at the beginning of July and the end of August. Conidial suspension of *A. placenta* at 1×109 conidia/ml caused 23.73% and 27.42% mortality of BPS population 14 and 21 days post inoculation, respectively (Dekle, 1976; Huang *et al*., 1988; Harrathi, 2008; Abd-Rabou, 2009; Homrahud *et al*., 2016; Jendoubi, 2018). The natural enemies' potential for the biological control of BPS appeared to be promising in the field. Authorities and farmers have to select specifically the control strategy goal (prevention, suppression, eradication) and give to pesticide applicators the best strategy and tactics in controlling BPS populations, with the least risk to the environment.

In Tunisia, it is compulsory to manage and pick up the monitoring techniques in the field at the farmer level in order to make the treatment periods appropriate and increase the efficacy of agro-chemicals products. In Algeria, the incidence of BPS is significant in commercial citrus orchards, justifying a regular application of a rational chemical control program to keep the pest population below the economic injury level. Applied research studies should be firstly focusing on the population dynamic of the species in Algerian citrus orchards and their interactions with environment and auxiliaries in order to determine the population peaks and the appropriate treatment periods. While in Egypt, the situation is better kept under control, infestation areas are reduced with time; research areas which are in need of more work in Egypt are the biological control and integrated control strategy.

Conclusion

BPS is clearly one of the most successful invader diaspid in new citrus areas, established in 92 countries, where citrus is grown. The species has reached a large distribution in countries from Africa and Asia, showing a high adaptation potential to different climates mainly to tropical and Mediterranean climates. BPS was not a key insect pest of citrus in many countries until the 1970s. The pest has been established on citrus as primary host in Asiatic areas (China, Iran and Taiwan) and then has spread within the crop expansion to different areas through the trade movement of citrus plant materials. It has been introduced and extended into most Mediterranean countries (Algeria, Egypt, France, Italy, Portugal, Spain and Tunisia) in Europe and then has been rapidly spreading northward and eastward.

Populations have been now established in the southern Mediterranean citrus areas, reaching major pest status. BPS is currently widespread within northern African countries in Algeria, Egypt, and Tunisia where control status presents several difficulties. Inconsistent data about this pest still exist in the literature and no contribution to Integrated Pest management strategy (IPM) has been developed in the new invaded citrus growing countries by scientists or growers. Here, a starting point of references is given to researchers and pest management decision-makers. Potential future research lines should be delineated for BPS in the infested areas. More directed works in these regions should be focused on field studies adapted to actual citrus crop conditions (seasonal dynamic in citrus orchards, insect resistance) and advanced control tools (monitoring tools, biocontrol, IPM approaches, bio-models based on weather data and pest's life cycle).

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References

Abd-Rabou S. (2009). Evaluation of *Aphytis melinus* De Bach (Hymenoptera: Chalcidoidea: Aphelinidae) in citrus orchards as a biocontrol agent of black scale, *Parlatoria ziziphi* (Lucas) (Hemiptera: Coccoidea: Diaspididae) in Egypt. Journal of Biological Control, 23 (1), 37-41.

Amin A. H., Salem Y. S. (1978). Population studies on scale insect species *Parlatoria ziziphus*, a new pest of citrus trees in Egypt (Homoptera, Coccoidea, Diaspididae). Proceedings of IV Conference of Pest Control Part I, Academy of Scientific Research and Technology and National Research Center, Cairo, Egypt, pp. 40-48.

Balachowsky A. S. (1953). Les cochenilles de France d'Europe, du Nord de l'Afrique, et du bassin Méditerranéen VII-Monographie des Coccoidea; Diaspidinae IV, *Odonaspidini*, *Parlatorini*. Actualités Scientifiques et Industrielles Entomologie Appliquée, 1202, 725-929.

Beardsley J. W., Gonzalez R. H. (1975). The biology and ecology of armored scales. Annual Review Entomology, 20, 47-73.

Belaribi F., Halladj M. (2015). Étude éco-éthologique des cochenilles des agrumes dans un verger d'oranger à la wilaya de Skikda. Master thesis in Sciences of Nature and Life, Faculty of Sciences of Nature and Life and Sciences of earth and Univers, University 8 Mai 1945 Guelma, Algeria, 86 pp.

Berrabah M. (2012). Contribution à l'étude de quelques ravageurs des agrumes à Tlemcen. Engineer thesis in Agronomy, University Abou Bekrbelkaid of Tlemcen, Algeria, 67 pp.
Bénassy C. (1975). Les cochenilles des agrumes dans le bassin méditerranéen. Annals of the Agronomic National Institute of Algeria, 4, 118-137.
Bénassy C., Soria F. (1964). Observations écologiques sur les cochenilles diaspines nuisibles aux agrumes en Tunisie. Annals of INRAT, 37, 193-222.
Blackburn V. L., Miller D. R. (1984). Pests not known to occur in the United States or of limited distribution, black parlatoria scale. Animal and Plant Health Inspection, 44, 81-45 and 1-13.
Bouazza I., Guenaoui Y. (2015). Density Infestations of *Parlatoria ziziphii* (Lucas) (Hemiptera: Diaspididae) on Citrus in the Northwestern Algeria. Proceedings of VI International Scientific Agricultural Symposium Agrosym, Dusan K., 15-18 October 2015, Jahorina, Bosnia, pp. 793-798.
Boukhobza L. (2015). L'effet des sels minéraux du sol sur l'écologie de *Parlatoria ziziphi* (Homoptera: Diaspididae) dans un verger d'oranger à Rouiba. Master thesis in Sciences of Nature and Life, High National institute of Agronomy El-Harrach, Algeria, 159 pp.
CABI Center for Agriculture and Bioscience International (1964). Distribution Maps of Plant Pests *Parlatoria ziziphus* (Lucas), Map 186. CAB International Publisher, Wallingford, United Kingdom.
Claps L. E., Wolff V. R. S., González R. H. (2001). Catálogo de las especies de Diaspididae (Hemiptera: Coccoidea) nativas de Argentina, Brasil y Chile. Insecta Mundi, 13, 239-256.
Clausen C. P. (1933). USDA Circular. In: Seasonal variation in the population of *Parlatoria zizyphus* (Lucas) at Giza region (El-Bolok, M. M., Sweilem, S. M., Abdel-Aleem, R. Y., Eds). Entomological Society Bulletin of Egypt, 65, 301-318.
Dekle G. W. (1976). Black parlatoria scale *Parlatoria ziziphi* (Lucas) (Homoptera: Diaspididae). Entomology circular, 171, 1-2.
Elsayed-Darwish A. A. F. (2016). Some ecological and behavioral aspects of the black parlatoria, *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae) and its parasites on mandarin trees. International Journal of Entomology Research, 1 (4), 33-38.
El-Bolok M. M., Sweilem S. M., Abdel-Aleem R. Y. (1985). Seasonal variation in the population of *Parlatoria zizyphus* (Lucas) at Giza region. Entomological Society Bulletin of Egypt, 65: 281-288.
EPPO European and Mediterranean Plant Protection Organization (2015). PQR database, www.eppo.int/.
Ferris G. F. (1937). Atlas of the scale insects of North America series IIII. In: Seasonal variation in the population of *Parlatoria zizyphus* (Lucas) at Giza region (El-Bolok, M. M., Sweilem, S. M., Abdel-Aleem, R. Y., Eds). Entomological Society Bulletin of Egypt, 65, 301-318.
Foldi I., Germain J. F. (2018). Checklist of the scale insects of France (Hemiptera, Coccomorpha). Entomological Society Bulletin of France, 123 (1), 7-18.
Garcia Morales M., Denno B. D., Miller D. R., Miller G. L., Ben-Dov Y., Hardy N. B. (2016). A literature-based model of scale insect biology and systematic. ScaleNet database, http://scalenet.info/catalogue/parlatoria%20ziziphi/.
Harrathi A. (2008). Le pou noir de l'oranger *Parlatoria ziziphi* Lucas (Hemiptera: Diaspididae): bioécologie et essai de lutte chimique. Ingineer thesis in Agronomic Sciences-Phytiatry, National Agronomic Institute of Tunisia, University of Carthage, Tunisia, 150 pp.

Henderson R. A. (2011). Diaspididae (Insecta: Hemiptera: Coccoidea). Fauna of New Zealand, 66, 1–273.
Homrahud D., Sopon U., Tipvadee A. (2016). Cultivation of *Aschersonia placenta* Berkeley and Broom and its efficacy for controlling *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae). Agriculture and Natural Resources, 50, 179-185. doi.https://doi.org/10.1016/j.anres.
Hosny M. (1943). Coccidae new to Egypt, with notes on some other species. Bulletin of Fouad premier Entomology, 23, 113-123.
Huang L. L., Wang D. W., Zhang Q. B., Lei H. D., Yue B. S. (1988). Study of bionomics and control of *Parlatoria zizyphus*. Acta Phytophylactica Sinica, 15 (1), 15-21.
Ipraloran J. C. (1971). Les agrumes: techniques agricoles et productions tropicale.- Maisonneuve and the Rose, Paris, France.
Jendoubi H. (2018). The scale insect fauna of citrus in Tunisia: A critical overview. International Journal of Fauna and Biological Studies, 5 (3), 169-178.
Jendoubi H., Grissa K. L., Suma P., Russo A. (2008). Scale insect fauna (Hemiptera, Coccoidea) of citrus in Cap Bon region (Tunisia). Bulletin IOBC/wprs, 38, 87-93.
Jendoubi H., Suma P., Russo A. (2021). The Black Parlatoria Scale, *Parlatoria ziziphi* (Lucas, 1853) (Hemiptera: Coccomporha: Diaspididae): a guide to field identification. Bulletin of Entomological Research, 111 (1), 39-48.
Longo S., Marotta S., Pellizzari G., Russo A., Tranfaglia A. (1995). An annotated list of the scale insects (Homoptera: Coccoidea) of Italy. Israel Journal of Entomology, 29, 113-130.
Lucas H. (1853). *Coccus ziziphi*.Annales de la Société Entomologique de France, 1, 28–29.
Mckenzie H.L. (1945). A revision of *Parlatoria* and closely allied genera. Microentomology, 10, 47-121.
Mckenzie H. L. (1956). The armored scale insects of California. Bulletin California Insect Survey, 5, 1-209.
Medjoub Y. (2014). Bio-écologie de la cochenille noire *Parlatoria ziziphi* (Homoptera, Diaspididae) sur les agrumes dans la station d'El Fhoul à Tlemcen. Master thesis in Agronomy, University of Abou Bekrbelkaid Tlemcen, Algeria, 73 pp.
Miller D.R., Davidson J.A. (2005). Armored Scale Insect Pests of Trees and Shrubs. Cornell University Press, New York, United States America.
Pagliano Th. (1938). Les parasites des vergers, des olivettes et des palmeraies. Central press, Tunis, Tunisia.
Pellizzari G. (2010). New Data on the Italian Scale Insect Fauna (Hemiptera, Coccoidea). Acta Phytopathologica et Entomologica Hungarica, 45 (1), 89-92.
Quilici S. (2003). Analysis of pest status risk (ARP), pest : *Parlatoria ziziphi* on citrus. CIRAD, Paris, France.
Ramos-Portilla A. A., Caballero A. (2017). Diaspididae on Citrus spp. (Rutaceae) from Colombia: New records and a taxonomic key to their identification. Revue of the National Faculty of Agronomy Medellín, 70 (2), 8139-8154.
Rosen D. (1990). Armored scale insects, their biology, natural enemies and control 4 A, World Crop Pests. Elsevier, Amsterdam, Netherlands.
Salama H. S., Abdel Salam A. L., Donia A., Megahed M. I. (1985). Studies on the population and distribution pattern of *Parlatoria ziziphus* (Lucas) in citrus orchards in Egypt. Insect Science and its Application, 6, 43-47.
Sigwalt B. (1971). Les études de démographie chez les cochenilles diaspines à l'Oranger en Tunisie. Cas particulier d'une espèce à générations chevauchantes : *Parlatoria ziziphi*

Lucas, applications à trois espèces nuisibles. Annals of Zoological and Ecological Animalia, 8 (1), 5-15.

Sweilem S. M., El-Bolok M. M., Abdel Aleem R. Y. (1985). Biological studies on *Parlatoria zizyphus* Lucas (Homoptera: Diaspididae). Bulletin de la Societé Entomologique d'Egypte, 65, 301-318.

Taibi A., Gacemi A., Medjdoub Y., Medjdoub I. (2016). Citrus Infestation by the Black Scale *Parlatoria ziziphi* Lucas (Homoptera: Diaspididae) in Tlemcen, Algeria. American Eurasian Journal of Agriciculture and Environmental Science, 16 (5), 928-933.

Talhouk A. M. S. (1975). Citrus pests throughout the world. Technical Monograph, 4, 1-21.

Tena A., Garcia Mari F. (2011). Current situation of citrus pests and diseases in the Mediterranean basin. Bulletin IOBC/wrps, 62, 365-378.

Tawfeek M. E. (2012). Distributions of armoured scale insects infesting citrus trees in different localities in Egypt. Journal of Entomology, 9, 429-434.

Tawfeek M. E., Abu-Shall A. M. H. (2010). Seasonal population dynamics of *Parlatoria ziziphus* (Lucas) (Homoptera: Diaspididae) in Menoufia Gavernorate. Alexandaria Science Exchange Journal, 31, 331-338.

IMPACT OF MACHINE LEARNING IN AGRICULTURAL RESEARCH

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Abstract

Each year, agronomists and farmers struggle with numerous questions regarding which seeds to plant, when to plant, how much input to apply to optimize yields, and when to harvest. Besides, the burden of analyzing and predicting the effects of a wide range of complex entities such as weather, disease, and varying consumer preferences is huge. The usual and yet unsolved question of food demand alarms everywhere. Although many claim that, several local crops could contribute to the global food supply, it is doubtful without further research and innovation. For decades agricultural researches, have been aiding farmers in improving agricultural systems and optimizing production. Agricultural research may include breeding improved seeds, enhanced plant protection, efficient irrigation, safe storage methods, new mechanizations, and others. In research, conducting extensive experiments to assure the credibility and quality of a product is necessary. However, in all these circumstances and research pathways, testing all possible research ideas and innovations is time-consuming and costly while the lack of sufficient and sustained funds is a key constraint in most developing countries. Hence, facilitating the diffusion of agricultural innovations and improved crop varieties in developing countries without disturbing the processes that lead to efficiency is timely and indispensable. In recent years, Machine learning techniques that can model complex inputs with complicated interactions have been widely used in agricultural activities. Thus, this article addresses and presents the importance, application, and impact of Machine learning in agricultural research, emphasizing facilitating research while reducing production costs and time.

Keywords: *Neural Network, Data, Agriculture.*

Introduction

Food consumption is growing at a higher pace than population growth, making meeting global food security demands a problem. The growing stress on food security and demand for high-quality nutritious foods, such as those derived from animal products, must be handled sustainably, without compromising environmental resources (Hobbs, 2007). Even though many people claim that several local crops could contribute extensively to the global food supply, it is doubtful that they will stand alone being adequate goods without further support from research and innovation (Calicioglu et al., 2019).

Agricultural research may be broadly defined as any research effort aiming at enhancing the yield and quality of crops and animals. For several decades agricultural research has been aiding farmers in optimizing their production to offset the ever-expanding population growth in many countries around the world through breeding programs, improved plant protection, irrigation,

storage systems, farm mechanization, efficient marketing, and better resource management (Zeigler et al., 2010).
However, in all these circumstances and research pathways, testing all possible research ideas and innovations is time-consuming and costly while the lack of sufficient and sustained funds is a key constraint in most developing countries. High-input, resource-intensive farming practices cannot provide sustainable food and agricultural production since they have resulted in huge deforestation, water scarcity, soil depletion, and high levels of greenhouse gas emissions. Innovative systems are required to maintain and enhance the natural resource base while simultaneously boosting production.
Though the lack of appropriate and long-term investments remains a key constraint Improved linkages that allow capacity building and technology flow are critical. Furthermore, many biotechnology products, such as novel crop types, are created by private firms with less commitment to public benefits. To make it easier for low-income nations to adopt agricultural biotechnologies and enhanced non-commercial staple crops without affecting the processes that lead to innovation (FAO, 2017). While there is no precise amount of agricultural research intensity, expending at least 1% of national GDP on science, technology and research is recommended. Countries in both the low-income and lower-middle-income groupings fall significantly short of this objective in the agricultural sector. Thus this article addresses and presents the importance, application, and impact of Machine learning on agricultural research with an emphasis on facilitating research while reducing production costs and time.

Materials and Methods

All around the world farms are striving to improve their efficiency to remain profitable. The constant expansion of the world population, and consequently the demand for an increasing amount of food, drives the need for efficiency (Godfray et al., 2010). To become more efficient, a farmer must make the proper decisions about when to inseminate, what to feed, and when to cure an animal, when and which seeds to plant, how much to irrigate, when to harvest — and analyze and predict the effects of a wide range of externalities such as weather, disease, changing consumer preferences among other things.
Every time, dozens of new concerns regarding optimizing agricultural production are prevalent. The majority of farmers base their technical decisions on their observations and experience. Nevertheless, acquiring experience takes time, and it is typically difficult to observe every action on a commercial farm over time along with complex variations. The application of sensors and automated decision-making has become crucial in the dairy industry, where herd sizes are constantly growing (Barkema et al., 2015).
Farmers are increasingly relying on data to obtain new insights, and they gather and analyze as much data as possible. Farms use a variety of sensors to collect various sorts of data. Motion sensors worn by cows and sensors attached to milking robots, for example, gather data on the health of cows. Because most farmers are unable to interpret raw data on their own, they rely on the functionality provided by Farm Management Information Systems (Slob et al., 2021). Farm management information systems used to be basic, but currently, some of them can handle comprehensive sensor data and provide significant decision-support capacity (Fountas et al., 2015). However, the full potential of data can only be exploited when machine learning techniques are used to assist or automate agricultural decision-making procedures.

Machine learning approaches typically involve a learning process to learn from experience or training data to do a certain task. Data employed in Machine learning contains a set of examples. Generally, an individual example is described by a set of features known as variables which may be nominal, binary, ordinal, or numeric. The performance of the ML model in a particular work is measured by various statistical and mathematical models. After the learning process, the trained model can be used to classify, predict, or cluster new data by using the skill gained through the training process (Liakos et al., 2018).

Fig 1. Typical Machine Learning Technique

Results and Discussions

Agronomy considers data analytics and machine learning to increase agricultural yields while reducing farmers' difficult work (Kim et al., 2014). Precision agriculture, also known as digital farming, is a technical method for developing high-quality agricultural output that makes use of contemporary data technology and expertise (Akhter and Ahmad, 2021).

In the agrarian sector, data analytics and machine learning approaches are playing an increasingly significant role in addressing the growing problems posed by weather and climatic variations such as temperature, rain, humidity, and other factors that negatively affect crop productivity. For a strong and expanding output, it is important to improve the accuracy of data analysis. The capacity to evaluate huge amounts of data can aid an organization in dealing with significant information that can have an impact on its operations (Russom et al., 2011).

Various traditional methods like manual detection of crop diseases and pests, calculations based on statistics to estimate the quantity and predict the production and loss of crops were generally burdensome before the advent of technology, these methods result in an error due to inspectors' lack of experience (Rumpf et al., 2010).

In the process of cultivation environmental features have the greatest impact on agricultural productivity. Crop growth and development are influenced by weather, which results in significant intra-seasonal yield fluctuation. Planting, fertilizer application, irrigation, tillage, and other crop agronomic management techniques can be employed to compensate for yield losses caused by weather. As a result, yield forecasting is a useful tool for maximizing crop output and assessing crop-area insurance contracts.

In agriculture, estimating yield performance for crop products is a major topic. In breeding programs, since it would take too long and be too expensive testing all likely hybrids generated by crossing two parents is not feasible. In breeding, hybrid performance is assessed through costly and time-consuming yield tests (Lanza et al., 1997).

Consequently, this undoubtedly indicates that there is a high demand for advanced techniques to forecast hybrid performance. A study that applied advanced machine learning approaches to develop a yield estimate model for corn hybrids found that employing machine learning models significantly reduces costs by reducing the number of hybrid breeding attempts. Moreover, results from field experiments are no longer required because results from the model can be provided in seconds. The entire breeding process is no longer time-intensive (Sarijaloo et al., 2021).

According to (Chlingaryan et al., 2018), fast advancements in sensing technology and machine learning approaches will create cost-effective and comprehensive solutions for better crop and environmental status assessment and decision-making. One of the goals of precision agriculture is to reduce the amount of herbicides used in the fields by employing site-specific weed management technologies. A Bayesian framework-based automatic decision making method for detecting weeds in maize crops was also shown to save cost and minimize pollution (Tellaeche et al., 2008).

Foughali et al. (2018) presented a unique decision support system based on sensor networks for the prevention of late blight. The model was found to be effective in combating potato late blight. The technology was able to calculate the proper amount of fungicide to be used. For the prediction of late blight, the forecast model incorporated weather-related data from weather stations as well as historical data. For the farmers, the method proved very efficient and cost-effective.

Conclusion

Because it feeds the whole population agriculture is essential to a country's economy. It links and interacts with all of the country's major enterprises in this way. In the majority of countries, agriculture is the primary source of employment. Agricultural research has profited from technology improvements, especially when it comes to combining industrial innovations into a sustainable agricultural production system. Technology has turned agriculture into a profitable business by revolutionizing every farming operation.

Contemporary application of machine learning algorithms such as evolutionary algorithms, neural networks, and others in agricultural systems have provided solutions to respective decision modeling issues. Hence it can be inferred that the adaptability, promotion, and lower costs of machine learning can greatly support in evaluating the complex relationship between the input and output of agricultural systems using analytical techniques that are characterized by non-linearity, time-variable characteristics, and a large number of unknown factors.

References

Akhter R., Ahmad S. (2021). Precision Agriculture using IoT Data Analytics and Machine Learning. Journal of King Saud University-Computer and Information Sciences. ISSN 1319-1578.

Barkema W., Keyserlingk M., Kastelic J., Lam T., Luby C., Roy J, LeBlanc S., Keefe G., Kelton D. (2015). Invited review: changes in the dairy industry affecting dairy cattle health and welfare. J. Dairy Sci. 98, 7426–7445.

Calicioglu O., Flammini A, Bracco S., Bellù L., Sims R. (2019). The Future Challenges of Food and Agriculture: An Integrated Analysis of Trends and Solutions. Sustainability. 2019, 11, 222.

Chlingaryan A., Sukkarieh S., Whelan B. (2018). Machine learning approaches for crop yield prediction and nitrogen status estimation in precision agriculture: A review. Comput. Electron. Agric. 151, 61–69.

FAO., (2017). The future of Food and agriculture: Trends and challenges. Rome.

Foughali K., Fathallah K., Frihida A. (2018). Using cloud iot for disease prevention in precision agriculture. Procedia Comput. Sci. 130, 575–582.

Fountas S., Carli G., Sørensen C., Tsiropoulos Z., Cavalaris C., Vatsanidou A., Liakos B., Canavari M., Wiebensohn J., Tisserye B. (2015). Farm management information systems: current situation and future perspectives. Comput. Electron. Agric. 115, 40–50.

Godfray H., Beddington R., Crute I., Haddad L., Lawrence D., Muir, J., Pretty J., Robinson S., Thomas S., Toulmin C. (2010). Food security: the challenge of feeding 9 billion people. Science 327, 812–818.

Hobbs P., (2007). Conservation agriculture: What is it and why is it important for future sustainable food production. J. Agric. Sci. 145, 127–137.

Kim D., Yang Y., Kang S., Kim K. (2014). On the design of beacon based wireless sensor network for agricultural emergency monitoring systems. Comput. Stand. Interfaces 36 (2), 288–299.

Lanza L., De Souza C., Ottoboni L., Vieira M., De Souza A. (1997). Genetic distance of Inbred lines and prediction of maize single-cross performance using rapd markers. Theor. Appl. Genet. 94, 1023–1030.

Liakos K., Busato P., Moshou D., Pearson S., Bochtis D. (2018). Machine Learning in Agriculture: A Review. Sensors. 18, 2674.

Rumpf T., Mahlein A., Steiner U., Oerke E., Dehne H., PlümernL. (2010). Early detection and classification of plant diseases with support vector machines based on hyperspectral reflectance. Comput. Electron. Agric. 74 (1), 91–99.

Russom P. (2011). Big Data Analytics, TDWI best practices report, The Data Warehousing Institute (TDWI) Research.

Sarijaloo F, Porta M, Taslimi B, Pardalos P. (2021).Yield performance estimation of corn hybrids using machine learning algorithms, Artificial Intelligence in Agriculture,Volume 5,Pages 82-89, ISSN 2589-7217.

Slob N, Catal C, Kassahun A. (2021). Application of machine learning to improve dairy farm management: A systematic literature review. Preventive Veterinary Medicine. 187.105237.

Tellaeche A., BurgosArtizzu, X., Pajares G., Ribeiro A. (2007). A vision-based classifier in precision agriculture combining bayes and support vector machines. In: IEEE International Symposium on Intelligent Signal Processing, pp. 1–6.

Zeigler R., Mohanty S. (2010). Support for international agricultural research: current status and future challenges, New Biotechnology. Volume 27, Issue 5, pp. 565-572.

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF MINT PLANT AGAINST SEED-BORNE BACTERIAL DISEASE AGENTS

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Abstract

Plant diseases caused by seed-borne bacterial pathogens place major constraints on vegetable crop production in Turkey and cause significant annual losses on a global scale. The aim of the present study was to investigate chemical composition and antibacterial activity of essential oil obtained from grapefruit fragrant Mint (*Mentha* x *piperita* f.sp. *officinalis* var. 'Aura') plant. Chemical composition of the essential oil was identified by Gas Chromatography coupled with Mass Spectrometer detector (GC-MS). According to the result of GC-MS analysis, 39 components were identified, accounting for 99.94% of the whole essential oil. The main components were linalool (57.86%), linalyl acetate (29.76%), α-terpineol (%3.69) and geranyl acetate (3.39%)). Antibacterial activity of essential oil was determined against five different economically important Gram-negative seed-borne plant bacterial disease agents such as bean halo blight disease agent *Pseudomonas syringae* pv. *phaseolicola*, tomato bacterial speck disease agent *Pseudomonas syringae* pv. *tomato*, tomato pith necrosis disease agents *Pseudomonas cichorii*, *Pseudomonas corrugata* and potato soft rot disease agent *Pectobacterium carotovorum* subsp. *carotovorum* by using paper disc diffusion assay. Based on inhibition zone diameter values, essential oils showed the highest antibacterial activities against *P. carotovorum* subsp. *carotovorum* (11.3 mm), followed by *P. syringae* pv. *phaseolicola* (10.7 mm), *P. cichorii* and *P. syringae* pv. *tomato* (8.7 mm). Essential oil did not displayed antibacterial activity against *P. corrugata.* Based on our results, the essential oil of mint plant collected from Hatay province has a potential to be applied as seed disinfectant against important seed-borne plant bacterial disease agents.

Keywords: *Antibacterial, Seed-borne, Essential oil, Mentha* x *piperita, Mint.*

Introduction

Plant diseases caused by certain plant bacterial pathogens are major constraints and cause huge yield losses in agriculture on a global scale. Depending on the infection stage, crop-pathogen interaction and environmental conditions, annual crop yield loss of agronomically important crops caused by certain bacterial pathogens may reach up to 5-50% (Sundin *et al.*, 2014). Currently, management bacterial diseases is mainly achieved by using cultural (such as use of clean certified seeds) or chemical treatment. Chemical control of disease relies upon the use of antibiotics (such as streptomycin) in USA or copper compounds in rest of the world and such control methods prevent bacterial multiplication but not always adequate control of seed-borne inoculum. Unfortunately, frequent use of antibiotics has led to the selection of resistant bacterial population against antibiotics. The high cost of pesticides, development of pesticides/antibiotic resistant pathogen isolates, governmental restriction on the use of antibiotics in European countries, including Turkey, and the interest of environmental consideration raise the need to find alternative control methods. Plant extracts and essential oils from several medicinal plant have been reported to possess antimicrobial activity against a wide range of fungal and bacterial plant pathogens (Burt, 2004). The biological activities of these plant oils have also been exploited in ethno-pharmacological and ethno-veterinary studies for the control of diseases and pests of humans and livestock. *Mentha* spp. (commonly known as mint) belong to the Lamiaceae family. The economic importance of *Mentha* spp. is evident from the fact that their essential oils and dried and fresh plant material are in daily use in many purposes such as confectionary, beverages, bakery, cosmetics, pharmaceuticals, and pesticides (Shaikh *et al.*, 2014). The genus Mentha, consists of about 25 species. Native from the temperate areas of the world is common in Eurasia, North America, southern Africa, and Australia, mints are widely distributed throughout. *Mentha* × *piperita* L. is cultivated several countries including USA, Morocco, Argentina, France, Hungary, Italy, and Switzerland (Chambers and Hummer, 1994). Although antibacterial activities of essential oils from different *Mentha spp* were reported on a variety of food and human pathogenic bacterial disease agents (Sivropoulou *et al.*, 1995), very few research has been conducted for the usage of essential oils from medicinal and aromatic plants against the seed-borne plant pathogenic bacteria.

Therefore, this research was carried out to evaluate the chemical composition and the antibacterial activity of essential oil from leaves of cultivated mint (*Mentha* x *piperita* f.sp. *officinalis* var. 'Aura') having grapefruit fragrant grown in a home garden in Hatay province against five different economically important Gram-negative seed-borne plant bacterial disease agents such as bean halo blight disease agent *Pseudomonas syringae* pv. *phaseolicola*, tomato bacterial speck disease agent *Pseudomonas syringae* pv. *tomato*, tomato pith necrosis disease agents *Pseudomonas cichorii*, *Pseudomonas corrugata* and potato soft rot disease agent *Pectobacterium carotovorum* subsp. *carotovorum* by using paper disc diffusion assay.

Material and Methods

Isolation of essential oils

Seedlings of grapefruit fragrant mint plant (*Mentha* x *piperita* f.sp. *officinalis* var. 'Aura' were purchased from a commercial company (N11.com) and cultivated in a local home garden in Hatay province. The essential oil was obtained from dried leaves at pre-flowering stage. A total of 25 g of plant samples was used and placed into a 1 L flask. Distilled water was then added until it covered the sample completely. Essential oils were obtained by hydrodistillation method using Clevenger-type distillation apparatus. The essential oils were dried over anhydrous sodium sulfate and stored in dark vial bottles at 4°C until analysis.

Determination of chemical composition of essential oil

The essential oil was analysed by a Thermo Scientific ISQ Single Quadrupole model gas chromatography-mass spectrometry (Kara *et al.*, 2020). The GC was equipped with a TRFAME MS column (60 m x 0.25 mm, 0.25 μm) column and helium carrier gas. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, mass spectra were recorded at 70eV, and the mass range was from 1.2-1200 m/z. The injection port temperature was 220°C, the column and oven temperature was initially 50°C. The oven temperature program was initially held at 50°C for 3 min and then programmed from 50°C by a ramp of 3°C/min up to 220°C. Helium (99.9 %) was used as a carrier gas (1.0 ml/min). The essential oil was diluted in hexane

(2 ml cyclohexane, 5 ml essential oil). The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9).

Test microorganisms and cultural methods

The antibacterial activity of mint essential oil was tested against bacterial disease agent *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas cichorii*, *Pseudomonas corrugata* and *Pectobacterium carotovorum* subsp. *carotovorum*. All of bacterial disease agent isolates were obtained from their respective host plants growing in the region. The bacterial cultures were maintained and tested on King B (KB) medium.

Determination of antibacterial activity of the essential oil

The antibacterial activity of the mint essential oil was determined by using the paper disc diffusion assay (Bozkurt *et al.*, 2020). The surface of KB plates was inoculated with 100 µl of overnight cultures of bacterial isolates. Sterile filter paper discs (6 mm in diameter) were treated with 5 µl of the essential oil and placed on the centre of the agar. The lid of each individual petri dish was replaced immediately to prevent unnecessary evaporation or interaction and sealed with parafilm and incubated at 25°C for 48 hr. In addition water amended discs was used for control treatment. Antibacterial activity of each essential oil against bacterial isolates was then determined by measuring the diameter of zones of inhibition, including the paper discs. Studies were performed in triplicate and repeated three times. SPSS statistic program was performed for all calculations, and the significance was determined by means of Duncan's Multiple Range Test ($P<0.05$).

Results and Discussion

The chemical components of essential oil from plant were identified by GC-MS analysis. Following GC-MS analysis, 39 different components were identified in essential oils, representing 99.94% of the essential oil. Among the components detected, linalool (57.86%), was found to be the major compound of the essential oil followed by linalyl acetate (29.76%), α-terpineol (3.69%) and geranyl acetate (3.39%) as major compounds in each essential oils investigated (Table 1). Several investigations have been carried out on the chemical composition of different samples of *Mentha* species from different geographical regions revealing that chemical composition and percentage varied depending upon the species and the harvesting time at different stages, and the geography as well as the extraction methods (Rohloff *et al.*, 2005). Some factors like physiological and environmental conditions, genetics and evolution also determine the chemical variability of *Mentha* essential oils (Figueiredo *et al.*, 2008). In a very recently published study, menthol (59.1%) in different *Mentha* spp., linalool (32.5-48.2%) in *M.* x *piperita* cv "Grenada", *M.* cf. x *gracilis* cv. "Ginger", and *M.* cf. x *gracilis* cv. "Aureus", cis-piperitone oxide, carvone, and menthofuran were reported to present in essential oil of *Mentha* spp. plants grown in a geographically small region of Estonia (Kapp *et al.*, 2020). However, in Korea, *M. piperita* leaves EO has different composition and include limonene (64.5 and 94.2%), 1,8-cineole (46.1%), p-menth-2-en-ol (34.5%), menthol (33.4%) and linalyl-acetate (28.2%) as main components (Yasukawa *et al.*, 1993).

Table 1. Chemical compositions of essential oil of mint plant used in this study.

RT	Compound Name	SI	RSI	Area %
6.61	β-Pinene	622	682	0.04
8.74	β-Phellandrene	873	953	0.02
9.02	β-Myrcene	977	986	0.19
10.08	Limonene	963	990	0.13
10.66	cis-Ocimene	985	994	0.31
11.42	trans-β-Ocimene	970	980	0.18
12.06	Eucalyptol	977	985	0.79
12.73	Cymene	818	853	0.03
13.67	Tetrahydroactinidiolide	643	702	0.02
15.07	Butanoic acid, 3-methyl-, pentyl ester	927	983	0.05
16.72	1-octen-3-yl acetate	978	988	0.46
17.15	1-Octen-3-ol	969	974	0.13
18.14	Cyclohexanone	894	965	0.03
18.79	Linalool oxide	906	961	0.17
20.62	**Linalool**	**991**	**993**	**57.86**
22.29	**Linalyl acetate**	**987**	**988**	**29.76**
24.12	trans-Caryophyllene	961	972	0.21
25.32	Bornyl acetate	920	952	0.03
25.99	Camphor	835	922	0.02
26.49	Isopinocamphone	833	894	0.13
26.98	**α-Terpineol**	**991**	**994**	**3.69**
28.22	Epoxylinalol	769	845	0.02
28.58	**Geranyl acetate**	**990**	**992**	**3.39**
28.95	Nerol	977	981	0.26
30.33	Geraniol	988	990	0.81
32.12	E-Citral	944	969	0.03
32.7	Acetic acid, phenethyl ester	902	931	0.02
34.04	3,6-Octadecadienoic acid, methyl ester	718	761	0.02
35.49	cis-3-Hexenyl iso-butyrate	905	931	0.04
38.67	cis-Jasmone	939	959	0.13
38.85	Veridiflorol	960	977	0.63
39.28	Eudesmol	752	788	0.02
39.99	Carvone	847	865	0.03
40.18	Caryophyllene oxide	877	931	0.02
40.41	Spathulenol	936	967	0.18
41.02	8-Hydroxylinalool	839	910	0.02
41.94	Guaiol	880	898	0.03
42.29	Torreyol	748	848	0.02

The antibacterial activities of mint essential oil and the response of seed-borne bacterial disease agents to the essential oil is presented in Table 2. Differences between bacterial disease agents were significant. Grapefruit fragrant mint essential oil had the highest inhibitory activity against *Pectobacterium carotovorum* subsp. *carotovorum* corresponding to 11.33 mm in the zones of inhibition over the control (sterile water). This bacteria was followed by *Pseudomonas syringae* pv. *phaseolicola* (10.67 mm), *Pseudomonas syringae* pv. *tomato* and *Pseudomonas cichorii*

(8.67 mm) respectively (Table 2). No antibacterial activity was, however, observed against bacterial disease agent *Pseudomonas corrugata*.

Table 2. Inhibitory effect of grapefruit fragrant mint essential oil on growth of seed-borne bacterial disease agents

Bacterial species	**Inhibition zone* (mm)**
Pseudomonas syringae pv. *phaseolicola*	10.67c**
Pseudomonas syringae pv. *tomato*	8.67b
Pectobacterium carotovorum subsp. *carotovorum*	11.33c
Pseudomonas corrugata	0.0a
Pseudomonas cichorii	8.67b
Control (sterile water)	0.0a

* Diameter of inhibition zone including disc diameter of 6 mm.
** Means in the column followed by different letters are significantly different according to Duncan Multiple Range Test (P<0.05).

Previous studies were conducted to assess antimicrobial activities of essential oils from different medicinal plants belonging to Lamiaceae family against some plant pathogenic bacterial disease agents such as *Agrobacterium tumefaciens, Clavibacter michiganensis* subsp. *michiganensis, Pseudomonas syringae pv. phaseolicola, Acidovorax avenae subsp. citrulli, Pseudomonas savastonoi pv. savastonoi, Pseudomonas syringae pv. tomato* (Soylu *et al.*, 2003; Daferera *et al.*, 2003; Soylu *et al.*, 2009; Mengüllüoğlu and Soylu, 2012; Bozkurt *et al.*, 2020). In many published studies, antibacterial effects of essential oils have been mainly investigated against human pathogens, food spoilage microorganisms and dermatophytes (Burt, 2004; Saeed *et al.*, 2006; Kaap *et al.*, 2020). To our knowledge there is no research on the evaluation of the efficacy of essential oil of mint against seed-borne plant pathogenic bacterial disease agents used in this study. Earlier studies have demonstrated the ability of different species of mint essential oils to retard and inhibit the growth of various plant pathogenic bacteria such as *Agrobacterium tumefaciens*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomanas savastanoi pv. savastanoi*, (Ben Hsouna *et al.*, 2019; Benali *et al.*, 2020; Siddique *et al.*, 2020).

Conclusions

In conclusion, the results of this investigation clearly showed that essential oils obtained from leaves of cultivated mint seedlings growing in the region has the antibacterial potential against the seed-borne plant pathogenic disease agents. The mode of action of essential oils against bacterium is unclear, however the involvement of essential oil components may disrupt the cell membrane of the bacterium and change its permeability. Essential oils of plants belonging Lamiaceae family were rich in phenolic compounds, which are believed to be responsible for the marked antimicrobial activity.

References

Ben Hsouna A., Touj N., Hammami I., Dridi K., Al-Ayed AS., Hamdi N. (2019). Chemical composition and *in vivo* efficacy of the essential oil of *Mentha piperita* L. in the suppression of crown gall disease on tomato. Journal of Oleo Science, 68: 419-426.

Benali T., Bouyahya A., Habbadi K., Zengin G., Khabbach A., Achbani E., Hammani K. (2020). Chemical composition and antibacterial activity of the essential oil and extracts of *Cistus ladaniferus* subsp. *ladanifer* and *Mentha suaveolens* against phytopathogenic bacteria and their eco-friendly management of phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology, 28, Article Number: 101696.

Bozkurt İ.A., Soylu S., Kara M., Soylu E.M. (2020). Chemical composition and antibacterial activity of essential oils isolated from medicinal plants against gall forming plant pathogenic bacterial disease agents. KSU Tarım ve Doğa Dergisi, 23: 1474-1482.

Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology, 94: 223-253.

Chambers H.L., Hummer K.E. (1994). Chromosome counts in the *Mentha* collection at the USDA- ARS National Clonal Germplasm Repository. Taxon, 43/3: 423-432.

Daferera D.J., Basil N., Ziogas N., Polissiou M.G. (2003). The effectiveness of plant essential oils on *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection, 22: 39-44.

Figueiredo A.C., Barroso J.G., Pedro L.G., Scheffer J.C. (2008). Factors affecting secondary metabolite production in plants: Volatile components and essential oils. Flavour and Fragrance Journal, 23: 213-226.

Kapp K., Püssa T., Orav A., Roasto M., Raal A., Vuorela P., Vuorela H., Tammela P. (2020). Chemical composition and antibacterial effect of *Mentha* spp. grown in Estonia. Natural Product Communications, 15/12: 1-14.

Kara M., Soylu S., Türkmen M., Kaya D.A. (2020). Determination and antifungal activities of laurel and fennel essential oils against fungal disease agents of cypress seedlings. Tekirdağ Ziraat Fakültesi Dergisi, 17: 264-275.

Rohloff J., Dragland S., Mordal R., Iversen T. (2005). Effect of harvest time and drying method on biomass production, essential oil yield, and quality of peppermint (*Mentha × piperit*a L.). Journal of Agricultural and Food Chemistry, 53: 4143-4148.

Shaikh S., Yaacob H.B., Rahim Z.H.A. (2014). Prospective role in treatment of major illnesses and potential benefits as a safe insecticide and natural food preservative of mint (*Mentha* spp.): a review. AJBPS. 4/35: 1-12.

Sivropoulou A., Kokkini S., Lanaras T., Arsenakis M. (1995). Antimicrobial activity of mint essential oils. Journal of Agricultural and Food Chemistry, 43/9: 2384-2388.

Soylu S,. Soylu E.M., Bozkurt İ.A., Kaya A.D. (2003). Antibacterial activities of essential oils from oregano, thyme, rosemary and lavender plants against *Pseudomonas savastanoi* pv. *phaseolicola*, the causal agent of halo blight of bean. Ovidius University Annals of Medical Science and Pharmacy, 1: 40-44.

Soylu S., Evrendilek G.A., Soylu, E.M. (2009). Chemical compositions and antibacterial activities of bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*) and dill (*Anethum graveolens* L.) essential oils against the growth of food-borne and seed-borne plant pathogenic bacteria. Italian Journal of Food Science, 21: 347-355.

Sundin G.W., Castiblanco L.F., Yuan X., Zeng Q., Yang C.H. (2016). Bacterial disease management: challenges, experience, innovation and future prospects, Molecular Plant Pathology, 17: 1506-1518.

Yasukawa K., Yamaguchi A., Arita J., Sakurai S., Ikeda A., Takido M. (1993). Inhibitory effect of edible plant extracts on 12-otetradecanoylphorbol-13- acetate-induced ear oedema in mice. Phytotherapy Research, 7/2: 185-189.

IDENTIFICATION OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* ISOLATES CAUSING BLACK ROT DISEASE ON CABBAGE IN NİĞDE PROVINCE OF TURKEY

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Abstract

Black rot disease caused by *Xanthomonas campestris pv. campestris* is one of the most hazardous disease of cabbage which is economically important vegetable cultivated in Turkey. Between October and November of 2019 year, 27 fields of cabbage were surveyed in Niğde province which is the largest producer of Turkey. Plants showing V-shaped necrotic lesions with surrounding yellow halo and blackened veins collected from 15 different fields with a disease prevalence of 55.5% for isolation. Infected leaf tissue containing veins was macerated and suspension was streaked on YPGA medium. Growth of yellow and mucoid colonies was checked after incubation at 28°C for 72 h. Isolated colonies (n=20) were Gram-negative, aerobic, non-fluorescent on King's medium B (KB), oxidase negative, catalase positive, caused hypersensitive reaction on tobacco,able to hydrolyse gelatine and aesculin. Fifteen bacterial isolates representing field surveyed produced a single and 619 and 445-bp PCR amplicons with DLH120/125 and ZUP2311/2312 primers specific for *X. campestris* pv. *campestris*. A bacterial suspensions (10^8 cfu ml^{-1}) of isolates were inoculated into leaves of three week old cabbage seedlings. Typical symptoms identical to those observed on naturally infected plants in the field appeared . According to biochemical, physiological, pathogenicity and molecular studies, identity of isolates was confirmed as *X. campestris* pv. *campestris.* All isolates could able to grow on KB medium amended with 100 ppm of streptomycin sulphate and 2.5 mM of copper sulphate.

Key words: *Identification*, *Bacterial disease, Black rot, X. campestris* pv. *Campestris.*

Introduction

Cabbage (*Brassica oleracea* var. *capitata* f. *alba*) is one of the most important economical crop grown in all over the world. It is most commonly grown cruciferous plant cultivated with a great socia-economic impact for direct human consumption and antioxidant based diet. Cabbage is under the infection of several viral, fungal and bacterial diseases (Aksoy, 2007; Şevik et al., 2016; Ayyaz et al., 2018). The main yield-limiting disease of cabbage is considered as Black rot caused by vascular plant pathogenic pathogen *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (*Xcc*) (Alvarez, 2020). *X. campestris* pv. *campestris* is a gram negative, obligate aerobic, rod shaped with yellows pigmented colonies in culture (Alvarez et al., 1994), Black rot disease was first noticed in USA on cabbage. Bacterium has been sporadically isolated in all continents wherever Brassicaceae plants are produced (Bradbury, 1986). It affects and widely distributed in many parts of the world described as a principal yield-limiting and destructive disease of cabbage, cauliflower, broccoli, Brussels sprouts, kale, radish, and some weeds (Bradbury, 1986; Jensen et al., 2010). Pathogen is primarily seed borne and can be transmitted

by infected plant material, soil, plant debris and weeds (Schaad et al., 1974; Williams, 1980). Especially, weeds are very important inoculum source for pathogen conservation throughout the year (Schaad and Dianese, 1981). Bacterium generally enters the plant tissue through hydathodes on the leaf margins and invades vascular tissue under favouruable conditions. The characteristic symptoms of the disease are described as V-shaped necrotic lesions starting from leaf margins and vascular movement progress towards the middle vein resulted darkening (Janse and Wenneker, 2002). The disease severity often depends on factors such as variety of host plant, climatal conditions that crop can suffer from stunning and death of young plants can occur (Taylor et al., 2002). Up to date, several reports indicated the occurance of black rot infections caused by *X. campestris* pv. *campestris* in Turkey. Pathogen was first identified in Mediterranean Region on cabbage, broccoli and Brussels sprouts by Aysan et al. (2006). Other reportes indicated that pathogen was responsible of black rot on cabbage (Aksoy, 2007), cauliflower (Aksoy et al., 2018) and red cabbage plants (Ozturk et al., 2019) grown in Black Sea Region.
Despite Niğde province located in the Central Anatolian Region of Turkey having economic importance on cabbage with the highest production value approximately 150.000 tons on 20.000 decares land, there was no current and descriptional information for black rot disease. So, the present research aimed at surveying, isolation, identification of the casual agent *X. campestris* pv. *campestris* on cabbage plants in Niğde.

Materials and Methods

Surveys were conducted between October and November of 2019 in Sazlıca, Bahçeli, Kaynarca and Bor districts in Turkey which are the major production areas. 54 leaf samples (two plants from each field) contained V-shaped necrotic areas on the leaves were collected from 27 cabbage fields. The leaves showing signs of typical symptoms were used for bacterial isolation. Lesions containing margins of healthy and diseased tissue were aseptically cut and replaced into a steriled extraction bag added 1 ml of sterile sodium chloride (0.85%), crushed using a sterile scalpel and left to bacterial releasing for 45 minutes. The extracts were plated on plated onto YPGA plates (Laala et al., 2015) and left for incubation at 26°C for 72 hours. Single yellow and mucoid *Xcc*-like colonies were purified on King’s B (KB) plates. All isolates were stored in Luria broth liquid (LB) medium supplemented with 40% glycerol at -20°C (Raghavendra et al., 2013). KOH, Kovacs oxidase reaction, oxidative and fermentative metabolism of glucose, hydrolysis of esculine, liquefaction of gelatin and induction of the hypersensitive reaction in tobacco leaves (cv. benthamiana) tests were performed (Lelliot and Stead, 1987; Klement et al., 1990; Schaad et al., 2001). Fluorescent pigment production was evaluated on KB plates on a UV transilluminator. Acid production from carbon sources were tested using methods described by Dye (1968). Salt tolerance was tested in LB nedium containing 5% NaCl. Reference *X. campestris* pv. *campestris* isolate Xc53 previously isolated from red cabbage (Ozturk et al., 2019) was used as positive control for each test. Primer pairs DLH120/DLH125 for detection *X. campestris* (Berg et al., 2005) and ZUP2311/ZUP 2312 (Rijlaarsdam et al., 2004) for detection of *X. campestris* pv. *campestris* isolates were used in PCR assay (Table 1). Suspected colony was picked up with a sterile tip and directly placed into the PCR reaction tube prepared in 25 μL final volume including 12.5 μL of 2x master mix, 1 μL of forward primer, 1 μL of reverse primer, 1 μL of DMSO and 9.5 μL sterile water. PCR parametres were set up on a thermal cycler (Bio-Rad T100 thermal cycler, USA) a protocol was used as recommended by ISTA (2013). The PCR products were separated on 1.5 % agarose gels in 1× TAE buffer, stained with ethidium bromide, and visualized using UV transilluminator.

Table 1. Primers used for the identification of black rot isolates

Primers	Sequence (5'-3')
DLH120	CCG TAG CAC TTA GTG CAA TG
DLH125	GCA TTT CCA TCG GTC ACG ATT G
ZUP2311	GCA AAG CCC TCG TTC ACG CAT
ZUP2312	GGT GGT GTG GCC GCT CTT CTC AT

Bacterial isolates were grown on KB plates incubated for 48 hours at 26°C. Bacterial inoculum was adjuested to 10^8 cfu mL^{-1} (OD_{600} = 0.1). The edges of leaves of two weeks old cabbage seedlings were cut by disinfected scissors and inoculated with bacterial cells from colonies using sterile tips. Plants were incubated in polyethylene humid chambers for 24 hours to faciliate bacterial enterance (Jensen et al., 2010; Raghavendra et al., 2013). Reactions of the inoculated plants were evaluated after 7-20 days of inoculation. A fullfill Koch's postulates were performed by symtomatic leaves onto YPGA medium and PCR was repeated for identidication of re-isolated bacterial colonies. Different concentration of copper sulphate (0.64, 1.28 and 2.56 mM) and streptomycin sulphate (20, 50 and 100 ppm) were streaked on KB medium amended with different concentration of copper and streptomycin and incubated at 28ºC for 72 hours. Bacterial isolates were classified for presence or absence of growth performance (Lelliot and Stead, 1987).

Results and discussion

Black rot disease was observed in Sazlıca, Bahçeli, Kaynarca and Bor districts of Niğde province during October and November of 2019. More samples were collected from Kaynarca and Sazlıca districts and found to be under the infection of pathogen caused very visible and heavy disease symptoms. The disease was recorded in each of the visited district. Typical V-shaped and blackened veins on leaves were found in the fields. In same cases heavy diseased plants were observed at mature head period including disease symptoms on vascular system when plant root cut in half. Identification tests were performed on 20 isolates showed *Xcc*-like morphological colonies on the YPGA medium. The bacterial colonies were round, yellow, mucoid, convex and shiny after 72 hour incubation.

They were Gram-negative (approved with KOH test), negative for oxidase, aerobic, non-fluorescent after 48 hours of incubation on King's B medium, hydrolyzed esculin, liqufied gelatin and utilized oxidatively D (+) glucose, L (+) arabinose, D (+) trehalose and D mannose as a carbon source (Popović et al., 2013; Corzo et al., 2019). They could not grow at 36ºC and 5% NaCl containing liquid medium. They had ability to cause hypersensitive reaction on tobacco leaves. Fifteen of twenty isolates were positive for DLH120/125 and ZUP2311/2312 primers that generated 619 bp and 445 bp PCR products, respectively (ISTA, 2013; Raghavendra et al., 2013; Laala et al., 2015) (Figure 2). All isolates presented the same results like the reference isolate Xc53 (Ozturk et al., 2019).

Figure 1. A. Typical V-shaped disease symptom on cabbage leaf in the surveyed region, **B.** Colony growth of *Xcc* cultures on YPGA medium, **C.** Artifically inoculated cabbage plant with *Xcc* produced V-shaped halo on leaf

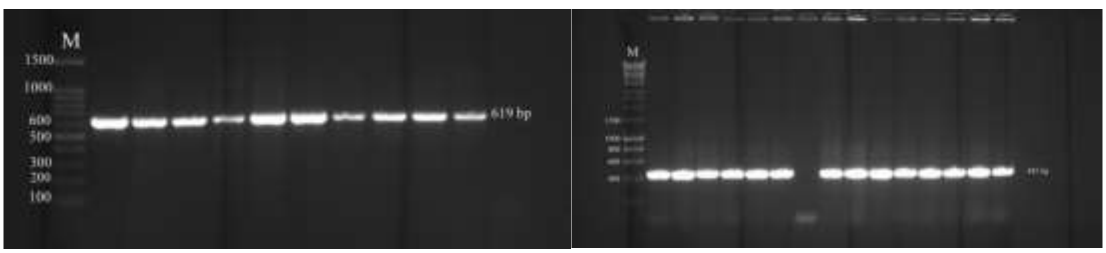

Figure 2. A. 619 bp PCR product generated by DLH120/DLH125 primer pair, **B.** 445 bp PCR product generated by ZUP2311/2312 primer pair

Fifteen isolates (NXcc1 to NXcc15) positive for PCR tests were pathogenic to cabbage plants. Typical disease symptoms were identical to those observed on naturally infected plants in the field. They initially caused small yellowish areas around the leaf in 7-10 days, followed by characteristic necrotic V-shaped lesions on the margins of leaves with chlorosis. Subsequently, blackening of vein and wilting of leaves occurred in 14–20 days (Alvarez et al. 1994; Popović et al., 2013). Plants inoculated with sterile water remained symptomless.
Reisolations were done onto YPGA and isolates showed the same colony morphology as original cultures. Pathogen was repsonsible of black rot of cabbage in the production areas of each of the Sazlıca, Bahçeli, Kaynarca and Bor districts. Pathogen is seed borne and spreads by several attemps such as movement of infected seeds, irrigation, rain splash infected plant debris left in field, weeds and mecanic damage. These factors increase the survival of pathogen in the region. It is indicated by growers even copper based products are commonly used there could not be offered an successful pathogen suppression in host plant. So, isolates were checked for copper resistance and they could grow on KB amended with 2.56 mM of copper sulphate. 100 ppm of streptomycin sulphate was also screened for pathogen survival and could not limit the growth of the isolates That can also be concluded according to the results obtained from to phenotypic tests, the isolates appear phenotypically similar.

Conclusions

Present research reports for the first presence of *X. campestris* pv. *campestris* on cabbage produced in Niğde province. Pathogenic isolates were identified by morphological, physiological, pathogenicity and molecular tests. The tests resulted identification of 15 *Xcc* isolates. The results obtained from this study provide certain information *Xcc* occurs on cabbage plants and confirms that black rot is widespread in the region. 15 of 27 visited individual fields were found to be under the infection of pathogen with a disease prevalence (55.5%). Several local varieties are dominant and preferred by growers. This local varieties are propogated by growers in their own fields and used for the next year. Especially, the fields sown by this local varieties were found to be under the infection of *X. campestris* pv. *campestris*. As indicated by Jensen et al. (2010) growers and workers observe black rot symptoms but mistakenly think it is a result of plant senescense. Certain precautions methods such as resistant/tolerant cabbage varieties, pathogen free seed and seedling material, certified pathogen-free propagative materials, alternative chemical compouds to supress pathogen viabilty in host tissue and crop rotation need to be taken by producers to prevent the spread of black rot disease in the region.

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References

Alvarez, A. M., Benedict, A. A., Mizumoto, C. Y., Hunter, J. E., & Gabriel, D. W. (1994). Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. Phytopathology, 84(12), 1449-1457.

Alvarez, A. M. (2000). Black rot of crucifers. In Mechanisms of resistance to plant diseases (pp. 21-52). Springer, Dordrecht.

Aksoy, H. Samsun ili bafra ilçesi'nde beyaz baş lahana alanlarinda siyah damar çürüklük etmeni *Xanthomonas campestris* pv. *campestris* [(Pammel 1895) Dowson 1939]'nin belirlenmesi. *Anadolu Tarım Bilimleri Dergisi*, 22(3), 297-300.553-557.

Aksoy, H. M., Ozturk, M., & Tufan, S. (2018). First report on Xanthomonas campestris pv. campestris causing bacterial black rot disease of cauliflower in Turkey. Journal of Plant Pathology, 100(1), 141-141.

Ayyaz, M., Bolat, V., & Serçe, Ç. U. (2018). Determination of cabbage (Brassica oleracea) virus infection in Nigde region of Turkey. *Journal of Entomology and Zoology Studies*; 6(2): 1118-1122

Berg, T., Tesoriero, L., & Hailstones, D. L. (2005). PCR- based detection of Xanthomonas campestris pathovars in Brassica seed. Plant Pathology, 54(3), 416-427.

Bradbury, J. F. (1986). Guide to plant pathogenic bacteria. CAB international.

Corzo, M., Quiñones, M. L., & Pauls, K. P. (2019). First report of Xanthomonas campestris causing black rot of chard in Cuba. *New Disease Reports*, 39(1), 13-13.

ISTA (2013). 7–019 Detection of *Xanthomonas campestris* pv. *campestris* on Brassica spp. (prepared by Roberts, S.J., Koenraadt, H.) International Rules for Seed Testing, Annexe to

Chapter7: Seed Health Testing Methods, Bassersdorf, Switzerland, International Seed Testing Association

Janse J.D., Wenneker M., 2002. Possibilities of avoidance and control of bacterial plant diseases when using pathogen-tested (certified) or treated planting material. Plant Pathology 51: 523–536.

Jensen, B. D., Vicente, J. G., Manandhar, H. K., & Roberts, S. J. (2010). Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable Brassica fields in Nepal. Plant Disease, 94(3), 298-305.

Klement, Z., Rudolph, K., & Sands, D. C. (1990). Methods in phytobacteriology. Akademiai Kiado.

Laala, S., Bouznad, Z., & Manceau, C. (2015). Development of a new technique to detect living cells of *Xanthomonas campestris* pv. *campestris* in crucifers seeds: the seed-qPCR. European Journal of Plant Pathology, 141(4), 637-646.

Laala, S., Cesbron, S., Kerkoud, M., Valentini, F., Bouznad, Z., Jacques, M. A., & Manceau, C. (2021). Characterization of *Xanthomonas campestris* pv. *campestris* in Algeria. Phytopathologia Mediterranea, 60(1), 51-62.

Lelliott, R. A., & Stead, D. E. (1987). Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications.

Popović, T., Jošić, D., Starović, M., Milovanović, P., Dolovac, N., Poštić, D., & Stanković, S. (2013). Phenotypic and genotypic characterization of *Xanthomonas campestris* strains isolated from cabbage, kale and broccoli. *Archives of Biological Sciences*, 65(2), 585-593.

Raghavendra, B. T., Singh, D., Yadava, D. K., Mondal, K. K., & Sharma, P. (2013). Virulence analysis and genetic diversity of *Xanthomonas campestris* pv. *campestris* causing black rot of crucifers. *Archives of Phytopathology and Plant Protection*, 46(2), 227-242.

Rijlaarsdam, A., Woudt, B., Simons, G., Koenraadt, H., Oosterhof, J., Asma, M., ... & De Koning, J. (2004, April). Development of specific primers for the molecular detection of *Xanthomonas campestris* pv. campestris. In EPPO conference on quality of diagnosis and new diagnostic methods for plant pests.

Ozturk, M., Eroglu, Z., & Secgin, Z. (2019). First report of *Xanthomonas campestris* pv. *campestris* as the causal agent of black rot disease on red cabbage in Turkey. Journal of Plant Pathology, 101(3), 795-795.

Schaad, N. W., & White, W. C. (1974). Survival of *Xanthomonas campestris* in soil. Phytopathology, 64(12), 1518-1520.

Schaad, N. W., Jones, J. B., & Chun, W. (2001). Laboratory guide for the identification of plant pathogenic bacteria (No. Ed. 3). American Phytopathological Society (APS Press).

Sevik, M. A. (2016). Viruses infecting brassica crops in the Black Sea region of Turkey. *Acta Agriculturae Scandinavica*, Section B—Soil & Plant Science, 66(7),

Schaad, N. W., & Dianese, J. C. (1981). Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. Phytopathology, 71(11), 1215-1220.

Taylor, J. D., Conway, J., Roberts, S. J., Astley, D., & Vicente, J. G. (2002). Sources and origin of resistance to *Xanthomonas campestris* pv. *campestris* in Brassica genomes. Phytopathology, 92(1), 105-111.

REMOTE SENSING AND GIS IN AGRICULTURAL PEST INSECT MONITORING: THE STATE OF THE ART IN TURKEY

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Abstract

Turkey with its geographical location and favorable climate has very rich fauna in terms of insect biodiversity, including especially agricultural plant pests. Agricultural pests destroy an estimated up to 30% of annual crop production worldwide. Keeping agricultural pests under control is very important to reduce the economic losses caused by insects in crop production. Monitoring and correct pest damage identification help us decide whether management is needed. The advanced techniques such as remote sensing and geographic information system (GIS) have become important tools for sustainable agricultural management. Recently, remote sensing and GIS techniques support a new approach for monitoring and determining the damage levels of insect pests in agriculture. The main objectives of these techniques are to collate data that help to estimate possible insect damage and make a decision for determining the most effective insect pest management. These systems have been used in collecting, mapping, analyzing the distribution of insect populations and predicting the damage of insect pests. There are several papers on the use of remote sensing and GIS techniques in agricultural pest control strategies. In this presentation, the some examples of remote sensing and GIS techniques in agriculture in the world are reviewed by focusing on monitoring and determining the damage levels of harmful insects that cause economic losses in the most common agricultural products such as hazelnuts, sunflowers, olives, cotton, cereals in Turkey.

Keywords: *GIS, Remote sensing, Insect pests, agriculture, Turkey.*

Introduction

Agricultural pests destroy an estimated 30% of annual crop production worldwide. It is very important to keep agricultural pests under control in order to reduce the economic losses caused by insects in crop production. Turkey is one of the ten largest agricultural producers in the world. Hazelnut, sunflower, olive, cotton and cereals are among the most produced agricultural products in Turkey. Turkey has a very rich fauna in terms of insect biodiversity, especially agricultural plant pests, with its geographical location and favorable climate. Recently, there has been an increasing interest in the use of remote sensing and GIS techniques in entomological studies (Dminić *et al.*, 2010; Nansen and Elliott, 2016). Remote sensing and GIS can be a rapid, accurate, and simple method for assessing pest damage on plants. Here we will focus on the most common agricultural products, their pests and the use of remote sensing and GIS techniques for monitoring insect pests in Turkey.

Agricultural crops

1. Hazelnut

Turkey is known as the homeland of hazelnut culture. Turkey is the largest producer of hazelnuts in the world with a total production area of 700 thousand hectares and approximately 600 thousand tons of product (İslam, 2018). However, insect pests constitute a major impediment to hazelnut production in Turkey. Although 150 insect and mite species have been determined from hazelnut, currently there are 7 hazelnut insects and mites could be classified as serious pests. This group includes *Curculio nucum, Gypsonema dealbana, Palomena prasina, Xyloborus dispar, Obera linearis* and *Hyphantria cunea* (Tuncer and Ecevit, 1997).

Of them, *Gypsonema dealbana, Xyloborus dispar, Obera linearis* and *Hyphantria cunea* cause damage on leaves, shoots, trunk and limbs. Due to the periodic and rapidly developing epidemic, it is not easy to estimate the damage of these pests and the actual loss. The hazelnut tree reaches 4-5 m and is usually 2-4 m tall and the appearance of a bush. The hazelnut has leaves 8-12 cm long and 6-10 cm wide. These features of hazelnut tree allow the use of remote sensing imagery as an efficient tool for identification, planning and monitoring hazelnut fields. There are several studies on the use of remote sensing and GIS techniques in hazelnut fields in Turkey (Reis and Yomralıoglu, 2006; Aydinoğlu, 2010; Taşdemir and Reis, 2011; Reis and Taşdemir, 2011; Sener *et al.,* 2013; Sarıoğlu *et al.,* 2013), but they are not related to insect damages. Multi-spectral images, acquired at a high radiometric and geometric resolution, by means of digital airborne systems or by satellite, can be useful for monitoring some diseases and insect damages of hazelnut trees (Fabi and Varvaro, 2009).

The fall webworm, Hyphantria cunea is one of the most important hazelnut pests in Turkey. Outbreaks caused by this pest that cause rapid and significant damage on leaves can be monitored by remote sensing techniques (Fig. 1). As seen in the fig.1, the insect pest damage in hazelnut fields can provide sufficient image data for remote sensing applications.

Fig 1. A hazelnut orchard severely damaged by *H. cunea* (Yaman, 2012)

In a case study, Fabi and Varvaro (2009) reports the initial results of the application of one of these systems, called "Advanced Spectroscopic Imaging System" for monitoring a bacterial disease of hazelnut by using aerial photographs taken during a decade of growing seasons, from 1996 to 2006. Furthermore, a protocol of investigation was set up, by means of vectorial GIS, in order to follow in time and in space the local spread of the bacterial disease on hazelnut trees

(Fabi and Varvaro, 2009). This study stimulates us to use Remote sensing and GIS techniques for monitoring insect pests in hazelnut fields in Turkey.

2. Sunflower

Turkey is one of the world's largest sunflower producing countries with an annual production of approximately 1.5 million metric tons and an area of approximately 569.000 ha. Sunflower is one of the most important oilseed crops in Turkey because the crude vegetable oil production is supplied by the sunflower (Konyalı, 2017). However, Turkey imports sunflower seeds, processed and raw sunflower oil for many years. Contrast, sunflower planted areas and production was decreasing in the last years. Therefore, it is necessary to increase the sunflower production areas and yield (Konyalı, 2017). On the other hand, several insect pests cause damage on sunflower leaves. In a research, Thripidae, Cicadellidae and Aphididae families were most important pests on sunflower leaves and Thripidae family was reported as the most abundant pest group (Apak *et al.*, 2017). Furthermore, a population fluctuation of these pests in sunflower fields was observed. According to that study, the highest population of Thripidae, Cicadellidae and Aphididae were observed on 19 July, 18 August and 25 August, respectively (Apak *et al.*, 2017).
There are some studies on the use of remote sensing and GIS techniques in sunflower fields in Turkey. Tunca *et al.* (2018) measured the spectral reflectance properties of sunflower using a spectroradiometer throughout the growing season. In another study, as a result of the classification of satellite images, sunflower cultivation areas were determined in the province of Edirne (Yerdelen *et al*., 2008). Narin *et al*. (2019) evaluated the performance of the Sentinel-2 satellite data in the monitoring of the phenological stages of the sunflower plant in Tokat province (Zile) in Middle Black Sea region of Turkey. Turhan *et al*. (2006) determined the effects of different salt concentrations on sunflower growth using spectral techniques and physiological-morphological characters. The number of similar studies can be increased, but we could not find articles on the use of remote sensing and GIS techniques on the monitoring of insect pests in sunflower fields in Turkey. In a study, Apak *et al.* (2017) observed a population fluctuation of insect pests in sunflower fields. It can be possible to demonstrate this population fluctuation as well as other insect pest damage by using remote sensing and GIS techniques.

3. Olive

Turkey ranks fifth among the world olive grower countries and has the potential of exporting a great majority of its production of olive fruit. In Turkey, pests are one of the most important problems in olive orchards (Bozbuğa and Elekçioğlu, 2008). In total, 8 pest species are of economic importance; *Bactoracera oleae*, *Prays oleae*, *Saissetia oleae*, *Parlatoria oleae*, *Euphyllura olivina*, *Calocoris trivialis*, *C. annulus* and *Pollinia pollini*. Of them, *Prays oleae*, one of twelve lepidopterous pest species known to occur in olive orchards in Turkey is economically important especially at the isolated olive plantations. The type of its damage depends on the attacked tissue. The damage (leaf drop) can be serious (Bozbuğa and Elekçioğlu, 2008).
There are studies on the use of remote sensing and GIS techniques in olive fields in Turkey. For example, Bolca ve Özen (2012) compared template method, a manual technique and OLICOUNT software, a semi-automatic counting modus to determine the number of olive tree presence. Kaleci et al. (2012) studied the determination of topographic characteristics of the olive field information system with remote sensing and GIS techniques. Although a variety

of insect pests can cause major damages to olive crop, the use of remote sensing and GIS techniques on olive pests are very limited. Vozikis et al. (2005) discussed the use of modern technologies (GPS, GIS and Geodata) for controlling olive tree pests. Kounatidis (2008) discussed the implementation of spatio-temporal analyses for management of the olive fly, *Bactrocera oleae* in detail. When compared the number of olive pest insect species in Turkey and the studies including these modern technologies, it is clearly seen that remote sensing and GIS techniques can be subjected to monitor olive pests in Turkey. Especially, *Prays oleae* can be subjected in these technologies because of that the type of its damage (leaf drop).

4. Cotton

Cotton has a very important place in Turkey's agriculture and economy. Turkey is among the eight countries that realize about 80% of cotton production in the World (URL-1). Cotton cultivation is carried out on an area of approximately 365.000 ha in Turkey and the annual cotton production is approximately 450.000 tons. Turkey is one of the leading countries in the world in terms of organic cotton production. In Turkey, insect pests are one of the most important problems in cotton fields. Among the several pest insects, 8 insect species are main cotton pests and they are of economic importance; *Aphis gossypii*, *Thrips tabaci*, *Empoasca decipiens*, *Asymmetrasca decedens*, *Tetranychus cinnabarinus*, *T. urticae* and *Bemisia tabaci* (Anonym, 2011).

Cotton is one of the most common plants subjected in the use of remote sensing and GIS techniques in agriculture. The use of remote sensing and GIS techniques are mostly focused on the determining the distribution of cotton fields in Turkey (Sarı *et al.*, 2007; Aydoğdu *et al.*, 2011). For example, Sarı *et al*. (2007) determined the spectral characteristics and distribution of the cotton growing areas in the West Mediterranean Region of Turkey using the digital data from the Landsat7 ETM Satellite. Contrast, the use of these technologies for monitoring cotton pests is being more popular in the World (Willers *et al*., 1999; Willers *et al.*, 2005; Verma *et al*., 2019). Verma *et al*. (2019) monitored changes in cotton acreage and alternate host crops of cotton bollworm using remote sensing and GIS. Chen *et al.* (2018) detected stress in cotton caused by aphids using leaf level hyperspectral measurements. Sudbrink *et al*. (2000) detected late-season pest damage to cotton & wild host plants of tarnished plant bug in the Mississippi Delta using remote sensing. Yang *et al.* (2011) used remote sensing for detecting and mapping whitefly (*Bemisia tabaci*) infestations. Several studies on the use of remote sensing and GIS on cotton pests stimulate us to use these technologies to determine and monitor the damage of cotton pests such as *Aphis gossypii* and *Bemisia tabaci.*

5. Cereals

Two cereals, wheat and barley, *Triticum aestivum* and *Hordeum vulgare* are very important and strategic food crops in Turkey as well as many other countries. These two crops are produced with 19 million mt and 8 million mt on approximately 9.4 million ha and 3.45 million ha in Turkey, respectively (Gül *et al.*, 2006). Wheat and barley are attacked by several insect pest species (Koçak and Babaroğlu, 2005). Sunn pests are known as a group of true bugs genera including *Eurygaster, Aelia, Carpocoris* and *Dolycoris*. Among them, *Eurygaster* species including *E. integriceps*, *E. maura*, *E. dilaticollis* and *E. austriaca* are the most important pest of cereals in Turkey. Their damage commonly results in yield losses of 20-30% in barley and 50-90% in wheat (Gül *et al.*, 2006).

There are some studies concerning the use of remote sensing and GIS in wheat and barley fields in Turkey (Özcan *et al.,* 2011; 2013; Şimşek *et al.*, 2016). However there is no study on the cereal pests in Turkey. Contrast, in the literature, several studies include the remote sensing and GIS techniques used to monitor cereal pests and their damage. There are considerable high number of studies on cereal crops (Wang *et al.* 2012; Mirik *et al.,* 2014; Bhattarai *et al.*, 2019). For examples, Wang *et al.* (2012) used the remote sensing techniques to monitor wheat stripe rust in China. Bhattarai *et al.* (2019) indicated that remote sensing data can be used to assess the areas of poor growth and health of wheat plants due to Hessian fly infestation. Contrast, numbers of such studies in Turkey is low. *Eurygaster* species, main pests of wheat and barley, can be monitored and their damages determined by the remote sensing and GIS techniques.

Conclusion

It is of great importance to monitor and estimate the damage caused by harmful insects in the agricultural areas with rapid techniques. Recently, remote sensing and GIS techniques support a new approach for monitoring and determining the damage levels of insect pests. However, a few studies have been focused on the use of remote sensing and GIS application in agriculture in Turkey. Furthermore, none of them is on the monitoring of insect damage.These limited studies stimulate us to use GIS and remote sensing applications to monitor agricultural pests and their damage. Among the most common agricultural pests in Turkey, hazelnut, grain and cotton pests are prominent for GIS and remote sensing applications.

References

Anonym, 2011. Cotton Integrated Handling Technical Instruction , Tagem, Ankara (in Turkish).

Apak K.F., Ilhan E., Tursun, N., Karaat F.G. (2017). The population fluctuation of insects in sunflower fields, Malatya, Turkey. International Conference on Agriculture, Forest, Food Sciences and Technologies, 15-17 May 2017, Nevşehir, Turkey.

Aydinoglu A.C. (2010). Examining environmental condition on the growth areas of Turkish hazelnut (*Corylus colurna* L.). African Journal of Biotechnology, 9(39), 6492-6502.

Aydoğdu M., Akçar H.T., Çullu M.A. (2011). Subsidy Payment for Cotton and Corn by Analyzing of Farmer Registration System Using Geographical Information System (GIS) and Remote Sensing (RS) Techniques: A Case Study for Şanlıurfa-Harran District, Jeodezi, Jeoinformasyon ve Arazi Yönetimi Dergisi, 3, Özel Sayı.

Bhattarai G.P., Schmid R.B., McCornack P.B. (2019). Remote Sensing Data to Detect Hessian Fly Infestation in Commercial Wheat Fields, Scientific Reports, 9,6109.

Bolca M., Özen F. (2012). A research of a suitable method on mapping olive tree fields with high resolution satellite images, Ege Üniv. Ziraat Fak. Derg., 49 (1), 63-70.

Bozbuğa R., Elekçioğlu Z. (2008). Pests and natural enemies determined in olive orchards in Turkey, Türk Bilimsel Derlemeler Dergisi, 1 (1), 87-97.

Chen T., Zeng R., Guo W., Hou X., Lan Y., Zhang L. (2018). Detection of Stress in Cotton (*Gossypium hirsutum* L.) Caused by Aphids Using Leaf Level Hyperspectral Measurements, Sensors (Basel, Switzerland), 18(9), 2798.

Dminić I., Kozina A., Bažok R., Barčić J.I. (2010). Geographic information systems (GIS) and entomological research: a review, Journal of Food Agriculture and Environment, 8(2),1193-1198.

Fabi A.,Varvaro L. (2009). Remote sensing in monitorıng the dieback of hazelnut on the 'Monti Cimini' district (Central Italy), Acta Hortic., 845, 521-526.

Gul A., Akbay C., Direk M. (2006). Sunn Pest Control Policies and Effect of Sunn Pest Damage on Wheat Quality and Price in Turkey. *Qual Quant* **40,** 469–480.

İslam A. (2018). Hazelnut culture in Turkey, Akademik Ziraat Dergisi, 7(2),259-266.

Kaleci N., İnalpulat M., Genç L., (2012). Determination of Topographic Characteristics of Olive Field Information System Using Remote Sensing and GIS Techniques: Geyikli Case Study. Presentation; I. Ulusal Sulama ve Tarımsal Yapılar Sempozyumu, May 24-25, 2012, 661-667, İzmir, Turkey.

Koçak E., Barbaroğlu N. (2005). The species of Sunn pest (Eurygaster spp.) (Heteroptera: Scutelleridae) in the overwintering areas of Central Anatolia Region of Turkey, Türk. entomol. derg., 29 (4), 301-307.

Konyalı S. (2017). Sunflower production, consumption, foreign trade and agricultural policies in Turkey, Social Sciences Research Journal, 6, 11-19.

Kounatidis I, Papadopoulos NT, Mavragani-Tsipidou P, Cohen Y, Tertivanidis K, Nomikou M, et al. (2008). Effect of elevation on spatio-temporal patterns of olive fly (*Bactrocera oleae*) populations in Northern Greece. J Appl Entomol 132: 722–733.

Mirik M., Ansley R.J., Steddom K. Rush C.M., Michels G.J., Workneh F., Cui S., Elliott N.C. (2014). High spectral and spatial resolution hyperspectral imagery for quantifying Russian wheat aphid infestation in wheat using the constrained energy minimization classifier. J. Appl. Rem. Sens. 8(1) 083661.

Nansen C., Elliott N. (2016). Remote sensing and reflectance profiling in Entomology, Annu. Rev. Entomol., 61, 139–158.

Narin Ö.G., Abdikan S., Şekertekin A., Delen A., Balik Şanli F. (2019). Performance Analysis of Sentinel-2 Satellite in Monitoring Sunflower Plants. Presentation; Tufuab Teknik Sempozyumu, April 25 – 27, 2019, 190-194, Aksaray, Turkey.

Özcan O., Musaoğlu N., Kurucu Y., Üstündağ B., Örmeci C. (2011). Investigation of Development Level of Wheat Plant in Different Planting Regions with Information Technologies. Presentation; TMMOB Coğrafi Bilgi Sistemleri Kongresi, 31 October - 04 November, 2011, Antalya, Turkey.

Özcan O., Musaoğlu N., Üstündağ B. (2013). Buğday Ekili Alanların Uzaktan Algılama ve CBS ile Mahsul Suyu İhtiyaçlarının Tahmini, Journal of Food, Agriculture & Environment, 11.

Reis S., Yomralıoglu T., (2006). Detection of current and potential hazelnut plantation areas in Trabzon, North East Turkey using GIS and RS, Journal of Environmental Biology, 27(4), 653-659.

Reis S., Taşdemir K. (2011). Identification of hazelnut fields using spectral and Gabor textural features, ISPRS Journal of Photogrammetry and Remote Sensing, 66(5),652-661.

Sarı M., Sönmez N., Yıldıran M. (2007). Determination of Quantitative Reflection Characteristics and Spatial Distribution of Cotton Plant by Satellite Data, Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 20(1),1-10.

Sarıoğlu F., Saygın F., Balcı G., Dengiz O., Demirsoy H. (2013). Determination of potential hazelnut plantation areas based GIS model case study: Samsun city of central Black Sea region, Eurasian Journal of Soil Science, 2 (1), 12-18.

Sener M., Altıntas B., Kurc H.C. (2013). Planning and Controlling of Hazelnut Production Areas with the Remote Sensing Techniques, KSÜ Doğa Bil. Derg., 16(1),16-23.

Sudbrink D., Harris F., Robbins J., Snodgrass G., Thomson S., Dugger P., Richter D. (2000). Remote sensing of late-season pest damage to cotton & wild host plants of tarnished plant bug in the Mississippi Delta, Presentation; Proceedings Beltwide Cotton Conferences, January 4-8, 2000, San Antonio, USA.

Şimşek F.F, Teke M., Altuntaş C. (2016). Controlling farmer product declarations in agricultural lands with remote sensing technique: Example of Harran plain. Presentation; Uzaktan Algılama-CBS Sempozyumu (UZAL-CBS 2016), October 5-7, 2016, Adana, Turkey.

Taşdemir K., Reis S. (2011). Land cover identification for finding hazelnut fields using WorldView-2 imagery. Presentation; *IEEE International Geoscience and Remote Sensing Symposium*, 158-161, doi: 10.1109/IGARSS.2011.6048922.

Turhan H., Genç L., Bostancı Y.B., Sümer A., Kavdir Y. (2006). Determination of the effects of salt stress on sunflower Helianthus annuus with the help of reflection techniques, UZAL-CBS.

Tunca E., Köksal E.S., Çetin S., Ekiz N.M., Balde H. (2018). Evaluation Spectral Properties of Sunflower Throughout the Growing Season, SDÜ Ziraat Fakültesi Dergisi, 12.

Tuncer C., Ecevit O. (1997). Current status of hazelnut pests in Turkey, In Köksal, A.İ., Okay, Y. & Güneş, N.T. (eds.), "Fourth Int. Sym. Hazelnut", Acta Hort., 445 ISHS.

URL-1: https://www.zmo.org.tr/resimler/ekler/8d437661d952917_ek.pdf Accessed on 12/07/2021.

Verma D., Kalubarme M.H., Saroha G.P., Mohan K.S., Ravi K.C., Brajendra Singh A.N. (2019). Monitoring Changes in Cotton Acreage and Alternate Host Crops of Cotton Bollworm Using Remote Sensing and GIS in Major Cotton Growing Regions of India. Presentation; The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, Volume XLII-3/W6, 2019 ISPRS-GEOGLAM-ISRS Joint Int. Workshop on Earth Observations for Agricultural Monitoring, February 18–20, 2019, New Delhi, India.

Vozikis E., Sidiropoulos N., Vozikis N.G. (2005). The use of modern technologies (GPS, GIS and Geodata) for controlling olive tree pests. Presentation; Pharaohs to Geoinformatics FIG Working Week 2005 and GSDI-8, April 16-21, 2005, Cairo, Egypt.

Wang H., Guo J., Ma Z. (2012). Monitoring wheat stripe rust using remote sensing technologies in China. In: Li D., Chen Y. (eds) Computer and Computing Technologies in Agriculture V. CCTA 2011. IFIP Advances in Information and Communication Technology, vol 370. Springer, Berlin, Heidelberg.

Willers J. L., Seal M. R., Luttrell R. G. (1999). Remote sensing, line-intercept sampling for tarnished plant bugs (Heteroptera: Miridae) in Mid-south cotton [Online], J. Cotton Sci, 3(4), 160-170. Available at http://www.jcotsci. org (verified 26 Aug. 2004).

Willers J. L., Jenkins J. N., Ladner W. L. (2005). Site-specific Approaches to Cotton Insect Control, Sampling and Remote Sensing Analysis Techniques, Precision Agriculture, 6, 431–452.

Yang C., James H., Everitt J.H. (2011). Remote Sensing for Detecting and Mapping Whitefly (*Bemisia tabaci*) Infestations. The Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants, 57-381.

Yerdelen A.A. Mermer F., Dedeoğlu H., Yıldız Y., Kaya S., Süzer M., Ocal B. (2008). Edirne İlinde Ürün Deseninin Coğrafi Bilgi Sistemleri ve Uzaktan Algılama Yöntemleri Kullanılarak Belirlenmesi ve Ayçiçeği Verim Tahmini, Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 17 (1-2), 12-18.

PROTIST PATHOGENS OF INDIAN MEAL MOTH *Plodia interpunctella* (LEPIDOPTERA: PYRALIDAE) IN TURKEY

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Abstract

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) is a common storage pest all over the world. The protection of stored products is very important both to ensure food safety and to contribute to the country economy. In addition, insect allergens emerging from foods contaminated with *P. interpunctella* pose a serious threat to human health. Different methods are used to control *P. interpunctella*. The most common of these methods is chemical control. However, chemical control is not considered as a right choice for environmentally safe, efficient and sustainable pest control. Therefore, interest in entomopathogenic organisms (EPOs) is increasing. Entomopathogenic organisms are important biological agents for the control of pest insect populations due to their common and high infection in insects. Natural enemies of *P. interpunctella* such as viruses, bacteria, protists, fungi, nematodes and parasitoids have been studied in detail. The present study includes the first records on the distribution and occurrence of protist patogens in the populations of *P. interpunctella* from 11 localities representing all Turkey between in the period 2019-2020. During the study, 3.286 of *P. interpunctella* samples including larvae, pupae and adults were dissected and searched for protist pathogens. We found three entomopathogenic protists, microsporidium, coccidian and neogergarine. The presence of the microsporidian pathogen was found in all 11 populations (33.7%), coccidian pathogen in two populations (5.55%) and neogregarine pathogen in three populations (3.12%). The distribution and occurrence of protest patogens in the populations of *P. interpunctella* were also given and discussed with literature.

Keywords: *Indian meal moth, Plodia interpunctella, entomopathogen, biological control.*

Introduction

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) is a common storage pest all over the world. The protection of stored products is very important both to ensure food safety and to contribute to the country economy. In addition, insect allergens emerging from foods contaminated with *P. interpunctella* pose a serious threat to human health (Hossain *et al.*, 2021; Mostafiz *et al.*, 2021). Different control methods are used against *P. interpunctella*. The most common of these methods is chemical control. However, chemical control is not considered the right choice for environmentally safe, efficient and sustainable pest control. Therefore, interest in entomopathogenic organisms (EPOs) is increasing (Lacey *et al.*, 2015; Jung *et al.*, 2021). Entomopathogenic organisms are important biological agent for pest insect populations due to its common and high infection in insects. For this reason, they are known as the most reliable and promising control agents against stored product pests (Yaman *et al.*, 2019; Freitas *et al.*, 2020;

Ghoneim *et al.*, 2021). In recent years, natural enemies of *P. interpunctella;* such as viruses, bacteria, protists, fungi, nematodes and parasitoids have been studied in detail (Adarkwah and Schöller, 2012; Batta, 2016; Cowan, 1986; Knell *et al.*, 1996; Malone, 1984a; Mbata and Shapiro-Ilan, 2005; Yaman *et al.*, 2016). In this study, distribution and occurrence of pathogens in the populations of *P. interpunctella* from eleven localities representing all Turkey between the years 2019-2020 is given.

Material and Methods

Insect Samples

During the study, 3.286 specimens including larvae, pupae and adults of *P. interpunctella* were collected from eleven regions (Aydin, Bolu, Denizli, Gaziantep, Isparta, İzmir, Malatya, Ordu, Samsun, Siirt, Trabzon) of Turkey (Table 1).

Table 1. Sampling localities and dates for *Plodia interpunctella* populations

Sampling populations	Sampling Date
AYD	12.06.2019, 02.07.2019, 22.07.2019, 18.06.2020, 30.06.2020
BOL	22.05.2019, 28.06.2019, 05.09.2019, 05.09.2019, 12.09.2019, 20.08.2019,11.03.2020, 23.03.2020, 30.04.2020, 01.06.2020, 13.07.2020
DNZ	01.06.2019, 28.06.2019
GZP	05.07.2019, 05.08.2019, 11.09.2019, 22.07.2020, 27.07.2020
ISP	02.05.2019, 13.07.2019, 06.08.2020, 31.08.2020
İZM	12.06.2019
MLT	13.06.2019, 21.06.2019, 12.09.2019
ORD	18.06.2019, 21.06.2020
SAM	10.06.2019, 10.07.2020
ST	28.06.2019
TRB	15.06.2019, 10.07.2020

(AYD: Aydın, BOL: Bolu, DNZ: Denizli, GZP: Gaziantep, ISP: Isparta, İZM: İzmir, MLT: Malatya, ORD: Ordu, SAM: Samsun, ST: Siirt, TRB: Trabzon)

Microscopic Examination

P. interpunctella larva, pupa and adult samples were dissected in Ringer's solution and then prepared wet smears including host fat body, malpighian tubules, gut and hemolymph were examined for presence of protist infections under a light microscope at a magnification of 400–1000 ×. When an infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope (Toguebaye, 1988).

Results and Discussion

During the study, 3.286 of *P. interpunctella* samples including larvae, pupae and adults were dissected and searched for protist pathogens such as microsporidia, coccidia and neogregarines in the eleven localities of Turkey between the years 2019-2020. Protist infections were confirmed by observation of their characteristic spores/oocysts.

Three different protist pathogens, microsporidium, coccidian and neogregarine were observed in *P. interpunctella* populations. These pathogens were diagnosed as *Vairimorpha plodiae*, *Adelina* sp. and *Mattesia* sp.

While *V. plodiae* was observed in all populations of *P. interpunctella*, *Adelina* sp. in two populations (Aydın and Ordu) and *Mattesia* sp. in three populations (Bolu, Malatya and Ordu) with the infection levels, 33.7, 5.5 and 3.1%, respectively (Tables 2, 3 and 4).

Table 2. Occurrence of *V. plodiae* in *P. interpunctella* populations.

Location	Number of larvae	İnfected larvae	Number of adult	İnfected adult	Number of pupa	İnfected pupa
Aydın	62	52	15	6		
Bolu	573	293	350	82	8	5
Denizli	14	6	5	1		
Gaziantep	353	18	4	3	10	1
Isparta	114	64	12	8	11	1
İzmir	37	9	8	6		
Malatya	316	45	71	3		
Ordu	-	-	159	15		
Samsun	145	125	39	35	8	8
Siirt	52	2	90	67		
Trabzon	90	4	-	-		
Total	1756	618	753	226	37	14
Infection rate (%)	35.19		30.01		37.84	
Total infection rate (%)	33.7					

Table 3. Occurrence of Adelina sp. in P. interpunctella populations.

Location	Number of larvae	İnfected larvae	Number of adult	İnfected adult	Number of pupa	İnfected pupa
Aydın	2	1	-	-	-	-
Ordu	34	1	-	-	-	-
Total	36	2	-	-	-	-
Total infection rate (%)	5.55		-	-	-	-

Table 4. Occurrence of Mattesia sp. in P. interpunctella populations.

Location	Number of larvae	İnfected larvae	Number of adult	İnfected adult	Number of pupa	İnfected pupa
Bolu			251	17		
Malatya	252	1	8	1		
Ordu	34	1	159	2		
Total	286	2	418	20		
Infection rate (%)	0.7		4.78			
Total infection rate (%)	3.1					

Microsporidium infections in *P. interpunctella* populations have been detected in previous studies. However, no record of *Adelina* and *Mattesia* infection was presented. In this study, three different protist pathogen records from Turkey are given. Detection of these pathogens in natural populations of the pest is important in terms of biological control against *P. interpunctella*.

Conclusions

Entomopathogens can be considered as a more effective and easy method, considering the harmfull of chemicals to the environment in Integrated Pest Management (IPM) (Mantzoukas *et al.*, 2021). Although commercial preparations of entomopathogenic protists for use in biological control are limited, they may play a role as suppressive factors in natural populations of the pest. The presence of three different protists in natural populations of *P. interpunctella* is promising in terms of suppression of the pest under natural conditions. The obtained results encourage the use of protists in biological control against *P. interpunctella*.

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References

Adarkwah C., Schöller M. (2012). Biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae) by single and double releases of two larval parasitoids in bulk stored wheat, *J*. Stored Prod. Res., 51, 1-5.

Batta Y.A. (2016). Recent advances in formulation and application of entomopathogenic fungi for biocontrol of storedgrain insects, Biocontrol Sci. Technol., 26, 1171-1183.

Cowan D.K., Vail P.V., Kok-Yokomi M.L., Schreiber F. C. (1986). Formulation of granulosis virüs of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae): efficacy, persistence and influence on oviposition and larval survival, J. Econ. Entomol., 79, 1085-1090.

Freitas A.C.O., Gigliolli A A.S., Caleffe R.T.C., Conte H. (2020). Insecticidal effect of diatomaceous earth and dolomite powder against Corn weevil *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), Turk J. Zool.,44, 490-497.

Ghoneim K., Hamadah K., Tanani M., Emam D. (2021). Quantitative and Qualitative Impacts of Selected Arthropod Venoms on the Larval Haemogram of the Greater Wax Moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae), Journal of Applied Life Sciences International, 24(2),1-26.

Hossain F., Hasan M., Khatun R. (2021). Impact of Host Density on the Performance of the *Bracon hebetor* (Say) (Hymenoptera: Braconidae) Parasitizing Indian Meal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), South Asian Journal of Parasitology, 5(1), 32-41.

Jung J.M., Nam Y., Jung S., Lee W.H. (2021). Spatial analysis of changes in *Plodia interpunctella* (Lepidoptera: Pyralidae) distribution depending on diets, Journal of Stored Products Research, 91.

Knell R.J., Begon M. J., Thompson D. (1996). Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: a test of the mass action assumption with an insect pathogen, The Royal Society, 263, 1366.

Lacey L.A., Grzywacz D., Shapiro-Ilan D. I., Frutos R., Brownbridge M., Goettel M. S. (2015). Insect pathogens as biological control agents: Back to the future, Journal of Invertebrate Pathology, 132, 1-41.

Malone L.A. (1984a). Factors controlling in vitro hatching of *Vairimorpha plodiae* (Microspora) spores and their infectivity to *Plodia interpunctella*, *Heliothis virescens* and *Pieris brassicae*, J. Invertebr. Pathol., 44, 192-197.

Mantzoukas S., Lagogiannis I., Ntoukas A., Tziros G.T., Poulas K., Eliopoulos P.A., Avtzis D.N. (2021). Could Gnomoniopsis castaneae Be Used as a Biological Control Agent against Insect Pests?, Appl. Sci., 11, 4066.

Mbata G.N., Shapıro-Ilan D.I. (2005). Laboratory Evaluation of Virulence of *Heterorhabditid* Nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), Entomological Society of America, 34, 676-682.

Mostafiz M., Hassan E., Acharya R., Kyoung Shim J., Yeoll Lee K. (2021). Methyl Benzoate Is Superior to Other Natural Fumigants for Controlling the Indian Meal Moth (*Plodia interpunctella*), Insects, 12(1), 23.

Toguebaye B.S., Marchand B., Bouix G. (1988). Microsporidia of Chrysomelidae. Petitpierre, E., Hsiao, T.H. & Jolivet, P.H. (eds.), "Biology of Chrysomelidae", Boston: Kluwer Academic Publishers, 399-416.

Yaman M., Güngör F. P., Güner B. G., Radek R., Linde A. (2016). First report and spore ultrastructure of *Vairimorpha plodiae* (Opisthokonta: Microspora) from *Plodia interpunctella* (Lepidoptera: Pyralidae) in Turkey, Acta Parasitologica**,** 61, 228–231.

Yaman M., Algı G., Radek R. (2019). Morphological, ultrastructural and molecular identification of a new microsporidian pathogen isolated from *Crepidodera aurata* (Coleoptera, Chrysomelidae), Turk J Zool., 43, 407-415.

APPLICATION OF MACHINE VISION AND IMAGE PROCESSING TECHNIQUES IN ASSESSING THE QUALITY OF NUT PRODUCTS

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Abstract

Nuts are seed kernels that are widely used in cooking or eaten as snack. They contain a high, inedible outer shell that usually needs to be cracked open to release the kernel inside. Nut products are always high in fat content and calories. The most commonly consumed nuts are: hazelnuts, peanut, walnut, almonds, cashew nuts, and pistachio nuts. Various factors can affect the quality of nut products during production, harvesting, post-harvest, and storage processes, such as: environmental factors, storage method, and corresponding storage conditions. These factors are mostly affecting the flavor, appearance, texture, color, and also the nutritional values of these products. This, in turn, affects the acceptability of the product by the consumers, as well as the marketability. Application of post-harvest quality control processes is very important to improve the quality and increase the product acceptability. Machine vision and image processing techniques are largely used as post-harvest quality control processes for classification, sorting, grading, and evaluating the quality of different agricultural products due to their consistency, objectivity, and reliability. In the current article, the general construction of the machine vision systems, application of machine vision and image processing techniques in evaluating the quality of different nut products are addressed.

Keywords: *Nuts, Almond, Hazelnut, Machine vision, Quality control***.**

Introduction

Nuts are some of the world's most popular snacks and come in many varieties, such as peanuts, pecans, pistachios, almonds, and hazelnuts. Many nuts are defined as seeds, while others are botanically defined as fruits. When eaten in moderation, nuts can be quite healthy and a fine source of protein, fat, fiber, etc. during 2020 and 2021 period over 5.3 million metric tons of tree nuts were produced worldwide (Shahbandeh, 2021). Within the type of nut trees, the production of almond (kernel basis) was the highest in the 2020/2021 production period as demonstrated in figure 1.

The harvest maturity and the procedures used for harvesting and harvest handling are factors affecting the quality of nut products. The most important nut quality factors include: color; texture (crispness), which is related to degree of dryness (moisture content); and flavor (oiliness, sweetness, absence of off-flavors due to development of staleness and rancidity). Deterioration rate is influenced by moisture content, incidence of decay-causing fungi, and insect damage (Kader, 2013). In general, Nuts in the shell have longer storage potential than shelled nuts. Since, broken pieces are more perishable than halves or whole kernels. In order to improve the quality of these products, post-harvest quality control operations need to be applied. These tasks have been largely performed using traditional approaches, which are very expensive, time-consuming, and ineffective approaches. In the current article, the general construction of the machine vision systems, application of machine vision and image processing techniques in evaluating the quality of different nut products are addressed.

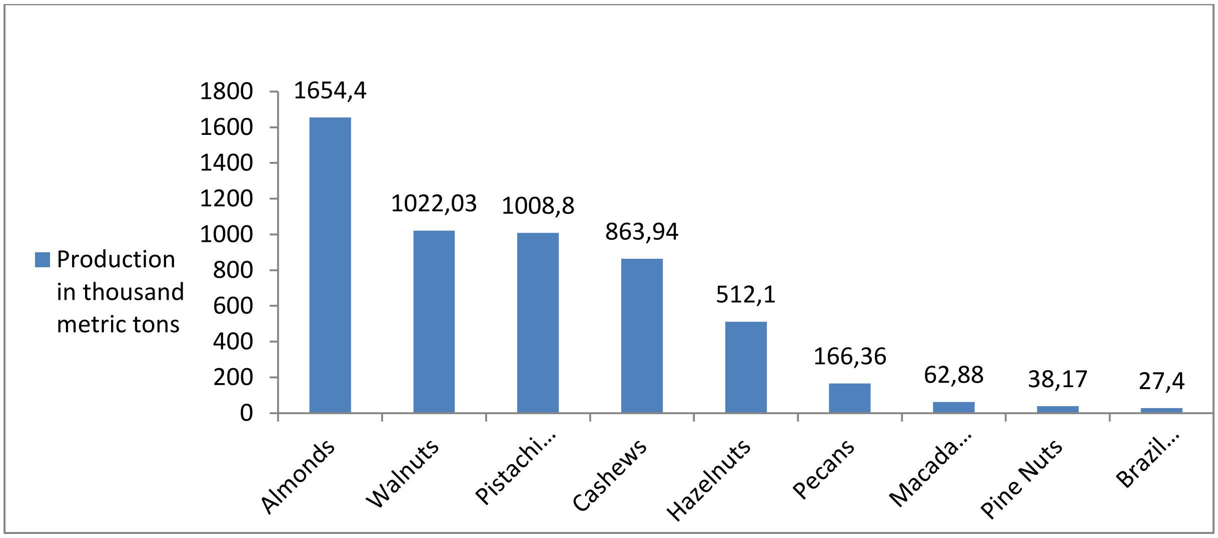

Fig. 1. Production of tree nuts worldwide in 2020/2021, by type (in 1,000 metric tons). (Statista, 2021)

Nut Quality Improvement

Improving the safety and quality of nuts is a comprehensive resource for food safety, product development and QA professionals using nuts in foods, those involved in nut growing, nut handling and nut processing, and researchers in food science interested in the area of improving the quality of these products using different methods(Casulli and Calhoun, 2013). Nut products, like many other agricultural products, require considerable amount of post harvest processing operations, such; separation, sorting, grating, defect detection and classification. Several techniques are currently used to perform these operations in order to improve the quality of nut products, like; image processing and machine vision systems. Digital image processing techniques as nondestructive quality control technique mainly derive from specific conditions in which researchers aim to mimic human vision and decision methodologies with artificial techniques in order to identify and classify objects without extracting samples from them, or permanently damaging them (Czimmermann et al., 2020).

Machine Vision System and Its Construction

Machine vision system is defined as a technology that has arisen from a union between camera and computer and try to imitate human vision in order to gather information from an object without requiring a physical interaction with that object (Sandoval et al., 2018). From other side of view (Timmermans, 1998) defined computer vision system as states that include capture, processing, and analysis of 2D images.

A typical machine vision system constructs of an image sensor, a frame grabber, lighting system, and a computer with suitable software and algorithms. An object image is captured using the image sensor, the analog signal generated by the sensor are digitized into a sequence of numbers and stored as an image into the computer, and then different type of image processing algorithms are used to extract a pattern from the image and represent the object. The extracted pattern is classified by classification algorithms which in turn may generate a signal to animate a trigger to direct the object into the required route. Figure 2 presents a block diagram of the software and hardware components and the inspection process of a typical machine vision system.

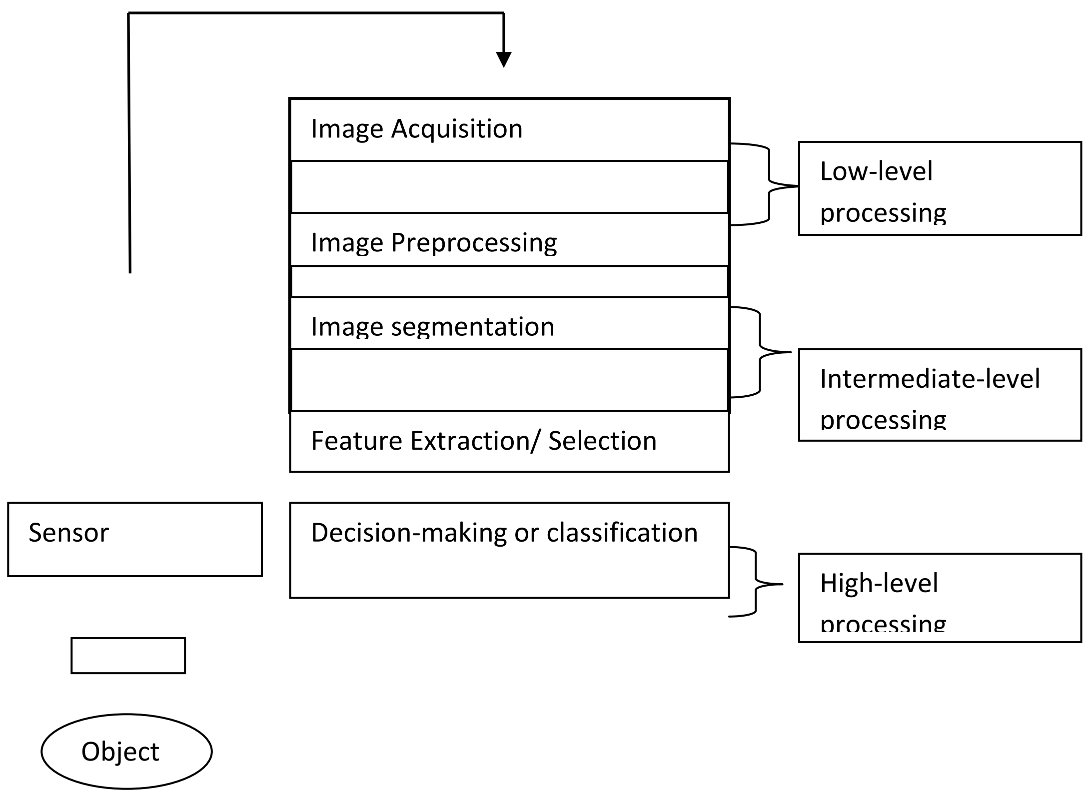

Fig.2.2 A schematic representation for inspection process by machine vision system

Machine Vision Image Acquisition

Obtaining good image of the object under inspection is the first problem in any automated task. In order to acquire all the useful information of the object, it is important to use a suitable light source to obtain the required quality of illumination required over the scene. As well as the performance of the illumination system can play an important role in the accuracy and efficiency of the machine vision system (Novini, 1995). Considering that the sensor used will receive the reflected light, the intensity of the illumination source should be limited by the sensitivity of the sensor.

The image sensor is the basic part of the imaging system within the camera for capturing images. In addition, the image sensor resolution must be taken into consideration, hence; it determines the amount of pixels from the image. There are many different sensors which can be used to generate an image, but self-scanned solid-state charge couple devices (CCD) are the most widely used image sensors (Brosnan and Sun, 2004). CCD cameras are widely used in computer vision system in industrial inspection problems in which a product moves along transport system require line-scan camera. Using these types of cameras, the image is carried out line by line as the object moves past the camera. Both the color and monochromatic cameras have been largely used in the agro-food industry, such as quality assessment, product selection, and product classification (Jhawar, 2016).

An image acquisition board or frame grabber is normally used to perform digitization task. The device is normally installed in a computer to digitize the signal received from the sensor in analog form by dividing the image into a tow dimensional grid containing picture elements defined as pixels. The analog signal of each sensor pixel is usually digitized by an 8bit resulting in a range of 0-255 for the signal (Narendra and Hareesh, 2010).

Inspection by Machine Vision Systems

Machine vision as nondestructive inspection method, is widely used in agricultural applications, including inspection and selection of fruits and vegetables (ElMasry et al., 2012).The visual aspect of the products considers an important factor for the agro-industry, since this parameter is used to determine the value of the product in the market. In addition, the appearance of agricultural products, i.e., their size, shape, and color, and the presence of stains or defects, negatively influence in the consumer perception and therefore determine the degree of acceptance prior to a purchase. The consumer also associates a certain internal quality with the appearance of the product, which affects future decisions in the purchases (Brosnan and Sun, 2004).

Machine vision inspection of agricultural and food products is more versatile than the existing optical and other traditional inspectors. Using machine vision techniques multiple-feature processing can be offered. These features can perform the signals sent by different sensors or obtained through different type of algorithms. The pattern classification algorithms implemented on a machine vision system provide multiple choices for agricultural products classification.

Mostly industrial inspection systems are designed to inspect only known objects at fixed position. The object needs to be appropriately illuminated in order to facilitate the acquisition of the previously known image features. Elias et al (2002) represent an intensive survey of machine vision applications in the industrial environment and they classified the industrial vision applications according to the inspected features of the product in four categories, namely: dimensional, structural, surface, and operational quality. They also can be classified according to the flexibility or degree of freedom of the system (DOFs), which is a measure of the ability of the inspection system to inspect independent features. DOF for any system could be increased, but sophisticated image classification approaches based on carefully selected models and algorithms must be employed. Which means that, the system can be improved to detect new types of defects if additional image processing and analysis functions are applied independently from the old ones to capture more image features.

Application of Machine Vision System in Nut Quality Evaluation

Application of machine vision system in quality evaluation begins with understanding the requirements of the application and also with the proceeds of selecting the appropriate machine vision hardware and software to solve the problem at hand. Mostly industrial inspection systems are designed to inspect only known objects at fixed position, and the object needs to be appropriately illuminated in order to facilitate the acquisition of the previously known image features.

The technology has been widely applied to determine the quality of nut products as illustrated in table 1. Recently, the technology was applied to identify hazelnut defects using RGB image analysis and color grams techniques (Giraudo et al., 2018). The half-cut RGB hazelnut images were acquired using digital camera after classified by industrial expert assessors into three reference categories, i.e. 'sound', 'rotten' and 'pest-affected'. The color grams were obtained by converting RGB images, and then Partial Least Squire Discriminant Analysis (PLS-DA) was developed and used as classification model. For better discrimination of the previously defined classes, Interval Partial Least Square Discriminant Analysis (IPLS-DA) feature selection algorithm by Nørgaard et al., (2000) was applied to select the most informative regions of the color gram signals. The whole defective hazelnuts have been also detected by Kivrak (2019) using image processing and machine learning techniques. The work was aimed to separate intact hazelnuts from damaged or imperfect ones. The images of hazelnut samples were captured by a mobile phone and processed using image tagging technique. Satisfactory results were obtained using supervised learning method.

As almonds are one of the most nutritious of all nuts and they have the highest protein content of any nut, increasing the quality of almond product by means of a new and reliable technique is a key factor in exporting and economic profitability of the final product. In order to evaluate the quality of the product, a robust method based on image processing and computational intelligence for almonds grading and classification was developed (Teimouri et al., 2015). Using artificial neural networks (ANNs) classifier, five classes of almond including normal almond (NA), broken almond (BA), double almond (DA), wrinkled almond (WA) and shell of almond (SA) were classified with very high accuracy. multiple researches have been performed to improve almond quality such; sweet and bitter almond classification (Abozar and Seyed, 2017), almond size prediction (Vidyarthi et al., 2020), and normal and broken almond classification (Teimouri et al., 2014).

Table 1. Application of machine vision system in quality inspection of nut products

Products	Application	Technology used	References
Almond	Defected, roasted, and foreign material classification	RGB Digital camera	(Delila Halac, 2017)
Almond	Pinholes detection	X-Ray imaging	(S. Kim and Schatzki, 2001)
Pistachio	Sorting different types of defects	X-Ray image histogram features	(Pearson et al., 2001)
chestnuts	Detection of insect infestation	NIR spectral tec.	(Moscetti et al., 2014)
chestnuts	defected chestnuts grading	NIR camera	(Zhan et al., 2010)
chestnuts	moldy chestnuts detection	near infrared spectra	(Zhou et al., 2009)
peanut	moldy peanut kernel detection	RGB camera, H,I,S color parameters	(Hong and Lirong, 2007)
Pecans	Damage inspection	X-Ray imaging	(Mathanker and Sunil, 2010)

Although chestnut is not widely spread around the world, it is of great importance in some countries such as China. The chestnut is one of China's important economic fruit and has rich nutrition and medicinal value. One of the most important defects that serially affect the overall quality of chestnuts is the existence of the worm-eaten chestnuts. Therefore, developing an effective, fast and accurate non-destructive testing (NDT) method for sorting chestnuts is an important process for the chestnut industry. For that reason a machine vision system was employed by Wang et al. (2011) to identify the worm-eaten chestnuts based on the edge image of the wormhole. The wormhole edges were obtained using the threshold method. The connected component of the binary images of the wormhole edge was labeled, and the first three longest components were considered as feature values of the worm channel. 100% recognition rate was obtained using back-propagation neural network model.

During the last decades, the application of machine vision technology in evaluating the quality of pistachio nuts have been increased, especially in grading and sorting systems. Recently, several researchers have conducted their efforts on developing automatic pistachio nut grading and sorting systems. Nouri-Ahmadabadi et al. (2017) developed an intelligent system based on machine vision and support vector machine (SVM) for sorting pistachio kernels. A color CCD camera was used for image capturing. HSV color space was gained from the RGB one and a feature vector containing 30 color features was extracted from the captured images. Using SVM classifier an accuracy of (99.17%) was achieved and applied in the online decision-making unit of the system.

Conclusion

This paper has summarized the important of machine vision in quality improvement and its construction. Also the application of machine vision and image processing techniques in evaluating the quality of nut products in order to increase the acceptability and marketability of these products are addressed in this article. However, there are still challenges in this topic that have to be overcome by researchers.

References

Abozar., Seyed-Hassan. (2017). Bag-of-Feature model for sweet and bitter almond classification. Biosystem Engineering, 156, 51-60.

Brosnan T., Sun D.W. (2004). Improving quality inspection of food products by computer vision—a review. Journal of Food Engineering, 61(1), 3-16.

Casull K. E., Calhoun S. (2013). Improving the Safety and Quality of Nuts (L. J. Harris Ed.). Food Science, Technology and Nutrition, 61(1), 3-16.

Czimmermann T., Ciuti G., Milazzo M., Chiurazzi M., Roccella S., Oddo,C. M., Dario P. (2020). Visual-Based Defect Detection and Classification Approaches for Industrial Applications-A survey. Sensors (Basel), 20(5).

Delila S., Emir T. (2017). Almonds classification using supervised learning. Journal of Food Engineering, 61(1), 3-16.

Elias G.M., E., Petrakisa., Michalis Z., Laurent P., Legat, J.D. (2002). A survey on industrial vision systems, applications and tools.pdf. Image and Vision Computing, 21 (2003), 171–188.

ElMasry G., Cubero S., Moltó E., Blasco J. (2012). In-line sorting of irregular potatoes by using automated computer-based machine vision system. Journal of Food Engineering, 112(1-2).

Giraudo A., Calvini R., Orlandi G., Ulrici A., Geobaldo F., Savorani F. (2018). Development of an automated method for the identification of defective hazelnuts based on RGB image analysis and colourgrams. Food Control, 94, 233-240.

Hong., Lirong (2007). Identification method for moldy peanut kernels based on neural network and image processing, . Transactions of the CSAE 23 (4) 158–161.
Jhawar J. (2016). Orange Sorting by Applying Pattern Recognition on Colour Image. Procedia Computer Science, 78, 691-697.
Kader. (2013). Impact of nut postharvest handling, de-shelling, drying and storage on quality. Food Science, Technology and Nutrition, 22-34.
Kivrak O., Gürbüz M. (2019). Detection of Defective Hazelnuts by Image Processing and Machine Learning.pdf. Natural and Engineering Sciences, 4(3), 100-106.
Mathanker ., Sunil. (2010). Development of a new local adaptive thresholding method and classification algorithms for X-ray machine vision inspection of pecans. desetation ofdoctora of philosophy, Oklahoma State University. (3443579)
Moscetti R., Haff R. P., Saranwong S., Monarca D., Cecchini M., Massantini R. (2014). Nondestructive detection of insect infested chestnuts based on NIR spectroscopy. Postharvest Biology and Technology, 87, 88-94.
Narendra., Hareesh. (2010). Prospects of Computer Vision Automated Grading and Sorting Systems in Agricultural and Food Products for Quality Evaluation. International Journal of Computer Applications 1 – No. 4, (0975 – 8887.
Nørgaard L., Saudland A., Wagner J., Nielsen J. P., Munck L., Engelsen S. B. (2000). Interval partial least-squares regression (iPLS): A comparative chemometric study with an example from near-infrared spectroscopy. . Applied spectroscopy, 54(3), 413–419.
Nouri-Ahmadabadi H., Omid M., Mohtasebi S. S., Soltani F. M. (2017). Design, development and evaluation of an online grading system for peeled pistachios equipped with machine vision technology and support vector machine. Information Processing in Agriculture, 4(4), 333-341.
Novini A. (1995). The latest in vision technology in todays food and beverage container manufacturing industry. Paper presented at the In Food Processing Automation IV Proceedings of the 1995 Conference, Michigan, USA:ASAE.
Pearson T. C., Doster M. A., Michailides T. J. (2001). Automated Detection of Pistachio Defects by Machine Vision. Applied Engineering in Agriculture, 17(5).
Kim S., Schatzki T. (2001). Detection of pinholes in almonds. American Society of Agricultural Engineers ISSN, 0001–2351.
Sandoval E. M., Rosas M. E. M., Sandoval J. R. M., Velasco M. M. M., Ávila H. C. D. (2018). Machine Vision system - A Tool for Automatic Color Analysis in Agriculture.
Shahbandeh M. (2021). Nut industry worldwide - statistics & facts.
Teimouri ., Omid ., Mollazadeh ., Rajabipour T. (2015). An artificial neural network-based method to identify five classes of almond according to visual features. J. Food Process.Eng, American Society of Agricultural Engineers ISSN, 0001–2351.
Teimouri N., Omid M., Mollazade K., Rajabipour A. (2014). A novel artificial neural networks assisted segmentation algorithm for discriminating almond nut and shell from background and shadow. Computers and Electronics in Agriculture, 105, 34-43.
Timmermans A. J. M. (1998). Computer vision system for on-line sorting of pot plants based on learning techniques. international society for horticultural science, 421, 91-98.
Vidyarthi S. K., Tiwari R., Singh S. K. (2020). Size and mass prediction of almond kernels using machine learning image processing. doi:10.1101/736348

Wang C., Li, X., Wang W., Feng Y., Zhou Z., Zhan H. (2011). Recognition of worm-eaten chestnuts based on machine vision. Mathematical and Computer Modelling, 54(3-4), 888-894.

Zhan H., Li., Xiaoyu.,Wang. (2010). Determination of chestnuts grading based on machine vision. Chinese Society of Agricultural Engineering (5), 327-331.

Zhou., Zhu Jie L., Li., Xiaoyu.(2009). Discrimination of Moldy Chinese Chestnut Based on Artificial Neural Network and Near Infrared Spectra. Transactions of the Chinese Society for Agricultural Machinery 26 (S1) 109–112.

DETERMINATION OF ESSENTIAL OIL COMPONENTS, MINERAL MATTER, AND HEAVY METAL OF CLARY SAGE (*SALVIA SCLAREA* L.) COLLECTED FROM CENTRAL ANATOLIA IN TURKEY

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Abstract

Clary sage (*Salvia sclarea* L.) is an important aromatic plant cultivated to obtain essential oil throughout the world. *Salvia sclarea* L. (Clary sage) is a biennial or perennial herb between 20-120 cm tall, branched at the top, with a thick, upright stem. It has lilac, white or pale blue flowers. The plant, which develops rosette leaves in the first year, blooms the following year. Usually, flowering starts in May and continues until the end of August. Clary sage is commercially grown mainly in Russia, Bulgaria, France and Morocco, with an annual production of about 150 tons of essential oil in these countries. Clary sage essential oil is obtained from the flower parts in full bloom. The aim of the study is to determine the quality of clary sage essential oil, and mineral matter content, growing in natural environment in Central Anatolia. In this study, samples were collected from natural environment in Yozgat (0751558D-4393790K 1837 m), Central Anatolia conditions, during flowering periods in 2017. Flower parts in full bloom period essential oil ratio were found as 0.10%. The main components and its ratio of clary sage essential oil Sclareol, Sclareoloxide, and geranyl-p-cymene were determined as 37.74%, 8.33%, and 4.58%, respectively. Macro elements of flower parts Ca, K, P, and S were determined as 104.419, 432.161, 43.169, and 21.363, respectively. Micro elements Fe, Mn, Cu, B, and Na were observed 3.185, 0.867, 0.414, 0.125, 0.524, and 2.574, respectively. Heavy metals Al, Cd, Co, and N, were found as 2.91543, 0.00021, 0.00643, and 0.00508, respectively.

Keywords: *Clary sage, Salvia sclarea L., essential oil, mineral matter, Sclareol.*

Introduction

Most of the Lamiaceae family members are rich in secondary metabolites, especially essential oils. Therefore, they are of great importance in various fields such as medicine, food, cosmetics and perfumery. Sage, which is a member of this family, is the general name of the species included in the genus *Salvia* (Dweck, 2000). There are 99 species of *Salvia* genus, 51 of which are endemic, naturally spread in the flora of Turkey (Güner ve ark., 2012). Although leaves, shoot tips, flowers and partly stems of *Salvia* species, which have been important medicinal plants since ancient times, are used, the most utilized part is the leaves. *Salvia* species have a very high market potential as they appeal to a wide consumer group (food industry, pharmaceutical and chemical industry, herbalists selling as retail products) due to their characteristics (Yaseen et al., 2015). One of these species, *Salvia sclarea* L. (Clary sage), is a biennial or perennial herb with a plant height of 20-120 cm, branched at the top, a thick, upright stem. It has lilac, white or pale blue flowers. Seeds are brown, rounded triangular in shape. The leaves are petiolate, heart-shaped, and there are gray glandular hairs on the plant. The plant, which develops rosette leaves in the first year, blooms the following year. Generally, its flowering starts in May and continues until the end of August. It is classified as early (less than 170 days), medium early (170-200 days) and late (more than 200 days) according to maturation time (Yaseen *et al*., 2014; Yaseen *et al*., 2015). *S. sclarea* is an economically important species that grows naturally in many countries around the world and is also cultivated. It is a common species in the Flora of Turkey. Clary sage is grown commercially in Russia, Bulgaria, France and Morocco, and annual essential oil production is approximately 150 tons in these countries (Džamić *et al.,* 2008; Hristova *et al.,* 2013; Yaseen *et al*., 2015). The inflorescences and leaves of *S. sclarea*, known as "misk adaçayı, pamuk otu, paskulak" in our country, are used in folk medicine for abdominal pain and constipation (Lahlou, 2004). During the full flowering period, essential oils ranging from 0.01-0.40% are obtained from the inflorescences (Verma 2010, Dogan *et al*., 2015). *S. sclarea* essential oil is rich in linalool and linalyl acetate. *S. sclarea* essential oil is an important commercial oil and is characterized as a colorless, brownish yellow or pale-yellow liquid with a characteristic odor in the European Pharmacopoeia (Aćimović *et al*., 2018). One of the main components of the essential oil, sclareol is the main bioactive compound that can also be used to produce Ambrox, a chemical compound in the tetralabdanoxide class, which is considered one of the most valuable perfumes of animal origin. In addition, the essential oil is used in the food industry to produce beer, tonic drinks, liqueurs, as well as Muscat and Vermouth type wines (Gonceariuc *et al*., 2016). Although intensive studies have been carried out on the essential oil composition of *S. sclarea*, very few studies on the mineral content have been found as a result of the literature reviews. It is now known that nutrients play a vital role in general health and the treatment of diseases. These elements are present in varying concentrations and in very small amounts in different parts of plants (root, seed, leaf, etc.) (Lokhandea *et al*., 2009). The aim of this study was to determine the essential oil composition and heavy metal and mineral content of *S. sclarea*.

Materials and Methods

Plant material: S. sclarea was collected from the natural area in full flowering. Location information and general view of the species are presented in Figure 1.

Location	0751558E-4393790N
Altitude	1837m
Collected Date	30.05.2017-23.06.2017
Province	Yozgat/TURKEY

Figure 1. *Salvia sclarea*

Determination of essential oil content

After the plants were collected from natural area, the flowering aerial parts were allowed to dry in a shady environment not exceeding room temperature. The amount of essential oil in the dried plants was determined by Clevenger distillation device. An average of 50 g of dried plant sample was distilled in 700 mL of water for 3 h. Essential oil values (%, v/w) were calculated as volume over dry matter. The obtained essential oils were placed in dark-colored flasks and stored at 4 °C in a refrigerator until they were analyzed (Oke *et al*., 2009; Baj *et al*., 2015).

Gas chromatography–mass spectroscopy analysis (GC/MS)

The compounds of the essential oil were detected with GC/MS (Shimadzu, QP2010 ULTRA) in the Science and Technology Application and Research Center of Yozgat Bozok University (Turkey). The analysis was performed according to the method described by Cosge Senkal et al. (2019).

Determination of heavy metal and nutrient contents

Plant samples were dried in the shade. From each sample,1 g was weighed and put into a porcelain crucible. The samples were burned at a maximum of 550 °C until gray ash was obtained, and 3 N HCl was added to these samples. Then they were filtered using filter paper (Whatman No.1), and distilled water was added up to a final volume of 10mL (Kacar and Inal, 2010). The heavy metals and nutrients (aluminum, cadmium, cobalt, chrome, nickel, phosphorus, potassium, calcium, sulfur, iron, copper, zinc, manganese, boron, and sodium) in the samples were detected using an iCAP-Qc ICP-MS spectrometer (Thermo Scientific) at the Science and Technology Application and Research Center (Yozgat Bozok University, Turkey. (Basaran *et al*., 2017).

Results and discussion

Compounds and contents of the essential oil

The amount of essential oil in the aerial parts of S. sclarea collected in full flowering stage was 0.1% (w/w) in our study. The amount of essential oil obtained from S. sclarea varies according to flowering maturity. The amount of essential oil, which is low before flowering, reaches its highest value during the full flowering period and decreases as the flowers begin to fade (Lattoo et al., 2006). The essential oil content of this species was reported as 0.4% by Torres et al. (1997), 0.03-2.5% by Aydogan (2006), and 1.36% by Sharkhiz (2009). Also, El-Gohary et al. (2020) obtained 0.1-0.2% essential oil in flowers of S.sclarea grown under culture conditions. The values obtained from the study are within the limits reported by the researchers. The GC-MS results of the essential oil obtained from the aerial parts of S. sclarea species are given in Table 1.

Table 1. Chemical composition of essential oil from aerial parts of *S.sclarea* (%)

No	Compounds	RT[1] (min)	(%)	No	Compounds	RT (min)	(%)
1	Linalool	5.148	3.63	21	Piperitenone	11.415	0.10
2	α-Terpineol	6.006	2.34	22	Sclaral (sclareolide lactol)	11.464	0.47
3	Nerol	6.257	0.93	23	2-Pentadecanone, 6,10,14-trimethyl ester	12.127	0.11
4	**Linalyl 2-methylpropanoate**	**6.438**	**13.06**	24	**Sclareoloxide**	**12.978**	**8.33**
5	Copaene	7.649	1.14	25	α-Springene	13.060	0.67
6	Caryophyllene	8.113	0.92	26	β-Springene	13.140	0.34
7	Germacrene D	8.690	2.53	**27**	**geranyl-p-cymene**	**13.389**	**4.58**
8	Bicyclogermacrene	8.838	0.47	28	geranyl-α-terpinene	13.523	1.73
9	Cadinene	9.017	0.42	29	Trans-Nuciferol	13.655	0.23
10	α-Elemol	9.288	0.12	30	Geranylgeranylacetate	13.693	1.10
11	α-Costol	9.365	0.10	31	Manoyl oxide	14.072	1.14
12	1,5-epoxysalvial-4(14)-ene	9.583	0.63	32	Manool	14.633	3.14
13	Spathulenol	9.686	3.71	33	Phytol	14.850	0.13
14	Caryophyllene oxide	9.775	2.46	34	Humulane-1,6-dien-3-ol	14.939	1.28
15	salvial-4(14)-en-1-one	9.863	0.30	35	13(16),14-labdien-8-ol	15.133	0.61
16	Bulnesol	10.358	0.34	36	cis-Sesquisabinene hydrate	15.485	0.29
17	β-Eudesmol	10.455	2.15	37	Farnesol	15.588	0.33
18	Limonen-6-ol, pivalate	10.628	0.53	38	**Sclareol**	**16.328**	**37.74**
19	Longiverbenone	10.710	0.60	39	Docosane, 11-butyl-	17.160	0.11
20	Isospathulenol	10.793	0.62	40	Pentacosane	18.284	0.57
TOTAL							**100.00**

[1]Retention Time

40 components were determined in the essential oil obtained from the plant. Of these components, sclareol constituted 37.74% of the essential oil. Linalyl 2-methylpropanoate (13.06%), sclareoloxide (8.33%) and geranyl-p-cymene (4.58%) were detected as other important components in the mentioned essential oil (Table 2). Farkaš et al. (2005), the essential oil obtained from S. sclarea flowers is characterized by high linalool, sclareol and linayl acetate content; Caissard et al. (2012) stated that sclareol is a high-value natural product obtained by solid/liquid extraction of sage clary sage inflorescences. The chemical composition of the essential oil obtained by water distillation from the aerial part of the plant grown under culture conditions in Iran was analyzed by GC and GC/MS and 50 components were determined. Among these components, linayl acetate (35.9%), germacrene D (13.3%), linalool (12.8%) and sclareol (9.27%) were reported to have the highest values (Sepldeh and Rowshan, 2013). Our findings are consistent with those of other researchers. However, there are some differences. It is known that many factors (genotype, ecological conditions, plant development period,

harvest/harvest time, drying, storage, working principles of the devices used, etc.) are effective on the chemical composition of essential oils. Dzumayev et al. (1995) emphasized that the chemical composition of essential oils obtained from S. sclarea varies according to the parts of the plant, the maturity of the plant, the location of the place where it is grown/collected, whether the plant is obtained from wild or cultivated conditions, the harvest year and the color of the bract leaves.

Mineral Matter and Heavy Metal Content

Some macro (Ca, K, P and S) and micro (Fe, Mn, Zn, Cu, B and Na) elements and heavy metals (Al, Cd, Co, Cr and Ni) analyzes in flowering aerial parts of *S. sclarea* were carried out and the results are presented in Table 2.

Table 2. Somme plant nutrients and heavy metal concentrations of S. sclarea (ppm)

Macrominerals		**Microminerals**		**Heavy metals**	
Ca	104.419	Fe	3.185	Al	2.91543
K	432.161	Mn	0.867	Cd	0.00021
P	43.169	Zn	0.414	Co	0.00277
S	21.363	Cu	0.125	Cr	0.00643
		B	0.524	Ni	0.00508
		Na	2.574		

Dietary minerals are accredited as an essential part of human nutrition due to their various beneficial functions. Major minerals include calcium, phosphorus, potassium, sulfur, sodium, chlorine and magnesium, and minor elements are iron, cobalt, copper, zinc, manganese, iodine, bromine and selenium (Yadav *et al*., 2017). In our study, K and Ca among the macro elements and Fe and Na among the micro elements had the highest values (Table 3). Ca, K, P, S, Fe, Mn, Zn, Cu, B and Na contents of *S.sclarea* collected during flowering period were recorded as 8844-13458 mg kg^{-1}, 22465-24267 mg kg^{-1}, 1852-3268 mg kg^{-1}, 2255-17437 mg kg^{-1}, 66.17- 198.5 mg kg^{-1}, 10.21-15.67 mg kg^{-1}, 17.34-31.99 mg kg^{-1}, 9.17-119.2 mg kg^{-1}, <0.2-6.35 mg kg^{-1}, and 45.96-168.9 mg kg^{-1}, respectively (Szentmihályi *et al*., 2004). *Salvia* species are rich in both major and minor minerals with nutritional properties. At the same time, there is great variation in mineral content among *Salvia* species. In a study conducted by Er et al. (2013), it was stated that Ca (12.402- 15.478 ppm) and S (1.034-2.257 ppm) contents of *S.halophila*, *S. tomentosa*, *S.heldreichiana* and *S. dichroantha* were found to be low compared to K (14.518-24.171 ppm) and P (1.385-1.910 ppm) levels. When our findings are compared with the literature values, it is observed that there are some differences. These differences are probably due to the development period of the plant, the organ used, genetic structure, growing conditions, climatic factors, species etc.

Determination of heavy metal contents is of great importance for the safe use of medicinal and aromatic plants collected from the natural environment (Baranowska *et al*., 2002). According to the analysis results, the heavy metal content of S sclarea was determined as Al > Cr > Ni > Co > Cd. The WHO/FDA breakpoints for "Cd, Cr and Ni" are 0.3, 0.02 and 1.63 ppm, respectively (Lone *et al*., 2003). The amounts of the three metals detected in this study are lower than the maximum allowable values

Conclusion

The chemical composition of essential oils is of great importance in determining the quality and usage areas of essential oils. The essential oil obtained from the flowering aerial parts of *S. sclarea* is rich in sclareol, an important component. Although the micronutrient content of the species is very low, it contains toxic heavy metal concentrations. There is limited research on the elemental content of *S. sclarea* in the literature. Therefore, our findings will be an important resource for future studies.

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References

Aćimović M., Kiprovski B., Rat M., Sikora V., Popović V., Koren A., Brdar-Jokanović M. (2018). *Salvia sclarea*: Chemical Composition and Biological Activity. Journal of Agronomy, Technology and Engineering Management, Vol. 1, p. 18-28.

Aydoğan M. F. (2006). *Salvia sclarea* L. Bitkisinin Uçucu Yağ Bileşimi ve Antimikrobiyal Etkisi Üzerine Araştırmalar. [Studies on Essential Oil Composition and Antimicrobial Effect of *Salvia sclarea* L.] Plant (Yüksek lisans tezi) [Master's thesis], Sağlık Bilimleri Enst., İnönü Üniversitesi, Malatya.

Baj T., Sieniawska E., Kowalski R., Wesolowski M., Ulewicz-Magulska B. (2015). Effectiveness of the Deryng and Clevenger-type apparatus in isolation of various types of components of essential oil from the *Mutelina purpurea* Thell. flowers. Acta Poloniae Pharmaceutica, Vol. 72, p. 507-515.

Baranowska I., Srogi K., Włochowicz A., Szczepanik K. (2002). Determination of heavy metal contents in samples of medicinal herbs. Polish Journal of Environmental Studies, Vol. 11, p. 467-471.

Basaran U., Akbik M., Mut H., Gulumser E., Copur Dogrusoz M. (2017). High-performance liquid chromatography with refractive index detection for the determination of inulin in chicory roots. Analytical Letters Vol. 51, p. 83-95.

Caissard J. C., Olivier T., Delbecque C., Palle S., Garry P. P, Audran A., Valot N., Moja S., Nicole F., Magnard J. L., Legrand S., Baudino S., Jullien F. (2012). Extracellular Localization of the Diterpene Sclareol in Clary Sage (*Salvia sclarea* L., Lamiaceae). PLoS ONE, Vol. 7, p. e48253.

Cosge Senkal B., Uskutoglu T., Cesur C., Ozavci V., Dogan H. (2019). Determination of essential oil components, mineral matter, and heavy metal content of *Salvia virgata* Jacq. grown in culture conditions. Turkish Journal of Agriculture and Forestry, Vol. 43, p. 395-404.

Dogan G., Hayta S., Yuce E., Bagcı, E. (2015). Composition of the Essential Oil of Two Salvia Taxa (*Salvia sclarea* and *Salvia verticillata* subsp. *verticillata*) from Turkey. Natural Science and Discovery, Vol. 1, p. 62- 67.

Dweck A. C. (2000). Sage, the Genus Salvia, Harwood Academic Publishers, Amsterdam.

Džamić A., Soković M., Ristić M., Grujić-Jovanović S., Vukojević J., Marın P. D. (2008). Chemical Composition and Antifungal Activity of *Salvia sclarea* (Lamiaceae) Essential Oil. Archives of Biological Sciences, Vol. 60, p. 233-237.

Dzumayev Kh. K., Tsibulskaya I. A., Zenkevich, G., Tkachenko K. G., Satzyperova I. F. (1995). Essental Oils of *Salvia sclarea* L. Produced from Plants Grown in Southern Uzbekistan. J. Essent. Oil Res., Vol. 7, p. 597-604.

El-Gohary A. E., Amer H. M., Salama A. B., Wahba H. E., Khalid K. A. (2020). Characterization of the Essential Oil Components of Adapted *Salvia sclarea* L. (Clary sage) Plant Under Egyptian Environmental Conditions. Journal of Essential Oil Bearing Plants, Vol. 23, p. 788-794.

Er M., Tugay O., Özcan M. M., Ulukuş D., Al-Juhaimi F. (2013). Biochemical properties of some Salvia species. Environmental Monitoring and Assessment Vol. 185, p. 5293-5198.

Farakaš P., Hollá M., Tekel J., Mellen S., Vaverková Š. (2005). Composition of the Essential Oils from The Flowers and Leaves of *Salvia sclarea* (Lamiaceae) Cultivated in the Slovak Republik. J. Essent. Oil Res., Vol. 17, p. 141-144.

Gonceariuc M., Balmus Z., Cotelea L. (2016). Genetic diversification of *Salvia sclarea* L. Quality by Increasing The Storage Capacity of The Essential Oil. Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii, Vol. 32, p. 29-36.

Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç M.T. 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler) [List of Plants of Turkey (Vascular Plants)], İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını.

Hristova Y., Gochev V., Wanner J., Jirovetz L., Schmidt E., Girova T., Kuzmanov A. (2013). Chemical Composition and Antifungal Activity of Essential Oil of *Salvia sclarea* L. from Bulgaria Against Clinical Isolates of Candida species. J. BioSci. Biotech., Vol. 2, p. 39-44.

Kacar B, Inal A. (2010). Plant Analysis, Nobel Yayıncılık, Ankara.

Lahlou, M. (2004). Essential Oils and Fragrance Compounds: Bioactivity and Mechanisms of Action. Flavour and Fragrance Journal, Vol. 19, p. 159-165.

Lokhandea R. S., Singarea P. U., Andhelea M.L., Acharyab R., Nairb A. G. C., Reddyb A. V. R. (2009). Analysis of Mineral Content of Some Medicinal Plants by NAA and AAS Techniques. Radiochemistry, 2009, Vol. 51, p. 321-325.

Lone M. I., Saleem S., Mahmood T., Saifullah K., Hussain G. (2003). Heavy metal contents of vegetables irrigated by sewage/tubewell water. International Journal of Agriculture and Biology, Vol. 5, pp. 533-535.

Lattoo S. K., Dhar R. S., Dhar, A. K., Sharma, P. R., Agarwal, S. G. (2006). Dynamics of Essential Oil Biosynthesis in Relation to Inflorescence and Glandular Ontogeny in *Salvia sclarea*. Flavour Fragr. J., Vol. 21, pp. 817-821.

Oke F., Aslım B., Öztürk S., Altundağ S. (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chemistry, Vol. 112, pp. 874-879.

Saharkhiz M. J., Ghani A., Hassanzadeh-Khayyat M. (2009). Changes in Essential Oil Content and Composition of Clary Sage (*Salvia sclarea*) Aerial Parts During Different Phenological Stages. Medicinal and Aromatic Plants Science and Biotechnology, Vol. 3, p. 90-93.

Sepldeh, N., Rowshan, V. (2013). Comparison of *S. sclarea* L. Essential Oil Components in Wild and Field Population. International Journal of Agriculture and Crop Sciences, Vol. 5, pp. 828-831.

Szentmihályi K., Héthelyi É., Virág V., Then M. (2009). Mineral elements in muscat sage plant (Salvia sclarea L.) and essential oil. Acta Biologica Szegediensis Vol. 53, pp. 35-38.

Torres M. E., Velasco-Negueruela A., Perez-Alonso M. J., Pinilla M. G. (1997). Volatile Constituents of two *Salvia* Species Grown Wild in Spain. Journal of Essential Oil Research, Vol. 9, pp. 27-33.

Vermani A., Prabhat N., Chauhan A. (2010). Physico-chemical analysis of ash of some medicinal plants growing in Uttarakhand, India. Nature and Science Vol. 8, pp. 88-91.

Yadav A., Joshi A., Kothari S. L., Kachhwaha S., Purohit S. (2017). Medicinal, Nutritional and Industrial Applications of Salvia species: A Revisit. Int. J. Pharm. Sci. Rev. Res., Vol. 43, p. 27-37.

Yaseen M., Kumar B., Ram D., Singh M., Anand S., Yadav H. K., Samad A. (2015). Agro Morphological, Chemical and Genetic Variability Studies for Yield Assessment in Clary Sage (*Salvia sclarea* L.). Industrial Crops and Products, Vol. 77, p. 640-647.

Yaseen M., Singh M., Ram D., Singh K. (2014). Production Potential, Nitrogen Use Efficiency and Economics of Clary Sage (*Salvia sclarea* L.) Varieties as Influenced by Nitrogen Levels Under Different Locations. Industrial Crops and Products, Vol. 54, p. 86-91.

THE NEW RACES OF *PUCCINIA HELIANTHI* SCHWEIN ON SUNFLOWER IN THE RUSSIAN FEDERATION

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Abstract

Over the past two decades, a wide spread of rust on sunflower in the Rostov, Saratov, Krasnodar, and other regions of the Russian Federation was observed. The identification of the racial structure of *P. helianthi* populations in Russia has not been done since the early 80s of the last century. At that time, races 100 and 300 were identified in Russia. In our recent study, we identified six races among the isolates of the rust pathogen collected between 2017 and 2019. In addition to the indicated races 100 and 300, we identified, for the first time, the new races: 700, 710, 722 and 772. The aim of this study was to determine the racial identity of 160 *P. helianthi* isolates collected in the period 2018-2020 in the Rostov, Saratov, and Krasnodar regions. We used eight standard differentiating lines of sunflower: SM-90, SM 29, R-386, HAR-1, HAR-2, HAR-3, HAR-4 and HAR-5. We used also the sunflower variety VNIIMK 8883 as a differentiator susceptible to all races of the pathogen. In addition to 6 races mentioned above, we identified 11 more new races for the first time: 304, 351, 352, 364, 704, 736, 740, 741, 745, 760 and 762. Race 700 prevailed among the isolates collected in the Rostov region in 2020; we also identified single specimens of races 760, 762. Thereby, we found 17 races of *P. helianthi* on sunflower in three regions of the Russian Federation. It is possible that other races are also present here, so further research is required.

Keywords: *sunflower, rust, Puccinia helianthi, races, regions.*

Introduction

The rust pathogen is an obligate parasite of sunflower. As a result of its vital activity, the fungus dries up the leaves, which has a negative effect the productivity and quality of the seeds of the crop (Sackston, 1962; Yang *et al.*, 1986; Gulya, 1990; Gulya, Maširević 1995). Yield losses can reach 60-80 % depending on weather conditions and the stage of plant development (Sackston, 1962; Markell *et al.*, 2009). Rust is common in all countries cultivating sunflower, including Russia (Gulya, 1997; Sendall *et al*., 2006; Friskop *et al*., 2012; Qi *et al*.,2011; Vypritskaya, 2015; Detsyna *et al*. 2018) (Fig. 1).

Figure 1. The sunflower affection by rust in the Krasnodar region in 2019 (orig).

The spread of the disease is directly related to the emergence of virulent races of the pathogen. The racial composition of *Puccinia helianthi* Schwein is not constant; sexual and asexual reproduction inherent in the pathogen, along with mutations, which leads to the rapid emergence of new virulent pathotypes It is known that in the USA in 2008-2009 there were found 38 races of rust pathogen. Of these, race 336 was predominant and race 777 was the most virulent. In 2011-2012, 29 races were identified among the samples from seven US states and one province of Canada. Among them, pathotypes 300, 304, and 324 were prevalent. There were differences in the occurrence of races by year, for example, in 2011, race 300 was prevalent in the sample of isolates, while in 2012 race 304 had the prevalence. The presence of races 324, 364, 704, and 736 in the sample of isolates of 2012 also increased (Jing et al., 2015; Friskop and Markell, 2016). There have also been significant changes in the racial structure of the rust pathogen population in Argentina. Since 1985, the physiological races of *P. helianthi* with virulence codes 100 and 300 have not been found there, but pathotypes 700, 704, 740, 744, 760 have been identified (Moreno et al., 2012). Our earlier studies showed that in three regions of the Russian Federation - the Saratov, Lipetsk, and Krasnodar regions, - we found 6 races of the sunflower rust pathogen. Of these, four races, - 700, 710, 722, and 772, - are highly virulent and have been identified in the Russian Federation for the first time. We also found the old races 100 and 300 (Antonova et al., 2020). This situation could arise under the influence of a combination of factors, such as: a widespread decrease of the time of return of a crop to its former place against the background of an increase in its crop acreage, as well as global warming. The monitoring of the racial structure of populations of the rust pathogen is a necessary condition for successful breeding in Russian Federation of sunflower varieties and hybrids resistant to this pathogen.

The aim of the study: to determine the racial identity of 160 P. helianthi isolates collected on sunflower fields in the Krasnodar and Saratov regions in 2018-2020, and in the Rostov region in 2020.

Materials and methods

We collected the leaves affected by rust from the sunflower plants of different genotypes in the Krasnodar, Saratov and Rostov regions and stored them in a refrigerator (+4 -+6 ^{o}C). The seeds of differentiating lines of sunflower resistance (SM 90, SM 29, R-386, HAR-1, HAR-2, HAR-3, HAR-4, HAR-5) and variety VNIIMK 8883, which was used as susceptible to all races of the pathogen, were sown in rows in flower boxes with a soil capacity of 6 kg. The boxes were placed in a climate chamber, where the plants were grown at a temperature of 23-25 ^{o}C in the daytime and 20 °C in the night-time with a 16-hour photoperiod until the appearance of the second pair of true leaves. Watering was carried out daily. The samples of leaves affected by rust (with uredinia) were placed in a humidity chamber for 24 hours to renew sporulation. Then the spores were washed off with a brush into distilled water. The amount (106 thousands) of urediniospores in the suspension was counted by using a Goryaev camera. Their optimal concentration for the affection of susceptible sunflower plants by 100 % should be 100 to 110 thousand pieces in 1 ml of water (Slyusar, 1981). The plants that formed the second pair of true leaves were sprayed with the prepared suspension and the boxes with them were placed in a humidity chamber at a temperature of 20-21 °C for 24 hours. Then they were returned to their previous conditions until the appearance of infection signs on the leaves. The incubation period was 9 days at 25 °C. After 9 days, the infection degree of differentiators was analyzed and classified as R (resistant) or S (susceptible) according to their response. The infectious type 3 or more was classified as a susceptible reaction on a 4-point scale proposed by E.E. Geshele (Geshele, 1971).

Results and discussion

There were identified a total of 160 rust pathogen isolates collected during the period of 2018-2020 in the Krasnodar, Saratov, and Rostov regions. Each tested *P. helianthi* isolate affected the differentiators susceptible to it with a degree higher than 3-4 points (Fig. 2).

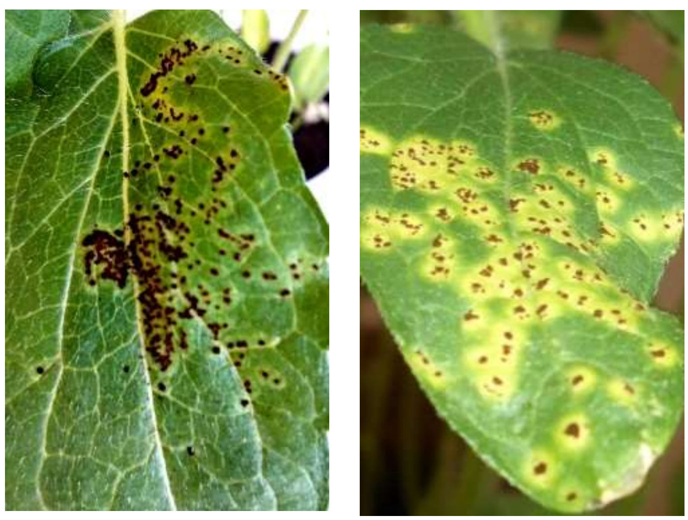

Figure 2. Uredopustules of *P. helianthi* isolate from the Rostovsky region with virulence code 700 on a leaves of differentiating line of sunflower resistance CM 90 (left) and CM 29 (right) (orig).

Among the isolates of the Saratov region collected in 2018, 5 races were identified, of which race 304 was detected for the first time. Races 300 and 700 were prevalent. Race 736 with the highest virulence code was found in a single sample (Fig. 3).

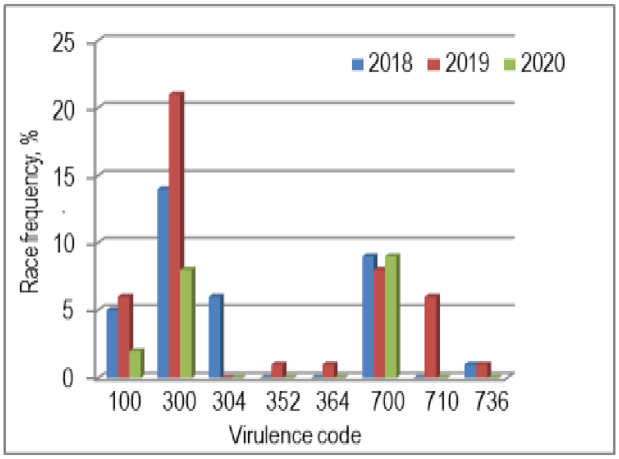

Figure 3. The distribution (%) of races of sunflower rust pathogen in isolate samples from the Saratov region, 2018-2020.

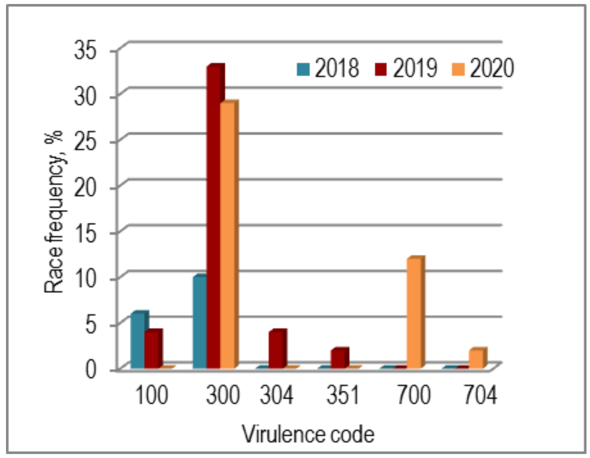

Figure 4. The distribution (%) of races of sunflower rust pathogen in isolate samples from the Krasnodar region, 2018-2020.

In the sample of isolates of 2019, a total of 7 races were identified, of which there were 3 new ones: 352, 364, and 710. Same as in the previous year, race 300 was prevalent, and race 736 was represented by one isolate. Races 700 and 300 dominated the sample of isolates of 2020. Two isolates represented race 100. The isolates collected in 2018 in the Krasnodar region were identified as races 100 and 300, with the prevalence of the latter (Fig. 4), while four races with the prevalence of race 300 as before represented the isolates of 2019. Races 100, 304, and 351 were represented by single isolates. Among these, race 351 was identified for the first time.
Among the isolates collected in the Krasnodar region in 2020, race 300 was also prevalent, but, for the first time in this region, there were identified races 700 and 704.
In the Rostov region, a strong affection of sunflower by rust occurred in 2020. In the sample of pathogen isolates collected there during this period from different sunflower genotypes, 7 races were identified, among which race 700 was prevalent (Fig. 5). For the first time in the Rostov region, there were identified races 740, 741, 745, 760, 762.

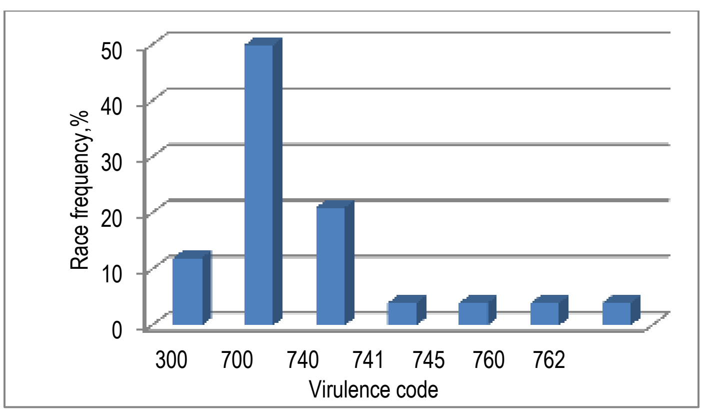

Figure 5. The distribution (%) of races of sunflower rust pathogen in isolate samples from the Rostov region in 2020.

In the combined sample of isolates from the indicated regions of the Russian Federation for 2018-2020, race 300 was prevalent (48 % of samples) (Table 1). Race 700 should also be noted (26 %). The small number of specimens of other races presented in Table 1 requires further monitoring of the frequency of their occurrence.

Table 1. The ratio of identified *P. helianthi* races among the isolates collected on sunflower in three regions of the Russian Federation in 2018-2020.

Region	Number of isolates, pcs.														
	Virulence code of isolate														
	100	**300**	**304**	**351**	**352**	**364**	**700**	**704**	**710**	**736**	**740**	**741**	**745**	**760**	**762**
2018															
Saratov	4	12	5	0	0	0	8	0	0	0	0	0	0	0	0
Krasnodar	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0
2019															
Saratov	5	18	0	0	1	1	7	0	5	1	0	0	0	0	0
Krasnodar	2	17	2	1	0	0	0	0	0	0	0	0	0	0	0
2020															
Saratov	2	7	0	0	0	0	8	0	0	0	0	0	0	0	0
Krasnodar	0	15	0	0	0	0	6	1	0	0	0	0	0	0	0
Rostov	0	3	0	0	0	0	12	0	0	0	5	1	1	1	1
Total number of isolates	16	77	7	1	1	1	41	1	5	1	5	1	1	1	1
The races ratio in the total sample of isolates, %	10	48	4.4	0.6	0.6	0.6	26	0.6	3.1	0.6	3.1	0.6	0.6	0.6	0.6

The fact that in the Russian Federation there are still races 100 and 300, identified in 1962 and 1981 of the last century (Sackston, 1962; Slyusar, 1981) can be explained by the wide cultivation of domestic varieties of both oil and confectionery sunflower in addition to hybrids of various origins. At the same time, the selection for rust resistance has not been carried out since 1983. As it is known, the physiological races of obligate parasites of agricultural crops disappear only with the removal from the cultivated range of crops of varieties and hybrids that feed them. Sunflower varieties that have not been bred for rust resistance can be called reserves for the preservation of old races of the pathogen in the Russian Federation.

In the last three decades, foreign-bred sunflower hybrids have been widely cultivated in the Russian Federation. It is logical to assume that the pathotypes of the rust pathogen in the Russian Federation will have the same triplet code as in other countries where a similar range of sunflower is grown. Thus, race 304 found in the Saratov and Krasnodar region was already present in several US states in 2011-2012 (Friskop et al., 2012). In the same period, races 700, 704, and 760 already existed not only in the United States but also in Argentina (Moreno et al., 2012). The identification of race 700 (26 % of the entire sample of isolates) in the three studied regions of the Russian Federation indicates the already significant prevalence of this pathotype in

the southern agrocenoses of Russia. In general, the racial identity of Russian isolates of the fungus is diverse, although the most virulent biotype 777 which was identified in the United States in 2008-2009 has not yet been found here.
The virulence diversity of *P. helianthi* isolates from the studied regions may be related to the growing intensification of sunflower cultivation with the widespread use of foreign-bred hybrids as sowing material, as well as with changes in climatic conditions. The races ratio in the total sample size may depend, in particular, on the number of isolates and varietal diversity of host plants from which the affected leaves were collected. The problem requires an elaborate approach to further study the ratio of rust pathogen pathotypes in the regions of the Russian Federation. Regular monitoring of the racial structure of the rust pathogen populations is required, both in the indicated regions of sunflower cultivation, and in others.

Conclusions

Thus, among the total studied sample of isolates of sunflower rust pathogen, collected in three regions of the Russian Federation in 2018-2020, we identified 17 races, races 300 (48 %) and 700 (26 %) prevailed. For the first time, there were identified 11 new pathotypes with the code numbers: 304, 351, 352, 364, 704, 736, 740, 741, 745, 760, 762.

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References

Antonova T., Araslanova N., Iwebor M., Saukova S., Pitinova Y. (2020). New races of *Puccinia helianthi* Schwein – sunflower rust pathogen in the Russian Federation. Vestnik of the Russian Agricultural Science, No 5, pp. 23-26. In Russian.

Detsyna A., Tereshchenko G., Illarionova I. (2018). The occurrence of rust on sunflower varieties in the conditions of the Krasnodar region. Oil crops. Scientific and technical bulletin of VNIIMK, Issue 2 (174), pp.101-106. In Russian.

Friskop A., Gulya T, Jordahl J., Ramsett M., Harveson R., Acevedo M., Markell S. (2012). Determination of *Puccinia helianthi* races in the Unites States Northern Great Plains. In: Proceedings of the 18th International Sunflower Conference, Mar del Plata & Balcarce, Argentina February 27–March 1, 2012, pp. 214–218.

Friskop A. Markell S (2016). Rust. pp. 23-25. In: R.M. Harveson S.G. Markell C.C. Block T.J. Gulya (eds.) Compendium of Sunflower Diseases and Pests. The American Phytopathological Society. St. Paul., Minnesota, U.S.A.

Jing L., Xu X., Jing J., Li L. Navi S. (2015). Determination of physiological races and evaluation of sunflower for resistance to *Puccinia helianthi* Schw. J. Phytopathol. 163 : 507-515.

Geshele E. (1971). The infectious process and rust pathogenesis. In the book Methodological guideline for phytopathological evaluation of cereals. Odessa, pp. 50-55. In Russian

Gulya, T. (1990). The sunflower rust situation in 1989. p. 106. In: Proc. Sunflower Research Workshop. Fargo, ND. 8-9 January 1990. National Sunflower Assoc., Bismarck, ND.

Gulya T. (1997). Sunflower rust races in the United States in 1996. Phytopathology, 87:S36.

.

Gulya T., Maširević S. (1995). Proposed methodologies for inoculation with *Puccinia helianthi* and for disease assessment. The FAO European Research Network on Sunflower. Bucharest, Romania, 25.-28.07.1995. pp. 31-48, M-33.

Markell S., Gulya T., McKay K., Hutter M., Hollingsworth C., Ulstad V., Koch R., Knudsvig A. (2009). Widespread occurrence of the aecial stage of sunflower rust caused by *Puccinia helianthi* in North Dakota and Minnesota in 2008. Plant Disease, vol. 93 (6), pp. 668-669. https://doi.org/10.1094/PDIS-93-6-0668C

Moreno P., Romano A., Romano M., Vergani P., Sposaro M., Bulos M., Altieri E., Ramos M., Sala C. 2012. A survey of physiological races of Puccinia helianthi in Argentina. In: Proceedings of the 18th International Sunflower Conference, Mar del Plata & Balcarce, Argentina February 27–March 1, 2012, pp. 278-281.

Qi L, Gulya T., Seiler G., Hulke B., Vick B. (2011). Identification of resistance to new virulent races of rust in sunflowers and validation of DNA markers in the gene pool. Phytopathology. 101. pp. 241-249.

Sackston. W. (1962). Studies on sunflower rust. III. Occurrence, distribution, and significance of races of *Puccinia helianthi* Schw. Canadian Journal of Botany, 40:1449-1458.

Sendall B., Kong G., Goulter K., Aitken E., Thompson S., Mitchell J., Kochman J., Lawson W., Shatte T., Gulya T. (2006). Diversity in the sunflower: *Puccinia helianthi* pathosystem in Australia. Australasia Plant Pathol. 35:657-670.

Slyusar E. (1981).The races of sunflower rust. The plant protection, № 11. p. 42. In Russian.

Vypritskaya A. (2015). Mycobiota of sunflower in the Tambov region: monography, Print-Service, Tambov. In Russian.

Yang S.,. Antonelli E., Luciano H., Lucinai N. (1986). Argentine and Australian sunflower rust differentials to four North American cultures of *Puccinia helianthi* from North Dacota. Plant Disease, 70 : 883-886.

CONTROL OF ELATERIDAE AND *GRYLLOTALPA GRYLLOTALPA* IN THE TOMATO CROPS

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Abstract

In order to obtain high and stable yields of tomato, one of the very important factors, is successful protection in the initial stage of the growing period against harmful insect species like Elateridae and *Gryllotalpa gryllotalpa*, which is achieved by insecticides application. The aim of the paper was to test the efficacy of insecticide tefluthrin in tomato protection against mentioned pests. The field trials were conducted in 2020 in the region of Vojvodina province at the localities Ruski Krstur (RK), Crvenka (C), Šajkaš (Š) and Budisava (B) on tomato crops, variety Amerikanac and Alparac, according to standard OEPP methods (PP 1/46; 1/152; 1/135). Insecticide based on tefluthrin (5 g a.s./kg, GR) at the rate of 12 and 15 kg/ha was applied, simultaneously with planting tomatoes. Plot size was 160 m^2 per variant and consisted of four replications. The effect of insecticide was derived based on the number of plants, from the four central rows, in the distance of 10m, as well as the number of damaged plants from Elateridae larvae and *G. gryllotalpa*. The first assessment was performed after 15 days of tomatoes planting. Efficacy (E%) of the insecticide was determined according to Abbott, while the significance of differences was evaluated by the LSD test (5%) by ANOVA. At the locality RK, insecticide treatment provided 18-27.9% higher number of plants in comparison to the control. The number of plants damaged by Elateridae and *G. gryllotalpa* was significantly reduced by insecticide use (1.5-5.3%) with regard to the control (18.9%). Efficacy of the tefluthrin was 71.9-92.1%, depending on the applied amount. Also, in the C locality, a significant reduction in the number of plants damaged by soil pests compared to the control (10.6%) was established after the use of tefluthrin (0.7-1.5%), while efficacy was in the range of 85.8-93%. At the locality Š, a significant reduction of the damaged plants percentage (0-1.5%) was registered compared to the control (8.5%), while 15 days after the treatment efficacy was in the range of 81.9-100%. A slightly lower reduction of damaged tomato plants was obtained by the use of tefluthrin in locality B where the efficiency was 72.8-83%, depending on the applied amount.

Key words: *tomato, Elateridae, Gryllotalpa gryllotalpa, tefluthrin.*

Introduction

Tomato (*Solanum lycopersicum* L.) represents one of the most common and economically important vegetables, grown in open fields and greenhouses. With an area of 20,000 ha (7% of total vegetable production) and a yield of 180,000 t, tomato is among the leading vegetable crops in the Republic of Serbia (SBS, 2018). However, average yields per hectare are low, and range around 9 t/ha. The reasons are not affected by the genetic potential of varieties and hybrids, but an inadequate and outdated growing technology, reduced and unsuitable application of mineral fertilizers and plant protection products (PPPs), as well as in unfavorable economic

circumstances (Takač et al., 2007). Application of chemical pesticides still takes the leading place in tomato protection. Intensive vegetable production with high yields is hardly sustainable without the use of pesticides. Pesticides should be used in a targeted manner, in order to control one or a group of pests, when other possible measurements have been exploited and when there is a threat of economically significant damage. In previous years, a large number of soil insecticides based on various active substances (methiocarb, chlorpyrifos, chlorpyrifos + imidacloprid, zeta-cypermethrin) were registered in the Republic of Serbia. However, after the ban of some of them, currently for the control of soil pests in vegetables, including tomato, insecticides based on tefluthrin and zeta-cypermethrin are used, with the remark that the use of zeta-cypermethrin is prohibited starting November 2021. The most destructive soil pests for tomatoes include *Gryllotalpa gryllotalpa* L. and larvae from the fam. Elateridae and Scarabeidae. European mole cricket (*G. gryllotalpa* L.) is an insect with high polyphagia which is harmful to many types of vegetables, as well to certain root crops. Also, larvae fam. Elateridae (beetles, wireworms) are one of the most important soil, polyphagous pests in agriculture. Elateridae are widespread in this region, so knowing their life cycle and adequate control is key to the survival of numerous crops. Mature larvae cause the greatest damage during hot and dry weather. The presence of wireworms is recognized by the perforated or completely bitten plants, which wither and dry. White grubs, larvae belonging to the Scarabaeidae family, periodically (every 2-3 years) attack the underground organs of tomatoes and endanger the survival of the entire plant by feeding on the roots. Other pests of tomatoes that damage the aboveground parts of tomatoes include: cotton bollworm (*Helicoverpa armigera* Hübn.), Aphids (Aphididae), tomato leafminer (*Tuta absoluta* Meyrick) and southern green stink bug (*Nezara viridula* L.) (Kereši et al., 2018).
The aim of this study was to test the effects of tefluthrin-based insecticides (5 g a.s./kg GR) for the control of soil pests under field production at four different localities, according to standard OEPP methods, as well as to determine the effectiveness of the mentioned insecticide in controlling the beetle larvae (*Agriotes* spp, Elateridae) and European mole cricket (*Gryllotalpa gryllotalpa*) in tomato.

Material and Methods

The experiments were conducted in the Vojvodina Autonomous Province (Serbia) at the four localities: Ruski Krstur, Crvenka, Šajkaš and Budisava, and were set up according to standard OEPP methods for the experimental design and data analysis (Anonimus, 2012), for the efficacy of insecticides in soil pest control (Anonimus, 2004) and for the phytotoxicity of the tested pesticides (Anonimus, 2014). The experiment was performed in 4 replications, while the type of experiment was a completely random block system. The size of the plot was 35 m² (6 rows x 0.7 x 10 m), the distance between the plants in the row was 30 cm. The application of insecticides was performed simultaneously with the planting: in Ruski Krstur and Crvenka, the planting was done on May 29, 2020., and in Šajkaš and Budisava on 05.06.2020. The PPP based on teflutrin (5 g a.s./kg GR) was applied in the amount of 12-15 kg/ha, by manual scattering of granules in previously made furrows, and immediately after that, the planting of tomatoes (American and Alparac variety) was done manually, followed by furrows burring. One assessment of the effects was performed in all tested localities, considering that the production of tomatoes was from seedlings, not from sowing. The assessment was performed 13, 15 and 16 days after planting, depending on the locality. The total number of plants was monitored, as well as the number of damaged plants from pests in the soil, in four central rows 10 m long. Standard deviation (Sd+), the significance of differences (LSD 5%) (ANOVA), relative values in relation to control (K=100%), and efficacy according to Abott were determined (Wentzel, 1963).

Results and Discussion

The results on the influence of teflutrin - based pesticide on the average number of plants per 10 m in length, as well as the number of damaged plants and the efficiency of insecticides at the tested localities are given in Tables 1-3. At the locality of R. Krstur, the average number of tomato plants per 10 m ranged from 25.8 to 33 (Table 1) in all examined variants. Regardless of the applied amount, the test plant protection product provided a significantly higher number of plants from 18.2 to 27.9% (Table 2) compared to the control. A significant reduction in the number of damaged plants from click beetles larvae and mole cricket compared to the control was found when using teflutrin in both tested amounts (Table 3). The efficiency of the tested insecticide ranged 71.9-92.1, depending on the applied amount. The average number of tomato plants at the locality Crvenka ranged from 29.0 to 33.8 in all tested variants (Table 1). The tested insecticide provided from 12.1 to 16.5% (Table 2) a higher number of plants compared to the control regardless of the amount applied. The insecticide tefluthrin applied in the recommended amounts provided a significant reduction in the number of damaged plants from click beetles larvae and mole cricket compared to the control (Table 3). The efficacy of the tested insecticide ranged from 85.8-93%.

Table 1. Average number of tomato plants per 10 m

Insecticide (kg/ha)	**Locality**			
	R. Krstur	Crvenka	Šajkaš	Budisava
	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd
tefluthrin (12)	**30.5**±1.29 b	**32.5**±1.73 b	**32.0**±1.82a	**30.8**±1.71a
tefluthrin (15)	**33.0**±0.82a	**33.8**±0.58a	**33.2**±0.96a	**32.3**±0.96a
control	**25.8**±0.85 c	**29.0**±1.41 c	**30.0**±0.82 b	**28.8**±0.96 b
LSD 5%	1.79	1.41	1.98	1.78

$\bar{x}$ – average number; ±Sd - standard deviation.

Table 2. Percentage of plants compared to control

Insecticide (kg/ha)	**Locality**			
	R. Krstur	Crvenka	Šajkaš	Budisava
	K 100%	K 100%	K 100%	K 100%
tefluthrin (12)	118.2	112.1	106.7	106.9
tefluthrin (15)	127.9	116.5	110.6	112.2
control	100.0	100.0	100.0	100.0

K 100% - relative value compared to control.

In the field at the Šajkaš locality, the average number of tomato plants per 10 m in length ranged from 30-33.2 (Table 1). Regardless of the amount applied, the tested PPP provided from 6.7 to 10.6% (Table 2) a higher number of plants compared to the control. After the application of tefluthrin, the number of damaged plants from the larvae of the click beetle (Agriotes spp.) and the mole cricket was significantly lower compared to the control (Table 3). The efficacy of the tested insecticide ranged from 81.9 to 100%, depending on the applied amount. The average number of tomato plants at the Budisava locality ranged from 28.8 to 32.3 in all tested variants (Table 1). Regardless of the amount applied, the tested insecticide provided from 6.9 to 12.2% (Table 2) a higher number of plants compared to the control. Also in the Budisava locality as in the previous localities, the applied insecticide provided a significant reduction in the number of damaged plants from the larvae of the click beetle and the mole cricket compared to the control (Table 3).

Table 3. Number of damaged tomato plants from the larvae of the click beetle and the mole cricket and efficacy of tested insecticide

Insecticide (kg/ha)	**Locality**							
	R. Krstur		Crvenka		Šajkaš		Budisava	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E %	$\bar{x}$ ±Sd	E %
tefluthrin (12)	1.75±1.26 a	**71.9**	0.50±0.58a	**85.8**	0.50±0.58 a	**81.9**	1.25±0.58 a	**72.8**
tefluthrin (15)	0.50±0.82 a	**92.1**	0.25±0.50a	**93.0**	0.00±0.00 a	**100**	0.75±0.96 a	**83.0**
control	6.25±1.5 b	/	3.50±1.29 b	/	2.75±0.9 b	/	4.50±1.2 b	/
LSD 5%	1.24		1.49		1.38		1.42	

$\bar{x}$ – average number; +Sd - standard deviation; E %- efficacy.

The application of tefluthrin-based PPP in amounts of 12 and 15 kg/ha in the localities R. Krstur, Crvenka, Šajkaš, and Budisava did not cause phytotoxicity, nor changes were registered in the form of deformations or leaf color on tomato plants of the American and Alparac varieties. Based on the available literature, there are no data on the occurrence of soil pest resistance to tefluthrin. However, in insect populations that are intensively controlled with insecticides based on tefluthrin or other insecticides from the group of pyrethroids, a change in sensitivity is possible, therefore the management of the anti-resistance strategy should be followed. Based on the study of Van Herk et al. (2008), larvae of *Agriotes obscurus* and *Limonius canus* were exposed to wheat seeds previously treated with insecticides based on imidacloprid and tefluthrin. Most larvae (> 80%) came into contact with seeds in both treatments. Teflutrin in contact with larvae (after <20 min) in all variants exhibited a repellent effect unless the larvae had previously died. Wireworm mortality was low in both variants (<50%). Hall (2003) conducted a biological test to assess the effects of tefluthrin and bifenthrin-based insecticides with the aim of controling *Melanotus communis* (Gillenhal). The tested insecticides significantly reduced the damage to the plants, which is in accordance with our study. The effect was primarily reflected as the repellent activity to wireworms of the tested PPP.

Conclusions

Based on the conducted study and the achieved results on the efficacy of product based on tefluthrin in tomato under field production with the aim of controlling the larvae of *Agriotes* spp., Elateridae and *G. gryllotalpa*, the following conclusion can be drawn:

- At the locality of Ruski Krstur, 15 days after planting, the product based on tefluthrin showed satisfactory efficacy (71.9-92.1%) and provided a significant increase in the number of plants from 18.2 to 27.9% compared to the control.
- At the Crvenka locality, 16 days after planting, the tested insecticide showed an efficiency of 85.9 to 93%, depending on the applied amount, and also provided a significant increase (12.1 - 16.5%) in the number of plants compared to the control.
- Thirteen days after planting and application of insecticides at the Šajkaš locality, tefluthrin showed high efficacy of 81.9-100%, and provided 6.7 to 10.6% more plants compared to the control.
- At the Budisava locality, 13 days after planting tomatoes and applying insecticides, efficacy ranged 72.8-83%. More plants (6.9 to 12.2%) were found compared to the control.

The insecticide tefluthrin, at all localities, provided satisfactory protection of tomato crops against the larvae of the click beetle (*Agriotes* spp., Elateridae) and the mole cricket (*G. gryllotalpa*).

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References

Anonimus (2004). Wireworms PP1/46(2). EPPO Standards, Guidelines for the Efficacy Evaluation of Plant Protection Products, 3, Insecticide and Acaricides, OEPP/EPPO, Paris, 71-74.

Anonimus (2012). Design and analysis of efficacy evaluation trials, PP 1/152(4), EPPO Bulletin, 42 (3): 367–381.

Anonimus (2014). Phytotoxicity assessment, PP 1/135(4), EPPO Bulletin, 44 (3): 265-273.

Hall, D. G. (2003). Laboratory Screening of Insecticides for Preventing Injury by the Wireworm Melanotus Communis (Coleoptera: Elateridae) to Germinating Sugarcane. Journal American Society of Sugarcane Technologists, 23, 8-19.

Kereši, T., Sekulić, R., Konjević, A. (2018). Special entomology 1 (Part - Insects in agriculture). University of Novi Sad, Faculty of Agriculture, Novi Sad.

RZS (2018). Republički zavod za statistiku, Republika Srbija. https://www.stat.gov.rs/sr-Latn/

Takač, A., Gvozdenović, Đ., Bugarski, D., Červenski, J. (2007). Modern tomato production. Institute of Field and Vegetable Crops Novi Sad.

Wentzel, H. (1963). Pflanzenschutz nachrichten Bayer. The basic Principles of Crop Protection Field Trials, 1963/3.

Van Herk, W. G., R. S. Vernon, B. Roitberg, D. (2008). Repellency of a Wireworm, *Agriotes obscurus* (Coleoptera: Elateridae), on Exposure to Synthetic Insecticides in a Soil-Less Bioassay, *Environmental Entomology*, 37(2):534–545.

DIFFERENTIATED HERBICIDES APPLICATION ON WINTER WHEAT CROPS USING OFFLINE INSTRUCTIONS MAP

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Abstract

Plant protection measures, including control of weeds, are significant part of production cost. The average annual growth of pesticides world market, during the period from 2010 to 2019, had showed 2.8 % rise , its volume reached in 2019 – 55,650.00 million dollars without VAT, while the Russian market showed an increase of more than three times -10.8 %, the market volume in 2019 was 2081.00 million dollars without VAT. The continuous use of herbicides negatively affects both the economic efficiency for agricultural crops cultivation and the environmental situation. The use of differentiated plant protection technologies, in terms of herbicides application may increase the efficiency of agricultural enterprises, as well as reduce the pesticide pressure on the environment. The research work was carried out on the experimental farm of Perm Agricultural Research Institute – division of Perm Federal Research Center. The experimental plots located on heavy loamy sod-podzolic soil. The object of research was winter wheat, variety "Skipetr". The study showed the effectiveness of differentiated application of herbicides on winter wheat in the Ural region, using the in offline mode. The grain yield reached 5.63 t ha -1 after differentiated application versus 5.10 t ha -1 with conventional technique, and 4.22 t ha -1 at the control. The herbicide pressure on cultivated areas was reduced by 46 % in average, and the economic efficiency of production increased by 40 % compared to areas where overall treatment was carried out with average recommended herbicide doses.

Keywords: *differentiated plant protection, herbicide, winter wheat, precision farming, task map.*

Introduction

Production of grain crops is traditionally the basic branch of agriculture in Russian Federation. Since 2015, the annual rise of grain production in Russia has increased by 15.1 %, in average and the record harvest in 2017 was 131.3 million tons. (ROSBIZNESKONSALTING, 2021) Winter wheat cultivation promoted the most of the gross yield. With production growth, the increase in the production costs also take place. One of the significant cost items of production is the organization of plant protection system, which includes herbicidal treatment of weeds. The average annual growth of the world market of pesticides, in the period from 2010 to 2019, showed 2.8 % rise, its volume reached – 55,650.00 million dollars without VAT in 2019, while the Russian market showed an increase of more than three times-10.8 %, the market volume for 2019 was-2081.00 million dollars without VAT. (Danilov, 2020)

In the conditions of modern agriculture, an urgent task is to increase the economic efficiency of production. Conventional technologies for the use of pesticides and agrochemicals, reckon for the application of the same dose over the field unit, without taking into account the density and level of distribution of pests, diseases, weeds and cultivated plants in its individual elementary areas (Gafurov e*t al.*, 2012).

One of the underestimated ways to reduce costs, as well as to increase environmental safety, is the use of differentiated plant protection (DPP) technologies. DPP is the technique based on the discretization of a biological object, comprehensive analysis of economic harmfulness threshold of negative factor with further decision about the impact on a discrete part of a biological object.

Differentiated application of herbicides in fields with heterogeneous weed infestation reduces herbicide consumption by 30-60 %, increases their payback by 1.3-1.7 times, and reduces the anthropogenic load on the environment. (Shpaar e*t al*., 2003; Gur'yanov and Artem'ev, 2018; Takács-György and Takács, 2009)

The purpose of the study is to evaluate the effectiveness of the technology of herbicides differentiated application on winter wheat using the offline task map.

Materials and Methods

Studies were conducted in 2020 on the basis of the Perm Agricultural Research Institute - the division of Perm Federal Research Center of Ural Branch of RAS, located in Perm Region in Russian Federation. Location of the experiment site: 57°50 '13.8" N 56°18'39.5" E (Fig. 1) The soil of the experimental site is sod-podzolic heavy loam, with following parameters: humus content in the upper soil layer 1.9 %, pH_{KCl}-5.0-5.2, P_2O_5-176-203, K_20-160-169 milligrams per 1000 g of the soil.

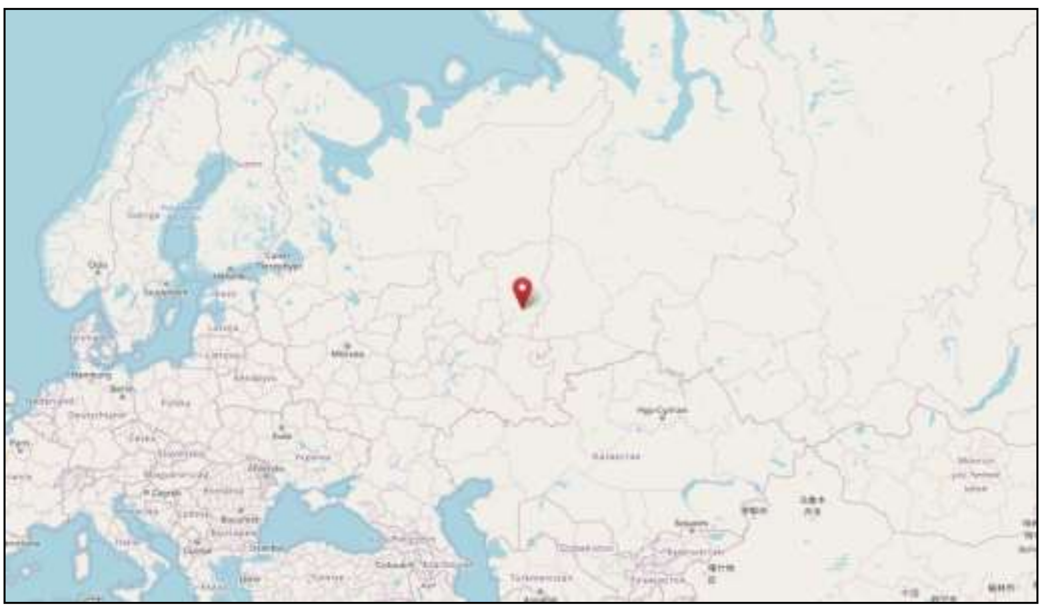

Figure 1. Location of the experiment site.

The technology of crops cultivation is generally accepted for the zone with the exception for studied questions. The predecessor was barley. Phosphorus and potassium fertilizers ($P_{60}K_{60}$) were used in autumn for pre-sowing cultivation, nitrogen fertilizer (urea) – split application (N_{30} before sowing cultivation, N_{30} - in spring as top dressing). The object of research is winter wheat *Triticum aestivum L*., cultivar "Skipetr". The seeding rate is 6 million of viable seeds per hectar. The sowing was carried out by a mechanical grain drill –John Deere 455. The sowing time was 1.09.2019.

Winter wheat "Skipetr" is characterized as a highly adaptive variety of universal type with high frost resistance. It is resistant to lodging on soils with low and medium level of fertility; the plant height is 84-96 cm, it overwinters perfectly in the phase of sprouts and seedlings. This cultivar has a very intense spring regrowth. It is also resistant to spring frosts (up to -12^{o}C).
The application of herbicides was carried out with an OP-2500 sprayer of the ARGO series with on-board computer "Bars 5" set on GAZ-66 vehicle (Fig. 3). The applied herbicide –
"Lintur" - (water-dispersible granules 0,165 kilogram per hectare (dicamba and triasulfuron)".

Figure 3. OP-2500 sprayer based on GAZ-66 vehicle

Experimental design:
1. Control, without herbicides.
2. Water treatment
3. "Lintur" overall application
4. "Lintur" differentiated application, using the task map.

Treatment placing was systematic, each treatment had three replications on the field, The area of each plot was 2.76 hectares, with 48 meters width. The total area of the experiment was 33.18 hectares.
For the purpose of precise positioning the sprayer machine on the ground the digital duplicate of real experimental field was created, where the experimental plots were showed (Fig. 4).

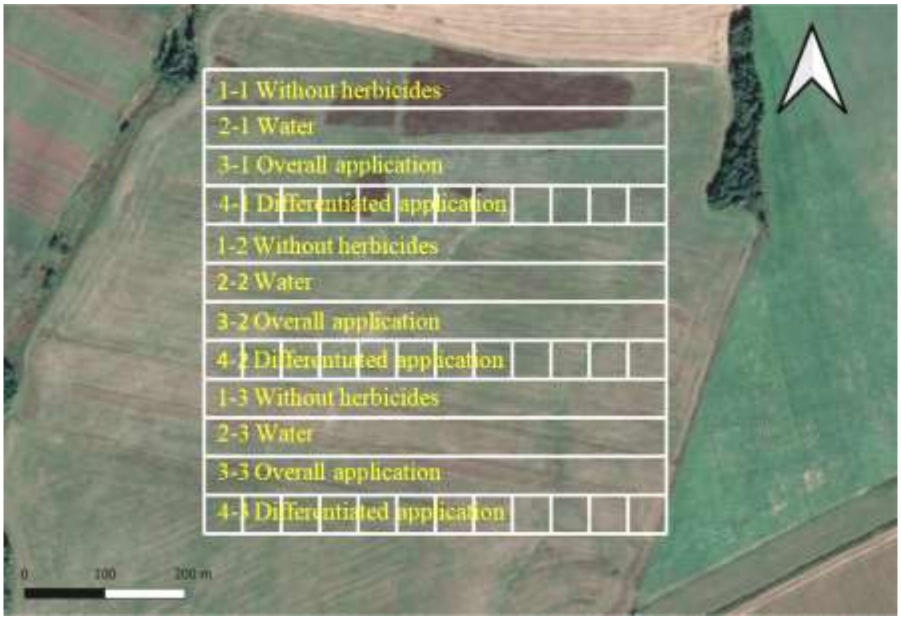

Figure 4. The diagram of the experiment options on the digital duplicate of the field.

Plots for herbicide differentiated application were additionally divided into 12 subplots, 48x48 meters each, where the weed infestation of crops was taken into account according to the numerical method for determining weed vegetation, by a continuous examination of crops and counting weeds on the calculated sites (1x1 meters). The number (A) of weed plants was calculated using the formula (1):

$$\mathrm{A} = \frac{\mathrm{a}}{S}, \qquad (1)$$

where: a - is the number of weeds encountered; S is the total registered area, m2. (Kamenskih and Samofalova, 2012; Fetyuhin e*t al.*, 2018). Analysis of the species composition of weeds was also fulfilled according Atlas of weeds (Syngenta, 2011).

Results and discussion

The weather conditions during the experiment time were following: the average daily air temperature in period "sowing – full sprouts" was 12.6^{o}C (average over the last 70 years temperature 12.5^{o}C); during tillering – 3.8 (multiyear average 11^{o}C), precipitation sum - 14 mm (multiyear average 35.2 mm) (Fig. 2). By the time of low temperatures, the plants reached the tillering phase.

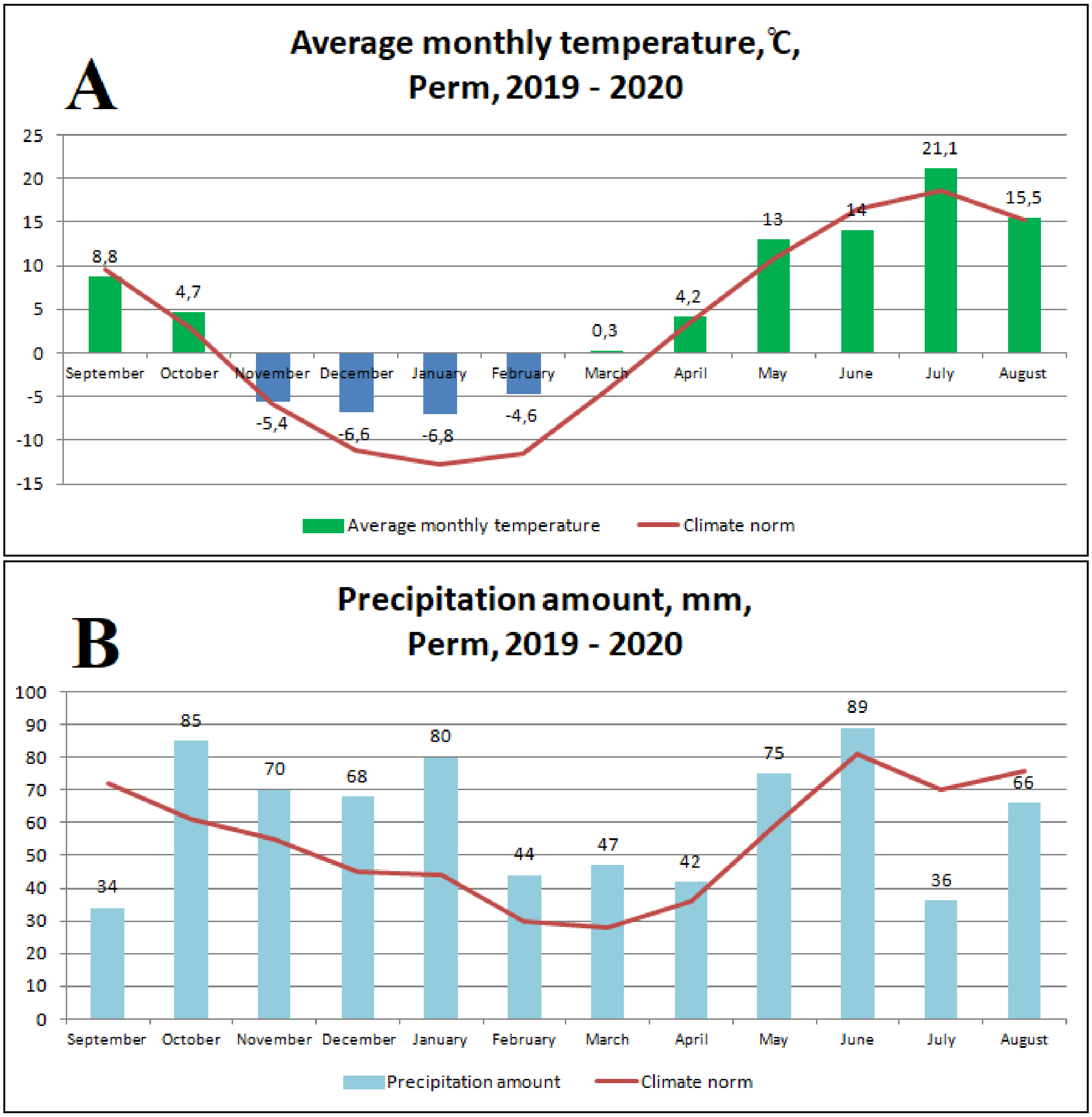

Figure 2. Agro-climatic conditions of the period 2019-2020,
A. average monthly temperature. B. precipitation amount

In the winter period of 2019-2020, the height of the snow cover reached 77 cm. The smelting of snow cover was noted in the first half of the April's decade - two weeks earlier compared with than with multiyear average.

May was characterized by unstable weather. An excess of the multiyear average emperature by 6 °C was observed in the first decade with new absolute maximum 28.2°C .The previous one was 27.7°C and was noted in 1962. In the second decade the decrease by -1...0°C was observed. The average air temperature for the month was 13°C, which is 2.3°C above the multiyear average. Precipitation sum was 127 % of the multiyear average sum. Most of it (65 mm) was noted in the third decade of May.

The summer of 2020 was generally characterized by air temperatures close to usual, with moderate precipitation deficiency. The average temperature of the summer season was higher compared with the multiyear average and 2017-2019 years. In the period from June 6 to June 11 extremely hot weather was observed with average daily temperatures within 19-24°C, that was 4-8°C higher than usual. The second and third decades were cold. The average monthly air temperature in June was 14.1°C, that was 2.5°C below the multiyear average. Precipitation sum for month was 89 mm that was slightly more compared with usual (110 %).

July was characterized by stable weather. The average monthly air temperature was 21.2°C, higher than the multiyear average by 3.1°C. Precipitation sum for the month was 36 mm – two times less compared with usual. The formation of the crop took place under conditions of increased heat and reduced moisture availability. The duration of the growing season was 342 days.

The qualitative and quantitative estimation of weeds on plots destined for differentiated application of herbicide (4-1, 4-2, 4-3), included the determination of weeds species, as well as the economic limit of harmfulness was calculated for the reasoned use of pesticides. After analyzing the results, a task map for the sprayer machine was created.

Analysis of the species composition of weeds in winter wheat crops showed that the most common were:

– dicotyledonous perennial weeds-*Convolvulus arvensis L., Taraxacum officinale Wigg*.), *Vicia cracca L*., (*Sonchus arvensis L*.);

– annual weeds-*Matricaria recutita L.,Chenopodium album L., Capsella bursa-pastoris L., Equisetum arvense L., Galeopsis tetrahit L., Viola arvensis Murr., Galium aparine L., Thlaspi arvense L., Stellaria media L., Geránium pretense.*

The share of perennials in total number of weeds was 9 %, annuals - 91 % (Fig. 5). The average indicator of weed infestation of winter wheat crops in the studied plots was 25 pcs/m^2.

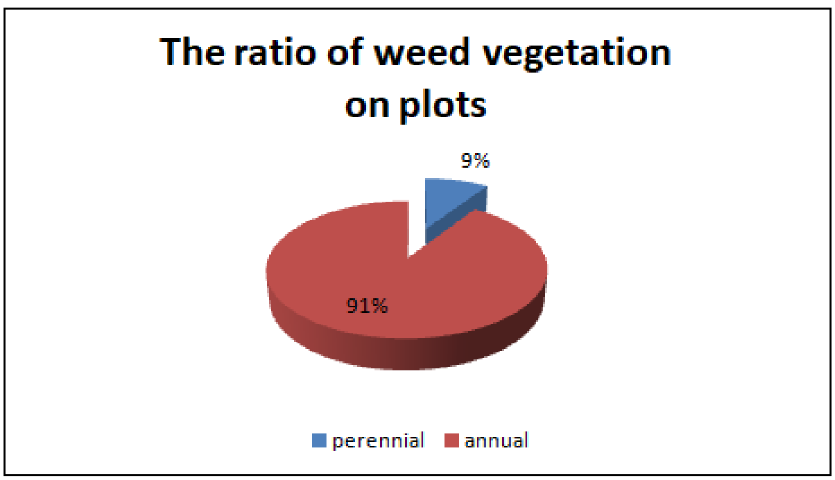

Figure 5. The ratio of weed vegetation on experimental plots.

After analyzing the weed species composition and studying the experience of using herbicides on winter grain crops (Mehmeti et al., 2018; Dvoreckij at al., 2012; Nikiforov et al., 2018), the most effective herbicide was selected - "Lintur, water-dispersible granules". Usual consumption rate of this herbicide is 0.15-0.18 kilogram per hectare, the spray liquid rate is 200-300 litres per hectare.

"Lintur, water-dispersible granules" has two active ingredients: dicamba and triasulfuron. Dicamba is a growth regulator, and triasulfuron inhibits the synthesis of essential amino acids. Both active components penetrate into plant through the root system and leaves.. The result of treatment is stunting, yellowing and necrosis of leaves and stems, with subsequent death of weeds. Symptoms appear on the 5th-7th day, and death occurs 2-3 weeks after treatment, depending on the type of weed and weather conditions. (Syngenta, 2019)

Analyzing the task map shows that the average treated area in the treatment with differentiated herbicide application was 1.46 hectares that was 46% less compared with the area of variant with overall application by the average recommended dose of the herbicide.

The economic assessment of herbicides consists from comparing the treatment cost and profit from the saved harvest. For grain crops, the income is determined only by the cost of saved crop. Herbicides prices, transportation and treatment expenses, staff salaries are taken into account in the consumable part of the assessment (Grin'ko, 2018).

Economic calculations carried out on the basis of the obtained biological crop yield of winter wheat demonstrated that with the herbicide differentiated application the profitability of the production of winter wheat "Skipetr" increased by 40% compared with the overall method. (Table 1)

Table 1. Economic efficiency of winter wheat cultivation.

Variant/ Name of indicator	**Biological crop yield ton per hectare**	**Costs per 1 hectare**	**Costs per 1 ton**	**Cost in rubles**	**Profit in rubles**	**Profitability %**
1.Control	4,22	33105,00	784,00	37600,00	4495,00	14
2.Water treatment	4,13	33066,00	801,00	36798,00	3732,00	11
3. Lintur, overall application, ,	5,1	35864,00	703,00	45441,00	9577,00	27
4. Lintur, differentiated application,	5,63	36293,00	645,00	50163,00	13870,00	38
LSD_{05}	0,31					

Conclusion

The results of fulfilled research work aimed to elaboration the technology of herbicides differentiated application on winter wheat in «offline» regime using the task map in mode allow us to make the following conclusions. Herbicides differentiated application provided the profitability rise by 40 % compared with the overall introduction with average recommended dose of herbicide. The herbicide load on the cultivated area was significantly reduced, including the herbicide consumption was reduced by 46 %. However, it is impossible to say with full confidence that these results will be applicable to other areas of winter wheat cultivation, since the species composition of weed vegetation is different. The research work requires further continuation.

References

Atlas of agricultural weeds, 2011. Syngenta, 117 p.

Danilov M.E. 2020. With us, the crops grow. Online newspaper "Field of August". 9 (203). Available from: https://www.avgust.com/newspaper/arh/detail.php?ID=7390 [Accessed 10th January 2021]

Dvoreckij S.A., Devyatkina T.F., Bochkarev D.V., Smolin N.V., 2012. «Effectiveness of herbicides and growth regulators in winter wheat crops». Journal Volga Region Farmland. № 4. pp. 15-19

Fetyuhin I.V., Avdeenko A.P., Avdeenko S.S., CHernenko V.V., Ryabceva N.A. 2018. Methods of accounting for the structure of the weed component in agrophytocenoses. Training manual. DON STATE AGRARIAN UNIVERSITY. Persianovski. pp. 76

Gafurov R.M., Sheptuhov V.A., Cimbalova V.A., Molodchuev A.A., 2012. «Features of the manifestation of intra-field variability of the quantitative and specific composition of weed vegetation in grain crops». Achievements and prospects of scientific support of the agro-industrial complex of the Central region of Russia. Collection of materials of the scientific and practical conference dedicated to the 80th anniversary of the Moscow NIISH «Nemchinovka». Nemchinovka. pp. 212-219

Grin'ko A.V. 2018. Economic assessment of the use of soil herbicides. Journal «ECONOMY AND BUSINESS: theory and practice». 2. pp. 44-46

Gur'yanov A.M. and Artem'ev A.A. 2018. Assessment of the contamination of agrocenoses and the effectiveness of differentiated use of herbicides in crop rotation. Journal «Agricultural Science Euro-North-East». 5 (66). pp. 83-89

Kamenskih N.Y. and Samofalova I.A. 2012. Development of adaptive landscape system of agriculture. Training manual. STATE EDUCATIONAL INSTITUTION OF HIGHER PROFESSIONAL EDUCATION THE PERM STATE AGRICULTURAL ACADEMY. Perm. pp.127

Mehmeti A., Pacanoski Z., Fetahaj R., Kika A. & Kabashi B. 2018, «Weed control in wheat with post-emergence herbicides», Bulgarian Journal of Agricultural Science, vol. 24, no. 1, pp. 74-79.

Nikiforov V.M., Silaev A.L., CHekin G.V., Smol'skij E.V., Nikiforov M.I., Nechaev M.M. 2018. The use of modern herbicides in the cultivation of spring wheat. VESTNIK of the Bryansk State Agricultural Academy. 1 (65). pp. 23-27

ROSBIZNESKONSALTING. 2021. Grain market in Russia: Exports ensure market balance. Available from: https://marketing.rbc.ru/articles/11446/ [Accessed 21th January 2021]

Shpaar D., Zaharenko A.V., Yakushev V.P. 2009. Precision agriculture. Pavel VOG. Saint Petersburg. pp. 392

Syngenta. 2019. Lintur, EDC. Available from: https://www.syngenta.ru/products/crop-protection/herbicides/lintur [Accessed 14th January 2021]

Takács-György K. and Takács I. 2009, «Economic analysis of precision weed management», Cereal Research Communications, vol. 37, no. 4, pp. 585-593.

INFLUENCE OF SULFUR DIOXIDE AND ASCORBIC ACID ON PHENOLIC ACIDS IN CABERNET SAUVIGNON WINE

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Abstract

Many reactions between O_2 and phenolic compounds or ascorbic acid in the wine induce an rapidly rate of consumption of O_2. If concentration of sulfur dioxide is sufficiently high in wine, the reaction products of O_2 and the wine components are mostly avoided. The impact of addition $K_2S_2O_5$ and ascorbic acid to phenolic composition in wine was investigated. Analysis of Cabernet Sauvignon wine was made by classical vinification and maceration (21 days) inoculated with pure yeast strain *Saccharomyces cerevisiae* (BDX, Lallemand, Canada). After six months of storage, in different samples of experimental wine increasing contents of $K_2S_2O_5$ (3g/hl, 5g/hl and 7g/hl) and ascorbic acid in concentration 200mg/lwere added. Control sample without any addition was the same for both of experiments. Phenolic acids in wines wereanalyzed by UPLC H-Class System.Thestatistically significant difference between control and wine samples added by 5 and 7g/hl of $K_2S_2O_5$for derivates of benzoic acidwas observed. Addition of $K_2S_2O_5$in all of added concentrations did not significantly changed content of derivates of cinnamic acids. SO_2 has an excellent abilitystabilise hydroxycinnamic acid polyphenols such as caffeic acid when exposed to conditions of oxidation. Influence of ascorbic acid wasnot statistically significant for content of benzoic and cinnamic acidsderivatesin wine. Among phenolic acids the most susceptible to oxidation are those containing an ortho-diphenol functional group, including caffeic acid and its derivatives and compounds with a triphenol group such as gallic acid.

Keywords: *Sulfur dioxide, Ascorbic acid, Phenolic compounds, Red wine.*

Introduction

Sulfur dioxide (SO_2) has been used for centuries by winemakers as a preservative due to its antimicrobial and antioxidant properties (Waterhouse, 2016). The central role of oxygen in the process of wine maturation has been long known. In recent years, a number of studies have described the influence of oxygen exposure on wine chemical and sensory characteristics, including changes of wine aroma, color and mouthfeel (Ferreira et al., 2015). Even in wines without SO_2 additions, low amounts of total SO_2 (typically 10–20 mg/L) are present at the end of alcoholic fermentation as a result of SO_2 formation during amino acid biosynthesis. However, in most wines, the majority of SO_2 is added exogenously either before or after fermentation, often in the form of potassium metabisulfite ($K_2S_2O_5$) or as SO_2 gas. Free bisulfite can be lost following wine oxidation, either by reaction with H_2O_2 or by forming adducts with acetaldehyde or other electrophiles. SO_2 adduct formation is reversible and thus formation and dissociation reactions are happening continuously (Waterhouse, 2016). The mechanisms of wine antioxidant

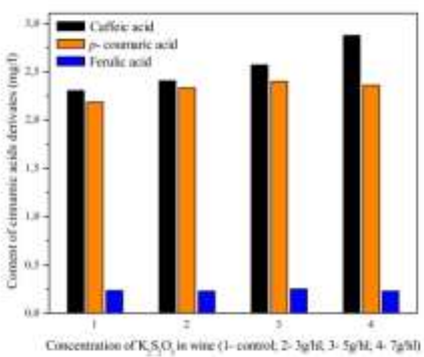
protection by SO_2 does not involve direct reaction with oxygen as long believed, but involves reaction with the quinones and hydrogen peroxide avoiding the generation of harmful hydroxyl radicals(Ferreira et al., 2015). The initial steps of oxygen consumption requires the presence of transition metal catalysts and good hydrogen donor molecules, particularly Fe(II) and *o*-diphenols. Indirect oxidation of *o*-diphenol (along with transition metals such as Cu or Fe) produces quinones and H_2O_2. Quinones are strong electrophiles that will then react with bisulfites and thus stop oxidation. If there was not enough bisulfite in the wine to bind the formed H_2O_2, Fe (II) -catalyzed Fenton reactions occurred which form aldehydes and other oxidized forms that can damage wine quality (Waterhouse, 2016). The oxidation of ascorbic acid generates dehydroascorbic acid and hydrogen peroxide. While hydrogen peroxide can lead to damaging spoilage reactions, especially in the presence of metal ions, dehydroascorbic acid is an unstable compound and is known to degrade into a wide range of products. Presence of sulfur dioxide is critical to ensure efficient scavenging of hydrogen peroxide and sulfur dioxide is recommended for use as a complementary antioxidant when utilising ascorbic acid especially in white wines (Barril et al., 2012).
Polyphenols also cannot react directly with oxygen in its normal triplet state. It is therefore proposed that transition metals, such as Fe and Cu, also play a key role in oxidative processes involving wine polyphenols. Phenols form complexes with metal ions such as Fe(III) and electron transfer occurs within the complex to reduce iron to the ferrous state and to oxidize the phenol to the semiquinone and than quinone. During that process oxygen is converted to hydrogen peroxide, which in the presence of Fe or Cu produce hydroxyl radicals. As mentioned above an action of SO_2 is to scavenge hydrogen peroxide, so preventing its destructive effect. Of the polyphenols found in wine, those containing a catechol system are the most readily oxidized, (Danilewich et al., 2007).
The combined use of ascorbic acid and sulfur dioxide therefore requires considerable care as oxidation of ascorbic acid leads to a loss of sulfur dioxide that, in turn, leads to a reduction in the main anti-microbial component. Ascorbic acid can act as a pro-oxidant leading to elevated levels of oxidative spoilage, even in the presence of sulfur dioxide (Bradshaw et. al., 2004). According to Bradshaw et. al. (2004) addition of ascorbic acid to a wine-like system resulted in an increased rate of browning of (+)-catechin, the oxidisable polyphenolic compound used in the wine model system.
The main focus of this paper is to investigate influence of addition $K_2S_2O_5$ and ascorbic acid to wine phenolic composition specifically benzoic and cinnamic acids derivates.

Material and Methods

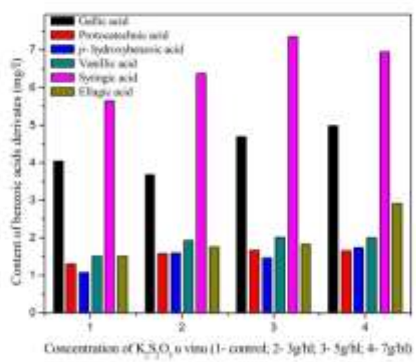
The experiments were performed in laboratory conditions on volume of 200 ml of wine, that was produced in winery on the experimental field of Agriculture faculty, Radmilovac in Belgrade. Vinification with maceration lasted 21 days with EXV enzyme preparation (Lallemand, Canada) and inoculation with wine selected yeast BDX (Lallemand, Canada) and punching down twice a day. During the destemming and crashing grapes, the must was sulphited with 10 g of $K_2S_2O_5$ per 100 kg of must. During wine maturation the level of free SO_2 was about 30mg/l. In this research that wine was control sample which was compared with same wine with addition of increasing concentrations of $K_2S_2O_5$ (3g/hl, 5g/hl and 7g/hl). Other part of experiment was related with addition of 200mg/l in same produced wine. Before addition of ascorbic acid, lever of free SO_2 was adjusted to 30 mg/l.

Results and Discussion

The statistically significant difference between control and wine samples added by 5 and 7g/hl of $K_2S_2O_5$ for derivates of benzoic acid was observed. Addition of $K_2S_2O_5$ in all of added concentrations did not significantly changed content of derivates of cinnamic acids ($p \geq 0,05$). According to Gabriele et al. (2018), the largest increase of caffeic acid was observed passing from the intermediate sulfur dioxide values to higher values, up to 150mg/l that is in agreement with our results where caffeic acid in sample with the highest SO_2 content has the highest value (2,87mg/l). In most cases, moving from 0 to 50 mg/L SO_2, which is a relatively low level of sulfur dioxide, the increase in concentration of the antioxidant compounds is less pronounced (Gabriele et al., 2018). In Merlot wine samples with addition 50, 100 i 200 mg/l SO_2 did not noticed significant difference for caffeic acid comparing with control (Tao et al., 2007). In our samples there was a slight increase in the content of ferulic and *p*-coumaric acid starting from wine with 30 mg/l SO_2 to wine with 50 mg/l SO_2. In the literature, it was found that the concentration of *p*-coumaric and ferulic acid is slightly higher in wines with a content of about 50 mg/l than in wines without added SO_2 (Garaguso and Nardini, 2015). Syringic acid concentrations as well as other benzoic acid derivatives increased with increasing SO_2 concentrations ($p \leq 0,05$). A higher concentration of syringic acid and gallic acid was found in wines with 50 mg/l than in wines that do not contain SO_2 (Garaguso and Nardini, 2015; Pateraki et al., 2014).

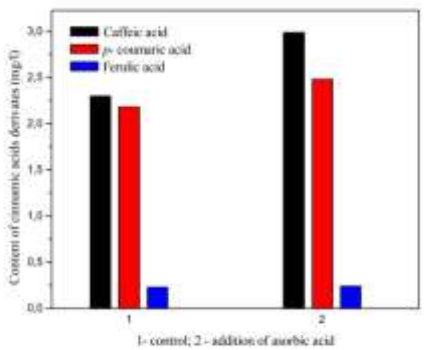


Graph 1. Impact of addition $K_2S_2O_5$ on cinnamic acids derivates.

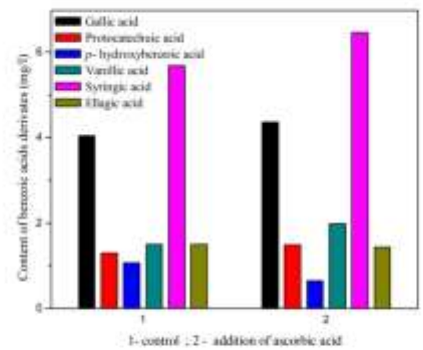


Graph 2. Impact of addition $K_2S_2O_5$ on benzoic acids derivates.

Influence of ascorbic acid was not statistically significant for content of benzoic and cinnamic acids derivates in our wines. It is assumed that the content of phenolic acids in wine samples with added ascorbic acid did not change significantly due to the presence of free SO_2, which at the beginning of the experiment in wine was ajust to 30 mg/l. According to Makhotkina and Kilmartin (2009), in a cyclic voltammetric study of wine antioxidants, it was concluded that ascorbic acid is negligible in terms of *o*-quinone reduction compared to SO_2 and glutathione. Caffeic acid quinones reacted with SO_2 the fastest compared to other quinones formed (Makhotkina and Kilmartin, 2009) and and so it can be explained unchanged or even a slight increase in the concentration of caffeic acid and other cinnamic acid derivates (*p*-coumaric acid and ferulic acid) in our wine samples where ascorbic acid and SO_2 were added. Among the benzoic acid derivates the most dominant were gallic and syringic acids in control as well as in experimental wine with ascorbic acid.



Graph 3. Impact of addition ascorbic acid on cinnamic acids derivates.



Graph 4. Impact of addition ascorbic acid on benzoic acids derivates.

Conclusion

Sulfur dioxide and ascorbic acid are all excellent reducing agents that are capable of interacting with polyphenol oxidation products. From the our results it may be concluded that sulfur dioxide in higher concentrations can readily protect phenolic acidsagainst oxidation. Addition of ascorbic acid in concentration 200mg/l did not show significant changes of cinnamic and benzoic acids derivates comparing with control sample.

References

Barril, C., Clark, A. C. & Scollary, G. R. (2012). Chemistry of ascorbic acid and sulfur dioxide as an antioxidant system relevant to white wine. Anal. Chim. Acta 732, 186–193.

Bradshaw, M. P., Scollary, G. R. & Prenzler, P. D. (2004). Examination of the sulfur dioxide-ascorbic acid anti-oxidant system in a model white wine matrix. J. Sci. Food Agric. 84, 318–324.

Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M. & Fernandez-Zurbano, P. (2015). Oxygen Consumption by Red Wines. Part I: Consumption Rates, Relationship with Chemical Composition, and Role of SO2. J. Agric. Food Chem. 63, 10928–10937.

Gabriele, M., Gerardi, C., Lucejko, J.J., Longo, V., Pucci, L., Domenici, V. (2018). Effects of low sulfur dioxide concentrations on bioactive compounds and antioxidant properties of Aglianico red wine. Food Chem. 245, 1105–1112.

Garaguso, I. & Nardini, M. (2015). Polyphenols content, phenolics profile and antioxidant activity of organic red wines produced without sulfur dioxide/sulfites addition in comparison to conventional red wines. Food Chem.179, 336–342.

Makhotkina, O. & Kilmartin, P. A. (2009). Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: Cyclic voltammetry. J. Electroanal. Chem.633, 165–174.

Pateraki, C. Paramithiotis, S., Doulgeraki, A. I., Kallithraka, S., Kotseridis, Y., Drosinos, E. H. (2014). Effect of sulfur dioxide addition in wild yeast population dynamics and polyphenolic composition during spontaneous red wine fermentation from Vitis vinifera cultivar Agiorgitiko. Eur. Food Res. Technol.239, 1067–1075.

Tao, J., Dykes, S. I. & Kilmartin, P. A. (2007) Effect of SO2 concentration on polyphenol development during red wine micro-oxygenation. J. Agric. Food Chem.55, 6104–6109.
Waterhouse, A. L., Sacks, G. L. & Jeffery, D. W. (2016). Understanding Wine Chemistry. Understanding Wine Chemistry. John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom.

SIDE EFFECTS OF SOME ESSENTIAL OILS ON *TUTA ABSOLUTA* (MEYRICK,1917) (LEPIDOPTERA, GELECHIIDAE) LARVAE UNDER LABORATORY CONDITIONS

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Abstract

Tomato leaf miner, *Tuta absoluta* (Meyrick,1917) (Lepidoptera, Gelechiidae), a highly devastating and invasive pest attacking tomatoes either in greenhouses or in open fields, is currently spreading throughout many countries in the world. However, it may be controlled by various measures, some of which involve cultural practices such as Integrated Pest Management (IPM) and others include the use of chemicals and other synthetic products such as pheromones. In this study, toxicity and repellent effect of essential oils (safflower, lavender, sage, clove) were tested on the third and fourth larval stages of *T. obsoluta* under laboratory conditions. Three different concentrations (1%, 3%, 5%) were used for treatments. Effects of essential oils were studied using contact method. Pure water and alcohol mixture were used in the control trials. Counts were recorded as dead and live larvae at 24th, 48th, and 72nd hours after application. The experiment was carried out in three replications. The most notable result of this study was that mortality effect (100%) of safflower and clove oil on larvae at concentrations of 5%. The other essential oils had a little larvicidal effect. At the 5 ml doses, the highest mortality rate was observed at 72nd hours. Lavender oil has a 30-35% mortality rate on tomato larvae as well as a repellent effect on the larvae. However, the effect of oils should also be studied on the natural enemies of *T. absoluta*.

Keywords: *Alternative control, essential oil, tomato, Tomato leaf miner, toxicity.*

Introduction

The main pest on tomatoes is the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) originating from South America (Garcia and Espul, 1982). This pest entered Europe from the east part of Spain, at the end of 2006 (Urbaneja *et al*., 2007). It spread to other Mediterranean and European countries [Potting,2009] and it has been reported in Greece (EPPO, 2011). It was first detected in 2009 in Turkey (EPPO, 2010a, Kılıç, 2010) and caused significant economic losses in product quality and quantity, after it was spread rapidly in tomato fields, in the recent years.

The main host plant of *T. absoluta* is tomato (*Lycopersicon esculentum*), but there has also been reported several solanaceous plants, including *Solanum nigrum*, *Datura stramonium* (Garcia and Espul,1982), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*) (Desneux *et al*., 2010) and potato crops as well (Pereyra and Sánchez, 2006). It has been high potential damage in tomato fields under favorable climatic conditions (EPPO, 2005).

One of the possible pathways for severe spreading of tomato leaf miner should be dependent on fruit importation and commercialization (Potting, 2009). This pest is transmitted over long distances with packages from countries contaminated with pests (EPPO, 2010b).

Struggle is not done in cases where the yield loss reaches 80-100% (Desneux *et al*., 2010). Different pest control methods (physical, culturel, trapping with pheromone and light traps, biological control, chemical or microbiological treatments) were applied (Desneux *et al*., 2010). Chemical control success rate decreases due to the endophytic behavior of the larvae and the rapid selection of resistant populations (Siqueira *et al*., 2000; Campos *et al*., 2015; Lietti *et al*., 2005, Silva *et al*., 2015; Roditakis *et al*., 2015). Chemical application methods were both harmful to the environment and very expensive.

As an alternative control method to pests, performing the studies on the effect of plant extracts, which has low risk of pest resistance development, is important in the sense of environmental and human health as well as the national economy. The number of studies on the effects of plant extracts on *T. absoluta* has been started to increase, recently (Gonçalves-Gervásio and Vendramim, 2004; Durmuşoğlu *et al*., 2011; Şenel, 2013; Adil *et al*., 2015; Bayındır *et al*., 2015; Chegini *et al*., 2017). Some essential oil showed oviposition deterrent and insecticidal activity on *T. absoluta* (Derbalah *et al*., 2012; Hussein *et al*., 2015; Campolo *et al*., 2017; Chegini and Abbasipour, 2017; Yarou *et al*., 2017). Otherwise, essential aromatic oils were used against pests seen in many plants. However, Gorski and Tomczak (2010) used citronella oil, basil oil, juniper oil, patchouli oil and eucalyptus oil in the control of *Aulacorthum solani* Kalt. on eggplant. Adverse effects of *Junglans regia, Juniperus excelsa*, *J. oxycedrus*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Foeniculum vulgare* and *Laurus nobilis* essential oils were showed on the reproduction ability of cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera:Aphididae) (Işık and Görür, 2009). Many essential oils from different plants such as lemon grass (*Cimbopogon winteriana*), rosemary (*Rosemarinus officinalis*), *Eulcalyptus globulus, Thymus vulgaris, Vetiveria zizanoides* and clove (*Eugenia caryophyllus*) are known for their pest control (Hussein *et al*., 2015).

For its insecticidal and repellent activities against many pests, clove essential oil has been widely studied, such as fire ants (Appel *et al*., 2004), *Diaphorina citri* (Mann *et al*., 2010), mosquitoes (Sutthanont *et al*., 2010), termites (Pandey *et al*., 2012), cockroaches (Omara *et al*., 2013), moths (Birah *et al*., 2010), weevils (Mishra *et al*., 2013) and *Cacopsylla chinensis* (Yang and Li) (Hemiptera: Psyllidae) (Tian *et al*., 2015).

For the reasons mentioned above, the study aimed to find out the effect of four different essential oils (safflower, lavender, sage and clove) on the larvae of *T. absoluta*. Hereby, increasing the use of plant extracts and oils, which has low risk of pest resistance development, will contribute to the reduction of negative effects on environmental and human health, and as a result of this, a more economical agricultural production will be carried out.

Material and Methods

The trials were carried out in 2019 in the laboratory of the Department of Entomology, Bursa Uludağ University (Turkey), Faculty of Agriculture, Department of Plant Protection.

The main material of the study consisted of tomato plant (*Solanum lycopersicum* L., Solanaceae) and the third and fourth instar larvae of *T. absoluta* collected from greenhouses nearby İzmir. Four different concentrations of the essential oils (safflower, lavender, sage and clove) were used in the experiment, including 1.00, 3.00 and 5.00% in concentrations. Also, distilled water (25 ml) +ethyl alcohol (% 96) (25 ml) was used as a control to compare with the different concentrations of the oils. All concentrations of the oils were applied by the contact or residual method by spraying.

The method used in this study was adapted from the method stated by Simon (2014). In this method, formulated essential oil was diluted in a solvent (distilled water 50%+ethyl alcohol 50%) and the solution filled in hand water spray container. In the experiments, plastic Petri dishes (10 cm diameter) with 25 small air holes were used in the upper cover. Firstly, water-soaked paper towel were placed at the bottom of Petri dishes in order to prevent the tomato leaves dry in a short time. Then the tomato leaves approximately 4 cm diameter were placed on paper towel one by one.

We separated the third or fourth instar larvae of tomato leaf miner from the leaves and transferred using a fine paintbrush on to the threatened tomato leaves. In all experiments, we surrounded the dishes by parafilm in order to avoid the larva escape. The control tomato leaves were treated only with distilled water. There were three replications for each doses of concentrations. The results were controlled after 24, 48, 72 hours and the number of live and alive individuals were noted.

The experiment was arranged in a completely randomized pot design with 3 replicates for each concentration of plant oils. The data obtained from the experiment were analyzed by ANOVA (variance analysis) method using JMP software. The LSD test was used to determine the difference between the means (Statistica, 1991).

Results and Discussion

The results of toxic effects of different concentrations of essential oils applied on *Tuta absoluta* larvae were given in Table 1. When each of the essential oils were examined separately, 5% dose of safflower and clove oils were found to be most effective. In this regard, (Casida, 1990) stated that the toxic effect of these oils might depend on its chemical composition and the level of insect sensitivity. Otherwise, Sabzalian *et al*. (2008) analyzed three different safflower plant species showed that stearic, linoleic, oleic and palmitic acids were constituting 96-99% of the total fatty acids. Similarly, Mihaela *et al*. (2013) determined that, major unsaturated fatty acids were oleic and linoleic acid. Likewise, Ramos-López *et al*. (2012) stated that linolenic and linoleic acid were found to have the insecticidal activities on *Spodoptera frugiperda*. A study conducted by Heba *et al*. (2013) showed that linoleic acid have high significant toxic effect on the two instars larva of *S. littoralis*. Ramsewak *et al*. (2001) supports this hypothesis and stated that oleic acid and linoleic acid isolated from the methanol fraction of the hexane extract of the *Dirca palustris* L. (Malvales: Thymelaeaceae) seeds cause larval mortality of the 4th instar of *Aedes aegypti* (L.) (Diptera: Culicidae) and reduced the growth of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), *Lymantria dispar* L. and *Orgyia leucostigma* (Smith) (Lepidoptera: Lymantriidae) larvae, likely due to the phagodeterrent action of these compounds. These results indicate that fatty acids may exhibit bioactivity on insects reported by Fatope *et al*. (2000).

The tested clove essential oil in this study showed high efficacy against *T. absoluta* and this agrees with the findings of Ebedah *et al*. (2016) who reported that treatment by clove oil caused 87.3% mortality against tomato leafminer. As well as Moawad *et al*. (2015) reported that mixed cloves, bitter orange and zinc sulfate had the highest toxic effect (97.0%). Similarly, Tian *et al*. (2015) reported that clove essential oil significantly reduced the population of *C. chinensis*. Also, lemon grass extract and essential aromatic oil have significantly insecticidal effect on *T. absoluta* (Hussein et al 2015). In addition, Campolo *et al*. (2017) reported that citrus peel essential oil nanoformulations suppress tomato moth. Another study conducted by Chegini and Abbasipour

(2017) showed that the essential oil of *Elettaria cardamomum* was toxic to *T. absoluta*. Adil *et al*. (2015) stated that after four hours of *Nigella sativa* essential oil exposure, they induced 100% of mortality of larvae and also had a strong repellent activity against the larvae of *T. absoluta*.

Table 1. Toxic effect of three different concentrations and duration of essential oils applied on *Tuta absoluta* larvae.

Oils	**Dose (%)**	**Mean Larvicidal Effect (%)**		
		24 hours	**48 hours**	**72 hours**
Clove oil	1	40 g	40 g	70 cd
	3	66 d	73 c	60 e
	5	100 a	100 a	100 a
Lavender oil	1	20 j	20 j	26.66 ı
	3	26.66 ı	40 g	26.66 ı
	5	26.66 ı	20 j	46.66 f
Sage leaf oil	1	20 j	13.33 k	26.66 ı
	3	20 j	20 j	33.33 h
	5	26.66 ı	33.33 h	46.66 f
Safflower oil	1	30 h-ı	50 f	58.33 e
	3	80 b	60 e	66.66 d
	5	100 a	100 a	100 a
Control	1	8.33 k-l	8 l	8.66 k-l
	3	8.33 k-l	9.33 k-l	10.66 k-l
	5	12.66 k-l	12.33 k-l	12.66 k-l

Toxic effects of four essential oils and different doses on *T. absoluta* larvae were shown in Figure 1. The highest mortality rate at 5% concentration of clove and safflower oil was 100%. The mortality rate of 60-70% was found in 3% doses. In sage oil, it was found that the concentration of 5% was 30-40%, the dose of 3 ml was 20-30% and the dose of 1 ml was 20%. It was found that there was no difference in lavender oil between 5 ml and 3 ml doses. The highest 30-35% mortality was observed in lavender oil. Moreover, tomato leafminer larvae have observed escape behavior to outside of the petri dishes that applied lavender oil. Similarly, Cosimi *et al*. (2009) tested essential oils extracted from bergamot, bay laurel and lavandin for repellency against *Sitophilus zeamais, Cryptolestes ferrugineus* adults and *Tenebrio molitor* larvae. In the control, mortality rates of around 10% were determined.

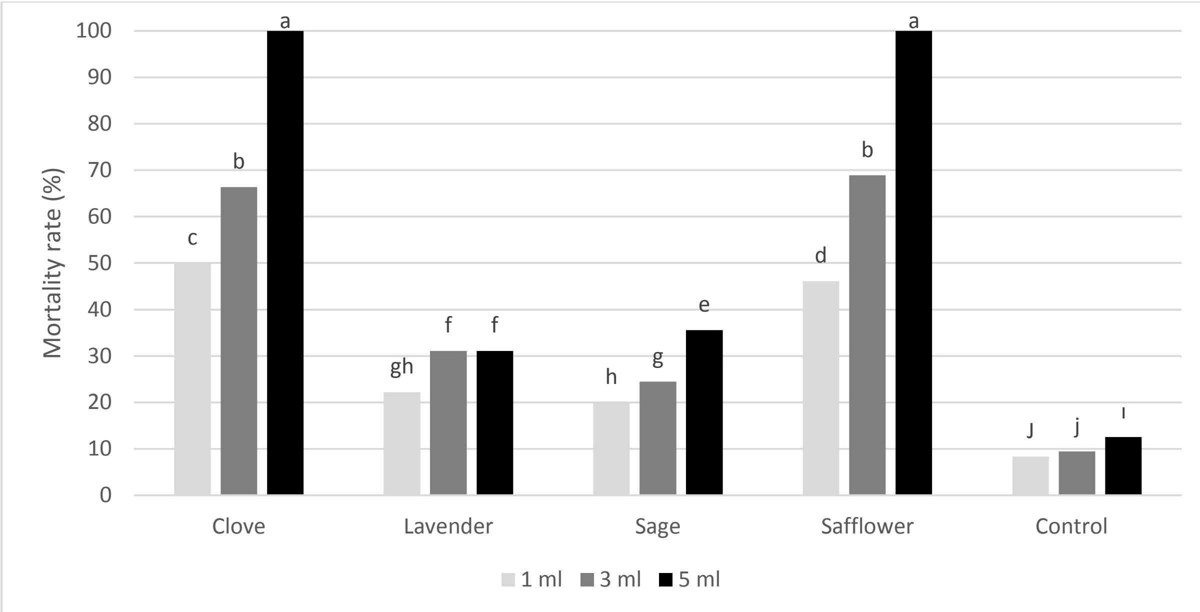

Figure 1. Toxic effects of four essential oils and different doses on *Tuta absoluta* larvae (F: 282,2679; DF: 44/90; P < 0.0001).

Toxic effects of essential oil doses and duration on *T. absoluta* larvae were given in Figure 2. Observations made in this study, the effect of duration on mortality rates was observed to be less than the effect of other parameters. At the 5 ml doses, the highest mortality rate was observed at 72nd hours and no statistically significant difference was observed at 24th and 48th hours. There were no statistically significant differences in the mortality rates at 24th, 48th and 72nd hours at 3 ml doses of oils. Mortality rate at the 72nd hour of the 1 ml dose was 35-40% and mortality rate at the 48th hour was 25-30% and at the 24th hour it was 20-25%.

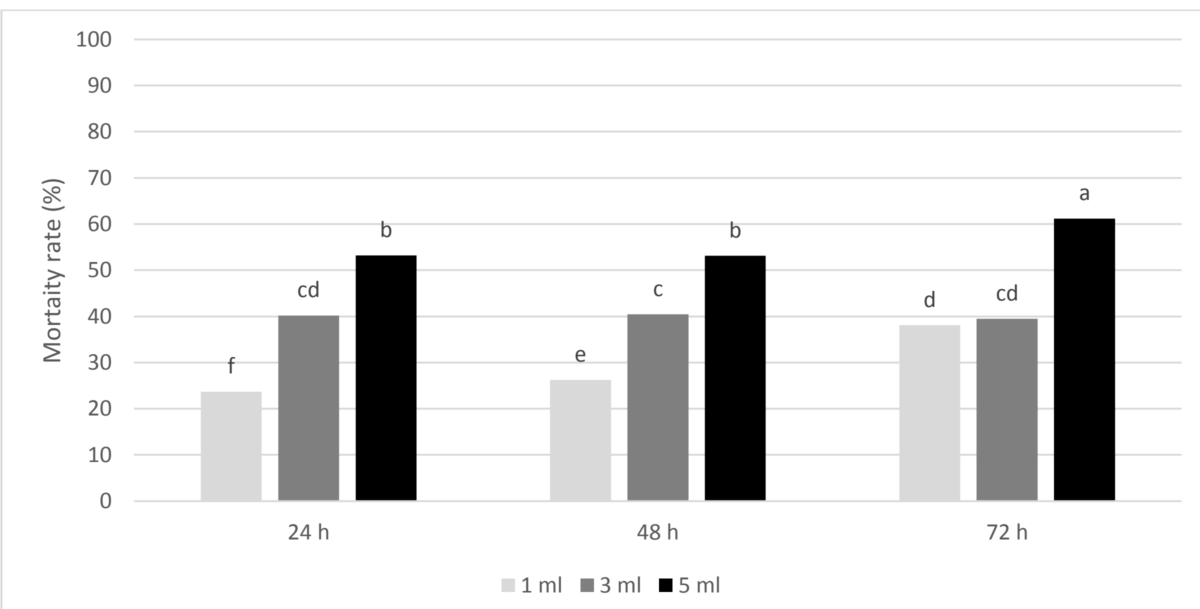

Figure 2. Toxic effects of oil doses and duration on *Tuta absoluta* larvae (F: 282,2679; DF: 44/90; P < 0.0001).

Conclusions

Generally, the extensive and unwise use of synthetic pesticides in the control programs against agricultural pests often creates major deleterious side effects. Fortunately, natural oils (safflower and clove) gave satisfactory results against *T. absoluta*, thereby; the use of thesee essential oils to protect tomato crops against this pest could thus be considered. This is why the in-vivo study of the insecticidal effect of the essential oils were necessary. It was concluded that the use of them for control mentioned tomato insect pests as alternatives to the classic pest control agents. The essential of oil lavender shows repellent activity as well as low insecticidal activity in vitro against the larvae of *T. absoluta*.

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References

Appel A. C., Gehret M.J. and Tanley M.J. (2004). Repellency and toxicity of mint oil granules to red imported fire ants (Hymenoptera: Formicidae). Journal of Economic Entomology, 97: 575-580.

Adil B., Tarik A., Abderrahim K. and Khadija O. (2015). Evaluation of the insecticidal effect of the essential oil of *Cinnamomum zeylanicum* against *Tuta absoluta* (Meyrick). International Journal of Innovative Research in Science, Engineering and Technology (An ISO 3297: 2007 Certified Organization) Vol. 4, Issue 9.

Bayindir A., Özger Ş., Karaca İ., Birgücü A.K. and Hassan E. (2015). Effects of some plant extracts on *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) under laboratory conditions. Advances in Food Sciences, 37(3): 132-137.

Birah, A., Sharma T.V.S., Singh S., and Srivastava R.C.(2010). Effect of aqueous leaf extract of cloves (*Syzygium aromaticum*) on growth and development of tobacco caterpillar (*Spodoptera litura*). Indian Journal of Agricultural Sciences, 80:534-537.

Campolo O., Cherif A., Ricupero M., Siscaro G., Grissa-Lebdi K., Russo A., Cucci L.M., Di Pietro P., Satriano C., Desneux N., Biondi A., Zappalà L. and Palmeri V. (2017). Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. Scientific Reports,7: 13036 | DOI:10.1038/s41598-017-13413-0

Campos M. R., Silva T., Silva W.M., Silva J.E. and Siqueira H.A. (2015). Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) Brazilian populations to ryanodine receptor modulators. Pest Management Science, 71: 537-544.

Casida J.H. (1990). Pesticide mode of action, evidence for implications of a finite number of biochemical targets. In: Casida J.E. (ed.). Pesticides and alternatives. Innovative chemical and Biological Approaches to Pest Control. Amsterdam: Elsevier, pp. 11-22.

Chegini S.G., Abbasipour H. (2017). Chemical composition and insecticidal effects of the essential oil of cardamom, *Elettaria cardamomum* on the tomato leaf miner, *Tuta absoluta*. Toxin Reviews, 36(1): 12-17.

Cosimi S., Rossi E., Cioni P.L. and Canale A. (2009). Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored product pests: Evaluation of

repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). Journal of Stored Products Research, 45: 125-132.
Derbalah A.S., Morsey S.Z. and El-Samahy M. (2012). Some recent approaches to control *Tuta absoluta* in tomato under greenhouse conditions. African Entomology, 20:1.
Desneux N., Wajnberg E., Wyckhuys K.A.G., Burgio G., Arpaia S., Narvaez-Vasquez C.A., Gonzalez-Cabrera J., Catalan Ruescas D., Tabone E., Frandon J., Pizzol J., Poncet C., Cabello T. and Urbaneja A. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, history of invasion and prospects for biological control. Journal of Pest Science, 83: 197-215.
Durmuşoğlu E., Hatipoğlu A. and Balcı H. (2011). Efficiency of some plant extracts against *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) under laboratory conditions. Journal of Turkish Entomology, 35(4): 651-663.
Ebadah I.M., Shalaby S.E.M. and Moawad S.M. (2016). Impact of certain natural plant oils and chemical insecticides against tomato insect pests. Journal of Entomology, 13 (3): 84-90.
EPPO (2005). European and mediterranean plant protection organization data sheets on quarantine pests. OEPP/EPPO Bulletin, 35: 434-435.
EPPO (2010a). First record of *Tuta absoluta* in Turkey (2010/208). EPPO Reporting Services 11(208). http://archives.eppo.int/EPPOReporting/2010/Rse-1011.pdf (Access Date: 15.05.2014).
EPPO (2010b). First report of *Tuta absoluta* in Hungary. EPPO Reporting Service, 3(052): 2.
EPPO (2011). First report of *Tuta absoluta* in Greece. EPPO Reporting Service, 4(071): 3.
Fatope M.O., Adoum O.A.and Takeda Y.(2000). C18 acetylenic fatty acids of *Ximenia americana* with potential pesticidal activity. Journal of Agricultural and Food Chemistry, 48: 1872-1874.
García M. F. and Espul J.C. (1982). Bioecology of the tomato moth (*Scrobipalpula absoluta*) in Mendoza, Argentine Republic. Revista de Investigaciones Agropecuarias, 17: 135-146.
Gonçalves-Gervásio R.C.R. and Vendramim J.D. (2004). Modo de ação de extratos de meliáceas sobre *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). Arquivos do Instituto Biológico, São Paulo, 71: 215-220.
Gonçalves-Gervásio R.C.R. and Vendramim J.D. (2007). Bioatividade do extrato aquosonde sementes de nim sobre *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) em três formas de aplicação. Ciência e Agrotecnologia, Lavras, 31(1): 28-34.
Gorski R. and Tomczak M. (2010). Usefulness of natural essential oils in the control of foxglove aphid (*Aulacorthum solani* Kalt.) occurring on eggplant (*Solanum melongena* L.) Ecological Chemistry and Engineerings, 17:3.
Heba Y., El-Lakwah S.F. and El Sayed Y.A. (2013). Insecticidal activity of linoleic acid against *Spodoptera littoralis* (Boisd.) Egyptian Journal of Agricultural Research, 91 (2): 573-580.
Hussein N.M., Hussein M.I., Hak S.H.G., Shaalan H.S. and Hammad M.A. (2015). Effect of two plant extracts and four aromatic oils on *Tuta absoluta* population and productivity of tomato cultivar gold stone. Journal of Plant Protection and Pathology, Mansoura Univ., 6 (6): 969-985.
Işık M., Görür G. (2009). Aphidicidial activity of seven essential oils against the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae). Munis Entomology and Zoology, 4 (2): 424-431.
Kılıç T. (2010). First record of *Tuta absoluta* in Turkey. Phytoparasitica, 38: 243-244.

Lietti M.M., Botto E. and Alzogaray R.A. (2005). Insecticide resistance in Argentine populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Neotropical Entomology, 34:113-119.

Mann R.S.,Tiwari S., Smoot J.M., Rouseff R.L.and Stelinski L.L. (2010). Repellency and toxicity of plant-based essential oils and their constituents against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Journal of Applied Entomology, 136: 87-96.

Mihaela P., Josef R., Monica N. and Rudolf Z. (2013). Perspectives of safflower oil as biodiesel source for South Eastern Europe (comparative study: Safflower, soybean and rapeseed). Fuel, 111, 114-119.

Mishra B.B., Tripathi S.P. andTripath C.P.M. (2013). Bioactivity of two plant derived essential oils against the rice weevils *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Proceedings of the National Academy of Sciences, India B.83:171-175.

Moawad S.S., Sharaby A., Ebadah I.M. and El-Behery H.(2015). Efficiency of zinc sulfate and some volatile oils on some insect pests of the tomato crop. Global Advanced Research Journal of Agricultural Science, 4: 182-187.

Omara S. M., Al-Ghamdi K.M., Mahmoud M.A.M. and Sharawi S.E. (2013). Repellency and fumigant toxicity of clove and sesame oils against American cockroach, *Periplaneta americana* (L.). African Journal of Biotechnology, 12:963-970.

Pandey A., Chattopadhyay P., Banerjee S., Pakshirajan K. and Singh L. (2012). Antitermitic activity of plant essential oils and their major constituents against termite *Odontotermes assamensis* Holmgren (Isoptera: Termitidae) of North East India. International Biodeterioration and Biodegradation, 75:63-67.

Pereyra P.C. and Sánchez N.E.(2006). Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotropical Entomology, 35: 671-676.

Potting R. (2009). Pest risk analysis, *Tuta absoluta*, tomato leaf miner moth. Plant protection service of the Netherlands, 24pp.

Ramos-López M. A., González-Chávez M.M., Cárdenas-Ortega N.C., ZavalaSánchez M.A. and Pérez G.S. (2012). Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. African Journal of Biotechnology, 11, 4274-4278.

Ramsewak R.S., Nair M.G., Murugesan S., Mattson W.J.and Zasada J. (2001). Insecticidal fatty acids and triglycerides from *Dirca palustris*. Journal of Agricultural and Food Chemistry, 49: 5852-5856.

Roditakis E.,Vasakis E., Grispou M., Stavrakaki M., Nauen R., Gravouil M. and Bassi A. (2015). First report of *Tuta absoluta* resistance to diamide insecticides. Journal of Pest Science, 88: 9-16.

Sabzalian M. R., Saeidi G. and Mirlohi A. (2008). Oil content and fatty acid composition in seeds of three safflower species. Journal of the American Oil Chemists' Society, 85(8): 717-721.

Silva W. M., Berger M., Bass C., Balbino V.Q., Amaral M.H., Campos M.R. and Siqueira H.A.(2015). Status of pyrethroid resistance and mechanisms in Brazilian populations of *Tuta absoluta*. Pesticide Biochemistry and Physiology, 122: 8-14.

Simon J.Y. (2014). The Toxicology and Biochemistry of Insecticides. CRC press, 1-380.

Siqueira H.A., Guedes R.N. and Picanco M.C. (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agricultural and Forest Entomology, 2: 147-153.
Şenel M. (2013). The effect of some plant extracts on the different biological stages of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). M.Sc. Thesis, Department of Plant Protection, Natural sciences institute, University of Adnan Menderes, Aydın, 53 pp.
Statistica (1991)**.** Complete Statistical System by StatSoft, Inc. 2325 East 13th Street, Tulsa, OK 74104
Sutthanont N., Choochote W., Tuetun B., Junkum A., Itpakdi A., Chaithong U., Riyong D. and Pitasawat B. (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and-resistant strains of *Aedes aegypti* (Diptera: Culicidae). Journal of Vector Ecology, 35:106–115.
Tian B.L., Liu Q.Z., Liu Z.L., Li P. and Wang J.W. (2015). Insecticidal potential of clove essential oil and its constituents on *Cacopsylla chinensis* (Hemiptera: Psyllidae) in laboratory and field. Journal of Economic Entomology, 108 (3): 957-961.
Urbaneja A., Vercher R., Navarro V., Porcuna J.L. and García-Marí F. (2007). La polilla del tomate, *Tuta absoluta*. Phytoma-España, 194: 16-24.
Yarou B.B., Thomas Bawin T., Boullis A., Heukin S., Lognay G., Verheggen F.J. and Francis F. (2017). Oviposition deterrent activity of basil plants and their essentials oils against *Tuta absoluta* (Lepidoptera: Gelechiidae). Environmental Science and Pollution Research, DOI 10.1007/s11356-017-9795-6.

OCCURRENCE OF OCHRATOXIN A IN RED WINE IN SERBIA IN 2020-2021

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Abstract

Wine production in Serbia has a long tradition and represents one of the significant branches of agricultural production. Wine produced in Serbia have for many years a reputation of good quality products. Health benefits of wine related mostly to cardiovascular diseases are well known. However, wine is also recognized as one of the main contributors to the dietary intake of ochratoxin A. Ochratoxin A is a widespread mycotoxin that is produced by several Aspergillus and Penicillium species and main mycotoxin occurring in wine. In this study, a total of 55 samples of red wine were collected from 18 producers from Serbia in the period 2020-2021. The samples were analysed for ochratoxin A (OTA) content by using an enzyme-linked immunosorbent assay Ridascreen® Ochratoxin A 30/15. The occurrence of OTA in the tested samples was 89%, with average concentration 0.47 μg kg^{-1} and maximum OTA content of 1.04 μg kg^{-1}. These results suggest very high level of occurrence of OTA in tested samples. The limit of 2.00 μg kg^{-1} imposed by Serbian legislation for OTA content in wine was not exceeded in any of the studied samples. It could be concluded that levels of OTA in wines from Serbia do not pose a risk for human health.

Key words: *wine, ochratoxin A, ELISA.*

Introduction

Ochratoxins are mycotoxins produced by several species of the fungal genera *Aspergillus* and *Penicillium*, and are among the most widely reported of the nearly 400 recognized mycotoxin contaminants of agricultural products (De Jesus et al., 2017).
Of all known variants, ochratoxin A (OTA) is the most prevalent cogener. Production of this mycotoxin was initially established with species *Aspergillus ochraceus*, *A. melleus*, *A. muricatus*, *A. petrakii*, *A. sclerotiorum* and similar. Furthermore, some species of *Penicillium* also produce OTA, of which the best known are *P. verrucosum* and *P. veridicatum* (Jović et al., 2009). Ochratoxin A (OTA) is a naturally occurring foodborne storage mycotoxin in temperate as well as in tropical areas (Devergowda et Murthy, 2009). OTA is found in a wide variety of agricultural commodities worldwide, ranging from cereal grains to dried fruits, wine and coffee (Bui-Klimke and Wu, 2015).
OTA has been shown to be potently toxic in various systems (De Jesus et al., 2018). OTA has been associated with Balkan Endemic Nephropathy (BEN) and the development of urinary tract tumors in humans (Abouzied et al., 2002; Shundo et al., 2006). Apart from a marked nephrotoxicity, ochratoxin A possesses teratogenic, immunotoxic and possibly neurotoxic properties (Burdaspal and Legarda, 2011). Some evidence exists in support of both genotoxic and non-genotoxic mechanisms by which OTA causescarcino genicity (Mally and Dekant, 2005; Pfohl-Leszkowicz and Castegnaro; 2005).

In 1993, the International Agency for Research on Cancer (IARC) classified OTA (Group B) as possible carcinogen to humans (IARC, 1993).
Among the agricultural products associated with OTA contamination, and of possible human health concern, is wine **(**De Jesus et al., 2018**)**. This product was identified as the second most important source of OTA after cereals in the European diet and among different types, red and sweet wine seemed to be the most contaminated (Miraglia and Brera, 2002).
The source of OTA contamination of wine is the raw material and therefore sanitary condition of grapes is a very important factor to avoid OTA presence in the final product (Gil-Serna et al., 2018). OTA levels in wine also depend on other factors such as latitude, environmental and weather conditions, managing conditions in the vineyards as well as on winemaking procedures (Silva et al., 2019). Many studies have been performed to unravel the most important ochratoxigenic species occurring in grapes. Several reports evidenced the contribution of Aspergillus section Nigri species to OTA contamination of grapes and their occurrence on the surface of healthy berries (Jessica Gil-Serna et al., 2018).
Wine production in Serbia has a long tradition and represents one of the significant branches of agricultural production. Therefore, the objective of this study was examination and determination of the presence of OTA in red wine in order to determine the levels of contamination. The reason for choosing only samples of red wine lays dawn in the fact that in previous years OTA was not detected in white wine samples at all.

Materials and Methods

Collection of samples

Present study examined 55 samples of red wine, collected from 18 producers from all over the Serbia in the period 2020-2021. Before analysis, the samples were stored at refrigerator (4-6ºC), protected from light.

Sample preparation

All collected samples were prepared and analyzed in accredited laboratory for testing food and feed safety „Jugoinspekt Beograd“. Samples of red wine were prepared according manufacturer's instructions. Prior to analysis, 5 ml of each sample was pipetted into a 50ml PP-tube and mixed with 20 ml of diluted ECO extractor. The mixture was shaked vigorously (manually) for 5 minutes. The extract was centrifuged for 5 minutes at 3500g, RT and 1 ml of supernatant was diluted with 1ml of ready to use wash buffer. According to manual instructions 50 µl of diluted sample was used in the test.

Ochratoxin A analysis and instrumental conditions

Determinaton and quantitive analysis of ochratoxin A was done by Enzyme Linked ImmunoSorbent Assay (ELISA) method, using RIDASCREEN® Ochratoxin A 30/15 (R-Biopharm) test kit. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s
Analysis were performed according to manufacturer's instructions (RIDASCREEN Ochratoxin A 30/15 Art.No.:1312). Limit of detection (LoD) was set on 0,015 µg kg^{-1} while limit of

quantification (LoQ) was calculated at 0,05 μg kg^{-1} . Measuring range was confirmed from 0,05 to 25 μg kg^{-1}, while recovery rate was ranging from 94 to 116 %. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the ochratoxin A concentration in the sample of red wine.

Statistical analysis

All obtained data were analyzed using SPSS 15.0 software (SPSS, IBM corporation, USA).

Results and Discussion

Ochratoxin A has been detected in 49 out of 55 (89%) samples of red wine, at levels ranging from 0.307 μg kg^{-1} to 1.04 μg kg^{-1}. Average concentration was 0.47 μg kg^{-1} and maximum OTA content of 1.04 μg kg^{-1}. In majority of the samples (48) OTA concentration ranged from 0.3 μg kg^{-1} to 1.00 μg kg^{-1} (Table 1). Only in 11% of examined samples OTA has not been detected. Most of the contaminated samples (18 out of 49) refer to 2020 year of the production which proves that aging has significant effect on OTA reduction in wine (Anli et al., 2011). Overall, this suggests very high level of occurrence of OTA in tested samples. High level of occurrence could be explained by the red wine making process. Maceration, which occurs just in red wine making process allows the migration of OTA from the grape to the must (Dachery et al., 2017).
Žurga et al. (2019) have found that 92% of studied samples of red wine were contaminated with ochratoxin A which is in correlance with this study (89%). Furthermore, in United States more than 85% of the samples evaluated were found to contain OTA (De Jesus et al., 2017).
The limit of 2.00 μg kg^{-1} imposed by Serbian legislation for OTA content in wine was not exceeded in any of the studied samples, which is comparable to those performed in some other studies. Belajova and Rauova (2007) have found that wines produced in Slovakia have OTA concentrations far below the proposed European limit (2.00 μg kg^{-1}). Furthermore, Žurga et al (2019) have reported that concentrations in all examinated samples of red wine were even 10 times lower than the maximum limit of contamination (2.00 μg kg^{-1}).
Although high level of occurrence was detected contamination levels were way below a provisional tolerable daily intake (PTWI) established for this toxin by JECFA (112 ng/kg bodyweight) (JECFA, 2007), which proves that wine from Serbia, consuming in reasonable amounts, are safe for consumption.

Table 1. Contamination range of analyzed samples

Year of the production	**Range of contamination (μg kg^{-1})/No. of analysed samples**		
	<0.3	0.3 – 1.00	> 1
2016	3	5	/
2017	3	5	/
2018	/	9	/
2019	/	12	/
2020	/	17	1

Conclusion

Ochratoxin A has been detected in majority of the analyzed samples. This leads to a conclusion that presence of funghi and its metabolites is natural and hardly can be avoided. Also, it was confirmed that the process of making red wine is more favorable to occurrence level of OTA. However, the levels of contamination did not exceed maximum limit imposed by the Serbian law which proves that with good agricultural practice the level of contamination can be managed. Furthermore, contamination levels were way below a provisional tolerable daily intake (PTWI) established for this toxin by JECFA which proves that wine from Serbia are safe for consumption.

References

Abouzied M. M., Horvath A. D., Podlesny P. M., Regina N. P., Metodiev V. D., Kamenova-Tozeva R. M., Niagolova N. D., Stein A. D., Petropoulos E. A., Ganev V. S. (2002): Ochratoxin A concentrations in food and feed from a region with Balkan Endemic Nephropathy. Food Aditives and Contaminants, Vol 19, 2002: 755-764.

Anli R.E., Vural N. and Bayram M. (2011): Removal of Ochratoxin A (OTA) from Naturally Contaminated Wines During the Vinification Process Journal of the Institute of Brewing, 117(3), 456–461, 2011

Belajova E., Rauova D. (2007): Determination of ochratoxin A and its occurrence in wines of Slovakian retail. Journal of Food and Nutrition Research, Vol 46, 2007: 68-74.

Bui-Klimke R. T., Wu F. (2015): Ochratoxin A and human health risk: A review of the evidence. Critical Reviews in Food Science and Nutrition, Vol 55, 2015: 1860-1869.

Burdaspal P., Legarda T. M. (2011): Occurrence of ochrantoxin A in sweet wines produced in Spain and other countries. Food Additives and Contaminants 24 (9), 2007: 976-986.

Dachery B., Veras F.F., Dal Magro L., Manfroi V., Welke J.E. (2017): Exposure risk assessment to ochratoxin A through consumption of juice and wine considering the effect of steam extraction time and vinification stages. Food and Chemical Toxicology 109 (2017) 237e244

De Jesus C. L., Bartley A., Welch Z. A., Berry P. J. (2017): High incidence and levels of ochratoxin A in wines sourced from United states. Toxins, 10, 1, 2018: 1-12.

Gil-Serna J., Vázquez C., González-Jaén M.T. and Patiño B. (2018): Wine Contamination with Ochratoxins: A Review Beverages 2018, 4, 6

IARC (1993): Ochratoxin A., IARC Monographs on the Evaluation of Carcinigenic Risks to Humans, Some Natturally Occuring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, Vol. 56; International Agency for Research on Cancer: Lyon, France, 1993: 489-521.

JECFA (2007): Joint Food and Agricultural Organization/World Health Organization. (FAO/WHO) Expert Committee on Food Additives, 2007. http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1905 (Accessed 30 August 2021)

Jović M. S., Petrović V. A., Marković R. N. (2009): Mycotoxins in wine with special attention on ochratoxin A. Zbornik Matice srpske za prirodne nauke. Matica Srpska Journal for Natural Sciences Matica srpska Novi Sad, 116, 2009: 91-100.

Mally A., Dekant W. (2005): DNA adduct formation by ochratoxin A: Review of the available evidence. Food Additives and Contaminants Supplement 1:65-74.

Miraglia M., Brera C. (2002): Task 3.2.7 Assessment of Dietary Intake of Ochratoxin A by the population of EU Member States. Reports on Tasks for Scientific Cooperation. Directorate General of Health and Consumer Protection. European Commission. Brussels : European Union).

Silva J. G. L., Rodrigues P. A., Pereira T. P. M. A., Lino M. C., Pena A. (2019): Ochratoxin A in the Portuguese Wine Market, Occurrence and Risk Assessment. Food Additives and Contaminants, Part B.

Pfohl-Leszkowicz A., Castegnaro M. (2005): Further arguments in favour of direct covalent binding of Ochratoxin A (OTA) after metabolic biotransformation. Food Additives and Contaminants Supplement 1:75-87.

Žurga P., Vahčić N., Pasković I., Banović M., Staver Malenica M. (2019): Occurence of Ochratoxin A and Biogenic Amines in Croatian Commercial Red Wines. Foods 2019, 8, 348: 1-14.

ANTIBACTERIAL ACTIVITY OF MANUKA HONEY WITH DIFFERENT MANUKA FACTOR

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Abstract

Honey has been a part of myths and legends for centuries, gift from heaven, but also as the food of the Gods. The history of honey is as long and rich as the history of bees, which are one of the oldest forms of insects. To man, honey is the oldest food and known medicine. Manuka honey is the most researched medium and the first registered 'medical honey'. It is monofloral honey that comes from the nectar of the Manuka plant, *Leptospermum scoparium*, which grows like a bush or tree in New Zealand and eastern Australia. Phytochemicals derived from plants from which bees collect nectar or honey dew can have a beneficial effect on human health. Compared to other types of honey, manuka honey contains more flavonoids and phenolic acids that act as antioxidants and anti-inflammatory, antiproliferative and antimicrobial. Methylglyoxal (MGO) is up to a hundred times more abundant in Manuka honey compared to other types of honey and is considered responsible for its strong antimicrobial action. Manuka honey has different biological effects and wide application in complementary, but also in conventional medicine. It acts antimicrobial on a wide range of bacteria, including multidrug-resistant strains, thanks to its peroxide and non-peroxide activity. It is a powerful antioxidant, has anti-inflammatory, immunostimulatory, cytotoxic and antiviral, antifungal, and anthelmintic effects. The aim of this study was to examine the activity of Manuka honey of different unique Manuka factor (UMF) on bacterial cultures: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Enterica* and *Salmonella Typhimurium* and *β-hemolytic Escherichia coli* isolated from clinical material and to determine the type of action on these pathogens. The agar diffusion method was used by placing disks with a diameter of 9 mm on a solid sterile agar (Müeller-Hinton-agar-MHA) where is 50 µl of honey (original honey and diluted samples in the proportion honey: deionized water 50:50 % and 75:25 %) was dropped with a micropipette. The results confirmed the antimicrobial activity of both Manuka honey on all tested bacterial strains with a range of 19.33mm to 36.66mm for Manuka UMF 5+ and a range of 21.66mm to 34.66mm for Manuka UMF 15+.

Key words: *antibacterial properties, manuka honey, pathogenic microorganisms.*

Introduction

Manuka honey is a product of the honeybee from the New Zealand area, where the evergreen shrub tree Manuka or New Zealand tea tree (lat. *Leptospermum scoparium*) grows. New Zealanders have long used it in traditional medicine for its proven healing properties (cleansing surgical wounds, abscesses, ulcers of various etiologies, burns, while the leaves of Manuka tree were used to make tea that is good for colds, for wound healing, but also as a sedative) (Wijesinghe et al., 2008;, Bonifacio et al., 2018).
Manuka honey consists of carbohydrates, minerals, proteins, fatty acids, phenolic and flavonoid compounds. Such compounds are also found in other types of honey, but Manuka honey is

unique due to the unusually high content of methylglyoxal (MGO- present in a concentration of 38 mg/ kg to 1541 mg/kg)) which is formed from dihydroxyacetone (DHA) which is correlated with antibacterial activity (Maube et al., 2013; Aiken et al., 2012; Atrott, 2009). Manuka honey is one of the most researched honeys. It is also evaluated using a classification system known as the unique Manuka factor (UMF). The UMF score of Manuka honey is strongly correlated with MGO equivalence and antibacterial activity, but this relationship has not yet been fully studied (Kato et al., 2014;, Molan, 2008).

The unique Manuka factor (UMF) depends on the content of methylglyoxal and is the important antibacterial action of honey along with other active components including hydrogen peroxide, pH, hyperosmolarity effect, bee defensin-1, etc., which act independently or with MGO and hydrogen peroxide (Atrott, 2013; Alvarez- Suarez, 2014; Szweda, 2017; Carnwath et al., 2014)

The main bioactive substances of manuka honey and the mechanisms responsible for its biological activity are currently being studied. This research supports its increased use in dermatology and the basis for the isolation and purification of substances for the development of biopharmaceutical products with antimicrobial properties and for wound healing (Alvarez-Suarez, 2014).

Manuka honey acts on a wide range of bacteria and bacteria that are resistant to first-line antibiotics are sensitive, such as *MRSA*, *Salmonella Enterica serovar Typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus epidermidis*, *Helicobacter pylori*, *Campylobacter spp*. (Ahmed - Suarez, 2013).

During a longer period of application, a reduced susceptibility of bacteria to Manuka honey was observed, but this phenomenon was not long-term, and no mutated resistant bacteria were found. The risk is low if honey is applied over a long period of time in high concentrations (Cooper et al., 2010). The need to find new antimicrobial drugs due to the increasing occurrence of resistant and multidrug-resistant bacterial strains to previously known antimicrobial drugs has prompted the scientific community to find safe and natural (alternative) drugs (Linder et al. 2016).

The aim of this study was to examine the activity of Manuka honey of different unique Manuka factor UMF on bacterial cultures: *Escherichia coli, Staphylococcus aureus, Salmonella Enterica* and *Salmonella Typhimurium* and *β-hemolytic Escherichia coli* isolated from clinical material.

Materials and methods

Manuka honey UMF 15+ purchased in commercial sales at the Žužemberk pharmacy (Grajski trg 41, Žužemberk) and Manuka honey UMF 5+ purchased in commercial sales in New Zealand were used as material.

Test microorganisms

Bacterial cultures were used to examine the antibacterial activity: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Enterica*, *Salmonella Typhimurium* and β-hemolytic *Escherichia coli* from the collection of isolates of the laboratory for microbiology (PI Veterinary Institute of Republic of Srpska "Dr. Vaso Butozan"). Cultures were seeded in nutrient broth and incubated for 18 hours at 37^0C. Petri dishes with a suitable medium (Müeller - Hinton agar) were seeded with 100 µl of bacterial suspension with a concentration of 10^5 CFU/ml.

Test method

The agar diffusion method was used by placing 9 mm diameter disks on a solid sterile agar (Müeller-Hinton-agar-MHA). 50 µl of honey (original honey and diluted samples in the proportion of honey: distilled water 50:50% and 75:25%) was instilled on the discs with a micropipette. As a control, 50 µl of sterile distilled water was added to the discs which was used to dilute the honey samples. The plates were left in the refrigerator for 30 minutes to diffuse the sample into the medium, then incubated for 24 hours at 37°C.

For each honey sample, three replicates were performed and after incubation from 18-24h, the results were read by determining the diameter of the inhibition zone and the mean value was calculated.

Type of action

To see if the honey was bactericidal or bacteriostatic, a small piece of agar was taken from the inhibition zones and added to the nutrient broth. Incubation was performed at 37ºC/24h. If the broth has become cloudy, the effect of honey is bacteriostatic and if the broth has remained clear after incubation, the action of honey is bactericidal.

Results and discussion

The antibacterial activity of Manuka honey with two different UMFs (UMF 15+ and UMF 5 +) was tested on five bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella Enterica*, *Salmonella Typhimurium* and *β-hemolytic Escherichia coli*) isolated from clinical material (poultry, throat swab, urine). The results are shown in Tables 1 and 2.

Table 1. Antibacterial activity of Manuka honey UMF 5+

Microorganisms	**Inhibition zones in mm**		
	Manuka honey UMF 5+ Original	***Manuka honey: distilled water 75:25%***	***Manuka honey: distilled water 50:50%***
Staphylococcus aureus	34.67±1.25	31.00±0.82	36.67±2.36
Escherichia coli	29.67±4.64	24.67±0.94	20,67±0.94
β- hemolitična Escherichia coli	26.33±2.62	20,00±2.45	22,00±2.16
Salmonella Enterica	27,33±0.94	25,67±0.47	19,33±0.47
Salmonella Typhimurium	24.00±1.41	29.33±0.94	25.00±0.00

Manuka honey UMF 5+ acted on all bacterial strains with different intensity of inhibition with a range of action from 19.33 mm to 36.66 mm. Manuka UMF 5 + showed the strongest action against *Staphylococcus aureus* in all combinations with a range of action of 36.66 mm (in the proportion of honey: distilled water 50:50%), 33.66 mm (pure honey) and 31.00 mm (in the proportion of honey: distilled water 75:25%). Pure Manuka honey had the strongest effect on *Escherichia coli*, *β-hemolytic Escherichia coli*, *Salmonella Enterica* with a range of 26.33 mm to 29.66 mm in relation to both combinations, while in the combination of honey: water 75:25% had the strongest effect on *Salmonella Typhimurium* (with a range of 29.33 mm), then honey: distilled water 50:50% (range of action 25.00 mm) and finally pure Manuka honey with a range of action of 24.00 mm.

Table 1. Antibacterial activity of Manuka honey UMF 15+

Microorganisms	**Inhibition zones in mm**		
	Manuka honey UMF 15+ original	***Manuka honey: distilled water 75:25%***	***Manuka honey: distilled water 50:50%***
Staphylococcus aureus	34.67±3.86	29.33±0.94	25.67±1.89
Escherichia coli	28.33±2.36	25.67±0.47	21,67±2.36
β- hemolitična Escherichia coli	25.33±2.05	23,67±1.25	19,00±2.94
Salmonella Enterica	28.33±2.05	24.33±0.94	20,00±1.63
Salmonella Tiphymurium	34.67±2.49	25.00±0.00	23.00±0.02

Manuka honey UMF 15+ had an inhibitory effect on the examined pathogens with a range of action from 19.00 mm to 34.66 mm. Pure Manuka UMF15 + had a stronger effect on all tested pathogens (range of action from 25.33 mm to 34.66 mm) compared to diluted honey in both proportions (honey: distilled water 50:50% and 75:25%). Also, Manuka UMF 15+ had a stronger effect on all tested pathogens in the proportion of honey: distilled water 75:25% (with a range of 22.66 mm to 33.33 mm) compared to the proportion of honey: distilled water 50:50% (range of action of 19.00 mm to 32.00 mm).

The disk diffusion method is most used for evaluation of the antibacterial activity of honey. The limiting factor is that different types of bacteria are differently sensitive to different types of honey. In addition, the agar-diffusion method evaluates the activity of honey according to the size of the growth inhibition zone, which depends on the antibacterial activity, but also on the rate of diffusion of antibacterial components through the agar. Due to relatively high molecular weight compounds that are limited in agar migration, the antibacterial activity of honey can be mistaken for low. (Kwakman, 2010; Gobin et al., 2014; Kalaba et al., 2020).

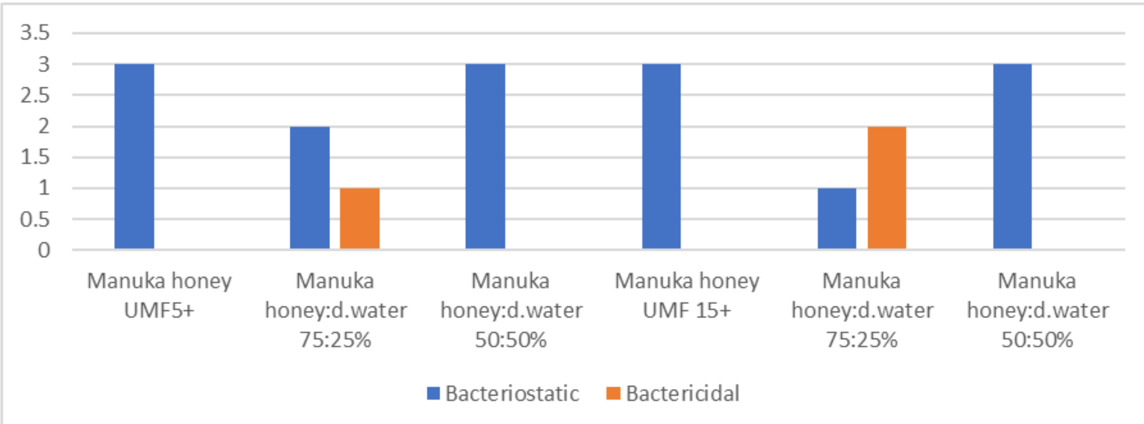
The results of the research are consistent with the results of other researchers and agree that Manuka honey with higher UMF has a stronger inhibitory effect. Manuka honey UMF 5+ contains 83mg/kg MGO, and Manuka UMF 15+ contains 250mg/kg MGO and is the reason for their stronger action in the fact that they have a higher total number of phenols that have the ability to clean free radicals (Alsara, 2009; Almasauddi et al., 2017; Lu et al., 2013; Jing et al., 2013). Also, there must be other components, which act independently or with the help of MGO and hydrogen peroxide (Tonks et al. 2007; Alvarez-Suarez, 2014). Due to the influence of various factors on the antimicrobial properties of honey it is not possible to predict exactly how much antimicrobial activity a honey will have.

The results of this study confirmed the stronger effect of Manuka honey on Gram-positive bacteria (*Staphylococcus aureus*) compared to Gram-negative bacteria (*Escherichia coli*, *Salmonella Enterica*, *Salmonella Typhimurium* and *β-hemolytic Escherichia coli*), which agrees with the results of other researchers (Irish et al., 2011; Kumar, 2014; Jing et al., 2013).

The effectiveness of the antibacterial action of honey on different types of bacteria is not the same due to different mechanisms of resistance. The outer membrane and the more complex three-layer cell membrane of Gram-negative bacteria in general causes increased resistance to some types of honey as well as to antibacterial drugs. Another mechanism of resistance involves the formation of extracellular biofilms, which represent physical barriers to antimicrobial activity (Pagès, 2009; Walsh, 2000; Hussein et al., 2012; Al-Vaili et al., 2012; Vallianou et al., 2014).

Honey has bacteriostatic and bactericidal activity in some pathogenic bacterial species such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, etc. Bacterial growth is inhibited by

low water activity and low pH. (Almasaudi et al., 2017 ; Lu J., et al., 2014 ; Kwakman and Zaat, 2012).
To see if the honey was bactericidal or bacteriostatic, a small piece of agar was taken from the inhibition zones and added to the nutrient broth. Incubation was performed for 24 hours at 37ºC.
Both Manuka med showed bactericidal activity on *E. coli*, *β-hemolytic E. coli*, *Salmonella Enterica* and *Salmonella Typhimurium* in all replicates. Both honeys showed bacteriostatic effect on the inhibition of *Staphylococcus aureus* growth in dilution with water in the ratio honey: water 75:25 in one replication in Manuka UMF 5+ and in two replicates in Manuka UMF 15+. These results agree with the results of other researchers (Almasauddi et al., 2017; Mohapatra et al., 2011; Vallianou et al., 2014; Kalaba et al., 2020).



Picture 1. Bacteriostatic and bactericidal action of Manuka UMF5 + and Manuka UMF15 +

Conclusion

The results confirmed the antimicrobial activity of both Manuka honey on all tested bacterial strains with a range of 19.33 mm to 36.66 mm for Manuka UMF 5+ and a range of 21.66 mm to 34.66 mm for Manuka UMF 15+.
Both Manuka honeys were bactericidal except in one replication in *Staphylococcus aureus* with Manuka UMF 5+ 75:25% and two replicates in Manuka UMF 15+ 75:25% when the honeys were bacteriostatic.
Manuka honey is a medical honey that confirms that there is a huge potential for the application of honey in the clinical environment. However, as the factors influencing the antimicrobial activity of honey are complex and numerous, it is important to continue research not only on those honeys known as antibacterial, but also on other locally produced and unexplored honeys.

References

Aiken A., Karuri D., Wanyoro A., Macleod J. (2012): Interventional studies for preventing surgical site infections in sub-Saharan Africa—a systematic review. *Int. J. Surg.* 2012;10:242–249.

Atrott J, Henle T. (2009): Methylglyoxal in Manuka honey—correlation with antibacterial properties. Czech J Food Sci. ;27:S163–S165.

Alvarez-Suarez J.M., Gasparrini M., Forbes-Hernández T.Y., Mazzoni L., Giampieri F. (2014): The composition and biological activity of honey: A focus on Manuka honey. Foods Jul 21 ;3(3):420-432

Alvarez-Suarez, J. M., Tulipani S., Diaz D., Estevez Y., Romandini S., Giampieri F., Damiani E., Astolfi P., Bompadre S., Battino M. (2010): Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem. Toxicol. 48, 2490-99.

Ahmed S., Othman N.H. (2013): Review of the Medicinal Effects of Tualang Honey and a Comparison with Manuka Honey. Malays J Med Sci. May; 20(3): 6–13.

Atrott J. (2013.) Methylglyoxal in Manuka-Honig (Leptospermum scoparium): Bildung, Wirkung, Konsequenzen. Dissertation

Almasaudi S.B.., Al-Nahari A.A.M., EI Sayd M., et al. (2017) Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi J Biol Sci. 2017;24:1255–1261

Alsarra I. (2009). Chitosan topical gel formulation in the management of burn wounds. Int J Biol Macromol. 2009;45:16–21

Al-Waili N., Al-Ghamdi A., Ansari M.J., Al-Attal Y., Salom K. (2012). Synergistic effects of honey and propolis toward drug multi-resistant *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* isolates in single and polymicrobial cultures. *Int. J. Med. Sci.* 2012;9(9):793–800.

Maria A Bonifacio, Stefania Cometa, Andrea Cochis, Piergiorgio Gentile , Ana M Ferreira , Barbara Azzimonti , Giuseppe Procino, Edmondo Ceci , Lia Rimondini, Elvira De Giglio (2018) Antibacterial effectiveness meets improved mechanical properties: Manuka honey/gellan gum composite hydrogels for cartilage repair. Carbohyd Polym. 2018;198:462–472.

Carnwath R., Graham E., Reynolds K., Pollock P. (2014) The antimicrobial activity of honey against common equine wound bacterial isolates. *Vet. J.* 2014;199:110–114.

Cooper R.A., Jenkins L., Henriques A.F., Duggan R.S., Burton N.F. (2010): Absence of bacterial resistance to medical-grade manuka honey. Eur J Clin Microbiol Infect Dis. 2010 Oct;29(10):1237-41.

Gobin, I., Vučković, D., Lušić, D. (2014): Antibacterial properties of honey. Medicina fluminensis 2014, Vol. 50, No. 2, p. 150-157

Hussein S.Z., Mohd Yusoff K., Makpol S., Mohd Yusof Y.A. (2012): Gelam honey inhibits the production of proinflammatory, mediators NO, PGE 2, TNF-α, and IL-6 in carrageenan-induced acute paw edema in rats. *Evid. Based Complement. Alt. Med*

Irish J, Blair S, Carter D. (2011): The antibacterial activity of honey derived from Australian flora. PLoS One. 2011;6:e18229

Johnston, M., McBride, M., Dahiya, D., Owusu-Apenten, E., Singh Nigam, P. (2018): Antibacterial activity of Manuka honey and its components: An overviewAIMS Microbiol. 2018; 4(4): 655–664.

Jing Lu, Dee A. Carter, Lynne Turnbull, Douglas Rosendale, Duncan Hedderley,Jonathan Stephens, Swapna Gannabathula, Gregor Steinhorn, Ralf C. Schlothauer, Cynthia B. Whitchurch, Elizabeth J. Harry (2013): The Effect of New Zealand Kanuka, Manuka and Clover Honeys on Bacterial Growth Dynamics and Cellular Morphology Varies According to the Species PLoS One. 2013; 8(2): e55898.

Kumar N. (2014): Comparison of the antibacterial efficacy of Manuka honey against *E. faecalis* and *E. coli*—An *in vitro* study. JCDR. 2014;8:ZC29–ZC31

Kwakman P.H.S., te Velde A.A., de Boer L., Speijer D., Vandenbroucke-Grauls C.M.J.E., Zaat S.A.J. (2010): How honey kills bacteria. FASEB J.; 24: 1-7.

Kwakman P. H., Zaat S. A.J. (2012): Antibacterial components of honey. IUBMB Life 64, 48-55.

Kalaba, V., Golić, B., Ilić, T. (2020): Antibacterial activity of different types of honey on pathogenic bacteria IX International Symposium on Agricultural Sciences AgroRe S 2020 PROCEEDINGS, pp 149.162

Linder K., Nicolau D., Nailor M. (2016): Predicting and preventing antimicrobial resistance utilizing pharmacodynamics: Part I Gram positive bacteria. Expert Opin Drug Met;12:267–280.

Lu J., Turnbull L., Burke C.M., Liu M., Carter D.A., Schlothauer R.C., Whitchurch C.B., Harry E.J. (2014): Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. *Peer J.*;2:e326

Lu J., Carter D.A., Turnbull L., Rosendale D., Hedderley D., Stephens J., Gannabathula S., Steinhorn G., Schlothauer R.C., Whitchurch C.B., Harry E.J. (2013):The effect of New Zealand kanuka, manuka and clover honeys on bacterial growth dynamics and cellular morphology varies according to the species**.** PLoS One. 2013; 8 (2): e55898. doi: 0.1371/Journal.pone.0055898. Epub 2013 Feb 13.

Molan P.C. (2008): An explanation of why the MGO level in Manuka honey does not show the antibacterial activity. New Zeal Beekeeper. 2008;16:11–13.

Moumbe F.G.P., Zambou F., Kaktcham M. (2013): Antimicrobial activity of probiotic strain *Lactobacillus plantarum* isolated from "SHA′A" and assement of its viability in local honey. *J. Microbiol. Biotechnol. Food Sci.* 2013;3:226–231.

Mohapatra D.P., Thakur V., Brar S.K. (2011): Antibacterial efficacy of raw and processed honey. *Biotechnol. Res. Int.* 2011;2011:917505.

Pagès J.M., Amaral L. (2009): Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. BBA-Proteins Proteom. 2009;1794:826–833.

Sherlock O., Dolan A., Athman R., Power A., Gethin G., Cowman S., Humphreys H. (2010): Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement. Alt. Med.* 2010;2:10–47.

Tonks A.J., Dudley E., Porter N. (2007): A 5.8-kDa component of Manuka honey stimulates immune cells via TLR4. J Leukocyte Biol. 2007;82:1147–1155.

Vallianou N., Gounari P., Skourtis A., Panagos J., Kazazis C. (2014). Honey and its anti-inflammatory, anti-bacterial, and anti-oxidant properties. *Gen. Med.* 2(1):132–137.

Walsh C. (2000): Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000;406:775–781.

Wijesinghe M., Weatherall M., Perrin K. (2009): Honey in the treatment of burns: a systematic review and meta-analysis of its efficacy. NZ Med J. 2009;122:47–60.

Yoji Kato, Rie Fujinaka, Akari Ishisaka, Yoko Nitta, Noritoshi Kitamoto, Yosuke Takimoto (2014) Plausible authentication of Manuka honey and related products by measuring leptosperin with methyl syringate. J Agr Food Chem. 2014;62:6400–6407.

ORGANIC AGRICULTURE

CHEMICAL-PHYSICAL PROPERTIES AND QUALITY OF HONEY FROM ORGANIC GROWING FROM THE AREA OF SOUTHERN HERZEGOVINA

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Abstract

Beekeeping, and thus honey production, has a very long tradition in Bosnia and Herzegovina. Due to the very favourable sub-Mediterranean climate conditions, and rich biodiversity, and especially a variety of medicinal plants, honey from the area of southern Herzegovina is a highly valued and sought-after product. On the list of 10 foods with the highest risk of counterfeiting, honey ranks sixth. This paper analyses samples of honey from organic farming from three locations in Herzegovina, namely Gornja Duboka, southeast of Stolac, Bančići (a village located between Stolac and Ljubinje) and Počitelj-brdo in the municipality of Čapljina, in order to determine their quality and originality product. The samples were analysed for the following parameters: water content, content of water-insoluble components, total ash, reducing sugar content, sucrose content, acid content, HMF content and electrical conductivity. The results of the analyses were compared with the reference values and statistically processed. The obtained results showed that all parameters, on which honey samples from all three localities were tested, were in accordance with the reference values prescribed by the Book of rules on honey and other bee products. From the obtained results of chemical and physical analyses, it can be concluded that all samples are natural, that they are not fake and that it is honey of very good quality.

Key words: *honey, location counterfeiting, parameters, chemical-physical properties, quality.*

Introduction

Honey is a mixture of relatively complex composition with over 70 chemical components been identified (Krell, 1996). Its chemical composition is influenced by agroecological, geographical, climatic conditions, as well as the characteristics of vegetation in a particular location. (Tewari and Irudayaraj, 2004). Also, the colour and taste of honey depend on the above mentioned factors (Persano Oddo and Piro, 2004). One of the most important characteristics by which its chemical composition can be described, is the variability of that composition, so it can be said that there are no two samples of honey that are completely identical. According to Mujić *et al.* (2014) the most common ingredient is invert sugar, actually a mixture of equal amounts of glucose and fructose formed by hydrolysis of sucrose and water. These authors state that the other ingredients in honey are proteins (including enzymes), minerals, vitamins, organic acids, nitrogen compounds, hydroxymethylfurfural, phenolic components, components that affect aromas. The most common are carbohydrates and water. Since different types of counterfeit honey can be found on the market, the aim of this study was to examine honey samples from three locations in southern Herzegovina for organic parameters to give a picture of the quality and originality of the product (Lazarević *et al* 2012).

Material and methods

Honey samples for this study were collected during 2019 from three locations from the areas of Stolac and Čapljina municipalities. All three locations are under influence of the sub-Mediterranean climate and are characterized as ecological areas. Samples are numbered 1, 2 and 3 as follows:

Sample 1- Spring honey; location: Gornja Duboka, southeast of Stolac at 12 km; geographical and pedological characteristics: hilly karst area with intensive forest areas in the lowlands; vegetation: sage, willow grass, dubačac, thyme, petteria and honeydew.

Sample 2 - Autumn honey; location: Bančići (village located between Stolac and Ljubinje); the apiary is located southeast of Stolac and southwest of Ljubinje; geographical and pedological characteristics: hilly area with individual karst zones; vegetation: heather, mint, white grass, ivy.

Sample 3 - Autumn honey; location: Počitelj – Gradina, municipality of Čapljina; geographical and pedological characteristics: hilly area, redness on karst; vegetation: dominant thorns, heather, sage.

The samples were tested for the following parameters:

- water content (drying method-standard procedure)
- content of water-insoluble components (gravimetric method)
- total ash (sample combustion method at 6000^{0}C)
- reducing sugar content (Fehling and Luff Schoorl method)
- sucrose content (hydrolysis and reduction method with Fehling solution)
- acid content (by neutralization method with phenolphthalein as an indicator)
- HMF content, hydroxymethylfurfural (Winkler photometric method)
- electrical conductivity (by a method of measuring electrical resistance that is reciprocal to electrical conductivity)

The analyses were performed in the laboratories of the Agromediterranean Institute in Mostar. The obtained results were compared with the maximum permitted quantities prescribed by the Book of rules on honey and other bee products in force in B&H (Official Gazette of BiH, No. 37, pp. 333 and 334), and statistically processed, using analysis of variance and Tukeytest.

Result and discussion

Tables 1 to 8 show the results of analyses for each examined parameter expressed as the mean value ± standard deviation (SD).

Table 1. Water content in honey samples (%)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Water content	**15.10 ± 0.05**	**15.30 ± 0.15**	**17.40 ± 0.04**	**20**

The stability of honey depends on the amount of contained water. In the case of increased water content, fermentation caused by osmophilic yeasts can be activated, which produces alcohol that turns into ethanoic acid and water, by oxidizing oxygen from the air, which results in a more acidic taste of honey (Zamora and Chirife, 2004). Honey that contains more than 20% of water is

more prone to fermentation. All three samples, which were the subject of the study, had a water content below the maximum allowed value.
Statistical analyses showed the existence of certain statistical significance in water content. By comparing Sample 3 to Sample 2 and for Sample 1, it indicated that significant difference was determined, while in comparison of Sample 2 with Sample 1 there was no significant difference.

Table 2. Acid content **(**milieqvkis/1000g)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Acid content	**30.10 ± 0.82**	**28.9 ± 0.77**	**26.30 ± 1.1**	**50**

The acids, which are an integral part of honey, are of quite different chemical composition. Out of all organic acids, gluconic acid is the most abundant, and tartaric, lactic, citric, acetic, oxalic, formic and malic are also present. Hydrochloric and phosphoric acids are the most abundant inorganic acids (Taranov, 2006). The acidity of honey is an indicator of its quality, and the pH is in the range of 3.2 to 6.5 (Mujić *et al.*, 2014). High acidity indicates fermentation where alcohol as a product is fermented into an organic acid. In all three tested samples, the acid content was lower than the maximum allowed. Analysis of variance showed that there was a statistical difference between the tested samples on acid content. Based on the Tukey test, the existence of significant difference in the comparisons of Sample 1 in relation to Sample 3 and Sample 2 in Sample 3 was established, while in the comparison of Sample 1 with Sample 2, no signifcant diference was determined for the examined parameter.

Table 3. Ash content (g/100g)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Ash	**0.477 ± 0.20**	**0.46 ± 0.16**	**0.587 ± 0.11**	**0.6**

When it comes to minerals, iron and aluminum predominate among all the other minerals in honey, such as potassium, sodium, calcium, phosphorus, sulfur, chlorine, magnesium. The most common microelements are copper, manganese, chromium, zinc, lead, arsenic, titanium, selenium. Out of the total mineral content, potassium is present in a percentage of 25% to 50%, and with sodium, calcium and phosphorus makes up at least 50% (Hernandez *et al.*, 2004). The higher ash content may indicate honey with addition of sugar molasses (Pohl, 2009). In all three tested samples, the ash content was below the maximum allowed value. Statistical analyses indicated that there was no statistical difference for the tested honey samples.

Table 4. Invert sugar content (g/100g)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Invert sugar	**F=33.697± 0.90 G=21.358±0.45 F+G=55.055**	**F=39.098± 0.98 G=26.860±0.62 F+G=65.958**	**F=37.839± 1.01 G=25.098±0.29 F+G=62.937**	**F +G Minimum 60**

F- Fructose, G- Glucose

The most common ingredient is an invert sugar, actually a mixture of equal amounts of glucose and fructose formed by hydrolysis of sucrose and water (Mujić *et al.*, 2014). The sweet taste of

honeycomes from carbohydrates. In addition, they affect the hygroscopicity, viscosity, density and tendency to crystallize. (Barhate *et al.,* 2003). The tendency for honey to crystallize can be determined based on the ratio of fructose to glucose. Honey is a supersaturated glucose solution and spontaneously goes into equilibrium by crystallizing excess of glucose in solution. Glucose loses water (becomes Glucose monohydrate) and turns into a crystalline form. Water, which was previously bounded to glucose, becomes free by increasing the water content in the non-crystallized parts of the honey. Due to that, honey becomes more prone to fermentation and spoilage. Fructose remains in a liquid state and forms a thin layer around glucose crystals. Honey changes colour, becomes lighter, it is no longer transparent, and changes taste (Batinić K. and Palinić D., 2014). The results showed that in Sample 1 the invert sugar content was slightly lower than the reference value, while Samples 2 and 3 were in accordance with the reference value.
Statistical analyses indicated statistical difference for the tested honey samples. Tukey test showed was performed and the existence of statistical significance in the comparisons of Sample 2 to Sample 3 and Sample 1 was proved, while in the comparison of Sample 3 with Sample 1 it can be concluded that no statistical significance was determined. Analysing the variance for glucose content of the tested honey samples, it can be concluded that there was a statistical difference for the tested honey samples. To determine the exact influence of the location on the quality of honey samples and which comparisons showed statistical significance, a Tukeytest was performed. Based on the Tukey test for glucose content, the existence of significant difference in the comparisons of Sample 2 to Sample 3 and Sample 1 was determined. Comparing Sample 3 with Sample 1 significant difference was also determined for the examined statistical parameter.
By analysing the variance for the fructose + glucose content of the tested honey samples, it can be concluded that there was a statistical difference for the tested honey samples. The influence of the location on this parameter was shown by the Tukeytest. Based on the Tukey test for F + G content, the existence of statistical significance in the comparisons of Sample 2 to Sample 3 and Sample 1 was proven. Comparing Sample 3 with Sample 1 significant difference for the examined statistical parameter was determined.

Table 5. Sucrose content (g/100 g)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Sucrose	**<0.6011**	**<0.6011**	**<0.6011**	**5**

The sucrose content of all three tested samples was the same in this case and it was slightly less than 0.6011 g/100 grams of sample, which is a satisfactory result since the maximum reference value for this parameter is 5 g/100 g of sample. Sucrose is important during the honey test because it provides an information about a counterfeit honey (feeding bees with sugar). The most of disaccharides and oligosaccharides are found in nectar and honey bees with their enzymes and organic acids reduce them to simple sugars.

Table 6. HMF content (hydroxymethylfurfural) (mg/kg)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
HMF (hydroxymethylfurfural)	**35.45±0.96**	**19.9± 0.60**	**37.31± 1.0**	**40**

According to its chemical characteristics, hydroxymethylfurfural belongs to cyclic aldehydes and can be formed by dehydration of fructose and glucose in an acidic environment. The amount of HMF in honey depends on the type of honey, pH value, acid concentration, amount of moisture, as well as the exposure of honey to light, actually on the method of storage. (Spano *et al*, 2005). HMF is naturally present in honey, its content is quite low in fresh honey, it is below 1 mg/kg, while it is high in honey treated with indirect heating during processing. (10 mg/kg). The high content of hydroxymethylfurfural in honey may indicate its forgery (Mujić *et al.,* 2014).
The HMF content was highest in sample number 3, in sample number 2. The results for all three samples are satisfactory comparing to the maximum reference value for this parameter. Anova test for the HMF content of the examined samples, indicated the statistical difference. Based on the Tukey test, there was a statistical significance in the HMF content comparing Sample 3 with Sample 1 and Sample 2. Statistical significance for the examined parameter was also indicated in comparison of Sample 1 with Sample 2.

Table 7. Content of water-insoluble components (g/100 g)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Water-insoluble components	**<0.01**	**<0.01**	**<0.01**	**max 0.1g**

The content of water-insoluble components for all three tested samples was same in this case and it amounts to slightly less than 0.01 g/100 g of sample, which means that the result is satisfactory comparing to the general maximum value of 0.1 g/100 g of sample.

Table 8. Electrical conductivity (mS/cm)

Parameter	Sample 1	Sample 2	Sample 3	Reference value
Electrical conductivity	**1.63 ± 0.08**	**1.33 ± 0.07**	**1.31± 0.04**	**Max 0.8**

Electrical conductivity is a characteristic that is defined in honey as the conductivity of a 20% solution of honey in water (20% is dry matter) at a temperature of 20° C. It depends on the amount of minerals and water, as well as dissociation of acids in such a way that the higher the value of these parameters, the stronger the conductivity. It is also used as a parameter to distinguish nectar honey from honeysuckle (Lazarević *et al.,* 2012). This parameter is also used to distinguish nectar types of honey from honeysuckle, but also to classify nectar honey according to botanical origin (da Silva *et al.,* 2016, Bogdanov *et al.,* 2004). Analysis of variance for electrical conductivity indicated that there was no statistical difference for the examined honey samples.

Conclusion

Based on the obtained results, and their comparison with the maximum permitted quantities, which are regulated by the Book of rules on honey and other bee products in force in B&H, and statistical analysis, it may be concluded that all three samples are original, unadulterated honey from organic farming of very good quality and can be recommended for consumption.

Reference

Batinić K., Palinić D. (2014). Handbook on honey, Federal Agro-Mediterranean Institute Mostar

Barhate, R. S., Subramanian, R., Nandini, K. E., Hebbar, H. U. (2003). Processing of honey using polymeric microfi ltration and ultrafi ltration membranes. J. Food Eng. 60, 49- 54.

Bogdanov, S., Ruoff, K., & Oddo, L. P. (2004). Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie, 35(Suppl. 1), S4- S17.

da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. Food Chemistry, 196, 309-323.

Hernandez, O.M., Fraga, J.M.G., Jimenez, A.I., Jimenez, F., Arias, J.J. (2004). Characterisation of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. Food Chemistry 43: 261-271.

Krell R. (1996). Value-added products from beekeeping. FAO Agricultural Services Bulletin No. 124.

Lazarević, K. B., Andrić, F., Trifković, J., Tešić, Ž., & Milojković-Opsenica, D. (2012). Characterisation of Serbian unifloral honeys according to their physicochemical parameters. Food chemistry, 132(4), 2060-2064.

Mujić I., Alibabić V., Travnjanin D. (2014). Processing of honey and other bee products, ISBN 978-953-6911-76-9, University of Rijeka

Persano Oddo L., Piro R. (2004). Main European unifloral honeys: descriptive sheets. Apidologie, 35: S38-S81

Pohl, P. (2009). Determination of metal content in honey by atomic absorption and emission spectrometries. Trends in Analytical Chemistry 28: 117–128.

Book of rules on Honey and Other Bee Products (Official Gazette of BiH, No. 37, pp. 333 and 334)

Spano, N., Casula, L., Panzanelli, A., Pilo, M.I., Piu, P.C., Scanu, R., Tapparo, A., Sanna, G. (2005). An RP-HPLC determination of 5-hydroximethylfurfural in honey. The case of strawberry tree honey. Talanta 68: 1390-1395.

Taranov, G.F. (2006.). Food and nutrition of bees, 4th edition, Neron, Bjelovar

Tewari J., Irudayaraj J. (2004). Quantification of saccharides in multiple floral honey using fourier transform infrared microattenuated total reflectance spectroscopy.Journal of Agricultural and Food Chemistry, 52 (11): 3237-43

Zamora, M.C., Chirife, J. (2004). Determination of water activity change due to crystallization in honey from Argentina. Food Control 38: 342-347.)

RESEARCH ON THE USE OF COVER CROPS FOR THE CONTROL OF DISEASES AND PESTS IN ORGANIC VINEYARDS

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Abstract

One new viticultural system capable of exploiting plant diversity was tested in the Murfatlar vineyard between November 2018 and September 2020. The experiment was set in a plot cultivated with Fetească neagră variety, in ecological system. The experiment plan consisted of four treatments, each having two sub-treatments: one treated according to the organic guidelines and the other untreated against pests and fungal diseases. The tested cover crops were a plant mix comprising *Lolium perenne* 50%, *Onobrychis* viciifolia 25%, *Trifolium repens* 25% (semi-permanent cover crops, mowed and mulched after flowering) and another mix represented by *Vicia sativa* 50%, *Sinapis* sp. 50% (mowed after flowering and incorporated into the soil) and *Tagetes sp.*, planted as seedlings under the vine row. The development of powdery mildew (as incidence and severity of symptoms on leaves and/or bunches) in the treatment with the plant mix *Vicia sativa* 50%, *Sinapis sp.* 50% made the degree of attack lower than the other treatments. On the other hand, the treatment with the plant mix *Lolium perenne* 50%, *Onobrychis viciifolia* 25%, *Trifolium repens* 25% showed mild forms of attack with *Plasmopara viticola*, *Guignardia bidwellii* and *Botrytis cinerea.* The treatment with *Tagetes sp*. had a repellent effect for the main pest of the vine, where *Lobesia botrana* registered the lowest numbers of captured adults and larvae on flowers and bunches. Regarding the production quality, the first innovative treatment obtained the best results both in 2019 and in 2020 (267 g/l and 262.8 g/l sugars respectively). Overall, there are no significant differences between the treatments in terms of grape production.

Key words: *Ecological vineyards***,** *Plant diversity, Cover crops, Fungal diseases.*

Introduction

Soil management can enhance important aspects of the vineyard ecosystem, in particular pest control and soil health. There is a growing interest in establishing cover crops that promote useful fauna, improve soil structure, suppress weeds, control pests and foliar pathogens.

Any attempt to improve biodiversity is closely linked to the impact of chemicals applied in vineyards. Thus, chemicals can be more or less toxic in terms of their effect on soil organisms and on natural predators of vine pests. Pest control, in particular, is frequently achieved through a combination of chemical applications and the actions of natural predators, but their abundance is influenced by applied agrotechnical works, including the application of pesticides (Stark & Banks 2003; Thomson & Hoffmann 2006). Identifying and adopting practices that enhance biodiversity, help reduce the need for chemical treatments and therefore promote agricultural sustainability. The term biodiversity refers to the biological diversity of an ecosystem, and its accurate measurement is difficult due to the complexity of natural systems. However, it is generally accepted that the more diversified a system is, the more resilient or self-adjusting it will be. A wide range of potential natural enemies coexist with vineyard pests and contribute to pest control through predation and parasitism. (Thomson și colab. 2007). This paper presents the results of the introduction of an innovative system, based on intercropping, which aims to improve the control of diseases and pests in organic vineyards.

Material and Methods

Location and variety

The new viticultural system capable of exploiting plant diversity was tested in the Murfatlar vineyard, within the Murfatlar Research Station for Viticulture and Oenology. The experiment spanned over 2 years, from November 2018 to September 2020. The variety cultivated in the experimental plot is Fetească Neagră (cultivated in ecological system).

Experimental design

Three experimental treatments were configured, using cover crops in comparison with a tilled conventionally control plot. Each experimental treatment had a subtreatment plot in which the first was phytosanitary treated and the other was left untreated. (table 1).

Table 1. The experimental treatments

Experimental treatments	**The used plants in cover crop**
V1 Inovativ treated 1	Mix Lolium perenne 50%, Onobrychis viciifolia 25%, Trifolium repens 25%. Semi-permanent cover crop, mowed and mulched after flowering. Applied organic phytosanitary treatments.
V1.2 Inovativ untreated 1	IDEM V1. No treatment.
V2 Inovativ treated 2	Mix of Vicia sativa 50%, Sinapis sp. 50%. mowed and incorporated after flowering. Applied organic phytosanitary treatments.
V2.1 Inovativ untreated 2	IDEM V2. No treatment.
V3 Inovativ treated 3	Tagetes sp. Under vine trunks. Applied organic phytosanitary treatments.
V3.1 Inovativ untrated 3	IDEM V3. No treatment.
V4 (Control) Traditional, bare soil treated	Tilled control plot. Applied organic phytosanitary treatments.
V4.1 (Control)Traditional, bare soil untreated	IDEM V4. No treatment.

Monitoring methods

- Phytosanitary control technique

During the season, the vineyards were inspected periodically to monitor the evolution of the disease epidemic, at least once a week.

To determine the resistance to diseases: downy mildew, powdery mildew, gray rot and grape black rot, the degree of attack was calculated for each pathogen. The frequency of attack (F) is expressed as a percentage of the total number of plants observed. The intensity of the attack (I) is given on the basis of grades, from 1 to 7, depending on the severity of which the plant is affected. Practically, the percentage was estimated by the number and area of the spots in relation to that of the leaves on a single plant, calculating the average of the figures obtained, based on which the degree of the intensity of the attack is given. In general, the rating of the intensity of the attack was assessed as follows: Degree 1 - No attack present; 2 - if 3% of the plants are attacked and there are rare spots or pustules on the leaves; 3 - if 6% of the plants have rare spots or pustules; 4 - when 12% of the plants are attacked; 5 - if 25% of the plants are attacked; 6 - if 50% of the plants have spots or pustules; 7 - when 75% of plants are attacked (Derived from Buffara et al., 2014; Derived from Caffi et al., 2010).

Knowing the frequency (F) and intensity (I) with which a disease attacks plants, the degree of attack was calculated according to the formula:

$$DA\% = \frac{F * I}{100}$$

- Grapevine moth (*Lobesia botrana*)

  A visual inspection of 100 inflorescences/bunches per treatment was performed in order to observe the presence or absence of deteriorated flowers/berries, each week, throughout the vegetation period. Particular attention was paid to grape bunches attacked by the grapevine moth during its most harmful generation (BBCH 83-85).

  Pheromone traps were used to monitor the adult density of the vine moth (*Lobesia botrana*), the economic damage threshold being established by the number of adults caught during a week. The traps were placed for all tested treatments, and the pheromone tablets were replaced at the emergence of the next generation.
- Grapes quality evaluation

  For quality and quantity assessment, standardized techniques were used, (upon OIV). Thus, physical analyzes were performed on grapes, specifying: average production/plant, mass of 100 berries, total sugar content, total acidity and malic acid.
- The statistical analysis was performed using the programs: SPSS 26.0 and XLSTAT.

Results and discussion

Following the observations and determinations that were made in the field, the severity of the attack of pathogens was established, as well as the number of affected organs (leaves and bunches).

Thus, in the wine year 2019 downy mildew, gray rot and grape black rot diseases recorded an insignificant value of the degree of attack, for all the observed treatments. This was possible due to climatic conditions that did not favor the development of these diseases. In contrast, the pathogen *Uncinula necator* - powdery mildew, recorded a significant degree of attack, especially in the case of the innovative plots and also in the untreated control treatment. The disease was manifested as an attack on the bunches.

In the wine year 2020, the pathogens *Uncinula necator* - powdery mildew and *Guignardia bidwellii* - grape black rot were mainly manifested. Downy mildew registered a moderate degree of attack, and manifested only on the leaves. Due to the dry climatic conditions, the pathogen *Botrytis cinerea* - gray rot did not manifest itself in the experimental plots.

Regarding the degree of attack of powdery mildew (*Uncinula necator*) in both study years, the untreated control treatment V4.1 registered the highest degree of attack, followed by the innovative untreated treatments V3.1, V1.1, V.2.1. For the other treated innovative treatments, where phytosanitary treatments were applied, there was a low degree of attack, being categorized as insignificant. The treated innovative treatment with the lowest attack value was V2 - *Vicia sativa* 50%, *Sinapis sp*. 50% (Figure 1).

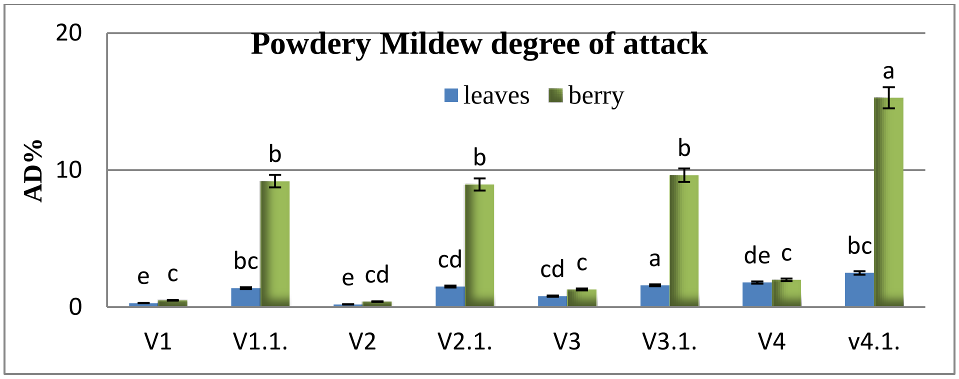


Figure 1. Degree of attack caused by *Uncinula necator* for each subtreatment

Downy mildew manifested itself on the leaves in an insignificant percentage for 2019, and in 2020 it registered a slight manifestation at leaf level. The innovative treatment that recorded the lowest degree of attack was V1 - *Lolium perenne* 50%, *Onobrychis viciifolia* 25%, *Trifolium repens* 25%, followed by V2 and V3, at short distance from V4 - control. In the case of untreated innovative treatments, the lowest degree of attack was recorded by V1.1, followed by V2.1 and V3.1, the untreated control treatment registering the highest degree of attack for both treated and untreated innovative treatments (Figure 2).

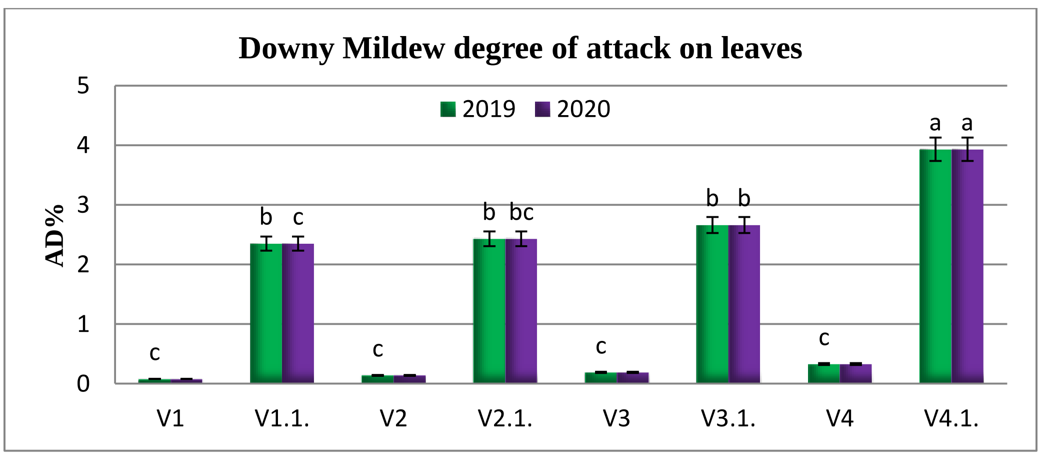


Figure 2. Degree of attack caused by *Plasmopara viticola* for each subtreatment

The attack of the grape black rot did not manifest itself in 2019, instead it was present and recorded significant values, in 2020. It had manifestations on both leaves and bunches. The untreated control treatment V4.1 being by far the most affected treatment, followed by the innovative untreated treatments V3.1, V2.1 and V1.1. In the case of the treated innovative treatments, the V3 treatment registered the highest degree of attack, followed by V2 and V1. The control treatment V4 is located between V3 and V2 (Figure 3). In the case of this pathogen, the innovative treated treatment that obtained a lower value of the attack degree is V1 - *Lolium perenne* 50%, *Onobrychis viciifolia* 25%, *Trifolium repens* 25%.

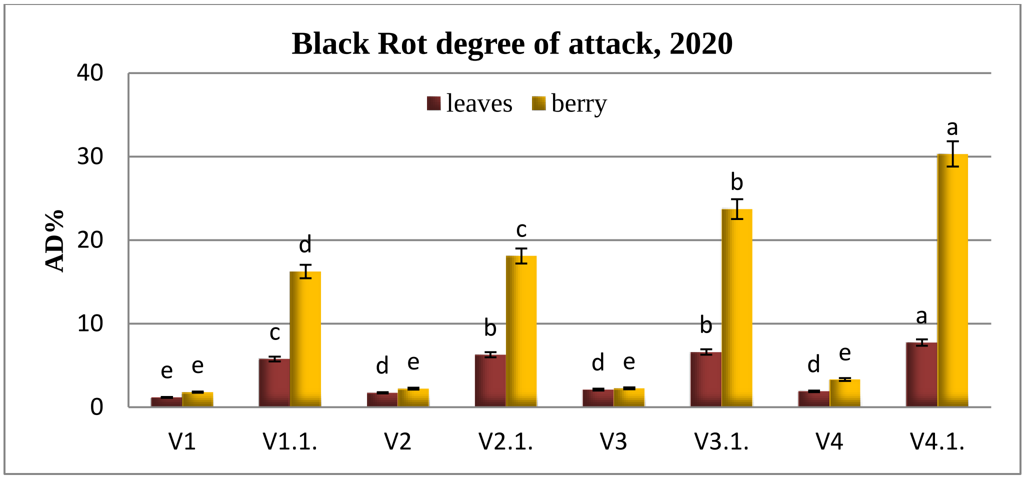
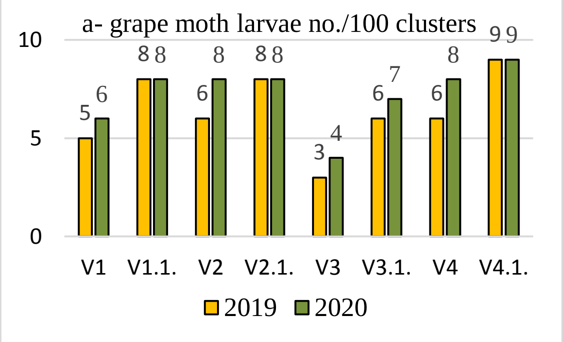


Figure 3. Degree of attack caused by *Guignardia bidwellii* for each subtreatment

There is a clear distinction between treated and untreated treatments, however the distance in the case of untreated treatments gives us a vision and a direction towards future research.

Grapevine moth (*Lobesia botrana*)
The presence of moth larvae was identified in the experimental plots, but the economic damage threshold was not reached, for both of the years 2019 and for 2020 (10 larvae per 100 bunches analyzed). The most affected treatment was V4.1, in both years. Among the innovative treated treatments, V3 and V1 recorded the lowest number of larvae, and for the untreated ones, V3.1 and V1.1 (Figure 4 a).
The number of adults caught in 2020 recorded a value of 3080, exceeding by 75% the value recorded in the previous year, with 1762 adults. Thus, although the economic damage threshold was not attended, the pressure of the adults of this pest was very high. The flight dynamics of this pest, as seen in the graph, recorded the highest values in the untreated control treatment V4.1 - 2019 and 2020. In the case of the other innovative treated treatments, the study recorded significat differences between them and the untreated treatments. Thus, the V3 treatment registered the lowest number of adults in both 2019 and 2020, followed by the V1 and V2 treatments, and for the untreated innovative treatments, V3.1, followed by V1.1 and V2.1 (Figure 4 b)
Thus, we can see the influence of *Tagetes sp.* plants on useful fauna, which made us have a very small number of larvae and especially adults in the experimental plots. But also, the other innovative treatments have positively influenced this balance.



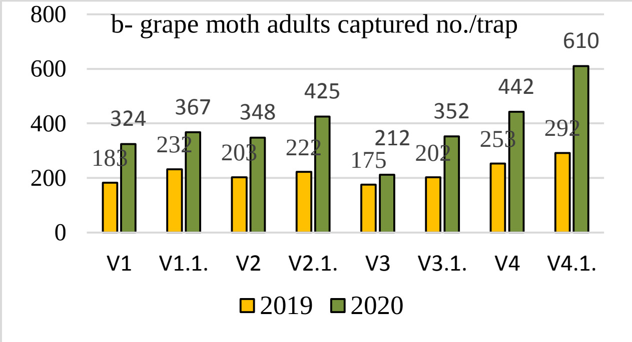


Figure 4. Larvae number (a) and dynamics of *Lobesia Botrana* adult population (b)

Regarding the **production quality**, the first innovative treatment obtained the best results both in 2019 and in 2020 (267 g/l and 262.8 g/l sugars respectively). Overall, there are no significant differences between the treatments in terms of grape production. Harvest data assessment is presented in table 2.

Table 2. The grapes quality

Specifica-tion	year	Treatment						Control	
		Innovative tratatend			Innovative Untreated			Treated	Untreated
		V1	V2	V3	V1.1.	V2.1	V3.1	V 4	V 4.1
Location and variety		MR/ Feteasca neagra							
Date of harvest		06.09.2019/14.09.2020							
Average production/vine (kg)	'19	1,86±0,3 (a)	1,76±0,2 (a,b)	1,79±0,2 (a,b)	1,57±0,3 (a,b)	1,53±0,1 (a,b)	1,68±0,2 (a,b)	1,85±0,3 (a)	1,41±0,1 (b)
	'20	1,06±0,2 (a)	1,04±0,2 (a)	1,02±0,2 (a)	0,98±0,1 (a)	0,93±0,2 (a)	0,945±0,1 (a)	1,04±0,2(a)	0,77±0,1 (a)
Average weight of 100 grains (g)	'19	97±2,8 (a)	96±2,4 (a)	96±2,2 (a)	95±2,3 (a)	95±2,4 (a)	95±2,1 (a)	96±2,2 (a)	95±2,0 (a)
	'20	90±2,0 (a)	88±2,2 (a)	80±2,0 (b)	75±1,2 (c)	71±1,3 (d,e)	74±1,5 (c,d)	88±2,2 (a)	70±1,7 (e)
Sugar content (g/l)	'19	267±3,2 (b)	261,7±3,0 (b,c)	259,6±3,2 (c)	261,7±3,5 (b,c)	260,6±3,0 (c)	260,6±3,2 (c)	273,5±3,3 (a)	257,4±3,4 (c)
	'20	262,8±2, 2(b)	225,7±2,4(f)	262,8±2,6 (b)	255,3±2,5(c)	245,8±2,4 (e)	258,5±2,6 (b,c)	268,1±2,6 (a)	242,6±2,1 (d)
Total acidity (tartaric) (g/l)	'19	4,54±0,2 (b)	4,54±0,3 (b)	4,85±0,3 (b)	4,69±0,2 (b)	5,46±0,4 (a)	5,46±0,5 (a)	4,54±0,1 (b)	4,54±0,1 (b)
	'20	3,75±0,4(b)	4,55±0,5 (a)	3,36±0,3 (b)	3,56±0,3 (b)	3,56±0,4 (b)	3,46±0,4 (b)	3,75±0,3 (b)	3,46±0,2(b)

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p<0,05. For the same compound, a common letter for 2 or more variants shows no significant difference among them.

Conclusions

Three experimental plots were tested using cover crops in comparison with control plots. Each treatment had two subtreatments, one that was phytosanitary treated and another that was not.

During the vegetation period, the vines were inspected repeatedly for epidemic control, in order to determine their resistance to the main diseases of the grapevine: downy mildew, powdery mildew, gray rot and grape black rot. At the same time, plants were inspected for the grapevine moth attack – *Lobesia botrana*.

In 2019, downy mildew, gray rot and grape black rot registered attacks below the threshold. Powdery mildew was an exception in this case, exceeding the threshold, especially for the treated treatments, as well as the untreated control plot.

In 2020, the main manifested diseases consisted of gray rot, grape black rot and downy mildew, the latter causing a moderate attack. Powdery mildew did not manifest in the experimental plots.

Summing up 2019 and 2020, the untreated 4.1 subtreatment registered the highest degree of attack, followed by the rest of the untreated plots. The treated treatments did not register severe degrees of attack.

For the grapevine moth, in both 2019 and 2020, degrees of attack exceeding the threshold were not registered.
From the perspective of production, 2019 registered higher results regarding grape quantity and quality in the treated treatments rather than the untreated treatments. In 2020, the effect of the drought and high temperatures rendered the effect produced by cover crops ineffective, thus obtaining a subpar production in comparison with the year 2019.

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References

Buffara C.R.S., Scapin C.R., et al. (2014). Elaboration and validation of a diagrammatic scale to assess downy mildew severity in grapevine. Ciência Rural, 44: 1384-1391.
Caffi T., Rossi V. and Bugiani R. (2010). Evaluation of a warning system for controlling primary infections of grapevine downy mildew. Plant disease, 94.6 : 709-716.
Stark J.D. and Banks J.E. (2003). Population-level effects of pesticides and other toxicants on arthropods. Annual review of entomology, 48.1: 505-519.
Thomson L.J. and Hoffmann A.A. (2006). Field validation of laboratory-derived IOBC toxicity ratings for natural enemies in commercial vineyards. Biological Control, 39.3: 507-515.
Thomson L.J. and Ary A.H. (2007). Effects of ground cover (straw and compost) on the abundance of natural enemies and soil macro invertebrates in vineyards. Agricultural and Forest Entomology, 9.3: 173-179.

CHARACTERIZATION OF COLORED MAIZE SEED FRACTIONS USING FLUORESCENCE SPECTROSCOPY AND MULTIVARIATE ANALYSIS

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Abstract

Application of fluorescence spectroscopy combined with chemometrics algorithms provides rapid and non-destructive screening method in seed quality estimation, widely used in the agricultural industry and crop breeding. Fluorescence spectroscopy is a technique capable of detecting differs fluorophores among various colored maize seed cultivars and through different seed fractions. In the present study, we used the Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm to analyse the excitation-emission matrices (EEMs) of various cultivars of colored maize (*Zea mays* L.) seeds and its fractions. The EEMs were recorded as a set, with the excitation ranging from 280 nm to 330 nm and the emission spectra ranging from 300 nm to 550 nm. The MCR-ALS analysis yielded two major fluorescence components for all of the analysed samples. Both position and shape of component 1 (C1) varied among the samples. On the other hand, the position and shape were similar for component 2 (C2). C1 could be used as a marker for the discrimination of colored seeds and their fractions. The observed variations in C1 between the analysed seeds may be due to the presence of their individual fluorophores, assigned to anthocyanins, proteins, and phenolics. In conclusion, the MCR-ALS analysis of the seed emission spectra has a great potential for the rapid and non-expensive characterization of various cultivars of colored seeds.

Keywords: *maize seed, fluorescence, Multivariate Curve Resolution-Alternating Least Squares.*

Introduction

Maize (*Zea mays* L.) is considered one of the major food sources worldwide. Health benefits correlated to consumption of the whole grain are related not only to nutrients like carbohydrates, proteins, dietary fiber, vitamins, and minerals but also to the presence of various phytochemicals (Siyuan, Tong, and Liu 2018). Properties of these phytochemicals contribute to the high antioxidant activities of the maize seeds (Del Pozo-Insfran et al. 2006). It is well known that phenolic compounds, mainly phenolic acids, flavonoids, and tannins are major phytochemicals abundant in seeds, with different composition and distribution within the seed fractions (Ndolo and Beta 2014). Polyphenolics like ferulic and p-coumaric acid found in white maize as well as their derivatives, have antioxidant and anticarcinogenic effects according to reported studies. Red colored maize seeds on the other hand have higher anthocyanins content which also expresses antioxidant activity (Del Pozo-Insfran et al. 2006).

Fluorescence spectroscopy is a sensitive, non-destructive and rapid technique, which doesn't require complex sample pretreatment and preparation. It has been used in analysis of various kinds of food, such as cereals food, dairy products, wine, honey and other samples (Sádecká and Tóthová 2007). Fluorescence spectra of cereals are dependent on the species and the cultivar (Zandomeneghi 1999). Cereal food contains a large number of fluorescent molecules

(fluorophores) such as proteins, phenolics and others. The fluorescence spectrum of a food sample is complex, composed of the signals of the contributing fluorophores. In combination with suitable statistical analysis, fluorescence spectra are useful tools for various applications (Sádecká and Tóthová 2007). Applications of the fluorescence spectroscopy combined with Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) for food analyses have been reported previously in many studies (Bartolić et al. 2018; Stanković et al. 2019, 2021).
We developed methods for the measurement and analysis of emission spectra with MCR-ALS of macromolecules composed of different kinds of monomers such as proteins and polyphenols for characterization of different cultivars of pigmented maize seeds (white and red).

Material and Methods

The pigmented (white and red) maize seeds were purchased from the local market in Belgrade, Serbia. The two fractions, inner and outer, were separated using the corresponding laboratory sieves after homogenisation in a mill and subsequently with liquid nitrogen in a mortar with a pestle. Obtained powder samples were used without any further processing before the fluorescence measurements.
The front-face fluorescence measurements of the red and white maize fractions were recorded by an Fl3-221 P spectrofluorimeter (JobinYvob, Horiba, France), equipped with a 450 W Xe lamp and a photomultiplier tube. The ranges of the excitation spectra were 280 - 330 nm, while the range for the recorded fluorescence emission spectra was 300 - 550 nm. A spectral bandwidth of 2 nm was used for excitation and emission slits.
The Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm was applied to analyse the excitation-emission matrices (EEMs) of studied samples. The MCR–ALS has been used to decompose the overlapping mixture of spectral signals into individual components (Stanković et al. 2019).

Results and Discussion

The representative excitation-emission matrices (EEMs) for the inner and outer seed fractions of the various cultivars of pigmented *Zea mays* L. seeds, are presented in Figure 1 (A-D). The differences were observed in their EEMs, which could be explained by the presence of their individual fluorophores, assigned to anthocyanins, proteins, and phenolics (Sádecká and Tóthová 2007). The EEMs of white seeds (Figure 1 A and B), showed considerably weaker emission signal in the range 330 - 360 nm for the excitation 280 - 295 nm, compared to red seeds.

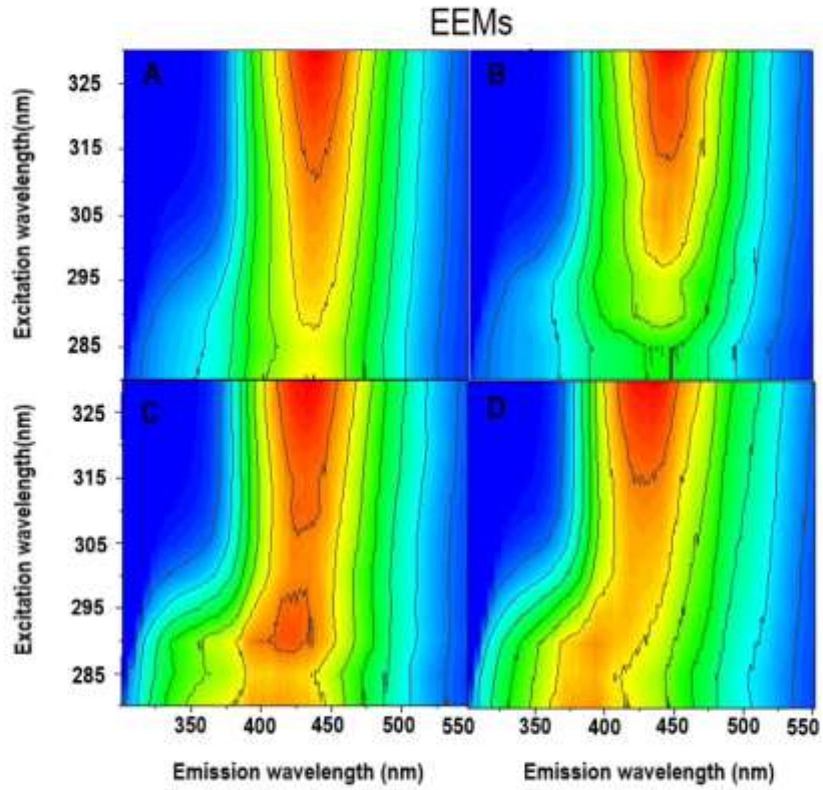

Figure 1. EEMs of the *Zea mays* L. seeds' fractions: inner (A-White and C-red) and outer (B-white and D-red).

Results of MCR-ALS analysis yielded two major fluorescence components. The position and shape of components C1 and C2 varied among the analysed samples.

As shown in Figure 2, the peak position of component 1 (C1) and component 2 (C2) were found around 360 nm and 450 nm, respectively. According to literature data, the fluorescence of the first component (C1) with the emission maximum at 360 nm originates from the aromatic amino acids present in cereal proteins (Zandomeneghi, 1999). Among the analysed samples, the C1 component of the red seeds fraction exhibited the highest relative intensity. Position of the emission maximum of C2 component, assigned to phenolic compounds, varied among the samples.

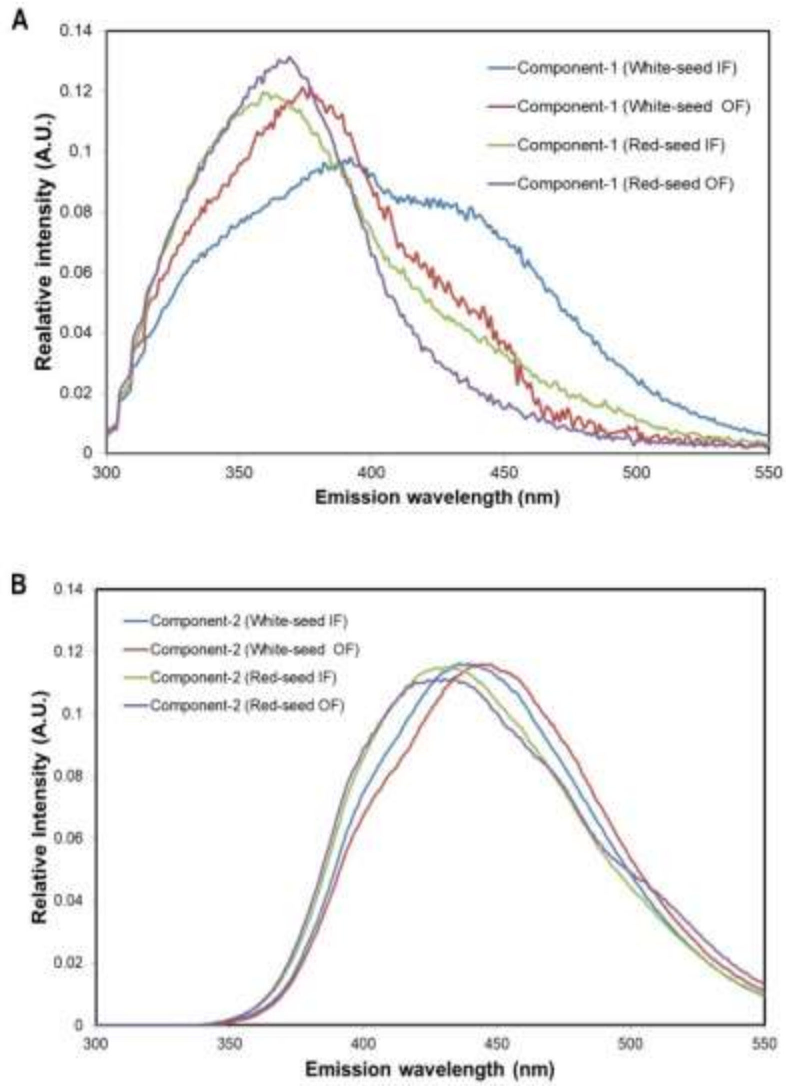

Figure 2. Emission profiles of the spectral components A) component 1 (C1) and B) component 2 (C2) obtained using the MCR-ALS method.

Conclusions

Our results imply that fluorescence spectroscopy combined with the MCR-ALS method could be applied to the rapid, simple, non-expensive characterization of various cultivars of colored seeds and their fractions. As the seed quality depends on different conditions, such as processing, storage, and others, MCR-ALS-derived components may be useful indicators for the screening of cereal seeds' health effects.

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References

Bartolić D, Stanković M, Mutavdžić D, Stanković S, Jovanović D, Radotić K (2018). Multivariate Curve Resolution - Alternate Least Square Analysis of Excitation-Emission Matrices for Maize Flour Contaminated with Aflatoxin B1, Journal of Fluorescence, 28 (3), 729–733.

Ndolo VU, Beta T (2014). Comparative Studies on Composition and Distribution of Phenolic Acids in Cereal Grain Botanical Fractions, Cereal Chemistry, 91 (5), 522–530.

Del Pozo-Insfran D, Brenes CH, Serna Saldivar SO, Talcott ST (2006). Polyphenolic and Antioxidant Content of White and Blue Corn (Zea Mays L.) Products, Food Research International, 39 (6), 696–703.

Sádecká J, Tóthová J (2007). Fluorescence Spectroscopy and Chemometrics in the Food Classification - A Review, Czech Journal of Food Sciences, 25 (4), 159–173.

Siyuan S, Tong L, Rui Hai L (2018). Corn Phytochemicals and Their Health Benefits, Food Science and Human Wellness, 7 (3), 185–195.

Stanković M, Bartolić D, Šikoparija B, Spasojević D, Mutavdžić D, Natić M, Radotić K (2019). Variability Estimation of the Protein and Phenol Total Content in Honey Using Front Face Fluorescence Spectroscopy Coupled with MCR–ALS Analysis, Journal of Applied Spectroscopy, 86 (2), 256–263.

Stanković M, Bartolić D, Mutavdžić D, Marković S, Grubić S, Jovanović NM, Radotić K (2021). Estimation of Honey Bee Colony Infection with Nosema Ceranae and Varroa Destructor Using Fluorescence Spectroscopy in Combination with Differential Scanning Calorimetry of Honey Samples, Journal of Apicultural Research 0 (0), 1–7. DOI: 10.1080/00218839.2021.1889803.

Zandomeneghi, M (1999). Fluorescence of Cereal Flours, Journal of Agricultural and Food Chemistry, 47 (3), 878–882.

THE STATE OF ORGANIC GRAIN PRODUCTION IN SERBIA

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Abstract

Areas under organic plant production in Serbia have been permanently increasing. In 2019, of the total area under this type of production (21265.4 ha), the greatest share was under orchards (5324.4 ha), and cereals (21265.4 ha). Considering that organic cereal production has been growing continuously, the aim of this study was to show the range of areas under organic cereals in Serbia and to show the regional distribution for the period 2016-2019. Data were obtained from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. The following methods were used in the study: desk research, content analysis, comparative analyses and analyses of base and chain indices. The largest production of cereals was recorded in the region of Vojvodina (3914.3 ha), and then in the regions of southern and eastern Serbia (708.6 ha). In 2019, the organic wheat production was predominant (2264.9 ha), maize (481.6 ha) and silage maize (323.5 ha) ranked second and third, respectively, while the smallest area was under millet (only 3.3 ha). Considering that Serbia has exceptionally favourable natural conditions for cereal production, it is evident that natural potentials are not used enough, although needs for healthy, organic cereals in the world market are unlimited.

Key words: *organic production, cereals, areas.*

Introduction

An excessive, uncontrolled application of chemicals and fertilisers in conventional agriculture has resulted in numerous adverse consequences for the environment, natural resources, water and soil quality, biodiversity, soil fertility, as well as food safety and quality (Kristiansen et al., 2006). In contrast to conventional agriculture, organic farming is a sustainable system of healthy food production, without residues of pesticides and additives, and with preservation of not only human health, but, also, of the health of plants, animals, soil, agroecosystem and the entire environment (Šeremešić et al., 2010). According to the Codex Alimentarius Commission (2001), '*organic agriculture is a holistic production management system that avoids use of synthetic fertilizers, pesticides and genetically modified organisms, minimizes pollution of air, soil and water, and optimizes the health and productivity of interdependent communities of plants, animals and people*'. An insignificant percentage (<1%) of the worldwide arable land is organically cultivated. Moreover, the global population considerably consuming organically produced food is also insignificant in number. Based on these pieces of information, it is evident that organic food is intended just for niche markets. The set goals of organic farming are far from being achieved in farms throughout the world. Yields obtained in organic agriculture are relatively low (Rahman et al., 2016) and still lower than those achieved in conventional agriculture (Reganold and Wachter, 2016). However, the organic products are more profitable and environmentally friendly, equally or more nutritious and contain less or do not contain at all pesticide residues in comparison with conventionally produced food. In order to achieve objectives of the organic agriculture, farmers have to implement many practices, such as: crop rotation with the use of various crops, different combinations of plants and livestock, leguminous plants (because of nitrogen fixation), organic manure and biological control of pests. Furthermore, not only that these practices should be applied, but the local accessible resources have to be used in the best possible way. In this regard, organic systems are naturally adjusted to a given locations and limitations (Scialabba and Müller-Lindenlauf, 2010). Globally, the increase in the organic agricultural land has been evident for many years (http://www.fao.org). The same trend has been observed in Serbia: the greatest areas have been cultivated with organic fruits (5324.4 ha), followed by cereals (4788.8 ha), industrial plants (2229.6 ha), fodder plants (1797.9 ha), medicinal and aromatic plants (258.5 ha), and the smallest areas are cultivated with organic vegetables (184.2 ha) (Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije/Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2021).

Material and Methods

Considering the increase in the areas under organic farming in the Republic of Serbia, the production of organic fruits ranks first and is followed by organic cereal production. Using literature data referring to organic agricultural production, as well as data available from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the organic cereal production in Serbia in the 2015-2019 period was analysed and graphically presented (using the Microsoft Excel 2010 program) in this study. The study encompassed the following methods: desk research, content analysis, comparative analyses and analyses of base and chain indices. The value of areas under organic cereal production in 2015 was used as a base.

Results and Discussion

Organic plant production has been increasing permanently not only in the world but also in Serbia. According to the data of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (2021), organic plant production was performed on the area of 21265.4 ha in 2019, while this area amounted to 15298 ha in 2015, which was an increase of 5967.4 ha or 39% for the four-year period. Although these areas increased in 2018 and 2019, the decrease of the areas was recorded in 2016 (by 6.1%) and 2017 (by 12.3%) (Table 1).
According to the 2019 reports of FiBL and FAO (http://www.fao.org), organic production took place in 187 countries on the area of 72.3 million hectares, while global sales of organic food and drink reached more than 106 billion euros. Moreover, according to the same reports, 3.1 million organic producers were registered globally and only 513 in Serbia (Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2021). Considering the global distribution of areas under organic farming in 2019, Oceania is a region with the most organic agricultural land (35.9 million ha), followed by Europe (16.5 million ha), Latin America (8.3 million ha), Asia (5.9 million ha), North America (3.6 million ha), while the smallest areas are in Africa (only 2 million ha). Europe is a region with a steady, long-term growth of organic agricultural land; it has over 23% of the world's organic agricultural land and is followed by Latin America (12%). Ten countries with the largest areas of organic agricultural land in 2019 were: Australia (35.69 million ha), Argentina (3.67 million ha), Spain (2.35 million ha), USA (2.33 million ha), India (2.3 million ha), France (2.24 million ha), China (2.22), Uruguay (2.14

million ha), Italy (1.99 million ha) and Germany (1.61 million ha) (Willer et al., 2021). According to Willer et al. (2021), in EU, in 2019, the organic market again grew faster than organic areas, and the EU organic food market increased to more than 41 billion euros or by 8%, whereas the farmland grew by 6%.

Table 1. Areas under organic plant production in Serbia in the 2015-2019 period

Year	Conversion period	Base indices	Organic status	Base indices	Total(ha)	Base indices
2015	7669.5	100	7628.5	100	15298.0	100
2016	6966.5	90.8	7391.4	96.9	14358.0	93.9
2017	5895.6	76.9	7527.6	98.7	13423.1	87.7
2018	6599.9	86.1	12654.7	165.9	19254.6	125.9
2019	7539.4	98.3	13726.1	179.9	21265.4	139.0

*Source: The author's presentation according to data of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (2021)

Organic production in Serbia varied in the analysed period from 4251.9 ha in 2015 to 4607.3 ha in 2016, so areas increased by 355.4 ha. In 2017 and 2018 areas decreased to 3661.7 ha and 3613.6 ha, respectively, while in 2019 the increase by 1175.2 ha was recorded and areas under organic production amounted to 4788.8 ha (Figure 1). Considering global cereal production in 2019, it took place on the area of 5073137 ha, ranking the first, while oilseed production on the area of 1676502 ha ranked second. The largest areas under organic cereal production are in Europe (2958165 ha), followed by Asia (1253310 ha), North America (582255 ha), Latin America (163769 ha), Africa (74344 ha) and Oceania (41293 ha) (Willer et al., 2021).

Figure 1 shows areas under organic production of nine plant species, with wheat ranking first (on average for the analysed period from 2015 to 2019) (41.4%).

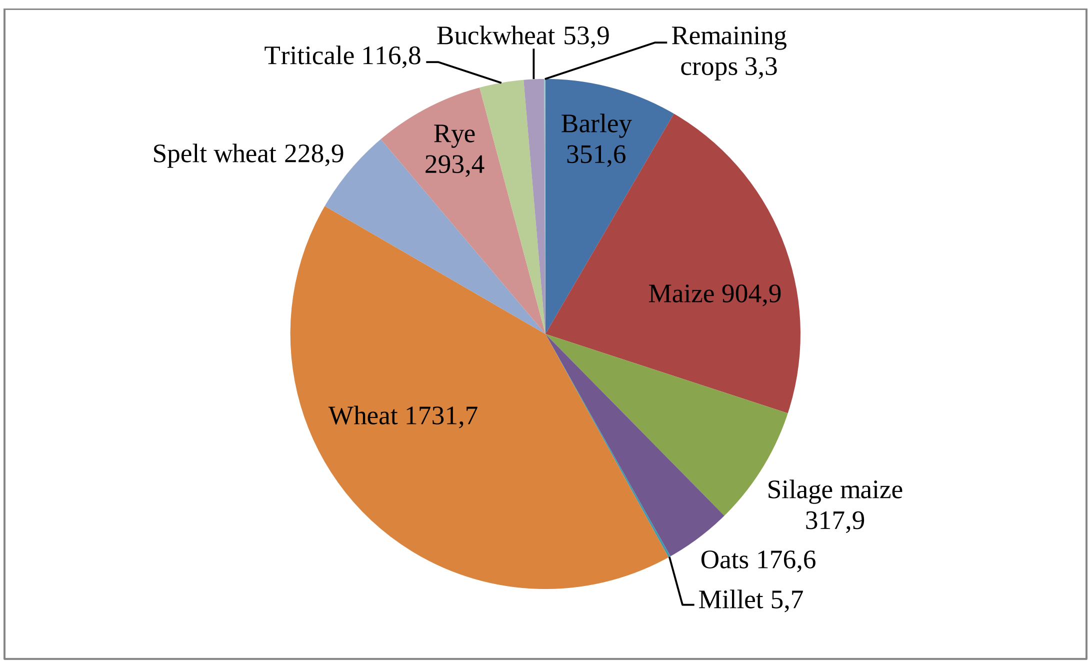

Figure 1. Areas (ha) under certain cereal crops in the organic system in Serbia (average for the 2015-2019 period)

The second largest areas were under maize (21.6%). Smaller areas were cultivated with barley (8.4%), silage maize (7.6%), rye (7%) and spelt wheat (5.5%), while less than 5% of areas were cultivated with oats (4.2%), triticale (2.8%) and buckwheat (1.3%).
Figure 2 presents the trend of areas under organic grain crops in Serbia in the 2015-2019 period.
In regard to the regional distribution of production, Vojvodina with the share of 3914.3 ha in 2019 ranked first and was followed by regions of south and eastern region Serbia, where production areas significantly varied over the four-year period.

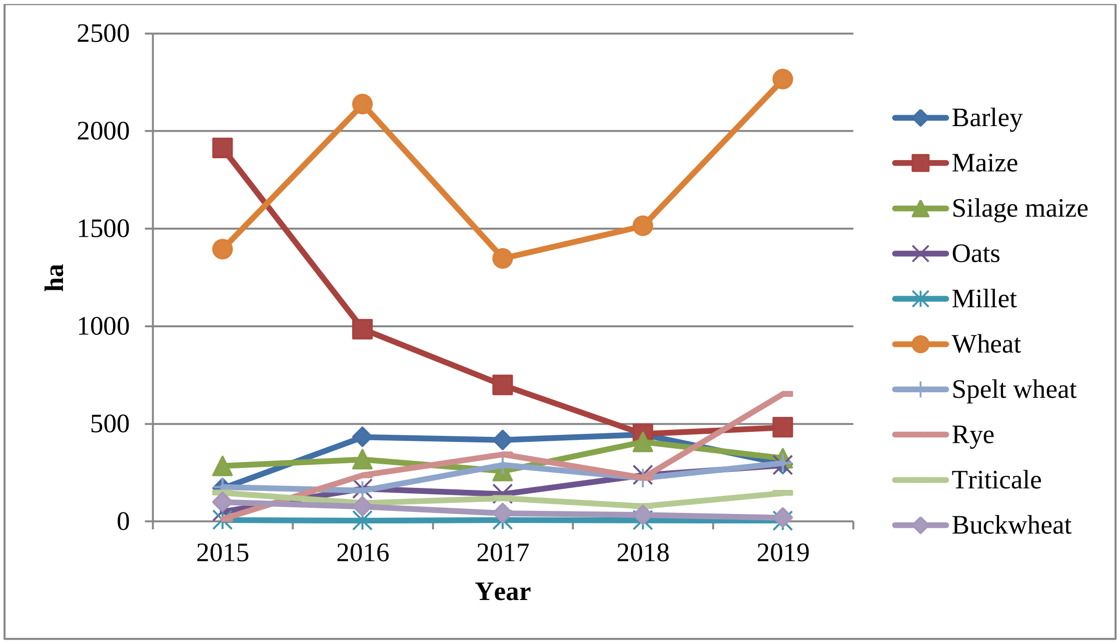

Figure 2. Areas (ha) under certain cereal crops in the organic system in Serbia in the 2015-2019 period

Namely, total of 158.8 ha under organic cereal production in 2015 increased seven times in 2016 (1182.8 ha). Then, during the following three years, these areas were reduced and amounted to only 708.6 ha in 2019 (Figure 3.). Even smaller production was registered in the region of Šumadija and western Serbia, where areas varied from 16.2 ha in 2015 to 160.4 ha in 2019. The smallest total area under organic cereal production was in the region of Belgrade and amounted to only 5.4 ha.

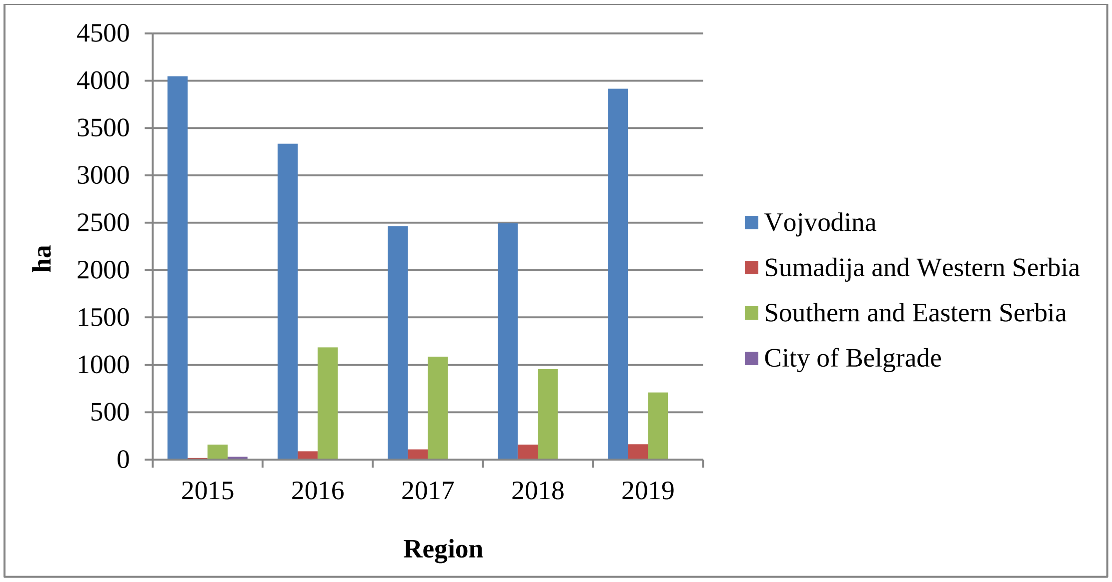

Figure 3. Areas under organic production in Serbia, over regions in the 2015-2019 period

In regard to the distribution of the global organic cereal area over a cereal type, wheat ranked first with 33.32%, and was followed by grain maize and silage maize with 14%, rice with 12.66%, oats with 11.63%, barley with 9.99%, rye with 5.47%, and millet with only 0.46% (Willer et al., 2021). The dynamics of changes in areas under certain grain crops in Serbia in the 2015-2019 period was presented by base and chain indices (Table 2).

Table 2. Dynamics of changes in areas under certain grain crops in Serbia in the 2015-2019 period

	Base indices (2015 = 100)				Chain indices			
Cereal crop	**2016**	**2017**	**2018**	**2019**	**2016**	**2017**	**2018**	**2019**
Barley	254.9	245.6	262.4	173.3	254.9	96.3	106.8	66.0
Maize	51.5	36.5	23.4	25.2	51.5	70.9	64.2	107.5
Silage maize	111.8	90.9	143.4	114.0	111.8	81.3	157.8	79.5
Oats	335.9	281.2	476.9	578.8	335.9	83.7	169.6	121.4
Millet	49.8	98.8	76.2	42.1	49.8	198.2	77.1	55.3
Wheat	153.2	96.6	108.5	162.4	153.2	63.1	112.3	149.6
Spelt wheat	89.7	164.8	126.1	170.0	89.7	183.6	76.6	134.8
Rye	2070.2	3008.6	1957.5	5730.1	2070.2	145.3	65.1	292.7
Triticale	64.1	80.7	53.3	98.8	64.1	125.9	66.0	185.5
Buckwheat	76.8	42.5	33.1	19.3	76.8	55.3	78.0	58.3

*Source: The author's presentation according to data of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (2021)

The drastic increase in the areas of 478.8% and 5630.1% was recorded in oats and rye, respectively in comparison to the base year (2015). A noticeable increase of approximately 70% was observed in 2019 in barley, wheat and spelt wheat. A pronounced decrease in areas were recorded in 2019 in buckwheat (by 80.7%), maize (by 74.8%) and millet (by 57.9%) in comparison to 2015. The areas of organic production of silage maize and triticale noticeably oscillated in the observed period, but were approximately the same.

Conclusions

The total area under organic cereal production in Serbia ranked second after areas under organic fruits. In regard to the regional distribution of organic production, Vojvodina encompassed the largest areas and the regions of south and eastern Serbia followed. Wheat, among nine cereal crops organically grown in the 2015-2019 period ranked first. The extreme increase in areas in relation to the base year was recorded in oats (478.8%) and rye (5630.1%), and then in barely, wheat and spelt wheat. On the other hand, the pronounced decrease in the areas was recorded in buckwheat, maize and millet. Serbia, with extremely favourable climate conditions, large areas with unpolluted land, great number of farmers, is a country with a high potential for production of organic cereals. Nevertheless, the number of farmers and the size of production areas, despite all favourable conditions, are still small.

References

Codex Alimentarius Commission (2001). *Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods*. First Revision. Joint Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) Food Standards Program, Rome, Italy. Available at Web site http://www.codexalimentarius.net/download/standards/360/CXG_032e.pdf

FiBL and FAO (2021). http://www.fao.org/family-farming/detail/en/c/1378841/ Available at 30.5.2021.

Kristiansen, P., Taji, A., Reganold, J. (Eds) (2006). Organic Agriculture A Global Perspective. Published by CSIRO PUBLISHING, Collingwood Victoria, Australia.

Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije (2021). http://www.minpolj.gov.rs/organska/?script=lat Available at 30.5.2021

Rahmann, G., Reza Ardakani, M., Bàrberi, P., Boehm, H., Canali, S., Chander, M., Zanoli, R. (2016). Organic Agriculture 3.0 is innovation with research. Organic Agriculture, 7(3), 169–197.

Reganold, J., Wachter, J. (2016). Organic agriculture in the twenty-first century. Nature Plants, 2, 15221.

Scialabba, N. E.-H., Müller-Lindenlauf, M. (2010). Organic agriculture and climate change. Renewable Agriculture and Food Systems, 25(02), 158–169.

Šeremešić, S., Vojnov, B., Manojlović, M., Milošev, D., Ugrenović, V., Filipović, V., Babec, B. (2017). Organska poljoprivreda u službi biodiverziteta i zdravlja. Letopis naučnih radova, 41 (2), 51-60.

Wiiler, H., Trávníček, J., Meier, C., Schlatter, B. (eds.) (2021). The World of Organic Agriculture Statistics and Emerging Ttrends 2021. Research Institute of Organic Agriculture FiBL, Frick, and IFOAM – Organics International, Bonn.

THE INFLUENCE OF ORGANIC FERTILIZERS ON THE SEED YIELD AND SEED QUALITY OF BUTTERNUT SQUASH (*CUCURBITA MOSCHATA*) GROWN ON DIFFERENT TYPES OF SOIL

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Abstract

Butternut Squash (*Cucurbita moschata*) is grown in Serbia on relatively small areas, despite its extraordinary nutritional properties that place it among the species with significant potential for the food industry. However, in the future, due to climate change, it is expected to expand its production in our country and region, primarily due to tolerance to high temperatures and drought, but also due to significant tolerance to diseases. In order to improve the technology of growing butternut squash in accordance with organic principles, a field experiment was conducted on the two soil types i.e. two different locations, with aim to investigate the effects of application of two different fertilizers on the seed yield and seed quality. Both locations are situated in the basin of the river Velika Morava, on two different types of soil (vertisol soil type and brown forest soil). At both locations, the pre-crop was corn and the applied agro-technical measures were in accordance with the principles of organic production. The average yield of Butternut Squash seeds varied from 678,1 kg/ha, as recorded on the brown forest soil, on the control treatment without fertilization to 918.75 kg/ha as recorded on the treatment with organic fertilizer NP 1 on the vertisol. Significant differences were also observed in seed germination which ranged from 84.67% in the control treatment on the brown forest soil to 98.33% as recorded in the treatment with the organic fertilizer NP2 on vertisol.

Keywords: *fertilizers, organic production, seed quality*.

Introduction

Butternut Squash (*Cucurbita moschata*) is an annual vegetable species belonging to the *Cucurbitaceae* family with vining growth, large and dark green leaves with bright patterns and big yellow flowers. It is grown for its specifically shaped fruits with characteristic color. In a human diet, it is appreciated for its distinctive taste, high provitamin A content and low fat content (Armesto *et al*. 2020). Thanks to these properties it has a low caloric content and it is interesting for the control of body weight, as well as cholesterol and triglyceride levels in the blood (Choi *et al*., 2007). Despite its extraordinary nutritional properties that place it among the species with significant potential for the food industry, Butternut Squash is grown on relatively small areas in Serbia, mostly like a few plants in farmers own gardens, rarely as a cash crop. However, In the future, due to climate changes, it is expected to expand its production in our country and region, primarily due to tolerance to high temperatures and drought (Ara *et al*. 2013, Shen and Yuan, 2020), but also due to significant tolerance to diseases and pests (Cavanagh *et al*., 2009; King *et al*. 2010).

The exact information about the butternut squash harvested area in the world is not available. However, the area on which the pumpkins, squash and gourds were cultivated ranged from 1.561 to 2.07 million ha in the last decade. In that period the average yields of mentioned species altogether ranged from 13.07 to 14.9 t/ha (FAOSTAT DATA 2021). According to some scientific works, the yields of butternut squash ranged from 23.03 to 78.61 for different varieties grown in the Slovak republic (Andrejiova *et al*., 2018), from 19.4 to 34.9 t/ha for different crop densities (Rangarajan *et al*. 2003), from 18.9 to 31.3 t/ha for different chemical and organic fertilizers in the agroecological conditions of Iraq (Ali *et al*., 2019), from 59.4 to 79.8 t/ha for different irrigation regimes and mulching in the agroecological conditions of northern India (Mishra, 2017). However, there is a lack of data concerning butternut squash seed yields and seed quality (Sajjan and Prasad, 2009). The Republic Statistical Office of the Republic of Serbia does not have available data on surfaces, yield and production of butternut squash in Serbia (Republic of Serbia, 2021a). According to National list of registered varieties, at the moment, only two different varieties of butternut squash are registered (Republic of Serbia, 2021b).
The two most frequent soil types in the central Serbia are the vertisol and the brown forest soil. Vertisols are soils with high silt and clay content that shrink and swell extensively upon changing soil moisture conditions (thanks to its clay content). They occur worldwide under various parent material and environmental conditions. Vertisol exhibits unique morphological charachteristic such as the presence of wedge-shaped aggregates so the shrink-swell phenomena are the dominant pedogenic processes in vertisols. That causes the changes in interparticle and intraparticle porosity when the moisture content is changing. In central Serbia, field and vegetable production is very prevalent on this type of land (Coulombe *et al*. 1996, Jelić *et al*. 2011; Dugalić and Gajić, 2012).
The brown forest soils occur in Europe, North America, Russia, China and elsewhere in the world in broadleaf forests of the temperate zone and agricultural land on which the previous forests were cleared sometime in the past. They have some good productive properties for field and vegetable crops production but in Serbia they are used more frequently for the production of fruits and vines (Dugalić and Gajić, 2012; Shishkov and Kolev, 2014).
One of the most important tasks in expanding the production of butternut squash is to provide sufficient amounts of seeds for planting and apply all knowledge that can affect production improvement. To that end, the aim of this work is to determine the optimal model of fertilizer application on these two most prevalled types of land in central Serbia.

Material and Methods

The field experiments were performed at two locations with different soil types, the first one near Jagodina town on the vertisol soil type (44°01′55.23″ N 21°15′18.55″ E, 108.0 m above sea level) and the second one in Žabari municipality in Serbia on the brown forest soil (44°37′38.56″ N 21°11′21.08″ E, 222 m above sea level). The basic chemical properties of different soil types were shown in Table 1.

Table 1. Soil chemical properties at the two experimental locations

Location	pH H_2O (KCl)	$CaCO_3$ (%)	Humus (%)	Total N (%)	P (ppm)[1]	K (ppm)[1]
Žabari	6.61 (5.15)	0.0	2.30	0.115	196.3	283.0
Jagodina	7.32 (5.59)	0.0	2.92	0.15	195.8	361.4

[1-] Available P an K

The previous crop was field corn and the harvest residues were chopped and plough into the soil. A mineral fertilizer (100 kg/ha, N-46%, UREA) was added. However, at the Žabari location about a half of harvested residues were colected and removed from the experimental field. At the late autumn, additional amount of mineral fertilizer (200 kg/ha N:P - 11:52) was applied and the soil is further tilled using disc harrow.
At the mid April, examined fertilizers (8.0 t/ha of organic fertilizer NP 1 – dried chicken manure with N, P and K content of approximately 4, 4, and 4 percent; 15 t/ha of organic fertilizer NP2 – with declared N, P and K content of approximately 4, 7, and 8 percent, respectively) were applied on the marked experimental plots and incorporated in to the surface soil layer using two wheel tractor with rotary tiller. Up to 4 seeds were sown manualy on the marked places (1.5m distance betveen rows and 0.6 distance betveen plants in the row) on the April the 18th (Žabari) and April the 19th (Jagodina).
There were 9 elementary plots randomly distributed on the Žabari location and also in another location the same (Jagodina). The size of elementary plot was 6.0 x 4.2 m, consisted of 4 rows each. The surface area of each elementary plot was 25.2 m^2.
After shoot emerging, excessive plants were removed and the space betveen the rows were cultivated several times in order to supress the apearance of weeds. Weed control within rows was performed manually. All measures applied except the mineral fertilization (farmyard of acceptable quality and allowed mineral fertilizers were not available) were in accordance to the Law on Organic Production and the accompanying regulations (Republic of Serbia 2021c).
Data on climate parameters for the nearest meteorogical station (Veliko Gradište) were provided through the internet portal of the Republic Hydrometeorological Service of Serbia (RHSS 2021). However, due to the specifically modified weather conditions at the Žabari experimental field site, during some summer and autumn months, precipitation was measured with the help of a round vessel with a graded scale, and the results are shown in parentheses (Table 2).

Table 2. Temperature (T) and precipitation (P) parameters for Butternut squash crop at two locations during the trial (2020), with long-term averages

Months	Žabari				Jagodina			
	2020		1981-2010		2020		1981-2010	
	T ^oC	P (mm)*	T ^oC	P (mm)	T ^oC	P (mm)	T ^oC	P (mm)
Apr	12,2	2	11,8	55.9	12.3	23.1	11.4	52.9
May	15,8	93	17	73.6	16	81.1	16.2	78.7
Jun	20,4	89,6 (78)	19,9	87.6	20.2	68.5	19	87.5
Jul	22	110,6 (89)	21,9	67.7	22.4	54.5	20.4	60.7
Aug	23	62 (43)	21,5	56.7	22.9	86.7	20.1	43.4
Sep	19,5	30,3 (37)	16,8	50.3	19.4	49.1	16.4	47.7
Oct	12,9	101,8 (86)	11,7	41.2	13.1	83.1	11.1	37.8
Nov	6,4	15,7 (12)	6	47.3	6.4	17.3	5.9	52.9
Av/Sum	16.5	505(440)	15.8	480.3	16.6	463.4	15.06	461.6

*- values within parentesis are related to the seasonal precipitation corection for the Žabari experimental site.

The Butternut Squash fruits were harvested during the late october of 2020. Samples per each treatment replication were taken (inner rows were used for sampling in order to avoid border effects). Collected fruits were weight on the field using hand scale (the data not shown) and the seeds were taken out from each fruit. The seed yields (natural seeds without processing) were measured using the technical scale after few days of drying in the tin layers in the ventilation barn. For each seed sample (three repetitions) the percentage of germination was determined in the acredited seed laboratory. The calculated percentage of germination data were transformed by arcsine $\sqrt{(x/100)}$ prior to analysis. Two way ANOVA and further LSD-testing were performed using Statistika 7 software package for Microsoft Windows.

Results and Discussion

Conventional farming agricultural production, without farmyard application, have been practiced at both experimental sites and strong anthropogenic factor with long-term rotation of corn, wheat and meadow/clover was present. The experiment was transition to organic agricultural practice. Chemical analysis of the examined soils from Žabari and Jagodina experimental sites indicate similar values of basic parameters with smaller differences in pH values, humus and potassium content (Table 1.). On the other side, there were differences concerning temperature conditions and precipitations (Table 2.). Despite the fact that the year was with enough rainfall, severe spring drought in the pre-sowing period and after sowing on the Žabari experimental site, slowed down the germination and emerging of butternut squash plants which later affected the crop homogeneity and possibly the results of this trial. Also the precipitation schedules during the vegetation period were diverse on the both localities. However, the examined effects of locality and applied fertilizers significantly affected butternut squash seed yields and germination rate (Table 3.).

Table 3. Mean squares (MS) from ANOVA for the butternut squash seed yield and the percentage of germination on two examined localities and different treatments (Fertilizers).

Effect	d.f.	Seed Yield	Percentage of germination
Locality (L)	1	35529**	29.4*
Fertilizers (F)	2	39366**	59.1**
L / F	2	89	112.4**
Error	12	357.41	3.61

*,** – significant at the 0.05 and 0.01 levels of probability, respectively

Table 4. Butternut squash seed yields (t/ha) and the percentage of germination on two examined localities and different treatments (Fertilizers)

Locality	Fertilizers	Seed yield (t/ha)	Germination (%)
Žabari	Control	678.10	84.67
	NP1	786.50	98.33
	NP2	834.07	96.00
Jagodina	Control	762.23	96.67
	NP1	884.27	93.33
	NP2	918.73	96.67
	$lsd_{0.05}$	19.42	1.95
	$lsd_{0.01}$	27.22	2.74

During the experiment, the average butternut squash seed yield on both sites was 810.65 kg/ha, but on vertisol (Jagodina), higher average yields were achieved compare to brown forest soil (Municipality of Žabari). The highest butternut squash seed yield was achieved at the locality of Jagodina when NP2 fertilizer has been applied (918.73 kg/ha) and the lowest (678.1 kg/ha) on the control treatment on the Žabari experimental site (Table 4.).

According to results of Sajjan and Prasad (2009), *Cucurbita moschata* seed yields can be doubled using fertilizers and plant growth stimulators. The fruit yields achieved are significantly below the yields reported by Mishra (2017) and significantly below the highest yields reported by Andrejiova *et al*. (2018) (the data not shown). Knowing the approximate share of seeds in butternut squash fruit yields, our results varied in accordance with results of Rangarajan *et al*. (2003) and Ali *et al*., (2019), but were higher compare to some other research (Sajjan and Prasad, 2009).

The percentage of germination is one of the key parameters concerning seed quality. The *Cucurbita moschata* percentage of germination is usually above 90% (Valdez-Melara *et al*., 2009) and all treatments were in that range except control treatment in Žabari, probably due to presence of some fruits with sturdy and immature seeds.

Conclusions

Yields that were significantly below genetic potential indicate that agroecological conditions were not optimal despite sufficient amounts of precipitation. Based on the results of the experiment, a better choice for butternut squash seed production is vertisol. In order to enable high seed yields as well as sustainable organic production and production according to principles of organic butternut squash cultivation during the conversion period it is necessary to use high doses of organic fertilizers. The NP2 fertilizer is recommended, regardless of the type of soil on which the production is organized.

When it comes to the basic seed quality parameter, the percentage of germination, it is not possible to give definitive instructions on this issue, because despite statistically significant differences between treatments, logical conclusions cannot be made and further investigation is needed.

Acknowledgement

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References

Ali, A. F., Alsaady, M. H. M., Salim, H. A. (2019). Influence of Magnetized Water and Nitrogen Bio-fertilizers on the Quantity and Quality Features of the Butternut Squash Cucurbita moschata. Iraqi Journal of Science, 2398-2409.

Andrejiova, A., Hegedusová, A., Maťová, A., Vargová, A. (2018). The possibility of butternut squash growing in conditions of Slovak Republic. International Journal of Agriculture Forestry and Life Sciences, 2(2), 116-121.

Ara N., Nakkanong K., Lu W., Yang J., Hu Z., Zhang M. (2013). Antioxidant enzymatic activities and gene expression associated with heat tolerance in the stems and roots of two cucurbit species ("*Cucurbita maxima*" and "*Cucurbita moschata*") and their interspecific inbred line "Maxchata". International journal of molecular sciences, 14(12), 24008-24028.

Armesto, J., Rocchetti, G., Senizza, B., Pateiro, M., Barba, F. J., Domínguez, R., Lorenzo, J. M. (2020). Nutritional characterization of Butternut squash (*Cucurbita moschata* D.): Effect of variety (Ariel vs. Pluto) and farming type (conventional vs. organic). Food Research International, 132, 109052.

Cavanagh, A., Hazzard, R., Adler, L. S., Boucher, J. (2009). Using trap crops for control of *Acalymma vittatum* (Coleoptera: Chrysomelidae) reduces insecticide use in butternut squash. Journal of economic entomology, 102(3), 1101-1107.

Choi, H., Eo, H., Park, K., Jin, M., Park, E.-J., Kim, S.-H., Kim, S. (2007). A water soluble extract from Cucurbita moschata shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. Biochemical and Biophysical Research Communications, 359(3), 419–425. https://doi.org/10.1016/J. BBRC.2007.05.107

Coulombe, C. E., Wilding, L. P., & Dixon, J. B. (1996). Overview of Vertisols: characteristics and impacts on society. Advances in Agronomy, 57, 289-375.

Dugalić G., Gajić B. (2012): Pedologija. Univerzitet u Kragujevcu, Agronomski fakultet u Čačku (Pedology-Soil Sciente, University of Kragujevac, Faculty of Agronomy, in Serbian), 1-295.

FAOSTAT DATA 2021 - http://www.fao.org/faostat/en/#data/QC

Jelić, M. Ž., Milivojević, J. Ž., Trifunović, S. R., Đalović, I. G., Milošev, D. S., & Šeremešić, S. I. (2011). Distribution and forms of iron in the vertisols of Serbia. Journal of the Serbian Chemical Society, 76(5), 781-794.

King, S. R., Davis, A. R., Zhang, X., Crosby, K. (2010). Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Scientia horticulturae, 127(2), 106-111.

Mishra, A. C. (2017). Effect of irrigation systems and mulching on soil temperature and fruityield of marrow (Cucurbita pepo) in temperate Himalaya of Uttarakhand. Vegetable Science, 44(1), 66-70.

Rangarajan A., Ingall A., Orzolek D., Otjen L. (2003). Moderate defoliation and plant population losses did not reduce yield or quality of butternut squash. HortTechnology, 13(3), 463-468.

RHSS 2021 - Republic Hydrometeorological Service of Serbia, http://www.hidmet.gov.rs/

Republic of Serbia 2021a – Statistical Office of the Republic of Serbia https://www.stat.gov.rs/

Republic of Serbia 2021b – Plant protection Directorate, Department for the Plant Varieties Registration, Ministry of Agriculture, Forestry and Water Management) http://www.sorte.minpolj.gov.rs/sites/default/files/rsprilogom_3.pdf

Republic of Serbia 2021c – Grupa za orgasnku proizvodnju, MPŠV, (Organic Production Group, Ministry of Agriculture, Forestry and Water Management) http://www.minpolj.gov.rs/organska/?script=lat

Sajjan A., Prasad M. (2009). Effect of fertilisers and growth regulators on seed yield and quality in pumpkin (*Cucurbita moschata* poir.). Agricultural Science Digest, 29(1), 20-23.

Shen, C., Yuan, J. (2020). Genome-wide characterization and expression analysis of heat shock transcription factor family in pumpkin (*Cucurbita moschata*). BMC plant biology, 20(1).

Shishkov T., Kolev N. (2014). Brown Forest Soils. In The Soils of Bulgaria (pp. 115-125). Springer, Dordrecht.

Valdez-Melara M., García A., Delgado M., Gatica-Arias A., Ramírez-Fonseca P. (2009). In vitro plant regeneration system for tropical butternut squash genotypes (*Cucurbita moschata*). Revista de Biología Tropical, 57, 119-127.

THE INFLUENCE OF SOME BIO-PRODUCTS ON GERMINATION AND PROTECTION OF *CHAMOMILLA RECUTITA* (L.) RAUCH SEEDS

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Abstract

The effects of six biological plant protection products, permitted in organic production ('Extrasol F', 'Polyversum', 'Timorex gold',' Vegard', 'Ozoneem trichul', 'EcoBooster Calcium'), in addition to two herbal preparations ('LAB 3' and 'LAB 4'), were examined on germination and seed quality of *C. recutita* cv. "Banatska". The aim of study was to discover the most effective bio-product with beneficial effects on quality and health of German chamomile seeds. Seed germination testing was conducted according to the standard procedure suggested by a Rule book on seed quality control, while the seed health was examined by the filter paper method. The seeds were treated with 15 ml of bio-product solution, while the same amount of distilled water was used as a control. The experiment was conducted in triplicates, and the seeds were observed on 14^{th} day following the treatment. In comparison to control, seed germination rate was increased in following treatments: 'LAB 4' by 11%, 'Extrasol F' by 9%, 'EcoBooster Calcium' and 'LAB 3' by 2%. Based on the symptoms observed on the seed surface, presence of *Alternaria* sp. was confirmed. Compared to control, the infection of seeds was reduced by 1% in treatments with 'Vegard', 'LAB 3' and 'LAB 4'. However, doubling the concentration didn't reduce infection but reduced germination in comparison to control. Since 'LAB 4' and 'Extrasol F' affected the most germination and to a certain extent prevented seed infection, both bio-products could be recommended for safe application on chamomile seeds.

Keywords: *German chamomile*, *germination and seed health*, *biological products*, *Alternaria* sp., *organic.*

Introduction

Chamomilla recutita L. is one of the most famous medicinal plants in Serbia, belonging to the Asteraceae family. It is cultivated for its inflorescences. Once properly dried, they represent well known herbal drug *Chamomilae flos*, which is widely used in pharmaceutical and cosmetic industries. It contains 0.2-1% essential oil (*Aetheroleum chamomilae*) whose color, depending on temperture, varies from dark blue to green (Glamočlija et al., 2015). As the plant is well-known for it antiseptic, antibacterial and antiviral properties the demands for its herbal drug is continuosly increasing. Apart that German chamomille is used in various herbal preparations for plant nutrition and protection it is also used in the composting procedure (Oljača et al., 2020). Therefore, it is quite important to enable conditions for production of healthy and safe herbal raw material, free from pesticide residues, heavy metals and economically harmful pests and pathogens. In order to produce a sufficient amount of such raw material, healthy and good quality seeds of German chamomile should be used.

Apart to commonly applied conventional measures, in attempt to increase seed germination various bio-agrotechnical measures use to be examined, particularly the "bio-products". The term bio-products stands primarily for toxicologically harmless and ecologically completely acceptable products. They might not be as effective as synthetic chemicals but they use to keep the level of harmful microorganisms below the threshold of economic harmfulness and within acceptable limits (Milenković, 2015). Among them stand out, 'Extrasol F' is a bio-product composed of rhizosphere nitrogen-fixing bacteria (*Bacillus subtilis* strain Č13) intended to improve seed germination. The bio-fungicide 'Polyversum' is based on the fungus *Pythium oligandrum* and is recommended for protection of seeds from economically important phytopathogenic fungi. The active ingredient of 'Timorex gold' bio-product is a tea tree essential oil (*Melaleuca alternifolia*) dissolved in paraffin oil (15×10^{10} CFU/ml), and apart to conventional it could be also used in organic production for protection against fungal pathogens causing symptoms of gray mold and potato blight (Kołodziejczik, 2018).
The application of bio-products based on beneficial fungi, bacteria and plant extracts can prevent the appearance of phytopathogenic fungi causing decay of cultivated plants but also damping of seedlings if the plantation use to be established via nursery plants. Soil pathogens such as *Pythium debarianum*, *Fusarium* spp., *Sclerotinia* spp., *Phytophtora* spp., *Aspergillus* spp., *Alternaria* spp., *Rhizoctonia* spp. and others are mainly causing of such symptoms.
The aim of study was to examine the influence of various bio-products - biofungicides, biostimulants and bioinsecticides, and some herbal preparations on quality and health of German chamomile seeds.

Material and methods

The study was conducted in the laboratory of Agricultural Research and Development of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Belgrade. The two-years old seeds of German chamomile (*C. recutita* L. Rauch.) cv. "Banatska", produced at the experimental field of the Institute, have been subjected to testings on seed germination and seed health, during 2021. The details on six bio-products (from the list of plant protection and plant nutrition products and the list of soil improvers, permitted in organic production) and two herbal preparations (created in the Institute), are provided in Table 1.

Table 1. Bio-products used in the treatments of German chamomile seed

Bio-product	Active substances		Short business name	
	name	**content**	**manufacturer**	**representative**
Extrasol F	*Bacillus subtilis* strain Č13	1×10^8 CFU/cm^3	BioGenesis, Bačka Topola and Jugo Hem, Leskovac	-
Polyversum	*Pythium oligandrum*	3% (1×10^6 - 10^7 oospore/g)	Biopreparaty, Czech Republic	Vins 2000, Belgrade
Timorex gold	tea tree oil *(Melaleuca alternifolia)*	222.5 + 194.5 g/l	Stockton Israel, Israel	Stockton, Belgrade

	+ paraffin oil			
Ozoneem trichul 1% EC	Azadirachtin	10 g/l	Ozone Biotech, India	BioGenesis, Bačka Topola
EcoBooster Calcium®	organic nitrogen fertilizer of animal and plant origin	3-3.5% total, 1.5-2% organic N, 5% organic C, CaO min. 2.5%, max. 12 C/N	Ekopatent, Vrbas	Ekopatent, Vrbas
Vegard	fertilizer and special products	amino acids 1-2%, fulvic acids 5%, humic acids 5%, other organic materials 20%	Beijing Kingbo Biotech, China	Timings, Belgrade
LAB3	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 20%, horsetail 20%, valerian 20%, dandelion 20%, yarrow 10%, nettle 10%, comfrey 5% and liquorice 5%	Institute for Medicinal Plants Research "dr J. Pančić", Belgrade	
LAB4	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 50% and valerian 50%	Institute for Medicinal Plants Research "dr J. Pančić", Belgrade	

Testing of bio-products on the quality of German chamomile seeds

The energy and total seed germination were examined as suggested by the Rule book on seed quality control („Official Gazette of the Socialist Federal Republic of Yugoslavia", no. 47/87, 60/87, 55/88 and 81/89, „Official Gazette of the Socialist Republic of Yugoslavia", no. 16/92, 8/93, 21/93, 30/94, 43/96, 10/98, 15/2001 and 58/2002 and „Official Gazette of the Republic of Yugoslavia", no. 34/2013). In short, the seed germination testing was conducted with 100 seeds on filter paper in Petri dishes, in triplicates. The seeds were previously treated with 15 ml of previously prepared solutions of the following single bio-products prepared in two concentrations: 'Extrasol F' (0.20 and 0.40 ml/kg), 'Polyversum' (0.50 and 1 g/kg), 'Timorex gold' (1 and 2 ml/kg), 'Ozoneem trichul' (1 and 2 ml/kg), 'EcoBooster Calcium' (0,50 and 1 ml/kg), 'Vegard' (0,50 and 1 ml/kg), 'LAB 3' (1 and 2 ml/kg) and 'LAB 4' (1 and 2 ml/kg), all being dissolved in 100 ml of distilled water. The same amount of distilled water (15 ml) was used in the control treatment. The number of germinated seeds was counted by the use of binocular loupe on 4th and 14th day from the day of setting up the experiment (ISTA, 2010).

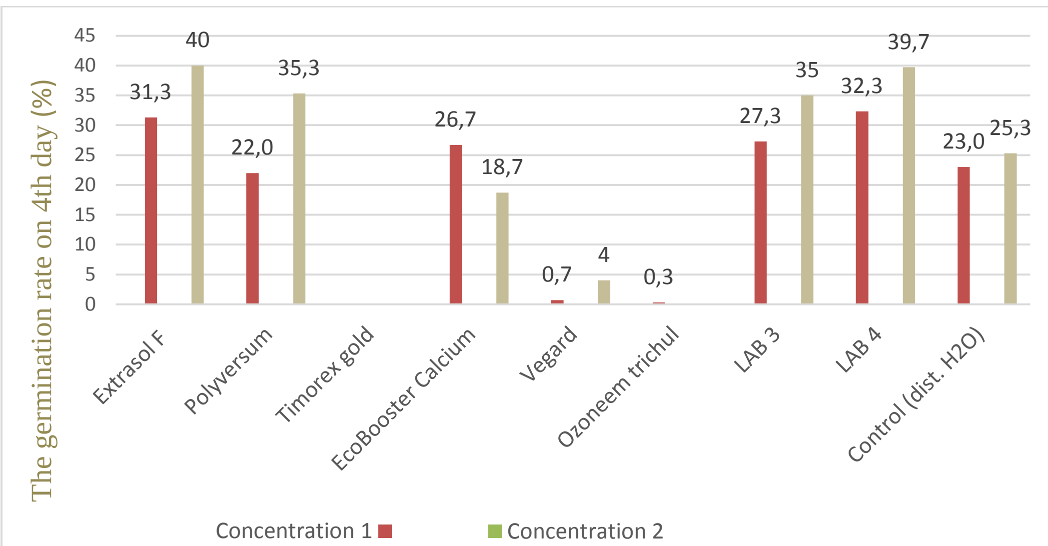
The seed health status of German chamomile

The seed health status was examined by the filter paper method. The experiment was conducted on previously sterilised (110 °C i 1h) and moistened filter paper, using 100 unsterilised seeds, in triplicates. Macroscopic and microscopic seed examinations were performed 14th day following their incubation in humidity chamber at T 20±2 °C. An Olympus CX43 microscope (Olympus, Hamburg, Germany) was used to observe the microscopic characteristics of the phytopathogenic fungi developed on the seeds, and the photographs were taken with an Axiocam ErC.5s (Zeiss, Göttingen, Germany).

Results and discussion

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on germination rate of German chamomile seeds, observed on 4th experimental day, are given in Graph 1.

Graph 1. The germination rate of German chamomile seeds observed on 4th day of the experiment (%).



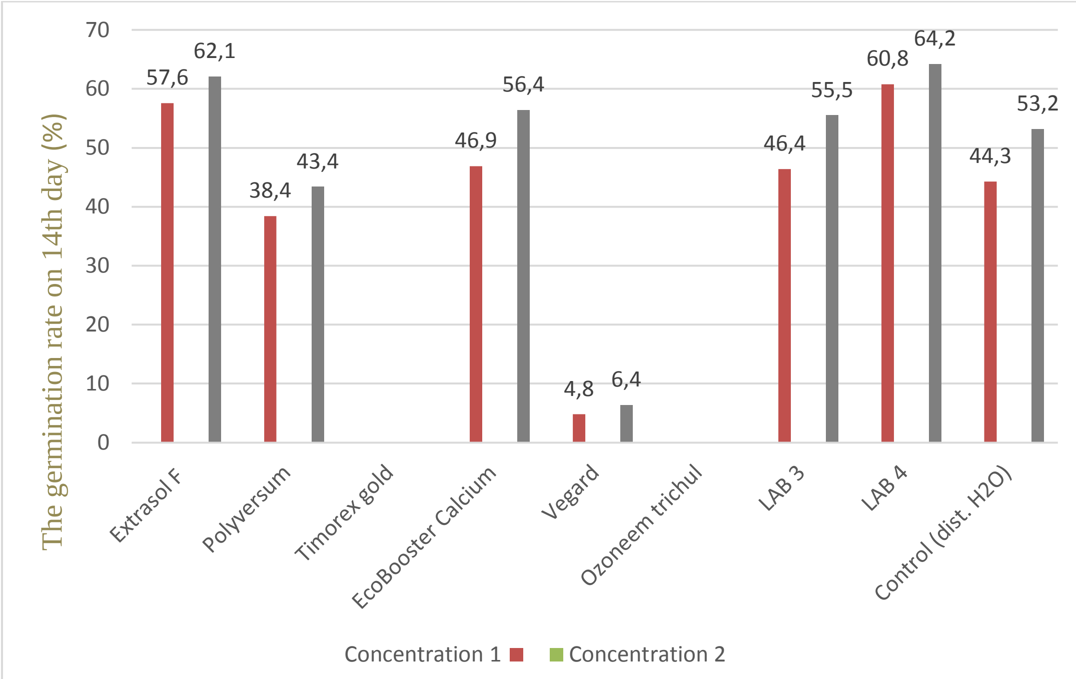
In seeds treated with the first (lower) concentrations of tested bio-products, the germination rate proved to be the highest in 'LAB 4' (averagely 32.3%) although similar results were also obtained with 'Extrasol F' (31.3%) and somewhat lesser with 'LAB 3' (27.3%).

The seeds treated with the second (higher) concentration of bio-product, generally had higher EG values (on average by 3.8%) compared to those achieved by the first concentration. In the treatment with second (higher) concentration of bio-product, the highest germination rate was achieved with 'Extrasol F' (40.0%) followed by 'LAB 4' (39.7%), although 'Polyversum' did not lag behind them a lot (35.3%).

The product 'Extrasol 55' stopped the development of economically significant plant diseases in grain crops (Jevtić et al., 2005), while the product of 'Polyversum' significantly affects on the germination rate of bean and soybean seeds (Horoszkiewicz-Janka et al., 2013).

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on the total germination of German chamomile seeds, observed on 14th experimental day, are given in Graph 2.

Graphs 2. The total germination of German chamomile seeds observed on 14th day of the experiment (%).



In the treatments with two concentrations of bio-products, the highest total of germination was achieved with 'LAB 4' (60.8% and 64.2%) followed by 'Extrasol F' (57.6% and 62.1%), while 'EcoBooster Calcium' had an effect (46.9% and 56.4%). The bio-products 'Timorex gold' and 'Ozoneem trichul' did not effect on seed germination. Filipović et al. (2014) showed that herbal preparation 'LAB 1' is shown beneficial effect on the EG and TG of two medicinal plant species, white origano (*Origanum heracleoticum* L.) and marjoram (*Origanum vulgare* L.). The biofungicides 'Extrasol F' and 'Polyversum' applied in the recommended concentrations (0.20 ml/kg and 0.50 g/kg, respectively), had an effect on fennel seed germination 73.3% and 64.7% (Filipović et al., 2021).

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on the occurence of German chamomile seeds pathogens, observed on 14th experimental day, are given in Graph 3.

Graphs 3. The occurence of pathogens on German chamomile seed observed on 14th day of the experiment (%).

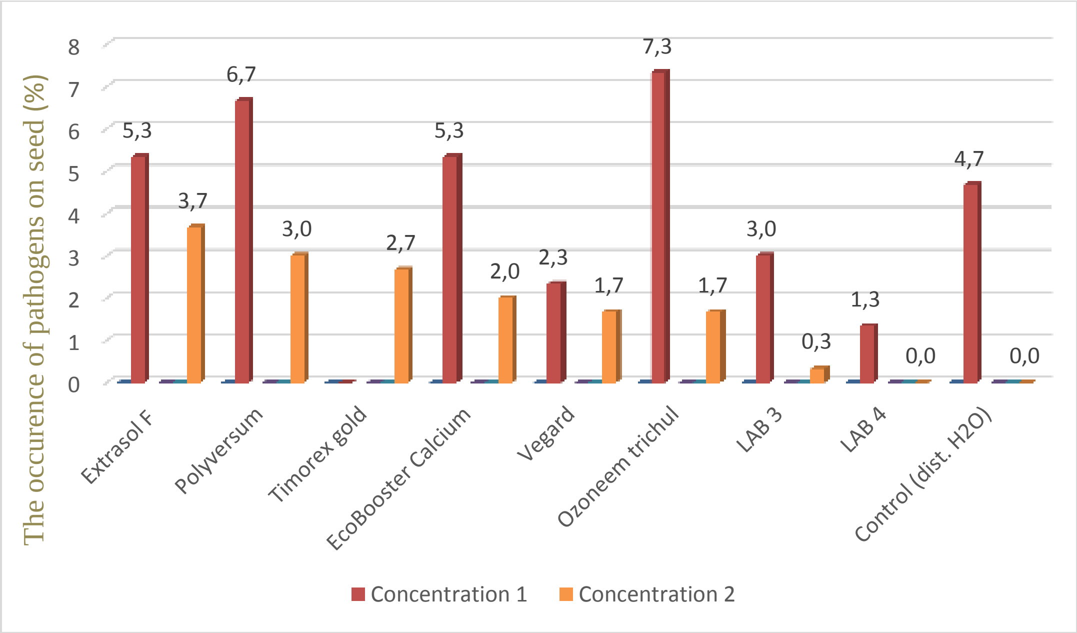


Photo 1. The symptoms of dark mycelia on the seed surface

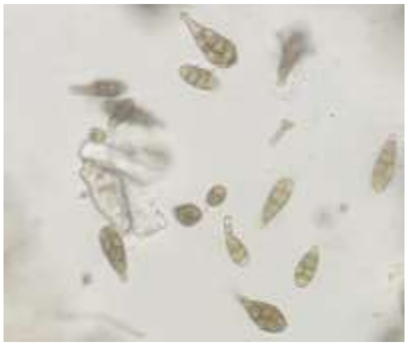


Photo 2. *Alternaria* sp.

Macroscopic examinations revealed the symptoms of dark mycelia on the seed surface, while the microscopic confirmed the presence of *Alternaria* sp. 'Timorex gold' in a lower concentration was prevented the occurence of seed infection, while the most infected seeds were recorded in the treatment with bioinsecticide 'Ozoneem trichul' (7.3%). In the treatment with higher concentration of 'LAB 4', the pathogens of genus *Alternaria* did not appear on the seed. Similar effect was achieved with 'LAB 3' (0.3%). The occurence of *Alternaria* sp. on the seed was confirmed after the treatment with 'Extrasol F' (3.7%), 'Polyversum' (3.0%) and in the control treatment (3.0%). In the research Filipović et al. (2021), the phytopathogenic fungi on fennel seed identified as *Alternaria* sp. The bio-products 'Extrasol F' and 'Polyversum' in the recommended concentrations (0.2 ml/kg i 0.5 g/kg) affected on reduce infected seeds. The higher concentrations did not reduce occurence of infected seeds.

Conclusions

The several bio-products showed positive effect on germination and reduction of pathogens on tested German chamomile seed cv. „Banatska". Bio-products 'LAB 4', 'Extrasol F' and 'EcoBooster Calcium' could be recommended for improvement of the germination rate and total seed germination of German chamomile seeds, while the 'Timorex gold' and 'LAB 4' could be recommended for the safe use in prevention of the seedborne diseases of *Ch. recutita* cv. „Banatska".

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References

Filipović, V., Popović, V., Aćimović, M., Marković, T., Protić, R., Ugrenović, V., Sikora, V. (2014). Stimulators seed germination of marjoram (*Origanum vulgare* L.) and white origano (*Origanum heracleoticum* L.). Medicinal raw materials 34 (pp. 81-91).

Filipović, V., Mikić, S., Ugrenović, V., Marković, T., Popović, V., Mrđan, S., Gordanić, S. (2021). The influence of biofungicides on germination and protection of the seeds *Foeniculum vulgare* L. In X International Symposium on Agricultural Sciences: AgroReS 2021 (pp. 41).

Glamočlija, Đ., Janković, S., Popović, V., Filipović, V., Kuzevski, J., Ugrenović, V. (2015). Alternative field plants in conventional and organic cultivation system, Institute for Applied Science in Agriculture, Belgrade, Serbia.

Horoszkiewicz-Janka, J., Jajor, E., Korbas, M. (2013). Control of horse bean and soybean root rot with selected agents recommended in ecological agriculture. Journal of Research and Applications in Agricultural Engineering, 58 (3), 209-213.

International Seed Testing Association (2010). ISTA Rules Proposals for the International Rules for Seed Testing 2011 Edition. Bassersdorf, Switzerland.

Jevtić, R., Marinković, B., Schaller, H. J., Jahn, M. (2005). Biological and alternative methods of parasite control in small grains. Proceedings of the Institute of Field and Vegetable Crops 41 (pp. 515-519).

Kołodziejczyk, M. (2018). An effect of biological, chemical and integrated potato protection against *Phytophthora infestans* (Mont.) De bary on the quality of tubers. Fragmenta Agronomica, 35 (4), 17-28.

Milenković, S. (2015). Plant protection products permitted in organic production. National Association for the Development of Organic Production Serbia Organica, Belgrade (pp. 1-38).

Oljača, S., Ugrenović, V., Filipović, V., Ognjenović, S., Bicikliski, O., Kolev, G., Jurjević, N., Kešelj, O. (2020). Handbook on organic farming. National Association for Organic Production Development "Serbia Organica", Serbia, Green Box Cultivation doo, North Macedonia, ProcertControl and Certification OKS, North Macedonia, NGO Eko-Zadar, Croatia.

VARIABILITY OF PLANT HEIGHT AND SPIKE CHARACTERISTICS OF DURUM WHEAT GROWING IN ORGANIC PRODUCTIONS

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Abstract

The aim of this study is to investigate phenotypic variability of yield components for seven different durum wheat genotypes (Windur, Žitka, KG Olimpik, KG-28-6, KG-3405-03, KG-43-33-1, and KG-44-3-1), which were grown during two years (2012/2013 and 2013/2014) at certified organic trial located in Mršinci, the Municipality of Čačak, Serbia. The field experiment was conducted in a randomized block design with three replications with plot of 5 m² on the soil belonging to the loamy clay type. Experiment was carried out by the organic technology of farming production of durum wheat.
For analysis of plant height and spike characteristics, 60 plants in full maturity stage were used (20 plants per replication). The primary tiller of plant was used for analyzed plant height, spike length and number of spikelets per spike.
Plant height, on average, ranged from 76.0 cm (Olimpik) to 92.1 cm (KG-3405-03), spike length from 6.9 cm (KG-44-3/1) to 8.2 cm (Windur) and number of spikelets per spike from 19.3 (Žitka) to 22.5 (Windur). Variability for plant height was similar for both of years (CV=3.6%, 3.9%, respectively), but for spike length (CV=5.5%, 6.6%, respectively) and for number of spikelets per spike (CV=5.4%, 6.2%, respectively) was lower in the first than in the second year.
Through variance analysis, a highly significant difference in mean values for all investigated components was established. Phenotypic analysis of variance indicated that ecological factors had higher impact in relations to genetic factors on expression of all three investigated traits.

Keywords*: durum wheat, organic production, plant height, spike characteristics, variability.*

Introduction

Durum wheat (*Triticum dururn* Desf.) is still globally considered a minor wheat crop and typically much of the research effort on durum is often conducted in conjunction with studies of bread wheat. Only a few regions in the world are capable of producing durum that meets the high standards for end-use suitability (Beres et al., 2020). In Serbia, there are generally conditions for growing durum wheat, although climatic conditions can be limiting factors for durum production. The production of winter varieties can be risky due to low frost resistance of durum wheat, especially in years with frost without snow cover. If spring varieties are grown, it is necessary to perform earlier sowing and provide the irrigation system, because high

temperatures and drought stress can significantly reduce the grain filling period, plant height, spike characteristics, yield components and yield (Pour-Aboughadareh et al., 2020).
Genotypes under organic agriculture need a high phenotypic plasticity to adapt to different and changing climatic conditions, should be more tolerant to abiotic and biotic stresses, need a proper root system to enable efficient nutrient taking and, therefore, produce high enough yields with less/low input (Pagnota et al., 2020). Durum wheat varieties do not only differ in their agronomic traits, but also in their response to diseases and insects (DePauw and Ruan, 2018). In organic production of durum wheat, among the principles with the greatest impact on disease management are selection of a resistant variety and crop rotation (Knox, 2018). Choice of cultivar can impact grain yield, grain quality, and the type of management practices that might be needed to optimize performance and profitability for a given environment. The interest of consumers and the food industry in organic products requires work on creating cultivars suitable for cultivation in the system of organic production.
The aim of this paper is to analyze the variability of durum wheat genotypes in organic production based on their behavior in different ecological conditions during the growing seasons.

Materials and Methods

Seven genotypes of winter durum wheat [Windur (Germany), Žitka, KG Olimpik, KG-28-6, KG-3405-03, KG-43-33-1, and KG-44-3-1 (Serbia)] were grown during two growing seasons (2012/2013 and 2013/2014) at certified organic trial parcel which is located in Mršinci, in the Municipality of Čačak, Serbia (20°30' E, 43°48' N, 220 m a.s.l.).
The field experiment was conducted in a randomized block design with three replications with plot of 5 m² on the soil which belongs to the loamy clay type. Experiment was carried out by the organic technology of scientific farming production of durum wheat. Soybean in the first and potato in the second year were used as the preceding crops. Sowing was done on November 6, 2012, and in October 25, 2013 with 600 seeds per square meter. The treatment of the crops during the growing season respected the principles of the organic farming.
For analysis of plant height and spike characteristics, 60 plants were used in full maturity stage (20 plants per replication). The primary tiller was used for analyzed plant height, spike length and number of spikelets per spike. The following parameters were computed: the average value ($\bar{x}$), the standard deviation (SD), the coefficient of variation (CV), the analysis of variance (ANOVA) and components of variance (σ^2). The significant differences between the average values were estimated by LSD-test values. The ANOVA was done according to a random block system with two factors using the MSTAT-C program (Michigan State University, 1990).

Results and Discussion

Plant height on average ranged from 76.0 cm in cultivar Olimpic to 92.1 cm for KG-3405-03, which plant height differed significantly to other genotypes. Variability on average was similar for both investigated years (3.6%, 3.9%, respectively). The highest plant height in the first year had cultivar Žitka (89.43 cm), while in the second year genotype KG-3405-03 (99.27 cm) had the highest plant height. Analysis of SD and CV showed that the examined genotypes showed low variability for plant height. Genotype KG-28-6 and Windur showed the highest variability of plant height in the first year (7.1%, 5.3%, respectively) and genotypes Windur and KG-44-3/1 in the second year (5.9%, 5.4%, respectively). Analysis of variance showed highly significant differences between genotypes and genotype-year interaction, while differences between years were significant (Table 1). In the total variance of this trait, the largest share of variance belonged to genotype-year interaction (64.18%) and genotype (25.99%). These results are in agreement with previous research (Falaki and Mohammed, 2011).

Table 1. Mean values, variability and ANOVA for plant height in durum wheat

Genotype	Year 2012/2013 $\bar{x}$ (cm)	SD	CV (%)	Year 2013/2014 $\bar{x}$ (cm)	SD	CV (%)	Average
Olimpik	77.30gh	2.58	3.3	74.70i	2.95	3.9	76.0
Windur	84.37d	4.44	5.3	83.70d	4.92	5.9	84.0
Žitka	89.43b	3.12	3.5	78.37fg	2.28	2.9	83.9
KG-28-6	78.43fg	5.54	7.1	76.27h	2.43	3.2	77.4
KG-44-3/1	79.60ef	1.79	2.2	85.97c	4.65	5.4	82.8
KG-43-33/1	80.57e	1.16	1.4	80.47e	2.78	3.4	80.5
KG-3405-03	85.0cd	2.32	2.7	99.27a	2.72	2.7	92.1
Average	82.1	2.99	3.6	82.7	3.25	3.9	-

LSD	**Genotype (G)**	**Year (Y)**	**G×Y**
0.05	0.9665	-	1.367
0.01	1.464	-	2.071

Analysis of variance

	Genotype (G)	Year (Y)	G×Y	Error	Total
DF	6	1	6	26	41
MS	169.811	3.486	94.032	0.468	-
F	362.9268**	7.4503*	200.9691**	-	-
σ^2 (%)	25.99	8.87	64.18	0.96	100.00

Means followed by different letter (s) within the columns differ significantly at 5% level of probability using LSD.

Spike length is an important component of wheat plant yield, which along with other components, significantly affects yield. In this study, the spike length of durum wheat genotypes ranged on average from 6.9 to 8.2 cm (KG-44-3/1, Windur, respectively). Variability of this component was higher in the second (6.6%) than in the first investigated year (5.5%). On average for all genotypes, the length of spike was similar in both years, about 7 cm, which means that this trait showed homogeneity over the years (Table 2). These results are in agreement with previous research reported by Matković et al. (2015) who also established average value of spike length at observed genotypes about 7 cm in organic production of durum wheat. Analysis of variance for spike length showed highly significant differences between genotypes and years as well as its interactions. In the total variance of this trait, the largest share of variance belonged to genotype-year interaction (80.22%) and year (10.63%). These results agree with previous research in durum wheat (Gorjanović and Kraljević-Balalić, 2006) and spelt wheat (Zečević et al. 2018). The number of spikelets per spike is shown in table 3. On average, this trait varied from 19.3 (Žitka) to 22.5 (Windur). Genotype KG-44-3/1 had also 22 spikelets per spike. On average for all genotypes, the number of spikelets per spike differed by only one per year (21 and 22). Similar results obtained by Gorjanović and Kraljević-Balalić (2006) who found 21-22 spikelets per spike.

Table 2. Mean values, variability and ANOVA for spike length in durum wheat

Genotype	Year						Average
	2012/2013			2013/2014			
	$\bar{x}$ (cm)	SD	CV (%)	$\bar{x}$ (cm)	SD	CV (%)	
Olimpik	7.43f	0.36	4.8	7.87d	0.41	5.2	7.6
Windur	8.17c	0.44	5.4	8.33b	0.73	8.8	8.2
Žitka	8.40b	0.47	5.6	7.50ef	0.56	7.5	7.9
KG-28-6	7.37f	0.47	6.4	6.97g	0.58	8.3	7.2
KG-44-3/1	6.20h	0.25	4.0	7.60e	0.48	6.3	6.9
KG-43-33/1	6.90g	0.25	3.6	8.37b	0.47	5.6	7.6
KG-3405-03	8.77a	0.76	8.7	7.37f	0.34	4.6	8.1
Average	7.60	0.43	5.5	7.70	0.51	6.6	-

LSD	**Genotype (G)**	**Year (Y)**	**G×Y**
0.05	0.1094	-	0.1548
0.01	0.1658	-	0.2345

Analysis of variance

	Genotype (G)	Year (Y)	G×Y	Error	Total
DF	6	1	6	26	41
MS	1.419	0.126	1.793	0.006	-
F	256.6142**	22.7716**	324.1970**	-	-
σ^2 (%)	8.34	10.63	80.22	0.81	100.00

Means followed by different letter(s) within the columns differ significantly at 5% level of probability using LSD.

On average, two sterile spikelets per spike were recorded, at basal and top position of spike. Wheat yield can be increased by decreasing the sterile basal and top spikelets, which can be achieved with appropriate plant density and by supplying sufficient nutrients, mainly nitrogen (Li et al., 2016). Analysis of variance revealed highly significant differences between the analyzed genotypes, years and genotype-year interaction. In the total variance of this trait, the largest share of variance belonged to the genotype-year interaction (53.45%) and then to the genotype (13.54%), table 3.

In organic durum wheat production, productivity of plants is lower than in conventional production because of low input of fertilization and protection against diseases and insects. Weeds are one of the largest contributors to wheat yield loss. Weed control and nitrogen supply are among the most important factors for durum yield and quality, especially in years with excess precipitation and low temperature during reproductive development of durum wheat in organic production system (Campiglia et al. 2015, Zečević et al., 2019). Resistance to disease continues to be a major factor in the maintenance or improvement of durum wheat yields, especially leaf rust, yellow or stripe rust and stem rust (Eversmeyer and Kramer, 2000).

Table 3. Mean values, variability and ANOVA for number of spikelets per spike in durum wheat

Genotype	Year 2012/2013 $\bar{x}$	SD	CV (%)	2013/2014 $\bar{x}$	SD	CV (%)	Average
Olimpik	21.0bc	0.96	4.6	22.0ab	0.81	3.7	21.5
Windur	23.0a	1.29	5.6	22.0ab	2.18	9.9	22.5
Žitka	19.0d	0.76	4.0	19.67cd	1.60	8.1	19.3
KG-28-6	22.0ab	1.02	4.6	21.0bc	1.71	8.1	21.5
KG-44-3/1	22.0ab	1.29	5.9	22.0ab	0.84	3.8	22.0
KG-43-33/1	20.33cd	1.24	6.1	23.33a	1.04	4.4	21.8
KG-3405-03	20.0cd	1.44	7.2	23.0a	1.31	5.7	21.5
Average	21.0	1.14	5.4	22.0	1.36	6.2	-

LSD	**Genotype (G)**	**Year (Y)**	**G×Y**
0.05	1.107	-	1.566
0.01	1.677	-	2.372

Analysis of variance

	Genotype (G)	Year (Y)	G×Y	Error	Total
DF	6	1	6	26	41
MS	6.040	6.881	4.214	0.614	-
F	9.8438**	11.2149**	6.8687**	-	-
σ^2 (%)	13.54	5.66	53.45	27.35	100.00

Means followed by different letter(s) within the columns differ significantly at 5% level of probability using LSD.

Conclusion

In this study, it was found that durum wheat genotypes behave differently in organic production for the tested plant traits. Differences between genotypes were significant for plant height and spike traits. The variability was low for all examined traits. Genotype-year interaction was expressed for all three examined traits (plant height, spike length and number of spikelets per spike). The largest impact of total variance belonged to the genotype-year interaction, above 50% for all investigated traits.

The analyzed genotypes possess low variability of the examined traits in different ecological conditions, which were expressed during the two vegetation seasons. These genotypes can be used as parents in breeding durum wheat for organic and conventional production.

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References

Beres, B.L., Rahmani, E., Clarke, J.M. Grassini, P., Pozniak, C.J., Geddes, C.M., Porker, K.D., May, W.E., Ransom, J.K. (2020). A Systematic Review of Durum Wheat: Enhancing

Production Systems by Exploring Genotype, Environment, and Management (G x E x M) Synergies. Front. Plant Sci. 11:568657. doi: 10.3389/fpls.2020.568657

Campiglia, E., Mancinelli, R., De Stefanis, E., Pucciarmati, S., and Radicetti, E. (2015). The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (*Triticum durum* Desf.) in the Mediterranean environment of Central Italy. Field Crops Res. 176, 34–44. doi: 10.1016/j.fcr.2015.02.021

DePauw, R.M., Ruan, Y. (2018). Sustainable production of durum wheat in Canada-Variety selection to meet farmer profitability. R.M. DePauw, editor. p. 10-15.

Eversmeyer, M. G., Kramer, C. L. (2000). Epidemiology of wheat leaf and stem rust in the central Great Plains of the USA. Annu. Rev. Phytopathol. 38, 491–513. doi: 10.1146/annurev.phyto.38.1.491

Falaki, A.M., Mohammed, I.B. (2011). Performance of some durum wheat varieties at Kadawa, Kano State of Nigeria. Bajopas, vol. 4(1), 48-51.

Gorjanović, B. and Kraljević-Balalić, M., 2006. Correlations among yield components in durum wheat. Genetika, 38(2), 115-120.

Knox, R.E. (2018). Sustainable production of durum wheat in Canada- Disease management to minimize crop losses and maximize quality. R.M. DePauw, editor. p. 58-62.

Li, Y., Cui, Z., Ni, Y., Zheng, M., Yang, D., Jin, M., Chen, J., Wang, Z., Yin, Y. (2016). Plant density effect on grain number and weight of two winter wheat cultivars at different spikelet and grain positions. PLoS ONE 11(5): e0155351. doi:10.1371/journal.pone.0155351

Matković, M., Bošković, J., Zečević, V., Knežević, D., Đurić, N. (2015). Influence of genotype on yield and quality components of durum wheat in organic production. Fifth International Symposium on Natural Resources management. Faculty of Management, Zaječar, Serbia, May 23, 2015, 17-22.

Pagnotta, M.A., Bonfiglioli, L., Forte, P. (2020). Selection of durum wheat lines under organic management - Preliminary results. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 71. Jahrestagung 2020, 23-24 November, Raumberg-Gumpenstein, pp 11-12.

Pour-Aboughadareh, A., Mohammadi, R., Etminan, A., Shooshtari, L., Maleki-Tabrizi, N., Poczai, P. (2020). Effects of Drought Stress on Some Agronomic and Morpho-Physiological Traits in Durum Wheat Genotypes. Sustainability 2020, 12, 5610; doi:10.3390/su12145610

Zečević, V., Milenković, S., Matković, M., Mićanović, D., Jauković, M., Luković, K., Bošković, J. (2018). Phenotypic variability of spike characteristics of spelt (*Triticum spelta* L.) in organic production. I domestic scientific-professional meeting, Sustainable primary agricultural production in Serbia-state, opportunities, limitations and chances, Megatrend University, Faculty of Biofarming, Backa Topola, 26.10.2018, Proceedings, 45-52.

Zečević, V., Milenković, S., Bošković, J., Matković Stojšin, M., Luković, K., Mićanović, D., Knežević, D. (2019): Investigation of yield and yield components of durum wheat genotypes in organic production. National scientific conference with international participation "Sustainable agricultural production - The role of agriculture in environmental protection". Megatrend University Belgrade, Faculty of Biofarming, Backa Topola, October 18, 2019, Belgrade. Proceedings, p. 113-120.

THE IMPORTANCE OF APPLYING ORGANIC PREPARATIONS IN THE PEPPER PRODUCTION

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Abstract

Pepper (*Capsicum annuum* L.), is one of the most important types of farmed vegetables. This plant is of huge economic significance in our country. It has been grown and used for a long time. The basic system of farming peppers is a conventional method of agricultural production which aims to achieve maximum production in both quality and quantity while keeping the expenses as low as possible. However, the conventional system has shown its disadvantages through adverse effect on peoples' health, the environment and its biodiversity. Organic production aims to produce safe food of high quality which production would be sustainable. The aim of this paper is to look into the possibilities, but first of all the advantages, of organic pepper production. Pepper is a very bold vegetable. It contains significant amounts of sugar, protein, minerals and vitamins. New technologies often have unintended consequences. High-yielding varieties, which require large amounts of water, artificial mineral nutrients and other agricultural chemicals, have caused environmental problems in many parts of the world. In some cases, high-yielding varieties have been shown to be less resistant to diseases and pests than traditional varieties. This requires greater use of pesticides, the excessive use of which has poisoned the soil and negatively affected biodiversity.

Key words: *peppers, agricultural production, organic production, high quality food.*

Introduction

Pepper (Capsicum annuum L.) is one of the oldest plant species in the world. It originated in South America, and in the fifteenth century the Spaniards brought it to Europe. Since it adapts and develops easily and quickly, it is estimated that there are about 50,000 species of cultivated peppers in the world.

In the Republic of Serbia, paprika is a whole surface plant and according to the procedures that are found after tomatoes. The application of a conventional system of cultivation and the use of modern greenhouses and hothouses have ensured that the fruit of fresh peppers will be available in the coming years. Mass consumption of pepper can be announced by its extraordinary nutritional and culinary properties. Also, they require a conventional system of agricultural production, significant contributions are made and the crop is economically very profitable.

The rules of organic farmers in the Republic of Serbia are defined by the Law on Organic Production of the Republic of Serbia, as well as the adopted standards and records of the European Union. In the period from 2010 to 2015, there was a significant increase in the area under organic products, amounting to 15,298 ha (Simić, 2017). According to the MAFWM, in the Republic of Serbia the area consists of only 1.3% of the area in relation to the total area under organic product. As for paprika, there is no precise data on the area on which it is grown, but it is known that the largest plantations with organic production plant are located in Vojvodina (Kikinda, Sombor, Fruška gora, Novi Sad, Zrenjanin, Bečej).

In the world, pepper has 1,600,000 ha, with an average height of 14.0 t/ha. It is mainly grown in the temperate zone and sporadically in the tropics. The largest producer in the world is China (20.0 t/ha), and the highest average yield is achieved by Spain (40.0 t/ha Gvozdenović, 2010).

In the structure of European production, our country participates with 4.4% and is in seventh place. Pepper is grown on 21,000 ha, with an average yield of 8.3 t/ha (Gvozdenović, 2010).

The areas on which the production is based are located in the plains of Vojvodina and in the basins and river valleys of central and southern Serbia. According to the procedures of surface culture, it is located in another place, next to tomatoes.

Pepper is an intensive culture and creation of high incomes per unit area. It is grown outdoors and outdoors. The price is completely used in technological (use) or physiological (botanical) maturity, fresh and processed. Pepper is a very bold vegetable. It contains significant amounts of sugar, protein, minerals and vitamins. The fruit contains 92% water, 6 g of carbohydrates, 0.99 g of protein, 0.3 g of fat and 1.2 g of fiber. It is especially rich in vitamin C, which has approximately 150 to 250 mg in botanically ripe fruits (Popović, 1991). Vitamins P, B1 and B2 and beta carotene, provitamin vitamin A are also available in peppers. The carotenoids capsatin and capsorbin give the fruit a red color (Lazić, 2008).

Organic agricultural production

New technologies often have unintended consequences. High-yielding varieties, which require large amounts of water, artificial mineral nutrients and other agricultural chemicals, have caused environmental problems in many parts of the world. In some cases, high-yielding varieties have been shown to be less resistant to diseases and pests than traditional varieties. This requires greater use of pesticides, the excessive use of which has poisoned the soil and negatively affected biodiversity. Also, the increasing mass genetic manipulation of cultivated plants and the elimination of diversity in order to maintain uniformity (monoculture), have contributed to the conventional cultivation system as an industrial process, in which plants and domestic animals are small factories (Kovačević, 2010).

Organic agricultural pepper production

Relation to land For planting peppers, flat soils are best, on which soil moisture and light conditions are uniform. A slight slope, up to 5%, is desirable for easier drainage of excess water. The optimal pH for growing peppers is 5.5-6.5, but it can also be grown on soils with a pH of 4.6-7.5 (Lazić, 2008). Pepper requires light and sandy soils, rich in organic matter. If the soil is not loose, a larger amount of sand is added to it.

Relation to temperature Since pepper is a thermophilic plant species, the optimal temperature for plant growth and development is 22-25 ºC. At the time of germination, flowering, formation and ripening of fruits and seeds, paprika requires 3-4 ºC higher temperature than stated. The soil temperature should be 3-4 ºC lower than the air temperature.

Relation to light Paprika is a heliophilic plant, short-lived. It does not tolerate shade and is successfully grown only in a period with plenty of light. For early varieties of peppers, a denser set of plants is desirable.

Nutrients Pepper has a great need for nutrients. Burnt manure is applied in autumn or spring just before planting. The manure is spread over the entire surface of the land, with slight loosening of the pitchforks. In this way it remains as mulch on the surface of the soil. 40% of compost manure was obtained from fresh manure by composting. On average, burnt manure contains 0.50% N, 0.25% P2O2, 0.60% K2O and 20% organic matter. The content of microelements is 10-20 mg / kg of boron, 150-200 mg of manganese, 15-20 mg of copper, 70-95 mg of zinc, 1-2 mg/kg of molybdenum and 13 mg/kg of cobalt, and creatine is present as a biostimulator, indole acetic acid and others (Lazić, 2011).

Compost is the most common basic organic nutrient. It can also be used for mulching (mulching) the soil. Different materials can be used for composting, and based on that, the compost was divided into compost manure, earthworm, compost from plant industrial waste and organic waste from the household.

In organic agriculture, other organic fertilizers can be used, provided that they have an organic certificate (earthworm, ash, peat, poultry manure).

Material and Methods

The experiment was set up in the village of Otanj near Požega, on the family farm. The plot is located at an altitude of 400 meters, with a slope of 1%.

Two greenhouses were used for the experiment, each measuring 40 × 8 m, which means that the total area of the plantations is 6.4 ares. The construction of the greenhouse is covered with polyvinyl chloride foil (PVC), which transmits up to 90% of the visible part of the spectrum and 80% of the ultraviolet part, and does not transmit the infrared part of the spectrum. The greenhouse has a built-in system for artificial watering according to the "drop by drop" system. Spring water is used for irrigation.

The following parameters were obtained by soil analysis:

- pH 6.05
- pH KCL 5.34
- humus 6.89%
- N 0.340%, P2O5 11.20 mg/100 and K2O 34.10 mg/100.

The medium-early variety of paprika Claudius (Claudius F1 500) was used for cultivation. The type is white babure, with a sharp tip. The fruits are very large (200 - 250 g) and sweet taste. Thanks to the well-developed root system and strong above-ground part, it is successfully grown even in weaker conditions. It is primarily recommended for growing in unheated greenhouses and in intensive production in the open field.

For the needs of the experiment, it was decided to grow peppers in one greenhouse according to the principles of conventional, and in the other greenhouse according to the principles of organic agricultural production. In both greenhouses, the basic tillage was done in the fall, and the pre-sowing preparation was done in the spring, a month before the plants were transplanted.

Based on the analysis of the soil, the need for nutrients was determined. Basic fertilization was done in the fall, just before tillage. Manure was applied in both greenhouses, with the application of mineral NPK fertilizer (400 kg/ha, formulations 1:2:3) in the system of conventional production.

Planting of plants was done in April, at a distance of 45 cm between rows and 25 cm in a row, at a depth of about 10 cm.

The first feeding was done fifteen days after transplanting the plants. The second feeding was in the phase of mass flowering and formation of the first fruits. For feeding peppers in the classical production system, KAN 27% N + 4.8 MgO was used, in the amount of 15 g/m^2. In organic production, the preparation Trianum P (Trihoderma harzianum T-22) was applied, by fertigation through a drip system, in the amount of 0.5 kg / ha. The application of Trianum P achieves a symbiotic relationship between this useful fungus and its roots. The reaction is the secretion of various aleopathic substances (hormones, vitamins, enzymes, phytoalexins, salicylic acid) that increase the resistance of the aboveground parts of the plant, but also various volatile and non-volatile antibiotic substances, which act on dangerous bacterial diseases.

Until the mass flowering, the plants were irrigated in the amount of 10-20 L/m^2, and with the formation of fruits, irrigation was every 5-7 days with 20-30 L/m^2.After watering and fertilizing, the plants were dug up.

The protection program was in line with the monitoring and occurrence of diseases and pests. A protective belt of velvet and basil plants has been planted around the greenhouse. At the beginning of the vegetation period, the plants were treated with copper preparations. An insecticide whose active substance is acetamiprid was used to control aphids in conventional production. In the organic production system, the plants were preventively treated with nettle macerate and garlic tea.

Results and Discussion

By measuring the mass of harvested paprika fruits, it was noticed that the fruits differ in weight, on average by about 30 g. The average value of the mass of paprika fruit in the conventional production system was 220-230 g, and in the organic about 200 g. The shape and color of the fruit were uniform in both cultivation systems. It was noticed that the fruits from the system of organic production had a more intense smell and taste.

Based on the comparison of the obtained results, it can be concluded that in the production plantation of peppers with the classical cultivation system, a higher yield per unit area is achieved. However, it is a known fact that the prices of organic primary and processed products, depending on the type, point of sale and season, have increased by about 50-300% compared to the prices of the same conventional products.

Conclusions

In relation to the total areas under organic agricultural production in the Republic of Serbia, organic pepper production is represented mainly on smaller areas (1.3%). Paprika belongs to the group of vegetables whose cultivation is the most profitable. One of the possibilities for increasing income per unit area is organic agricultural production. In order to successfully grow peppers in a protected area according to organic principles, preventive measures must be applied more strictly compared to the conventional production system. The fruiting period is shorter and the yield per unit area is lower. Disease and pest control is one of the key problems due to the significantly smaller number of pesticides available. Initial investments in this type of production are higher compared to conventional production, but in the long run they pay off. In the production process, energy consumption is significantly lower. The products obtained are of better quality and stay fresh longer. They are a protected trademark and are several times more expensive than products obtained by conventional means. Organic vegetable production is not a return to rural agriculture, but it is a modern form of agriculture and represents a complete management system. Organic agricultural production requires a great understanding of producers, primarily about the need to protect the human environment and the production of safe and biologically valuable products, without pesticide residues and other chemical substances.

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References

Simić I. (2017). Organic agriculture in Serbia 2017. National Association for the Development of Organic Production Serbia organika, Belgrade, pp. 12.
Gvozdenović Đ. (2010). Paprika. Novi Sad, Institute of Field and Vegetable Crops.
Popović M. (1991). Vegetables, Belgrade, Nolit, pp. 172-174.
Lazić B. (2008). Green garden all year round. Novi Sad, ABM Ekonomik.
Kovačević D. (2010). Environmental protection in field and vegetable growing, script, Belgrade, Faculty of Agriculture, Belgrade.
Lazić B. (2011). Organic vegetables. Belgrade, Andrejevic Endowment.

ORGANIC FARMING AND POLICIES APPLIED IN TURKEY

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Abstract

Agricultural activities are among the current topics in every period. Agriculture is indispensable in terms of its contribution to community nutrition. It is important for people to live a healthy life and to improve their living conditions. Organic farming is known as environmentally friendly in respect of agricultural sustainability. It preserves the ecological balance as it is applied within certain rules. The aim of study is to assess the organic farming potential of Turkey. In addition, it is to examine the state supports applied in this regard. Organic farming started with intensive agricultural supports in the European Union and Turkey. However, it could not show rapid development in Turkey. Today, there are serious increases in the growing area and production amount of organic products. Organic farming method is applied for many products in Turkey. In recent years, it has increased especially in feed crops, grains and nuts. In addition to organic herbal products, organic animal products have also increased. The number of farmers involved in organic crop production was 53,782 in 2019. In addition, there are 170 farmers engaged in organic animal production in Turkey. Organic farming also has an important role in world trade. Turkey has an organic product export value of over $ 200 million in 2019. Producers and consumers should be informed about this. In the future, organic farming activities are expected to increase in Turkey.

Keywords: A*griculture, environment, health, sustainability, market.*

Introduction

The rapidly increasing world population brings with it the demand for foodstuffs. However, it is impossible to increase the amount of land required for the current production. For this reason, production is tried to be increased with yield obtained from the unit area. However, as well as increasing productivity is important, the health of humans and other living things is also important (Kaya and Bay, 2020). The ecological destruction arising from the increased use of chemical inputs due to the rapid population growth and increasing food demand has caused increasing demand for organic products (Ayla and Altıntaş, 2017). Healthy life in a sustainable environment is possible with accessible food production. It is a method that cares about the environment and human health. Organic farming is an agriculture method that aims to evaluate the future of human and ecosystem as an indivisible whole with healthy plant and animal production (Akkurt et al., 2018). It is also an environmentally, socially and economically sustainable farming system (Willer et al., 2019). According to 2016 data in the world, it is known that organic production is made by 2.7 million producers in an area of 57.8 million hectares in 178 countries (Willer and Lernoud (Ed.), 2017). Organic farming in Turkey has started in line with demands from EU countries in 1985. Today, it has reached a sectoral status with more than 200 products that can be classified as herbal products, processed food and other organic products (Öztürk and Islam, 2014; Kaya and Bay, 2020). According to the report published by IFOAM

(International Federation of Organic Agriculture Movements) and FIBL (Research Institute of Organic Agriculture) Research Institute; the economic magnitude of the organic market globally is around $81.6 billion. Whereas the organic market share among the EU countries is around $27.1 billion. This would mean that one thirds of global organic market network belonged to the EU countries (Merdan, 2019). The number of farmers engaged in organic farming in Turkey is higher than the 80.000 in recent years (Kaya and Bay, 2020). Consumer demand for organic farming and food products has naturally increased the number of farmers who adopt organic farming (Demiryürek, 2011). However, Turkey's possible to be processed into products to the world market. Turkey has a competitive advantage. It has advantages such as geographical location, climate characteristics, product variety, soil quality, and high labor force working in agriculture. (Özbağ, 2010). The aim of this study is to evaluate the potential of organic farming in Turkey. In addition, it is to examine the state supports applied in this regard.

Material and Methods

Data from the Turkish Statistical Institute were used in the study. In addition, national and international studies were used. The change in Turkey's organic agriculture data has been examined over the years. In this context, organic products, number of farmers, cultivation area and production amount were evaluated in Turkey. In addition, the amount of support paid has been examined in Turkey. The supports are determined as unit price by the Ministry of Agriculture and Forestry.

Results and Discussion

Values related to Turkey's organic farming potential are given in Table 1. Organic crops have increased in Turkey in recent years. The data showed fluctuations over the years. While the number of organic products grown in 2005 was 205, it increased to 235 in 2020. There is an increase in the number of products by about 15%. In addition, organic farming producer has increased significantly. While there were 14 401 farmers in 2005, it has reached 52 590 farmers in 2020. Between 2005 and 2020, the number of farmers engaged in organic production has increased more than 3 times. In addition, the production area among the ails mentioned has almost doubled. While there was 203 811ha organic production area in 2005, it became 382 665 hectares in 2020. Turkey's organic product production potential has almost quadrupled in the same period (Table 1).

Table 1. Organic crop production in Turkey

Year	Number of crops	Number of farmers	Index (2005=100)	Area[1] (ha)	Index (2005=100)	Production (ton)	Index (2005=100)
2005	205	14 401	100	203 811	100	421 934	100
2010	216	42 097	292	510 033	250	1 343 737	318
2015	197	69 967	486	515 268	253	1 829 291	433
2016	238	67 878	471	523 777	257	2 473 600	586
2017	214	75 067	521	543 033	266	2 406 606	583
2018	213	79 563	552	626 885	308	2 371 612	562
2019	213	74 545	518	545 870	268	2 030 466	481
2020	235	52 590	365	382 665	188	1 631 943	387

Reference: TUIK, (2021b)

(1) Natural harvest areas are included.

The development and change of organic farming in Turkey can be seen in Figure 1. There has been a significant increase in organic farming in recent years. The area of organic farming has also increased in Turkey. However, the amount of production and the number of farmers increased more than area (Figure 1).

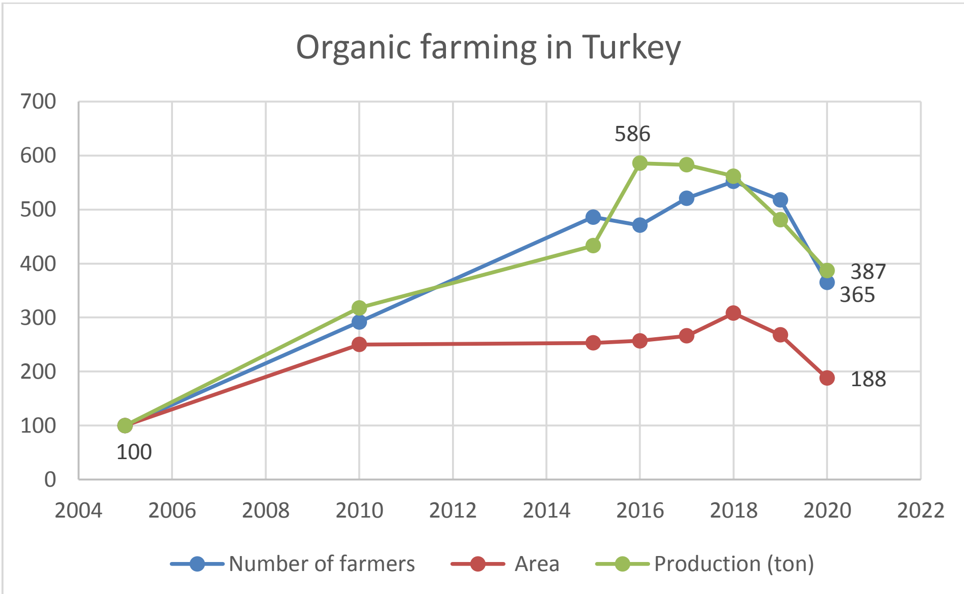

Figure 1. Change of organic farming data between 2005-2020

In 2019, there are 170 farmers within the scope of organic animal production. Egg chickens, ovine, bovine, carnivorous chickens are grown organically. In 2019, 865 781 animals were raised organically, and 819 tons of meat were obtained from these animals in Turkey. In addition, 5394 tons of organic milk was produced. On the other hand, 179 781 501 eggs were obtained from the reared laying hens. Beekeeping is also among the livestock activities. There are 249 farmers growing organic bees in Turkey. In addition, there are 50 100 hives. Organically, 577 tons of honey is obtained from these bees (TUIK, 2021a). Based on production guidelines, organic livestock farming has set itself the goal of establishing environmentally friendly production, sustaining animals in good health, realising high animal welfare standards and producing high quality products (Atasever and Adıgüzel, 2006).

Today, the increase in environmental and health problems increases the importance of healthy foods. Support for environmental policies should be increased. Many products are supported within the scope of agricultural policies in Turkey. Organic farming producers are also supported within the scope of the applied basin-based support system.

Organic farming support unit prices were obtained from the Ministry of Agriculture and Forestry. There are supports that encourage organic farming. These supports are given according to different categories. The supports are determined at 100 TL/da for the 1st category. It was determined as 70 TL/da for the 2nd category, 30 TL/da for the 3rd, and 10 TL/da for the 4th, respectively. In addition, there are beekeeping supports within the scope of organic livestock. In this context, the unit price of the beehive was determined as 10 TL/da. Not only organic plant production, but also organic animal production is supported.

Conclusion

Market share of organic farming should be increased in Turkey. Because environmental pollution and health problems have increased. In addition, the decrease in biodiversity, barren soils, decrease in water resources and water pollution constitute a serious problem. Consumption of organic foods in Turkey's domestic market should also be expanded. Producers and consumers should be made aware of the environment and health. Organic farming supports will increase Turkey's competitiveness. Organic farmers and markets should be brought together. Cooperatives should be established in this area.

References

Akkurt, M., Şenses, İ.M., Erdoğan, Ü. (2018). Organic viticulture recent status in Turkey and development, Turkish Journal of Agriculture - Food Science and Technology, 6(11): 1511-1516.

Anonymous (2021). Republic of Turkey Ministry of Agriculture and Forestry, Field-based supports/Organic farming support, Accessed: 05.11.2021, Available from: https://www.tarimorman.gov.tr/

Atasever, M., Adıgüzel, G. (2006). Quality of Organic Foods, Atatürk Üniv. Ziraat Fak. Derg. 37 (1), 117-122.

Ayla, D., Altıntaş, D. (2017). A Review of Organic Production and Marketing Issues, Kastamonu University Journal of Faculty of Economics and Administrative Sciences- Volume 19, Issue 4.

Demiryürek, K. 2011. The concept of organic agriculture and current status of in the world and Turkey, Journal of Agricultural Faculty of Gaziosmanpasa University, 28(1), 27-36.

Kaya, A., Bay, S. (2020). Organic grape production and producer status in Adıyaman province; example of Besni district, Turkish Journal of Agriculture-Food Science and Technology, 8(9): 1988-1993.

Merdan, K. (2019). Current situation and development potential of organic agriculture in European Union countries, Eurasian Journal of Researches in Social and Economics, 6(1): 167-186.

Özbağ, B.C. (2010). Economic Analysis of Organic Farming in Turkey, Uludag University, Insitute of Natural and Applied Science, PhD Dissertation, Bursa.

Öztürk, D.; İslam, A. (2014). Marketing Of Organic Products in Turkey, Journal of Social Sciences Studies. I, (2014): 75-94.

TUIK (2021a). Turkish Statistical Institute, Data Portal for Statistics, Accessed: 20.05.2021, Available from: https://data.tuik.gov.tr/

TUIK (2021b). Turkish Statistical Institute, Data Portal for Statistics, Accessed: 05.11.2021, Available from: https://data.tuik.gov.tr/

Willer and Lernoud, (2017). The world of organic farming: statistics and emerging trends 2017, Research Institute of Organic Agriculture (FiBL), Kitap, Switzerland.

Willer, H., Lernoud, J., and Kemper, L. (2019). The World of Organic Agriculture, eds H.Willer and J. Lernoud. Frick: FiBL.

CHEMICAL COMPOSITION OF MEDLAR (*Mespilus germanica*) AND WILD SERVICE TREE (*Sorbus torminalis*) – UNDERUTILIZED EDIBLE FRUITS FROM BOSNIA AND HERZEGOVINA

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Abstract

Medlar (*Mespilus germanica* L.) is an old fruit species that is not present in production plantations and on larger areas in B&H and neighboring countries. It can mostly be found in backyards, as a sporadic fruit tree. Wild service tree (*Sorbus torminalis* (L.) Crantz) is a species of semi-shade, a regular companion of oak forests, with a wide ecological valence. Apart from belonging to the same family (*Rosaceae*), their fruits are both edible and medicinal, can be consumed only if *bletted* (softened by frost) and are characterized by a similar taste. The fruits of wild service tree were harvested on the mountain Manjača, while the fruits of medlar were collected from an old orchard in the suburbs of Banja Luka. Fruits were analyzed on the following: moisture, dry matter, total sugars, total acidity, mineral content, pectin, vitamin C, elements (Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Pb, Se, Zn), total phenols, flavonols, flavonoids, non-flavonoids and anthocyanins. Analyzes were carried out in four parallel repetitions and the results are expressed as mean value ± standard deviations. The obtained results showed that the fruits of the observed species have a rather uneven elemental composition, with the exception of Na and P. Checker tree fruits contain more vitamin C (15.80 mg/100g) than medlars (4.44 mg /100g). They are also superior in terms of total phenols, flavonoids and non-flavonoids (2416.49, 1299.33, 1117.16 μgGAE/g, respectively) compared to medlars (530.3, 36.14, 513.5 μgGAE/g, respectively). The same is the case with total flavonols (323.51 vs. 174.97 μgQE/g). Checkers contain an average of 301.24 μg/g of anthocyanins, which are not registered in medlars.

Key words: *medlar, wild service tree, nutrition value, element concentration, bioactive compounds.*

Introduction

Wild service tree or checker (chequer) tree (*Sorbus torminalis* Crantz) is widespread from Western Europe to southwestern Asia, with part of its range in North Africa. In our country, it most often occurs in the belt of oak forests, individually or in small groups, as a partial-shade species. The fruit is a typical syncarpous stone, which contains two seeds; the fruits are also called "checkers ("chequers"). They are round or ellipsoidal in shape, with significant variations in size and shape. Kárpáti (1960) distinguishes seven different fruit shapes: *typus, sphaerocarpa, pisifera, macrocarpa, microcarpa, dolichocarpa* and *pomoida.* While young, the fruit is reddish-yellow and when fully ripe dark brown, sprinkled with white dots. Fruit is inedible until over ripe, *i.e*. begin to blet, which usually happens after the first frost, when they can be eaten fresh, but are usually processed into compotes and jams. Earlier, in pre-hops times, when hops (*Humulus lupulus*) were not a primary component of beer, they were used in the production of beer, and flour was obtained from dried fruits. The fruits of the wild service tree also have a healing effect, which is eloquently mentioned by the scientific name of the taxon - *torminalis*, which means "good for colic". In folk medicine, the young bark and subcortex are used to treat diabetes. In addition to fruits, wood also has a recognizable use value. It is one of the hardest European types of wood with excellent aesthetic and technical characteristics, which is used to make musical instruments: flute, harpsichord, etc. Wild service tree is also important for forest ecosystems. Bees like to visit its flowers, fruits are a favorite food for birds, game and other forest animals, and the leaves are especially loved by deer and roe deer. From an utilitarian point of view, it is important to note that wild service tree bears fruit every two years or three times in four years (Barengo et al., 2001; Oršanić et al., 2009).

Medlar (*Mespulus germanica* L .; syn. *Crataegus germanica* (L.) Kunze) is the only species of the genus *Mespilus*. It appears as a shrub or low tree. The spontaneous area of medlar includes a part of Persia, Transcaucasia, Armenia, and in Europe, the Strandža mountain in Bulgaria. Its introversion in southern Europe is debatable, where it is probably subspontaneous, arriving from the cultures that were raised by ancient peoples (Jovanović, 1956). In our country it grows as a cultivated species, and can very rarely be found in warmer positions within the belt of oak forests. The fruit is brown, edible medlar, 2-3 cm long, with 2-5 seeds.

Medlar is a fruit that the ancient Assyrians and Babylonians used to enjoy. Pliny spoke of it as a source of excellent juice, and in "recent" history it is mentioned by both Cervantes and Chaucer. Even Shakespeare in the greatest love story, about Romeo and Juliet, found a place for – medlar; Act 2, Scene1 citation:

"Now will he sit under a medlar tree,
And wish his mistress were that kind of fruit
As maids call medlars, when they laugh alone.
O Romeo, that she were, O that she were
An open-arse and thou a poperin pear! "

Medlars are used to prepare various medicinal and edible preparations, such as: teas, liqueurs, compotes, jams. In folk medicine, medlar is used as a powerful anti-inflammatory agent that has a beneficial effect on the whole organism. It has been used for centuries for indigestion - suppresses intestinal and gastric inflammation, eliminates bloating. It is a powerful natural laxative. Due to its antiviral and antibacterial activity, it helps alleviate tonsillitis and airway inflammation (Phipps et al., 2003; Tucakov 1964, 1984).

Chemical composition of chequers and medlars have not been sufficiently investigated in our literature. In addition to the works of Mrkonjić (2017), Šebek et al. (2017) and Veličković et al. (2013), there are few other sources that deal with this issue in our language area. Knowing the chemical composition of these fruits is not an end in itself. It should contribute to the reaffirmation of the chequers and medlars, once highly desirable and valued in our country and in Europe, and now forgotten. By by gathering of chequers in the wilderness and medlars in the orchards, the rural and peri-urban population is given the opportunity to gain additional income, either by selling fresh fruits or through their processing into healthy organic products.

Material and method

Chequers were harvested on the mountain Manjača, while medlars were collected from an old orchard in the suburbs of Banja Luka, Bosnia and Herzegovina. The fruit harvest was done in the first week of October 2020, when chequers and medlars were at the stage of full physiological maturity. Fruits were picked from different parts of the crown with the telescopic scissors. Fruits that have undergone a ripening period were not used for analysis, whether they were left lying on the ground under a tree or stored in storage until over-ripening. There are two reasons for such approach. The first reason is the complexity and for now insufficient determination of the processes that take place during the ripening period, which makes comparisons and conclusions difficult (Rop et al., 2011; Altunaş et al., 2013). Another reason is that the eventual industrial processing of these fruits would be based exclusively on physiologically ripe fruits, and not on fruits in which the process of enzymatic browning has begun or already taking place, which generally results in loss of nutritional, functional and organoleptic qualities (Romero-Rodriguez et al., 2000; Rop et al., 2011).

Prior to the chemical analysis, the fruits are washed, cut into chops, chopped and homogenized in a stainless steel rotating knife homogenizer (Vasilišin et al., 2015). Certain components of the chemical composition were determined by standard AOAC methods (AOAC, 2016). For each component of the chemical composition three parallel analyzes were performed, and the results are expressed as mean values. Total anthocyanins were determined by the spectrophotometrically modified "single" pH method (Sun et al., 2002). The content of total phenols was determined by the modified Folin-Ciocalte method (Wolfe et al., 2003). As a standard compound, gallic acid was used and the results were expressed as equivalents of gallic acid (GAE), i.e. mg GAE/g. Total flavonoids were determined by Kumaran method (Kumaran & Karunakaran, 2007), and the total flavonols by Ordonez method (Ordonez et al., 2006). Quercetin was used as the standard compound, and the results were expressed as mg quercetin (Qc)/g.

Two methods have been used to measure the antioxidant activity of the fruits studied: ABTS (2,2-azinobis(3-ethyl-benzothiazoline-6-sulphonate) and DPPH (2,2-diphenyl-1-picrylhydrazyl). The DPPH test is one of the oldest and the most frequently used methods for the determination of the antioxidant activity of food extracts (Ratty et al., 1988). Antioxidant activity against the DPPH radical was determined by the Liyana-Pathiranana and Shahidi method (Liyana-Pathiranana & Shahidi, 2005), and for the ABTS radical the modified method of Re and associates was used (Re et al., 1999). As a standard compound, Trolox was used. The results were presented with the TEAC value (Trolox equivalent of antioxidant activity), i.e. as μg Trolox/g_{FW}. The results are expressed as the inhibitory concentration - IC_{50}. It is the concentration of extract (mg/ml) required to scavenge 50 % of DPPH.

The elemental composition of the investigated fruits (Na, K, Ca, Mg, P, Zn, Cu, Cr, Fe, Cd, Pb, Al, Se, Mn, Mo) was determined as follows. Fruit samples are burned in a mixture of concentrated acids (nitrogen and perchloric acid) in the digestion thermoblock according to the prescribed temperature regime. After digestion, the solutes were filtered in 50 mL dilution flask, diluted with deionized water, with conductivity of 0.056 μS/cm and stored for analysis of mineral substances. Each sample was prepared in duplicate. Optical Emission Spectrophotometer (ICP OES) was used to determine the mineral content of samples. The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin Elmer auto-sampler model 510. Certificate reference standard solution, instrument calibration standard , with concentration of elements 100 mg/L was used for calibration of spectrophotometer. The concentrations of the elements for the calibration direction were different, determined according to preliminary measurements for the specified metals (Vučić et al., 2018). Linearity was performed by analysis of standard solutions. 20 standard solutions of five concentration levels depending on the analyse were determined. Limit of determination and quantification was performed by analysis of blanks.

Results and discussion

The results of the chemical analysis are shown in tables 1-4. Chequers contain 65.20 % of water and about 34.80% of dry matter, while medlars contain 78.79 % of water and 21.21 % of dry matter (Tab. 1). Medlars contain slightly more sugar (11.51 %), but also significantly less vitamin C (4.44 %), compared to chequers (7.38 % and 15.80 mg/100 g, respectively). However, compared to their wild relatives, fruit of the wild service tree is inferior in terms of vitamin C content. Thus, the whitebeam fruit (*Sorbus aria* Crantz) contains on average 37 mg of vitamin C/100 g and the rowan fruit (*Sorbus aucuparia* L.) 34 mg of vitamin C / 100 g (Vračarić et al., 1990). When it comes to total acidity and pectin content, the differences between chequers and medlars are negligible.

There is a significant difference in the content of total dry matter between the observed species. When we compare the content of total dry matter in our material with data from other areas, we notice a similar phenomenon. Thus, in the material from Straznica in the Czech Republic the content of dry matter in medlars is 29.88-37.15% (Rop et al., 2011), and in the material from Turkey 27.85% (Haciseferogullari et al., 2005), *i.e.* 28.20-33.68 (Altunaş et al., 2013).

Table 1: Chemical composition of fresh fruits of the wild service tree (chequers) and the medlar

	Water (%)	Total dry matter (%)	Total sugars (%)	Total acidity (%)	Pectin (%)	Vitamin C (mg/100 g)
Chequers	65.20	34.80	7.38	0.74	0.28	15.80
Medlars	78.79	21.21	11.51	0.60	0.27	4.44

Regarding the elemental composition, among the 15 analyzed elements, potassium is the most common in both types of fruits, followed by calcium and magnesium (Tab. 2). In checkers, Fe, Cd, Pb and Se were analyzed but not determined. In addition to the above, Cu and Mo have not yet been determined in medlars.

Table 2: Average element concentration (mg/kg_{DW}) in chequers and medlars

	Na	K	Ca	Mg	P	Zn	Cu	Cr	Al	Mn	Mo
Chequers	12.9	1892.2	213.97	115.8	80.6	11.0	0.7	0.10	3.11	0.42	0.009
Medlars	14.6	1096.7	1081.2	529.4	104.8	4.0	n.d.	0.21	1.68	1.18	n.d.

N.D. – analyzed but not detected

Chequers and medlars also contain chemical compounds from the category of phytochemicals, such as: phenols, flavonols, non-flavonoids and anthocyanins (Tab. 3). In all observed parameters, checkers are superior to medlars, especially when it comes to phenols and flavonoids, with the remark that anthocyanins are not even registered in medlars.

However, the content of phenols in our samples is almost 10 times lower than in the samples from the Belgrade Forest, Istanbul, Turkey (Gozde et al., 2015), where it is 20.44 mgGAE / g). Also our research confirmed the statement of the mentioned authors that water is the best solvent for extraction of phenols from chequers.

Table 3. Phytochemicals in chequers and medlars

	Phenols (µgGAE/g)	Flavonols (µgQcE/g)	Flavonoids (µgGAE/g)	Non-flavonoids (µgGAE/g)	Anthocyanins (µg/mg)
Chequers	2416.49	323.51	1299.33	1117.16	301.24
Medlars	520.3	174.97	36.14	513.5	N.D.

The antioxidant potential of the studied fruits species is shown in Table 4. Since lower IC_{50} values indicate greater free radical scavenging activity, the observed table shows that chequers have a significantly higher antioxidant capacity than medlars.
These results are in complete agreement with the data in Table 3 and the stated conclusion that chequers contain significantly more phenols and polyphenolic compounds, such as flavonoides, which are known to possess significant antioxidant activities (Rice-Evans et al., 1997; Rop et al., 2011; Gozde et al., 2015).

Table 4. Antioxidant activity of chequers and medlar fruits

Parameters	Chequers	Medlars
ABTS (IC_{50}) mg Trolox/ ml $_{F.W.}$	0.97 ± 0.15	5.58 ± 0.06
DPPH (IC_{50}) mg Trolox/ ml $_{F.W.}$	5.03± 0.57	46.39 ± 0.1

Notice: IC - inhibitory concentration required to scavenge 50 % of ABTS and DPPH

Conclusion

Ripe chequers and medlars are solid sources of minerals and trace elements. Checkers contain more vitamin C than medlars. They are also superior in terms of total phenols, flavonoids and non-flavonoids. Both species studied show enviable antioxidant potential, with checkers having a significantly higher antioxidant capacity than medlars.

References

Altunaş E., Gül N.E., Bayram M. (2013): The physical, chemical and mechanical properties of medlar (*Mespilus germanica* L.) during physiological maturity and ripening period. Journal of Agricultural Faculty of Gaziosmanpasa Universit, No. 30 (1). 33-40

Anonymus (2006): Toxicological profile for cyanide. Public Health Service Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. 1-341

AOAC (2016): Official methods of analysis of AOAC International - 20th ed., Editor: Latimer W. G. Research BLVD, Rockville, Maryland. 1-3172

Barengo N., Rudow A., Schwab P. (2001): Promotion of rare tree species on the north side of the Swiss Alps: the wild service tree [Förderung seltener Baumarten auf der Schweizer Alpennordseite: Elsbeere, Sorbus torminalis (L.) Crantz]. ETH Zürich/BUWAL. 1-5

Gozde H., Tugba Y.- O., Ayse C. (2015): Antioxidant and antiacetylcholinesterase activities of *Sorbus torminalis* (L.) Crantz (wild service tree) fruits. Journal of Food and Drug Analysis, 23. 57-62

Haciseferogullari H., Ozcan M., Sonmete M.H., Ozbek O. (2005): Some physical and chemical parameters of wild medlar (*Mespilus germanica* L.) fruit grown in Turkey. Journal of Food Engineering, 69. 1-7

Kárpáti Z. (1960): The Sorbus species of Hungary and the neighboring areas [Die Sorbus-Arten Ungarns und der angrenzenden Gebiete]. Feddes Repertorium 62 (2-3). 71-334

Kumaran A., Karunakaran R. J. (2007): In vitro antioxidant activities of methanol extracts of *Phyllanthus* species from India. Food Science and Technology, Vol. 40 (2). 344-352

Liyana-Pathirana M.C., Shahidi F. (2005): Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. Journal of Agriculture and Food Chemistry, Vol. 53 (7). 2433-2440

Mrkonjić Z. (2017): Phytochemical characterization and biochemical activity of fruits of genus *Sorbus* L. 1753 (*Rosaceae*, *Maloideae*) as natural source of nutraceutics . PhD Thesis. University of Novi Sad, Faculty of Sciences. 1-158

Ordoñez A.A.L., Gomez J.D., Vattuone M., Isla M.I. (2006): Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry, Vol. 97 (3). 452-458

Oršanić M., Drvodelić D., Jermić T., Mikac S. (2009): Variability of morphological and biological characteristics of Wild Service Tree (*Sorbus torminalis* (L.) Crantz) fruits and seeds from different altitudes. *Periodicum Biologoricum*. Vol. 111, No 4. 495–504

Phipps J.B., O'Kennon R.J., Lance R.W. (2003): Hawthorns and medlars. Royal Horticultural Society, Cambridge, U.K. Timber Press 1-180

Ratty K.A., Sunatomo J.M., Das P.N. (1988): Interaction of flavonoids with 1,1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. Biochemical Pharmacology, Vol. 37, Issue 6. 989-995

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Ewans C. (1999): Antioxidant activity applying and improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, Vol. 26 (9-10). 1231-1237

Rice-Evans C., Miller N., Paganga G. (1999): Antioxidant properties of phenolic compounds. Trends in Plant Science, Vol. 2, Issue 4. 152-159

Romero-Rodriguez A., Simal-Lozano J., Vazquez-Oderiz L., Lopez-Hernandez J., Gonzalez Castro M.J. (2000): Physical, physicochemical and chemical changes during maturation of medlards and persimmons. Deutsche Lebensmittel Rundschau, 96. 142-145

Rop O., Sochor J., Jurikova T., Zitka O., Skutkova H., Mlcek J., Salas P., Krska B., Babula P., Adam V., Kramarova D., Beklova M., Provaznik I., Kizek R. (2011): Effect of five different stages of ripening on chemical compounds in medlar (*Mespilus germanica* L.). Molecules, 16. 74-91

Sun J., Chu Y.F., Wu X., Liu R.H. (2002): Antioxidant and antiproliferative activities of common fruits, Journal of Agricultural and Food Chemistry, 50. 7449–7454

Šebek G., Pavlova V., Popović T. (2019): Biochemical and pomological characteristics of fruit of some commercial medlar cultivars (*Mespilus germanica* L.) grown in Bijelo Polje. Food and Environment Safety. Journal of Faculty of Food Engineering, Romania. Vol. XVIII, Issue 2, 97-104

Tucakov J. (1964): Pharmacognosy [Farmakognozija]. Zavod za izdavanje udžbenika Socijalističke Republike Srbije. 1-523

Tucakov J. (1984): Herbal treatment, Phytotherapy [Lečenje biljem, Fitoterapija]. Izdavačka radna organizacija "Rad". Beograd. 1-717

Wolfe K., Wu X., Liu R.H. (2003): Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry, Vol. 51 (3). 609-614

Vasilišin L., Lakić N., Vučić G., Petković B., Vojinović Đ. (2015): Fulfillment of RDA values for mineral substances when consuming strawberry fruit. Proceedings, XI Symposium "Contemporary Technology and Economic Development", Leskovac, Serbia. 117-126

Vasilišin L., Vučić G., Vojinović Đ., Kukrić Z. (2017): Mineral composition of different varieties of cherries (Oblačinska and Maraska). Proceedings 2, XXII Conference of Biotechnology with International Participation, Čačak, Serbia. 611-616

Veličković M.M, Radivojević D.D., Oparnica C.D., Nikićević N.J., Živković M.B., Đordević N.O., Vajs V.E., Tešević V.V. (2013): Volatile compounds in Medlar fruit (*Mespilus germanica* L.) at two ripening stages. Hemijska Industrija, 67. 437–441

Vračarić B., Bakić J., Čolić D., Lintner V., Micković M., Rajšić R., Stevanović D., Uvalin M. 1990): Nutrition in nature [Ishrana u prirodi]. Treće izdanje. Vojnoizdavački i novinski centar, Split. 1-366

Vučić G., Vasilišin L., Samelak I., Kukrić Z., Kukrić N. (2018): Content of mineral matter in *Allium ursinum* from different locations of Republika Srpska. Proceedings 2, XXII Conference of Biotechnology with International Participation, Čačak, Serbia. 530-535

ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

CSR STRATEGIES OF INVASIVE WEED FLORA IN VINEYARDS OF BOSNIA AND HERZEGOVINA

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Abstract

Invasions of invasive species pose one of the greatest threats to biodiversity on a global scale. Because of that research on invasive species has recently been intensified, putting the focus mostly on studying invasive species and their habitats, determining impacts on indigenous species and communities, as well as options for controlling and countering proliferation. The problem of invasive species in Bosnia and Herzegovina is a relatively new area, considering that the List of Invasive Species was published only in 2019. The aim of this study was to list invasive species that occur as weed species in vineyards in Bosnia and Herzegovina and to expand the existing knowledge on CSR life strategies of invasive flora, combining the existing data from different sources as a whole, and determining the appropriate strategy for those species for which it is not known. At 73 vineyard locations, 27 invasive species were determined and their CSR life strategy was defined. Plant material necessary for the analysis was collected by field research in the area of vineyards in Bosnia and Herzegovina. The material is analysed and life strategies are defined on the basis of values of seven life traits (i.e. height, dry leaf mass, leaf dry matter content, onset and flowering period, lateral spread, specific leaf area). The most frequent type of strategy among the invasive flora of vineyards in Bosnia and Herzegovina is the CR strategy with 11 species and the C strategy with 9 species. Life strategy R is represented by 4 species and CSR with one species. With the increase of invasive alien species in vineyards, especially C and CR strategy plants, there is a high possibility of spreading these species to the surrounding ecosystems, natural and semi-natural vegetation, and thus seriously endangering biodiversity.

Keywords: *invasive species, CSR strategy, vineyards, Bosnia and Herzegovina.*

Introduction

Although there are numerous definitions of the Invasive Alien Species (IAS), all the definitions are based on the four major criteria: the origin of the species, its ability to reproduce in the wilderness, spatial dispersion and the influence onto the environment (Richardson et al., 2000). Invasive alien species endanger biological diversity. On the global scale they are the second largest threat to the biodiversity, immediately after direct destruction of the natural habitat (Council of Europe, 2007). There are more and more such species that have negative effects onto human health, for example *Ambrosia artemisifolia* L.

The problem of invasive species came into the focus of scientific research in the last several decades. As a part of a huge European project DAISIE (Delivering Alien Invasive Species Inventories for Europe) more than 11.000 alien species have been recorded. The researchers included in this project have a task to monitor and deliver invasive species inventories (Lambdon et al., 2008.).

Numerous species are continuously being introduced into new regions. Controlling invasive species and lowering their impact onto the native species and ecosystems represent nowadays one of the biggest challenges in the protection of nature in Europe and in the world. It is impossible to remove the invasive alien species completely from the habitat to which it spread, because it is not cost-effective. Therefore, it is of huge importance to discover the presence of the possible invasive alien species in the ecosystem at an early stage. Invasive alien species are as a rule connected to the anthropogenic habitats. Their impact is the largest onto the human-controlled habitats. Agricultural land, such as vineyards, are extremely weak habitats with highly expressed disturbances that are suitable for invasive species and their further introduction into natural habitats.

Despite legislative acts, enough attention has not been given to invasive species in Bosnia and Herzegovina. Namely, there are no laws that govern monitoring, control and prevention of the negative consequences of the invasive species. Before the register of the Invasive alien species was done (2019), there had only been some sporadic papers in Bosnia and Herzegovina mentioning invasive species. Šumatić (1990) writes about a massive presence of the species *Ambrosia artemisiifolia* in the compositions of the association *Panico-Galinsogetum Tx et Becker*, 1942 in the area of central part of northern Bosnia. The same author (1997) emphasises a special part of the association *Panico-Galinsogetum ambrosiosum artemisiifoliae* in the region of Derventa. Similar results have been noted by Mitrić (2004) in the orchards in the region from Gradačac to Prijedor. Šarić et al. (1992) describe as invasive species the following: *Sorghum halepense*, *Ambrosia artemisiifolia* and*Galeopsis tetrahit*.

Šarić et al. (2000) write about the spread of the following new weed species in Bosnia and Herzegovina: *Abutilon theophasti* Medik., *Xanthium strumarium* L. and *Datura stramonium* L. Šilić and Abadžić (2000) write about the spread of neophyte species in Bosnia and Herzegovina, such as the americanophytes. Some of them are dangerous and invasive weeds like *Ambrosia artemisiifolia* L., *Amorpha fruticosa* L., *Conyza canadensis* (L.) Cronq., *Erigeron annuus* (L.) Pers., *Galinsoga parviflora* Cav., *Tagetes minuta* L. Šumatić and Janjić (2006) have noted a considerable increase of adventive weed species in Bosnia and Herzegovina (Republic of Srpska) with regard to the earlier research from the from the 1960s.

The very first list of the invasive species with their distribution in the Federation of Bosnia and Herzegovina was done in 2019 in the collaboration of the Faculty of Science and Mathematics of the University of Sarajevo and the Federal Ministry of Environment and Tourism. The List comprises 81 plant species. An official List of the invasive species for the Republic of Srpska still does not exist. Therefore, the knowledge of the contents of the invasive weed flora and its changes because of the geographical and climate conditions, is highly useful in inventorying, monitoring and managing invasive weeds. Vrbničanin et al. (2004) recorded 97 alien invasive weed species in the flora of Serbia, with dominance of the species from the family Asteraceae. The Croatian List (Boršić et al., 2008) contains 64 invasive alien weed species while 73 invasive alien plant species have been recorded in Montenegro so far (Alien and invasive alien species in Montenegro , 2021).

Materials and methods

During a three-year research time span (2018, 2019 and 2020), 73 localities of the vineyards in Bosnia and Herzegovina were observed (Figure 1). Each locality latitude, longitude and altitude were found using a GPS. Keys and iconography, standard for this region, were used for the analysis, determination and identification of the plant taxa. . In addition to the plant register and their distribution, each identified taxon (species, subspecies or variety) was enriched with a belonging flora element, life form and CSR life strategy.

Figure 5. The localities of the researched vineyards

A wish to classify plant species into specific categories according to their life strategies for the sake of easier understanding such organisms, has existed in research for a long time now. One such system was suggested by Grime (1979) in his book *Plant strategies and vegetation processes*. Grime proposed a theory according to which all the external factors that limit the amount of present live or dead plant material in any habitat can be divided into two categories: stress and disturbance.

Grime made a division of the species into three basic categories on the basis of adjustability of the plants onto the factors: (1) C-competitors, (2) S-stress tolerators, and (3) R-ruderals. Under competition he means a tendency of the neighbouring plants to use the very same light quantum, ion of mineral nutrients, molecule of water and air volume.

- Competitive plants (C strategy) grow best in conditions of low stress and disturbance. They are competitive to the other species in the habitat because they are morphologically adjusted to use the resources best.
- Stress tolerant plants (S strategy) are adjusted to the conditions of high stress but low disturbances. They mostly grow in habitats with extreme conditions like extremely low pH soil, low amount of light, etc.
- Ruderal plants (R strategy) can survive disturbances, but not the constant stress. Therefore, they are one year colonizers of the habitats with disturbed conditions. The simultaneous presence of the high amount of stress and disturbances is not helpful for the plant life.

In addition to the three basic categories, 16 types were established through further analysis. They are suited to different combinations of stress and disturbance in the habitat.
On the basis of collected data, a CSR analysis was conducted for the invasive weed flora in the vineyards of Bosnia and Herzegovina. Data on CSR strategies were withdrawn from the Internet bases Flora Croatica Database (Nikolić, 2010), BiolFlor (Klotz et al., 2002) and from the intern Lookup base of programmes for the CSR strategies determination (Hunt et al., 2004). For the species for which the CSR strategy was different in only one of the three sources, the strategy from the overlapping two sources was adopted. In case that data about the CSR strategy were accessible in two sources, and they differed, the species were taken for further examination.

Results and discussion

Through the floristic research done in the vineyards of Bosnia and Herzegovina on chosen locations, 27 invasive weed species were noted. They were classified in 15 families (Table 1). According to the numerical presence of the species, the largest presence is noted in the following families: *Asteraceae* (10 weed species), *Amaranthaceae*, *Fabaceae* and *Poaceae* (with 2 invasive weed species each), *Asclepiadaceae*, *Euphorbiaceae*, *Malvaceae*, *Simaroubaceae*, *Solanaceae*, *Phytolaccaceae*, *Plantaginaceae*, *Portulacaceae*, *Rosaceae* and *Urticaceae* (with one weed species each).

Table 1. List of invasive weed species in the vineyards of Bosnia and Herzegovina

Species	***Family***	**Life form**	**Origin**	**CSR**
Abutilon therophrasti Medik	*Malvaceae*	T	Asia	CR
Ailanthus altissima (Mill.) Swingle	*Simaroubaceae*	F	Asia	C
Amaranthus albus L.	*Amaranthaceae*	T	North America	R
Amaranthus retroflexus L.	*Amaranthaceae*	T	North America	CR
Ambrosia artemisiifolia L	*Asteraceae*	T	North America	CR
Artemisia vulgaris subsp. verlotorm (Lamotte) Bonnier .	*Asteraceae*	H	America	C
Asclepias syriace L.	*Asclepiadace*	G	North America	C
Datura stramonium L.	*Solanaceae*	T	North America	CR
Conyza bonariensis (L.) Cronquist	*Asteraceae*	T	America	CR
Conyza canadensis (L.) Cronq.	*Asteraceae*	T	North America and Central America	CR
Cuscuta campestris Yunck.	*Cuscutaceae*	T	North America	/
Elusine indicata (L.) Geartn.	*Poaceae*	T	Africa	C
Erigeron annuus (L.) Pers.	*Asteraceae*	T	America	CR
Euphorbia prostrata Aiton	*Euphorbiaceae*	T	America	R
Galinsoga parviflora Cav.	*Asteraceae*	T	America	CR
Lathyrus tuberosum L.	*Fabaceae*	G	Asia	C
Medicago sativa L.	*Fabaceae*	H	Eastern Europe, Asia	C/CSR
Phytolacca americana L.	*Phytolaccaceae*	G	North America	C
Picris eschioides L.	*Asteraceae*	H	South Europe	CR
Portulacea oleracea L.	*Portulacaceae*	T	Asia	R
Rubus ceasiues L.	*Rosaceae*	P	Europe and Asia	C
Sorghum halepense (L.) Pers	*Poaceae*	H	Africa and south-western Asia	C

Tagetes minuta L.	*Asteraceae*	T	South America	/
Urtica dioica L.	*Urticaceae*	H	Europe and Asia	C
Veronica persica Poir.	*Plantaginaceae*	T	Asia	R
Xanthium strumarium L.	*Asteraceae*	T	North America	CR
Xanthium strumarium ssp. italicum (Moretti) D.	*Asteraceae*	T	North America	CR

All of the above listed invasive species are not present at the official list of the Invasive alien species in Bosnia and Herzegovina, but they are invasive in the neighbouring countries – Croatia, Serbia and Montenegro. Due to the vicinity and the similarity in the biogeographical regions these species are listed in this table.

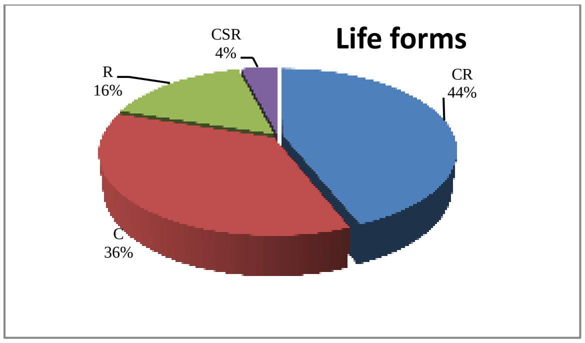

Figure 2. Life form of the invasive weed flora

Regarding life forms of the invasive weed species, life form Therophyte is dominant (17 weed species – 63 %) (Figure 2.). After Therophyte the next most numerous life form is *Hemicryptophyta* (5 weed species – 18 %), followed by *Geophyta* (three weed species – 11 %). They are followed by *Chamaephyta* and *Phanerphyta* with one (1) invasive weed species each
Regarding the origin, all the plants are introduced. The largest number of the plants was introduced from America (16 weed plants - 52 %), from Asia (9 weed plants – 29 %), four weed plants, whose native habitat was not in Bosnia and Herzegovina, arrived from other parts of Europe and two weed species from Africa.

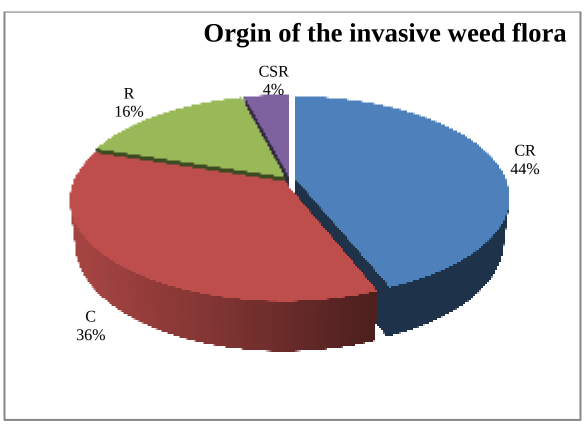

Figure 3. Origin of the invasive weed flora

The material was analysed on the basis of the collected invasive weed species and life strategies were established on the basis of 7 characteristics of life (height, mass of dried leaf, ratio of the dry matter of the leaf, the beginning and the duration of blooming, spatial spreading, specific leaf surface). The most frequent types of the strategy among the invasive flora in the vineyards of Bosnia and Herzegovina are CR strategy with 11 species and C strategy with 9 species. Life strategy R is present with 4 species and life strategy CSR with one species.

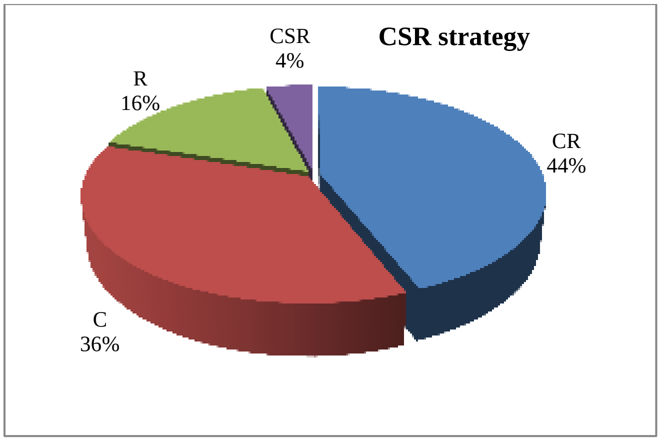

Figure 4. CRS strategy of the invasive weeds in the vineyards of Bosnia and Herzegovina

For some species CSR strategy was not done, because at the time of the fieldwork research some vegetative (*Cuscuta campestris* Yunck.) and generative (*Tagetes minuta* L.) parts were missing. As already said, CR and C strategies are dominant in this region, but also in the neighbouring countries, which is a matter of concern since these species have a negative impact on native species and diversity (Figure 4). The results of the research ask for further research of the CSR strategies of the invasive weed species that will contribute to better understanding of their spreading. Family *Asteraceae*, that has the largest number of invasive weeds, possesses some characteristics that can explain their superiority. *Asteraceae* are namely prone to the invasive behaviour due to their abundance of the life forms, due to dissemination adjustability and due to their huge numbers, which is the reason why they are highly present among the weeds. CSR system is based upon the influence of the environmental factors onto the flora so that the presence of a specific strategy can reveal data about the condition of the sheer ecosystem. To exemplify it, an increase of C type species in the habitat can be brought into connection with the anthropogenic habitat abandoning by the S type with an enlarged eutrophication, and by the R type with more frequent disturbances in the habitat (Hodgson et al., 1999).

Conclusion

The majority of the listed invasive families has a large number of herbaceous species with a short life cycle (Terophytes), summer development, good adjustability to the arable land. Also they are weed species that produce seeds in huge quantities *(Abutilon therophrasti* Medic., *Asclepias syriace* L., *Cuscuta campestris* Yunck., *Portulaceae oleraceae* L., et ct.).
According to the floristic research done at 73 locations in the vineyards of Bosnia and Herzegovina the most represented is the family *Asteraceae* with 10 weed species, whereas in the domain of life forms we have a dominance of the life form Therophyte with 17 weed species.

The largest number of invasive weeds comes from America. With the increase of invasive alien species in vineyards, especially C and CR strategy plants, there is a high possibility of spreading these species to the surrounding ecosystems, natural and semi-natural vegetation, and thus seriously endangering biodiversity.

References

Boršić, I., Milović,M., Dujmović,I., Bogdanović, S., Cigić,P., Rešetnik, I., Nikolić, T., Mitić.B., (2008) Preliminary check-list of invasive alien plant species (IAS) in Croatia. Nat. Croatica, vol. 17, No2: 55-71, Zagreb

Council of Europe: The Bern Convention (Convention on the conservation of European wildlife and natural habitats, Bern, 1979), Question and Answers, No. 9, November 2007

Grime J. P. (1979) Plant strategies and vegetation processes. John Wiley and Sons, New York

Hodgson, J. G. – Wilson, P. J. – Hunt, R. – Grime, J. P. – Thompson, K. (1999): Allocating C-S-R plant functional types: a soft approach to a hard problem, *OIKOS 85*, 282–294

Hunt R., Hodgson J. G., Thompson K., Bungener P., Dunnett N. P., Askew A. P. (2004) Lookup C-S-R database

Lambdon, P.W., Pyšek, P., Basnou, C., Arianoutsou, M., Essl, F., Hejda, M., Jarošík, V., Pergl, J., Winter, M., Anastasiu, P., Andriopoulos, P., Bazos, I., Brundu, G., Celesti-Grapow, L., Chassot, P., Delipetrou, P., Josefsson, M., Kark, S., Klotz, S., Kokkoris,Y., Kühn, I., Marchante, H., Perglová, I., Pino, J., Vilà, M., Zikos, A., Roy, D.,Hulme, P.E. (2008): Alien flora of Europe: species diversity, temporal trends,geographical patterns and research needs. Preslia 80: 101-149.

Mitrić, S. (2004): Proučavanje korova voćnjaka kao osnova za primjenu herbicida, Poljoprivredni fakultet, Magistarska teza, (Study of orchard weeds as a basis for herbicide application. Faculty of Agriculture, Master's thesis), Banja Luka

Richardson, D.M., Pyšek, P., Reymanek, M., Barbour, M.G., Panetta, F.D., West, C.J .(2006). Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distribution 6: 93-107.

Šarić, T., Đikić, M., Gadžo, D. (2000): Promjena korovske flore pod utjecajem agrotehnike Herbologica, (Change of weed flora under the influence of agrotechnics) Vol. 1, No.1, 15-27, Sarajevo.

Šumatić, N. (1990) Weed vegetation of northeastern Bosnia Korovna vegetacija sjeveroistočne Bosne", (Weed vegetation of northeastern Bosnia Scientific conference *Population, species, biocenosis,* abstracts) Sarajevo.

Šumatić, N., Janjić, N. (2006), Adventivne biljke u Bosni i Hercegovini, (Adventive plants in Bosnia and Herzegovina), Acta herbologica 15, 9-14

Vrbničanin,S.,Karadžić,B., Dajić-Stevanović,Z. (2004): Adventivne i invazivne korovske vrste u Srbiji, (Adventive and invasive weed species in Serbia), Beograd, Acta Herbologica, Vol.13, No.1, 1-12

Foreign and invasive alien species in Montenegro 2021 https://parksdinarides.org/docs/brosura_strane_i_invazivne_strane_biljne_vrste_u_crnoj_gori.pdf

TEMPORARY AND PERMANENT WET ZONES IN BOSNIA AND HERZEGOVINA

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Abstract

The goal of this research is to examine the spatial distribution of temporary and permanent wet zones in Bosnia and Herzegovina (B&H). In addition, the research refers to changes that occurred in the period 2012-2018 using the Copernicus Water and Wetness (WaW) and CORINE Land Cover (CLC) database. Regarding water resources, B&H is one of the richest European countries, with total amount to over 10 thousand m^3/per capita/per year. The article also shows the vertical distribution of temporary and permanent wet zones. The European digital elevation model (EU DEM) was used to divide the territory of B&H into three altitude zones: from 0-500 m, from 500-1000 m, and over 1000 m. As the country is at the contact of the Pannonian Plain in the north, the Dinaric Mountains in the central part and the Adriatic Sea in the south, the impact of large natural regions on wetness area distribution is examined.
The CLC database has been processed by extracting the wetland zones and calculating their spatial coverage and changes that happened during the period from 2000 to 2018. The research of WaW database shows that permanent wet areas occupy 0.1% and temporary wet areas occupy 2.5% of the B&H territory. According to CLC database wetlands cover 0.12% of the country's territory. So far, there has been no research on wetness zones based on the WaW high resolution layers database in B&H, which is the most significant contribution of this article.

Keywords: *Copernicus, Water and Wetness, regions, Bosnia and Herzegovina.*

Introduction

Soil moisture plays a pivotal role in vegetation dynamics, considering that soil water availability is a crucial limiting factor for plant photosynthesis (Proietti et al, 2019). Consequently, soil moisture maps provide an invaluable resource to quantify the effects of rainfall deficits on vegetated lands (Cammalleri et al, 2015). Soil moisture–atmosphere interactions are key elements of the regional climate system (Leutwayler et al, 2021). Wetlands are especially beneficial under extreme drought or flood conditions for their ability to retain water, reduce runoff, filter sediments, and provide water purification. The greatest impact to wetlands are from changes to hydrologic regimes (Hartig et al, 1997).
B&H is a country in the Western Balkans, with an area of 51209 km^2. The distribution of the total land area is as follows: 5% lowlands, 24% hills, 42% mountains, and 29% karst regions (TNC & SBUR of B&H, 2016). According to the last census from 2013, B&H has about 3.53 million inhabitants (ASB&H, 2016), or 69 per square kilometre. Annual precipitation varies from 800 mm in the north along the Sava River, to 2000 mm in the central and southeastern mountain regions (SNC B&H, 2013). The average annual rainfall in B&H is 1250 mm. Total renewable water resources per capita in B&H amount to 10592 m^3/per capita/per year (FAO,

2017). The total area of all water reservoirs and lakes cover 19921 ha (199.21 km^2) or 0.39% of the country's territory (Drašković & Gutalj, 2021). The river network density is 0.4 km / km^2.
The aim of the paper is to show the precise spatial distribution of temporary and permanent wet areas in B&H by altitude using the WaW and CLC databases. Wetness areas in B&H should be defined in the future as areas of high biodiversity, considering they provide important ecosystem services.

Materials and methods

The Copernicus Land Monitoring Service (CLMS) provides geographical information on land cover and its changes, land use, vegetation state, water cycle and earth surface energy variables to a broad range of users in Europe and across the world in the field of environmental terrestrial applications.
The main product of WaW database is a classified High-Resolution Layers (HRL), which contains defined classes of permanent water, temporary water, permanent wet, temporary wet, and dry areas, derived from water and wetness occurrences in the period 2012-2018. The Water Wetness Probability Index (WWPI) indicates the occurrence of water and/or wet areas throughout the entire observation period 2012-2018 for the 2018 product and is derived from frequencies of WATER, WET and DRY. The resulting product assembles the water and wet occurrence as an index on a scale between 0 (only dry observations) to 100 (only water observations) (see Figure 1). The HRL WWPI is provided in a pixel resolution of 10 m (CLMS, 2020).
WaW 2018 comprises the product in full spatial resolution of 10m x 10m (instead of the original 20m x 20m resolution of the WAW 2015 production). For the analysis of surface humidity, the WaW database from 2018 has been used, as an indicator of the spatial coverage of temporary and permanent wet areas in the period 2012-2018 (areas with 25% to 75% wet, including areas of changing soil moisture and intermittent wetlands).
Permanent water comprises e.g. rivers and lakes while temporary water include e.g. temporarily inundated areas. Among the wet zones, permanent wet areas comprises e.g. areas of permanently high soil moisture while temporary wet areas include e.g. areas of changing soil moisture (Langanke, 2016).
The CLC database contain wetlands information within the five main categories. The CLC database is available in both vector and raster format, while WaW is available only in raster format. The data are processed in GIS software and exported to Microsoft Excel, and then classified and calculated by individual types, areas and percentages.
The EU-Hydro database is available in the ESRI Geodatabase shapefile vector format. The river network is divided into two types: polygons (rivers wider than 50 m) and polylines (rivers narrower than 50 m). Digital elevation model (EU-DEMv1.1.) with a spatial resolution of 25 m has been used for distribution of wetness areas by altitude zones.

Results and Discussion

According to the WaW 2018 database, the areas under the category of permanent and temporary water occupy 33257 ha (0.64%) and 1464 ha (0.03%), which is a total of 0.67% of the territory of B&H (matches CLC 2018 data). Temporary wet areas cover 128680 ha (1286.8 km^2) or 2.5% and permanent wet cover 5222 ha (52.22 km^2) or 0.1% of the country's territory. CLC 2018 database shows that inland marshes cover 5242 ha and peatbogs 697 ha. So that, area of wetland cover 5939 ha (59.39 km^2).

Due to the increase in resolution from 20 m to 10 m and the reduction of the minimum cartographic unit from 1 ha in 2015 to 0.03 ha in 2018, these two databases are not completely comparable and the data may significantly differ by individual regions. According to WaW 2015 database temporary wet area cover 4.02% and permanent wet 0.1% of the B&H territory. From the same database permanent and temporary water cover respectively 0.44% and 0.13%, or 0.57% in total of country territory.

The largest temporary wet zones are karst fields at the west Bosnia region, and on the south at the region of Herzegovina. There are two main reasons: a large amount of precipitation (more than 1500 mm annually) and geological layers in which limestone rocks predominate that cannot accept all the water during rainy period. Another zone with large temporary wet areas is in the north-eastern part of the country, at regions of Posavina and Semberija. In this case, the reason for wetness is related to the relatively frequent outflow of large rivers and their tributaries from riverbeds and alluvial plains. Figure 1 shows wet occurrence by altitudes and in relation to geographical position of mayor rivers.

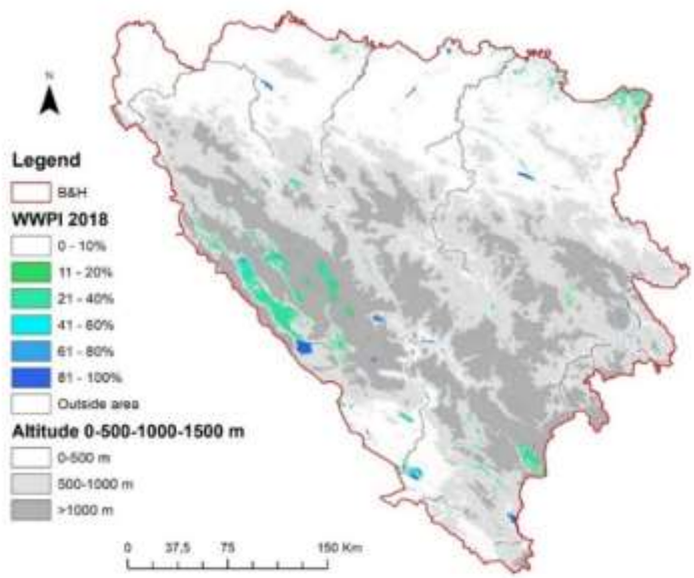

Figure 1. Wetness zones by percentage and altitude (Data source: WWPI 2018, EU-Hydro 2018 and EU-DEMv1.1)

Altitude and differences in vertical climate zonation certainly affect the appearance of temporary wet areas. Considering the altitude range from 0 to 2367 m, three altitude zones of 0-500 m (39.47% of the B&H territory), 500-1000 m (35.53%), and over 1000 m (25%) have been taken into account.

The WaW 2018 database shows that temporary wet area are not evenly distributed by altitude zones. They occur more within the zone of 500-1000 m (52.4%) than within the zone of 0-500 m (37.0%) and the zone over 1000 m (10.6%) (Table 1). This difference appear thanks to the karst fields in the west of the country whose altitude is between 500-1000 m. The only larger temporary wet area above 1000 m is at Kupreško karst field. The area in the north around the rivers Sava and Drina belongs to the zone from 0 to 500 m.

Table 1. Spatial distribution of temporary wet areas by altitude

Wetness zones	0-500 m		500-1000 m		>1000 m	
	P (km^2)	P (%)	P (km^2)	P (%)	P (km^2)	P (%)
Temporary wet	502.5	37.0	647.75	52.4	136.55	10.6
Permanent wet	27.16	52.1	24.56	47	0.5	0.9

*Data source: WaW 2018 and EU-DEMv1.1

When it comes to permanent wet area 52.1% is located within 0-500 m, 47% is within 1000-1500 m and 0.9% over 1000 m. Spatial distribution of permanent wet zones is similar to temporary wet zones, so lower wet zones are around Sava and Drina river, and higher are in the western part of the country.

Estimates show that B&H will be exposed to climate change impacts that could have consequences for its entire society. Studies of temperature change for the period 1961-2010 indicate that temperatures have increased in all areas of the country. A comparative seasonal analysis for 1981-2010 and 1961-1990 showed that the largest increases in average temperature during the summer months were observed in Herzegovina (Mostar 1.2°C). The largest decrease in annual precipitation was during the spring and summer seasons, in the region of Herzegovina (Mostar and Bileća, up to 20%) (SNC, 2013).

According to CLC Changes database, in period from 2000 to 2018 wetlands (4.1) reduced the area for 490 ha (4.9 km^2). Among them, inland marshes (4.1.1) were most endangered by other land cover types, primarily with water bodies (5.1.2) and non-irrigated arable land (2.1.1). Some areas with higher amount of precipitation caused expansion of water bodies over inland marshes, while part of wetlands has been converted to agricultural use. In future, some of remaining wetlands will be probably subject to agricultural drainage.

Runoff is identified as a key hydrological parameter affecting wetland function (Hartig et al., 1997). The average runoff coefficient in B&H is 0.57 (SNC B&H, 2013). Higher temperatures accompanied by lower precipitation will change the hydrologic regime enough to damage wetland functions in Herzegovina region. Other wet zones will be less affected. Wetland protection could assist to decrease vulnerability of climate change impacts.

Conclusions

According to WaW database in 2018 water cover 0.67% of territory of Bosnia and Herzegovina. Temporary and permanent wet area cover 2.5% and 0.1% respectively. The largest wetness zones are located in the western part of the country, at region of Livanjsko, Glamočko and Kupreško karst fields, and also Gatačko karst field at Herzegovina region. There are two main reasons for higher level of wetness. Firstly, area of karst fields receive over 1500 mm precipitations annually, and secondly, limestone geologic layers cannot accept all the water in humid period so water goes ascending and flooding the bottom of fields. Also, temporary wet zones appear along the alluvial plain, especially at Sava and Drina river basins in north-eastern part of the country.

The predicted climate changes in precipitation and air temperature will affect negatively on wetness areas in B&H. Aridity is expected to increase, because of reduced summer rainfall coupled with increased evaporation rates. The extent of future wetland losses will depend on land-use policies, socioeconomic conditions, and vulnerability to climate change.

References

Agency for statistic of Bosnia and Herzegovina (ASB&H) (2016). Census of Population, Households and Dwellings in Bosnia and Herzegovina, 2013. Database. http://www.statistika.ba/ (accessed November 15, 2020)

Cammalleri, C., Micale, F., Vogt, J. (2015). On the value of combining different modelled soil moisture products for European drought monitoring, *Journal of Hydrology* 525, 547–558.

Copernicus Land Monitoring Service (CLMS) (2020). Water and Wetness 2018, User manual. https://land.copernicus.eu/user-corner/technical-library/water-wetness-2018-user-manual.pdf (accessed 10 January 2021)

Copernicus Land Monitoring Service (CLMS) European Digital Elevation Model (EU-DEM) v1.1. Database. https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1/view (accessed 05 January 2021)

Copernicus Land Monitoring Service (CLMS) EU-Hydro, Database https://land.copernicus.eu/imagery-in-situ/eu-hydro (acces. 25 January 2020)

Copernicus Land Monitoring Service (CLMS) Water and Wetness, Database https://land.copernicus.eu/pan-european/high-resolution-layers/water-wetness (accessed 20 January 2020)

Drašković B., Gutalj M. (2021). Inland water in Bosnia and Herzegovina, Book of Proceedings «IOP Conference Series: Earth and Environmental Science 834», 012058, Perm, Russia. doi:10.1088/1755-1315/834/1/012058

FAO (2017). Database, AQUASTAT Website, available at: http://www.fao.org/aquastat/en/ (accessed on 13 February 2021)

Hartig, EK., Grozev, O., Rozenzweig, C. (1997). Climate change, agriculture and wetlands in Easteren Europe: vulnerability, adaptation and policy. *Climatic Change* volume 36, pp. 107–121.

Langanke T. (2016). Copernicus Land Monitoring Service – High Resolution Layer Water and Wetness: Product Specifications Document. Available at: https://land.copernicus.eu/user-corner/technical-library/hrl-water-wetness-technical-document-prod-2015

Leutwayler, D., Imamovic, A., Schär, C. (2021). The Continental-Scale Soil Moisture–Precipitation Feedback in Europe with Parameterized and Explicit Convection, *Journal of Climate*, Volume 34, Issue 13, pp. 5303–5320.

SNC B&H (2013). Second National Comunication of Bosnia and Herzegovina under the United Nations framework convention on climate changes. 196p.

TNC & SBUR of B&H (2016). Third National Communication and Second Biennial Update Report on Greenhouse Gas Emissions of B&H. 258p.

Proietti, C., Anav, A., Vitale, M., Fares, S., Fornasier, MF, Screpanti, A., Salvati, L., Paoletti, E., Sicard, P., and De Marco, A. (2019). A New Wetness Index to Evaluate the Soil Water Availability Influence on Gross Primary Production of European Forests, *Climate*, 2019, 7(3), 42.

WEED FLORA OF ARABLE CROPS IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract

This paper has studied weed population of arable crops in Herzegovina region (Bosnia and Herzegovina). On 34 research localities, overall 51 species of vascular plants from total of 25 families were noted. The highest share had family *Asteraceae* (counting 11 species) and *Poaceae* (counting 5 species). The analysis of biological spectrum of association *Panico- Portulacetum oleraceae* Lozanovski 1962. Terophyte-hemicriptophyte character could be determined, with slightly higher share of terophytes, and smaller share of hemicriptophytes, resulting exclusively from mechanical measures of weed control. In areal spectrum of association *Panico-Portulacetum oleraceae* Lozanovski 1962 seven groups of flora elements were asserted, among which elements of wide-spread dominate. Thirty two (32) plant species related to the cosmopolite group of flora elements. The stands of the *association Panico-Portulacetum oleraceae* Lozanovski 1962 in arable crops of Herzegovina contain 51 weed species, which represents a certain floristic wealth in relation to the eponymous association described in other localities. Special emphasis has to be set on species common for all localities, which are the typical species of syntaxonomic units (association, alliance, order and class): *Portulaca oleracea* L., *Echinochloa crus-galli* (L.) Beauv., *Setaria glauca* (L.) Beauv., *Amaranthus retroflexus* L., *Chenopodium album* L. and *Solanum nigrum* L. The most common type of strategy in weed flora is R (28.00%), followed by plants of CR strategy (16.00%).

Keywords: *weed flora, CSR strategies, floral diversity.*

Introduction

Openness of perennial crops as well as the methods of cultivating the land are favourable to the 'entry' of a high number of ruderal species, particularly invasive, into floristic composition of weed communities, which results in 'ruderalisation' of weed communities, significant change of floristic composition, pushing of segetal weeds and inability to plan adequate controlling measures for a long period of time (Kovačević *et al.* 2014). The composition of flora is affected by agro-technical measures (method of land tilling, fertilizer spreading, herbicides use, grass sowing, mowing) and method of cultivation (cultivation form, inter-tillage and tillage in a row) (Barić *et al.* 2021)

The weeds are spread around the world (*ubikwists*), they have an exceptional ability of survival, they are able to crop up in various soils and under various climate conditions, growth and development phases quickly pass by, their seed is able to postpone the period of cropping up (dormancy); furthermore, they are able to keep live and sproutable seed for very many years under field conditions, they bear fruits several times a year, they produce seed of equal weight and form as well as the cultivated species, they increase in number generatively (by seed) and/or by vegetative organs, which are fragile and do not let to be pulled out (they break). The underground organs are rich with reserve nutritious organs so they easily resist stormy weather. They have especially developed methods of spreading to new areas. With their root and vegetative organs they deeply get through the soil. They are able to develop resistance to certain groups of herbicides and because of that, they compete for the space, nutrients, water and light with agricultural and other (desired) plant species, damaging them directly and indirectly. (Ministry of Foreign Trade and Economic Relations, 2021)

CSR classification (Hodgson et al. 1999) is a practical method for the categorization of plants according to Grime's theoretical triangular scheme of competitor, stress tolerator and ruderal plant strategies (CSR theory). The CSR classification method uses various functional traits quantified *in situ* (or measured from material collected *in situ*). (Pierce et al. 2013) Three principal strategies represent viable trait combinations arising under conditions of competition, abiotic limitation to growth or periodic biomass destruction, respectively. Specifically, C-selected 'competitors' are said to survive in relatively stable, productive habitats via investment of resources in continued vegetative growth and rapid attainment of large individual and organ size to aid resource pre-emption. S-selected 'stress tolerators' protect metabolic performance in variable and resource-poor environments by investing mainly in capacity to retain resources and repair cellular components in dense, persistent tissues. R-selection, or ruderalism, involves investment of a large proportion of resources not in the individual but in propagules from which the population can regenerate in the face of repeated lethal biomass destruction events, or disturbances. (Pierce et al.2017)

The goals of this paper are to research, describe and systematize the weed plants of arable crops the Herzegovina; to determine their position as compared to the affiliation to certain plant communities as well as plant forms. Moreover, the objective is to determine the CSR life strategy of weed plants by determining factors of competitiveness, stress tolerance and ruderal factors.

Material and methods

Floristic–phytocoenological researches of weed flora and vegetation of arable crops were conducted during three vegetation seasons (2018, 2019 and 2020), from spring to the late autumn.

The research encompassed 34 localities and by means of GPS the geographical latitude, geographical longitude and height above sea level have been very precisely determined (with an error of few meters) for all localities. Also, for every researched locality, where the phytocoenological photos were taken, the dates of taking photos were recorded as well as the municipalities, which certain locality belongs to.

Floristic–phytocoenological researches were carried out upon principles and methods of Swiss-French (Zürich-Montpellier) phytocoenology school Braun Blanquet (1965), and they encompassed analytical and synthetic phase of research.

Processing and determination of plant species were carried out by means of the following floristic works: Flora Europaea I-V (Tutin, ed., 1964-1980, 1993), Flora Bosne i Hercegovine – *Flora of Bosnia and Herzegovina* (Beck, 1903, 1906-1923), Flora SR Srbije I-IX – *Flora of Federal Republic of Serbia I-IX* (Josifović, ed., 1970-1977), Flora Hrvatske – *Flora of Croatia* (Domac, 1994), Ilustrovana korovska flora Jugoslavije – *Illustrated Weed Flora of Yugoslavia*

(Čanak et al., 1978), Ikonographie der Flora des Südöstlichen Mitteleuropa (Javorka et Csapody, 1979) and Flora Italiana (Fiori et Paoletti, 1921).
Nomenclature of plant species has been aligned according to the works: Flora Europaea (Tutin et al. 1964 –1980, 1993), then Flora Croatica Database (Nikolić, 2015), and only partly according to Pignatti (1982) and Trinajstić (1975 –1986).
Numerousness and superficial feature as well as integration in the analysed forest stands have been defined upon the principles and methods of Swiss-French phytocoenology school (1965 (Zürich-Montpellier), combined with the scale per Braun-Blanquet (1965).
Field testing, phytocoenological photos were taken at the areas of 100 m^2.
The phytocoenological photo represents a list of plant species in a certain forest stand, in process of which a combined assessment of numerousness and superficial feature has been obligatorily defined for every species, and often also the assessment of integration or social feature. The first and the second assessment are mutually divided by point. For every phytocoenological photo the general and ecological data on forest stand are recorded. All photos have been taken prior to the conduction of agro-technical measures (plowing, tilling with a rotary hole, inter-tillage, use of herbicides).
Syntaxonomic overview of vegetation is shown according to the following publications: Sintaksonomski pregled vegetacije Srbije - *Syntaxonomic overview of vegetation of Serbia* (Kojić et al., 1998) and Pflanzensoziologische Exkursionaflora für Deutschland und angrenzedne Gbiete (Oberdorfer, 2001).
Comparison of a large number of regularly developed species of some community defines the frequency of the presence of some plant species in a certain community. This analysis is the most successfully carried out based on the analytical phytocoenological table-sheet. A degree of the presence is shown in percentages showing in how many researched forest stands of some community certain plant species is present. The presence is expressed in Roman numerals I to V, i.e.:
I = species present in 1 - 20% of the photos,
II = species present in 21 - 40% of the photos,
III = species present in 41 - 60% of the photos,
IV = species present in 61 - 80% of the photos,
V = species present in 81 - 100% of the photos.
Grime CSR strategy of plants has been made. Grime has made exactly based on plants adaptiveness to the factors the classification into three basic categories: competitive (C), stress-tolerant (S) and ruderal (R) species. The competition assumes the tendency of neighbouring plants to use the same quantum of light, ion of mineral substances, molecule of water and volume of air.
The existing data on CSR strategies have been taken from the Internet databases Flora Croatica Database (Nikolić, 2010), BiolFlor (Klotz et al., 2002) and internal Lookup database of the programme for determining the CSR strategies (Hodgson et al., 1999).

Results and discussion

At the researched localities there are 51 plant species recorded with 25 families so the most numerous ones are the family *Asteraceae* with 11 species and *Poaceae* with 5 species.

Table-sheet 1. Localities of arable crops where the phenocoenological photos of the association *Panico – Portulacetum oleraceae* Lozanovski 1962 were taken.

Photo no.	Date of photo taking	Municipality	Locality	N	E
1.	8 Aug 2018	Stolac	Domanovići	43°05'05,7''	17°55'32,1''
2.	25 Jul 2019	Stolac	Domanovići	43°05'05,7''	17°55'32,1''
3.	28 Jul 2019	Stolac	Domanovići	43°05'05,7''	17°55'32,1''
4.	9 Aug 2018	Stolac	Domanovići	43°05'05,7''	17°55'32,1''
5.	18 Aug 2020	Stolac	Opličići-Osmankuša	43°05'05,0''	17°55'32,0''
6.	18 Aug 2020	Stolac	Opličići-Osmankuša	43°05'05,0''	17°55'32,0''
7.	30 Sep 2019	Čitluk	Potpolje	43°12'31,0''	17°32'18,0''
8.	30 Jul 2018	Čitluk	Potpolje	43°12'31,0''	17°32'18,0''
9.	25 Jul 2019	Čitluk	Potpolje	43°12'31,0''	17°32'18,0''
10.	23 Jul 2020	Čitluk	Potpolje	43°12'31,0''	17°32'18,0''
11.	9 Aug 2018	Čitluk	Međugorje	43°12'31,8''	17°32'18,0''
12.	25 Jul 2019	Grude	Klobuk	43°17'23,7''	17°26'16,2''
13.	18 Aug 2020	Grude	Klobuk	43°17'23,7''	17°26'16,2''
14.	8 Aug 2018	Grude	Klobuk	43°17'23,7''	17°26'16,2''
15.	9 Aug 2018	Grude	Klobuk	43°17'23,7''	17°26'16,2''
16.	25 Jul 2020	Grude	Grude	43°17'23,0''	17°26'16,0''
17.	10 Aug 2020	Grude	Grude	43°17'23,0''	17°26'16,0''
18.	30 May 2019	Široki Brijeg	Trn	43°22'41,0''	17°33'23,2''
19.	10 Aug 2019	Široki Brijeg	Trn	43°22'41,2''	17°33'23,5''
20.	12 Aug 2019	Široki Brijeg	Trn	43°22'41,2''	17°33'23,5''
21.	12 Aug 2018	Čitluk	Međugorje	43°12'31,8''	17°32'18,0''
22.	15 Jul 2019	Stolac	Gorica	43°05'05,7''	17°55'32,1''
23.	29 Sep 2019	Stolac	Gorica	43°05'05,7''	17°55'32,1''
24.	30 Sep 2018	Grude	Klobuk	43°17'23,7''	17°26'16,2''
25.	28 Sep 2018	Grude	Klobuk	43°17'23,7''	17°26'16,2''
26.	15 Oct 2018	Trebinje	Agrokop	42°41'17,4''	18°19'59,9''
27.	11 Aug 2018	Trebinje	Agrokop	42°41'17,4''	18°19'59,9''
28.	10 May 2019	Trebinje	Agrokop	42°41'17,4''	18°19'59,9''
29.	4 Oct 2019	Trebinje	Agrokop	42°41'17,4''	18°19'59,9''
30.	8 Aug 2018	Široki Brijeg	Knešpolje	43°22'41,0''	17°33'23,5''
31.	12 Jul 2018	Široki Brijeg	Knešpolje	43°22'41,0''	17°33'23,5''
32.	15 Jul 2018	Široki Brijeg	Knešpolje	43°22'41,0''	17°33'23,5''
33.	19 Sep 2019	Široki Brijeg	Široki	43°22'41,2''	17°33'23,6''
34.	3 Oct 2018	Široki Brijeg	Široki	43°22'41,0''	17°33'23,5''

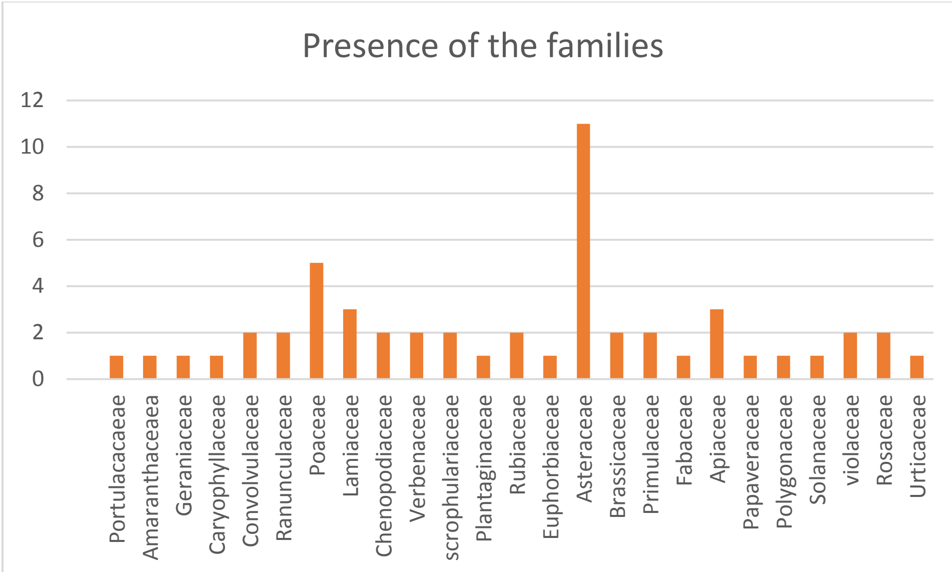

Figure 1. Presence of the families at the researched localities of arable crops

In the spectre of life forms, the life form of terophyte dominates (27 – 53%). After terophytes, the most numerous are the hemicryptophytes (19 species – 37 %) and geophytes (5 species – 10%).

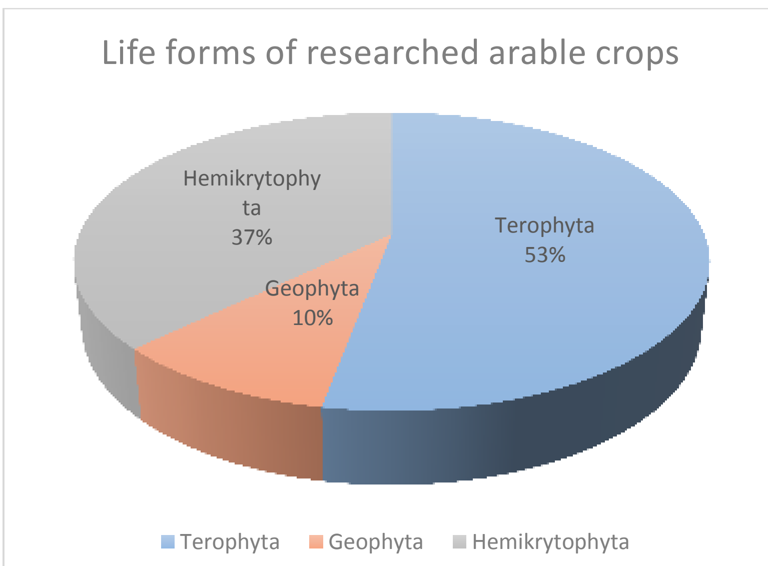

Figure 2. Overview of life forms of researched arable crops

In the areal spectre of the association *Panico-Portulacetum oleraceae* Lozanovski 1962 there are 7 groups of flora elements defined. A cosmopolitan group of flora elements dominates and 33 plant species (62%) belong to this group.

Forest stands of the association *Panico-Portulacetum oleraceae* Lozanovski 1962 in the arable crops of Herzegovina make 51 weed species, which represents a certain floristic richness in comparison to the association of the same name, described at other localities. Joint species at all localities are to be particularly pointed out which belong to the characteristic species of syntaxonomic units (association, alliance, order and class): *Portulaca oleracea* L., *Echinochloa crus-galli* (L.) Beauv., *Setaria glauca* (L.) Beauv., *Amaranthus retroflexus* L., *Chenopodium album* L. *and Solanum nigrum* L. Based on the collected weed flora, the material has been analysed so the life strategies have been defined on the basis of 7 life characteristics (height, weight of dry leaf, share of the leaf substance, starting point and period of flowering, side spreading, specific leaf surface). The most frequent type of the strategy among the weed arable crops is the R strategy with 15 species and CR strategy with 8 species.

Table-sheet 2. Allocation of CSR strategies

Strategy	Arable crop
R	15
CSR	3
CR	8
C/CSR	3
CS	2
SR/CSR	1
R/SR	3
C/CR	2
R/CSR	4
C	3
R/CR	6
S/CSR	1
TOTAL	51

Šinžar and Živanović (1993) studied the weed community of the vineyards in Banat (Vojvodina). Based on the results, the weed communities from the associations Panico – Portulacetum oleraceae in Banat are found to be danker habitat of the northern slopes of the peak mountains and they concord with 18 species in Bosnia and Herzegovina. Arable Crops of Strumica Valley (Lozanovski and Piperkovska, 1992) have shown significant differences in floristic composition and they are the result of more intensive anthropogenic impact, small floristic richness and sub-Mediterranean character of this area.

Conclusion

For the presence and extent of weed plants and constitution of weed communities, in addition to the abiotic factors, a significant share belongs also to the orographic factors. The majority of them acts indirectly by changing the others, in the first place the climate and edaphic factors. Out of the edaphic factors, for the cultivated plants and weeds as well as their followers, the biggest importance belongs to the height above sea level and exposition. If the agro-technical measures are more intensive, the weed community is kept more on the level of weed communities of wide-row plough-field arable crops with the dominating terophytes and weaker presence of geophytes and hemicryptophytes. Weed suppression with agro-technical measures substantially raises the price of production and it has a relatively short effect so therefore the use of herbicides has enabled that the weeds could be efficiently supressed for a long period of time with the increase of cost-effectiveness of the production.

References

Barić, K., Ostojić, Z., Šoštarčić, V. Šćepanović, M. (2021). Weed flora of vineyards. Glasilo biljne zaštite, 408-410

Beck, G. (1891). Flora of South Bosnia and the adjacent Herzegovina, Natural-history museum Vienna, . 6,3:332 (90), Vienna.

Beck, G. Maly, K. Bjelčić, Ž. (1967). Flora of Bosnia and Herzegovina 4, Symposium 2, 1–110, Sarajevo.

Beck, G. Maly, K. Bjelčić, Ž. (1983). Flora of Bosnia and Herzegovina 4, Symposium 4, 1- 188, Sarajevo.

Council of Europe: The Bern Convention (Convention on the conservation of European wildlife and natural habitats, Bern, 1979), Question and Answers, No. 9, November 2007, Croatian encyclopaedia, web publication, Weeds, Leksikografski zavod Miroslav Krleža. Accessed on 1 July 2021 http://www.enciklopedija.hr/Natuknica.aspx?ID=33253

Kovačević, Z., Mitrić, S., Klečević, B. (2014). Representation of grasses (Poaceae) in the communities of perennial crops of Bosnia and Herzegovina. Acta herbologica,

Kovačević, Z., Kojić, M., Šumatić, N., Janjić, V., Mitrić, S., Đikić, M., Petrović, D., Kelečević, B., Čekić, S. (2016). Diversity of weed flora and vegetation in agro-phytocenosis of Bosnia and Herzegovina, Vrdnik, X Congress on Weeds, Herbology Association of Serbia, Collection of abstracts, p.24-25

Ministry of Foreign Trade and Economic Relations (n.d.), Agriculture, nourishment forestry and rural development in Bosnia and Herzegovina, Ministry of Foreign Trade and Economic Relations of Bosnia and Herzegovina. Accessed on 1 July 2021 http://www.mvteo.gov.ba/Content/Read/poljoprivreda-prehrana-sumarstvo-ruralni-razvoj?lang=hr

Nikolić, T. (2015). Flora Croatica database, Faculty of Science, University of Zagreb, https://hirc.botanic.hr/fcd/

Pierce, S. *et al.* (2013). Allocating CSR plant functional types: the use of leaf economics and size traits to classify woody and herbaceous vascular plants, Functional Ecology 27, p.1002-1010

Pierce, S. *et al.* (2017). A global method for calculating plant CSR ecological strategies applied across biomes world-wide, Functional Ecology 31, p.444-457

ASSESMENT OF THE DIVERSITY AND DISTRIBUTION OF HONEY PLANTS BY HABITATS IN THE CENTRAL PARTS OF KRIVODOL MUNICIPLAITY (NORHERN BULGARIA)

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Abstract

Melliferous plants are key to the ecosystem pollination service. The melliferous plants are under strong anthropogenic pressure in the conditions of intensive agriculture and modern climate changes in southern Europe. The study is aimed at identifying and spatial distribution of honey plants species in traditional agricultural areas of northern Bulgaria: following the example of Vratsa district, Krivodol municipality. The study covers the period 2019-2021. Special attention is paid to the semi-natural and natural fragments in the landscape - headlands, hay meadows and pastures. The study presents the results of field observations conducted by the Braun-Blanquet method in 120 phytocenological descriptions of representative areas in the plain-hilly terrain. 14 habitat types according to the EUNIS classification have been identified and the projective cover and the number of honey-bearing species in habitats have been assessed, and the species with over 10% projective cover have been extracted and described. In the course of the research, landfills with invasive melliferous plants were visited and described. There is destruction of hay meadows and pastures and their conversion into arable land, which leads to loss of melliferous plants and habitats.

Key words: *melliferous plants, habitats, Northern Bulgaria, agricultural landscapes.*

Introduction

Melliferous vegetation (pollen- and nectar-rich plants) is an important component of modern biodiversity (IPBES, 2016). It contributes significantly to maintaining pollinator activity and limiting the degradation of natural and semi-natural habitats subjected to the combined impacts of urbanization, intensive agriculture and climate change (Perennes, 2021). Melliferous vegetation is an essential prerequisite for the spatial distribution and abundance of pollinating insects and plays a determining role in the spatial organisation of agriculture, productivity and sustainability in cultivated plants and the effectiveness of good agricultural practices (Regulation (EU) 2017/2393). Understanding the role of Melliferous vegetation in the structure of natural and cultivated habitats is essential for developing spatial patterns of pollination of cultivated and wild plants, clarifying the vulnerability of native pollinators (primarily bees) and developing adaptive land management practices.
The ecosystem service of pollination provided by honey bees is crucial for the normal functioning of different ecosystems: natural, semi-natural and agrarian. After the key ecological prerequisites for pollination processes to take place is the presence of honey bee vegetation in the

structure of landscapes. Contemporary challenges in land management pose as acute the issue of maintaining beekeeping traditions in areas dominated by agricultural activity as a guarantee of their sustainability.

The EU Pollinators Initiative (2018) identifies land-use change, intensive agriculture and pesticide use, pollution, invasive alien species, disease and climate change as major threats to the distribution of melliferous plants and associated pollinators.

The balance of honey bee vegetation and pollinators is perennially in the focus of the scientific community, managers and decision makers (IPBES, 2016). It is widely acknowledged that declines in pollinators and pollination lead to losses in biodiversity (over 85% of wild flowering plants depend on pollination) and serious problems in crop yields - annual food production from agriculture worth nearly $577 billion is formed with the direct involvement of pollinators (IPBES, 2016). Melliferous vegetation, as a major input for pollinator dispersal, is a key factor for ecosystems and humanity in terms of biodiversity, agriculture, climate change adaptation, and all other pollination-related ecosystem services. Pollinator conservation must be included in ecosystem and habitat restoration processes and to promote their long-term sustainability (Christmann, S., 2019).

The aim of the present study is to determine the distribution and diversity of melliferous plants in different habitat types in part of Northern Bulgaria. The region is known for its traditional agricultural landscapes for the Bulgarian geographical conditions – wide area arable lands, hay meadows and pastures, fragmented forest vegetation. An important sub-task of the study is the identification of current anthropogenic pressures on the territory that negatively affect the distribution of melliferous vegetation - structural changes in landscapes, land use change, invasive species, etc., and hinder the provision of pollination services.

Material and methods

As a result of a preliminary geographical analysis, the municipality of Krivodol, Vratsa region was selected as the pilot area (Fig. 1). The total area of the territory included in the study amounts to 25 km^2. The spatial extent and shape of the territories selected for field analyses was tailored to the biological and physiological capacities of the honey bee for activity relative to the location of the hive (Tsankov, 2015). Field surveys were conducted between 2019 and 2021, and phytocenological descriptions were collected in different ecosystem types. The descriptions were made in July, when the maximum in vegetation development occurs, following the methodological approach of Brown-Blanke (Westhoff & van der Maarel, 1973). 120 phytocenological descriptions were carried out.

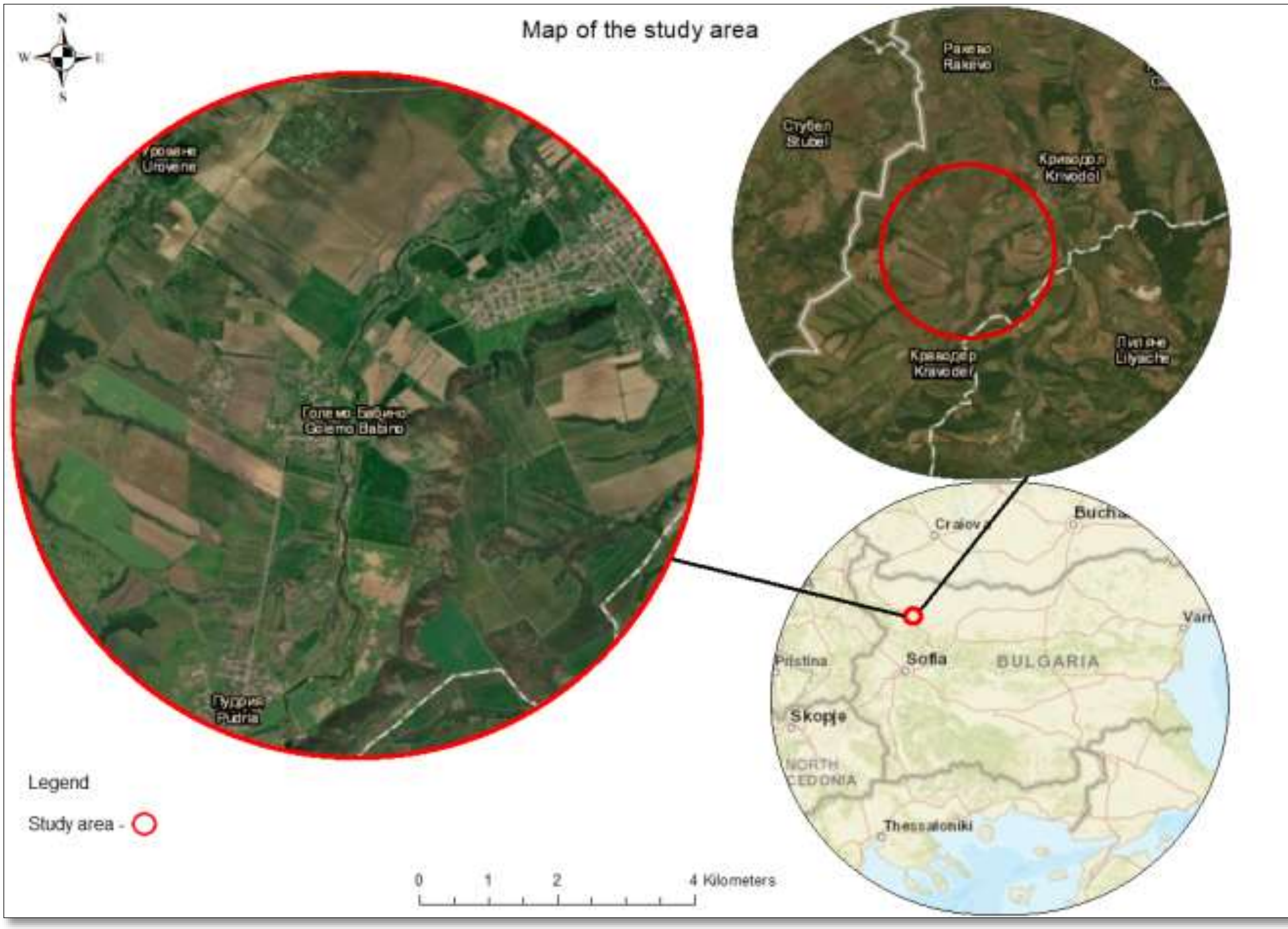

Fig. №1 Study area

Field studies include the abundance and diversity of melliferous plants species in natural habitats, collecting samples and specimens. Sample plots were laid out in homogeneous and representative plots of the plant communities, following the methodology of Chytry & Otypkova (2003), namely for herbaceous vegetation - $16m^2$; for shrub vegetation - $64m^2$, for forest vegetation - $225m^2$. All vascular plant species were noted within the boundary of each sample plot. Using a previously prepared honey plant list from which were extracted all honey plant species an their abundance and cover within the phytocenological descriptions boundaries were estimated using the Braun-Blanquet scale (Braun-Blanquet, 1965). For each phytocenological description, the following types of data on environmental conditions were collected: elevation, exposure, soil power, and grazing intensity.

Results and discussion

As a result of the surveys conducted and the subsequent analysis of the honey plant richness, 14 natural habitat types have been identified, corresponding to the EUNIS classification. The assessment of their composition, structure and ecological characteristics is based on 120 phytocenological descriptions.

Ruderal and herbaceous phytocenoses are the most widespread, followed by forest and shrub phytocenoses. In the last 15-20 years, as a result of the reduction in grassland use, the cover of shrub vegetation is also increasing. The remaining vegetation types are of limited distribution - hygro- and hydrophilous phytocenoses.

Forest vegetation

Euro-Siberian steppe *Quercus* woods (G1.7A1): Abiotic characteristic and distribution: this vegetation type is characterized by remnant forests that have been highly modified due to prolonged anthropogenic impact. They occupy limited areas in the land of the villages of Golemo Babino, Pudria and Urovone, with altitudes ranging from 200 to 220 m above the sea level. The soils are medium to relatively deep, and shallow in places where there is outcrop of bedrock. Composition and structure: Within this vegetation category, there is a distinct tree, shrub, grass and moss floor in the vertical structure. The overall projective vegetation cover is 100%, with tree floor cover ranging from 80-100%. Shrub and herbaceous floor cover are 60-70% and 70-85%, respectively. A total of 30 species of melliferous have been identified in the species composition of the habitat phytocenoses, forming a cover of 90-100%. The species with >10% cover are *Quercus cerris*, *Q. pubescens*, *Q. frainetto*, *Crataegus monogyna*, *Acer campestre*, *A. tataricum*, *Fraxinus ornus*, *Buglossoides purpuruleaceae*, *Ligustrum vulgare*, *Hedera helix* and *Glechoma hederacea*.

Riverine willow woodland (G1.11): Abiotic characteristics and distribution: This habitats has a limited distribution in this area. It forms banded polygons along the river channel. The soils are alluvial, well moistened. Composition and structure: The overall projective vegetation cover is 100%. Coverage of the shrub and herbaceous floors are 30-80% and 80-85%, respectively. In the species composition of the phytocenoses of the habitat, a total of 22 species of melliferous plants have been identified, forming a cover of 95-100%. The species with >10% cover are *Salix fragilis, Acer campestre, Cornus sanguinea, Clematis vitalba, Aegopodium podagraria, Crataegus monogyna, Hedera helix, Prunus avium, Rubus caesius, Ulmus glabra, U. minor*.

Robinia plantations (G1.C3): Abiotic characteristic and distribution: This vegetation has a fragmented distribution in the study area, with strips around arable land and artificial plantations along villages Golemo Babino, Urovene and Krivodol. *Robinia pseudoacacia* has secondary widespread as the species occurs in a variety of ecological conditions, including highly disturbed areas. Composition and structure: In the species composition, 11 species of melliferous plants are found, which form a cover of 95-100%. The species with >10% cover are *Robinia pseudoacacia, Crataegus monogyna, Prunus cerasifera.*

Shrub vegetation

Moesian oriental hornbeam thickets (F3. 2431): Abiotic characteristic and distribution: The vegetation has a fragmented distribution in the study area in the around of the villages of Golemo Babino, Urovene and the town of Krivodol. The soils are shallow to medium in depth. Composition and structure: this vegetation occurs on eroded terrains or on the site of highly degraded forest phytocenoses. After the processes of deforestation and erosion, *Carpinus orientalis* has spread significantly and formed its modern cover. In the species composition, 18 species of melliferous plants are found, which form a cover of about 50%. The species with > 10% cover are *Cornus mas, Fraxinus ornus*.

Medio-European rich-soil thickets (F3.11): Abiotic characteristic and distribution: this vegetation has a fragmented distribution in the study area in the margins of arable land, grassland, etc. The soils are of medium depth. Composition and structure: Coverage of the shrubs are 90-100%. The herbaceous floor is well developed, but in 80% of the cases it is strongly influenced by the density of the shrubs. There are 29 species of melliferous plants in the species composition, forming a cover of about 80-100%. The species with > 10% cover are *Acer campestre, Buglossoides purpuruleacea, Clematis vitalba, Cornus sanguinea, Crataegus monogyna, Prunus spinosa, Rubus caesius.*

Grass vegetation

Moesio-Carpathian steppes (E1.222): Abiotic characteristic and distribution: this vegetation is widespread in the study area, comprising predominantly grassland. Soils are medium to shallow in depth. Composition and structure: It is a herbaceous vegetation with a semi-enclosed horizontal structure (90-100%). Characterised by a rich species composition. The species composition includes 65 species of melliferous plants which form a cover of about 10-100%. The species with > 10% cover are *Agrimonia eupatoria, Dorycnium herbaceum, Galium verum, Medicago falcata, Origanum vulgare, Plantago lanceolate, Potentilla argentea, Xeranthemum cylindraceum, Trifolium repens.*

Permanent mesotrophic pastures and aftermath-grazed meadows (E2.1): Abiotic characteristic and distribution: This vegetation has a local distribution on the leveled areas in the area of the village of Pudria and the town of Krivodol. The soils are powerful. There is a pasture use regime. Composition and structure: These phytocenoses have a closed horizontal structure with a projective cover of 90-100%. The species composition is poor and is composed of species resistant to trampling. In the species composition, 18 species of melliferous plants have been identified, forming a cover of about 30-80%. The species with > 10% cover are *Trifolium repens, Mentha spicata, Xeranthemum cylindraceum.*

Low and medium altitude hay meadows (E2.2): Abiotic characteristic and distribution: This vegetation has a local distribution on the flattened terrain and waterlogged land in the village of Kravoder. The soils are powerful. Composition and structure: These phytocenoses have a closed horizontal structure with a projective cover of 100%. In the species composition 7 species of honey plants are found which form a cover of about 3%. There are no species of these with a cover > 10%.

Continental humid meadows (E3.46): Abiotic characteristic and distribution: This vegetation has a local distribution between the villages of Golemo Babino and Pudria. The soils are powerful, waterlogged periodically during the year. Composition and structure: In the species composition, 9 species of melliferous plants are found, which form a cover of about 65-95%. The species with > 10% cover are *Epilobium hirsutum, Lythrum salicaria*.

Water-fringing reedbeds and tall helophytes other than canes (C3.2): Abiotic characteristic and distribution: This vegetation has a local distribution on floodplains and waterlogged habitats in the area of the villages of Golemo Babino, Pudria, Urovone and the town of Krivodol. Soils are waterlogged and some localities are permanently submerged in water. Composition and structure: This vegetation has a closed horizontal structure with a projective cover of 100%. In the species composition, 11 species of melliferous plants were found to form very little or no cover. The only exception is a description with 100% projective coverage. Only *Galega officinalis* is a species with > 10% cover.

Fox sedge tussocks (D5.219): Abiotic characteristic and distribution: this vegetation has a local distribution on the land of the village of Pudria. The soils are waterlogged. Composition and structure: This vegetation has a closed horizontal structure with a projective cover of 100%. In the species composition 2 species of honey plants are found, which form a cover of about 3,2%. There are no species with > 10% cover.

Iris beds (C3.24B): Abiotic characteristic and distribution. This vegetation is native to the area of Krivodol. It occupies an area of several square meters. The habitat is waterlogged, with a water depth of 20-25 cm. Composition and structure. In the species composition, 1 species of melliferous plant (*Lythrum salicaria*) is identified, which has a cover of about 0.5%. There are no species with > 10% cover.

Anthropogenic herb stands (E5.1): Abiotic characteristic and distribution: This vegetation combines ruderal and weedy vegetation that is widespread in the study area (crops, landfills, roadsides, orchards, hedgerows, etc.). This unit is very difficult as a polygon site because of the small areas it occupies and the fact that some of the phytocenoses develop within the range of other habitats of the EUNIS classification, such as cropland. Composition and structure: This vegetation has a semi-open to closed horizontal structure with a projected cover of 80-100%. Species with greater abundance and cover are highly heterogeneous. In the species composition, 76 species of melliferous plants have been identified, forming a cover of 5-100%. The species with > 10% cover are *Verbascum densiflorum, Trifolium arvense, Stachys annua, Sambucus ebulus, Salvia verticillata, Rumex acetosa, Rubus caesius, Potentilla supina, Portulaca oleracea, Polygonum aviculare, Plantago major, Persicaria lapathifolia, Malva sylvestris, Daucus carota, Cichorium intybus, Chamomilla recutita, Carduus acanthoides.*

Intensively-farmed crops interspersed with strips of natural and/or semi-natural vegetation (X07): Located within the entire study area. They are formed on the sites of natural forest, grass and shrub vegetation that was widespread in the past and destroyed by man. Arable farmland is included in this unit. Communities of mapping unit E5.1 develop adjacent to or at certain times of the year on areas of arable land.

The pooled data show that the abundance and cover of honey plants varies substantially across the identified natural habitats. In terms of the minimum projected cover of honey plants, the data are similar. For mean and maximum values, the variation is significant. The highest values are found at E3.46, G1.11, F3.11, G1.C3 and G1.7A1, which is due to the dominance of species in the phytocenoses that are melliferous plants. On the other hand, the lowest values were at X.07, E.2.2 and D5.219.

In terms of environmental factors, there are disturbances to the natural vegetation in the area that pose a potential risk to beekeeping. Most of the semi-natural forest vegetation has been destroyed as a result of agricultural development over the last century. The few remaining areas of forest vegetation are of very limited extent, mainly on steep valley slopes. Their fragmentation and degradation impair the provision of full ecosystem services. The forest habitats support a rich diversity of honey bee plants, some of which (*Quercus cerris*, *Q. pubescens*, *Q. frainetto, Robinia pseudoacacia*) have over 75% projective cover and provide abundant natural grazing for bees. Much of the moisture-loving woody vegetation (*Salix fragilis, Populus nigra*) along the bed of the Botunia River has been destroyed in recent years and no measures have been taken to restore it. This has led to changes in the river bed and also to several spring floods of the land around the river.

The problem facing beekeeping is the destruction and "degradation" of grasslands and pastures. In the past, these habitats were widespread due to livestock farming by the local population.

They are the habitats of many numbers and types of melliferous plants that are important for bees in periods without major grazing by agricultural crops. Destroying them and converting them to arable land reduces the richness and diversity of food sources for bees. In places, the destruction of these grasslands leads to flooding, landslides, compaction of the topsoil and accelerated soil erosion. The destruction of weed vegetation in the ecotone zones around the fields is often observed when agricultural areas are treated. Another adverse effect on the diversity of melliferous plant species, and especially on bee activity, is the narrowing and destruction of hayfields as a result of ploughing. Hedgerows are the 'corridors' along which honey bees move (nectar corridors).

Conclusion

In the selected pilot area of traditional agricultural landscapes in northern Bulgaria, as a result of the research conducted, 14 habitat types have been identified (EUNIS). This is the first time such a study has been carried out in this territory. The highest concentration of melliferous plants (64-74 species) was found in habitats E1.222 and E5.1. There is a limited presence of honey bees (2-3 species) in the structure of E.2.2. and D5.219. The areal cover of melliferous plants varies widely: differences in minimum projected cover of 61 species range from 0.1-1%. The highest values for the mean projective cover are reported for habitats G1.11 and E3.46 and it is 21.5%, while the lowest values are reported for habitat D5.219 (- 1.07%. There is a strong fragmentation of semi-natural habitats and the destruction of hedgerows (nectar corridors) and ecotone zones near arable areas, which is a major threat to the presence of melliferous vegetation in the structure of landscapes.
The study will be used as a basis for extending the scope of the study to melliferous vegetation and the ecosystem services associated with it.

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References

Braun-Blanquet, J. 1965. Plant Sociology. The Study of Plant Communities. Hafiner Publishing Company. New York and London.

Christmann, S., 2019. Do we realize the full impact of pollinator loss on other ecosystem services and the challenges for any restoration in terrestrial areas? Restoration Ecology. Doi: 10.1111/rec.12950.

Chytry, M., Otypkova Z., 2003. Plot sizes used for phytosociological sampling of European vegetation. Journal of Vegetation Science 14: 563-570.

EU Pollinators Initiative. 2018. SWD(2018) 302 https://ec.europa.eu/environment/nature/conservation/species/pollinators/documents/EU_pollinators_initiative_evidence_base.pdf

IPBES. 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. S. G. Potts, V. L. Imperatriz-Fonseca, and H. T. Ngo, (eds). Bonn, Germany. 552 pages.

Perennes et al.. 2021. A hierarchical framework for mapping pollination ecosystem service potential at the local scale, Ecological Modelling 444.2021, 109484, ISSN 0304-3800, https://doi.org/10.1016/j.ecolmodel.2021.109484.

REGULATION (EU). 2017/2393, OJ L 350/34 OF 29.12.20, https://eur-lex.europa.eu/legal-content/en/txt/pdf/?uri=celex:32017r2393&from=fr

Tsankov, G., 2015. 41 unique honey-bearing plants, ed. Enyovche, S (in Bulgarian).

Westhoff, V., van der Maarel, E., 1973. The Braun-Blanquet approach. In: Whittaker, R.H., Ed., Ordination and Classification of Communities, Dr. W. Junk, Dordrecht, 617-626.

RISK SPATIALIZATION OF AGRICULTURAL PHYTOSANITARY PRACTICES: CASE STUDY IN SOUTH-WEST FRANCE

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Abstract

Despite the political efforts, France is among the main consuming countries of pesticides in the world and the first at European level with a consumption of more than 85,900 tons/year (BNV-D[1], 2019). The preservation of natural resources through the management of diffuse pollution related to pesticide use is considered as a major challenge in France and based on the identification of the highest risk areas. Our work aims to map the phytosanitary practices impact on human health and on natural resources, based on indicators of phytosanitary pressure (TFI: Treatment Frequency Index) and risk (IRSA: Indicator of Risk on the Applicator's Health, and IRTE: Indicator of Toxicity Risk on the Environment). These indicators are calculated with the EToPhy[2] software. This approach allows to present the spatial distribution of risk and phytosanitary pressure in the south-west of France (Gimone watershed). This mapping process is based on the scores of phytosanitary pressure and risk assigned to each plot, calculated from the applied dose of pesticides and their toxicity degree.

The spatialization of the health and environmental impact of farmers' phytosanitary practices enable us to identify the plots that represent the highest risk and their location to natural resources such as streams. The result could be used to improve the management of agricultural phytosanitary practices, taking into account the proximity of treated plots to different natural resources.

Keywords: *Risk, phytosanitary practices, spatialization, indicators, natural resources.*

Introduction

In the early 1960s, a period known as the "Green Revolution", agricultural production methods shifted towards the intensification of cropping systems with the aim of ensuring food security. Following this agricultural revolution, the use of pesticides was increased in France and all over the world (Guichard *et al.*, 2017; Sharma *et al.*, 2019). Thus, the types of plant protection products and the methods of application have diversified.

Indeed, the excessive use of phytosanitary products in agriculture has generated the contamination of surface and groundwater which constitutes the resources intended for human consumption. This pollution leads several problems of public health and on the environment such

[1] **BNVD:** National Bank of Plant Protection Sales by approved distributors, https://bnvd.ineris.fr/

[2] **EToPhy software (2020)**, APP deposit n°: IDDN.FR.001.090003.000. S.P.2020.000.31500. developed by CIHEAM-IAMM https://etophy.fr/

as the quality of water resources and agricultural products (Aouadi *et al.,* 2018). Monitoring and reducing the diffuse phytosanitary pollution have become major issues for water resources, human health and the balance of natural ecosystems.
Several research studies have been carried out to analyze the health and environmental impact of pesticide use and the contribution of agricultural practices to the diffuse pollution (Mghirbi *et al.*, 2015; Mghirbi *et al.*, 2018; Kanj 2018; Grimene *et al.*, 2021).
Our research is oriented towards the same perspective of risk assessment related to agricultural phytosanitary practices based on indicators of phytosanitary pressure (TFI: Treatment Frequency Index) and risk (IRSA: Indicator of Risk on the Applicator's Health and IRTE: Indicator of toxicity Risk on the Environment). These indicators make it possible to illustrate the spatial distribution of the phytosanitary pressure and the toxicity risk related to pesticide use at plots level on Gimone watershed in the south-west France.

Materials and methods

The adopted approach in this work consists of a spatial risk assessment of agricultural phytosanitary practices through the Indicator of Risk on the Applicator's Health (IRSA) and the Environment (IRTE), calculated by EToPhy. The EToPhy tool makes it possible to refine the analysis of the health and environmental impact of pesticide use through the disaggregation of the IRSA and IRTE into 2 sub-indicators of risk to human health (IRSA acute, IRSA chronic) and 3 environmental sub-indicators relating to the three environmental compartments: water, soil and air (IRTE aquatic, IRTE terrestrial invertebrate, IRTE bird) (Mghirbi *et al.,* 2015; Mghirbi, 2016). These indicators are then integrated into a GIS in order to design spatial distribution maps of the health and environmental impact of agricultural phytosanitary practices. These maps will allow to identify the plots with high risk to human health and to the environment in the Gimone watershed, which extended over the two departments of Gers and Tarn-et-Garonne, located in the south-west of France (Figure 1).

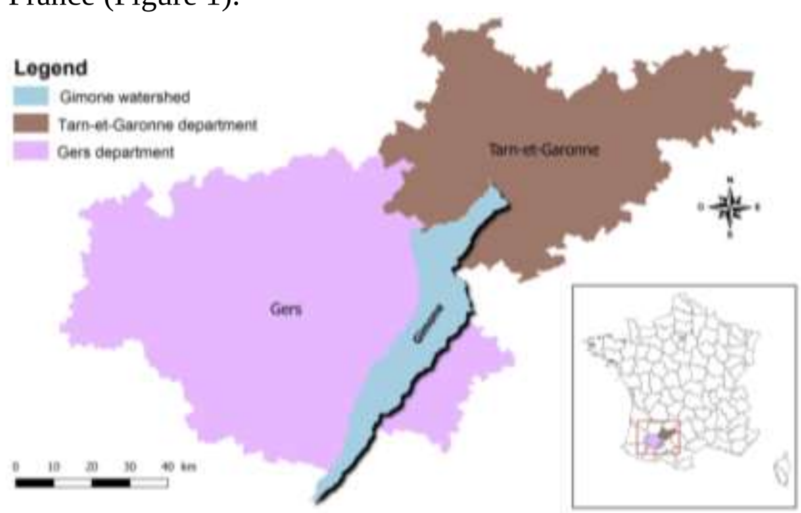

Figure 1. Study area location: the Gimone watershed in south-west France (sources: IGN GEOFLA 2017, Carthage Database 2013).

The territory of the Gimone watershed is largely occupied by agriculture, about 57,400 ha of the UAA[3] (i.e. around 70% of its surface area). The land use is almost homogeneous with a predominance of agricultural areas dedicated to annual crops (wheat, sunflowers, soybeans, corn). Cereal crops dominated by soft wheat and durum wheat represent the main crops, followed by sunflower and soybeans.

Given the dominance of agriculture at the watershed level, it turns out that agricultural practices, including the phytosanitary products applied by farmers have an impact on the water quality. The Gimone watershed is considered to be a territory with high agricultural pressure and low potential for resilience to diffuse pollution given its physical characteristics which play a negative role in the pollutant transfer.

Our study is based on two types of data:

- A geographic database: represents the parcels data from the study area and which contains all the geo-referenced parcels from the geographic data sources (Graphical Parcels Register RPG).
- A phytosanitary practices database: represents all phytosanitary treatments (pesticide applications) on the surveyed plots and all parameters related to the application of the plant protection products (farm identifier, plot identifier, plot area, treated area, TFI, approved dose, applied dose, IRSA and its sub-indicators, IRTE and its sub-indicators).

The phytosanitary practices database will used to calculate risk indicators for the different surveyed plots. The IRSA is a scoring indicator which assesses the acute and chronic toxicity of plant protection products taking into account the physico-chemical and toxicological properties of the active ingredients. The IRTE assesses the toxicity risk of phytosanitary products on non-target living organisms in each environmental compartments (water, air, soil) based on the eco-toxicological and physico-chemical characteristics of the active ingredients. In order to carry out this analysis of agricultural phytosanitary practices, a sample of 161 farms (3,340 plots) was selected on the Gimone watershed, illustrated in the following table.

Table 1. Distribution of crops according to the plots surveyed in the Gers and Tarn-et-Garonne departments

Crop categorie	Crop	Plot number	Area (ha)
	Soft wheat	1,113	5,699
	Sunflower	685	3,049
	Grain corn	209	1,093
	Rape	200	1,113
	Durum wheat	182	882
	Barley	164	577
	Soya	103	517
Field crops	Sorghum	38	134
	Triticale	35	97
	Oilseed flax	32	167
	Chick pea	29	148
	Grassland	11	30
	Lentils	9	32
	Protein pea	9	33
	Horse bean	7	48

[3] **UAA :** Utilised Agricultural Land

	Forage corn	7	34
	Coriander	3	7
	Rye-grass	3	12
	Oat	1	4
	Subtotal	**2,840**	**13,673**
Arboriculture	Apple	308	216
	Organic apple	12	6
	Cherry	50	8
	Plum	32	22
	Organic plum	6	2
	Peach	8	1
	Organic pear	8	2
	Kiwi	3	1
	Organic kiwi	3	1
	Apricot tree	3	0.1
	Subtotal	**433**	**259**
Market gardening	Garlic	53	109
	Onions	2	1
	Carrot	1	4
	Subtotal	**56**	**114**
Viticulture	Vine	11	7
Total	**30 crops**	**3,340**	**14,053**

Results and discussion

Analysis of the agricultural phytosanitary practices

The analysis of agricultural phytosanitary practices enabled us to draw up graphs which illustrate the risk variability between the different crops (Figure 2) and also to compare the risk between the different production methods, conventional/integrated and organic (Figure 3).
The graph below shows an overall analysis of the risk and pressure indicators between some crops from four crop categories (field crops, arboriculture, market gardening and viticulture) in order to compare the health and environmental impact of phytosanitary practices according to the products applied by farmers. The results of the analysis show a remarkable difference between the risks related to different crop categories. The tree crops (arboriculture) presented the highest treatment frequency and risk, especially on human health. The risk is higher in the plots of apple trees with a treatment frequency more than 26.

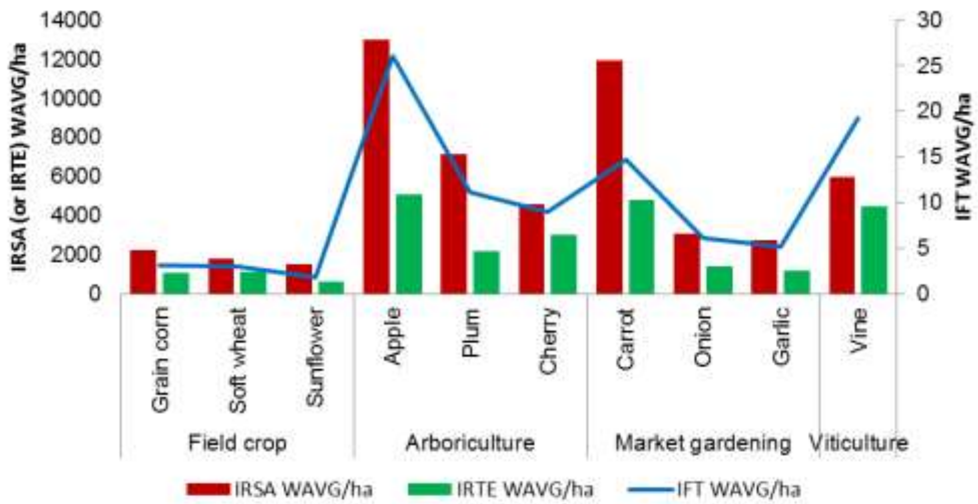

Figure 2. Variability of indicators according to crop categories from conventional/integrated production (values expressed as weighted average per hectare)

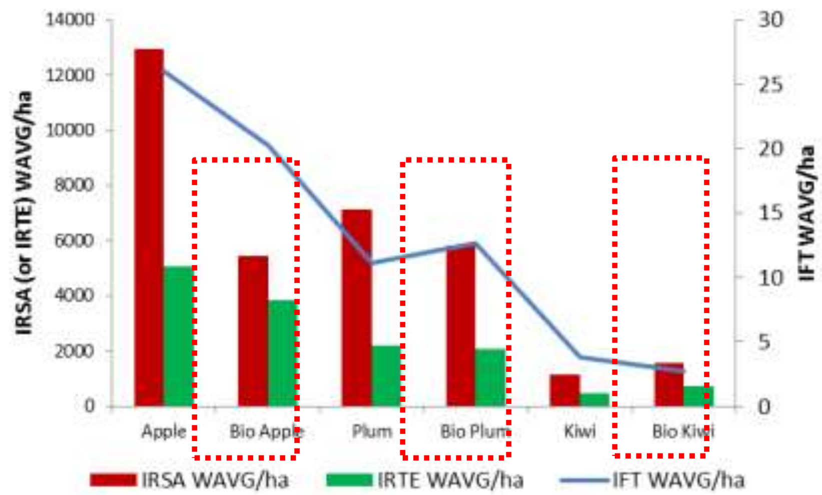

Figure 3. Variability of indicators according to the production mode (conventional/integrated and organic) between tree crops (values expressed as weighted average per hectare)

According to the production mode, the average TFI/ha in organic farming is lower than in conventional/integrated. This indicate that the treatment frequency of organic crops remain always lower than that of conventional production. The risk is lower in organic plots, but it is not negligible either.

The use of risk sub-indicators to human health and the environment makes it possible to refine the analysis of the toxicity degree of plant protection products in order to improve the management of plant protection practices and the pesticide choice. The graphs 4 and 5 show a comparison of the toxicity part between different crop categories.

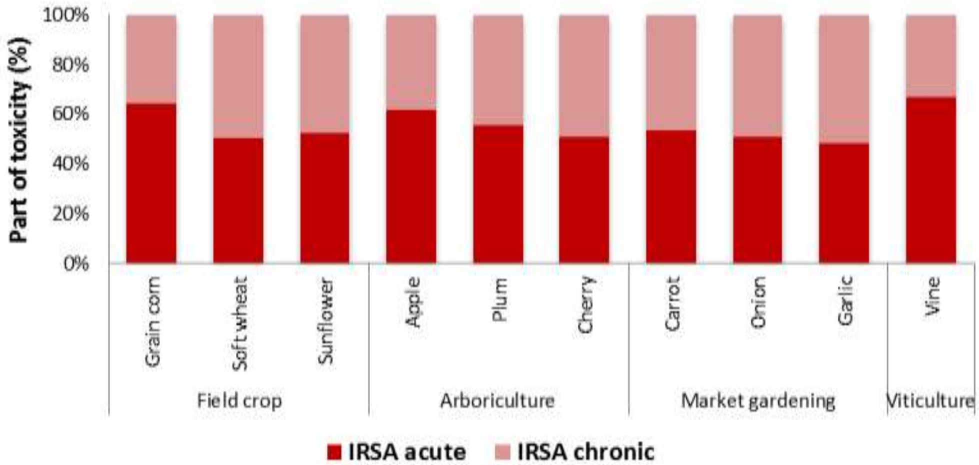

Figure 4. Comparison of acute and chronic toxicity part of plant protection practices between crops

Regardless of the crop, the acute toxicity risk of phytosanitary practices is greater than 50%. The majority of the products used on the surveyed plots have more acute health risk (irritation risk and risk via inhalation, dermal or oral route) than chronic (RMC risk, neurotoxicity and endocrine-disrupting effect).

The environmental risk sub-indicators presented in figure 5 show the toxicity part of phytosanitary practices for each environmental compartment: soil, air and water (IRTE terrestrial invertebrate, IRTE bird and IRTE aquatic) according to crops.

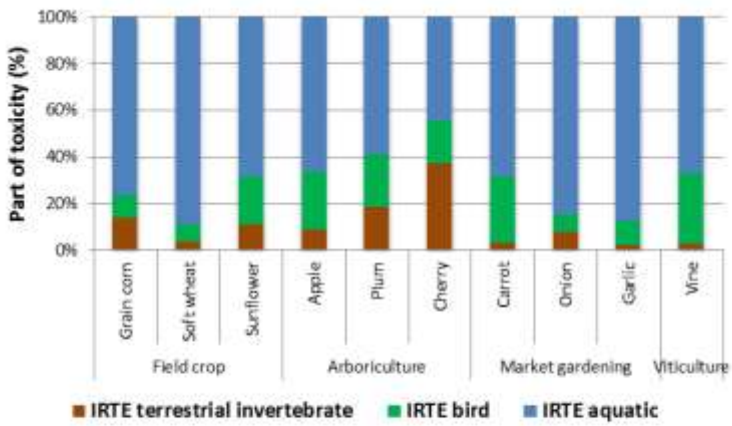

Figure 5. Comparison of the toxicity part of plant protection practices for each environmental compartment between crops

The proportion of the toxicity risk to the aquatic environment is over 70% for field crops, market gardening and in viticulture, which leads us to study in more detail the risk of phytosanitary diffuse pollution on the aquatic environment.

Risk mapping of agricultural phytosanitary practices

Risk mapping is carried out at plot scale which associated to a crop. Each crop has an average toxicity risk value/ha. The risk indicators are represented according to 3 classes from low to high, from green to red, respectively (Figure 6). The spatial analyse of the toxicity risk of phytosanitary practices on human health reveals that the medium to high health risk patches cover 40% of the total UAA of the catchment area (Figure 6A). This analysis clearly shows us the issue of health security for farmers and their neighbourhood through the identification of areas with high risk on human health. The risk of aquatic toxicity varies between medium and high scores and represents around 30% of the total UAA of the Gimone area. The issue associated with this medium-high toxicity risk is the proximity of the treated plots to the streams or rivers, which makes it possible a direct transfer of pollutants towards the aquatic environment (Figure 6B). This spatial analyse leads us to act on phytosanitary practices through the improvement of the active ingredients/products choice by farmers depending on the proximity of plots to the natural environment, in order to replace the phytosanitary products with a high toxicity risk to the aquatic environment with less harmful one.

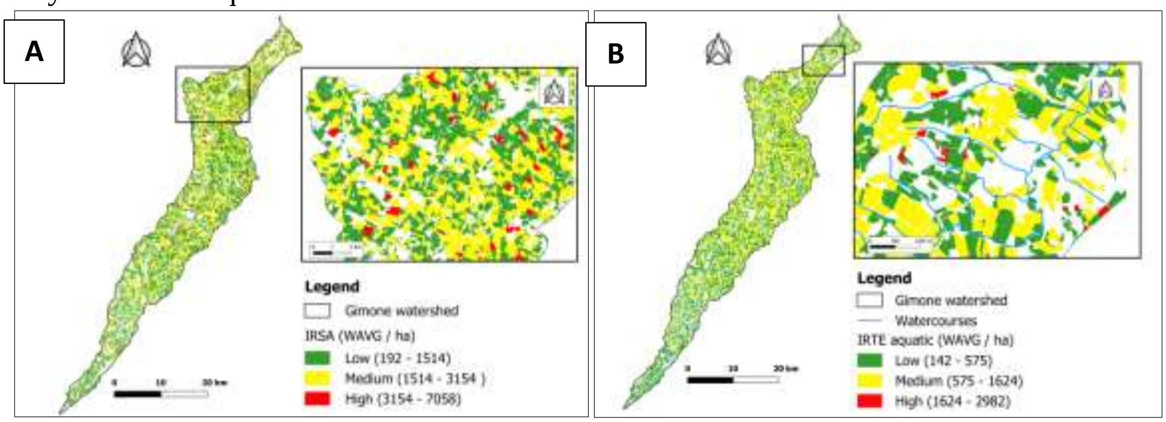

Figure 6. Map of human health risk related to agricultural phytosanitary practices on the plots of the Gimone watershed (A); Map of aquatic toxicity risk related to agricultural phytosanitary practices on the plots of the Gimone watershed (B) (Sources: Carthage DB, RPG 2017)

Conclusion

The evaluation of phytosanitary practices is based on the complementarity between TFI, IRSA, IRTE and the risk sub-indicators used to determine the toxicity degree of plant protection products on human health and on the 3 environmental compartments: soil, air and water.

An improved management of phytosanitary practices can be implemented through the right choice of products depending on the proximity of plots to natural environment and using decision support tools to analyse the health and environmental impact of pesticide use.

An increasing orientation of several famers towards the organic production mode cannot ensure a reduction in the toxicity risk associated to phytosanitary practices because the organic farming is not safe to human health and to the environment.Spatial analysis of the health and environmental impact of agricultural phytosanitary practices using GIS is considered as a decision support tool to improve the management of the pesticide use at plots and farms level. Furthermore, this spatial analysis provides to the natural resources managers a reflection support tool to improve the management of the diffuse phytosanitary pollution through the identification of areas with high risk on non-target living organisms in the different environmental compartments (water, air and soil). These tools make it possible to set up agri-environmental measures and action plans at different scales from plot to the watershed. These measures aim to reduce the toxicity risk of agricultural phytosanitary practices on human health and on the environment.

Acknowledgment

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References

Aouadi, N., Macary, F., Gaillard, J., Devier, M. H., & Budzinski, H. (2018). Évaluation des risques de contamination des eaux de surface par les produits phytosanitaires: application à un bassin versant viticole (projet PhytoCOTE) (*Risk assessment of surface water contamination by phytosanitary products: application to a wine-growing watershed*).

Grimene, C., Mghirbi, O., Louvet, S., Bord, J. P., & Le Grusse, P. (2021). Spatial characterization of surface water vulnerability to diffuse pollution related to pesticide contamination: case of the Gimone watershed in France. Environmental Science and Pollution Research, pages (1-23).

Guichard L., Dedieu F., Jeuffroy M.-H., Meynard J.-M., Reau R., Savini I. (2017). Le plan Ecophyto de réduction d'usage des pesticides en France: décryptage d'un échec et raisons d'espérer (*The Ecophyto plan to reduce the use of pesticides in France: deciphering a failure and reasons for hope*). Cahiers Agricultures 26(1):14002.

Kanj F. (2018). Outils et méthodes pour une politique territoriale de gestion raisonnée des pratiques agricoles: cas d'application dans la région de la Béqaa au Liban (*Tools and methods for a territorial policy of reasoned management of agricultural practices: application case in the Béqaa region in Lebanon*). PhD dissertation, Université Paul-Valéry Montpellier 3. https://tel.archives-ouvertes.fr/tel-01983155

Mghirbi O. (2016). Résilience des exploitations agricoles face au changement des pratiques phytosanitaires: Conception d'outils de gestion des risques liés aux pesticides – Cas du bassin versant de l'étang de l'Or en France (*Resilience of farms to changes in phytosanitary practices: Design of pesticide risk management tools - Case of the Etang de l'Or watershed in France*). PhD dissertation, Université Paul Valéry Montpellier 3.

Mghirbi O., Bord J.-P., Le Grusse P., Mandart E., Fabre J. (2018). Mapping for the management of diffuse pollution risks related to agricultural plant protection practices: case of the Etang de l'Or catchment area in France. Environmental Science and Pollution Research, vol(25), pages (14117–14137).

Mghirbi O., Ellefi K., Le Grusse P., Mandart E., Fabre J., Ayadi H., Bord J.P. (2015). Assessing plant protection practices using pressure indicator and toxicity risk indicators: analysis of the relationship between these indicators for improved risk management, application in viticulture. Environmental Science and Pollution Research, Vol. (22), pages (8058-8074).

Sharma A., Kumar V., Shahzad B., Tanveer M., Sidhu G. P. S., Handa N., ... & Thukral A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. SN Applied Sciences, 1(11), pages (1-16).

ETOPHY: WEB PLATFORM FOR MANAGING THE IMPACTS OF PLANT PROTECTION PRODUCTS USED IN AGRICULTURE

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Abstract

EToPhy is a web application/platform which aims to assess and control the impacts related to the phytosanitary products used in agriculture on the environment and on human health. The EToPhy web platform is presented as a dashboard to determine the phytosanitary footprint of agricultural phytosanitary practices based on indicators of phytosanitary pressure (TFI: Treatment Frequency Index), and of risk (IRSA: Indicator of Risk on the Applicator's Health, IRTE: Indicator of Toxicity Risk on the Environment). These risk indicators break down into acute and chronic IRSA and IRTE sub-indicators for terrestrial invertebrates, birds, and aquatic organisms. This tool provides two web applications to improve the management of pesticide use related risk to the applicator's health and to the environment for professionals involved in the management of plant protection products (PPPs):
- EToPhy *Simulateur* aims to assess the toxicity degree of PPPs and to find alternatives that are less harmful according to the crop and pest to be treated.
- EToPhy *Analyses* makes it possible to analyze the health and environmental impact of farmer phytosanitary practices at different scales (product, field, crop and farm).
The phytosanitary pressure and risk indicators have enabled us to analyze the impact of plant protection practices and to compare the cropping systems of conventional, integrated and organic agriculture.

Keywords: *EToPhy web platform, human health, environment, indicators, plant protection products.*

Introduction

The use of pesticides in agriculture has been steadily increasing in recent years in France and around the world, despite efforts by political and public decision-makers to implement sustainable strategies and measures for reducing the use of plant protection products (Auberto *et al.*, 2005; Guichard *et al.*, 2017; Sharma *et al.*, 2019). Several studies have shown the health and environmental impact of plant protection practices and the effect of frequent exposure to pesticides on public health and the environment (Alavanja, 2009; Hallenbeck & Cunningham-Burns, 2012; Baldi *et al.*, 2013; Mahmood *et al.*, 2016). As a result, the management of diffuse

pollution related to the use of plant protection products has become a concern for the different stakeholders involved in pesticide management (policy makers, land and natural resource managers, agricultural technicians/advisors and farmers).
These different stakeholders have expressed their need for decision support tools to improve the management of risks due to plant protection products used in agriculture to human health and the environment. Several tools have been developed based on technological and/or technical solutions in order to control the risks of phytosanitary diffuse pollution at different spatial scales, from the plot to the watershed or agricultural region (Bockstaller & Girardin, 2003; Bockstaller *et al.*, 2008; Houdart *et al.*, 2009; Ossard *et al.*, 2009; Ayadi *et al.*, 2014; Mghirbi *et al.*, 2015, 2017, 2018; Lammoglia *et al.*, 2017; Kanj, 2018; Juan *et al.*, 2018). The development of the EToPhy[4] tool by the CIHEAM-IAMM research team, as part of the TRam research project (Ayadi, 2013, Le Grusse *et al.*, 2014, Mghirbi, 2016), adds to the list of research work carried out to meet the expectations of the different stakeholders by improving the management of phytosanitary practices and reducing their sanitary and environmental impact.
EToPhy was initially developed as desktop software for use in teaching and research. This format was not operational enough for professionals involved in pesticide management, hence the idea of developing a simple and practical web version as a collaboration between the CIHEAM-IAMM and the R&D company Ecoclimasol within the GesPPEIR collaborative research project.

Materials and methods

Design of the EToPhy web platform

A first step towards the identification of potential user needs was carried out through repeated workshops with technicians from agricultural cooperatives, and farmers from the south-west of France, as part of the GesPPEIR project. The objective of this participatory approach was to build a web platform according to the needs of professionals involved in the management of phytosanitary practices. This platform consists in evaluating and controlling the risks linked to the use of pesticides to human health and the environment at the level of plots and farms.
Indeed, the EToPhy web platform is a dashboard for determining the phytosanitary footprint of pesticides and agricultural phytosanitary practices based on pressure indicators such as the TFI (Treatment Frequency Index, an official French indicator used within the Ecophyto plan which characterises the intensity of pesticide use) and risk indicators such as the IRSA (*Indicateur de Risque sur la Santé de l'Applicateur*: Indicator of Risk on the Applicator's Health) and the IRTE (*Indicateur de Risque de Toxicité sur l'Environnement*: Indicator of Toxicity Risk on the Environment). Moreover, the EToPhy tool makes it possible to refine the analysis of the health and environmental impact of pesticides through the disaggregation of the IRSA and IRTE into 2 sub-indicators of risk to human health (IRSA acute, IRSA chronic) and 3 environmental sub-indicators relating to the three environmental compartments: water, soil and air (IRTE aquatic, IRTE terrestrial invertebrate, IRTE bird) (Mghirbi *et al.*, 2015; Mghirbi, 2016).
The IRSA and IRTE indicators were mainly developed based on the work of researchers from Quebec (Samuel *et al.*, 2012) and Norway (Spikkerud, 2000; Spikkerud *et al.*, 2004), as well as the work of European focus groups (Boesten *et al.*, 1997; Linder *et al.*, 2001; Klein *et al.*, 2003). These risk indicators differ from those used in Quebec in that they are adapted to European

[4] **EToPhy software (2020)**, APP deposit n°: IDDN.FR.001.090003.000. S.P.2020.000.31500.

standards, and take into account European approvals and regulations (Balderacchi & Trevisan, 2010).
IRSA is a generic scoring indicator that can be modified according to the application context. It assesses the acute and chronic toxicity of plant protection products by considering the physicochemical and toxicological properties of the active ingredients. It also expresses the potential risk associated with the use of the product by taking into account the exposure related to the type of formulation (Samuel *et al.*, 2012; Ayadi, 2013; Mghirbi, 2016).
IRTE is a scoring indicator, determined by the sum of six variables that evaluate eco-toxicological impacts on non-target living organisms (terrestrial invertebrates: earthworms and honeybees; herbivorous birds: Virginia quail, and granivorous birds: mallard; aquatic organisms: fish, daphnia, algae and aquatic plants) and physico-chemical behaviours in the receiving environment (mobility, persistence in soil and bioaccumulation). It assigns a weight to these variables (a score from 0 to 8 representing the level of toxicity), and then integrates them into the calculation, based on a toxicity/exposure ratio (Samuel *et al.*, 2012; Ayadi, 2013; Mghirbi *et al.*, 2015; Mghirbi, 2016).
These scoring indicators (IRSA and IRTE) are generic and can be modulated according to phytosanitary practices (commercial preparation, physicochemical and eco-toxicological characteristics), spatial scale (place of application: open field, greenhouse, garden, etc.), as well as according to the conditions of the physical environment and/or receiving environment (crop interception factor, drift, runoff and drainage potential) (Samuel *et al.*, 2012; Ayadi, 2013).
The calculation of these indicators is based on the use of 2 databases (updated several times a year): (i) one on the commercial characteristics and uses of registered plant protection products, Basagri[5] (provided by the company Lexagri), (ii) and one on the physico-chemical, toxicological and eco-toxicological properties of active ingredients, Footprint[6](Lewis et al., 2016). The databases were structured on the Ecoclimasol servers to automatically manage the regular updates of the two databases, as well as their possible changes in format. It was also necessary to structure the pairings between the Basagri parameters and those of Footprint (PPDB) to calculate the indicators.

Functionalities of the EToPhy web platform

Two applications have been developed on the "EToPhy web[7]" platform (also entitled "Dephyto"): EToPhy *Simulateur* and EToPhy *Analyses* (Figure 1). EToPhy *Simulateur* aims to assess the toxicity of plant protection products and active ingredients marketed (from the Basagri and Footprint databases) and to find alternatives that are less harmful to human health and the environment depending on the crop and the target/pest to be treated.
EToPhy *Analyses* makes it possible to analyze the sanitary and environmental impact of farmer phytosanitary practices at different levels (product, plot, crop and farm) in order to reduce the risk linked to the use of pesticides to human health and non-target organisms in the 3 environmental compartments: water, air and soil. This application works by means of 2 services used successively. First, the user should locate these plots on a map via the GEOLOC service. Then, the phytosanitary interventions of each plot must be stored in a record book via the LOGBOOK service. The database associated with this service is able to store information from

[5] https://www.lexagri.com/service_basagri.php
[6] http://sitem.herts.ac.uk/aeru/ppdb/
[7] https://www.etophy.fr

different databases, such as Basagri (database of commercial products used in EToPhy *Simulateur*), but also databases usually used by farmers and agricultural cooperatives in France (e.g. the Télépac[8] database for CAP declarations). Once all the information is filled in, EToPhy *Analyses* makes it possible to compare phytosanitary practices between plots, crops and farms. This operation makes it easier for users to identify the product(s) that have too high impact on health and the environment, in order to substitute them by using the risk comparator of plant protection products on EToPhy *Simulateur*. In addition to these EToPhy *Analyses* functions, the user can refine his/her choice of plant protection products according to environmental issues, and to the characteristics of the natural environment through the geolocation of plots via the GEOLOC service. Several other comparison queries provide users with a relatively rich field of analysis of phytosanitary practices according to agricultural campaigns, production methods (conventional, integrated or organic) and specificities of pesticide choices between synthetic and bio-control products.

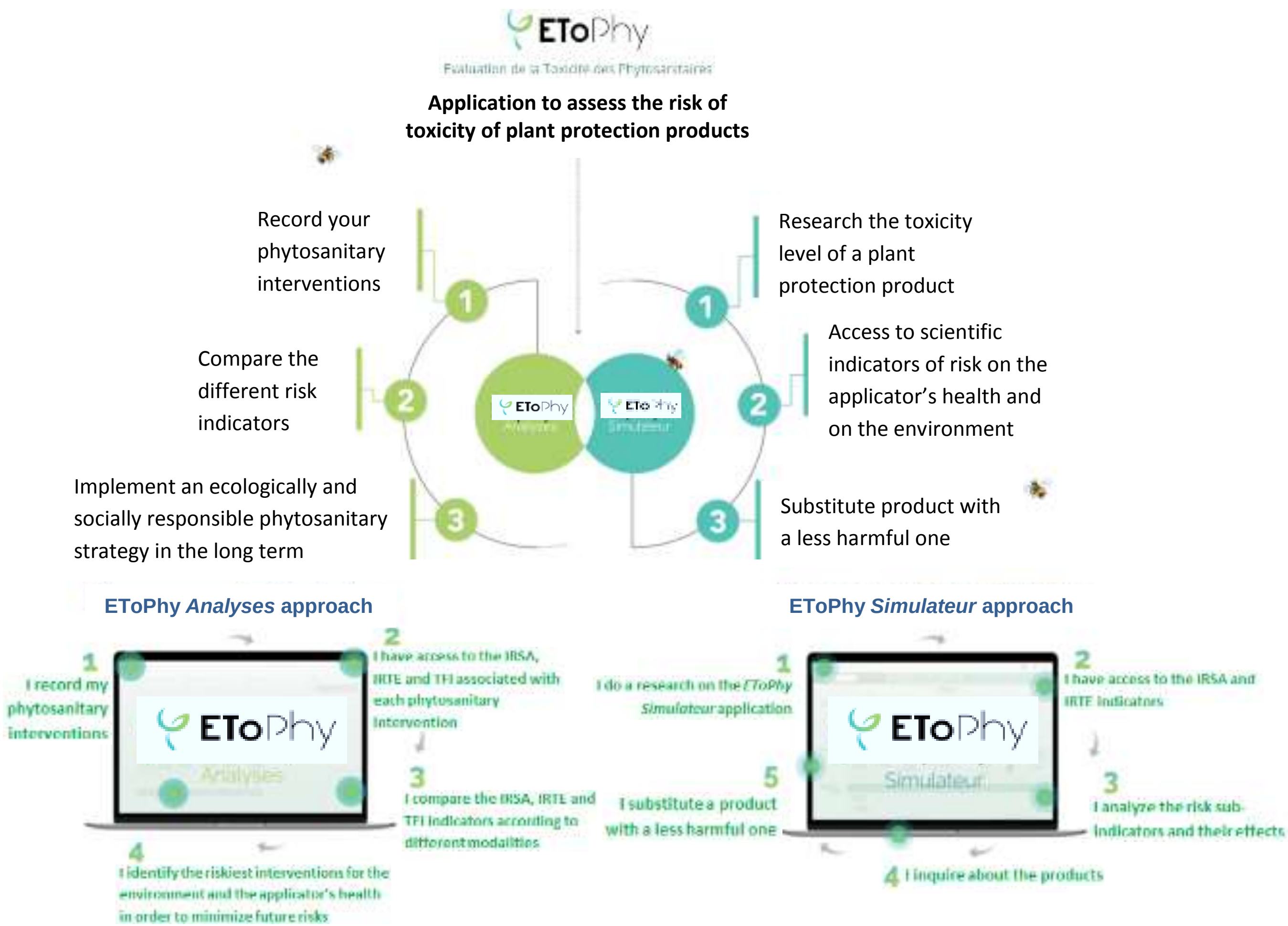

Figure 1. Illustrative diagram of the "EToPhy web" functionalities on the Dephyto platform

[8] https://www.telepac.agriculture.gouv.fr

Results and discussion

EToPhy *Simulateur*

The EToPhy *Simulateur* web application takes the form of a search engine. It calculates the IRSA and IRTE risk indicators linked to the use of a plant protection product based on a search by product name (Figure 2), by active ingredient name, or for a specific target on a given crop. It provides regulatory information on the product uses (Figure 2) and details of the risks to the environment such as the eco-toxicological impact on non-target organisms (earthworms, bees, birds, aquatic plants, fish, etc.), as well as to human health, such as chronic and acute toxicity (Figures 3 and 4). Finally, it allows the comparison of the toxicity of different products and helps the user to choose products that are less toxic to human health and to the 3 environmental compartments: soil, air and water (Figure 5).

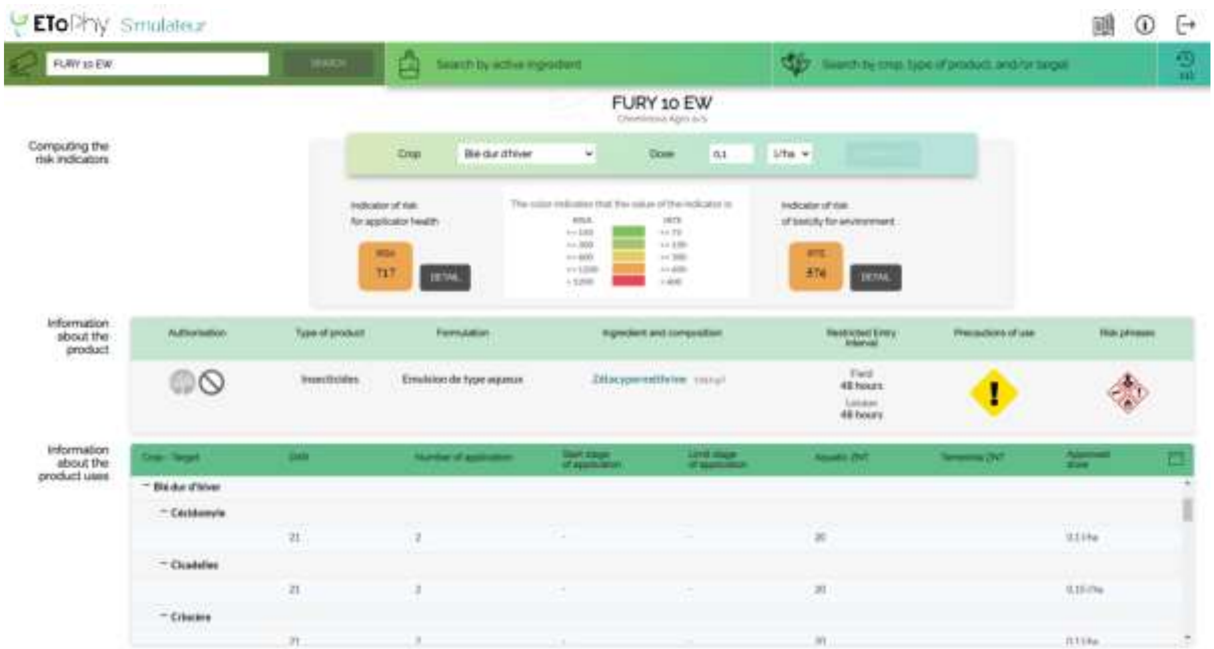

Figure 2. EToPhy Simulateur screenshot – Visualisation of the applicator health risk indicator (IRSA) and environmental toxicity risk indicator (IRTE) for the Fury 10 EW product with a 0.1 l/ha dose, and regulatory information on these uses.

Figure 3. EToPhy Simulateur screenshot showing the details of the applicator health risk indicator (IRSA) for Fury 10 EW with a 0.1 l/ha dose.

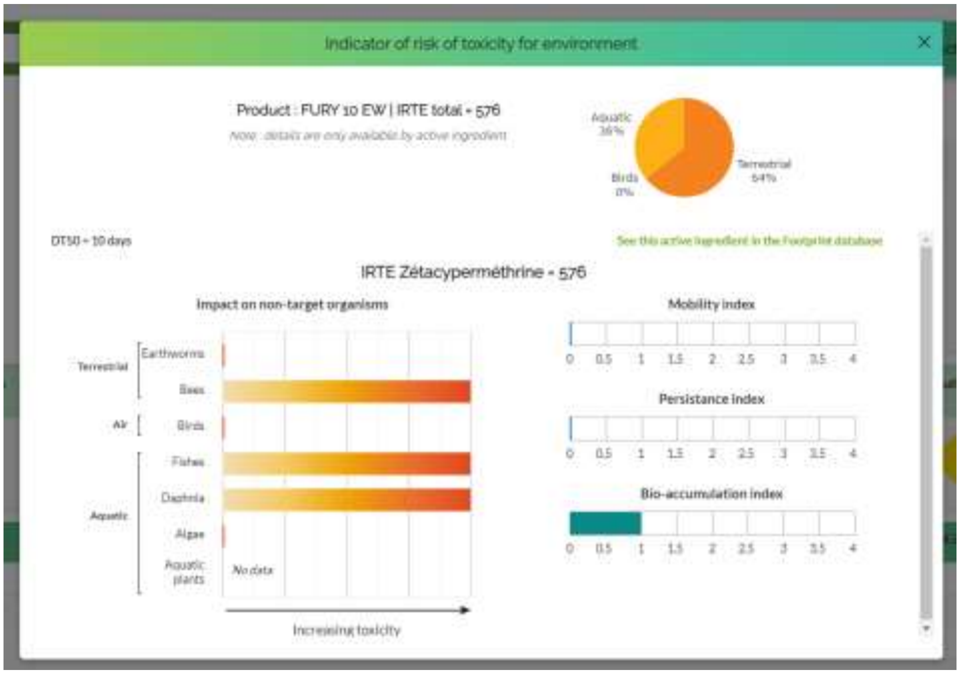

Figure 4. EToPhy Simulateur screenshot showing the details of the environmental toxicity risk indicator (IRTE) for Fury 10 EW with a 0.1 l/ha dose.

Figure 5. EToPhy Simulateur screenshot showing the comparative table of the toxicity risk of different phytosanitary products for a given crop-target (Wine grape vine - Powdery mildew).

EToPhy *Analyses*

EToPhy *Analyses* calculates the phytosanitary footprint of each phytosanitary intervention applied on the plot in question and recorded in LOGBOOK (Figure 6). The phytosanitary footprint is expressed via the IRSA and IRTE risk indicators (and the sub-indicators), and the TFI. EToPhy *Analyses* consists of a tool for selecting farms, crop years, plots and crops, for which the user wishes to visualise the results of the risk indicators and the TFI (Figure 7). Based on this selection, graphs offer a comparison of TFI, IRSA and IRTE indicators between the applied products (synthetic or bio-control), between plots or crops, between seasons and between farms, while taking into account the production mode (conventional, integrated or organic). Figure 7 shows an example of the contribution to the phytosanitary footprint (indicators in values/ha and in %) of the different products applied on a farm (conventional/integrated production for durum wheat, winter oilseed rape and maize plots). The user can then evaluate the environmental performance according to the levels of analysis (product, plot, crop), identify practices to be improved, as well as simulate crop treatment practices that are less toxic to human health and the environment.

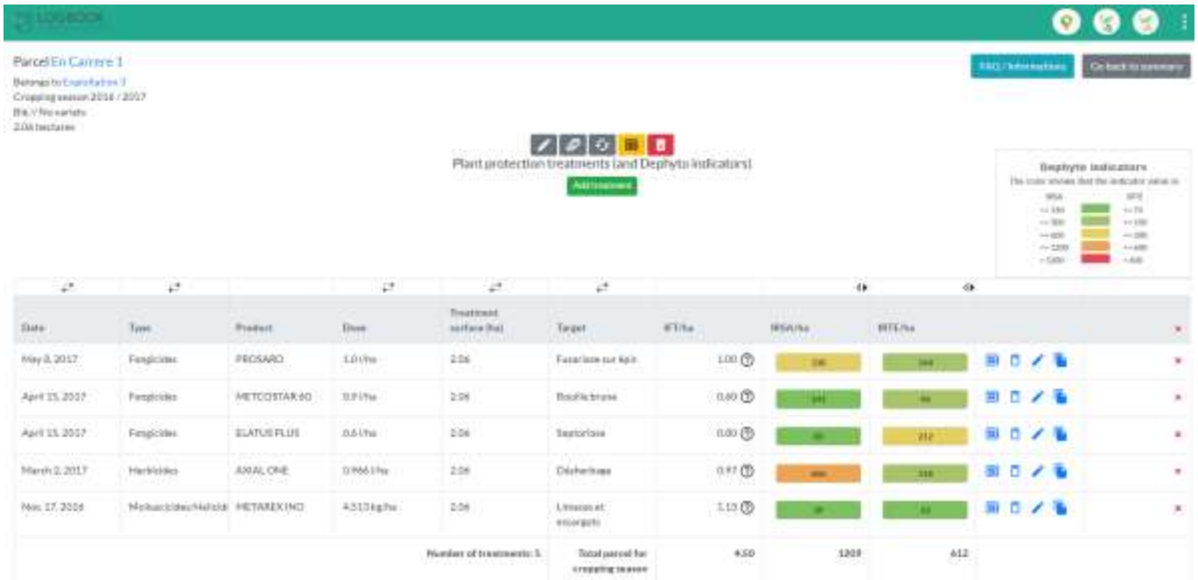

Figure 6. Screenshot of the LOGBOOK agricultural practice recording service – Visualisation of phytosanitary interventions applied on a plot of winter durum wheat, as well as the TFI, IRSA and IRTE indicators (values/ha) related to each intervention.

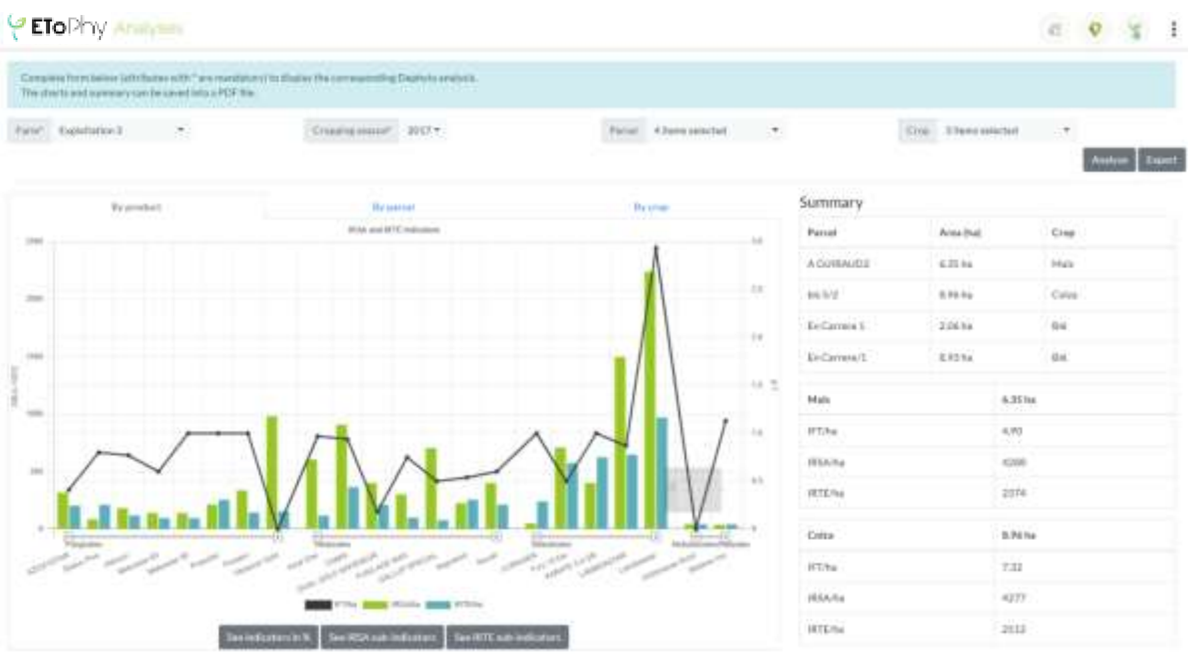

Figure 7. Screenshot of the EToPhy *Analyses* service - Contribution to the phytosanitary footprint (indicators in values/ha and in %) of the different products used on a farm with durum wheat, rape and maize plots.

Conclusion

The development of the EToPhy web platform, as a decision support and risk analysis tool for pesticide use, is part of the precision agriculture and smart agriculture concepts to improve the management of agricultural phytosanitary practices. The deployment of big data technology makes it possible to manage the significant number of parameters (characteristics of commercial products and active ingredients), in order to simplify user access to information from databases. The pressure and risk indicators calculated on the platform represent a decision support dashboard for the better integrated management of the health and environmental impact of plant protection products applied on plots. The functionalities of the EToPhy web platform (EToPhy *Analyses* and EToPhy *Simulateur*) play a dual role in the monitoring and the control of the diffuse phytosanitary pollution, depending on the spatial scale of phytosanitary practice management: at a local level (plot/farm) and at a territorial level (catchment area, agricultural region).

Acknowledgments

EToPhy web developed by CIHEAM-IAMM and the R&D Company Ecoclimasol as part of the collaborative research project GesPPEIR "*Gestion eau, Phytosanitaires, Prévisions et Indicateurs de Risques*" funded by the ERDF and the Occitanie region, France (2016-2021). https://etophy.fr/

References

Alavanja, M. C. (2009). Pesticides use and exposure extensive worldwide. Reviews on environmental health, 24(4), pp. 303-309.

Aubertot J.-N., Barbier J.-M., Carpentier A., Gril J.N., Guichard L., Lucas P., Savary S., Savini S., Voltz M. (2005). Pesticides, agriculture et environnement. Réduire l'utilisation des pesticides et limiter leurs impacts environnementaux. Rapport d'expertise scientifique collective, INRA et CEMAGREF.

Ayadi H. (2013). Outils de gestion de la pollution phytosanitaire diffuse au niveau d'un territoire : cas d'application zone humide Ramsar de la Merja Zerga au Maroc. Thèse de doctorat en Géographie et Aménagement de l'Espace, spécialité Agronomie: Université Montpellier 3.

Ayadi H., Le Bars M., Le Grusse P., Mandart E., Fabre J., Bouaziz A., Bord J.P. (2014). SimPhy: a simulation game to lessen the impact of phytosanitaries on health and the environment-the case of Merja Zerga in Morocco. Environmental Science and Pollution Research, vol. 21, pp. 4950-4963.

Bockstaller C., Girardin P. (2003). How to validate environmental indicators. Agricultural Systems, vol. 76, pp. 639-653.

Bockstaller C., Guichard L., Makowski D., Aveline A., Girardin P., Plantureux S. (2008). Agri-environmental indicators to assess cropping and farming systems. A review. Agronomy for Sustainable Development, vol. 28, pp. 139-149.

Guichard L., Dedieu F., Jeuffroy M.-H., Meynard J.-M., Reau R., Savini I. (2017). Le plan Ecophyto de réduction d'usage des pesticides en France: décryptage d'un échec et raisons d'espérer. Cahiers Agricultures 26(1):14002.

Hallenbeck W.H., Cunningham-Burns K.M. (2012). Pesticides and human health. Springer Science & Business Media.

Houdart M., Tixier P., Lassoudière A., Saudubray F. (2009). Assessing pesticide pollution risk: from field to watershed. Agronomy for sustainable development, 29(2), pp. 321-327.

Baldi I., Cordier S., Coumoul X., Elbaz A., Gamet Payrastre L. et al. (2013). Pesticides : Effets sur la santé. [Rapport de recherche] Institut national de la santé et de la recherche médicale (INSERM). Paris : Inserm : Editions EDP Sciences (ISSN : 1264-1782).

Juan G., Barataud F., Billy C., Bouchet L., Carpentier A., Gouy V., Le Hénaff G., Voltz M. (2018). Référentiel sur les outils de la recherche pour réduire les pollutions de l'eau par les pesticides. Rapport final, convention INRA-AFB.

Lammoglia SK., Moeys J., Barriuso E. et al. (2017). Sequential use of the STICS crop model and of the MACRO pesticide fate model to simulate pesticides leaching in cropping systems. Environ Science and Pollution Research, vol. 24, pp. 6895–6909.

Le Grusse P., Mandart E., Bouaziz A., Le Bars M., Bord J-P., Fabre J. (2014). Gestion de la toxicité en zone Ramsar (TRam). Rapport scientifique du programme pesticides: Programme évaluation et réduction des risques liés à l'utilisation des pesticides. APR Pesticide 2009.

Mahmood I., Imadi S.R., Shazadi K., Gul A., Hakeem K.R. (2016). Effects of Pesticides on Environment. In: Hakeem K., Akhtar M., Abdullah S. (eds) Plant, Soil and Microbes, pp. 253-269.

Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). Effects of pesticides on environment. In Plant, soil and microbes, pp. 253-269.

Mghirbi O., Ellefi K., Le Grusse P., Mandart E., Fabre J., Ayadi H., Bord J.P. (2015). Assessing plant protection practices using pressure indicator and toxicity risk indicators: analysis of the relationship between these indicators for improved risk management, application in viticulture. Environmental Science and Pollution Research, vol. 22, pp. 8058-8074.

Mghirbi O. (2016). Résilience des exploitations agricoles face au changement des pratiques phytosanitaires: Conception d'outils de gestion des risques liés aux pesticides – Cas du bassin versant de l'étang de l'Or en France. PhD dissertation, Université Paul Valéry Montpellier 3.

Mghirbi O., Le Grusse P., Fabre J., Mandart E., Bord J.-P. (2017). OptiPhy, a technical-economic optimisation model for improving the management of plant protection practices in agriculture: a decision-support tool for controlling the toxicity risks related to pesticides. Environmental Science and Pollution Research, vol. 24, pp. 6951–6972.

Mghirbi O., Bord J-P, Le Grusse P, Mandart E, Fabre J (2018) Mapping for the management of diffuse pollution risks related to agricultural plant protection practices: case of the Etang de l'Or catchment area in France. Environmental Science and Pollution Research, vol. 25, pp.14117–14137.

Ossard A., Galan M. B., Boizard H., Leclercq C., Lemoine C. (2009). Évaluation des impacts environnementaux des pratiques agricoles à l'échelle de la parcelle et de l'exploitation en vue de l'élaboration d'un plan d'actions: une méthode de diagnostic spatialisé fondée sur des indicateurs, le DAE-G. Ingénieries, (59-60), pp. 71-87.

Samuel O., Dion S., ST-Laurent L., April M.H. (2012). IRPeQ, Indicateur de risque des Pesticides du Québec. 2 ed. Québec: Ministère de l'Agriculture, des Pêcheries et de l'Alimentation / Ministère du Développement durable, de l'Environnement et des Parcs / Institut national de santé publique du Québec.

Sharma A., Kumar V., Shahzad B., Tanveer M., Sidhu G. P. S., Handa N., ... & Thukral A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. SN Applied Sciences, 1(11), pp. 1-16.

ASSESSING THE POTENTIAL USE OF GFS-ANL MODEL WEATHER DATA IN FIELD-SCALE PRECISION AGRICULTURE IMPLEMENTATION

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Abstract

Implementation of precision irrigation (PI) approaches requires accurate meteorological data to estimate the evaporative demand of the atmosphere and thus the potential crop water needs. Consequently, extensive networks of meteorological stations are needed if such practices are to be integrated within farmers management practices. However, the establishment of such networks are costly. Free meteorological forecast models (e.g., global forecast model, GFS) can be used as an alternative free source of data. These models, except from a weather prediction for the next days, offer databases with historic meteorological data based on re-analysis (e.g., GFS-ANL). Nevertheless, their spatial resolution of 0.25×0.25 degrees (~ 28×28 km), is quite large and questions the reliability of the obtained data. In this framework, data from three weather stations located within the water district of Thessaly in central Greece and the corresponding GFS-ANL time series were collected. The parameters that were obtained were air temperature, wind speed, relative humidity and precipitation. Successively, a statistical analysis was performed to evaluate the model capability to simulate satisfactorily the measured weather data. The results indicated that the GFS-ANL model could simulate the air temperature quite fair (R^2 ~0.97; RMSE < 1.77 °C), but that was not the case for the rest or the parameters.

Keywords: *irrigation, precision agriculture, weather model, reference evapotranspiration.*

Introduction

All the precision irrigation approaches, except maybe only those that are using almost-real-time field soil moisture measurements, exploit meteorological data to estimate the reference evapotranspiration (ET_0), a process that describes the way that the water is transferred from earth to the atmosphere via evaporation from the soil surface (or other surfaces e.g., plant leaves) and through transpiration by plants (Allen et al., 1998; Tsakmakis et al., 2017).

While there is a substantial number of formulas proposed for the estimation of ET_0, like Blaney-Criddle, Penman, Priestley-Taylor, Turk and Hargreaves (Liu et al., 2017), the most popular and reliable formula for ET_0 estimation is FAO-56 Penman-Monteith, a modified version of the formulas originally proposed by Penman and Monteith (Allen et al., 1998). The latter is utilizing the meteorological parameters of maximum temperature, mean temperature, minimum temperature, maximum relative humidity, mean relative humidity, minimum relative humidity, mean wind speed and mean solar shortwave radiation (Rs) or net radiation to estimate ET_0.

Moreover, precipitation height, even though it's not required for the ET_0 estimations, constitutes a vital meteorological parameter in the overall PI programming and implementation, as it can postpone irrigation events and change the initial scheduling. It is obvious that field specific measurements of the above-mentioned parameters would guarantee accurate and precise

estimations but that's not always the case. Specifically, in the region of Thessaly, in central Greece, the network of meteorological stations is not dense and in most of the cases the fields are about 20-30 km from the closest station. Moreover, the data acquisition from the existing stations is laborious and difficult, as at the moment there is not available a national unified Application Programming Interface (API) that will provide easy and fast access to the metrological stations network.
As an alternative solution, data from free meteorological forecast models maybe can be used (e.g., global forecast model, GFS). These models, except from a weather prediction for the next days, offer databases with historic meteorological data based on re-analysis (e.g., GFS-ANL). The drawback of these databases is that they refer to a global grid with a cell spatial resolution of 0.25×0.25 degrees (~ 28×28 km). Thus, the question arising is; are these large-scale data suitable for the implantation of a PI field-scale approach? To answer this question an analysis was performed between data obtained from a meteorological station located within the region of Thessaly and the corresponding historic data-series from GFS-ANL model.

Material and Methods

The data used for this analysis were obtained from three meteorological stations that are installed nearby the villages Achilion, (Lat: 39.548138°, Long: 22.685881°, Alt:48 m), Chalki (Lat: 39.57083°, Long: 22.54333°, Alt:75 m) and Agia (Lat: 39.71615°, Long: 22.76201°, Alt:167 m). All three villages are located within the water district of Thessaly (EL08), in central Greece. The meteorological stations in the villages of Achilion and Agia were able to measure air temperature, relative humidity, wind speed and direction, solar shortwave radiation (Rs) and precipitation height, while the station in Chalki village recorded air temperature, wind speed and precipitation. The stations were installed and have been in operation for different time periods (e.g., station in Achilion was installed on 2020, in Chalki from 2017 etc.). For the purpose of this study the time period between March to August 2020 was selected, as during this time (a) all the three stations were in operation and (b) it corresponds to the summer arable crops cultivation period.
The GFS-ANL data for the same period was downloaded from the archives of National Oceanic and Atmospheric Administration center (NOAA). Specifically, the retrieved data was referred to the model run of 00:00 and the predictions of 00:00, 06:00, 12:00 and 18:00. The grid cell that corresponded to the study area had latitude and longitude equal to 39.50° and 22.75°, respectively. Retrieved meteorological parameters were air temperature, relative humidity, wind speed and precipitation height. Successively, the retrieved 6-hours temporal analysis data were resampled to daily values. It should be mentioned that substantial gaps were observed in NOAA archived data and thus the retrieved time series were not complete. Subsequently, the meteorological stations' time series were filtered to match the available NOAA archived dates.
Once the data was correctly prepared, a statistical analysis was performed to evaluate the simulation capability of the GFS-ANL model. The statistical parameters that were used to assess the potential differences between the field-stations measured data and the GFS-ANL historic time series were (a) the coefficient of determination (R^2) as a measure of the goodness-of-fit between the measured and the modeled data and (b) Root Mean Square Error as an index of the residual error among the field-measurements and the large-scale modeled data (RMSE; Eq. 1)

$$RMSE = \left[\frac{\sum_{i=1}^{n}(Si-Oi)}{n}\right]^{0.5} \quad (1)$$

Results and Discussion

The fluctuations of the meteorological stations measured and the GFS-ANL modeled air temperature, relative humidity, wind speed and precipitation height are illustrated in Figure 1. At first glance the measured and modeled air temperature data seems to follow the same pattern for all the three stations (Figure 1a, 1b, 1c). This trend is confirmed by statistical indices, with R^2 exhibiting values equal to 0.92, 0.97 and 0.97 in the cases of Achilion, Chalki and Agia meteorological stations, respectively (Table 1). Moreover, the RMSE was found to be 2.39 °C in the case of Achilion station, but less than 1.58 °C in the cases of Chalki and Agia stations. When the data from the three stations were aggregated, the R^2 and RMSE were found to be 0.97 and 1.77 °C, respectively, indicating a quite satisfactory match between the modeled and measured time series. The corresponding aggregated data scatter plot (Figure 2a), showed that the residual difference between the two series is almost evenly distributed around the 1:1 line, with just a few cases where the GFS-ANL model overestimated substantially the air temperature values. Similar results have been reported by previous studies (Sinha and Chandrasekar, 2010; Fairman et al., 2011).

In the case of wind speed, the obtained results were not satisfactory. The pattern between the measured and modeled data was found to vary considerably among the stations (Figure 1d, 1e, 1f). Specifically, in Agia, the GFS-ANL model was found to overestimate the wind speed consistently, whilst in the locations of Achilion and Chalki the measured and modeled wind speed data were arbitrarily blended during spring, but GFS-ANL tent to underestimate wind speed values during the summer (June to August). These trends were described by the statistical analysis quite well, with R^2 be equal to 0.36 in Agia, where model constantly overestimated the wind speed values, and 0.10 and 0.03 in the cases of Achilion and Chalki, respectively (Table 1). Additionally, the RMSE was fluctuating between 0.66 and 1.09 m/s, among the stations, confirming the significant variations among the measured and modeled values. As a result of the poor model performance observed in the individual stations, the aggregated wind speed data implied a poor model capability to provide robust wind speed datasets, with R^2 and RMSE values equal to 0.03 and 0.85 m/s, respectively. The corresponding aggregated data scatter plot (Figure 2b), illustrates clearly the inconsistency between the measured and modeled values, unveiling a subtle model trend to overestimate wind speed.

Looking closely to the precipitation measured and modeled time series, its' clearly illustrated that during 2020 cultivation period there were just three events where more than 3 mm of water fell, on April 4, June 16 and August 8 (Figure 1g, 1h, 1i). It is worth to mention that in the unique case that the GFS-ANL model reported an event with more than 6 mm of water (April 4th), the very same day all the three stations recorded a precipitation event with more than 6 mm. However, that's not the case for the other two events. On June 16, the precipitation event reported by GFS-ANL was recorded by Achilion and Chalki weather stations, but not by the weather station in Agia. More strikingly, on August 8, while all the weather stations recorded a precipitation event with more than 10 mm, the GFS-ANL model does not show rain at all. Moreover, various minor precipitation events (less than 3 mm of water) that were recorded by Achilion weather station, were not reported by GFS-ANL model or recorded by the other two stations. As a result, the R^2 in the case of Achilion was found to be 0.03 (Table 1), while for the other two stations was remarkably higher, equal to 0.69 and 0.53 for Chalki and Agia weather stations, respectively. Accordingly, the RMSE for Achilion was 2.42 mm, whilst for the other two stations was calculated less than 1.79 mm. When all the stations data was aggregated the overall model, performance was found to be poor (R^2 0.12; RMSE 2.04 mm), but this is mainly attributed to the discrepancies between GFS-ANL and Achilion station time series. Lastly, the aggregated data scatter plot (Figure 2c), implied that the model tends to underestimate the precipitation height.

Figures 1j and 1k, illustrated that GFS-ANL consistently underestimated the relative humidity values, both in Achilion and Agia stations. The magnitude of underestimation found to be similar in both cases (RMSE ~ 17.80 %). Accordingly, the correlation between the modeled and measured data was poor for both stations, with R^2 showing values lower than 0.4. When the data of both stations was aggregated the R^2 improved slightly (0.42) but the RMSE remained the same (17.80%). The overestimation trend that was illustrated in Figures 1j and 1k, is reconfirmed buy the aggregated data scatter plot (Figure 2d), where the overwhelming majority of the scatter dots are observed to be located left of 1:1 line (y axis).

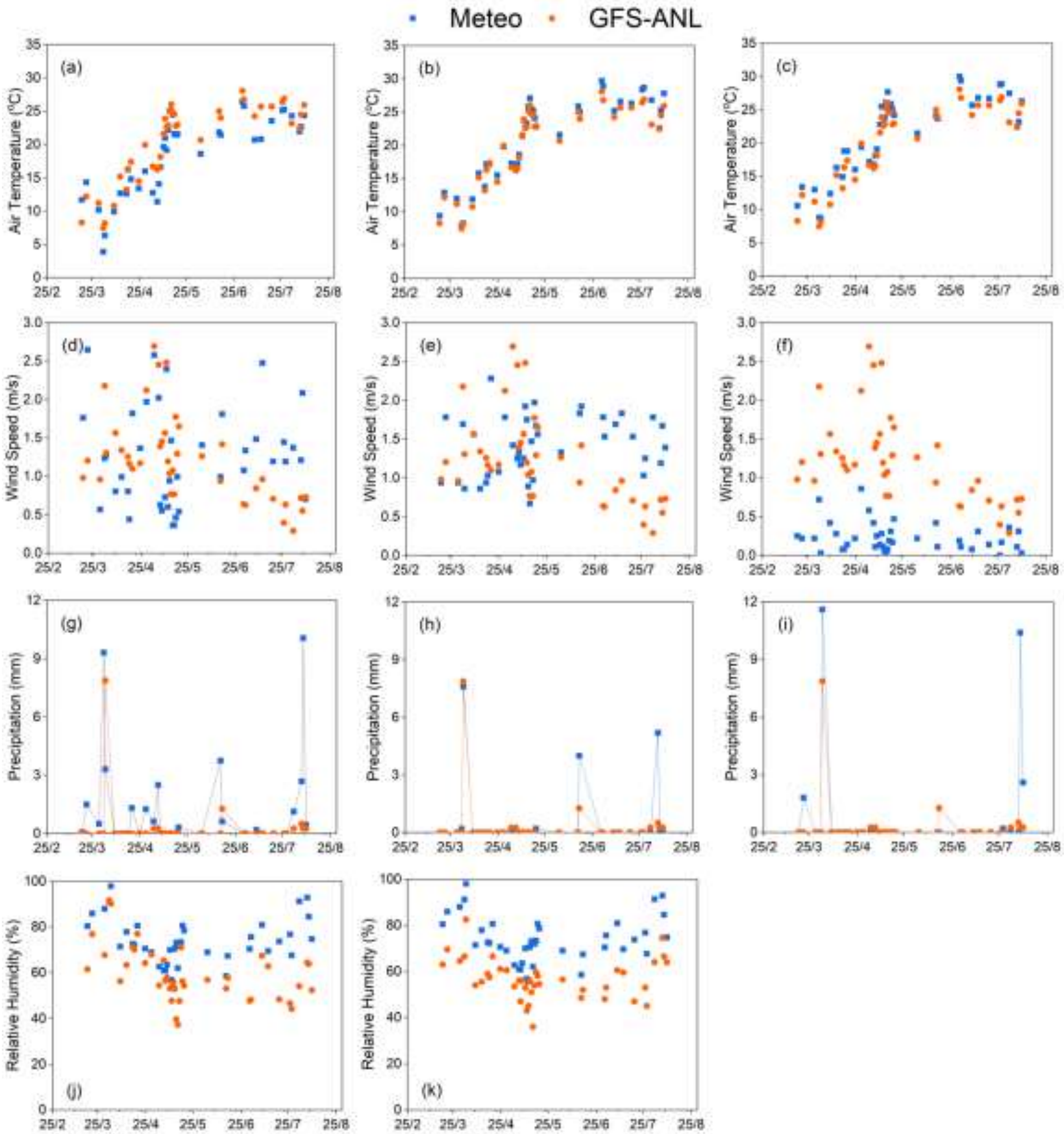

Figure 1. Time series of meteorological station measured and GFS-ANL modeled air temperature in (a) Achilion, (b) Chalki, and (c) Agia; wind speed in (d) Achilion, (e) Chalki and (f) Agia; precipitation height in (g) Achilion, (h) Chalki and (i) Agia; relative humidity (j) Achilion and (k) Agia.

Table 1. Statistical analysis results between the meteorological stations and GFS-ANL modeled meteorological parameters.

Meteorological Parameter	R^2	RMSE
Achilion Meteorological Station		
Air Temperature (°C)	0.92	2.39
Relative Humidity (%)	0.30	17.80
Wind Speed (m/s)	0.10	0.73
Precipitation (mm)	0.03	2.42
Chalki Meteorological Station		
Air Temperature (°C)	0.97	1.10
Relative Humidity (%)	-	-
Wind Speed (m/s)	0.03	0.66
Precipitation (mm)	0.69	0.87
Agia Meteorological Station		
Air Temperature (°C)	0.97	1.58
Relative Humidity (%)	0.67	17.89
Wind Speed (m/s)	0.36	1.09
Precipitation (mm)	0.53	1.79
Aggregate Meteorological Station Data		
Air Temperature (°C)	0.97	1.77
Relative Humidity (%)	0.42	17.84
Wind Speed (m/s)	0.03	0.85
Precipitation (mm)	0.12	2.04

R^2=coefficient of determination; RMSE=root mean square error

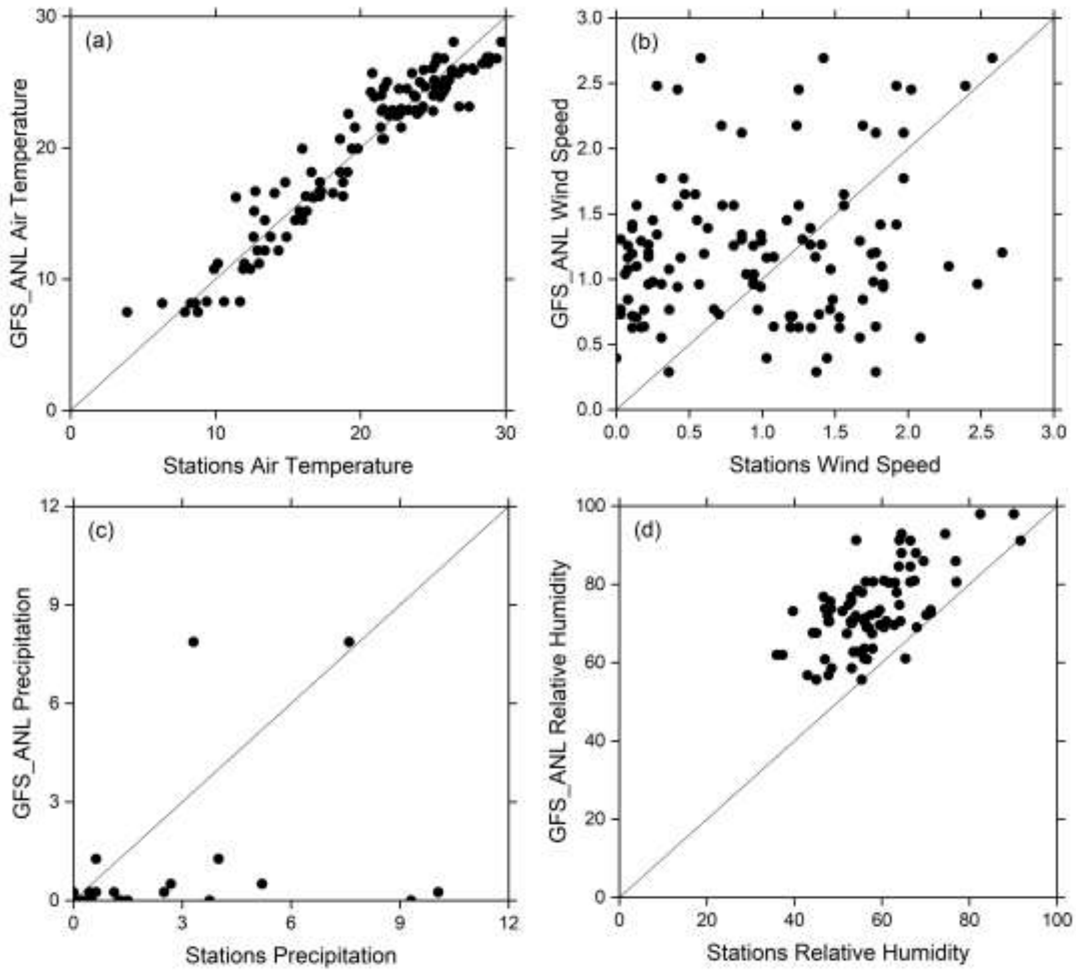

Figure 2. Scatter plots between aggregated meteorological station measured and GFS-ANL modeled (a) air temperature; (b) wind speed; (c) precipitation and (d) relative humidity.

Conclusions

Even though the analysis performed in this study concerns only three meteorological stations and a short time period, its main findings are considered to be indicative of the GFS-ANL model capability to simulate with accuracy and precision the meteorological conditions that prevail in field scale. The results showed that while the air temperature data can be used with pretty fair accuracy for PI scheduling, the relative humidity, wind speed and precipitation data could not be used for such a purpose. In the cases of precipitation and wind speed, the unique differences observed among the modeled and measured data for each station, may imply that the variations of land relief could have a crucial impact in the actual precipitation and wind speed values and thus could not be simulated satisfactorily by a model with a 28x28 grid cell spatial analysis. As for the relative humidity, the constant GFS-ANL underestimation indicates that a statistical correction, based on long time series of measures and observed data, may improve the model capacity to simulate the former. This conclusion highlights the need of the establishment of an extensive meteorological station web, capable to provide the PI algorithms and models with reliable data and thus increasing their robustness and reliability.

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References

Allen R., Pereira L.S., Raes D., Smith M. (1998) Crop evapotranspiration: Guidelines for computing crop requirements. Irrigation and drainage paper 56, FAO, Italy.

Jonathon G., Fairman Jr., Udaysankar S.N, Sundar A.C., Mölg T. (2011) Land use change impacts on regional climate over Kilimanjaro. Journal of geophysical research, Vol 116(D03).

Sinha P.K., Chandrasekar A. (2010) Improvement of Mesoscale Forecasts of Monsoon Despessions Through Assimilation of QuichSCAT Wind Data: Two Case Studies Over India. The Open Atmospheric Science Journal, Vol 4, pp. 160-177.

Liu X., Xu C., Zhong X., Li Y., Yuan X., Cao J. (2017) Comparison of 16 models for reference crop evapotranspiration against weighing lysimeter measurement. Agricultural Water Management, Vol 184, pp. 145–155.

Tsakmakis I., Kokkos N., Pisinaras V., Papaevangelou V., Hatzigiannakis E., Arampatzis G., Gikas G.D., Linker R., Zoras S., Evagelopoulos V., Tsihrintzis V.A., Battilani A., Sylaios G. (2017) Operational Precise Irrigation for Cotton Cultivation through the Coupling of Meteorological and Crop Growth Models. Water Resources Management, Vol 31, pp. 563-580.

DECISION SUPPORT SYSTEM FOR WASTEWATER AND BIOSOLIDS SAFE REUSE IN AGRICULTURAL APPLICATIONS

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Abstract

Since the appearance of humans on the earth, various wastes started being produced, such as human or animal solid or liquid excreta and liquid or solid wastes produced by man's domestic, agricultural, and industrial activities. Today, vast quantities of wastewater and biosolids are being produced the world over, and they are either disposed of in the surface water systems or accumulate in the soil, causing environmental problems. Both the wastewater and biosolids contain valuable constituents such as water, plant nutrients, organic matter (OM), and energy. Safe reuse of treated wastewater and sludge in agriculture could reduce the inorganic fertilizers usage, decreasing unexploited rejected wastewater and sludge, contributing sustainably to the environment. However, the decision-making for safe and rational reuse of wastewater and sludge is a complex task. A computer-aided Decision Support System (DSS) contribution seems to offer an attractive solution for safe reuse and effective management of the effluents mentioned above. The present work describes such a DSS developed by the HOU Laboratory of Sustainable Waste Management Technologies. To demonstrate the DSS function based on the reuse of wastewater and biosolids, two examples are given of reuse of treated municipal wastewater (TMW) and biosolids, one with low nutrient content and a second with high nutrient content. From the DSS reports, it is concluded that by using wastewater and biosolids with higher nutrient concentrations, considerable gains, up to 100% of nutrients are achieved.

Keywords: *wastewater, sludge, biosolids, safe reuse, decision support system.*

Introduction

Since the appearance of human beings on the earth, various wastes started being produced and spread in the environment, such as human or animal solid or liquid excreta originating from the physiological activities of the organism, as well as liquid or solid wastes produced by the domestic, agricultural, and industrial activities of man.

Of course, these wastes in ancient times, were in balance with the environment as they were generated in lower quantities and they generally were natural, nontoxic byproducts as those of the present time. They were basically friendly to the environment. The increase of the human population during the course of history, led inevitably to the disturbance of this balance, and consequently the various environmental problems started appearing gradually and steadily. Thus, for example the production of the wastewater worldwide has reached 5.6×10^9 m^3, of which is reused for the irrigation of crops, only a small fraction, corresponding to <1% of the total natural irrigation water (Jiménez & Asano, 2008; Puma & Cook, 2010). This marginal water is mainly used in arid to semiarid regions. For example in Jordan 38% (Alfarra et al., 2011), in California 46% (Sato et al., 2013) and 85% in Israel (Goldstein et al., 2014). Similarly, large quantities of

biosolids are being produced annually in the world. Thus, only in the European Union (EU) 10.275.697 t of biosolids have been produced (Zorpas, 2017).
Both the wastewater and biosolids contain valuable constituents such as water, plant nutrients, organic matter (OM), and energy. Especially, the biosolids are carriers of both high levels of OM and plant nutrients; the average range of N P K composition for anaerobically digested sludge being: N 4.2 - 4.8%, P 2.7 - 3.0%, K 0.3 - 0.4%, and N 4.8%, P 2.7%, and K 0.4% for aerobically treated sludge (Sommers, 1977), and OM 22.6 – 28.4%, Zn 219.0 – 313.1 mg/kg, Cu 11.4 – 109.4 mg/kg, Fe 184.8 – 427.5 mg/kg, Mn 10.5 – 208.5 mg/kg (Dimitrelos, 2015).
In the meantime, the human population is increasing in such rates that according to estimations of the United Nations (UN) it is expected that it will increase by 2.1 billion people till the 2030, especially in the developing countries and consequently there will be an increase of the wastewater and biosolid production. This situation is indeed alarming and the authorities are hardly trying to find ways to dispose these outputs in a safe and productive way.
The decision-making for safe and rational reuse of wastewater and sludge is a complex task. Among the recent developments that have been accomplished in relation to the exploitation of wastewater and biosolids, is the contribution of a computer-aided system, which seems to open new innovative paths for effectively and efficiently facing the severe problem of safe reuse. The research team of the School of Science and Technology of the Hellenic Open University developed a DSS, which could be used as a very useful tool in approaching the safe reuse of wastewater and biosolids in Agriculture (Koukoulakis et al, 2019). This paper aims to demonstrate the DSS's ability for reusing wastewater and biosolids based on examples with real-world data inputs.

Material and Methods

The current version of the DSS used in this work is built as a web application with PHP, MySQL and JavaScript. The DSS calculates the optimum nutrient dose for the crop, based on the nutrient inputs contained in soil in the form of residual nutrients, in wastewater, and in biosolids and the nutrient losses due to leaching, denitrification, fixation and removal via crop harvesting. It is also able to evaluate the quantity of inorganic fertilizer-carrier corresponding to the optimum nutrient dose, and the quantity to be applied during the different crop growth stages (early growth, flowering, fruit set etc) and it guides the user about the proper time (Fall, Winter, Spring), and the method of application of the fertilizer.
The DSS calculates the the value of the soil pollution index and can give quantitative information about the level of soil pollution with heavy metals. It can also determine the quality of the wastewater being used, and it may exclude it from reuse if its concentrations in heavy metals are higher than the international standards.
The knowledge base of the DSS contains rules with recommended maximum heavy metal concentrations in wastewater. The system estimates Elemental Pollution Index before and after wastewater and biosolids application and it can detect highly acidic soils and calculates the optimum rate of liming material needed for the desired pH. Its knowledge base also contains a large set of rules to calculate optimum nutrient dose and to provide fertilization advices for every supported crop.
To demonstrate the DSS function based on the reuse of wastewater and biosolids, two examples are given of reuse of treated municipal wastewater (TMW) and biosolids, one with low nutrient content and a second with high nutrient content. The examples utilize analytical input data from

the same potato crop. Also, the same soil data are used in both cases, and an equal volume of wastewater (3500 m^3/ha) and biosolids weight (10000 kg/ha) was applied, respectively. The DSS evaluates input data and suggests optimal nutrient doses in every scenario.

Results and Discussion

The following two examples are given of reuse, first with low nutrient content of treated municipal wastewater (TMWW) and biosolids and second with high content, respectively.
Example 1: Application to potato crop of 3500 m^3/ha wastewater of low plant nutrients and soil heavy metal content, respectively. Also of 10000 kg/ha biosolids, similarly of low nutrient and heavy metal content. The physical – chemical and elemental content of the soil are reported in Table 2 and
Table 3. The wastewater and biosolids data are reported in
Table 4. The level of the heavy metal soil pollution is evaluated by the pollution indexes in Table 5. Optimum nutrient doses are calculated by the DSS suggested for application to potato crop, as well as the attained nutrient dose gains are reported in Table 6.
Example 2: Application to the potato crop of equal TMWW volume and biosolid weight to the above quantities per ha of wastewater and biosolids respectively but of higher content in plant nutrient (
Table 4). The analytical data of soil is unchanged because the same soil is used. The evaluation of the heavy metal soil pollution level is given in Table 5. The nutrient doses and gains suggested by the DSS are given in Table 6.

Table 2. Physical and chemical properties of soil

pH	$CaCO_3$ (%)	Org. Matter (%)	EC (mS/cm)	Clay (%)
7.10	5.00	2.66	0.55	26.00

Table 3. Macro-micronutrients and heavy metals of soil (mg/kg)

	mg/kg		mg/kg
N	0.00	Mn	6.00
P	10.00	Zn	0.80
K	130.00	Cu	0.20
Ca	775.00	Cd	0.01
Mg	25.00	Co	0.05
Na	15.00	Cr	0.00
B	0.89	Ni	1.00
Fe	4.00	Pb	1.20

Table 4. Macro-micronutrients and heavy metals of TMWW and biosolid

	Example 1			Example 2	
	TMWW (mg/l)	Biosolid (mg/kg)		TMWW (mg/l)	Biosolid (mg/kg)
N	0.00	3.50	N	0.00	3.50
P	0.10	150.00	P	1.50	450.00
K	2.00	120.00	K	12.50	3333.00

	Example 1			Example 2	
	TMWW (mg/l)	Biosolid (mg/kg)		TMWW (mg/l)	Biosolid (mg/kg)
Mg	5.46	35.00	Mg	35.00	309.00
Na	0.00	0.00	Na	0.00	0.00
B	0.00	0.50	B	0.70	0.50
Fe	0.04	150.00	Fe	0.25	121.00
Mn	0.00	35.00	Mn	0.10	43.60
Zn	0.01	20.00	Zn	0.50	70.00
Cu	0.00	6.00	Cu	0.04	29.00
Cd	0.00	0.19	Cd	0.00	0.36
Co	0.01	0.52	Co	0.01	0.60
Cr	0.00	0.25	Cr	0.00	0.40
Ni	0.05	4.51	Ni	0.04	0.37
Pb	2.74	125.00	Pb	0.19	302.30

Table 5. Evaluation of soil pollution level

Soil pollution index	Value of pollution index before irrigation with treated wastewater	Value of pollution index after irrigation with treated wastewater	Evaluation of pollution level
EPI Example 1	0.1698	0.1956	No pollution
EPI Example 2	0.1698	0.2022	No pollution

Table 6. Nutrient doses calculated by the DSS suggested for application to potato crops for the two examples respectively

Nutrient	**Example 1 Nutrient dose$_1$ (kg/ha)**	**Example 2 Nutrient dose$_2$ (kg/ha)**	**Suggested nutrient dose gain [1] (kg/ha)**	**Percent of suggested nutrient dose gain (%)**
N	101.28	91.06	10.22	10
P_2O_5	22.64	0.00	22.64	100
K_2O	93.02	0.00	93.02	100
MgO	0.00	0.00	-	-
Fe	1.12	0.31	0.81	72
Zn	1.43	0.00	1.43	100
Mn	9.80	9.38	0.42	4
Cu	1.79	1.59	0.20	11
B	1.34	0.00	1.34	100

[1] Suggested nutrient dose gain = Nutrient dose$_1$ – nutrient dose$_2$

In both examples, the DSS calculated low soil pollution index after irrigation with treated wastewater and decided that the wastewater was safe for reuse (Table 4).
In Example 1, the DSS calculated extra nutrient doses for N, P_2O_5, K_2O, Fe, Zn, Mn, Cu and B. This is due to application of low plant nutrients content wastewater and biosolids (Table 5).

In Example 2 with the application of higher plant nutrients content wastewater and biosolids, the DSS calculated lower extra nutrient doses for N, Fe, Mn and Cu (Table 5). For the rest of the nutrients the DSS calculated that the applied wastewater and biosolids nutrients content is sufficient and suggested that no extra fertilization required (Table 5).
Based on the data of table 5, it is clear that by using wastewater and biosolids with higher nutrient concentrations, considerable gains, up to 100% of nutrients are achieved.

Conclusions

The DSS can evaluate soil pollution levels and alert the user for possible implications such as for example increase of soil heavy metal accumulation level, and appearance of toxicity phenomena in the growing plants. The examples mentioned above also demonstrate that using the DSS could lead to significant nutrient gains of up to 100%, leading to less inorganic fertilizers consumption and therefore contributing to the farmer's economy. The use of the DSS could effectively alleviate the problems related to the reuse until a cost-effective removal of toxic heavy metals will be able to transform the TMWW and biosolids in agriculture and generally in the ecosystem as safe as possible, protecting the human life and the environmental quality.

References

Alfarra, A., Kemp-Benedict, E., Hötzl, H., Sader, N., & Sonneveld, B. (2011). A Framework for Wastewater Reuse in Jordan: Utilizing a Modified Wastewater Reuse Index. *Water Resources Management*, *25*(4), 1153–1167. https://doi.org/10.1007/s11269-010-9768-8

Dimitrelos, I. (2015). *Determination of heavy metals and crystalline structure of the constituents sludge wastewater treatment and assessing the degree of availability of metals* [Thesis]. http://nemertes.lis.upatras.gr/jspui//handle/10889/9753

Goldstein, M., Shenker, M., & Chefetz, B. (2014). Insights into the Uptake Processes of Wastewater-Borne Pharmaceuticals by Vegetables. *Environmental Science & Technology*, *48*(10), 5593–5600. https://doi.org/10.1021/es5008615

Jiménez, B., & Asano, T. (2008). *Water Reuse: An International Survey of current practice, issues and needs*. IWA Publishing. https://doi.org/10.2166/9781780401881

Koukoulakis, P., Kyritsis, S., & Kalavrouziotis, I. (2019). Decision Support System for Wastewater and Biosolids Reuse in Agricultural Applications (Version 1.5) [Software]. Available from http://wdss.eap.gr.

Puma, M., & Cook, B. (2010). *Effects of irrigation on global climate during the 20th century* (Vol. 115). https://doi.org/10.1029/2010JD014122

Sato, T., Qadir, M., Yamamoto, S., Endo, T., & Zahoor, A. (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agricultural Water Management*, *130*, 1–13. https://doi.org/10.1016/j.agwat.2013.08.007

Sommers, L. E. (1977). Chemical composition of sewage sludges and analysis of their potential use as fertilizers. *J. Environ. Qual.; (United States)*. https://doi.org/10/bmrzth

Zorpas, A. A. (2017). Bio solids composting and soil applications. In I. K. Kalavrouziotis (Ed.), *Wastewater and Biosolids Management* (pp. 107–120). International Water Association. https://doi.org/10.2166/9781780408231_0107

SOIL INORGANIC NITROGEN AND COTTON YIELD SIMULATION USING THE CropSyst MODEL: A PRELIMINARY CASE STUDY IN GREECE

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Abstract

Agricultural management practices on fertilizer nitrogen are vital to soil sustainability and crop productivity, as well as to reducing global greenhouse gases and environmental pollution. An increasing number of models have been adapted for specific purposes and scales of application which may lead to better understanding of crop response under different environmental conditions and management practices in agriculture. One of the greatest challenges is the capability of models to simulate adequately crop growth and soil nitrogen dynamics under different management practices. Among the crop simulation models that have been developed over the last decades, CropSyst model was selected for the present study. CropSyst (Cropping Systems Simulation Model) is a multi-year multi-crop simulation model developed to study the effect of cropping systems management on productivity and environment and has been widely used to simulate the growth of several crops with generally good results in many parts of the world. The objective of this study was the evaluation of CropSyst model's ability to simulate the soil inorganic nitrogen and cotton production in two locations in Greece, Larissa (central Greece) and Orestiada (northern Greece). The simulated results were compared with the measurements of soil inorganic N and crop yield of the two experimental fields throughout the growing season. It is concluded that the amount of soil inorganic nitrogen as well as the cotton yield were generally simulated well for both locations, indicating an overall satisfactory model performance. Hence, CropSyst model can be an important tool in agricultural research to assess the environmental impact and agronomic benefit of fertilizer N applications to irrigated areas.

Keywords: *CropSyst Crop Growth Simulation Model, Soil nitrogen simulation, Cotton yield.*

Introduction

During the last few decades, cropping systems modeling for simulating crop growth and development has been evolving along with the progress of computer technology aiming to provide tools for understanding and analyzing the main processes characterizing the agroecosystems behavior (Todorovic *et al.*, 2009). The evolution of the modeling efforts is influenced by the changing goals, target users and policies over the years ranging from models with a scientific insight at plant scale to those focused on practical applications and management practices impacts (Sinclair and Seligman, 2000). Those models result from the mathematical representation of the processes that explain the crop-soil interaction driven by weather and management (van Ittersum and Donatelli, 2003) and thus, they are suitable for testing hypotheses, for the evaluation of the impacts of alternative management practices (Acutis *et al.*, 2000) and climate scenarios (Confalonieri *et al.*, 2006).

Nitrogen (N) is a critical resource for the development of the best management practices in agriculture and cannot be analyzed independently of weather, soil characteristics, hydrology, crop characteristics, management practices and other factors of the complex soil-plant-atmosphere system (Stöckle *et al.*, 1994). Cropping systems models are powerful tools for investigating the processes related to N movements and transformation in soils and they can be used to provide information as regards the ability of given management practices to increase productivity while minimizing the environmental impact (Stöckle *et al.*, 1994) for increasing the agroecosystem efficiency.

CropSyst (Stöckle *et al.* 2003) is a multi-year, multi-crop simulation model developed to evaluate the effects of soil, weather and management on crop growth and environmental impact. The model is particularly suitable for the simulation of complex cropping systems and can be used for simulating a succession of different crops. Furthermore, several tillage, irrigation and N fertilization options are implemented in this model and a reduced number of inputs are needed with respect to other approaches (Confalonieri and Bocchi, 2005). CropSyst has been tested for simulating crop yields and N balances for different cropping systems and appears promising as a tool to analyze management practices for N. For this study, CropSyst model was used to simulate the soil inorganic N and cotton production in two experimental fields in central and northern Greece.

Materials and methods

In the current study, for the simulation of cotton yield and soil inorganic N in both locations in Greece, CropSyst model was used. CropSyst simulates the soil water budget, soil-plant N budget, crop growth and development, residues production and decomposition, soil erosion by water, and salinity as influenced by weather, soil features, crop characteristics and cropping system management options (Stöckle *et al.*, 2003). The inorganic N budget in CropSyst model includes separate budgets for nitrate and ammonium and the processes used are N transformations (net ammonification, nitrification, and denitrification), ammonium sorption, symbiotic N fixation, crop N demand and crop N uptake (Stöckle *et al*., 2003).

Nitrogen transformations follow the approach presented by Stöckle and Campbell (1989) using first-order kinetics. The amount of organic N mineralized is calculated by the equation:

$$\mathrm{MIN} = \mathrm{MIN_{pot}}\left(\mathrm{MIN} - \mathrm{MF} \times e^{(-\mathrm{MRATE} \times \Delta t)}\right) \quad (1)$$

where MIN (kg N ha^{-1}) is the amount of organic matter N mineralized into ammonium in time t, MIN_{pot} (kg N ha^{-1}) is the potential amount of organic matter N available for mineralization, MF is a soil moisture function and MRATE (day^{-1}) is the mineralization rate constant depending on soil temperature.

The daily amount of nitrified ammonium is estimated by:

$$\mathrm{NIT} = \mathrm{NH_4}\left(1 - e^{(-\mathrm{NRATE} \times \Delta t)}\right) \times \mathrm{MF} \quad (2)$$

where NIT (kg NH_4-N ha^{-1}) is the amount of ammonium N transformed into nitrate in time t, NH_4 (kg NH_4-N ha^{-1}) is the amount of ammonium N available for nitrification, NRATE (day^{-1}) is the nitrification rate constant and MF is the soil moisture function.

The daily amount of denitrification is calculated as follows:

$$DEN = NO_3\left(1 - e^{(-DRATE \times \Delta t)}\right) \quad (3)$$

where DEN (kg NO_3-N ha^{-1}) is the amount of nitrate N transformed into gaseous N in time t, NO_3 (kg NO_3-N ha^{-1}) is the amount of nitrate available for denitrification, DRATE (day^{-1}) is the denitrification rate constant dependent on the denitrification rate value at 15°C.

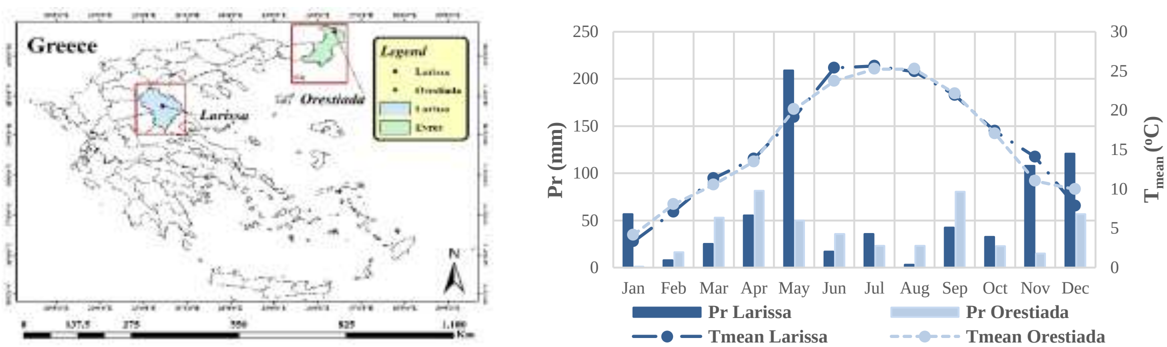
Crop yield is calculated as the total biomass at physiological maturity (B_{PM}) multiplied by the unstressed harvest index (HI).

$$Y = B_{PM} \times HI \quad (4)$$

$$HI = \frac{\text{Harvestable Yield}}{\text{Above Ground Biomass}} \quad (5)$$

where Y (kg ha^{-1}) is the crop yield and B_{PM} (kg ha^{-1}) is the total biomass accumulated at physiological maturity. The harvest index can be adjusted at the calibration phase to account for sensitivity to water and nitrogen stress during flowering and/or grain filling.

Two cotton producing fields were selected to evaluate the CropSyst model's ability to simulate crop yield and soil inorganic N through the crop growing season. The first field was located in Larissa, central Greece, whereas the second field in Orestiada, northern Greece (Figure 1a). Climate parameters of the two areas are also presented in Figure 1b. Soil quality properties of the two fields are presented in Table 1. The field in Larissa was classified as clayey soil throughout its soil profile, whereas the field in Orestiada as a clay loam.



(a) Experimental Fields Mapping **(b) Climate Parameters of the Experimental Fields**

Figure 1. (a) Location of the experimental fields in central and northern Greece; (b) Monthly precipitation (Pr, mm) and mean temperature (T_{mean}, °C) of the experimental fields.

Table 1. Soil physical and chemical properties of the fields in Larissa and Orestiada.

	Sand (%)	Silt (%)	Clay (%)	pH	OM (%)	CEC ($cmol_c$/kg)	EC_e (dS/m)	Olsen-P (mg/kg)	Exch. K (mg/kg)
Larissa									
0-30 cm	7.6	36.5	55.9	8.0	2.2	39.1	0.77	15.9	274.4
30-60 cm	8.9	33.4	57.7	8.2	1.3	37.8	0.76	3.3	142.2
60-90 cm	10.7	31.9	57.5	8.5	0.9	36.5	0.74	0.8	122.3
Orestiada									
0-30 cm	34.0	37.2	28.8	7.6	1.2	41.1	0.43	9.6	142.8
30-60 cm	32.0	35.2	32.8	7.5	0.7	39.1	0.33	2.2	112.6
60-90 cm	26.0	37.2	36.8	7.6	0.7	38.6	0.31	1.5	155.8

Cotton production and soil inorganic N fluctuations in Larissa were simulated for the 2019 growing season. Cotton seeding was on the 4th of May 2019 and harvest on the 16th of October 2019. The total amount of fertilizer N applied was 149.7 kg ha^{-1} (Table 2). The total amount of precipitation and irrigation water applied during the growing season was 575 mm. Soil samples were collected on May 20, July 1, August 20 and September 23, 2019, at three sampling depths (0-30, 30-60 and 60-90 cm).

Cotton production and soil inorganic N fluctuations in Orestiada were simulated for the 2020 growing season. Cotton seeding was on the 30th of April 2020 and harvest on the 16th and 23rd of October 2020. The total amount of fertilizer N applied was 201.0 kg ha^{-1} (Table 2). The total amount of precipitation and irrigation water applied during the growing season was 445 mm. Soil samples were collected on May 18, July 21 and October 23, 2020, at three sampling depths (0-30, 30-60 and 60-90 cm).

In both cases, the collected soil samples were air-dried, ground, sieved (2 mm) and analyzed for NH_4-N and NO_3-N by extraction with KCl (2M) and spectrophotometric determination. Soil inorganic N was calculated as the sum of NH_4-N and NO_3-N. Cotton seed fresh yield was determined at harvest.

Table 2. Fertilizer N application to cotton fields (dates, rates and methods of application): in Larissa (growing season 2019) and Orestiada (growing season 2020).

	Larissa			*Orestiada*	
Date	Rate (kg N ha^{-1})	Method	Date	Rate (kg N ha^{-1})	Method
30-Apr-19	45	Surface broadcasting	23-Apr-20	86	Surface broadcasting
14-Jun-19	31.3	Fertigation	25-Jun-20	115	Surface broadcasting
14-Jul-19	44.4	Fertigation			
22-Jul-19	19	Fertigation			
29-Jul-19	10	Fertigation			

Results and discussion

Cotton yield was well simulated in both locations by the CropSyst model (Figure 2). In both cases, cotton yield simulated values were slightly underestimated (by less than 1%), compared to the observed ones.

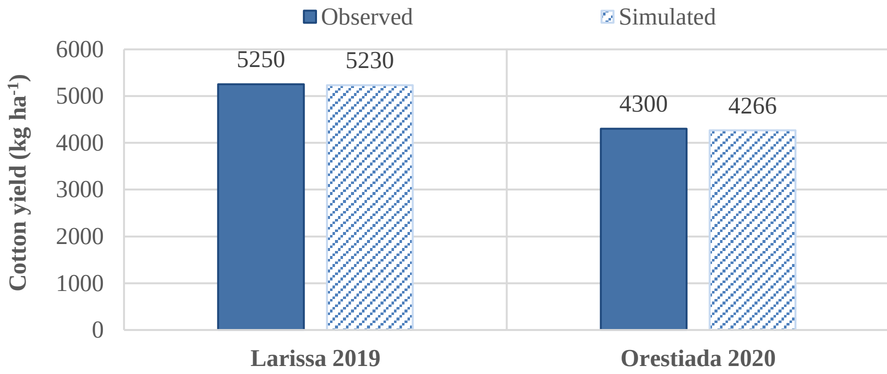
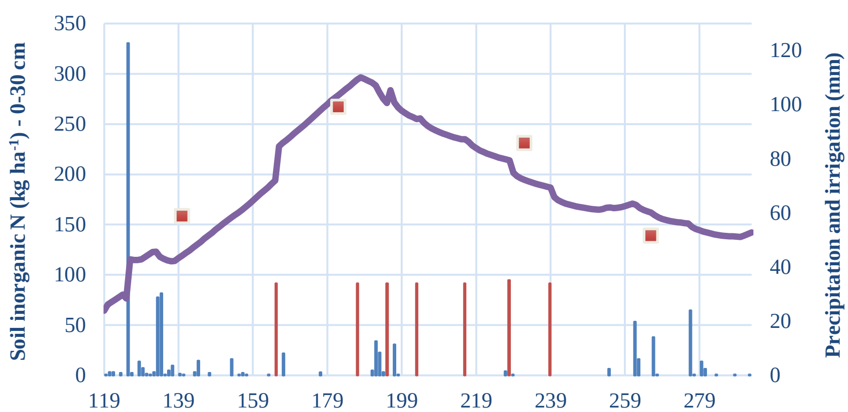


Figure 2. Cotton yield in Larissa, 2019 (left) and Orestiada, 2020 (right); observed and simulated values by the CropSyst model.

As far as the soil inorganic N fluctuations through the soil profile during the crop growing season in Larissa 2019, it can be seen in Figure 3 that the model simulated well the soil inorganic N within the top 30 cm depths. In lower depths (30-90 cm), however, model simulation overestimated, in comparison to the measured value, soil inorganic N towards the end of the growing season indicating higher potential for N losses over time.

Model simulation of soil inorganic N fluctuations through the soil profile during the cotton growing season in Orestiada 2020 (Figure 4) showed a tendency to overestimate the soil inorganic N content within the topsoil (0-30 cm) during the period of high cotton N requirements. Since crop yield was well simulated by the model, this finding may probably be attributed to an underestimation of N losses (predominantly through ammonia volatilization and/or nitrate leaching) during this period, especially taking into consideration that the amount of fertilizer N applied was rather high (201 kg N ha^{-1}), well above cotton N requirements.



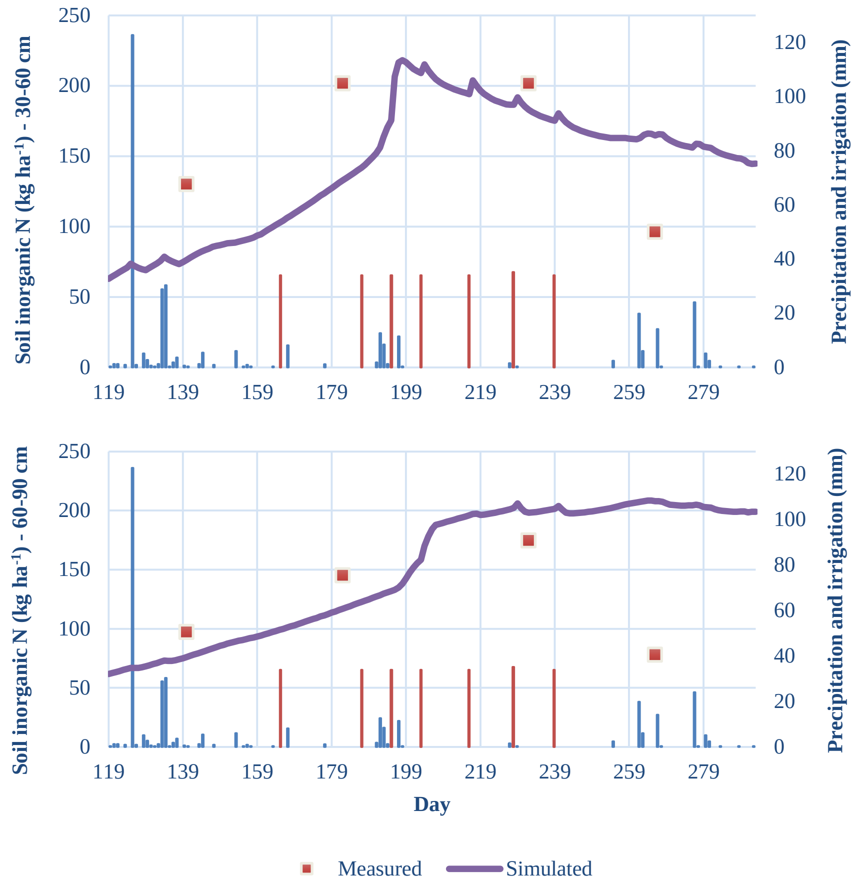
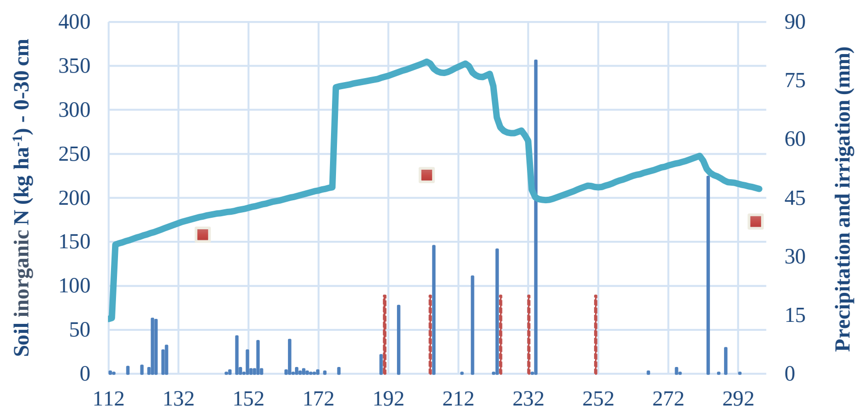


Figure 3. Soil inorganic N (sum of NH_4-N and NO_3-N) within the 0-30 cm depths (top), 30-60 cm depths (middle) and 60-90 cm depths (bottom), during cotton growing season in Larissa, 2019; measured and simulated values by the CropSyst model. The blue bars show precipitation, whereas the red bars irrigation water applied.



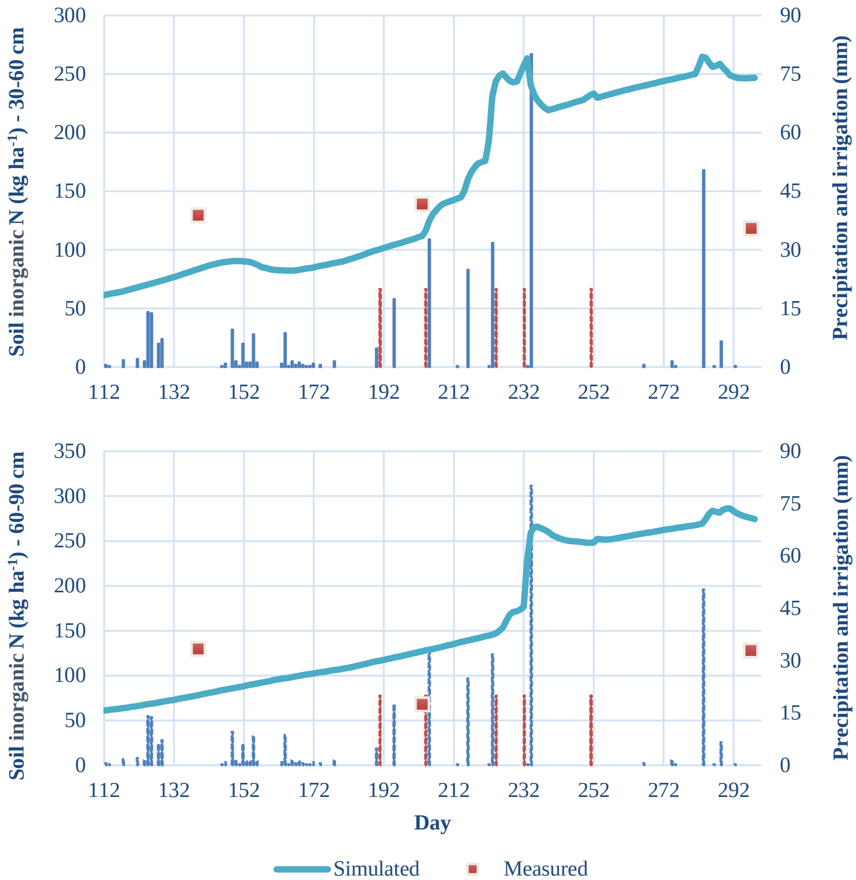


Figure 4. Soil inorganic N (sum of NH_4-N and NO_3-N) within the 0-30 cm depths (top), 30-60 cm depths (middle) and 60-90 cm depths (bottom), during cotton growing season in Orestiada, 2020; measured and simulated values by the CropSyst model. The blue bars show precipitation, whereas the red bars irrigation water applied.

As in the case of Larissa, also in Orestiada in lower depths (30-90 cm) soil inorganic N simulation showed higher values, than the measured ones, towards the end of the growing season indicating higher potential for N losses over time. The mismatch between measured and simulated soil inorganic N values towards the end of the growing season within the 30-90 cm depths may not necessarily mean that the model overestimated the total amount of N losses as some of these losses may have occurred earlier in the growing season (e.g. in the form of ammonia volatilization). Also, it is possible the simulated higher N losses potential to be partially attributed to the model simulation of the N mineralization due to soil incorporation of cotton residues after harvest.

In general, although differences between measured and simulated values were observed in some cases, the distribution of soil inorganic N during the crop growing season was simulated satisfactorily by CropSyst model in both locations.

Conclusion

In this study CropSyst model was used to simulate cotton yield and soil inorganic nitrogen under high nitrogen inputs in two locations in central and northern Greece. Cotton yield was well simulated in both locations. Soil inorganic N distribution during the crop growing season was generally satisfactorily simulated in both locations. Thus, CropSyst model can be considered as an important tool to assess the environmental impact and agronomic benefit of fertilizer N applications to irrigated areas under Mediterranean conditions.

Acknowledgement

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References

Acutis, M., Ducco, G. and Grignani, C. (2000). Stochastic use of LEACHN model to forecast nitrate leaching in different maize cropping systems, European Journal of Agronomy, Vol. 13, 191-206.

Confalonieri, R., Gusberti, D., Bocchi, S. and Acutis, M. (2006). The CropSyst model to simulate the N balance of rice for alternative management, Agronomy for Sustainable Development, Vol. 26 (4), 241-249.

Sinclair, T. R. and Seligman, N. (2000). Criteria for publishing papers on crop modelling, Field Crops Research, Vol. 68, 165-172.

Stöckle, C. O. and Campbell, G. S. (1989). Simulation of crop response to water and nitrogen: an application example using spring wheat, Transactions of ASAE, Vol. 32, 66-74.

Stöckle, C. O., Donatelli, M. and Nelson, R. L. (2003). CropSyst: a cropping systems simulation model, European Journal of Agronomy, Vol. 18, 289-307.

Stöckle, C. O., Martin, S. A. and Campbell, G. C. (1994). CropSyst, a cropping systems simulation Model: water/nitrogen budgets and crop yield, Agricultural Systems, Vol. 46, 335-359.

Todorovic M., Albrizio, R., Zivotic, L., Abi Saab, M.-T., Stöckle, C. O. and Steduto, P. (2009). Assessment of AquaCrop, CropSyst, and WOFOST Models in the simulation of sunflower growth under different water regimes, Agronomy Journal, Vol. 101, 509-521.

van Ittersum, M. K. and Donatelli, M. (2003). Modelling cropping systems - highlights of the symposium and preface to the special issues, European Journal of Agronomy, Vol. 18, 187-197.

VULNERABILITY ASSESSMENT OF SOIL SALINIZATION BY IRRIGATION WITH SALINE WATER: APPLICATION IN THE COASTAL AREA OF RHODOPE, NE GREECE

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Abstract

The water scarcity has led to a significant decline of water table in many coastal aquifers of the Mediterranean basin. This has also led to salinization of groundwater reserves due to seawater intrusion, which will eventually affect the irrigated agricultural land due to salt accumulation by irrigation with saline waters. To cope with this problem, MEDSAL Project (www.medsal.net) aims to evaluate the vulnerability of soil to salinization by irrigation with saline waters through its intrinsic ability to leach salts via water percolation below the root zone with the aid of precipitation. The specific approach is based on the existing formula LOSW-P, which evaluates the precipitation water losses by percolation below the root-zone of 30 cm from a theoretical reference crop surface considering soil physical properties, topography and mean long-term climate conditions. The application of LOSW-P was performed for the Rhodope pilot area of MEDSAL project, as well as all for the total of irrigated lands in Greece. The results showed that ~80% of irrigated lands (including Rhodope) present LOSW-P values <100 mm·year^{-1} (only through precipitation), which are relatively low considering that these regions require irrigation rates >600 mm·year^{-1}. At least for the case of Greece, the proposed methodology of the MEDSAL project indicated that the use of water with relatively higher salinity may not be a solution to most irrigated lands (including Rhodope coastal area) and further solutions shall be evaluated.

Keywords: *soil vulnerability to salinization, MEDSAL project, Greece.*

Introduction

The water scarcity, mainly caused by droughts and overexploitation of the available groundwater recourses used for irrigation and domestic use has led to a significant decline of water table in many coastal aquifers of the Mediterranean basin. This phenomenon is anticipated to be further impacted by the foreseen climate change in the forthcoming years (Stigter *et al*., 2014; Martín-Arias *et al*., 2020). As a result of the water scarcity in coastal areas, the salinization of groundwater reserves due to seawater intrusion and/or other potential factors (e.g. trapped saline lenses, evaporitic formations, hydrothermal brines, etc) will eventually affect the soil resources of the irrigated agricultural land due to salt accumulation by irrigation. The use of brackish/saline water for irrigation creates consequent threats for agricultural soils due to the increase of their salinity-sodicity, which could eventually be a step towards a consequent desertification of Mediterranean agricultural lands (Okur & Örçen, 2020).

In the context of MEDSAL Project (www.medsal.net), which aims to provide holistic approaches for securing the availability and quality of groundwater reserves in the Mediterranean coastal areas, the additional component of soil quality is also highly regarded, since the afore described pressures and the imminent climate change has already led in some cases and will eventually lead to further use of brackish/saline water resources for irrigation. To efficiently cope with the adverse effects of this phenomenon, a suite of methodological steps and actions should be carefully developed structuring proactive management approaches, which could prevent and/or minimize the impact of soil salinity. The first step of such approaches, which is the subject of this work, is to propose a method for evaluating the intrinsic ability of agricultural land to leach salts via water percolation below the root zone with the aid of precipitation. This approach is based on the assumption that the less water is percolated below the root zone due to precipitation, the less salt is leached below the root zone and therefore the more vulnerable is the soil to salt accumulation due to irrigation with brackish/saline waters. The specific approach is based on the existing formula LOSW-P (Aschonitis *et al.*, 2012), which was developed to assess the intrinsic vulnerability of agricultural land to water losses by percolation below the root-zone of 30 cm of a theoretical reference crop (Penman-Monteith concept) considering soil physical properties, topography and mean long-term climate conditions.

Material and methods

The proposed LOSW-P methodology considers a uniform surface of actively dense cover with clipped perennial grass, whose rates of evapotranspiration under non-water stress conditions are equivalent to the reference crop evapotranspiration of American Society of Civil Engineers (ASCE)-standardized/Food Agriculture Organization of the United Nations (FAO)-56 concept (Allen *et al.*, 2005). The use of grass as a reference surface is used to remove the effect of different land uses. The LOSW-P index was calibrated based on the inputs and outputs of simulation scenarios performed by the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) model (Knisel and Davis, 2000). The simulations covered: (i) Different soils with respective hydraulic characteristics of the four hydrological soil types A, B, C and D of USDA–NRCS (2007); (ii) Different topography by using different slopes of grass surfaces using the curve number vs. the slope of the grass surface relationship of Getter *et al.* (2007); (iii) Different climate conditions from Greece, Italy and USA; (iv) Irrigation or non-irrigation conditions. Irrigation was applied automatically by the GLEAMS model when the soil moisture was falling to 20% of available soil moisture (ASM) in order to return it to 100% ASM corresponding to field capacity conditions. All scenarios were performed for the first 30 cm of the soil profile, which included the largest percentage of the rootzone. The results were used to develop and calibrate LOSW-P index, which describe annual water losses by percolation below the 30 cm according to the following equation (Aschonitis *et al.*, 2012):

$$LOSW\text{-}P = \begin{Bmatrix} 0.0941\sqrt{K_s} - \ 0.761\sqrt{SL} + 0.4185\sqrt{P} \\ - \ 0.0487\sqrt{ET_o} + 0.0903\sqrt{IR} \end{Bmatrix}^2 \tag{1}$$

where *LOSW-P* is the annual water losses by percolation under the 30 cm of a reference grass (mm $year^{-1}$), K_s is saturated hydraulic conductivity (mm day^{-1}), *SL* is soil surface slope (%), *P* is annual precipitation (mm $year^{-1}$), ET_o is the annual reference evapotranspiration (mm $year^{-1}$) and *IR* is the annual irrigation to cover the deficit of reference evapotranspiration (mm $year^{-1}$). It has to be noted that when the formula inside the brackets of Eq.1 leads to negative values, the value of *LOSW-P* is set equal to 0. The above equation can be used either for $IR = 0$ or $IR \neq 0$. For the latter case, the annual irrigation *IR* for covering the deficit of annual reference evapotranspiration is calculated as follows (Aschonitis *et al*., 2013):

$$IR = \sum_{1}^{12} IR_i \text{ , where } IR_i = ET_{o,i} - P_i \text{ when } ET_{o,i} > P_i \text{ else } IR_i = 0 \qquad (2)$$

where *IR* is the annual irrigation required for covering the deficit of annual reference evapotranspiration (mm year^{-1}), IR_i is the monthly irrigation for covering the deficit of reference evapotranspiration of the month i (mm month^{-1}), $ET_{o,i}$ is the monthly reference evapotranspiration (mm month^{-1}) (estimated in this study by FAO-56 method), P_i is the monthly precipitation and i is the month. The use of larger *IR* values from the values of Eq.2 for over-irrigation analysis using Eq.1 is not indicated. In this study Eq.1 was applied for *IR*=0 in order to assess the amount of precipitation water (clean water) that percolates below the root zone and is responsible for salt leaching.

The ET_o is estimated using the ASCE-standardized method (former FAO-56) for short reference crop by the following function (Allen *et al*., 2005):

$$ET_o = \frac{0.408\Delta(R_n - G) + \dfrac{\gamma u_2(e_s - e_a)C_n}{(T + 273.16)}}{\Delta + \gamma(1 + C_d u_2)} \qquad (3)$$

where ET_o is the reference crop evapotranspiration (mm d^{-1}), R_n is net radiation at the crop surface (MJ m^{-2} d^{-1}), u_2 is mean daily wind speed at 2 m height (m s^{-1}), T is the mean daily air temperature (°C), G is soil heat flux density at the soil surface (MJ m^{-2} d^{-1}), e_s is the mean daily vapor pressure (kPa), e_a is the mean daily actual vapor pressure (kPa), Δ is the slope of the saturation vapor pressure–temperature curve (kPa °C^{-1}), γ is the psychrometric constant (kPa °C^{-1}) and C_n and C_d are constants of 900 and 0.34, respectively.

The LOSW-P for the annual water losses by percolation under the 30 cm of a reference grass was estimated using the following four databases:

- The database of Hijmans *et al.* (2005) provides gridded data of mean monthly precipitation P and mean monthly temperature T for the period 1950–2000 (WorldClim version 1.2) at 30 arc-sec (~1×1 km) spatial resolution. Their mean annual values are given in Fig.1a,b, respectively.
- The database of Aschonitis *et al.* (2017) (10.1594/PANGAEA.868808) provides gridded data of mean monthly reference evapotranspiration ET_o (Eq.3) of the period 1950–2000 at 30 arc-sec (~1×1 km) spatial resolution (Fig.1c) (this database is built using temperatures from the WorldClim version 1.2 database). Using the ET_o and precipitation, the irrigation map of the reference crop *IR* is built according to Eq.2 (Fig.1d).
- The surface slope (Fig.1e) was obtained by the digital elevation model of GTOPO30 (pixel analysis of 30 arc-sec, ~1×1 km) as it is given by the USGS (United States Geological Survey).
- The European Soil Database provided by the European Commission Joint Research Centre (Hiederer *et al*., 2013) provides soil data (% sand, % silt, % clay, % gravel, % organic carbon) with spatial analysis (~1×1 km). These data are used to estimate the saturated hy-draulic conductivity Ks according to the respective pedotransfer function (PTF) of Saxton and Rawls (2006), taking into account the gravel and organic matter effect (Fig.1f).
- The Corine Land Cover 2018 (https://land.copernicus.eu/pan-european/corine-land-cover/clc2018) was used to derive irrigated lands.

The application of LOSW-P formula was performed for the Rhodope pilot area of MEDSAL project, as well as all for the total of irrigated lands in Greece for performing comparisons at national scale.

Results and discussion

The *LOSW-P* ranged between 21-241 mm·year^{-1} (mean annual water losses by percolation only by precipitation) with an average value of 82 mm·year^{-1} at country level, based on all irrigated lands of Greece. The respective values of *LOSW-P* for the coastal region of Rhodope ranged between 31-91 mm·year^{-1} with an average value of 81 mm·year^{-1}, which approximates the average value of *LOSW-P* at a country level. The respective irrigation requirements for covering the reference crop evapotranspiration after removing precipitation ranged between 320-1122 mm·year^{-1} at country level and 645-676 mm·year^{-1} for Rhodope region (Fig.2).

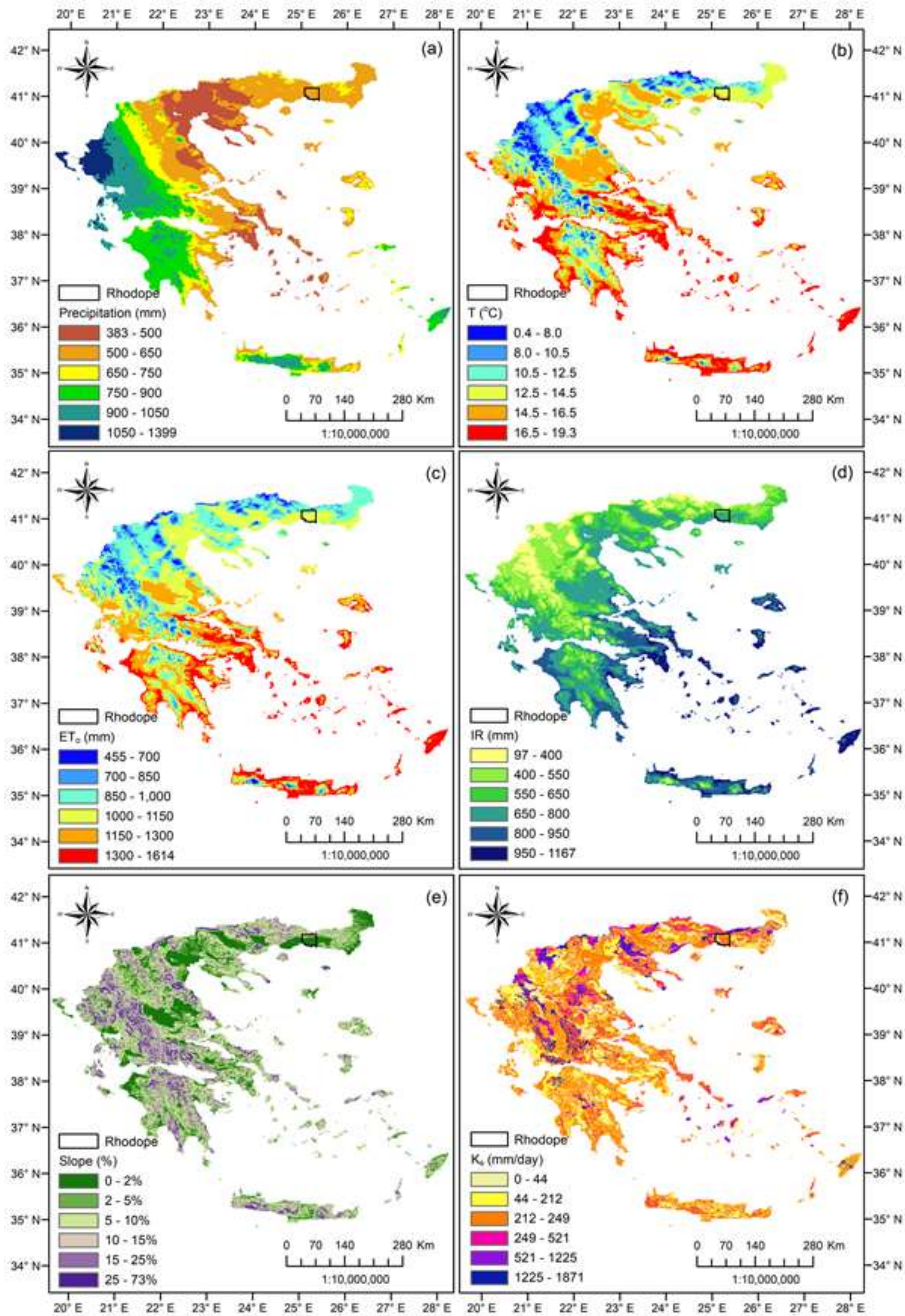
(a)
Rhodope
Precipitation (mm)
383 - 500
500 - 650
650 - 750
750 - 900
900 - 1050
1050 - 1399
(b)
Rhodope
T (°C)
0.4 - 8.0
8.0 - 10.5
10.5 - 12.5
12.5 - 14.5
14.5 - 16.5
16.5 - 19.3
(c)
Rhodope
ETo (mm)
455 - 700
700 - 850
850 - 1,000
1000 - 1150
1150 - 1300
1300 - 1614
(d)
Rhodope
IR (mm)
97 - 400
400 - 550
550 - 650
650 - 800
800 - 950
950 - 1167
(e)
Rhodope
Slope (%)
0 - 2%
2 - 5%
5 - 10%
10 - 15%
15 - 25%
25 - 73%
(f)
Rhodope
Ks (mm/day)
0 - 44
44 - 212
212 - 249
249 - 521
521 - 1225
1225 - 1871
0 70 140 280 Km
1:10,000,000
20° E 21° E 22° E 23° E 24° E 25° E 26° E 27° E 28° E
42° N 41° N 40° N 39° N 38° N 37° N 36° N 35° N 34° N

Figure 1. Mean annual values of (a) precipitation, (b) temperature, (c) reference evapotranspiration according to ASCE-standardized for short reference crops, (d) irrigation required for covering the deficit of annual reference evapotranspiration for the period 1950–2000, (e) surface slope and (f) saturated hydraulic conductivity in Greece.

The results of the study showed that ~80% of irrigated lands of Greece (including Rhodope) present *LOSW-P* values <100 mm·year^{-1} (Fig.2), which are relatively low considering that these regions require irrigation rates >600 mm·year^{-1} (Fig.1d). This indicates that the use of high salinity waters at amounts larger than 500 mm·year^{-1} would increase the soil salinity due to the inadequate salt leaching by precipitation water. The problem of inadequate salt leaching is expected to be more intense in soils with higher percentages of clay and lower values of saturated hydraulic conductivity. For these regions, the LOSW-P maps should also be combined with current soil salinity maps and water salinity monitoring of available water sources for irrigation (groundwater and reclaimed wastewater) in order to build integrated water resources management for combatting soil desertification by the salt accumulation by irrigation.

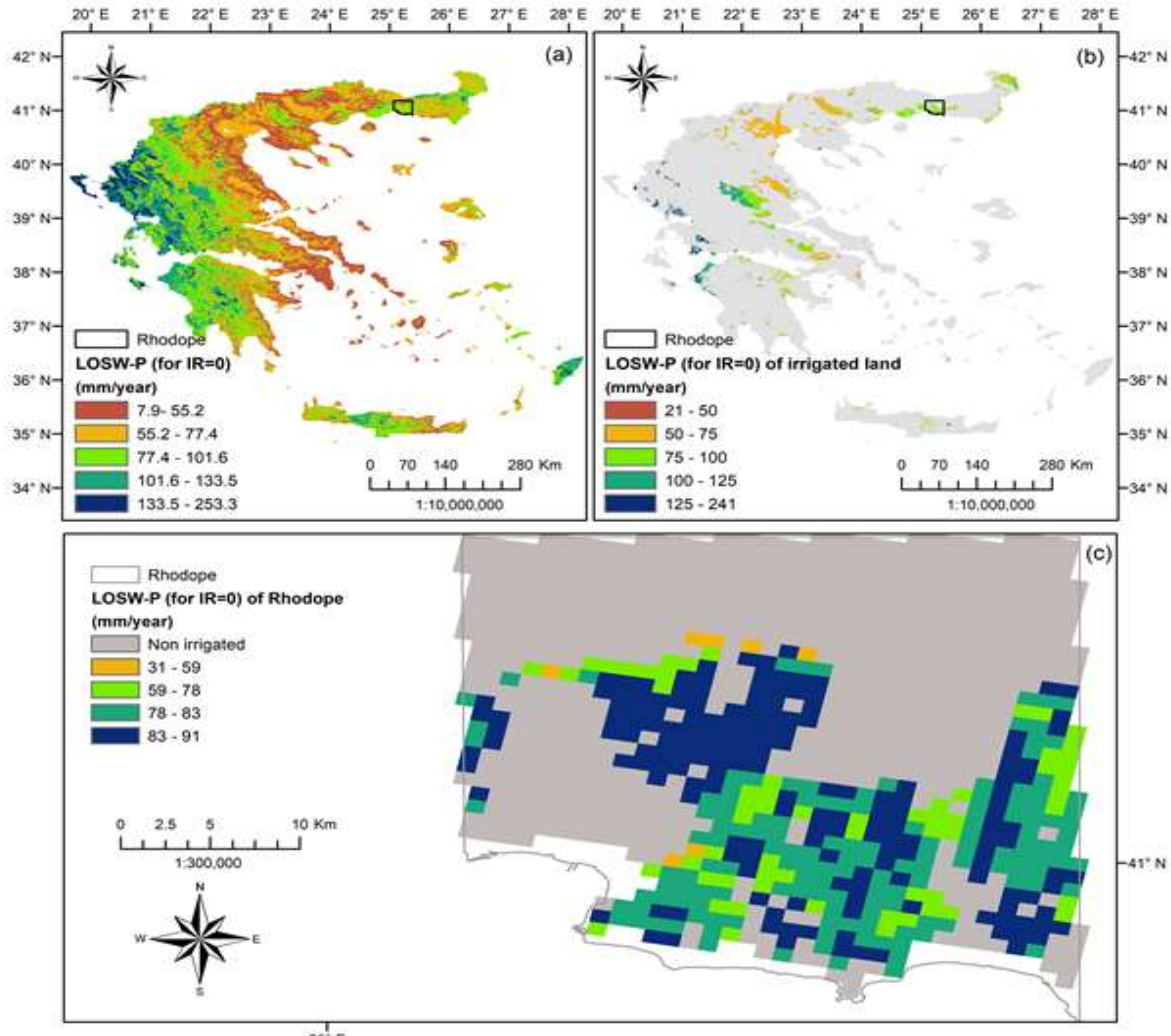

Figure 2. Mean annual values of (a) *LOSW-P* (for *IR*=0) for the whole Greece, (b) *LOSW-P* (for *IR*=0) for all the irrigated lands of Greece, (c) *LOSW-P* (for *IR*=0) for all the irrigated lands of Rhodope.

Conclusions

In this study, the *LOSW-P* methodology was proposed in the context of MEDSAL project for evaluating the intrinsic ability of agricultural land to leach salts via water percolation below the root zone with the aid of precipitation. At least for the case of Greece, the proposed methodology indicated that the use of water with relatively higher salinity may not be a solution to most irrigated lands (including Rhodope coastal area) due to the low *LOSW-P* values and further solutions shall be evaluated.

Acknowledgement

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References

Allen R.G., Walter I.A., Elliott R., Howell T., Itenfisu D., Jensen M. (2005) The ASCE Standardized Reference Evapotranspiration Equation; Final Report (ASCE-EWRI); Environmental and Water Resources Institute, Task Committee on Standardization of Reference Evapotranspiration of the Environmental and Water Resources Institute: Reston, VA, USA.

Aschonitis V.G., Mastrocicco M., Colombani N., Salemi E., Kazakis N., Voudouris K., Castaldelli, G. (2012). Assessment of the intrinsic vulnerability of agricultural land to water and nitrogen losses via deterministic approach and regression analysis. Water Air Soil Poll., 223: 1605-1614.

Aschonitis V.G., Salemi E., Colombani N., Castaldelli G., Mastrocicco, M. (2013). Formulation of indices to describe intrinsic nitrogen transformation rates for the implementation of best management practices in agricultural lands. Water Air Soil Poll., 224: art.no. 1489.

Aschonitis V.G., Papamichail D., Demertzi K., Colombani N., Mastrocicco M., Ghirardini A., Castaldelli G., Fano E.-A. (2017). High-resolution global grids of revised Priestley-Taylor and Hargreaves-Samani coefficients for assessing ASCE-standardized reference crop evapotranspiration and solar radiation. Earth Syst. Sci. Data, 9: 615-638.

Getter K.L., Rowe D.B., Andresen J.A. (2007). Quantifying the effect of slope on extensive green roof stormwater retention. Ecol. Eng., 31: 225-231.

Hiederer R. (2013). Mapping Soil Properties for Europe - Spatial Representation of Soil Database Attributes; EUR26082EN Scientific and Technical Research Series; Publications Office of the European Union: Luxembourg.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. (2005). Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25: 1965-1978.

Knisel W.G, Davis F.M. (2000). GLEAMS, Groundwater Loading Effects from Agricultural Management Systems V3.0; Publ. No. SEWRL-WGK/FMD-050199; U.S.D.A.: Tifton, GA, USA.

Martín-Arias J., Martínez-Santos P., Andreo B. (2020). Modelling the effects of climate change and population growth in four intensively exploited Mediterranean aquifers. The Mijas range, southern Spain. Journal of Environmental Management, 262: art. no. 110316.

Okur B., Örçen N. (2020). Chapter 12 - Soil salinization and climate change. Editor(s): Majeti Narasimha Vara Prasad, Marcin Pietrzykowski, Climate Change and Soil Interactions, Elsevier, pp. 331-350.

Saxton K.E., Rawls W.J. Soil water estimates by texture and organic matter for hydrologic solutions. Soil Sci. Soc. Am. J. 2006, 70, 1569–1578, doi:10.2136/sssaj2005.0117.

Stigter, T.Y., Nunes, J.P., Pisani, B., Fakir, Y., Hugman, R., Li, Y., Tomé, S., Ribeiro, L., Samper, J., Oliveira, R., Monteiro, J.P., Silva, A., Tavares, P.C.F., Shapouri, M., Cancela da Fonseca, L., El Himer, H., 2014. Comparative assessment of climate change and its impacts on three coastal aquifers in the Mediterranean. Regional Environmental Change, 14 (SUPPL.1), pp. 41-56.

USDA–NRCS. National Engineering Handbook; Hydrologic Soil Groups, (210–VI–NEH); NRCS: Washington, DC, USA, 2007; Chapter 7.

EVALUATION OF WINTER WHEAT YIELD USING SATELLITE IMAGERY

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Abstract

Estimating biomass or crop yield can improve farm management and optimize precision agriculture applications. Satellite data provides good information from the farms. In this regard, this research was aimed at finding a fast method with acceptable accuracy in order to predict the yield of winter wheat by using NDVI and LAI indices on the five farms located in Neyshabur, Khorasan Razavi province. The MODIS images of the TERRA satellite were conducted during the years 2012-2013 to 2014-2015. The results showed that there was a high correlation between NDVI and LAI indices (88 to 99%) over the years. The correlation between the observed performances with the LAI index varied between 2012-2013 and 2014-2015 so that in some years there was a strong correlation and in some years, a weak correlation. The correlation coefficient between observed wheat yield and NDVI index was 85.63, 15, and 48% in 2012-2013, 2013-2014 and 2014-2015 respectively. Results showed that vegetation indices changed in different fields and at different times during the growing season, so that the trend of changes in the indexes increased with approaching June and then decreased with approaching the wheat harvest time. Based on the results of this study, the sum of squared errors (RMSE) for the 10 calibrated farms was calculated to be 1.38 ton/ha, and the total squared errors (RMSE) was validated for 5 farms equal to 1.45 ton/ha. Based our results, wheat yield can be well predicted using regression between vegetation indices in flowering and filling stage and yield using regression analysis.

Key words: *Yield estimation, Remote sensing, Leaf area index, Normalized difference vegetation Index,*

Introduction

Crop growth and yield information are critical for the government in guiding and readjusting the macro planting system and improving agricultural operation and management (Guo, 2007). The Global population continues to rise and will reach between 7.4 and 10.6 billion people by the year 2050 (United Nations, 2003). With the growing population and rising per-capita consumption, the demand for cereals and grains is expected to increase 1.3% per year (Duvick and Cassman, 1999). Wheat is one of the most important staple crops and is widely grown throughout the world, mainly in Asia, Europe, and America (Sui et al., 2018). Remote sensing data, owing to their non-contact, timely, and repetitive coverage at a regional scale, provides the opportunity for crop-production estimation and growth monitoring prior to harvest (Thorp et al., 2012). Normalized Difference Vegetation Index (NDVI) is the most extensively used parameter to statistically correlate to crop growth and yield for spring and winter wheat, corn, rice, sorghum and soybeans across the world, such as the USA, Brazil, Argentina, Greece, Morocco, Zimbabwe, China, Mongolia, India, Poland and other countries (Groten, 1993; Gumma et al., 2014; Kogan et al., 2012; Maselli et al., 1993; Rasmussen, 1992; J.H. Zhang et al., 2014). NDVI is the most used for the yield estimation. Compared with the leaf area index (LAI) and the fraction of photosynthetically active radiation (FPAR), the NDVI is more relevant to the potato yield (ITA, 2002) and (Jiao et al., 2014) built a regression model between the regional yield and the accumulated MODIS NDVI at the booting–heading stage of winter wheat, and the wheat yield was well estimated with the relative error of 5%. (Becker-Reshef et al., 2010). LAI is a key biophysical variable influencing land surface processes such as photosynthesis, transpiration, and energy balance and is a required input for various ecological models (Bonan, 1993). Either direct or indirect methods have been used to estimate in situ LAI. Assessment of crop leaf area index (LAI) in agricultural landscapes is of importance for addressing various agricultural issues such as: crop growth monitoring, vegetation stress, crop forecasting, yield predictions and management practices. Indeed, LAI is a canopy biophysical variable that plays a major role in vegetation physiological processes.

Huang et al. (Conway, and Toenniessen, 1999) assimilated the LAI derived from Landsat thematic map (TM) and MODIS data into the world food studies (WOFOST) crop growth model for predicting the regional-scale winter wheat yield of China's Hebei Province, and the improved predicting accuracy with the root mean square error (RMSE) of 151.92 kg/ha was obtained. (Sui et al., 2018). Therefore, the current research aims to evaluate the potential of MODIS-derived measures of greenness and leaf area index (LAI), and information related to the actual yield of wheat to estimate wheat production and yield in the region of Neyshabour as a case study.

Material and methods

Cereal production and yield at a national scale from 2012–2015 were obtained from the Ministry of Agriculture Jahad (https://www.maj.ir/Index.aspx). Wheat yields were measured as tons per hectare of land harvested regionally and in different farms, and the total harvest is calculated accordingly. The Study area located between 58°13'N to 59º3′N Latitude and 35º4′E to 36º39′E Longitude. The maximum and minimum elevation of the basin is 1520 m and 1213 m above MSL, respectively. The weather data were collected at the weather station by the Iran Meteorological Administration and made available on the Islamic republic of Iran Meteorological Organization (http://irimo.ir). They included: temperature, rainfall, relative humidity, and wind speed. The performance of the 5 farms was measured (Table 1).

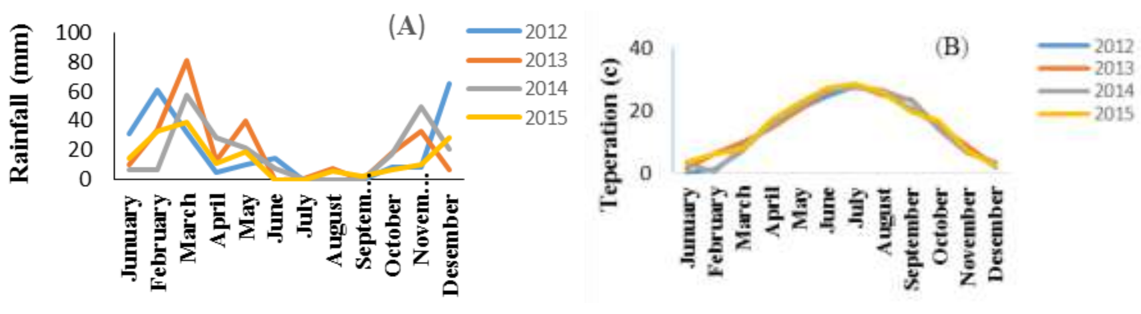


Fig 1. 365 days mean rainfall (A) temperatures (B) for the Neyshabur study site, for the wheat crop cycles December to 30 November) of 2012 (blue), 2013 (brick red), 2014 (Gray), 2015 (Red).

Table 1. Wheat yield in 5 farms during 2012-2013 till 2014-2015 crop years.

Farms number	Owners	Yields (Tons/ hectare)			Area cultivation (hectares)
		2012-2013	2013-2014	2014 -2015	
1	Site 1	3.7	6	4	50
2	Site 2	3	3.5	4	70
3	Site 3	5.7	5.3	5.7	13
4	Site 4	4	4.5	5	50
5	Site 5	4	4.5	5	50

Results and discussion

The results showed that LAI index had a correlation coefficient of 73% with yield in 2012-2013 crop year (Fig. 2-A). The correlation between the LAI index and yield in the crop year of 2013-2014 and 2014-2015 was 8.03 and 26.73.7%, respectively (Fig. 2-B, and 2-C). Sarvad et al. (2014), reported a positive and significant correlation between leaf area index and grain yield and biological yield. In general, it can be stated that the correlation between the LAI index and the observed performance was variable, which may be due to farm management quality such as fertilizer management, irrigation or weed management and weather condition in different years.

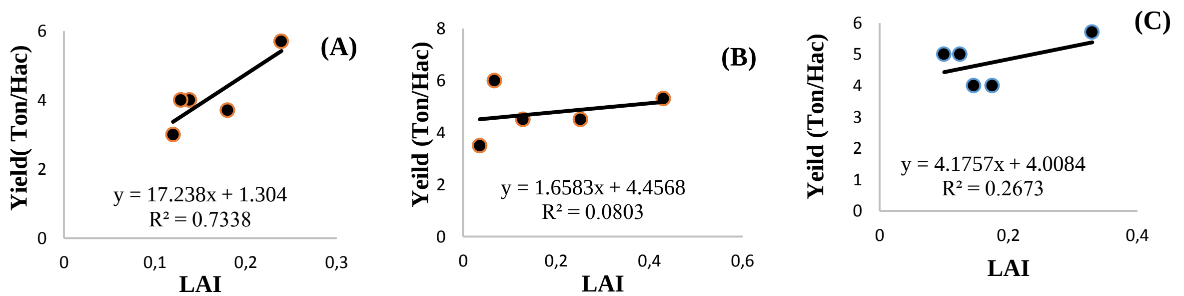


Fig. 2. The regression between the LAI and yield in three crop years, (A) 2012-2013, (B) 2013-2014, (C) 2014-2015.

The correlation between NDVI and yield in the growing season 2012-2013 showed that in this time, the correlation was 85.63% (Fig. 3-A). Although, in the other years, the correlation was not high. For example: in crop year 2013-2014 between NDVI and yield was negative (-15%), While in the growing season of 2014-2015 the index showed a correlation of 48% with yield (Figures 3-B and 3-C). The images used in the flowering stage of wheat had a high correlation between spectral indices and wheat biomass.

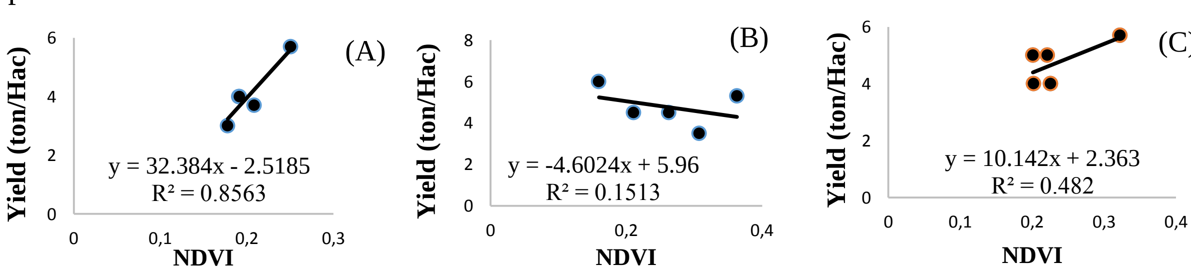


Fig. 3. The regression between the NDVI and yield (A). 2012-2013. (B) 2013-2014. (C) 2014-2015.

The study of regression between vegetation index indicates that a strong linear relationship was been established between LAI and NDVI in May to July. By examining the regression relations between vegetation indexes within 2012 to 2015 years, it can be argued that these indicators have a good relationship with each other. For example: In 2012-2013, the correlation between these two indicators was between 88 to 92 percent. As well as, in 2013-2014, the range of correlations was between 92 till 99 percent which reflects the high correlation between these two indicators. However, in 2014-2015, the high correlation between the indicators was between 98 to 99 percent. In general, the trend of changes in vegetation indices shows that, as the flower season of winter wheat approaches, the vegetative index has increased and as we approach the harvesting season, the trend is decreasing (Fig. 4).

Zhao et al (2011) revealed that renormalized difference water index (RDWI) and MODIS near-infrared reflectance had the highest correlation with yield at grain-filling stages. Thus, considering that June is the season of flowering and filling of wheat in Neyshabur region, the high correlation between LAI and NDVI obtained at this stage not far from expectation.

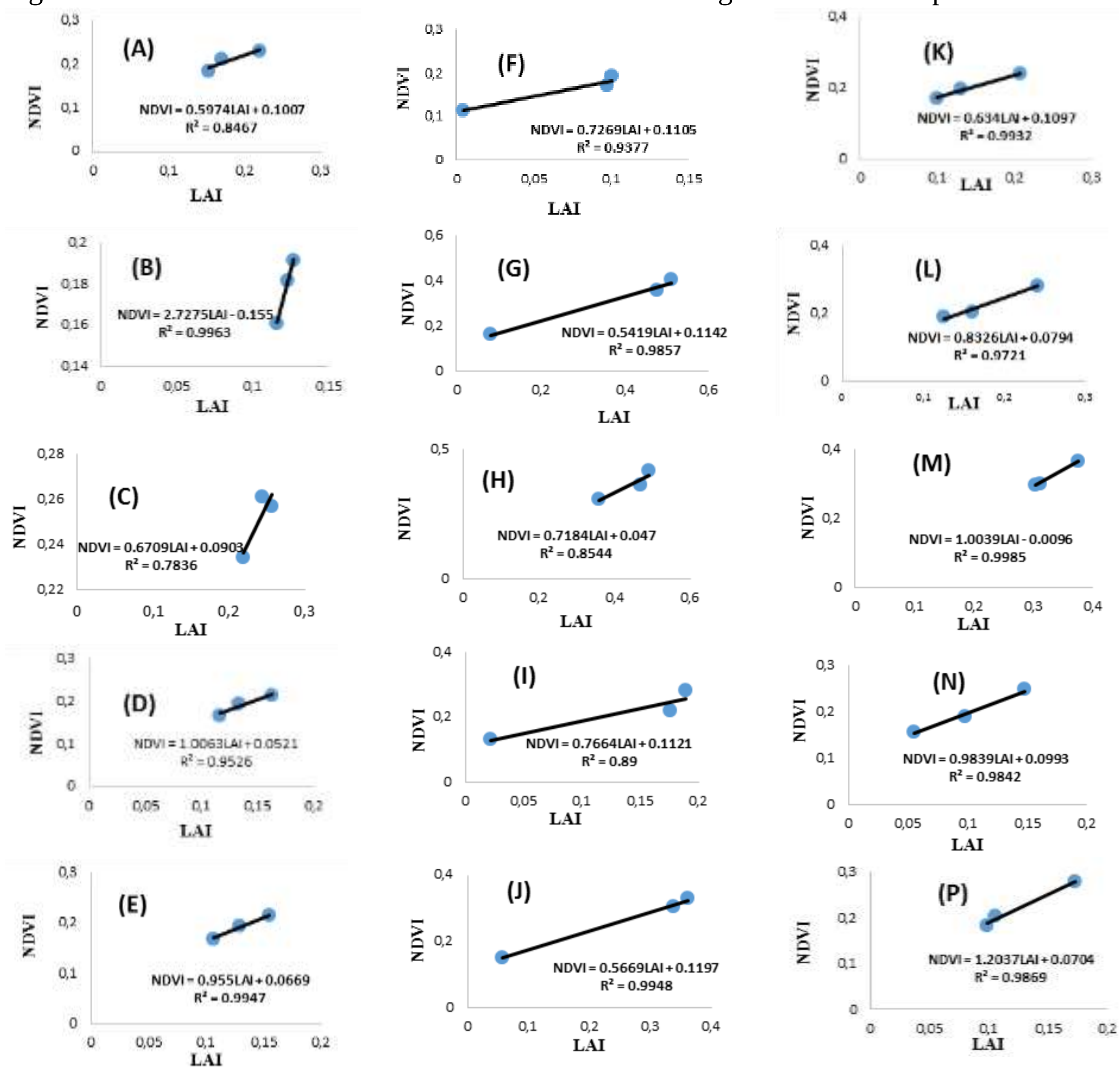
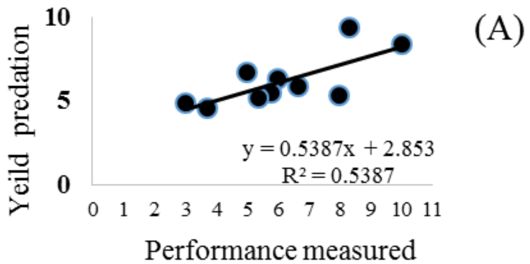


Fig 4. The regression between LAI and NDVI in three years, A to E charts 2012-2013 crop year, F to J charts 2013-2014 crop year and K to P charts 2014-2015.

The regression relationship between the predicted values and the observed actual yield of wheat was also linear and acceptable. The results obtained using of predicted wheat yield data were acceptable with 53% accuracy. In this study, in order to determine the error coefficient, a correlation was established between actual performance and predicted performance. Finally, it was found that the calibrated farms with square root error of 1.38 tons per hectare and 5 validated farms with square root error of 1.45 tons per hectare can be a very good model for predicting wheat yield with the help of the above indicators and satellite images (Fig. 5).



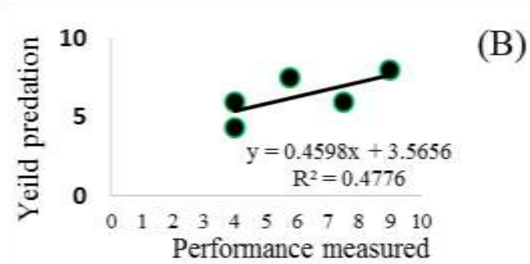


Fig. 5. (A) Ten calibration figure farms, (B) Five Validation figure farms.

Conclusion

Using remote sensing data can eliminate various barriers for managers to make decisions, with the technology being able to estimate wheat yield significantly. Our results showed that wheat yield was varied between years and fields duo to farm management quality and weather condition in different years. The correlation between LAI index and NDVI with wheat yield was varied from year to year. The maximum correlation between growth indices was found at the flowering and filling stages of winter wheat. Based our results, wheat yield can be well predicted using regression between vegetation indices in flowering and filling stage and yield using regression analysis.

References

Becker-Reshef I., Justice C., Sullivan M., Vermote E., Tucker C., Anyamba A., Small J., Pak E., Masuoka E., Schmaltz J., Hansen M., Pittman K., Birkett C., Williams D., Reynolds C., Doorn B. (2010). Monitoring global croplands with coarse resolution earth observations: the global agriculture monitoring (GLAM) project. Remote Sens. 2, 1589–1609.

Bonan, G.B. (1993). Importance of leaf area index and forest type when estimating photosynthesis in boreal forests. Remote Sensing of Environment,43, 303– 314.

Conway G., Toenniessen G. (1999). Feeding the world in the twenty-first century. Nature 402, C55–C58.

Duvick D.N., Cassman K.G. (1999). Post-green revolution trends in yield potential of temperate maize in the north-central United States. Crop Sci. 39, 1622–1630.

Food and Agriculture Organization of the United Nations (FAO). FAO Statistical Databases. (2014). Available online: http://faostat.fao.org (accessed on 28 November 2017).

Groten S.M.E. (1993). Ndvi–crop monitoring and early yield assessment of burkina-faso. Int. J. Remote Sens. 14, 1495–1515.

Gumma M.K., Thenkabail P.S., Maunahan A., Islam S., Nelson A. (2014). Mapping seasonal rice cropland extent and area in the high cropping intensity environment of Bangladesh using MODIS 500 m data for the year 2010. Isprs J. Photogrammetry Remote Sens. 91, 98–113.
Guo J.M. (2007). Simulation of Regional Winter Wheat Growth Using Remote Sensing Data and Crop Growth Model. Nanjing University of Information Science & Tech-nology, Nanjing, pp. 1–138 (in Chinese).
ITA, (2002). ICARE: Integrated Crop Area Estimates, Final MARS/JRC Report.
Jiao X.F., Kovacs J.M., Shang J.L., McNairn H., Walters D., Ma B.L., Geng X.Y. (2014). Object-oriented crop mapping and monitoring using multi-temporal polarimetric RADARSAT-2 data. ISPRS Journal of Photogrammetry and Remote Sensing. 96, 38-46
Kogan F., Salazar L., Roytman L. (2012). Forecasting crop production using satellite-based vegetation health indices in Kansas, USA. Int. J. Remote Sens. 33, 2798– 2814.
Maselli F., Conese C., Petkov L., Gilabert M.A. (1993). Environmental monitoring and crop forecasting in the Sahel through the use of Noaa Ndvi data –a case-study– Niger 1986–89. Int. J. Remote Sens. 14, 3471–3487.
Rasmussen M.S. (1992). Assessment of millet yields and production in Northern Burkina-Faso using integrated Ndvi from the Avhrr. Int. J. Remote Sens. 13, 3431–3442.
Sui J., Qin Q., Ren H., and Sun Y., Zhang T., Wang J., Gong S. (2018). Winter wheat production estimation based on environmental stress factors from satellite observations. Remote Sensing. 10(6):962.
Thorp K.R., Wang G., West A.L., Moran M.S., Bronson K.F., White J.W., Mon J. (2012). Estimating crop biophysical properties from remote sensing data by inverting linked radiative transfer and ecophysiological models. 124, 224–233.
Zhang J.H., Feng L.L., Yao F.M. (2014). Improved maize cultivated area estimation over a large scale combining MODIS-EVI time series data and crop phenological information. Isprs J. Photogrammetry Remote Sens. 94, 102–113.
Zhao C., Bao Y., Cheng M., Huang W., Liu L. (2011). Use of Landsat TM and EOS MODIS imaging technologies for estimation of winter wheat yield in the North China Plain. International journal of remote sensing, 33(4), p 1029-1041.

PERFORMANCE OF A PILOT-SCALE CONSTRUCTED WETLAND SYSTEM AND REUSE OF TREATED WASTEWATER IN AGRICULTURAL IRRIGATION

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Abstract

In recent years, climate change has greatly affected rainfall and air temperature levels leading to a reduction of water resources in Southern Europe. Agriculture has been hit hard by the fall in water supply and a negative impact on irrigated crops has been observed. The treated wastewater (TWW) reuse for agricultural irrigation can represent a solution for farmers, allowing to reduce the consumption of freshwater (FW) and the need of mineral fertilisers. In the rural environment, constructed wetland systems are the most promising nature-based solutions to obtain TWW. This paper reports a two-year study on the wastewater treatment performance of a pilot-scale horizontal subsurface flow system in Sicily (Italy) and the effect of two sources of irrigation water (FW and TWW) on tomato plants and soil characteristics. The system had a total surface area of 100 m^2 and was planted with giant reed and umbrella sedge. An experimental field of tomato was set up close to the system. Results highlighted a high pollutant removal efficiency (RE) of the system. In particular, RE levels of *Escherichia coli* was found to be higher than 85% on average. The production and qualitative parameters of tomato fruits were significantly affected by the different irrigation treatments. The total yield of fruits was higher than 70 t ha^{-1} in the TWW-irrigated plots. Microbial contamination was high in fruits which were in contact with bare soil. The source of irrigation water did not vary the chemical composition of the soil. Our findings confirm that TWW provides an additional water source and nutrients where the supply of FW is limited.

Keywords: *Constructed wetland*; *Treated wastewater reuse; Tomato; Savings.*

Introduction

In the Mediterranean region, agricultural production has been lately affected by climate change. Higher air temperatures, less distribution of rainfall events and more frequent periods of drought have caused a significant reduction of crop yields in many areas of Southern Europe. In this scenario, the reuse of treated wastewater (TWW) for irrigation purpose represents a useful practice for sustainable water management due to various agronomic reasons. It increases the water resource in the agricultural sector, saving higher quality water for human consumption and lower quality water for irrigation (Leto *et al*., 2013). It meets growing water demand and reduces discharge of wastewater (WW) in soil and water bodies (Pedrero *et al*., 2010; Licata *et al*., 2019). It represents a source of mineral and organic nutrients and its application can increase crop yields and reduce the use of chemical fertilisers (La Bella *et al*., 2016). However, despite these benefits, the use of TWW irrigation could affect the chemical soil characteristics and increase the level of pathogens in plant and soil (Rusan *et al*., 2007; Castro *et al*., 2011). As a consequence, efficient WW treatment is needed in order to safeguard the environment and, at the same time, provide benefits for agriculture. In some Italian regions, conventional treatment systems are outdated and do not always meet legislative requirements as they do not perform all treatments needed to ensure high water quality (Licata *et al*., 2017). In this situation, constructed wetlands (CWs) can play a key role in the treatment and reuse of various types of WW due to their characteristics. CWs represent an example of a nature-based solution and can be integrated into wastewater treatment plants, as tertiary-treatment technology, to complete the purification process of WW. A number of studies demonstrate the potential of CWs in the agricultural sector and make in evidence the application of CWs for obtaining TWW. In particular, various authors have examined the effects of TWW on horticultural crops, evaluating how TWW affects plant and soil characteristics both in the short and long-term (Cirelli *et al*., 2012; Gatta *et al*., 2018). Among open field crops, tomato represents the most investigated crop for the Mediterranean area as a major dietary component in many countries. Tomato is, in fact, a key element in the human diet due to its high nutritional and health properties. In recent years, research on this species has focused on the effects of TWW irrigation on yield and quality highlighting the increase in marketable yields (MY) and fall in health and hygiene standards in the fruits (Al-Lahman *et al*., 2003; Gatta *et al*., 2015). The aim of this paper was to assess the WW treatment performance of a pilot-scale horizontal subsurface flow system (HSSFs) CW in Sicily (Italy) and the effect of freshwater (FW) and TWW on tomato plants and soil characteristics in the short-term.

Materials and methods

Tests were carried out in two years from 2016-2017 in the area of the HSSFs CW in Raffadali, located in Western Sicily (Italy - 37°24'N – 1°05'E, 446 m a.s.l.). The CW was used to treat urban WW produced from the municipal treatment plant. The CW had a total area of 100 m^2 and included two parallel units which were separately planted with giant reed (*Arundo donax* L.) and umbrella sedge (*Cyperus alternifolius* L.). The main functional and technical characteristics were described by Leto *et al*. (2013) in a previous study.

WW samples were taken monthly at the inlet and outlet pipes from April to September of each year. Total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) levels were determined in accordance with Italian water analytical methods. Total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS) and *Escherichia coli* (EC) levels were determined in accordance with APHA standard methods for water testing. For each planted-unit, pollutant removal efficiency (RE) was based on pollutant concentration and calculated by guidelines of the International Water Association.

An experimental field of tomato was set up close to the HSSFs CW. A cultivar was transplanted in the third 10-day-period of April 2016 and 2017, using a plant density of 2.2 plants m^{-2}. The single plot size was 50 m^2. A split-plot design for a two-factor experiment was adopted with three replications. Irrigation was applied from April to June twice a week for 1 h and from July to September twice a week for 3 h. Irrigated-plots received 80 kg N ha^{-1}, 130 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} through fertigation. Total yield (TY) of fruits was calculated Fruit diameter (D), fruit colour (Co), fruit soluble solids content (SSC) and titratable acidity (TA) of tomato juice were determined on a sample of 20 marketable fruits from each plot. Dry matter (DM) content was also calculated by drying the fruits in a ventilated oven at 70 °C. Fruits in 300 g samples were harvested a week before irrigation from each plot to determine the levels of FC, FS and EC in both fruit skin and flesh.

Regarding soil, samples were taken at a depth of 0-30 cm from each plot and analysed for pH, electrical conductivity, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), total calcium carbonate ($CaCO_3$), phosphorus (P), potassium (K) and sodium (Na) content.
Statistical analyses were performed using the package MINITAB 17 for Windows. For WW composition, all the representative values were shown using mean ± standard deviation calculations. For plant and soil parameters, analysis of variance was carried out and the difference between means was investigated out using the Tukey test.

Results and discussion

Removal efficiency of pollutants in the HSSFs CW

Table 1 summarises the RE values of chemical pollutants. For TSS, differences in RE values between the two CW planted units can be explained by the diverse root length density of the species and time of contact between WW and roots (Vymazal, 2009). In general, BOD_5 and COD RE values were high and stayed within a range consistent with previous HSSFs CW studies treating urban WW (Kadlec et al., 2000). TN RE was lower than the organic matter removal due to low oxygen levels in the system. TP RE average values were lower and probably due to the gradual filling of the sorption sites by the plant roots, the presence of undecomposed plant parts around the substrate surface and the adsorption properties intrinsic to the substrate (La Bella et al., 2016).

Table 1. Chemical composition of the TWW from inlet to outlet in the HSSFs CW. RE from April to September 2016/2017. Two-year average values (± SD) are shown.

Parameter	Influent	Effluent[1]	Effluent[2]	RE (%)[1]	RE (%)[2]
TSS (mg L^{-1})	42.11 ± 1.9	10.31 ± 0.4	11.40 ± 0.4	78.91	71.30
BOD_5 (mg L^{-1})	34.31 ± 0.7	13.42 ± 0.3	13.92 ± 0.3	61.21	59.65
COD (mg L^{-1})	55.92 ± 1.4	18.62 ± 0.2	19.43 ± 0.3	66.41	65.23
TN (mg L^{-1})	21.32 ± 0.4	10.92 ± 0.3	11.21 ± 0.2	49.12	47.21
TP (mg L^{-1})	8.02 ± 0.2	4.85 ± 0.1	4.99 ± 0.1	39.52	37.78

[1] giant reed-planted unit; [2] umbrella sedge-planted unit.

In the case of microbiological level (Table 2), the good aerobic conditions in the planted units eased the production of a greater bacteria biofilm and promoted removal of pathogens, as stated by El-Khateeb *et al*. (2009). For each parameter, RE levels were found to be above 80.00% in accordance with the findings of other studies carried out under similar operating conditions at the HSSFs CW.

Table 2. Microbiological composition of the TWW from inlet to outlet in the HSSFs CW. RE from April to September 2016/2017. Two-year average values (± SD) are shown.

Parameter	Influent	Effluent[1]	Effluent[2]	RE (%)[1]	RE (%)[2]
TC (CFU 100 ml^{-1})	4.44 ± 1.5[3]	3.41 ± 0.9	3.48 ± 1.2	90.58	88.81
FC (CFU 100 ml^{-1})	4.29 ± 1.1	3.36 ± 1.4	3.41 ± 1.3	88.31	87.11
FS (CFU 100 ml^{-1})	3.99 ± 2.5	3.27 ± 1.02	3.38 ± 1.1	81.32	74.60
EC (CFU 100 ml^{-1})	3.21 ± 0.5	2.25 ± 1.2	2.28 ± 0.9	88.91	87.13

[1] giant reed-planted unit; [2] umbrella sedge-planted unit; [3] values are shown as units of Log_{10}.

In this study, average chemical and microbiological parameters results at outlet of the HSSFs CW, such as TP and EC, were not always within the legal limits of the Italian Decree 152/2006 concerning the reuse of TWW for irrigation purposes.

Effects of freshwater and treated wastewater irrigation on yield and quality of tomato fruits

The main factors did not determine significant changes for all plant parameters (Table 3). Significant differences were found between FW- and TWW-irrigated plants concerning TY of fruits. Our findings were in agreement with results obtained by Gatta *et al*. (2015) who stated that the genetic constitution of cultivars, the type of TWW applied and the climate and soil characteristics can play a fundamental role. The highest pH average values were recorded in the FW-irrigated fruits. This was confirmed by other studies (Al-Lahman *et al*. 2003; Cirelli *et al*., 2012; Gatta *et al*., 2015) and highlights that pH is significantly affected by source of irrigation water. Concerning other qualitative parameters of fruit, significant differences were found between the irrigation treatments for SSC, DM, D and Co.

Table 3. Production and qualitative parameters of tomato fruits in response to year and irrigation water. Average values of 2-year tests are shown.

Factor	TY (t ha^{-1})	pH	SSC (° Brix)	TA (g 100 ml^{-1})	DM (%)	D (cm)	Co (a^*/b^*)
Y_1	70.83 b	4.57 a	4.78 a	0.26 a	5.50 a	1.21 a	2.53 a
Y_2	74.78 a	4.56 a	4.78 a	0.27 a	5.49 a	1.21 a	2.54 a
IW_1	66.13 b	4.68 a	4.81 a	0.28 a	5.53 a	1.19 b	2.53 ab
IW_2	75.51 a	4.54 b	4.78 b	0.26 a	5.47 b	1.21 ab	2.52 b
IW_3	76.78 a	4.58 b	4.76 b	0.26 a	5.47 b	1.22 a	2.53 a
Y x IW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**

Means followed by the same letter are not significantly different according to Tukey's test: ** significant at $p \leq 0.01$; n.s = not significant.
Y_1 = 2016; Y_2 = 2017; IW_1 = FW; IW_2 = TWW from giant reed-planted unit; IW_3 = TWW from umbrella sedge-planted unit.

Fruit fresh and skin had different microbial levels. Fruit flesh was uncontaminated whereas fruit skin was greatly contaminated by microorganisms. In both years, a different contamination levels were found in August and September and were probably due to diverse microbial levels in the TWW produced from HSSFs CW (Table 4).

Table 4. Microbial levels in the TWW-irrigated fruits during 2016-2017. Average values are shown.

Parameter	Period			
	August		September	
	Skin	Flesh	Skin	Flesh
FC (CFU 100 g^{-1})	425	0	538	0
FS (CFU 100 g^{-1})	670	0	765	0
EC (CFU 100 g^{-1})	127	0	101	0

In this study, it was observed that the most contaminated fruits were those in contact with the bare soil. As affirmed by Bastos and Mara (1995), the increased soil moisture from TWW irrigation could allow for bacterial re-growth, whilst Irénikatché Akponikpè *et al*. (2011) state that the rough surface of the tomato fruit facilitates microbial contamination, especially in fruits which touch the soil. Al-Lahman *et al*. (2003) sustain that weather conditions, such as moisture, solar radiation and temperature, can significantly affect the microbial contamination on fruit skin.

Effects of freshwater and treated wastewater irrigation on soil characteristics

Soil chemical characteristics as affected by year and irrigation water are shown in Table 5. The main factors did not determine significant changes for all soil parameters. No significant variations in topsoil pH of the FW- and TWW-irrigated plots were recorded due to short-term application of TWW, as sustained by Rusan *et al*. (2007). Soil salinity increased over the two years and was found to be higher on average in the TWW-irrigated soil compared to the FW-irrigated soil and this was probably due to original salt levels in the WW, as reported by Mohammad and Mazahreh (2003). TWW-irrigated soil had a higher average values of TOC with respect to FW-irrigated soil due to higher nutrient content and organic compounds in TWW, as confirmed by various authors (Heidarpour *et al*. 2007; Rusan *et al*. 2007).

Table 5. Chemical characteristics of soil in response to year and irrigation water. Average values of 2-year tests are shown.

Factor	pH	EC ($\mu S\ cm^{-1}$)	TOC ($g\ kg^{-1}$)	TKN ($g\ kg^{-1}$)	$CaCO_3$ ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K (ppm)	Na (ppm)
Y_1	7.66 a	191.32 b	7.78 a	1.27 b	1.33 a	0.04 a	555.95 a	93.65 a
Y_2	7.63 b	200.53 a	7.82 a	1.29 a	1.33 a	0.03 a	560.93 a	92.87 a
IW_1	7.65 a	188.76 b	7.70 b	1.24 b	1.32 b	0.03 a	541.73 b	91.05 c
IW_2	7.64 a	198.77 a	7.82 a	1.29 a	1.34 a	0.03 a	563.30 a	93.53 b
IW_3	7.65 a	200.25 a	7.88 a	1.32 a	1.33 a	0.03 a	568.29 a	95.21 a
Y x IW	n.s.	n.s.	**	**	n.s.	n.s.	n.s.	**

Means followed by the same letter are not significantly different according to Tukey's test: ** significant at $p \leq 0.01$; n.s = not significant.
Y_1 = 2016; Y_2 = 2017; IW_1 = FW; IW_2 = TWW from giant reed-planted unit; IW_3 = TWW from umbrella sedge-planted unit.

Concerning N, differences between the FW- and TWW-irrigated soil were probably due to the fact that, in the short-term, the effect of leaching and plant uptake on N content was not significant. In our study, it was observed that Na content did not significantly increase during the 2 years. However, TWW-irrigated soil had higher Na content in comparison with FW-irrigated soil. It is possible to say that, also in the short-term, the continuous use of TWW irrigation could increase the accumulation of Na in the topsoil. Consequently, periodic applications of good quality irrigation water seem necessary to avoid any risk to the soil structure and tomato plant.

Conclusions

In two-year tests, most chemical and microbiological parameters of wastewater were found to be highly compatible with Italian standards for irrigation reuse due to HSSFs CW performance. It was observed that treated wastewater obtained from constructed wetlands can be a source of water for the irrigation of tomato, especially in areas with prolonged water shortage. However, a continuous irrigation with treated wastewater could increase the level of sodium in the topsoil and be negative for soil productivity and plant growth. A careful management and periodic monitoring of soil fertility are, therefore, essential. Concerning tomato, in the short-term, treated wastewater irrigation determined an increase of total yield of fruits in comparison with freshwater irrigation. But additional disinfection techniques seem necessary in order to avoid any kind of health risk for humans due to high level of microbial contamination of fruits. Further research is, then, required to assess the effect of treated wastewater on plant and soil characteristics in the medium and long-term.

References

Al-Lahman O., El Assi N.M., Fayyad M. (2003). Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit, Agricultural Water Management, Vol. 61(1), pp. 51-62.

Bastos R.K.X., Mara D.D. (1995). The bacterial quality of salad crops drip and furrow irrigated with waste stabilization pond effluent: an evaluation of the WHO guidelines, Water Science and Technology, Vol. 31(12), pp. 425-430.

Castro E., Mañas M.P., De Las Heras J. (2011). Effects of wastewater irrigation on soil properties and turfgrass growth, Water Science and Technology, Vol. 638, pp. 1678-1688.

Cirelli, G.L., Consoli S., Licciardello F., Aiello R., Giuffrida F., Leonardi C. (2012). Treated municipal wastewater reuse in vegetable production, Agricultural Water Management, Vol. 104, pp. 63-170.

El-Khateeb M.A., Al-Herrawy A.Z., Kamel M.M., El-Gohary F.A. (2009). Use of wetlands as post-treatment of anaerobically treated effluent. Desalination, Vol. 245(1-3), pp. 50-59.

Gatta G., Libutti A., Gagliardi A., Beneduce L., Brusetti L., Borruso L. (2015). Treated agro-industrial wastewater irrigation of tomato crop: Effects on qualitative/quantitative characteristics of production and microbiological properties of the soil, Agricultural Water Management, Vol. 149, pp. 33-43.

Gatta G., Gagliardi A., Disciglio G., Lonigro A., Francavilla M., Tarantino E., Giuliani M.M. (2018). Irrigation with treated municipal wastewater on artichoke crop: Assessment of soil and yield heavy metal content and human risk. Water, Vol. 10(255), pp. 1-18.

Heidarpour M., Mostafazadeh-Fard B., Abedi Koupai J., Malekian R. (2007). The effects of treated wastewater on soil chemical properties using subsurface and surface irrigation methods. Agricultural Water Management, Vol. 90, pp. 87-94.

Irénikatché Akponikpè B., Wima K., Yacouba H., Mermoud A. (2011). Reuse of domestic wastewater treated in macrophyte ponds to irrigate tomato and eggplant in semi-arid West-Africa: Benefits and risks. Agricultural Water Management, Vol. 98, pp. 834-840.

Kadlec R.H., Knight R.L., Vymazal J., Brix H., Cooper P., Haberl R. (2000). Constructed wetlands for pollution control: processes, performance, design and operation, International Water Association publisher, London, United Kingdom.

La Bella S., Tuttolomondo T., Leto C., Bonsangue G., Leone R., Virga G., Licata M. (2016). Pollutant removal efficiency of a pilot-scale Horizontal Subsurface Flow in Sicily (Italy) planted with *Cyperus alternifolius* L. and *Typha latifolia* L. and reuse of treated wastewater for irrigation of *Arundo donax* L. for pellet production - Results of two-year tests under

Mediterranean climatic conditions. Desalination and Water Treatment, Vol. 57, pp. 22743-22763.

Leto C., Tuttolomondo T., La Bella S., Leone R., Licata M. (2013). Growth of *Arundo donax* L. and *Cyperus alternifolius* L. in a horizontal subsurface flow constructed wetland using pre-treated urban wastewater – a case study in Sicily (Italy). Desalination and Water Treatment, Vol. 251(40-42), pp. 7447-7459.

Licata M., La Bella S., Virga G., Leto C., Tuttolomondo T. (2017) Management of irrigation water and nutrient demands of tomato (*Lycopersicon esculentum* Mill.) using urban treated wastewater from a pilot-scale horizontal subsurface flow system constructed wetland in Sicily (Italy). Desalination and Water Treatment, Vol. 73, pp. 422-439.

Licata M., Gennaro M.C., Tuttolomondo T., Leto C., La Bella S. (2019). Research focusing on plant performance in constructed wetlands and agronomic application of treated wastewater – A set of experimental studies in Sicily (Italy), Plos One, Vol. 14(7), pp. 1-27.

Mohammad M.J., Mazahreh N. (2003). Changes in soil fertility parameters in response to irrigation of forage crops with secondary treated wastewater, Communications in Soil Science and Plant Analysis, Vol. 34(9-10), pp.1281-1294.

Pedrero F., Kalavrouziotis I., Alarcón J.J., Koukoulakis P., Asano T. (2010). Use of treated municipal wastewater in irrigated agriculture – Review of some practices in Spain and Greece. Agricultural Water Management, Vol. 97, pp. 1233-1241.

Rusan M.J.M., Hinnawi S., Rousan L. (2007). Long-term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination, Vol. 215(1-3), pp. 143-152.

Vymazal J. (2009). The use constructed wetlands with horizontal subsurface flow for various types of wastewater. Ecological Engineering, Vol. 35, pp. 1-17.

SUGGESTED SOLUTIONS FOR WATER PROBLEM IN LIBYA

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Abstract

The problem of water in Libya is caused by the increasing demand for fresh water. The groundwater supply is limited. The water supply will become more problematic with rapidly increasing population and low rainfall. After discovery of hug quantity of fresh groundwater in the deserts the south part of the country, the Libyan Government planned the Great Manmade River project. The Libya authorities designed and installed the hydraulic infrastructure needed to withdraw and transport this fossil water to various demand cites in the northern part of the country where most people live and majority of agriculture activities, Libya such as many Mediterranean countries, especially those in the south of the Mediterranean. facing by problem of the water because of population growth it is so high compare with north of Mediterranean countries. The problem of water – its quantity and quality as well – in the south is exacerbated further by the rapid growth of population, the competition among agriculture, industry and tourism sectors and the extension of irrigation and industrial development. Water scarcity a fundamental problem in Libya and most parts of the world Water shortage are often due to problems of uneven distribution and the management of existing Water supplies in Libya could be improved, Like most countries in the Middle East and North Africa, The Great Man-Mad River project was carried out to transport Fresh water from underground reservoirs in south Libya to more fertile and cultivable land where most people live, through a network of pipes that are buried at a depth of 7 meters under the ground, The pipe is 1.600 km long and its inner diameter is 4 meters, After the termination of all its network the pipes will be approximately 4.000 km long which make it the largest artificial irrigation network in the world The growth of Water demand has a marked impact on the water resources of Libya which suffered serious depletions and quality deterioration

Key worlds: *Mediterranean, Libya water problem, population growth.*

Introduction

Libya is a North Africa country on the south coast of the Mediterranean Sea with a coastline of about 1.900 Kilometers. Apart of Mediterranean Sea coast, Libya has frontiers with six Arabic and non-Arabic African countries, namely, Algeria and Tunisia in the west Egypt in the east, Sudan on the southeast, and Chad and Niger in the south. The country has a small population. The population was about 1.52. million inhabitants in 1954. It occupies a relatively large area of about 1,760,000 square kilometers. It is the fourth-largest country in Africa and seven times as large as Great Britain and Northern Ireland. Approximately 95 % of total land area in the country is desert, while 4 % is grassland suitable for grazing animals, and 1 % is forest (FAO 2012). The most important agricultural zones include Jabal al Akhdar and Jabal Nafusah, Jifara plain, Kufrah and the desert mountains to the south, Croplands cover only 355,000 ha of the total area, pasture and rangeland comprise approximately 13,300,000 ha, and forestlands encompass about

547,000 ha (Alldrissi et al., 1996). Approximately half of all crops are grown in Jabal al Akhdar, and the other half is grown in Jabal Nafusah, Kufrah, and the desert mountains to the south. with 90 % being less than 20 ha, 9 % between 20–100 ha, and 1% larger than 100 ha (Lariel, 2015). Farms that are larger than 100 ha are mostly owned by the government (Laytimi, 2005). Because of the arid conditions in the country, irrigation is becoming a common pursuit to supply water to crops

Fig (1) MAP of Libya Source: http://www.ewpnet.com/libya/river.htm

Libya Like Many countries in the World the Agriculture sector consumes about 80 % of the water, then comes Domestic water in the second level (27%) and Industry sector the last one (3%) , However majority of Libyan people looks the water as free item (DONOT PAY FOR USE WATER), especially Libyan farmers who consume about 80% of the water in Libya, especially after 2011 Arab Spring (Ehdadan_ 2018) .**Water resources in Libya conventional water resources Surface water** Libya's surface water resources are prone to drought in much the same way as in other countries across North Africa, the average annual rainfall for the years 1945–2010 in the eastern region was 350 mm, 254 mm in the western region, 168 mm in the central region; and 22 mm in the Sahara region (an average of 72 mm for north Sahara and 2 mm for south Sahara). Libya is one of the driest countries in the world. Temperatures are very high (Edwin & Ronny, 2005). **Groundwater** Libya depends heavily on grounder water, which accounts for more than 97% of water use (Omar, 2007). The demand for water has more than doubled between 1977 and 1994, thus intensifying pressure on this finite resource (Alghraiani, 2003). Libya has five principal regions with substantial water resources. They are Jifara plain and Jabal Nafusah region the Middle zone; Aljabal al akhdar region; Fezzan region and Kufrah and Assaris region. These regions constitute the transition between Jifarah plain in the west and Al Jabal Al Akhdar and Fezzan and Alharuj Aswad in the south. The current water use is around 400 million m3/yr. Described and illustrated the Groundwater in Libya as the main source of water supply in Libya's five basins; three of them in northern Libya, namely, Jifara plain, EL Jabal Al Akhdar, and EL Hamada, and two in southern Libya, namely, Murzuq and EL-Kufra-Serir **Non-conventional water resources** Due to the increasing demand for water, there is growing competition between the agricultural and industrial sectors for freshwater supplies. Therefore, wastewater has increasingly become the most predominant low cost and reliable alternative to conventional irrigation water in many countries, especially in the arid and semi-arid regions. Reuse of wastewater in urban and peri-urban agriculture is already a widespread practice in different parts of the world (Jiménez et al., 2010a, Winpenny, et al., 2010). It is estimated about 10% of the global population consumes foods grown with wastewater irrigation (WHO, 2006). The Libyan Government has responded to the issue of water scarcity predominately through utilizing fossil groundwater, with only a minor emphasis on integrating non-conventional water such as desalinated seawater, wastewater reuse and the GMMR into national water management. **Desalination** is the second of the non-conventional water resources adapted in Libya (Bashir, 2017). Many desalination plants of different sizes were built near large municipal centres and industrial complexes**. wastewater** According to data obtained from the General Company for water and wastewater (GCWW), there are around twenty-three wastewater treatment plants distributed all over the country. Only ten plants out the total number are working, and in operation, eight plants are out of service, and five plants are being maintained by the company's management teams. (Bashir, 2018) **Great Manmade River Project. (G M R P)** In 1959, oil was discovered in Libya by ESSO, USA company (Later renamed EXXON) and production and export of oil in commercial quantities began in 1961 (Wright, 1981; Vansewll, 1998), and as a result, the Libyan economic situation changed dramatically. The Libyan economy became primarily dependent on the revenues from the oil sector at the same time this America discovered hug of quantity of groundwater. In 1960s as oil exploration moved further south into the Libyan Desert in search of new oil fields, drilling rigs revealed the presence of immense underground water reserves. This discovery was an important trigger for the establishment of a major civil engineering project in the country, the so-called "Great Man-Made River Project". Moreover, the expanding economy led to a growing population along the fertile coastal strip, although the traditional water supplies in the region were inadequate for the needs of the growing population.

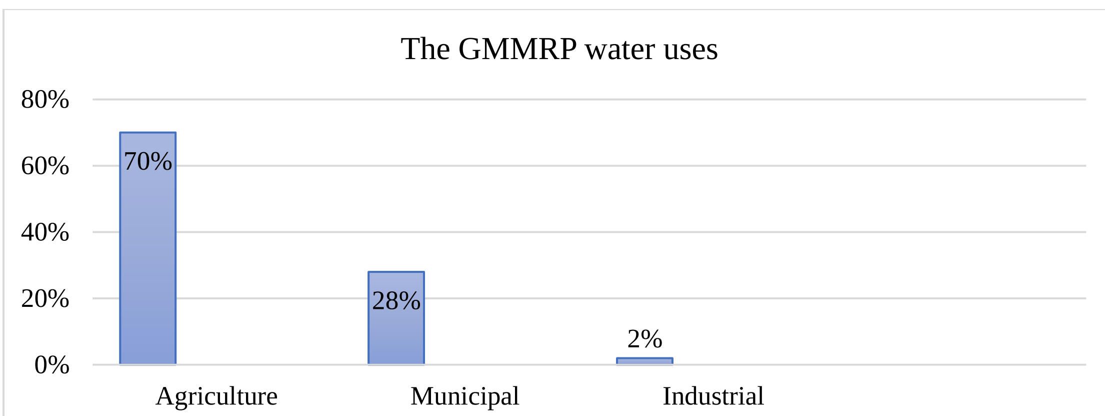

Figure (2) usage of water from the GMMRP. **SOURCE** The Impact of Great Man Made River Project On Libya Agriculture Activities and the Environment PhD Thesis ZIDAN, University of Technology Malaysia 2007.

This project has been divided into five phases.

Phase I of the project is to provide water for Benghazi, the second largest city of Libya. The plan started in 1990 and was completed in 1994. The system can supply 2.000.000 m^3 /day of the water to Benghazi.

Phase II of the project was finished in September 2000. It was the section for supplying 2.5 million tons of water every day to the main city of Tripoli.
Phase III will increase the water flow in Phase I system by 1,680,000 m^3 per day.
Phase IV will provide 1,000.000m^3 per day of water through a pipeline to Tobruk.
Phase V will connect Phases I and II and install two power stations to pump 1,000,000 m3/day of water, and links Phases I and II with a pipeline from Sirt to Tripoli

Material and Methods

The method adopted by the paper is to clarify the water problem in Libya **by knowing the water sources in this country**, which constitute groundwater the largest proportion of its water sources, as well as knowing the solutions undertaken by previous governments to confront this problem in countries where the desert constitutes 90% of its area. And give an overview of the man-made river project, which transports 6 million cubic meters of water per day to coastal areas such as Tripoli and Benghazi the biggest Cities in Libya, the /7/7need for water and the level of its consumption depends on the climate and lifestyle (Gleick, 1996). According to WWAP (2003) a person needs 20 to 50 litres of water every day. For the very basic needs, this quantity reduces to 1.8–5.0 litres per day (Gleick, 1996), and increases to 6–15 liters per day to preserve hygiene (washing the body to avoid contracting skin disease).

Table 1. Water requirement amounts for meeting basic human needs

Basic Human needs	Water requirement
Basic water consumption for survival	2–5
Basic sanitation	8–20
Bathing or showering	6–15
Cooking	4–10

*Source, Gleick, 1996, 1998; Wallace, 2000; WWAP, 2003

Results and Discussion

The demand for water in the country such as Libya 90% is desert it is so high because of the nature of this country, limited of the water resources groundwater share about 97% of the water resources in the country, Libya does not have a permanent river , very little rainfall occurs in north part on Libya 200-250 mm .in south part of Libya the average of rainfall is 2 mm such as Sabha and Murzuq The main reasons for increased desertification in Libya are low vegetation cover resulting from rising air temperatures, and decreasing precipitation . The demand for water has more than doubled between 1977 and 1994, thus intensifying pressure on this finite resource relatively high due to the warm climate. Demand also keeps increasing with time because of the high growth rate of the population. Since the surface water resources of many arid countries are limited, and the management of these sources are still incomplete, there is a growing need to fulfil some part of the demand from groundwater resources. Experience has shown that groundwater abstraction. Libya experiences scarcity of water due to its arid location (Pastel et al., 1996). During the journey to search for oil in the depths of the Libyan desert, underground water reservoirs were discovered, and from here came the idea of transporting water through pipelines for 4000 km to coastal cities such as Tripoli and Benghazi. Northern part of the country, majority of Libyan people looks the water as free item especially Libyan farmers who consume about 80% of the water in Libya, especially after 2011 Arab Spring

Conclusion and suggestions

Considering that there is increasing water demand and decline in water availability and/or reliability, Adoption of other economic tools or agricultural policies in addition to water pricing also will help increase optimization of water use for food production, which will promote effective use of water and reduce the water scarcity problem. These tools such as adoption of modern methods of irrigation, e.g., drip irrigation; planting drought-tolerant crops; reducing the use of chemical fertilizers that require large amounts of water to be useful; encouraging the trend towards organic farming that needs less water; altering the production methods; and using wastewater for supplemental irrigation will positively affect the water use in agriculture and help in developing and improving the existing water resources. On the other hand, the current findings of this study reveal that there are two factors which negatively affect the success of water pricing. These are water consumption and cost of water pumping. Moreover, there is the role of the government in successfully using water pricing as a policy to address the problem of water scarcity. And last but not least, the water price must be set by the authorities in Libya for agricultural, domestic and industrial water, and everyone must pay the value of the water he consumes.

References

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Alghariani, S. A. (2003). Future perspectives of irrigation in Southern Mediterranean Region: Policies and management issues. In M. Al-Rasheed, V. P. Singh & M. M. Sheriff (Eds.), *Proceedings of the International Conference on Water Resources Management in Arid Regions*, (pp. 313–320).

Alldrissi, M., Sbeita, A., Jebriel, A., Zintani, A., Shreidi, & Ghawawi, H. (1996). *Libya: Country Report to the FAO International Technical Conference on Plant Genetic*s

Brika, B. (2017). Water resources and desalination in the Libyan perspective: A review. Conference Paper at the *12th Gulf Water Conference Manama – Kingdom of Bahrain*

Barik, B. (2018). *Water Resources and Desalination in Libya: A Review* Advanced Laboratory of Chemical Analysis, Authority of Natural Science Research and Technology. Tripoli Libya

Edawin & Ronny.V. (2005). An alternative solution of the water shortage problem in Libya. Water Resources Manage *21*, 961, 982

Ehdadan Jamal. The Problem of the water at the present time and the future in Libya, PHD thesis Pisa University, Italy .2018

Food and Agriculture Organization. (2012). *Libya: FAO in emergency*. Retrieved from: http://www.fao.org/emergencies/countries/detail/en/c/161512/.

Gleick, P. H. (1996). Basic water requirements for human activities: Meeting basic needs. *Water International*, *21*, 83–92.

Lariel, N. (2015). *Challenges in the sustainability of Libyan agriculture: Opportunities for the Libyan seed system* (Ph.D. dissertation). Colorado State University, US.

Laytimi, A. (2005). *Market and trade policies for Mediterranean agriculture: The case of fruit/ vegetable and olive oil*. Retrieved from: http://medfrol.maich.gr/documentation /view/reports/wp1-asr/Libya.pdf.

Pastel, S. L., Daily, G. C., & Ehrlich, P. R. (1996). Human appropriation of renewable fresh water. *Science, 271*, 785–787.

Salem, O. M. (2007). Water resources management in Libya. *Workshop on Integrated Water Resources Management.* Tripoli Libya.

Winpenny, J. T., Heinz, I., Koo-Oshima, S. (2010). *The wealth of waste: The economics of wastewater use in agriculture*. Food and Agriculture Organization of the United Nations.

Wheida, E., Verheven, R. (2004). Desalination as a water supply technique in Libya. *Desalination*.*165*, 89–97

DETERMINATION OF ORGANOCHLORINE PESTICIDES IN SURFACE WATER FROM TIMIȘ AND SIBIU COUNTIES IN ROMANIA

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Abstract

Organochlorine pesticides (OCPs) are continually detected in the environment due to their increasing applications in agriculture and industry. The presence of OCPs in the environment is not desirable since they are well known to have negative impact in humans, animals and birds. Thus, there has been a continual demand to monitor the presence of OCPs within the environment.
Surface waters play an important role in long range transport of pesticides. Organochlorine pesticides are most often found in the water sources due to their increased persistence in the external environment. In this study were determined some organochlorine pesticides in Târnava Mare River from Sibiu County and Bega River from Timiș County. For the analysis of OCPs was used gas chromatographic method with electron capture detection (GC-ECD) for separation and quantification. The results obtained were below the maximum concentration admitted by Law 458/2002 republished in 2017 (national law, 2017) regarding drinking water quality and according with the requirements of the Drinking Water Directive (Council Directive 98/83/EC/1998) intended for human consumption. The solution to reduce risk of pesticides use is ecological agriculture, which gains increasingly more ground in Romania too.

Keywords: *organochlorine pesticides, surface waters, agriculture, ecological.*

Introduction

Global chemical pollution has become a matter of great concern with the increase in public awareness of environmental problems. Among a large number of man-made chemicals, greater attention has been focused on semi-volatile and persistent organochlorines, such as DDT, HCH, Heptachlor, Aldrin, and their metabolites or degradation products, on account of their high bioaccumulation potential and harmful biological effects. Organochlorine pesticides (OCPs) are among the most widespread and persistent environmental pollutants. They are toxic, highly resistant to degradation and they tend to bioaccumulate through the food chain. (Derouiche *et al.*, 2007) Humans and animals exposed to OCPs can develop various health problems including cancer, genetic variation, diseases of the immune system, reproductive toxicity (Wang *et al.*, 2009), being also potential endocrine disruptors (Zhang *et al.*, 2012). Nowadays, these compounds are forbidden around the world, but due to their physicochemical properties they can still be found at trace levels in the environment (Yuan *et al.*, 2013). Aqueous media can be a suitable compartment to study any type of contamination within a specified geographic area because of its ubiquity and peculiar physical-chemical properties. Monitoring the trace levels of organochlorine pesticides in water is important for human health protection and environmental control (Lu *et al.*, 2011).
The purpose of this study is the drinking water quality assessment, in terms of contamination with organochlorine pesticides in Timiș and Sibiu counties. The concentrations of some OCPs

were determined in surface water samples collected from Bega River (Timiș County) and Târnava Mare River (Sibiu County), using a combined extraction method followed by gas chromatography coupled with electron capture detector (GC-ECD). The study was carried out in different seasons in order to generate a representative database, which can improve our knowledge about the contamination status in these water systems. The study targets the assessment of organochlorine pesticide residues in water bodies of agriculture intensive areas and comparison of these residues in surface water. These measurements can be used as baseline levels to monitor the future changes and to predict their future impact on the population of the area.

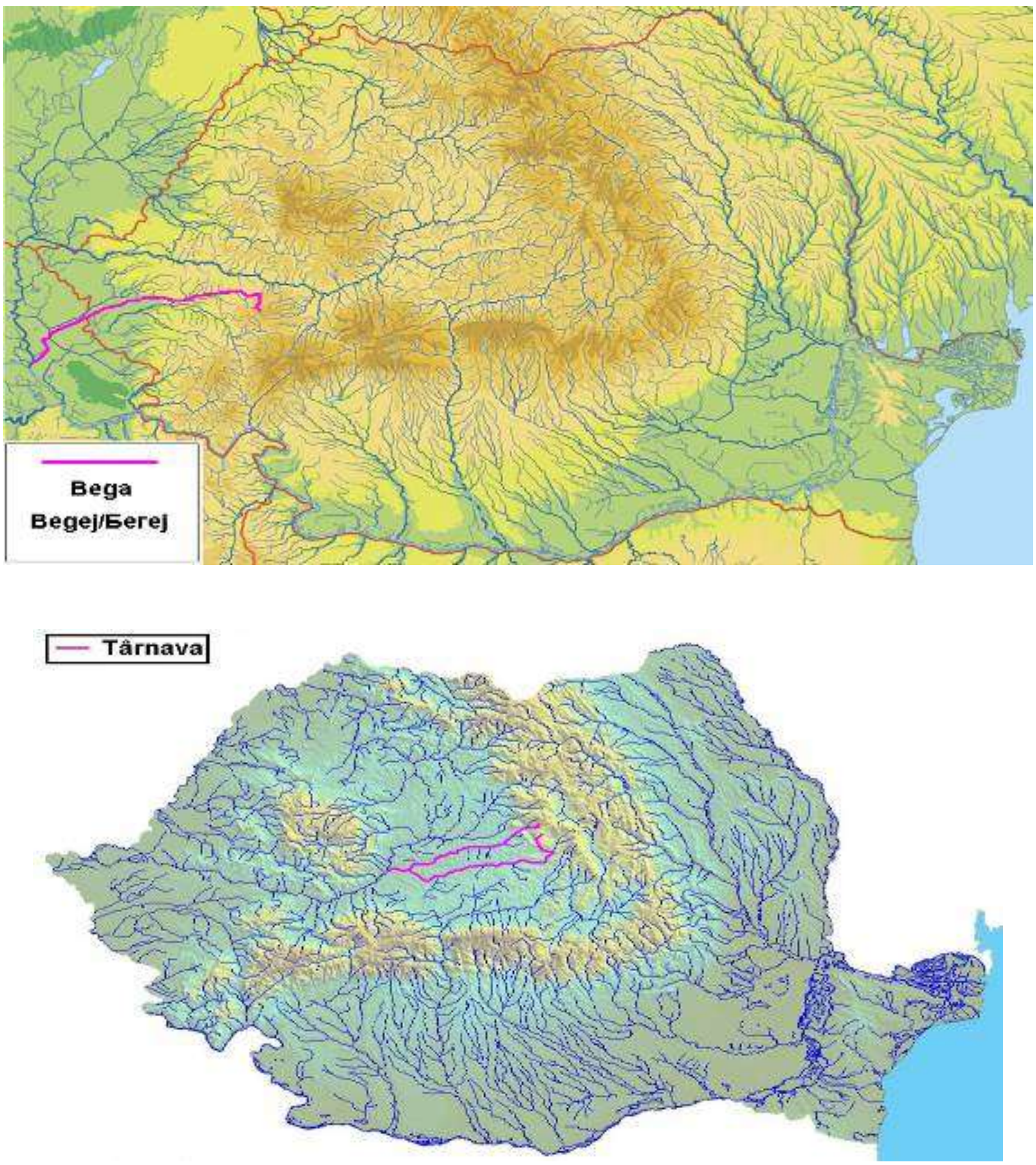

Figure 1. Map of the sampling locations

Materials and methods

Solvents were gas chromatography grade (Merck, Darmstadt, Germany). Mix standard solution 10 μg/ml for each component from LGC Standards, Germany. Ultrapure water was prepared using a Milli-Q water purification system from Millipore (Bedford, MA, USA). The instrumentation used consists in an Agilent Technologies 6890N gas chromatograph (GC) with electron-capture detector (μ-ECD). The schematic diagram of gas chromatography is presented in figure 2. Preparation and bringing to working conditions of the gas chromatograph and column are performed according to the instructions manual for the device.

Water sampling is a very important step in the process of pesticides determination, because collected samples must be representative and must not cause changes in the water composition and quality due to defective techniques or improper conditions of material preparation. Before sampling, the polyethylene recipients are prepared to be clean, rinsed with distilled water, dried and rinsed with dichloromethane, then dried again. Drinking water samples were collected and transported in insulated packages at 6 – 10 ºC to protect them against changes of their chemical properties and deterioration.

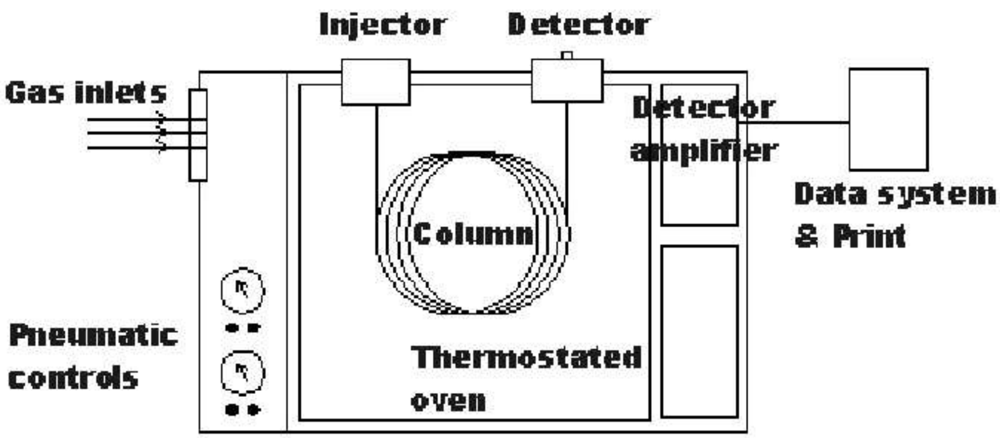

Figure 2. Schematic diagram of gas chromatography

Results and Discussions

Calibration of the method used was performed using external standard calibration curve with different concentration levels of OCPs, spiked in n-hexane (10 ml) using the same procedure for SPME extraction and chromatographic determination, starting with solvent conversion. The working standard solutions were prepared by diluting accurate volumes of mix standard solution of OCPs in n-hexane. The method linearity was studied in the range 0.0010 - 50 μg/l, and the correlation coefficients were higher than 0.985 for all the investigated compounds.

The blank test was performed using 2 l ultrapure water passed through the entire procedure. The detection limits of the entire method varied between 0.0050 ng/l and 0.150 ng/l. Because the analytes are concentrated on the cartridge and fiber, and then are rapidly delivered to the column, minimum detection limits are achieved. The accuracy of the method was determined in terms of recovery experiments by extracting the OCPs from spiked water samples, prepared by adding adequate volumes of mix standard solution to 2 l of blank matrix. The precision, expressed as the repeatability (% RSD) was determined by three consecutive extractions of

OCPs from spiked water samples. The values obtained for recovery and for RSD ranged between 36.5 - 112.2 % and 6.7 - 20.4 %, respectively.
The surface water samples were collected from the two rivers in 2 l pre-cleaned brown glass bottles with glass stoppers.
Upon arrival at the laboratory the samples were filtered through 0.45 μm filter membranes and preserved at 4 °C prior to analysis.
The samples were subjected to the SPE, HS-SPME extractions and then to the GC-ECD analyses, respecting the conditions described in the instructions manual for the device. Fiber blanks were measured before each sample to check the carry-over effect. The results of OCPs concentrations in the studied surface water samples are shown in Table 1.
The results shows that drinking water from the rivers of the two counties (Timiș and Sibiu) have an appropriate quality for the indicator of total organochlorine pesticides, well below the maximum admitted concentration according to National Law 458/2002 (national law, 2017) regarding drinking water quality. This law which regulates drinking water quality in Romania, transposes Directive 98/83/EC on drinking water.
The following organochlorine pesticides were analyzed in the water samples: α – HCH, β – HCH, δ – HCH, γ – HCH, Heptachlor, Aldrin, Heptachlor epoxide, 4,4' – DDE, Dieldrin, Endrin,
β – Endosulfan, 4,4' – DDD, Endosulfan sulphate, 4,4' – DDT.
Hexachlorocyclohexane is a cyclic saturated chlorinated compound that persists in the environment and has eight possible isomers. Despite being a banned toxic substance in most countries, HCH is regularly detected in the aquatic environment.455Only four isomers were of the most commercial importance (α-HCH, β-HCH, δ-HCH and γ-HCH). The latter isomer, gamma-hexachlorocyclohexane (γ-HCH) was a purified form which was widely utilized as a pesticide, due to its potent insecticidal properties. Consequently, when an HCH containing pesticide product contained more than 99% γ-HCH, the substance was termed as lindane.
Heptachlor is applied as a soil treatment, as a seed treatment (maize, small grains and sorghum) or directly to foliage. It is used to control ants, cutworms, maggots, termites, thrips, weevils, wireworms and many other insect pests in both cultivated and uncultivated soils. Heptachlor also controls household insects and pests of humans and domestic animals. In many countries, heptachlor is banned or applied only by subsurface injection. Heptachlor epoxide is not commercially available but is an oxidation product of heptachlor.
Endosulfan is an insecticide still in widespread use in many countries, on crops like cotton, soy, coffee, tea and vegetables, but also banned in 55 countries because of high toxicity to humans and nearly all other organisms, and its persistence in the environment. The principal metabolite is endosulfan sulphate and is regarded as being equally toxic and of increased persistence in comparison with the parent isomers.
Aldrin is a pesticide used to control soil insects such as termites, corn rootworm, wireworms, rice water weevil, and grasshoppers. It has been widely used to protect crops such as corn and potatoes, and has been effective to protect wooden structures from termites. Aldrin is readily metabolized to dieldrin by both plants and animals.
Endrin is a foliar insecticide used mainly on field crops such as cotton and grains. It has also been used as a rodenticide to control mice and voles. It is rapidly metabolized by animals and does not accumulate in fat to the same extent as other compounds with similar structures. It can enter the atmosphere by volatilization and can contaminate surface water from soil run-off. (Lu *et al.*, 2011)

In the European Union (EU), the maximum admissible concentrations (MACs) of OCPs in both the environment and drinking water are 0.1 µg/l for a single compound and 0.5 µg/l for the total concentration of all OCPs (Zhang *et al.*, 2012).

Table 1. OCPs concentrations in the studied surface water samples

<table>
<tr><th>Organochlorine pesticides</th><th>Analysis results in water from Bega River</th><th>Analysis results in water from Târnava Mare River</th><th>Maximum admitted concentration according to Law 458/2002</th></tr>
<tr><td>α - HCH</td><td><0,01</td><td><0,01</td><td rowspan="14">0,10 µg/l</td></tr>
<tr><td>β - HCH</td><td><0,01</td><td><0,01</td></tr>
<tr><td>δ - HCH</td><td><0,01</td><td><0,01</td></tr>
<tr><td>γ - HCH</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Heptachlor</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Aldrin</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Heptachlor epoxide</td><td><0,01</td><td><0,01</td></tr>
<tr><td>4,4’ – DDE</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Dieldrin</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Endrin</td><td><0,01</td><td><0,01</td></tr>
<tr><td>β – Endosulfan</td><td><0,01</td><td><0,01</td></tr>
<tr><td>4,4’ – DDD</td><td><0,01</td><td><0,01</td></tr>
<tr><td>Endosulfan sulphate</td><td><0,01</td><td><0,01</td></tr>
<tr><td>4,4’ – DDT</td><td><0,01</td><td><0,01</td></tr>
</table>

Conclusions

Unprecedented benefits have been obtained using pesticides in controlling disease causing organisms and insects, weeds, and other pests. The mechanism of action that makes them efficient also makes them hazardous to human, animals and environment. It is therefore important to understand associated risks and to limit use of pesticides to minimize possible damage to human health and environment.

The authorities should educate farmers and the public regarding the safe use and handling of pesticides. Lastly, the public should minimize the level of exposure to pesticide residues in food-by-food processing techniques such as washing, peeling, canning, cooking, etc.

The content of pesticides in the water samples analyzed from the two research areas was below the detectable level of < 0.001 µg/l. According to the results obtained in this study, the drinking water quality obtained from the two rivers from Timiș and Sibiu counties does not represent a risk factor for the population health in terms of exposure to organochlorine pesticides.

Good results were achieved and the method used in this study was validated with certified reference materials of OCPs in drinking water.

References

National Law 458/2002 republished (2017), regarding drinking water quality.

Council Directive 98/83/EC/1998 (1998), regarding the quality of water intended for human consumption.

Derouiche, A., Ridha Driss M., Morizur J.-P. and Taphanel M.-H. (2007). Simultaneous analysis of polychlorinated biphenyls and organochlorine pesticides in water by headspace solid-phase microextraction with gas chromatography – tandem mass spectrometry. J. Chrom. A, 1138,
pp. 231 - 243.

Wang Y.-R., Zhang M., Wang Q., Yang D.-Y., Li C.-L., Liu J., Li J.-G., Li H,,Yang X.-Y. (2009). Exposure of mother–child and postpartum woman–infant pairs to DDT and its metabolites in Tianjin, China. Sci. Tot. Environ, 396, pp. 34-41.

Zhang, Y. and H. K.Lee. (2012). Application of ultrasound-assisted emulsification microextraction based on applying low-density organic solvent for the determination of organochlorine pesticides in water samples. J. Chrom. A, 1252, pp. 67-73.

Yuan, L., Qi, S., Wu, X., Wu, C., Xing, X. and Gong X. (2013). Spatial and temporal variations of organochlorine pesticides (OCPs) in water and sediments from Honghu Lake, China. J. Geochem. Explor., 132, pp. 181–187.

Lu, L. C., Wang, C. I. and Sye W. F. (2011). Applications of chitosan beads and porous crab shell powder for the removal of 17 organochlorine pesticides (OCPs) in water solution. Carbohydr. Polym., 83, pp. 1984–1989.

COMPARISON OF CELL WALL STRUCTURE OF DIFFERENT WESTERN BALKAN PLANT SPECIES AS A SOURCE FOR BIOFUELS

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Abstract

Understanding of composition and connections between the building macromolecules of plant biomass, such as cellulose, hemicellulose and lignin, is main key for their better utilization in biofuels industry. We compared four different plant species which are abundant in the region of the Western Balkans. We investigated the structure of the cell walls, as the main constituent of plant biomass, isolated from branches of softwood (*Picea omorika* (Pancic) Purkine), hardwood (*Acer platanoides L.*), maize stem (*Zea mays L.*) as examples of crop species, and *Paulownia tomentosa* tree as a fast-growing species with a huge biomass yield. For our investigation, we combined Fluorescence-detected linear dichroism (FDLD) method and X-ray Diffraction. We obtained data for anisotropy and crystallography which are a base for prediction of the best and appropriate plant species for easy deconstruction of its biomass. Our results show that Acer branch as a hardwood shows the highest anisotropy and the lowest crystallinity compared to the other species while *Picea Omorika* needles show opposite results as the lowest anisotropy and the higher crystallinity. The results for maize show that the stems are easier for utilization than leaves. The isolated cell walls from leaves of *Paulownia tomentosa* show similar results and good correlation between anisotropy and crystallinity, thus we can conclude that this plant is easy to use in biofuel industries.

Keywords: *cell wall, biofuels, anisotropy, crystallinity.*

Introduction

Plant cell walls are the most abundant renewable and biodegradable composite material on the Earth. The secondary cell walls are rich in biopolymers such as cellulose, hemicellulose and lignin, which are of great importance in the biofuel industry.
The chemical composition and interconnection of the polymers in plant cell walls vary between different plant species and directly affects their application and accessibility for use (Pauly and Keegstra, 2008; Ragauskas et al., 2006). Cellulose consists of linear chains of glucose units linked by β-1,4-glycosidic bonds and is the most abundant component in plant cell walls. A large amount of hydroxyl groups in cellulose is involved in complex intra- and inter-molecular hydrogen bonds and so directly form high-order (crystalline) or low-order (amorphous) regions (Kondo, 2004; Wang, 2008). Amorphous regions in cellulose interact with hemicellulose chains (Atalla et al., 1993; Cosgrove, 2005). Hydroxyl group distribution of the cellulose surface affects a hydrophilic character of this molecule (Perez and Mazeau, 2005). Different hemicelluloses interact with cellulose and lignin, and the strength of the structure depends on this interaction

(Atalla et al., 1993; Barakat et al., 2007; Rueland Joseleau, 2005; Scheller and Ulvskov, 2010). Lignin is the second most abundant polymer on the Earth. It has a complex polymer structure composed of polyphenolic units (coniferyl, syringyl, coumaryl). Each plant species has a specific lignin composition. Trees like softwood and hardwood have lignin content from 30% to 34%, the higher content being in the softwood species. Lignin in herbaceous species is rich in syringil units. Lignin is a major source of large-scale biomass utility.

In our study, we compared the structure of four different plant species: spruce wood (*Picea omorika (Pančić) Purkine*) as an example of softwood, maple wood (*Acer platanoides L.*) and *Paulownia tomentosa* wood as a hardwood species, and maize stems (*Zea mays* L.) as a herbaceous plant and widely used agricultural plant.

In this investigation we measured Fluorescence-detected linear dichroism (FDLD) of cellulose labeled with Congo Red by using differential polarization laser scanning microscope (DP-LSM), which provides information of structural order of cellulose fibers. We also measured cellulose crystallinity in the isolated cell walls from branches and leaves by X-ray diffraction (XRD) method. These two methods have not been combined before for structural characterization of biomass. Thus cell wall structural characteristics and polymer interactions were monitored through structural order of cellulose fibers.

Materials and methods

Plant material: Four different plant species were used. The cell walls were isolated from branches of spruce (*Picea omorika* (Pančić) *Purkyne*, Gymnospermae, conifer), maple (*Acer platanoides L.*, Angiospermae, Dicotyledones, deciduous species), *Paulownia tomentosa* wood, and from maize stems (*Zea mays L.*, Angiospermae, Monocotyledones, grass family).

Isolation of plant cell walls: The extractive-free cell wall material was obtained from the spruce, maple, paulownia and maize. Plant material (1g) was homogenized in 10 mL of 80 % methanol in 50-mL Big Clean tubes filled with a stainless steel matrix for 45 s at a speed of 4.5 m/s, using a FastPrep-24 apparatus (MP Biomedicals, Santa Ana, CA, USA). After stirring for 5 min at room temperature, the sample was again subjected to FastPrep homogenization at the same speed. Such obtained plant material was dried for 72 h at 80 C. Dry homogenates of plant material were ground into a fine powder. To obtain cell walls, 400 mg of powder was homogenized for 5–10 min in 10 mL 80 % methanol. The homogenate was slightly stirred for 1 h at room temperature and centrifuged for 5 min at 1500 x g. Further, the resulting precipitate was extracted twice with 10 mL 80 % methanol. The precipitate was subjected to the following washing steps, according to Strack et al. (1988) and Chen et al. (2000):1 x (1 M NaCl, 0.5 % Triton X-100), 2 x distilled water, 2 x 100 % methanol, 2 x 100 % acetone (each step in 20 mL, 30 min). In each washing step, the sample was homogenized in 20 mL of corresponding solvent and then subsequently stirred for 10 min at room temperature and then centrifuged at 1500 x g for 10 min. The supernatant was subsequently removed. A FastPrep-24 System (MP Biomedicals, Santa Ana, CA, USA) was used in each isolation step for more efficient extraction of the cell wall material. The FastPrep-24 instruments and matrix tubes provide rapid and thorough, automated disruption of plant cell walls, which are difficult to homogenize/lyse.

The X-ray measurements of dry isolated cell walls were carried out by Siemens D-500 powder diffractometer. CuKa radiation was used in conjunction with a CuKb nickel filter. Measurements were repeated for each type of sample (species), and representative diffractograms were shown. The percentage crystallinity of the samples was calculated from the ratio of the area under the

diffraction peaks to the total area under the whole diffraction pattern (Georget et al., 1999; Hermans and Weidinger, 1948). Amorphous background patterns were generated, fitted to and subtracted from each diffraction pattern by using OriginPro 7.5.
In the DP-LSM method, the confocal fluorescence intensity images were recorded on a Zeiss LSM 410 laser scanning microscope (Carl Zeiss Jena, Jena, Germany) equipped with a differential polarization (DP) attachment (Garab et al., 2005; Steinbach et al., 2009). Briefly, the DP attachment modulates the polarization state of the excitation laser beam at 100 kHz between horizontally and vertically linear polarization, using a photoelastic modulator (PEM-90, Hinds Instruments). The FDLD signal, proportional to the fluorescence intensity difference, elicited by two orthogonally plane-polarized beams, was obtained from the demodulation circuit, and images were recorded on the LSM. Using the runtime calculation, the FDLD imaging needs only a single scan and it avoids all the artifacts from the multiple scans (such as sample and light intensity stability, bleaching). The provided pixel values are the average of more than ten cycles of modulation. The images were in resolution of 512 x 512 dots, covering the area of 50 x 50 and 64 x 64 lm. Each image consists of two channels: FDLD channel and fluorescence emission channel. FDLD values for dipoles oriented along the Y axis correspond to 1, while the values of dipoles oriented along the X axis correspond to -1. The samples of isolated cell walls were stained with freshly prepared 2 % (w/v) solution of Congo Red (Merck, Darmstadt, Germany) for 30 min, followed by rinsing in distilled water three times. Congo Red has earlier been used to determine the mean cellulose fibril orientation in plants (Verbelen and Kerstens, 2000). The samples (isolated cell wall fibrillar fragments) stained with Congo Red were excited at 488 nm, and fluorescence emission was observed above 560 nm.

Results and Discussion

Cellulose crystallinity in the samples was followed by X-ray diffraction. Based on the data obtained from the diffraction patterns of the cell wall samples for acer, spruce, Paulownia and maize, by Nara & Komiya (1983); it is obvious that there is a difference in the overall crystallinity of the samples. Table 1 shows that crystallinity is nearly the same (42 %) in the cell walls of softwood and harwood species. Crystallinity of the cell wall of maize is much higher (58 %). The peaks obtained from the diffractograms are typical for cellulose I and are located at $2\Theta \approx 14.9^o$, 16.49^o, 22.84^o (Marchessault and Sundararajan 1983). The peak at $2\Theta \approx 23^o$ generally is described as "highly crystalline" region of cellulose, while a broad peak at $2\Theta \approx 16^o$ is characteristic of the less organized polysaccharide structure (graphs not shown) (Fig. 3). The crystallinity of maize is significantly higher because it contains a lower proportion of lignin compared to woody species, which results in a better packaging and a more ordered distribution of cellulose microfibrils. A percentage of crystallinity of approximately 42% was found in all trees, which corresponds to that calculated by Hulleman et al. (1994) for cellulose in cotton and wood of 40 - 48%.

Table 1. Calculated crystallinity of cellulose for the samples of four plant species

Acer branch %	42.39
Picea branch %	42.08
Maize stem %	57.80
Paulownia branch %	41.84

The FDLD observed by DP-LSM imaging is a suitable tool for mapping of the optical anisotropy of cellulose (marked by Congo red) in the cell wall, that corresponds to the cell wall linear dichroism (Steinbach et al., 2008). The anisotropy of each sample was calculated from the images by Image J program. Results are shown in Fig. 1. High anisotropy was obtained for maple branch, Paulownia wood and maize stems. We found a lower anisotropy for Picea branch. One can assume that high anisotropy indicates a simpler structure and more regular packing of cellulose molecules in hardwood species than in the softwood species.

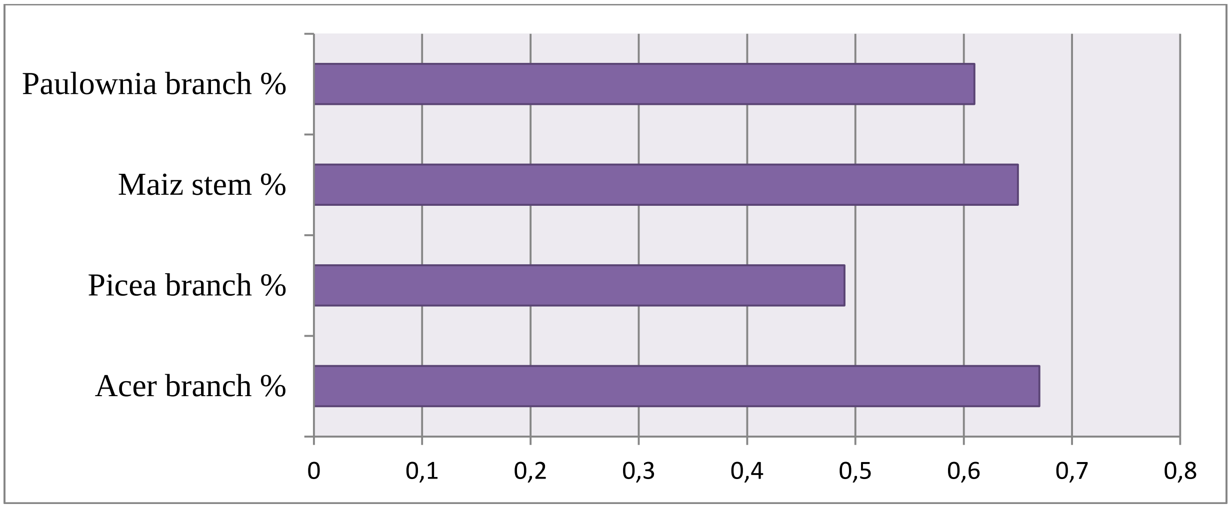

Figure 1. Anisotropy data of the cell walls isolated from the Acer branch, the spruce branch and maize stem calculated from DPLS images

Conclusions

All four different plant species have different chemical compositions and different interactions between the building macromolecules. Through microscopic visualization and X-ray diffraction, we obtained data for anisotropy and crystallography which are a base for prediction of the best and appropriate plant species for easy deconstruction of its biomass structure. Our results show that Acer branch as hardwood shows the highest anisotropy and the lowest crystallinity compared to the other species while spruce needles show opposite results as the lowest anisotropy and the highest crystallinity. The results for maize show that the stems are easy for utilization. The isolated cell walls from *Paulownia tomentosa* show similar results and good correlation between anisotropy and crystallinity, thus one can conclude that plants such as maize and hardwood species, especially Paulownia tree, may be suitable to use in biofuel industries.

Acknowledgement

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References

Atalla RH., Hackney JM., Uhlin I., Thompson NS. (1993). Hemicelluloses as structure regulators in the aggregation of native cellulose. Int J Biol Macromol, 15:109–112.

Barakat A., Winter H., Rondeau-Mouro C., Saake B., Chabbert B., Cathala B. (2007). Studies of xylan interactions and cross-linking to synthetic lignins formed by bulk and end-wise polymerization: a model study of lignin carbohydrate complex formation. Planta 226: 267–281.

Chen M., Sommer A., McClure JW. (2000). Fourier transform—IR determination of protein contamination in thioglycolic acid lignin from radish seedlings and improved methods for extractive-free cell wall preparation. Phytochem Anal, 11:153–159.

Cosgrove DJ. (2005). Growth of the plant cell wall. Nat Rev Mol Cell Biol, 6:850–861.

Garab G., Galajda P., Pomozi I., Finzi L., Praznovszky T., Ormos P., van Amerongen H. (2005). Alignment of biological microparticles by a polarized laser beam. Eur Biophys J., 34: 335–343.

Georget DM., Cairns P., Smith C., Waldron KW. (1999). Crystallinity of lyophilised carrot cell wall components. Int J Biol Macromol, 26: 325–331.

Hermans PH. and Weidinger A. (1948). Quantitative X-Ray investigations on the crystallinity of cellulose fibers. A background analysis. J Appl Phys, 19: 491–506.

Hulleman HD., Van Hazendonk JM., Van Dam JEG. (1994). Determination of crystallinity in native cellulose from higher plants with diffuse reflectance Fourier transform infrared spectroscopy. Carbohydr Res, 261: 163–172.

Kondo T. (2004). Hydrogen bonds in cellulose and cellulose derivatives. In: Dumitriu S (ed.), Polysaccharides: Structural diversity and functional versatility, New York, USA. ISBN 3-540-37102-8,

Marchessault RH. and Sundararajan PR. (1983). Cellulose. In: Aspinal G 0. (ed.), polysaccharides, vol. 2. Academic Press Inc., New York, pp 12–95.

Pauly M. and Keegstra K. (2008). Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J., 54: 559–568.

Perez S. and Mazeau K. (2005). Conformation, Structures, and Morphologies of Celluloses. In: Dumitriu S. (ed), Polysaccharides: structural diversity and functional versatility, 2nd ed. CRC, New York, pp. 41–68.

Ragauskas AJ., Williams CK., Davison BH. et al (2006). The path forward for biofuels and biomaterials. Science, 311:484–489.

Ruel K. and Joseleau J. (2005). Deposition of hemicelluloses and lignins during secondary wood cell wall assembly. In: Entwistle KM, Walker JCF (eds.), The hemicelluloses workshop 2005. University of Canterbury, Christchurch, pp. 103–113.

Scheller HV and Ulvskov P (2010). Hemicelluloses. Annu Rev Plant Biol, 61: 263–289.

Steinbach G., Pomozi I., Zsiros O., Pay A., Horva´th GV, Garab G (2008). Imaging fluorescence detected linear dichroism of plant cell walls in laser scanning confocal microscope. Cytom Part A J Int Soc Anal Cytol, 73: 202–208.

Steinbach G., Pomozi I., Zsiros O., Menczel L., Garab G. (2009). Imaging anisotropy using differential polarization laser scanning confocal microscopy. Acta Histochem, 111: 316–325.

Strack D., Heilemann J., Momken M., Wray V. (1988). Cell wall conjugated phenolics from coniferous leaves. Phytochemistry, 27: 3517–3521.

Verbelen J. and Kerstens S. (2000). Polarization confocal microscopy and Congo Red fluorescence: a simple and rapid method to determine the mean cellulose fibril orientation in plants. J Microsc, 198: 101–107.

Wang Y. (2008). Cellulose fiber dissolution in sodium hydroxide solution at low temperature: dissolution kinetics and solubility improvement. Thesis. Georgia Institute of Technology, USA.

HUMUS CONTENT IN SERBIA TO THE MAPPING INVESTIGATED PITCH

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Abstract

Soil is one of the most important resources of each state. The research in this study was conducted in order to preserve this very important resource. Humic substances have a positive effect of water-air maintenance and total biological properties of the soil which have a direct impact on plant nutrition and and in the first place on the nutrition of plants with nitrogen.
Humus content is the basic parameter of soil fertility. The paper analyzes humus content of 21 localities in central Serbia. Total is examined 5.313 samples. Results on humus content indicate that there are soils from insufficiently humus to extremely humus. In 92% of the examined soil samples are weak and medium humus, 7% of the soil is very humus a only 1% insufficiently humus. The obtained results can be related to the natural properties of soil types on the examined plots, but also to the way of applying fertilizers on them. Natural climatic processes and the impact of anthropogenic influence, man continuously affect changes in soil quality. All plots are mapped and based on the obtained results recommendations are given agricultural producers on repair measures and application of organic and mineral fertilizers.

Key words: *Soil, conservation of resources, humus content, repair measures, mapping.*

Introduction

Land is one of the most important natural resources of any country. As the basic means of agricultural production, its economic importance is determined by fertility, which is the ability of the soil to provide plants with the necessary nutrients, water, air, heat and other factors during the vegetation period. In order to achieve high and stable yields and increase the quality of fruits in crop, vegetable and fruit production, it is important to know both the characteristics of the soil and the requirements of cultivated plants. Knowledge of these parameters enables the correct application of agrotechnical and ameliorative measures (Janković et al., 2020).
Mapping the fertility of agricultural land with digital sampling records and software platform for recommendations-tips on fertilizer use, represents the application of science for soil conservation of the Republic of Serbia and reducing the negative effects of uncontrolled fertilizer application with digital soil sampling records and electronic database of chemical analyzes and recommendations to farmers use of fertilizers (Janković et al., 2021). Humus is a guarantee of soil fertility, it is formed in the process of decomposition of soil organic matter by microbiological means. The content of humus in the soil is conditioned by the type of soil because different types of soil and processes in them determine the degree of decomposition of organic matter and conversion into humic compounds. The humus content in the soils is different and therefore the soils are divided into: insufficiently humus 1% humus, poorly humus 1-3%

humus, medium humus 3-5% humus, highly humus 5-10% humus, extremely humus 10 and more% humus (according to Kotzman).

Mineralization of humus releases plant nutrients in an accessible form and a significant amount of energy important for soil processes. Previous research has shown that humus has several times higher absorption capacity than mineral colloids. Humus particles have an electric charge on the surface, which has an attractive force towards the subject (aqueous solutions of soil minerals and compost decomposition products) due to the opposite charge. The solution on the surface of humus particles, which binds the cohesive force of the electric charge, does not leach into the lower layers of the soil, but remains in the root zone of plants (Popović, 2002; Aziz 2009; Gajić, 2006; Dugalić and Gajić, 2012; BIO IS, 2014;).

Cultivation and uptake of organic constituents affect many soil characteristics including its humus as one of the most sensitive properties, as well as group and fractional composition. Changes in the content and group and fractional composition of humus affect the current pedogenetic processes and other soil characteristics (Molnar, 1997; 2001; Islam and Weil, 2000; Kastori et al., 2006; FAO, 2006; EC 2010; Cupać et al., 2007).

Agricultural advisory and professional services of Serbia have been working for many years on the control of soil fertility on the farms of agricultural producers and give them recommendations for proper fertilization and soil repair. This measure is important for: preservation of the land of the Republic of Serbia; environmental protection; reduction of negative effects of uncontrolled application of fertilizers; education of agricultural producers for proper use of fertilizers; development and planning of agricultural production on the principles of sustainable development. On the territory of Serbia, the representation of types of agricultural land ranges from very fertile (chernozem, grove, alluvium) without limiting factors in agricultural production to those of medium and low fertility (smonica, pseudogley, semigley) (Marschner et al., 2001; Fraga et al., 2014; Stevanović et al., 2016). The aim of this study was to determine the humus content at 21 sites in Serbia in order to ensure the proper use of mineral and organic fertilizers.

Materials and methods

Fertility control of arable agricultural land in central Serbia in 2020 and advising agricultural producers on the basis of control was carried out by the Agricultural Advisory and Expert Services of the Republic of Serbia in advisory work, to determine the level of nutrients in agricultural land, in order to ensure proper use of mineral and organic fertilizers. Chemical analysis of the samples was performed by accredited laboratories that have a certificate of laboratory accreditation issued by the Accreditation Body of Serbia in accordance with the requirements of the valid standard SRPS ISO / IEC 17025. Determination of humus in the laboratory was done according to Kotzman, volumetric method. Division of soils into categories according to humus content is shown in Table 1.

Table 1. Division of humus according to Kotzman, volumetric method

Humus content	% of humus
Insufficient humus	1
Low humus	1-3
Medium humus	3-5
High humus	5-10
Extremely humus	10 and more

The paper analyzes 21 localities in central Serbia by the Agricultural Advisory and Expert Services of Central Serbia. Based on the available data in the software platform for the needs of research in this study, the humus content in the Republic of Serbia was determined with the mapping of the examined plots. The average area of sampled plots by PSSS and municipalities was 0,63 ha and the maximum was 1,58 ha, which clearly indicates the fragmentation of plots in central Serbia. For the research in this paper, for each of the 21 localities, 253 samples were used, which were distributed by categories from insufficiently humus to extremely humus soil. The total number of soil samples on which the research was conducted was 5.313.

Field research included soil sampling, which was performed using an Android application on a mobile phone with automatic recording of GPS coordinates. The sampling procedure was performed in the field according to a unique methodology with the accompanying necessary documentation in accordance with the standards. Data were used from the software platform, with data on sampler, number and area of cadastral parcel, cadastral municipality and land class, sampling depth, GPS sampling coordinates, crop, test results, planned yield, recommended amounts of pure nutrients, lime material, minerals and organic fertilizers, recommendations-tips for fertilization and land repairs for field, vegetable and fruit crops for a particular agricultural producer and PSSS. Laboratory tests included analysis of the basic chemical properties of agricultural soil: acidity, carbonate content, humus content, total nitrogen, and readily available forms of phosphorus and potassium. Fertility control of agricultural land was done with digital sampling records and a software platform for given recommendations-tips on the use of fertilizers.

The results of the performed research are presented on the basis of mathematical-statistical methods and procedures that were applied for the processing of the original data and for the analysis and evaluation of the obtained and arranged results. This approach includes all applied research methods in laboratory conditions and field materials. In relation to the methods of experimental research, the presentation of the results is of an analytical nature. This enables a more detailed and complete presentation of all achieved research results. The results of the research performed in this paper are presented in a table according to the applied processing of the original data. The results of the research are grouped and presented as follows: Number of samples by categories and average values by categories.

Data analysis. All data for evaluation were presented in average. Excel 2013 software was applied to create the database and figure.The percentage of humus content in soil was tested using multiple regression models.

Results and Discussion

Table 2 and Figure 1 shows the number of samples by categories of humus content for 21 examined localities, as well as the cumulative and percentage share of each category for all examined localities.

Out of a total of 5.313 samples examined for all examined localities, there were: insufficiently humus 52, weakly humus 2.403, medium humus 2.481, highly humus 360, extremely highly humus 17 samples.

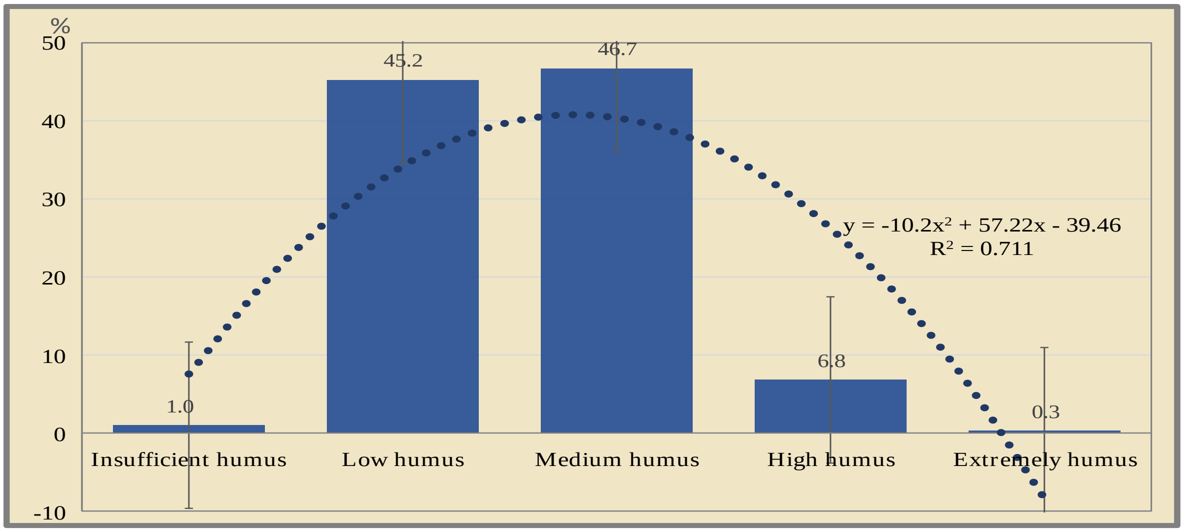

Figure 1. Percentage share of each category humus content in examined soil

The percentage share of samples by categories for all examined localities was: insufficient humus 1%, low humus 45.2%, medium humus 46.7%, high humus 6.8%, extremely humus 0.3% of samples. The most represented samples were in the category of medium humus 2.481, ie. 46.7%. In the category of extremely humus, there were at least 17 samples, that is 0.3%.

Table 2. Number of samples by categories of humus content

PSSS	**Categories**					
Parameters	**insufficient humus**	**low humus**	**medium humus**	**high humus**	**extremely humus**	**Total number of samples**
Belgrade	1	145	107			253
Valjevo	1	179	73			253
Vranje	11	155	79	8		253
Zaječar		174	79			253
Jagodina	14	122	95	19	3	253
K.Mitrovica		92	138	23		253
Kragujevac	1	90	142	15	5	253
Kraljevo	1	60	153	38	1	253
Kruševac	1	128	92	32		253
Leskovac	8	199	43	3		253
Loznica		33	194	26		253
Negotin	1	184	67		1	253
Niš	1	77	159	16		253
Novi Pazar		39	214			253
Pirot		37	179	37		253
Poljoservis	1	146	104	2		253
Prokuplje		35	200	18		253
Smederevo		218	35			253
Užice		46	105	96	6	253
Čačak	5	87	133	27	1	253
Šabac	6	157	90			253
Total	**52**	**2403**	**2481**	**360**	**17**	**5313**
% category participation	1.0	45.2	46.7	6.8	0.3	-

Table 3 shows the average values of humus content by categories for each individually examined locality, as well as the average values for all localities by categories. Observed for all localities, the lowest average value in humus content of 2.41% was recorded for the locality PSSS Smederevo. Observed for all localities, the highest average value in humus content of 4.84% was recorded for the PSSS Uzice locality.
Observed by categories, the average values of humus content were as follows: insufficient humus 0.58%, low humus 2.33%, medium humus 3.72%, strong humus 6.02%, extremely humus 12.50%.

Table 3. Average values by categories of humus content

PSSS	**Categories of humus**					
Parameters	**insufficient humus**	**low humus**	**medium humus**	**high humus**	**extremely humus**	**Average**
Belgrade	0.99	2.47	3.56			2.92
Valjevo	0.16	2.31	3.47			2.64
Vranje	0.65	2.21	3.58	6.02		2.69
Zaječar		2.23	3.35			2.58
Jagodina	0.47	2.34	3.64	6.02	10.24	3.09
K.Mitrovica		2.32	3.87	5.75		3.48
Kragujevac	0.87	2.35	3.71	6.77	13.44	3.59
Kraljevo	0.93	2.43	3.81	6.09	19.00	3.87
Kruševac	0.10	2.35	3.78	5.73		3.29
Leskovac	0.74	2.24	3.59	5.16		2.46
Loznica		2.86	3.56	5.91		3.71
Negotin	0.95	2.31	3.41		12.90	2.64
Niš	0.97	2.41	3.80	5.74		3.49
Novi Pazar		2.55	3.92			3.71
Pirot		2.73	3.93	5.42		3.97
Poljoservis	0.13	2.36	3.43	5.42		2.82
Prokuplje		2.69	3.83	5.50		3.79
Smederevo		2.26	3.37			2.41
Užice		2.54	4.03	6.43	11.00	4.84
Čačak	0.32	2.29	3.82	6.15	16.77	3.52
Šabac	0.65	2.25	3.64			2.71
Average	**0.58**	**2.33**	**3.72**	**6.02**	**12.50**	**3.25**

In accordance with our research are the results of Sekulić et al. (2010) where the authors state that it is an analysis of over 77,000 samples of plowland in AP Vojvodina indicated that 39% of the samples belong to the category of low humus soils with the organic matter content ranging from 1% to 3%. The category of soils well provided with humus (3% to 5%) included 60% of the analyzed plowland of Vojvodina. Organic matter content in Vojvodina soils is under strong anthropogenic influence, and it is not advisable to remove harvest residues and use them as biomass without prior soil analysis.

Conclusion

The content of humus in the soil is conditioned by the type of soil because different types of soil and processes in them determine the degree of decomposition of organic matter and conversion into humic compounds.

The most represented samples were in the category of medium humus 2,481, ie. 46.7%. In the category of extremely humus, there were at least 17 samples, ie. 0.3%.

Observed for all localities, the lowest average value in the humus content of 2.41% was recorded for the PSSS Smederevo locality. Observed by categories, the average values of humus content were as follows: insufficient humus 0.58%, low humus 2.33%, medium humus 3.72%, strong humus 6.02%, extremely humus 12.50%.

Observed for all localities, the highest average value in humus content of 4.84% was recorded for the PSSS Uzice locality.

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References

Aziz I., Mahmood T., Raut Y., Lewis W., Islam R., Weil R.R. (2009): Active organic matter as a simple measure of field soil quality. ASA Internation Meetings, Pittsburg, PA.

BIO Intelligence Service (2014): Study on soil and water in a changing environment. BIO Intelligence Service with support from Hydrologic. Final report to European Commission - DG Environment.

Cupać S., Đorđević, A., Jovanović, Lj. (2007) Effect of land use on group and fractional composition of humus in rendzina soils in Serbia. Journal of Agricultural Sciences, vol. 52, No 2, 2007, 145-153.

Dugalić G. i Gajić B.: (2012) Pedology. University of Kragujevac, Faculty of Agriculture, In Serbian: Pedologija. Univerzitet u Kragujevcu, Agronomski fakultet, Čačak.

Gajić B.: (2006): Soil physics. University of Belgrade, Faculty of Agriculture, Belgrade. In Serbian: Fizika zemljišta. Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Dobbelaere S., Vanderleyden J., Okon Y. (2003): Plant growth-promoting effects of diazo-trophs in the rhizosphere. Crit. Rev. Plant Sci. 22, 107-149.

FAO, Rome (2006): Guideline for soil description

Fraga H., Malheiro A., Moutinho-Pereira J., Cardoso R., Soares P., Cancela J., Pinto J., Santos J. (2014): Integrated Analysis of Climate, Soil, Topography and Vegetative Growth in Iberian Viticultural Regions. PLOS ONE. 9 (9): e108078.

European Commision (2010). Technical guidelines for the collection of soil erosion and soil organic carbon data for Europe through Eionet. Directorate General JRC.

Islam K. R. and Weil R. R. (2000): Land use effects on soil quality in a tropic forest ecosystem of Bangladesh. Agriculture, Ecosystems& Environment. 79: 9-16.

IUSS Working Group WRB (2014): World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps.World Soil Resources Reports No. 106. FAO, Rome.

Kastori R., Kadar I., Sekulić P., Bogdanović D., Milošević N., Pucarević M. (2006):Sampling of land and plants from unpolluted and contaminated habitats. Scientific Institute of Field and Vegetable Crops. Vassal, Novi Sad. In Serbian: Uzorkovanje zemljišta i biljaka nezagađenih i zagađenih staništa. Naučni Institut za ratarstvo i povrtarstvo. Vazal, Novi Sad.

Marschner P., Yang C.H., Lieberei R., Crowley D.E. (2001): Soil and plant specific effect on bacterial community composition in the rhizosfere, Soil biology and Biochemistry 33, 1437-1445.

Molnar I., Milošev, D., Kurjački, I., Gajić, V., Dozet, D. (1997): Influence of crop rotation and fertilization on changes in chemical properties of chernozem. Land management, use and preservation, Belgrade-Novi Sad: Yugoslav Society for the Study of Land. In Serbian: Uticaj plodoreda i đubrenja na promene hemijskih osobina černozema. Uređenje, korišćenje i očuvanje zemljišta, Beograd-Novi Sad: Jugoslovensko društvo za proučavanje zemljišta, 320-328.

Janković S., Simić D., Vujović B., Rahović D., Stanković S., Pantelić Lj., Radulović A., Brzaković N., Đoković A., Jerinić S., Pavlović G. (2020): Fertility of agricultural land with recommendations for growing agricultural crops on the territory of the municipality of Uzice - Study. Agricultural Advisory and Expert Service of Uzice and the Institute for the Application of Science in Agriculture. In Serbian: Plodnost poljoprivrednog zemljišta sa preporukama za gajenje poljoprivrednih kultura na teritoriji opštine Užice- Studija. Poljoprivredna savetodavna i stručna služba Užice i Institut za primenu nauke u poljoprivredi, 1-39.

Popović V. (2002): Determination of residual amounts of heavy metals of the selected locality in order to protect the environment. Master's thesis. University of Novi Sad, Faculty of Technical Sciences, Center for University Studies, Environmental Engineering. In Serbian: Određivanje rezidualnih količina teških metala odabranog lokaliteta u cilju zaštite životne sredine. Magistarski rad. Univerzitet u Novom Sadu, FTN, Centar za Univerzitetske studije, Inženjerstvo za zaštitu životne sredine, Novi Sad, 1- 88.

Sekulić P., Ninkov J., Hristov N., Vasin J., Šeremešić S., Zeremski-Škorić T. (2010). Organic matter content in Vojvodina soils and the possibility of using harvest residues as renewable source of energy. Ratarstvo i povrtarstvo / Field and Vegetable, 47, 2, 591-598.

Stevanović P., Popović, V., Glamočlija, Đ., Tatić M., Spalević, V., Jovović, Z., Simić, D., Maksimović, L. (2016): Influence of nitrogen nutrients on soybean nodulation (Glycine max) on chernozem and pseudogley. XXX Conferences of agronomists, veterinarians, technologists and agroeconomists. Padinska Skela. In Serbian: Uticaj azotnih hraniva na nodulaciju soje (*Glycine max*) na černozemu i pseudogleju. XXX Savetovanja agronoma, veterinara, tehnologa i agroekonomista. Padinska Skela. Belgrade. 22. 1-2, 67-76.

URBAN GREENERY RESEARCH: LEARNING TRENDS FROM THE PAST DECADE

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Abstract

Cities play an enormous role in societies and daily human lives. Around 68% of the world population is projected to live in urban areas by 2050. Rapid urbanization has separated human beings from nature exposure, and one way of bringing nature back and integrating it into urban infrastructures is the careful planning and management of urban green resources (e.g. urban gardens, parks, woodlands, etc.). The overall goal of this study was to examine urban greenery research focus and trends using bibliometric methods. A search was conducted using Scopus to identify English language papers on urban greenery published in peer-reviewed journals between 2010 and 2020. In order to identify themes focuses, content analysis was conducted to analyze the keywords, specifically the frequency of occurrence of the articles' keywords. Overall, 7045 publications related to urban greenery research were obtained as the data for this study, where more than 60% of all papers published, fall into the category of environmental science. Evaluation of the keywords used in papers published over the last decade shows that the tendencies of the body of literature related to this topic are mostly oriented towards land use planning. Observed publication trends and publication characteristics, as well as disciplinary contributions, are further discussed.

Key words: *urban greenspace, key word, Scopus, article.*

Introduction

Spending time in natural environments has been found to promote human health and well-being. Particularly important are contacts with nature in urban surroundings, where providing natural green elements is of the utmost importance. According to UN report (2019), 55% of the world's population is currently living in urban areas, and if we look at the projected data, the percentage goes even higher, as 70% of the world population is projected to live in urban areas by 2050. As urban areas expand, understanding what benefits urban greenery brings to overall biodiversity and society has become increasingly important. Biodiversity is of the utmost importance in human-managed ecosystems. Urban cities comprise a range of habitat types with different kind of anthropogenic pressure, and such variability affects species composition (Lepczyk et al., 2017), where cities often contain more threatened species per unit area than non-urban areas (Ives, 2016). Urban greenery was found to contribute also to the indoor environment via the influence on the climate, energy use, air quality, sonic environment as well as aesthetic quality (Wang et al., 2014), playing an important role in urban planning and design.
Many studies have already reported a positive relationships between urban natural elements and human health and well-being (Groenewegen et al., 2006; Gopal and Nagendra, 2014; Wang et al., 2019), where researchers even tried to quantify the ideal amount of time spent outside in natural environments (120mins per week) to boost overall health (White et al., 2019)

As urban green spaces supply different social and environmental ecosystem services that benefit local people (Kabisch et al., 2015), scientists in the various fields focus on these important parts of the urban environment, particularly those coming from urban planning, environmental management and public health. In this study, we aim to analyze the research on urban greenery (based on the online database) over the last 10 years. Specifically, the objective is to discuss publication trends and describe publication characteristics, as well as disciplinary contributions.

Materials and methods

In order to establish the number of published papers in urban greenery related topics, we used Scopus database. This database has a broad coverage and many authors have confirmed the advantages of using Scopus to implement a bibliometric analysis (Montoya et al., 2018; Giraldo et al., 2019). The search terms for the data were set as " urban greener* OR urban park* OR urban woodland* OR urban forest OR urban green element* OR urban green space* OR urban tree* OR urban green area*. The terms were searched within the "article title, abstract, keywords" option and the results were refined by year (2010–2020). We limited our search to English language only, as it is considered to be the most widely used language in scientific publications. Data collection was conducted on May 17, 2021. We limited our search only to articles, and we excluded other document types.

Scientific production over the given time frame was analyzed both by absolute and relative number of papers extracted from the database, bearing in mind scientific production in general is constantly expanding from year to year, with growth rates from less than 1% up to the middle of the 18th century, to 8 - 9% to 2012 (Bornmann and Mutz, 2015). To get the relative values, we divided the number of papers per year (with our combination of search terms) by the total number of published papers per year. In order to analyze keywords used by the authors, we firstly calculated the frequency of occurrence of the articles' keywords, and then ranked them.

Results and Discussion

Overall, 7045 publications related to urban greenery research were obtained as the data for this study. Scientific output on urban greenery is growing, as shown by the relative number of papers in each year (published from 2010 to 2020) where the trend line clearly demonstrates an upward trend (Fig. 1).

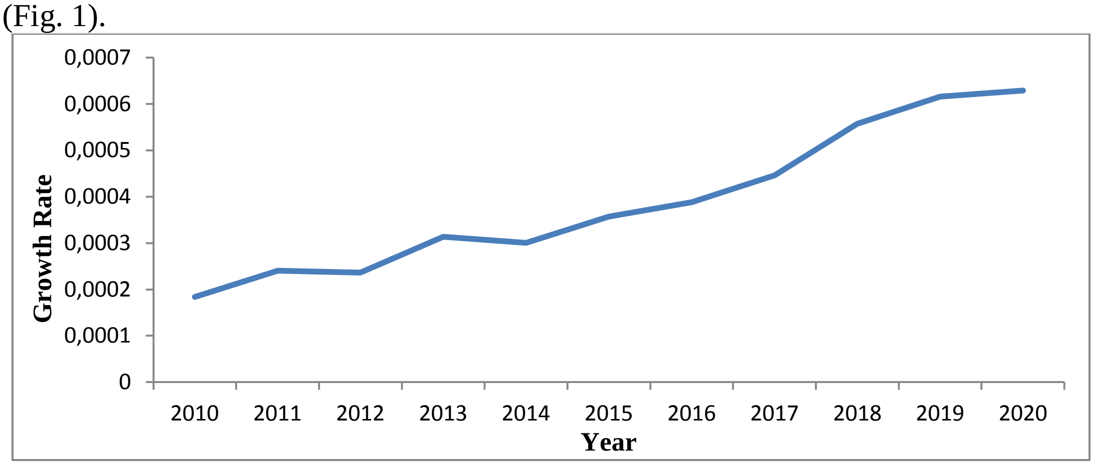

Figure 1. Relative growth rate of scientific production on urban greenery (the number of papers on urban green research divided by the total number of published papers per year).

More than 60% of all papers published, fall into the category of environmental science (Table 1). However, this is a very broad category, so looking more closely at specific journals in which the papers were published can offer a better understanding of disciplinary contributions when it comes to urban greenery research.

Table 1. Number and percentage of papers broken down by SCOPUS subject categories (decreasingly ordered by the number of papers). Only the top 10 Scopus subject categories are shown.

Subject area	Number of papers (%)
Environmental Science	4405 (62.5)
Social Sciences	2251(32.0)
Agricultural and Biological Sciences	1687(23.9)
Engineering	946(13.4)
Energy	745 (10.6)
Earth and Planetary Sciences	714 (10.1)
Medicine	532 (7.6)
Business, Management and Accounting	303(4.3)
Biochemistry, Genetics and Molecular Biology	287(4.1)
Computer Science	210(3.0)

All retrieved papers were published in a wide range of 160 different scientific journals (Fig. 2). The top 3 most active journals are *Urban Forestry And Urban Greening*, *Sustainability* and *Landscape And Urban Planning*.

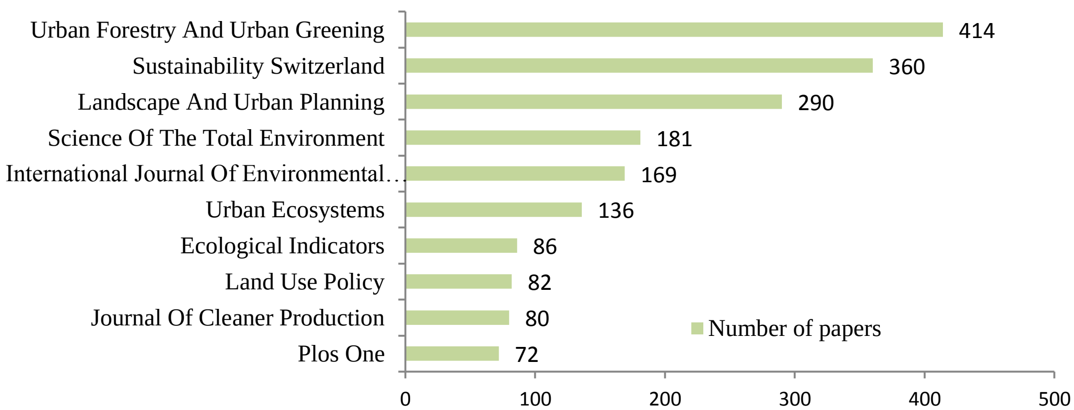

Figure 2. Number of papers published in different journals over the last decade

The most cited article on urban greenery was published by the Solar Energy journal in 2014 and it is focused on green roof mitigation technologies to fight heat island and improve comfort in urban environments. Next two highly cited papers are also related to ecosystem services important for urban planning and economic-decision making, published in Ecological Economic and Science, respectively. Bearing in mind that academic impact is frequently measured through citation counts (Tennant et al., 2016), findings show that research on regulating ecosystem services provided by urban greenery currently has the strongest impact. This is in accordance with the recent bibliometric analysis regrading global research of greenery systems carried by Chàfer et al. (2021), which revealed that green roofs is the main topic studied over the 1974–2019 period, as a result of increased awareness of environmental issues. Interestingly, almost

40% of papers are open access papers. Taking into account that open access papers often get easier into societal discussion, such papers could have greater societal impact (Tennant et al., 2016), thus influencing public and local policies.
Keywords analysis revealed "urban planning" can be found in more than 1300 articles, while at the same time "human" is not in the top five keywords (Fig. 3).

Figure 3. Distribution of different keywords in research papers dealing with urban greenery topics

When speaking about urban planning and sustainable development, in order to design more sustainable cities, one should be sure that needs of local people are met (Derkzen et al., 2015). A recent study found that the cultural services are the major feedback variables of the public supporting green spaces distributing in urban areas (Chang et al., 2017), which has shown to be of the utmost importance during the COVID-19 pandemic, when citizens strongly expressed the need for urban green areas integrated within the urban fabric (Ugolini et al., 2020).

Conclusion

Urban greenery has a wide variety of ecological, social and economic benefits. Evaluation of the keywords used in papers published over the last decade shows that the tendencies of the body of literature related to this topic are mostly oriented towards land use planning (taking into account regulating ecosystem services as primary).

Acknowledgement

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References

Bornmann, L., & Mutz, R. (2015). Growth rates of modern science: A bibliometric analysis based on the number of publications and cited references. *Journal of the Association for Information Science and Technology, 66*(11), 2215-2222.

Chang, J., Qu, Z., Xu, R., Pan, K., Xu, B., Min, Y., ... & Ge, Y. (2017). Assessing the ecosystem services provided by urban green spaces along urban center-edge gradients. *Scientific reports*, *7*(1), 1-9.

Chàfer, M., Cabeza, L. F., Pisello, A. L., Tan, C. L., & Wong, N. H. (2021). Trends and gaps in global research of greenery systems through a bibliometric analysis. *Sustainable Cities and Society*, 65, 102608.

Derkzen, M., van Teeffelen, A. & Verburg, P. (2015). Quantifying urban ecosystem services based on high-resolution data of urban green space: an assessment for Rotterdam, the Netherlands. Journal of Applied Ecology, 52(4), pp.1020-1032. DOI: 10.1111/1365- 2664.12469

Giraldo, P., Benavente, E., Manzano-Agugliaro, F., & Gimenez, E. (2019). Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy*, *9*(7), 352.

Gopal, D., & Nagendra, H. (2014). Vegetation in Bangalore's slums: Boosting livelihoods, well-being and social capital. Sustainability, 6(5), 2459-2473.

Groenewegen, P. P., Van den Berg, A. E., De Vris, S., & Verheij, R. A. (2006). Vitamin G: effects of green space on health, well-being, and social safety. BMC public health, 6(1), 1-9.

Ives, C. D., Lentini, P. E., Threlfall, C. G., Ikin, K., Shanahan, D. F., Garrard, G. E., ... & Kendal, D. (2016). Cities are hotspots for threatened species. *Global Ecology and Biogeography*, *25*(1), 117-126.

Kabisch, N., S. Qureshi, and D. Haase. 2015. Human-environment interactions in urban green spaces — a systematic review of contemporary issues and prospects for future research. *Environmental Impact Assessment Review*, 50:25-34.

Lepczyk, C. A., Aronson, M. F., Evans, K. L., Goddard, M. A., Lerman, S. B., & MacIvor, J. S. (2017). Biodiversity in the city: fundamental questions for understanding the ecology of urban green spaces for biodiversity conservation. *BioScience*, *67*(9), 799-807.

Montoya, F. G., Alcayde, A., Baños, R., & Manzano-Agugliaro, F. (2018). A fast method for identifying worldwide scientific collaborations using the Scopus database. *Telematics and Informatics*, *35*(1), 168-185.

Tennant, J. P., Waldner, F., Jacques, D. C., Masuzzo, P., Collister, L. B., & Hartgerink, C. H. (2016). The academic, economic and societal impacts of Open Access: an evidence-based review. F1000Research, 5.

Ugolini, F., Massetti, L., Calaza-Martínez, P., Cariñanos, P., Dobbs, C., Ostoić, S. K., Sanesi, G. (2020). Effects of the COVID-19 pandemic on the use and perceptions of urban green space: An international exploratory study. *Urban forestry & urban greening*, *56*, 126888.

United Nations, Department of Economic and Social Affairs, Population Division (2019). World Urbanization Prospects: The 2018 Revision (ST/ESA/SER.A/420). New York: United Nations.

Wang, Y., Bakker, F., De Groot, R., & Wörtche, H. (2014). Effect of ecosystem services provided by urban green infrastructure on indoor environment: A literature review. Building and environment, 77, 88-100.

Wang, R., Helbich, M., Yao, Y., Zhang, J., Liu, P., Yuan, Y., & Liu, Y. (2019). Urban greenery and mental wellbeing in adults: Cross-sectional mediation analyses on multiple pathways across different greenery measures. *Environmental research*, *176*, 108535.

White, M. P., Alcock, I., Grellier, J., Wheeler, B. W., Hartig, T., Warber, S. L., ... & Fleming, L. E. (2019). Spending at least 120 minutes a week in nature is associated with good health and wellbeing. *Scientific reports*, 9(1), 1-11.

SURVIVAL OF MICROORGANISMS IN CRUDE OIL POLLUTED SOIL

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Abstract

Soil pollution by crude oil can occur naturally via spillage, accidents or improper disposal of crude oil and related products. Indigenous soil microorganisms such as bacteria, yeasts and filamentous fungi can use crude oil hydrocarbons as food and source of energy. They transform the complex organic compounds into less toxic or non toxic substances such as carbon dioxide, water and fatty acids. The aim of this research was to follow the survival of microorganisms in crude oil polluted soil in *in vitro* conditions. Two dosages of crude oil were tested (5 and 20 g kg^{-1}). Total number of bacteria, actinobacteria, fungi, aminoheterotrophs and azotobacter were monitored during six months period. Addition of crude oil negatively affected only the number of actinobacteria and azotobacter and the number decreased in relation to the control at the beginning of the experiment. Six months later, the total number of bacteria did not change significantly (log No 7.83 to log No 8.96 g $soil^{-1}$, respectively), regardless of the amount of added crude oil. Number of fungi decreased (log No 3.79 g $soil^{-1}$) as well as the number of azotobacter (log No 3.88 g $soil^{-1}$). The obtained results suggested that the autochthonous soil microorganisms adapted well and had high survival rate.

Key words: *microorganisms, crude oil, survival, pollution.*

Introduction

Soil pollution with crude oil and its products can occur during exploitation, processing, storage, transport and by accidental spillage (Pinedo *et al.*, 2013; Veerapagu *et al.*, 2019). When it happens, a huge environmental threat emerges. However, for microorganisms the crude oil could be a substrate for growth, an energy source and sources of carbon, nitrogen and sulfur (Talaiekhozani *et al.,* 2015). The most commonly used hydrocarbons are C1-C40 alkanes, cycloalkanes, benzene, ethylbenzene, toluene, numerous heterocyclic compounds (Beškoski *et al.*, 2012). Microbes transform the complex organic compounds into less toxic or non toxic substances such as carbon dioxide, water and fatty acids. Microbial degradation of hydrocarbon could take place under aerobic and anaerobic conditions and is influenced by temperature, soil pH, nutrients availability etc. On the other hand, the influence of oil on microorganisms is a gradual process, in which the microorganisms with the ability of salt tolerance, anaerobic metabolism or oil degradation potential continue to flourish in culture (Shaoping *et al.,* 2021).

Dominant bacterial species that use oil carbohydrates belong to the genus *Nocardia, Pseudomonas, Bacillus Acinetobacter, Flavobacterium, Micrococcus, Arthrobacter, Corynebacterium, Achromobacter, Rhodococcus* while *Rhodotorulla, Candida, Fusarium, Aspergillus, Mucor, Penicillium, Trichoderma* and *Phanerochaete* are the most important fungi (Koshlaf and Ball, 2017; Piñón-Castillo *et al.*, 2017). Ikhajiagbe and Ogwu (2020) found six bacterial and nine fungal species in crude oil-polluted soil from 16 different sites in their study.

Bacterial count in crude oil-polluted sites ranged between 1.51 and 5.43×10^5 CFU g^{-1} soil while fungal count varied between 0.44 and 3.49×10^5 CFU g $soil^{-1}$. Common microorganisms' species were *Bacillus subtilis* and *Aspergillus niger*.
The presence of indigenous microbial population, their survival and reproduction in crude oil reservoirs is advantageous for the implementation of methods known as Microbial Improved Oil Recovery-MIOR (Wood, 2019). *Pseudomonas aeruginosa, Bacillus licheniformis, Xanthomonas campestris* and *Desulfovibrio desulfuricans* are some of the common bacterial species used in oil recovery processes (Singh et al., 2007). Isolation and characterization of microorganisms with high survival rate and crude oil degrading ability is important in order to remediate the polluted sites with these organisms by means of bioaugmentation.
Having that in mind, the aim of the research was set up. The survival of autochthonous microorganisms in crude oil polluted soil in *in vitro* conditions was monitored during six months in order to screen for potential crude oil degraders.

Material and methods

Soil for the study was humofluvisol. Control treatment was only watered while in other treatments 5 and 20 g kg^{-1} of crude oil was added. Experiment was carried out in 12 L pots. 70% FWC was maintained throughout the experiment. Each treatment had three repetitions. The experiments lasted six months, starting May till October 2019 in *in vitro* conditions. First sampling was carried out one week after the addition of crude oil. The soil samples for microbiological analyses were collected and placed in sterile polyurethane bags and kept at a temperature of 4°C. Laboratory measurements were performed in the microbiological laboratories of the Faculty of Agriculture, Novi Sad (Serbia). The microbiological analyses included determining the total number of bacteria, actinobacteria, fungi, aminoheterotrophs and azotobacters. The number of microorganisms was determined using the dilution method (Trolldenier, 1996). The following nutrient media were used (Hi Media Laboratories Pvt. Limited, Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for the number of actinobacteria, potato dextrose agar for the number of fungi, meat peptone agar for the number of aminoheterotrophs, and mannitol salt agar for the number of azotobacters. Inoculated Petri dishes were placed in thermostat and incubated at constant temperature of 28 °C. The incubation period depended on the tested group of microorganisms and varied between two and 10 days. After incubation, the plates were inspected. The number of microorganisms was calculated using formula A×B×C/D, where A is average number of microorganisms per 1 gram of absolutely dry soil, B is coefficient of correction per 1 ml (2—when 0.5 ml of soil suspension was inoculated; 5—when 0.2 ml of soil was inoculated), C is soil dilution, D is mass of 1 g of absolutely dry soil-drying at 105°C for 4 h (Jarak and Djuric, 2006). The number of microorganisms was calculated in one gram of absolutely dry soil ($CFUg^{-1}$ soil) and expressed in a *logarithmic* graph.

Results and discussion

The number of investigated groups of microorganisms was high at the beginning of the experiment (Fig. 1). Addition of crude oil only affected the number of actinobacteria and azotobacter and they number decreased in relation to the control. Total number of bacteria, aminoheterotrophs and fungi were higher than the control treatment.

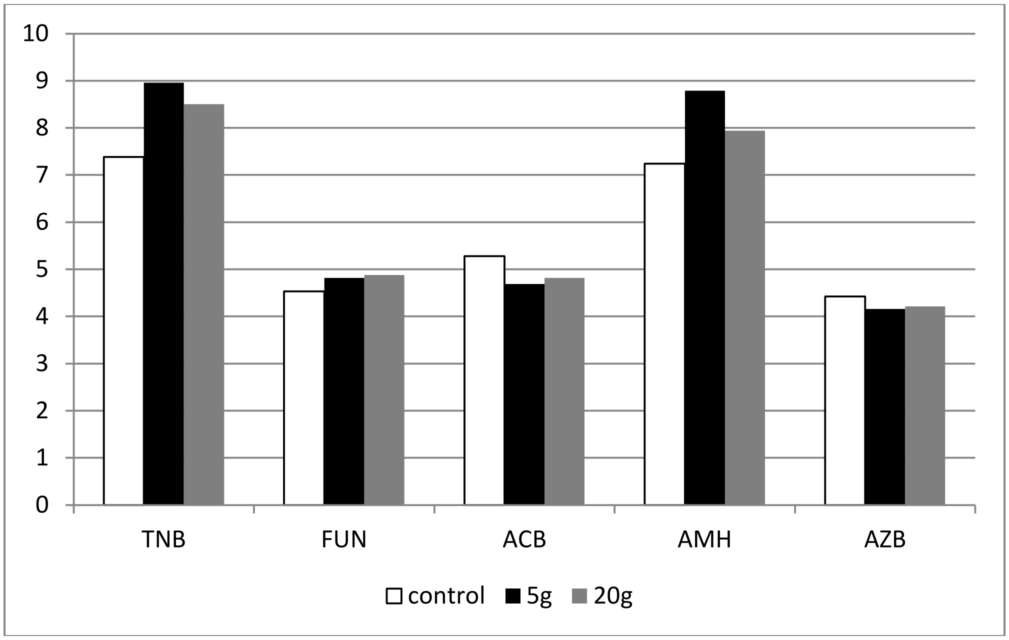

Fig. 1. Number of microorganisms in soil at the beginning of the experiment (log No g^{-1} soil)
TNB-total number of bacteria; FUN-fungi; ACB-actinobacteria; AMH-aminoheterotrophs; AZB-azotobacters

Six months later, the total number of bacteria did not change significantly, regardless of the amount of added crude oil (Fig. 2). Number of fungi decreased as well as the number of azotobacter. The number of actinobacteria and aminoheterotrophs were a little higher than the number in the watered treatment.

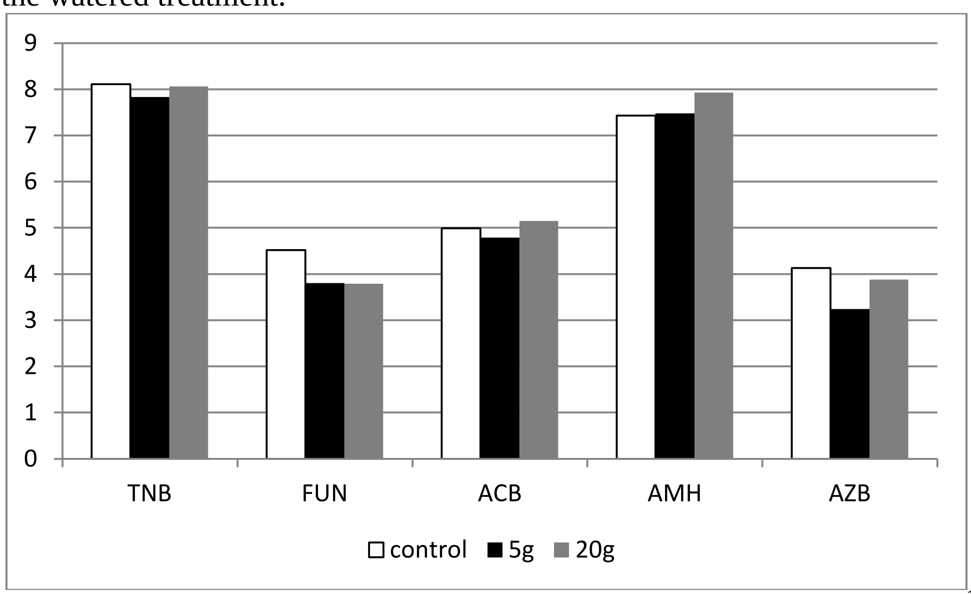

Fig. 2. Number of microorganisms in soil at the end of the experiment (log No g^{-1} soil)
TNB-total number of bacteria; FUN-fungi; ACB-actinobacteria; AMH-aminoheterotrophs; AZB-azotobacters

These results are in accordance with the results obtained by Li *et al.* (2000). They conducted an *in-situ* experiment in eastern China with polluted soil with petroleum hydrocarbons at concentrations up to 200 g kg^{-1} dry soil. The qualitative assessment of bacterial microflora identified dominant bacteria species from genus's *Xanthomonas*, *Bacillus* and *Hyphomicrobium*. The number of bacteria was of order 10^7 CFU g^{-1} soil. Bacteria contain different enzymes that breakdown hydrocarbons in soil. Voiculescu *et al.*, (2003) identified 23 taxonomic groups of bacteria belonging to the family *Enterobacteriaceae* and genera *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Aeromonas*, *Vibrio*, *Microbacterium*, *Flavobacterium*, *Corynebacterium*, *Mycobacterium*, *Arthrobacter*, *Brevibacterium*, *Nocardia*, *Bacillus*, *Micrococcus*, *Staphylococcus* and *Sarcina*. Filamentous fungi that were actively engaged in crude oil degradation in soil were species from genus *Aspergillus spp.*, *Penicillinium sp.*, *Paecilomyces sp. and Fusarrium* sp. Agu *et al.*, (2015) isolated heterogeneous bacteria from oil polluted soil. They belonged to the genus *Aeromonas* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Corynebacterium spp.*, *Pseudomonas spp.*, *Aeromonas spp.*, *Staphylococcus spp.*, *Micrococcus spp.*, *and Bacillus spp*. The total count ranged from 0.22×10^7 to 2.83×10^7 CFU g^{-1} soil. They argued that the ability of the microorganism to survive is associated with its ability to use crude oil as carbon source but were also affected by abiotic factors such are temperature, nutrients availability and oxygen content in soil.

In this research, the amount of degraded hydrocarbons was not monitored. However, it can be assumed that degradation processes occurred and certain amount of end products were used and/or released by the microorganisms. Usually, microorganisms adapt very well in short period of time and start the degradation pathways through gradual transformation of contaminants into intermediates of the central metabolism. Hyun *et al.* (2008) and Nam *et al.* (1998) found that the degradation rate of petroleum hydrocarbon pollutants is generally higher during the early stage when the pollutants are easily available. In later stages the contaminants bioavailability is limited as a result of hydrocarbons sequestration. It means that once that stage has been reached no further degradation occurs during remediation.

This experiment showed that the indigenous soil microorganisms were not affected negatively by the addition of different amount of crude oil. They adapted to the presence of the contaminant, probably used it as energy and nutrient source and consequently had high survival rate. Some of the microorganisms could be screened for their oil degrading ability and potentially used as remediation agents

Conclusions

Addition of crude oil affected the number of actinobacteria and azotobacter and the number decreased in relation to the control at the beginning of the experiment. Six months later, the total number of bacteria did not change significantly (log No 7.83 to log No 8.96 g $soil^{-1}$, respectively), regardless of the amount of added crude oil. Number of fungi decreased (log No 3.79 g $soil^{-1}$) as well as the number of azotobacter (log No 3.88 g $soil^{-1}$). The obtained results suggested that the autochthonous soil microorganisms adapted well and had high survival rate.

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References

Agu K.C., Edet B.E., Ada I.C., Sunday A.N., Chidi O.B., Gladys A.C., Uche E.C., Uchenna O.M., Chinedu O.A. (2015). Isolation and Characterization of Microorganisms from Oil Polluted Soil in *Kwata*, Awka South, Nigeria. American Journal of Current Microbiology 3:46-59.

Beškoski V.P., Gojgić-Cvijović G.Đ., Milić J.S., Ilić M.V., Miletić S.B., Jovančićević B.S., Vrvić M.M. (2012). Bioremediation of soil polluted with crude oil and its derivatives: Microorganisms, degradation, pathways, technologies. Hemijska Industrija 66 (2) 275–289. DOI: 10.2298/HEMIND110824084B

Hyun S., Ahn M.Y., Zimmerman A.R., Kim M., Kim J.G. (2008). Implication of hydraulic properties of bioremediated diesel-contaminated soil. Chemosphere 71: 1646–1653.

Ikhajiagbe B., Ogwu M.C. (2020). Hazard quotient, microbial diversity, and plant composition of spent crude oil polluted soil. Beni-Suef University Journal of Basic and Applied Sciences 9: 26. https://doi.org/10.1186/s43088-020-00052-0

Jarak M., Djurić S. (2006). Praktikum iz mikrobiologije (Manual of microbiology). , Novi Sad. (In Serbian).

Koshlaf E., Ball A.S. (2017). Soil bioremediation approaches for petroleum hydrocarbon polluted environments. AIMS Microbiology, 3(1): 25-49. DOI: 10.3934/microbiol.2017.1.25

Li G., Huang W., Lerner D. N., Zhang X. (2000). Enrichment of degrading microbes and bioremediation of petrochemical contaminants in polluted soil. Water Research 34 (15), pp. 3845-3853.

Nam K., Chung N., Alexander M. (1998) Relationship between organic matter content of soil and the sequestration of phenanthrene. Environmental Science & Technology 32: 3785–3788.

Pinedo J., Ibáñez R., Irabien Á. (2013). Assessment of Soil Pollution Based on Total Petroleum Hydrocarbons and Individual Oil Substances. Journal of Environmental Management 130, 72–79.

Piñón-Castillo H.A., Gutiérrez D.L., Zavala-Díaz de la Serna F.J., Hernández-Castillo D., Muñoz-Castellanos L.N., Rivera-Chavira B.E., Nevárez-Moorillón G.V. (2017). Laboratory-Scale Biodegradation of Fuel Oil No. 6 in Contaminated Soils by Autochthonous Bacteria, In Advances in Bioremediation and Phytoremediation, Ed. Naofumi Shiomi, IntechOpen, DOI: 10.5772/intechopen.70350.

Shaoping K., Zhiwei D., Bingchen W., Huihui W., Jialiang L., Hongbo Sh. (2021). Changes of sensitive microbial community in oil polluted soil in the coastal area in Shandong, China for ecorestoration. Ecotoxicology and Environmental Safety 207: 111551.

Singh A., Van Hamme J.D., Ward, O.P. (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnology Advances 25: 99-121.

Talaiekhozani A., Jafarzadeh N., Fulazzaky M.A. Talaie M.R., Beheshti M. (2015). Kinetics of substrate utilization and bacterial growth of crude oil degraded by *Pseudomonas aeruginosa*. Journal of Environmental Health Science and Engineering 13, 64. https://doi.org/10.1186/s40201-015-0221-z

Trolldenier G. (1996). Plate Count Technique. In: Schinner F, Kandeler E, Ohlinger R, Margesin R (eds) Methods in soil biology. Springer, Berlin, 20–26.

Veerapagu M, Jeya K.R, Kalaivani R, Jeyanthi K.A., Geethanjali S. (2019). Screening of hydrocarbon degrading bacteria isolated from oil contaminated soil. The Pharma Innovation Journal 8(6): 69-72.

Voiculescu A.R., Dumitru M., Toti M. (2003). Decontaminarea Solurilor Poluate cu ComDecontamination of Soils Polluted with Organic Compounds). Ed. SITECH, Craiova, ISBN 973-657-939-5.

Wood D.A. (2019). Microbial improved and enhanced oil recovery (MIEOR): Review of a set of technologies diversifying their applications. Advances in Geo-Energy Research 3(2): 122-140. doi:10.26804/ager.2019.02.02

INFLUENCE OF ENVIRONMENTAL FACTORS ON INSECT POLLINATORS IN NORTHERN SERBIA

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Abstract

One of the major consequences of anthropogenic influence is the evident decrease in the diversity and number of insect pollinators. Being highly vulnerable ecosystem service, mainly due to agricultural intensification, the spread of diseases and parasites, and pesticide pollution, loss of pollinator diversity may lead to loss of other species that directly or indirectly rely on them and could have significant economic impacts by reducing crop pollination services. In this study, we examined the relationship between environmental variables (altitude, distance from the nearest agricultural area, grazing intensity, and mowing) on the composition and abundance of pollinator species. Pollinators were sampled on the territory of the Autonomous Province of Vojvodina in the Republic of Serbia, on four types of habitats categorised according to the CORINE land cover classification as classes 2.1.1 Non-irrigated arable land, 2.4.3 Land principally occupied by agriculture, with significant areas of natural vegetation, 3.1.1 Broad-leaved forest and 3.2.1 Natural grassland. Our results showed a significant correlation between the composition of pollinator species and the overall set of environmental factors. We found that forest, followed by grassland habitats, had the most diverse pollinator communities. Localities under grazing and mowing appeared to be less diverse in pollinators, whereby agricultural areas had the lowest pollinator diversity. Our findings suggest that the diversity of pollinators is highly influenced by land use and confirm the importance of forest habitats in agricultural landscapes such as Vojvodina Authonomous Province in Serbia as important refuge habitats for foraging and nesting of pollinators.

Keywords: *pollinator diversity, pollinator abundance, habitat types, land use.*

Introduction

Insect pollinators provide essential ecosystem services by pollinating over 85% of all known plant species (Grimaldi and Engel, 2005; Ollerton et al., 2011). A wide range of plants whose survival depends on pollination include crops, fruits, nuts, vegetables and cereals (Gordon and Davis, 2003; Klein et al., 2007). Furthermore, many plant species are more sensitive to the deficiency of pollinators than to the lack of other resources (Burd, 1994; Ashman et al., 2004). Insect pollination is vital for agricultural food production and sustenance of 80% of wild plant species (Potts et al., 2010). When it comes to the impact of pollination on agricultural production, Williams (1994) states that 84% of the crops grown in Europe are directly dependent on pollination, and Klein et al. (2007) claim that 87 crops, which is 70% of the 124 key plant species that humans use directly in their diet worldwide, have a dependence on insect pollinators. Habitat and climate changes caused by anthropogenic factors have a negative impact on many taxa, including pollinators. Given the declining diversity and abundance of pollinators (Dias et

al., 1999; Klein et al., 2007) and the identified decline in at least one region or country on every continent (except Antarctica), this issue has been the subject of research for many years (AllenWardell et al., 1998; Kremen and Ricketts, 2000; Biesmeijer et al., 2006, Potts et al., 2010). Other factors that lead to a decrease in the number of pollinators are: intensification of agricultural production, cultivation of monocultures, spread of diseases and parasites, use of pesticides, urbanisation and reduction of available niches necessary for feeding and reproduction of pollinators (Allen-Wardell et al., 1998; Kevan and Phillips, 2001; Biesmeijer et al., 2006).
Different anthropogenic disturbances and the intensive management of agroecosystems have a severe impact on the biodiversity of pollinators. In order to secure pollinator-friendly habitats and advance pollinator conservation it is essential to investigate the impact of influential abiotic traits on important pollinator groups.
In this paper, we analysed the relationship between environmental variables and insect pollinator composition in Vojvodina region (North Serbia). The following abiotic traits were considered: altitude, distance from an agricultural area, grazing intensity, and mowing.

Material and Methods

The paper is based on a sample of pollinators (bees and hoverflies) that were collected during the three years of fieldwork (2011-2013) on four habitat types (Mudri-Stojnić et al., 2012; Markov et al., 2016). A map with sites is shown in Figure 1.

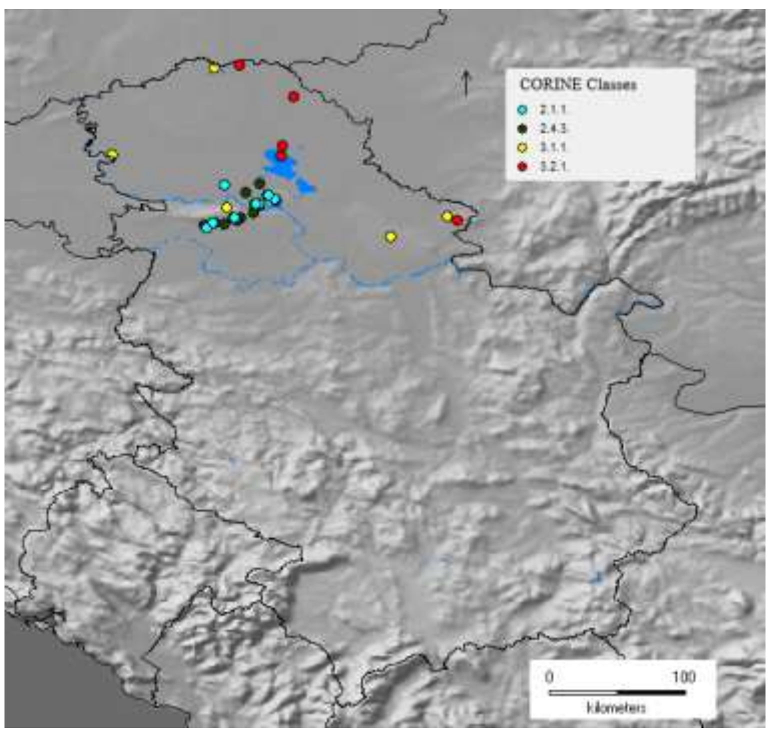

Figure 1. Sampling sites.

To examine the strength of the relationship between the explanatory variables and the species data set, multivariate analysis was conducted in the software package CANOCO 4.5 (Ter Braak and Smilauer, 2002). The dependent variables were tested by indirect gradient length analysis

using the DCA method to determine the most appropriate ordination method (detrended correspondence analysis) (Leps and Smilauer, 2003).
Taking into account the gradient length obtained by DCA analysis (2.89), a limited (constrained) canonical redundancy analysis (RDA) was performed. The influence of four environmental variables on the composition and distribution of pollinator species was tested: altitude, distance from the agricultural area (DAA), grazing intensity (grazing) and mowing. Different CORINE Land Cover habitats (2.1.1., 2.4.3., 3.1.1. and 3.2.1. codes) were considered as qualitative, categorical variables. A Monte-Carlo permutation test with 499 permutations was used to determine whether the examined environmental variables had a significant effect on the composition of pollinator species (Ter Braak and Smilauer, 2002).

Results and Discussion

The RDA diagram (Figure 2.) shows the sample areas (26 in total) and examined environmental variables. RDA analysis showed a significant correlation between the composition of pollinator species and the total set of environmental predictors ($F = 3.52$; $P = 0.002$ with 499 permutations). The variables that generated the longest arrows are DAA (distance from agricultural area) and mowing.

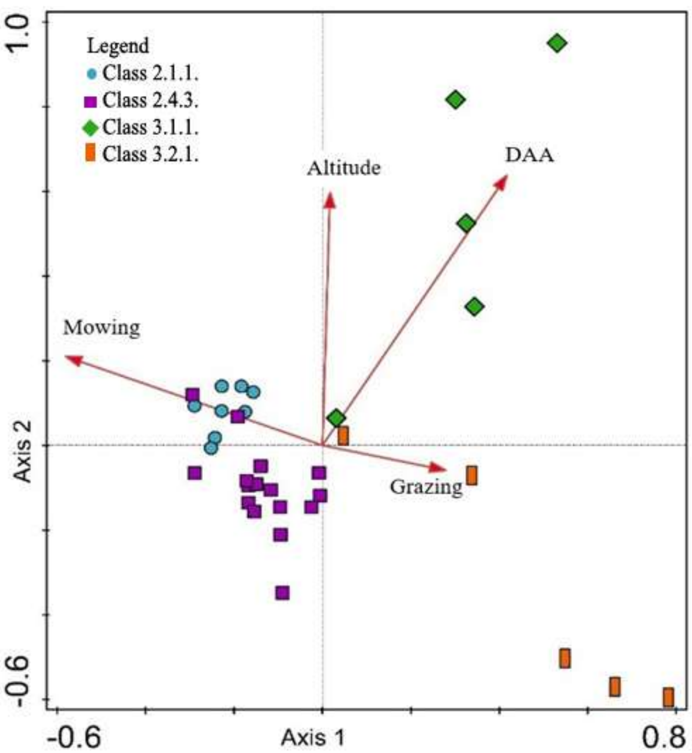

Figure 2. RDA ordination plot with statistically significant environmental variables and species diversity within sample areas (CORINE habitat types).

In Figure 3. it can bee observed that the first two ordinates, or dimensions (Dim 1 and Dim 2), explain 64% (cumulative percentage) of variability in relation to pollinator species abundance and environmental factors. The first ordinate represents the gradient of spatial variables (altitude

and distance from the agricultural habitat), and the second one represents the gradient of management activities (grazing and mowing).
The largest number of insect pollinator species was found on the land cover type broad-leaved forest (CORINE code 3.1.1), followed by natural grasslands (CORINE code 3.2.1), and land principally occupied by agriculture, with significant areas of natural vegetation (CORINE code 2.4.3). The least number of species was found on non-irrigated arable land (CORINE code 2.1.1). Ricarte et al. (2011) investigated the diversity of hoverflies in different habitat types (forest, shrubby and grassland areas) and their results also demonstrated a greater abundance of species in forest habitats. Forests provide a wide range of resources and microhabitats, acting as a shelterbelts in grassland-dominated areas (Ricarte et al., 2011). Within the agricultural setting of Vojvodina, Fruška gora and Vršački breg, can be considered as key reservoirs providing abundant resources for pollinators.

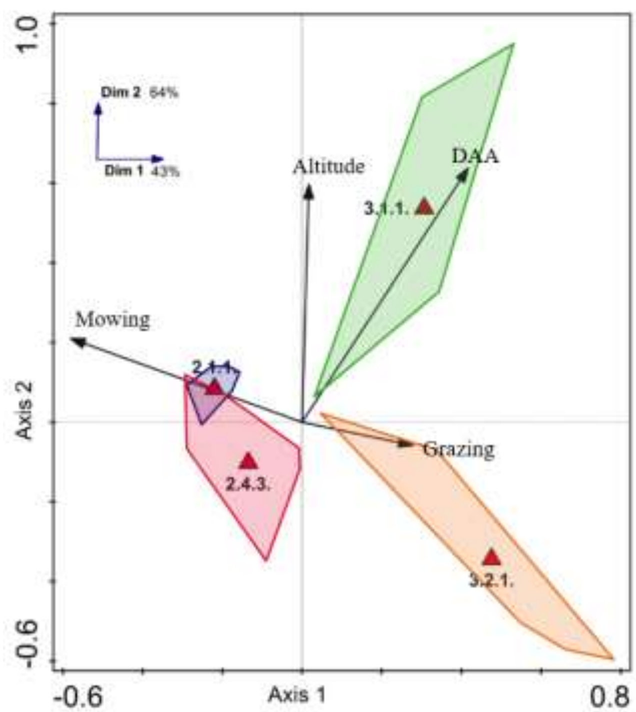

Figure 3. RDA ordination plot of environmental variables and grouped CORINE habitat types. Categorical variables (CORINE habitat types) are represented by red triangle.

The greatest diversity of pollinator species in forest habitats (Fig 2., Fig 3.), can be explained by the preference of hoverflies towards more heterogeneous habitats. Investigated forest habitats of high complexity and microhabitat diversity provide a wide range of food resources, nesting sites and meet different specific requirements of pollinator species. For example on Fruška gora site, pollinators were collected within forest stands and marginal forest habitats near the streams. In Vršački breg, pollinators were sampled in habitats dominated by mixed stands of downy oak (*Quercus pubescens* Willd.), Hungarian oak (*Quercus frainetto* Ten.), Turkey oak (*Quercus cerris* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.), pedunculate oak (*Quercus robur* L.), and stands comprised of common hornbeam (*Carpinus betulus* L.), silver lime (*Tilia tomentosa* Moench.) and common beach (*Fagus sylvatica* L.) Sites on Subotica sands are characterised by acacia forest with a ground floor consisting of species from the buttercup family (Ranunculaceae). In Deliblato sands, pollinators were sampled in habitats dominated by acacia forest with ground cover species from the grass family (Poaceae), pea family (Fabaceae) and teasel family (Dipsacaceae) such as *Knautia sp*..

Grazing sites demonstrated significant species diversity, lower than forest habitats, however far higher compared to agricultural sites. Open habitats are an excellent food source for many species of bees and hoverflies, due to their dependence on the flowering plants, and essential nesting sites for ground nesting pollinator species. Species diversity in open habitats as well as statistically significant impact of grazing and mowing on the pollinator species composition, indicate the importance of preserving these habitat types and preventing succession, i.e. excessive growth of woody plants.

Although there is a relatively small difference in altitude between investigated sites (range from 69m to 347m), statistical analysis showed that altitude significantly impacts the composition of pollinator species. Considering that landscape heterogeneity increases with altitude, it is expected that sites at higher altitudes demonstrate greater species richness. Additionally, forest sites are within protected areas with significantly reduced human impact, while sites at lower altitudes are under intensive agricultural production, monoculture fields, and herbicide use.

Although monocultures (e.g. sunflower) are a suitable food source for certain pollinator species, the conversion of wild flower habitats into monocultures results in significant depletion of food sources for a large number of hoverfly and bumblebee species (Osgathorpe et al., 2011). In addition, specialised species are generally more sensitive to changes in land-use patterns within agricultural areas (Kleijn and Raemakers, 2008), whereby pesticides (e.g. neonicotinoids) lead to adverse changes in pollinator behaviour, which is manifested in the reduced search for food (Whitehorn et al., 2012).

Agricultural intensification usually results in expansion of infrastructural structures, such as roads, which can lead to isolation of pollinator populations over several years. This might explain statistically significant correlation between the composition of pollinator species and the distance from the agricultural area.

Conclusions

Our study demonstrates that the composition of pollinator species on four types of habitats in Vojvodina is closely related to environmental factors (altitude, distance from the nearest agricultural area, grazing intensity, and mowing), where forest had the most diverse pollinator communities, while agricultural areas had the lowest pollinator diversity. Our findings suggest that the diversity of pollinators is highly influenced by land use and confirm the importance of forest habitats in agricultural landscapes such as Vojvodina as important refuge habitats for foraging and nesting of pollinators. Additional field studies are necessary to improve the assessment of pollinators responses to abiotic drivers.

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References

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P.,Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P.G., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P., Walker, S. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields, Conservation Biology, Vol. 12: 8-17.

Ashman, T.-L., Knight, T. M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R., Mazer, S.J., Johnston, M., Mitchell, R.J., Morgan, M.T., Wilson, W.G. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences, Ecology, Vol. 85: 2408-2421.

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands, Science, Vol. 313: 351-354.

Burd, M. (1994). Batemans principal and plant reproduction: The role of pollen limitation in fruit and seed set, Botanical Review, Vol. 60: 83-139.

Dias, B.S.F., Raw, A., Imperatri-Fonseca, V.L. (1999). International Pollinators Initiative: The São Paulo Declaration on Pollinators, Brasilian Ministry of the Environment, Brasilia, Brazil. Available at: https://www.cbd.int/doc/ref/agr-pollinator-rpt.pdf Accessed: 23.05.2021.

Gordon, J., Davis, L. (2003). Valuing honeybee pollination, Rural Industries Research and Development Corporation Paper 03/077, Canberra, ACT, Australia.

Grimaldi, D., Engel., M.S. (2005). Evolution of the insects, Cambridge University Press, New York.

Kevan, P. G., Phillips, T.P. (2001). The economic impacts of pollinator declines: an approach to assessing the consequences, Conservation Ecology, Vol. 5: 8-15.

Kleijn, D., I. Raemakers, (2008). A retrospective analysis of pollen host plant use by stable and declining bumble bee species, Ecology, Vol. 89(7): 1811-1823.

Klein, A. M., Vaissière, B., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. (2007). Importance of crop pollinators in changing landscapes for world crops, Proceedings of the Royal Society of Biological Sciences, Vol. 274: 303-313.

Kremen, C., Ricketts, T. (2000). Global perspectives on pollination disruptions. Conservation Biology, Vol. 14: 1226-1228.

Leps, J., Smilauer, P. (2003). Multivariate Analysis of Ecological Data Using CANOCO, Cambridge University Press, Cambridge.

Markov, Z., Nedeljković, Z., Ricarte, A., Vujić, A., Jovičić, S., Jozan, Z., MudriStojnić, S., Radenković, S., Ćetković, A. (2016). Bee (Hymenoptera: Apoidea) and hoverfly (Diptera: Syrphidae) pollinators in Pannonian habitats of Serbia, with a description of a new Eumerus Meigen species (Syrphidae), Zootaxa, Vol. 4154(1): 027-050.

Mudri-Stojnić, S., Andrić, A., Józan, Z., Vujić, A. (2012). Pollinator diversity (Hymenoptera and Diptera) in semi-natural habitats in Serbia during summer, Archives of biological sciences, Vol. 64(2): 777-786.

Ollerton, J., Winfree, R., Tarrant, S. (2011). How many flowering plants are pollinated by animals? Oikos, Vol. 120: 321-326.

Osgathorpe, L., Park, K., Goulson, D., Acs, S., Hanley, N. (2011). Trade-offs between agriculture and biodiversity in marginal areas, Ecological Economics, Vol. 70: 1162- 1169.
Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers, Trends in Ecology and Evolution, Vol. 25 (6): 345-353.
Ricarte, A., Marcos-García, M.A., Moreno, C.E. (2011). Assessing the effects of vegetation type on hoverfly (Diptera: Syrphidae) diversity in a Mediterranean landscape: implications for conservation, Journal of Insect Conservation, Vol. 15: 865–877.
Ter Braak, C.J.F., Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca.
Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production, Science, Vol. 336: 351-352.
Williams, I. H. (1994) The dependences of crop production within the European Union on pollination by honey bees, Agricultural Zoology Reviews, Vol. 6: 229-257.

RASPBERRY PRODUCTION AND ECONOMIC VALUE OF INSECT POLLINATION OF RASPBERRY IN SERBIA

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Abstract

The Republic of Serbia ranks among the largest producers of raspberry on the global market, with an annual production of circa 120,000 tons and an export of 80,000 tons. Although commercial raspberry cultivars are largely self-fertile and self-pollinate autogamously, without insect pollination the fruits are small or crumb. Insects that are mainly used to pollinate commercial raspberries are honeybees. In this research, we analyse the production of raspberries in the Republic of Serbia, for the whole country and separately for the northern and southern region during the period from 2010 to 2020, as well as estimate the economic value of insect pollination (EVIP) ecosystem service of raspberry in the same decade. Likewise, we presented the total value of raspberry crop (TVC) to emphasize the importance of insect pollination to the yield. In Serbia, the annual yield of raspberries ranged from 70,000 tons in 2012 to 127,000 tons in 2018, but over 90% of the yield is produced in the southern region. Considering producer price per metric ton, annual raspberry production and dependence upon the insect, the estimated EVIP ranged from approximately 48 million EUR in 2011 to 117.5 million EUR in 2020. In the observed period, the TVC has been followed the EVIP's trend to conclude, insect pollinators represent a significant share of TVC; they have a substantial positive impact on raspberry production, therefore conservation activities and good management practices are needed to maintain and improve the ecosystem pollination service in relation to this Serbian brand.

Keywords: *pollination service, EVIP, raspberry yield, honeybees*.

Introduction

Globally, the production of pollinator-dependent crops has increased during the last several decades, even more rapidly than pollinator-independent crops (Aizen et al., 2008; Garibaldi et al., 2011). Growing need for food has resulted in expanding of both markets and agricultural production. Increased food demand is also reflected in the production of raspberries worldwide, where the global harvested area has been more than doubled since 1986, reaching 127,578 ha in 2019 (FAOSTAT, 2021). The Republic of Serbia is one of the world's leading raspberry producers, with 80,000 tonnes of annually exported raspberry and steady expansion of land under raspberry cultivation (Stojanović et al., 2018).

Red raspberry (*Rubus idaeus* L.) grown for commercial purposes is predominantly self-fertile (Żurawicz, 2016), but due to the specific morphology of the flower, complete self-fertilization is not possible (Shanks, 1969). However, in the presence of insect pollinators and their raspberry flower visitation, the fruit has a greater weight and the increased number of drupelets (Shanks, 1969; Cane, 2005).

Unfortunately, agricultural intensification has a negative impact on many wildlife species (Robinson and Sutherland, 2002; Benton et al., 2003), including insect pollinators (Winfree et

al., 2009; Garibaldi et al., 2011). The abundance and diversity of wild insect pollinators have decreased in many regions, mainly due to agricultural land expansion and intensification (Potts et al., 2010). Because of apparent declines in a range of pollinator species worldwide, and potential pollinator deficit to produce entomophilous crops, there is a rising concern about the sustainability of pollination services (Steffan Dewenter et al., 2005).
In order to support pollination services in agroecosystems, it is necessary to develop appropriate management practices that can serve to conserve pollinators (Velthuis and Van Doorn, 2006). Economic valuation of insect pollination is of big importance when it comes to informing conservation efforts, providing information needed for prioritizing species and their habitats. Ecosystem services valuation can help identifying the benefits provided by ecosystems and biodiversity that are often left unrecognized and/or underestimated when it comes to decision - making in natural resource management. Due to the above-mentioned reasons, assessing the economic value of ecosystems and their services has been gaining significant attention in the scientific community worldwide (Perez-Verdin et al., 2016; Picanço et al., 2017; Porto et al., 2020).
This study aims to (i) provide an overview of raspberry production in the past decade in Serbia and (ii) estimate and analyse the economic value of insect pollination (EVIP) of the raspberry production in Serbia, which could assist in providing guidelines for the conservation of ecosystem pollination service. EVIP of raspberry is calculated to emphasize the economic importance of pollinators for agricultural production. The value obtained in this way should serve decision-makers but also other stakeholders as a guideline for conservation.

Material and Methods

Economic value of insect pollination of raspberry is calculated according to Gallai et al. (2009) methodology. The dataset used contains information on producer price, annual raspberry production as well as on the impact of insect pollination on raspberry. According to Klein et al. (2007), the ratio of raspberry dependence on pollination was estimated to be 65%.
This value indicates that due to the lack of pollination, raspberry yields would be reduced between 40% and 90%, so pollination is specified as "essential".
EVIP is calculated by multiplying the quantity of produced crop, the producer price per unit mass and the ratio of raspberry dependence on pollination. The result of multiplication of the first two parameters represents the producer price. The output data of this analysis are the value of the annual production of raspberries, the value of the contribution of insect pollination and EVIP.
Data on producer price and production for the whole country and separately for Serbia's northern and southern region from 2010 to 2020 were provided from the Statistical Office of the Republic of Serbia (SORS, 2021). For comparative purposes, the prices were converted into euros (EUR) according to the value of the national currency for a given period in the archives of the National Bank of Serbia (NBS, 2021).

Results and Discussion

The production area and total raspberry yield in Serbia, (as well as for the northern and southern part of the country depicted separately), are shown in Figures 1 and 2. Production area in Serbia increased from 11,500 ha in 2010 to 24,000 ha, as registered in 2020 (Fig. 1). The northern and southern part of Serbia also showed an upward trend when it comes to the production area. In the northern part the area increased from 471 ha in 2010 to 2,242 ha by 2020, while in the southern part, from 11,119 ha (2010) to 21,786 ha (2020). When it comes to the total raspberry yield, it was 83,870 t at the beginning of the observed period, afterwards in 2012 a decline to 70,320 t can be noticed, followed by an increase in yield. Another decline was recorded in 2017 (109,742 t), after which the yield of raspberries increased to 118,674 t by 2020. Similar oscillations of raspberry yield occurred in the northern and southern part of the country (Fig. 2). Despite a growing production area, the total yield did not follow this upward trend, which can be primarily attributed to the poor weather conditions over specific years with decreasing yield.

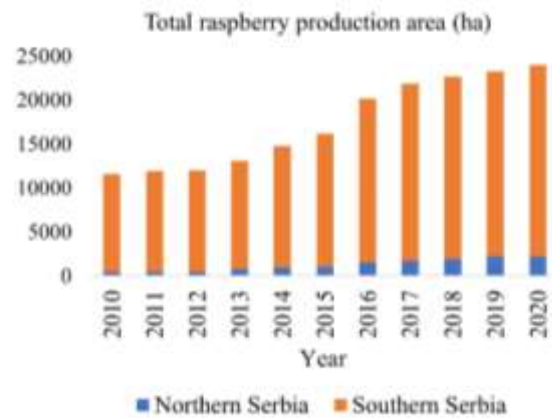

Figure 1. Total production area of raspberry (ha) in Serbia and separately in northern and southern part of country for the period 2010-2020.

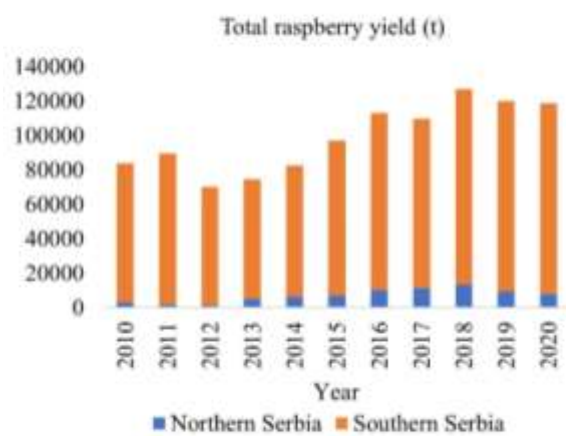

Figure 2. Total yield of raspberry (t) in Serbia and separately in northern and southern part of country for the period 2010-2020.

According to the bioeconomic approach, the EVIP for raspberries (in EUR) was estimated to range from approximately 48 million in 2011 to 117.5 million in 2020. To emphasize the importance and contribution of insect pollination to the yield of raspberries, the value of the Serbian raspberry crop is presented in Figure 3. The value was the lowest in 2011 when it was around EUR 74 million, while the highest recorded value was in 2020 (approximately EUR 181 million).

If the pollination services were to decline, the quality of the fruit would be drastically reduced (Andrikopoulos and Cane, 2018), which would consequently have a negative impact on individual producers and the economy in general. For raspberry, floral visitors promote increased quality of the fruit, which is an indirect and complex benefit to measure, however extremely important for the agricultural market. The obtained values of insect pollination of raspberries, combined with production data, represent a guideline for maintaining the ecosystem service of raspberry pollination and planning appropriate management practices. In this case, only the direct use value for agriculture is included in calculating EVIP. Nevertheless, the assessment result indicates that the investment in maintaining the services provided by insect pollinators to raspberries is justified, given that pollinators are threatened by various factors such as the use of harmful pesticides (Walker et al., 2017) or habitat destruction (Ollerton et al., 2014).

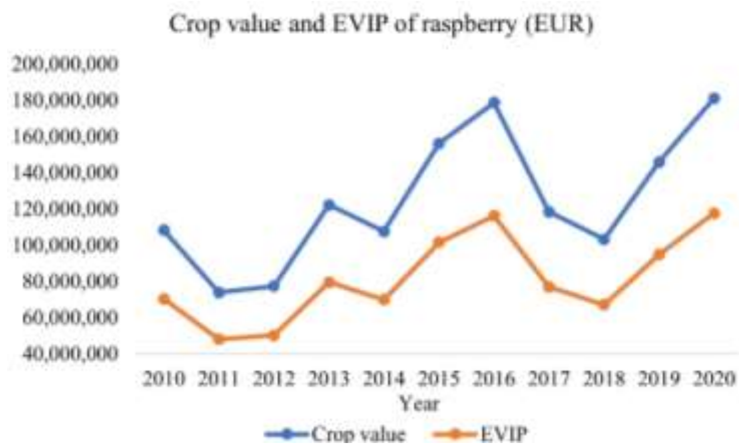

Figure 3. Crop value and Economic value of insect pollination (EVIP) of raspberry in EUR for period 2010-2020

Acknowledging the benefits of insect pollinators for the cultivation of raspberries, reflected in EVIP (even in such a simplified way), brings additional attention to this group of organisms in conservation practice. The EVIP cannot be used as the only source of information, but it can be implemented in a more comprehensive program that would aim to maintain the ecosystem service of raspberry pollination and improve yields. In this case, it means providing financial resources for the protection of wild insects pollinators and thus meeting their environmental requirements as well as enabling the direct benefit of their service through agriculture (de Snoo and de Leeuw, 1996). This aspect of (legal) protection of wild pollinators, specifically wild bees, is especially neglected in Serbia, where the Regulation on the declaration and protection of protected and strictly protected wild species of plants, animals and fungi contains only one bee species (Službeni glasnik RS, broj 36/09).

Wild pollinators can increase the production of raspberries and, in that sense, thay could be considered as an essential natural resource (Sáez et al., 2018; Andrikopoulos and Cane, 2018). However, wild pollinators are not numerous enough for adequate pollination when it comes to intensive raspberry production. In this paper, only the direct use value of insect pollinators regarding raspberry production is presented, which limits providing specific management guidelines for increasing and maintaining pollination services. Nevertheless, according to the obtained EVIP for raspberries and the apparent importance of this insect group, some general guidelines can be provided:

- to maintain raspberry yield, it is necessary to understand and meet appropriate habitat requirements for specific pollinator species (Shuler et al., 2005), e.g. nesting sites in forest patches or dead trees left (Westrich et al., 1996);
- in order to provide enhanced food supplies for insect pollinators, a diversity of flower resources should be locally present (Westrich et al., 1996; Goulson, 2003);
- raspberry fields should be enriched with flower paths and hedges around the fields, small forest patches and/or least individual trees, which increases the likelihood of colonization of many bee species (Steffan-Dewenter et al., 2002);
- insecticides, especially those that can contaminate pollen and nectar should be avoided, preventing the loss of entire pollinator populations (Delaplane and Mayer, 2000).

The financial burden in implementing these and other similar recommendations can be mitigated through agri-environmental incentive programs where farmers who apply biodiversity protection strategies are adequately compensated for this activity.

Conclusions

Additional research on benefits of insect pollinators together with conservation activities could help increase and stabilize insect pollination services and increase its capacity with positive externalities on agricultural raspberry production. Additionally, better understanding of the benefits that wild insects bring to the agricultural production could lead to easier implementation of sustainable and cost-effective agricultural production by the farmers (e.g., investing less in renting hives). Moreover, many users of other ecosystem services that indirectly depend on pollinators would also benefit. Finally, conservation efforts to maintain insect communities that have impact on raspberry yields would also positively affect a range of ecosystems' processes. By assessing the value of insect pollination services regarding raspberries production, the need for investment in its preservation and maintenance becomes obvious and justified.

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References

Aizen, M. A., Garibaldi, L. A., Cunningham, S. S., and Klein, A. M. (2008). Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency, Current Biology, Vol. 18, 1572–1575.

Andrikopoulos, J. C. and Cane, H. J. (2018). Comparative Pollination Efficacies of Five Bee Species on Raspberry, Journal of Economic Entomology, Vol. 111(6), 2513–2519.

Benton, T. G., Vickery, J. A. and Wilson. J. D. (2003). Farmland biodiversity: is habitat heterogeneity the key?, Trends in Ecology and Evolution, Vol. 18, 182–188.

Cane, J. H. (2005). Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries (*Rubus*: Rosaceae), Hortscience, Vol. 40, 1705–1708.

de Snoo, G. R. and de Leeuw, J. (1996). Non-target insects in unsprayed cereal edges and aphid dispersal to the adjacent crop, Journal of Applied Entomology, Vol. 120, 501–504.

Delaplane, K. S. and Mayer, D. F. (2000). Crop pollination by bees, New York, CABI Publishing.
Gallai, N., Salles, J. M., Settele, J. and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline, Ecological Economics, Vol. 68, 810–821.
Garibaldi, L.A., Aizen, M.A., Klein, A.M., Cunningham, S.A. and Harder, L.D. (2011). Global growth and stability of agricultural yield decrease with pollinator dependency. Proceedings of the National Academy of Science of the United States of America, Vol. 14, 5909–5914.
Goulson, D. (2003). Conserving wild bees for crop pollination, The Journal of Food, Agriculture and Environment, Vol. 1, 142–144.
FAOSTAT (2021) http://www.fao.org/faostat/en/#data/QC Accessed: 25.05.2021.
Klein, A. M., Vaissière, B., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A. and Kremen, C. (2007). Importance of crop pollinators in changing landscapes for world crops, Royal Society of Biological Sciences, Vol. 274, 303–313.
NBS (2021) https://nbs.rs/en/drugi-nivo-navigacije/statistika/ks_stat/ Accessed: 07.05.2021.
Ollerton, J., Erenler, H., Edwards, M., and Crockett, R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes, Science, Vol. 346 (6215), 1360–1362.
Perez-Verdin, G., Sanjurjo-Rivera, E., Galicia, L., Hernandez-Diaz, J. C., Hernandez-Trejo, V., and Marquez-Linares, M. A. (2016). Economic valuation of ecosystem services in Mexico: Current status and trends, Ecosystem Services, Vol. 21, 6–19.
Picanço, A., Gil, A., Rigal, F., & Borges, P. A. (2017). Pollination services mapping and economic valuation from insect communities: a case study in the Azores (Terceira Island), Nature Conservation, Vol. 18, 1.
Porto, R. G., de Almeida, R. F., Cruz-Neto, O., Tabarelli, M., Viana, B. F., Peres, C. A., and Lopes, A. V. (2020). Pollination ecosystem services: A comprehensive review of economic values, research funding and policy actions, Food Security, 1-18.
Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers, Trends in Ecology and Evolution, Vol. 25, 345–353.
Robinson, R. A., and Sutherland, W. J. (2002). Post-war changes inarable farming and biodiversity in Great Britain, Journal of Applied Ecology, Vol. 39, 157–176.
Sáez, A., Morales, J. M., Morales, C. L., Harder, L. D., Aizen, M. A. (2018). The costs and benefits of pollinator dependence: empirically based simulations predict raspberry fruit quality, Ecological Applications, Vol. 28(5), 1215–1222.
Shanks Jr, C. H. (1969). Pollination of raspberries by honeybees, Journal of Apicultural Research, Vol. 8, 19–21.
Shuler, R. E., Roulston, T. H. and Farris, G. E. (2005). Farming practices influence wild pollinator populations on squash and pumpkin, Journal of Economic Entomology, Vol. 98, 790–795.
Službeni glasnik RS, broj 36/09, Accessed: 04.06.2021. www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2010/5/3/reg
SORS (2021). https://data.stat.gov.rs/?caller=SDDB&languageCode=en-US 04.05.2021.
Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C. and Tscharntke, T. (2002). Scale-dependent effects of landscape structure on three pollinator guilds, Ecology, Vol. 83: 1421–1432.

Stojanović, Ž., Ristić, B., Filipović, J. (2018). Monograph chapter on Serbian organic raspberry, Faculty of Economics, University of Belgrade, Belgrade.

Velthuis, H. H. W., and Van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination, Apidologie, Vol. 37, 421–451.

Walker, L., and Wu, S. (2017). Pollinators and pesticides. In International Farm Animal, Wildlife and Food Safety Law (pp. 495–513). Springer, Cham.

Westrich, P., Matheson, A., Buchmann, S.L., O'Toole, C., Westrich, P. and Williams, H. (1996). Habitat requirements of central European bees and the problems of partial habitats. In The conservation of bees pp. 1–16. Linnean Society of London and the International Bee Research Association by Academic Press London, UK.

Winfree, R., Aguilar, R., Vazquez, D. P., LeBuhn, G. and Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance, Ecology, Vol. 90, 2068–2076.

Żurawicz, E. (2016). Cross-pollination increases the number of drupelets in the fruits of red raspberry (*Rubus idaeus* L.), Acta Horticulturae, Vol. 1133, 145–152.

ACTIVITY OF SOIL MICROBIAL COMMUNITY AS AN INDICATOR OF PLANT SPECIES INVASION ON SOIL ECOSYSTEM

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Abstract

Invasive alien species pose a significant threat to biodiversity. Non-native plants often have dramatic impacts on the resident vegetation by modifying its composition and structure, decreasing native species abundance and richness via competition, predation, hybridization and indirect effect. The aim of our study was to report that selected invasive plants (*Helianthus tuberosus*, *Heracleum mantegazzianum* and *Fallopia japonica*) altered soil microbial indices and physicochemical properties. The research was carried out during a vegetation season (summer 2017) on Lekárovce and Opátka localities of South-Eastern Slovakia from the depth of 0.15 – 0.20 m. Soil reaction, soil moisture, soil organic carbon, soil basal respiration and soil enzyme activities (urease, FDA, beta-glucosidase, acid and alkaline phosphatases) were determined. Our study showed changes in soil samples with the observed invasive plants compared to the control sites. The results suggest that studied non-native plants have ability to change content of soil moisture. Soil reaction was also affected by invasion, while soil organic carbon was not significantly changed. Determining the intensity of soil respiration is of great importance for assessing the biological activity and the rate of mineralization processes in soil ecosystems. Our findings show reduced soil basal respiration in the presence of invasive plants, especially in *F. japonica* vegetation. The results of soil enzyme activity showed significant changes between invaded and control sites. Based on the results we assume that biology of the invasive plants had the high impact on soil systems and microbial indices might be considered as an indicator of environment disturbances.

Keywords: *Microbial indices, Invasive plants, Physicochemical properties.*

Introduction

Human activities (such as travel, artificial planting, etc.) has contributed to the spread of non-native plant species in ecosystems, and some of them have become invasive. The invasion of non-native plant species is one of the most important components of global change. The number of invasive plant species their distribution are increasing in many parts of the world. Invasive plants are found to spread even in the ice-free Islands of Antarctica despite the Antarctica treaty. Many species have been introduced accidentally (e.g., in water ballast, in soil, or as crop seed "contaminants"), but some have been intentionally introduced as ornamentals, food, or fiber products. Plant invasions can reduce the stability of the ecosystems by changing the supply of resources or by changing the trophic structure and its relationships (Čerevková *et al*., 2019; Fitoussi *et al*., 2016). The negative impact of non-native species on ecosystems is evident (Vitousek *et al*., 1996), but several authors have shown some positive features of exotic plants

(Dudek *et al*., 2016). Invasion of exotic plant species might alter many ecosystem properties, including important soil function and characteristics. Moreover, the same species can have the different impact on soil system, depending on local conditions (Dassonvile *et al.*, 2008). Because invasive plants can survive in harsh conditions and their ecology in polluted sites is very important, the study of Bobuľská *et al*. (2018) showed that some invasive species might be considered as a potential accumulator of risk elements. Relationships between alien plant species and their aboveground effects have been relatively well studied, but little is known about the effects of invasive plants on diversity and activity of microbial population. Microbiological characteristics are very often and effectively used as indicators of soil quality, because the large surface area, reactivity, distribution, generation time and diversity of the soil microflora allow a virtually immediate response to any changes (Kubát *et al.*, 2002). Microbial parameters are useful in monitoring environmental changes, but the determination of only one attribute is of no significance for the soil and its changes in the environment. Several studies show that non-native plant species might alter soil conditions such as soil moisture, organic matter, as well as other chemical and physical soil indices (Bobuľská *et al*., 2016; Duda *et al*., 2003).
The aim of this study was to investigate the relationship between selected biochemical soil properties and invasive plants on two studied localities.

Material and Methods

Sites description
The present study was carried out in two localities of Košice Basin (Lekárovce and Opátka) on Southeast of Slovakia (Figure 1) in June 2017. Both localities have invasion of *Helianthus tuberosus*, *Heracleum mantegazzianum* (Lekárovce locality) and *Fallopia japonica* (Opátka locality). The dominant soil type is Fluvisol that is a genetically young soil in alluvial deposits. Apart from river sediments, they also occur on alluvial plains, river fans, valleys and tidal marshes on all climate zones (Jordanova, 2017). Fluvisols belong to a group of high productive arable lands on which cereals, root crops and industrial crops are grown. In some parts, fluvial soils are used as a vegetable land. They are located on the floodplains of rivers, and their development is repeatedly disrupted by floods. Thus, the soil profile is enriched with the sludge sediments (Vilček and Zverková, 2015).

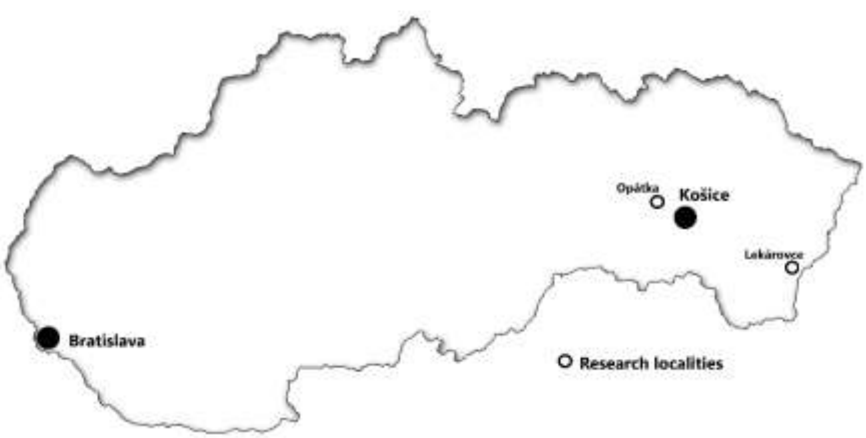

Figure 1. Experimental localities of the studied area in Košice Basin.

Soil sampling

All three selected invasive species formed dense patches on the research localities. Thus, invaded and non-invaded (control) sites were clearly recognized. After removal of surface litter, soil samples were collected in three replicates at the depth of 0-20 cm from each site and transported to the laboratory. In laboratory conditions, the soils were sieved using 2 mm mesh. Sieved soil samples were divided into two parts. Air-dried soil samples were selected to analyse chemical properties and another part stored at 4°C for soil physical and biological indices.

Soil analyses

Air-dried soil samples were used to measure soil reaction and organic carbon content. Soil pH was detected in a 1:3 mixture of soil and 0.01 M $CaCl_2$ solution using a digital pH meter. Soil organic carbon was determined by the Turin's method (Fiala *et al.*, 1999). Gravimetric soil moisture was calculated on 10 g of fresh subsamples after drying in a 105°C oven for 24 h. Soil basal respiration was measured by the CO_2 released from 100 g samples of field moist soil in 500 mL hermetically sealed flasks at 25°C for 48 h. CO_2 was captured by its reaction with NaOH (1mol L^{-1}) and titrated with HCl (0.5 mol L^{-1}) after the addition of $BaCl_2$ (1 mol L^{-1}). The concentration of the released carbon was estimated in μg C-CO_2 g soil dry^{-1}day^{-1} (Alef and Nannipieri, 1995). Enzymatic activity was determined using field-moist soil and each enzyme assay was performed as described in Table 1. Statistical analyses were done in R studio program (R Core Team).

Table 1. Incubation condition of selected enzymes with biochemical indicators.

Enzyme	Incubation condition					Reference
	Substrate	Buffer (pH)	Temperature (°C)	Soil (g)	Time (h)	
URE	Urea	6.7 (PB)	37	5	24	(Khaziev, 1976)
FDA	Fluoroscein diacetate	7.6 (PPB)	30	1	1	(Green *et al.*, 2006)
BGL	4-Nitrophenyl glucopyranoside	6.0 (MUB)	37	1	3	(Eivazi and Tabatabai, 1988)
$PHOS_{AL}$	p-Nitrophenyl phosphate	5.0 (AB)	37	5	3	(Grejtovský, 1991)
$PHOS_{AC}$	p-Nitrophenyl phosphate	10.0 (BB)	37	5	3	(Grejtovský, 1991)

URE (urease activity); FDA (fluorescein diacetate activity); BGL (beta-glucosidase activity); $PHOS_{AL}$ (alkaline phosphatase activity); $PHOS_{AC}$ (acidic phosphatase activity); PB (phosphate buffer); PPB (potassium phosphate buffer); MUB (modified universal buffer); AB acetate buffer); BB (borate buffer).

Results and Discussion

Soil physico-chemical properties and microbial indices from selected localities that present invaded and non-invaded (control) ecosystems are shown in Table 2. In our study, the status of invasion had significant impact on soil physico-chemical properties and microbial indices on soil ecosystem.

Table 2. Means ± standard errors of soil physico-chemical properties and microbial indices in the soil samples in invades and non-invaded (control) sites.

Status/Parameters	pH	SM	C_{ox}	SBR	URE	FDA	BGL	$PHOS_{AL}$	$PHOS_{AC}$
H. tuberosus	7.1 ±0.2	16.4 ±1.2	1.9 ±0.4	153 ±25	0.31 ±0.02	14.4 ±0.6	14.3 ±1.9	339 ±19	215 ±27
HT (control site)	6.5 ±0.2	15,8 ±1.6	1.7 ±0.3	212 ±	0.19 ±0.01	25.6 ±0.4	18.6 ±1.0	306 ±14	579 ±35
H. mantegazzianum	7.1 ±0.1	18.7 ±1.0	1.8 ±0.4	183 ±31	0,07 ±0.01	12.2 ±0.7	14.8 ±1.5	358 ±21	279 ±36
HM (control site)	6.5 ±0.3	17.6 ±1.3	1.6 ±0.2	219 ±19	0.22 ±0.01	17.9 ±0.3	17.2 ±0.7	426 ±14	387 ±26
F. japonica	7.7 ±0.1	17.4 ±1.1	3.8 ±0.5	132 ±36	0.43 ±0.03	37.7 ±0.5	38.2 ±1.1	430 ±25	309 ±33
FJ (control site)	7.4 ±0.1	16.4 ±2.6	3.7 ±0.3	329 ±41	0.22 ±0.01	19.6 ±0.1	37.7 ±0.6	360 ±31	275 ±28

SM (soil moisture, %); C_{ox} (organic carbon, %); SBR (soil basal respiration, μg C-CO_2/g soil /24 h); URE (urease activity, μg NH_4/g soil/24 h); FDA (fluorescein diacetate activity, μg FS/g soil/1 h); BGL (beta-glucosidase activity, μg pNP/g soil/1 h); $PHOS_{AL}$ (alkaline phosphatase activity, mg P/g soil/3 h); $PHOS_{AC}$ (acidic phosphatase activity, mg P/g soil/3 h).

The results show that soil moisture is higher in all invaded systems compare to the control sites. The study of Ehrenfeld (2003) also confirmed that invasive plant species have ability to alter soil moisture content. This might be explained by the fact that invasive plants have shallow root system and lower water supply. Soil pH is one of the most important chemical parameter that affects the solubility of soil substances, and therefore their usability by living organisms. According to Sheahan *et al*. (2012), soil pH is important factor of soil fertility, despite the fact that it changes dynamically depending on the internal and external factors. Increased soil acidity inhibits the activity of beneficial bacteria, thereby reducing the activity of the soil ecosystem. The results revealed that all invasive species are able to increase the value of pH, which was also reported in several other studies (Bobuľská *et al*., 2019; Čerevková *et al*., 2019; Zhang *et al*., 2009). Content of organic carbon was slightly higher in invaded ecosystems, but this trend was not statistically observed. Generally, invasive species form dense covers, they are fast growing, produce large amount of litter and therefore are considered to alter nutrient cycling and significantly change soil organic matter (Metcalfe *et al*., 2011). Some studies show that invasive species were able to increase the content of organic carbon (Tong *et al*., 2011; Wang *et al*., 2018), but the results of Feng *et al*. (2019) show the opposite trend.
Determining of intensity of soil respiration is of great importance for estimation the biological activity in soil ecosystem and the rate of mineralization processes. The soil basal respiration did not statistically vary between invaded and control sites (with the exception for *F. japonica* sites), but had tendency to be higher in natural habitats. The FDA measures the enzyme activity of a microbial population and can provide an estimate of the total microbial activity in environmental samples. Activity of FDA showed higher values in native ecosystems with the exception of *F. japonica* that provided higher activity of this parameter. Activity of beta-glucosidase was significantly lower in sites for *H. tuberosus* and *H. mantegazzianum*, while this enzyme did not significantly change in *F. japonica* sites. The opposite trend was observed for activity of soil urease, where its value increased in invaded systems, except *F. japonica*. Urease activity

depends on soil moisture, soil reaction, content and quality of humus, as well as the total content of soil nitrogen (Ehrenfeld, 2003). Both phosphatases, acid and alkaline, showed the changes due to the status of ecosystems. In soils with low phosphorus content, soil phosphatase activities are very important. The activity of soil phosphatases is a condition for making organic phosphorus available to plants. Phosphatases have different optimal pH values and are divided into acidic and alkaline.

The effects of invaded plant species on soil properties were generally significantly different between invaded and non-invaded sites (Table 3).

Table 3. The values of Mann-Whitney nonparametrical test for comparison soil properties between invasion status (invaded vs. non-invaded) regardless to invasive plant species

Parameter		**U**	**z**	P value
pH	Between invaded and non-invaded sites	30	4.58	0.0002 *
SM		103	−2.61	0.009 *
C_{ox}		100	−2.69	0.89
SBR		200	0.02	1.0
BGL		68	3.55	0.008 *
FDA		186	−3.6	0.009 *
URE		55	−3.9	0.0001 *
$PHOS_{AC}$		161	−1.04	0.29
$PHOS_{AL}$		90	2.96	0.36

SM (soil moisture), C_{ox} (soil organic carbon), SBR (Soil basal respiration), FDA (fluorescein diacetate activity), BGL (beta-glucosidase activity), URE (urease activity), $PHOS_{AL}$ (alkaline phosphatase activity), $PHOS_{AC}$ (acidic phosphatase activity), * $P < 0.05$.

Conclusions

In this study, we estimated the changes occurring in soil physico-chemical properties and microbial indices due to exotic invasion in two localities of southeast Slovakia. It was observed that selected non-native plant species (*Helianthus tuberosus*, *Heracleum mantegazzianum* and *Fallopia japonica*) significantly affected soil physico-chemical properties (soil moisture, soil reaction) and microbial indices (activity of beta-glucosidase, urease and FDA). Thus, we can conclude that invasive species augment soil properties and stablish themselves. Moreover, our results also suggest that activity of soil enzymes, as well as other soil physico-chemical properties can be widely used as an indicator of changes in natural ecosystems.

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References

Alef, K., Nannipieri, P. (1995). Methods in applied soil microbiology and biochemistry. Academic Press, London.

Bobuľská, L., Čekanová, K., Demková, L., Oboňa, J., Sarvaš, J. (2018). Evaluation of the phytoremediation properties of the invasive species *Solidago* genus. Analele Universității din Craiova - Biologie, Horticultura, Tehnologia Prelucrarii Produselor Agricole, Ingineria Mediului, vol. 23, pp. 314-320.

Bobuľská, L., Demková, L., Čerevková, A., Renčo, M. (2019). Invasive Goldenrod (*Solidago gigantea*) influences soil microbial activities in forest and grassland ecosystems in central Europe. Diversity, vol. 11, pp. 134.

Bobuľská, L., Macková, D., Malina, R., Demková, L. (2016). Occurrence and dynamics of *Impatiens parviflora* depending on various environmental conditions in the protected areas in Slovakia. European Journal of Ecology, vol. 2, pp. 87-98.

Čerevková, A., Miklisová, D., Bobuľská, L., Renčo, M. (2019). Impact of the invasive plant *Solidago gigantea* on soil nematodes in a semi-natural grassland and a temperate broadleaved mixed forest. Journal of Helminthology, vol. 94, pp. e51.

Dassonville, N., Vanderhoeven, S., Vanparys, V., Hayez, M., Gruber, W., Meerts, P. (2008). Impacts of alien invasive plant on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia, vol. 157, pp 131-140.

Duda, J., Freeman, D., Emlen, J. (2003). Differences in native soil ecology associated with invasion of the exotic annual chenopod, *Halogeton glomeratus*. Biology and Fertility of Soils, vol. 38, pp. 72–77.

Dudek, K., Michlewicz, M., Dudek, M., Tryjanowski, P. (2016) Invasive Canadian goldenrod (*Solidago canadensis* L.) as a preferred foraging habitat for spiders. Arthropod-Plant Interaction, vol. 10, pp. 377–381.

Ehrenfeld, J.G. (2003). Effects on exotic plant invasion on soil nutrient cycling processes. Ecosystems, vol. 6, pp. 503-523.

Eivazi, F., Tabatabai, M.A., (1988). Glucosidases and galactosidases in soils. Soil Biology and Biochemistry, vol. 20, pp. 601-606.

Feng, J., Wang, S., Wang, S., Ying, R., Yin, F., Jiang, L., Li, Z. (2019). Effects of invasive *Spartina alternifolia* Loisel. and subsequent ecological replacement by *Sonneratia apetala* Buch.-Ham. on soil organic carbon fraction and stock. Forests, vol. 10, pp. 171.

Fiala, K., Barančíková, G., Brečková, V., Búrik, V., Houšková, B., Chomaničová, A., Kobza, J., Litavec, T., Makovníková, I., Pechová, B., Váradiová, D. (1999). Partial monitoring system – soil. Binding methods. Soil Science and Conservation Research Institute, Bratislava.

Fitoussi, N., Pen-Mouratov, S., Steinberger, Y. (2016). Soil free-living nematodes as bio-indicators for assaying the invasive effect of the alien plant *Heterotheca subaxillaris* in a coastal dune ecosystem. Applied Soil Ecology, vol. 102, pp. 1-9.

Green, V.S., Stott, D.E., Diack, M. (2006). Assay for fluorescein diacetate hydrolysis activity: optimization for soil samples. Soil Biology and Biochemistry, vol. 38, pp. 693-701.

Grejtovský, A. (1991). Influence of soil improvers on enzymatic activity of heavy alluvial soil. Plant, Soil and Environment, vol. 37, pp. 289-295.

Jordanova, N. (2017). Soil Magnetism: *Applications in Pedology, Environmental Science and Agriculture*. Academic Press, London.

Khaziev, F.K. (1979). Soil enzyme activity. Nauka, Moscow.

Kubát, J., Nováková, J., Cerhanová, D. (2002). Výskyt a aktivita půdních mikroorganizmů ve dlouhodobých polních pokusech na orné půdě, In: Tesařová, M., Záhora, J. (eds), Biologické indikátory kvality půd (in czech), Mendel University in Brno, pp 18-25.

Metcalfe, D.B., Fisher, R.A., Wardle, D.A. (2011). Plant communities as drivers of soil respiration: Pathways, mechanisms, and significance for global change. Biogeosciences, vol. 8, pp. 2047-2061.

R Core Team. (2018) R: A Language and Environment for Statistical Computing, 1st ed., R. Foundation for Statistical Computing. Vienna, Austria, available online: https://www.r-project.org/ (accessed on 12 February 2020).

Sheahan, C.M., Bray, D.B., Bhat, M.G., Jayachandran, K. (2012). Ecological, economic and organizational dimension of organic farming in Miami-Dade country. Journal of Sustainable Agriculture, vol. 36, pp. 83-106.

Tong, C., Zhang, L., Wang, W., Gauci, V., Marrs, R., Liu, B., Jia, R., Zeng, C. (2011). Contrasting nutrient stocks and litter decomposition in stands of native and invasive species in a sub-tropical estuarine marsh. Environmental Research, vol. 111, pp. 909-916.

Vilček, J., Zverková, M. (2015). Pedogeografia (*in slovak*). Prešovská univerzita v Prešove, Prešov.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R. (1996). Biological invasions as global environmental change. American Journal of Science, vol. 84, pp. 218–228.

Wang, W., Sardans, J., Wang, C., Asensio, D., Bartrons, M., Peñuelas, D. (2018). Species-specific impacts of invasive plant success on vertical profiles of soil carbon accumulation and nutrient retention in the Minjiang River Tidal Estuarine wetlands of China. Soil Systems, vol. 2, pp. 5.

Zhang, C.B., Wang, J., Qian, B.Y., Li, W.H. (2009). Effects of the invader *Solidago canadensis* on soil properties. Applied Soil Ecology, vol. 43, pp. 163-169.

MERCURY POLLUTION IN FORMER MINING AREA (SLOVAKIA) AND THEIR INFLUENCE ON BIOLOGICAL AND CHEMICAL SOIL PROPERTIES

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Abstract

Current and former mining activities in Slovakia and related industrial activities focused on ore processing have significantly affected the quality of the environment. Pollution of such sites affects all components of the environment, the health of living organisms, including humans. The research was carried out in Nižná Slaná former mining area during the summer of 2019. The soil samples were taken from the vicinity of mining bodies – tailing pond and abandoned area of the ore processing plant. The aim of the study was to determine the level mercury pollution in the surrounding of mining bodies in the Nižná Slaná former mining area, and to evaluate the influence of pollution to the selected biological and chemical soil properties. The total content of mercury was determined by a direct mercury analyzer (AMA-254). Nutrients (Ca, Mg, K and Na), the activity of soil enzymes (urease, acid, and alkaline phosphatase, FDA, and *β*-glucosidase), and soil pH was determined in laboratory conditions. Mercury pollution in the evaluated area was expressed by contamination factor (C_f). Limit value of mercury in soil samples exceeded the permissible limit value at all sampling sites. Contamination factor reached extremely high values, expressing very high contamination at all sampling sites. Higher values of mercury in soil ecosystem have an inhibitory effect to the soil enzymes, but nutrient seems to be resistant. The soil reaction was higher (alkaline), which is probably due to the presence of sludge from the sludge in the soil environment.

Keywords: *Tailing pond, activity of soil enzymes, nutrients, contamination factor*.

Introduction

Mining and smelting activities have a long tradition in Slovakia (Rybár et al.,2006). It was first mentioned in documents from the 4th century, with the biggest expansion during 13. – 14. century. From the beginning it was only surface mining, later it was switched to deep extraction, which had a much greater impact on the landscape. In the places where mines occurred, were created processing plants areas and the number of mining bodies usually used for secondary (waste) material storage (Dudek, 2005).Tailing ponds and the areas of former processing plants represent the remains of mining activities in former mining areas. In addition to negatively changing the appearance of the landscape, they are also a source of hazardous elements - heavy metals, that enter the environment and change its properties (Demková et al., 2020). Bad soil quality in the former mining areas, predominantly in the vicinity of the mining bodies is long time serious environmental problem (Guo et al., 2017). Mercury is considered one of the most dangerous heavy metal, because of toxicity, high persistency in ecosystem and bioaccumulation ability in the food chain (Árvay et al., 2017). Because of its toxic effect on human health, mercury contamination is a significant concern worldwide **(**Beckers and Rinklebe, 2017). Mining and smelting activities contribute to the increase of mercury content in the environment (Liu et al., 2021). It has been shown several times that soil properties, such as soil reaction, the activity of soil enzymes, or the content of nutrients are changing under the influence of environmental loads, which has a negative effect on the quality of the soil environment (Demková et al., 2019). Additionally, soil enzymes are very often used as bioindicators of soil quality because they react quickly to environmental stress, and they are very sensitive to the changes in the soil's ecosystem (Wyszkowska et al., 2002). It also has been found that soils polluted by risk elements have reached a low value of nutrients what leads to the decreased soil fertility and health (Fazekašová and Bobuľska, 2012). The aim of the study was i) to determine the content of the mercury in the soil samples sampled in the vicinity of the two mining bodies – tailing pond and area of the former processing plant, ii) to evaluate the level of pollution by mercury using the contamination factor (C_f), iii) evaluate the influence of the soil pollution by the mercury to the selected soil properties – the activity of soil enzymes, soil nutrients and the soil pH, iv) to determine the differences in the soil properties between two different types of sampling sites (tailing pond and area of former processing plant).

Materials and methods

Mining activities in the Nižná Slaná village in Slovakia, which is the object of the study, were focused on iron and precious metals extraction. The need to process the extracted ore material required the construction of a processing plant. After more than six centuries of intensive mining and processing activities, the mining activity in Nižná Slaná was definitively finished in 1996. The remnants of mining activities in this area include an extensive tailings pond, several heaps, and an abandoned area of an ore processing plant. The mentioned mining bodies are a source of undesirable substances such as heavy metals. Soil (substrate) samples (0-10 cm) were taken during summer 2019 from the tailing pond and its surrounding (n=6) and from the area of the processing plant (n=6) in the cadastre of Nižná Slaná village (Slovakia). Subsequently, the soil was manually cleaned of dead plant parts, dried at room temperature, sieved (<2mm), homogenized, and stored for analysis. Soil reaction was determined as follows: 25 mL of 0.01 M CaCl2 was added to the 5g of soil. After one hour of shaking the pH was measured using InoLab pH 720-WTW. The content of nutrients (Ca, K, Mg, Na) was measured using ICP-OEC Agilent 720 (Agilient Technologies, Germany). The activity of acid (ACP) and alkaline (ALP) phosphatase was determined by Grejtovský (1991), the activity of urease (URE) by Khaziev (1976), the activity of ß-glucosidase was determined by Eivazi and Tabatabai (1988), and activity of fluorescein diacetate (FDA) was determined by Green et al. (2006). The level of soil pollution by mercury was expressed by contamination factor (C_f) which was calculated as follows (Hakanson, 1980):

$$C_f^i = \frac{C_{0-1}^i}{C_n^i} \qquad (1)$$

where: C_{0-1}^i is the total concentration of the mercury in soil and C_n^i is the background level of mercury, which was 0.08 mg.kg^{-1}(Čurlik and Šefčík 1999). Hakanson (1980) divided contamination factor values into 4 classes, listed in the Table 1.

Table 1 Classes of the contamiantion factor values according Hakanson (1980).

contamination factor value (C_f)	Level of contamination
C_f^i<1	low contamination
if 1≤ C_f^i<3	moderate contamination
3≤ C_f^i<6	considerable contamiantion
C_f^i ≥6	very high contamination

All statistical analyses were performed using R studio (R Core Team 2012). Non-parametrical Mann-Whitney U test was used to express the significant differences in soil characteristics between two types of sampling sites (tailing pond – TP and processing plant areas – PP). Spearman's correlation coefficient was used to determine the relationships between soil characteristics determined in soil samples.

Results and discussion

The limit value of mercury for Slovak soils is set by Act. No 220/2004 Coll of Laws to 0.50 $mg.kg^{-1}$. Based on the results obtained we can conclude, that at all sampling sites above the limit value of mercury was determined (Table 2). According to mercury pollution, it is very often linked to mining activities and the industrial activities associated with it (Higueras, 2014). The area of the Nižná Slaná is included in the list of environmental burdens in Slovakia, mainly due to the danger arising from the tailings pond located above the village (Enviroportal, 2014). Soil pollution usually has an inhibitory effect on the activity of soil enzymes, although different enzymes have been found to respond differently to soil pollution (Duan et al., 2018). All evaluated soil enzymes gave a negative (not significant) correlation with the mercury content (with exception of ACP) (Table 3). Acid phosphatase gave a positive correlation with mercury content what is a sign, that acid phosphatase is the most resistant to mercury pollution. Zheng et al. (2019) consistently with our results, confirmed that soil phosphatase activity was least affected by the mercury content of the soil. For soils contaminated with heavy metals is also typical, that the values of the soil reaction are in the lower values (Hohl and Varma 2010). In our case, the soil pH values ranged between 3.73 – 7.36, which are relatively high values for this type of soil. On the contrary, Martinez et al. (2020) have documented that the pH values of sewage sludge use to reach strongly alkaline values, which was confirmed by our results. Additionally, the correlation between soil pH and mercury content was found positive, which is also not obvious in polluted soils. All evaluated soil enzymes gave a significant positive correlation between themselves and a negative correlation with soil pH. Consistently with our results, Taylor et al. (2002) have found a negative correlation between pH and soil enzymes. Soil pH gave a significant positive correlation with Ca and Mg ($p<0.01$**), and a significant negative correlation with K and Na ($p<0.01$**).

Table 2. Total content of Hg [mg kg^{-1}], nutrients (Ca, K, Mg and Na [mg kg-1] soil pH, and soil enzymes: FDA [µg FS/ g soil h], BG [µg p NP/ g soil 1h], URE [mg NH_4^+Ng^{-1}24h^{-1}], ACP [mg P q^{-1} 3h^{-1}], ALP [mg P q^{-1} 3h^{-1}] determined in soil samples sampled in the surrounding of tailing pond (TP) and processing plant area (PP).

No.	Sampling site	Hg	FDA	BG	URE	ACP	ALP	pH	Ca	K	Mg	Na
1	TP	16.7	28.2	132	0.63	275	184	5.25	6705	6910	5340	506
2	TP	20.6	8.83	0	0.19	30.6	40.5	7.36	48106	5394	15727	351
3	TP	1.02	22.3	41	0.38	139	177	4.45	534	9509	1757	1145
4	TP	7.81	24.9	165	0.61	185	123	5.05	1888	8521	2508	826
5	TP	1.88	25.7	175	0.69	123	156	6.82	24215	6193	10468	313
6	TP	3.37	37.8	236	0.47	260	198	4.00	695	6525	2602	618
7	PP	1.61	15.7	105	0.43	69.4	52.3	3.73	371	9689	2342	433
8	PP	15.5	20.9	76,2	0.50	118	53.3	4.88	825	11241	1278	235
9	PP	4.75	17.3	35,9	0.26	53.0	61.5	7.02	30311	4992	14722	359
10	PP	4.39	27.1	103	0.27	45.4	96.1	7.07	51211	4499	23768	498
11	PP	2.81	16.4	41,0	0.35	46.1	68.8	7.10	11802	2358	22168	224
12	PP	1.50	21.8	95,5	0.35	132	112	6.97	1280	4026	1364	252

Table 3. Spearman's correlation relationships between soil characteristics determined in Nižná Slaná former mining area.

	FDA	BG	URE	ACP	ALP	pH	Ca	K	Mg	Na
Hg	-0.26	-0.26	-0.01	0.09	-0.24	0.09	0.25	0.17	0.02	-0.2
FDA		**0,85****	**0,53***	**0,77****	**0,81****	-0,39	-0,30	0,08	-0,29	0,32
BG			**0.70****	**0.72****	**0.66***	-0.44	-0.36	-0.15	-0.38	0.16
URE				**0.68***	**0.57***	-0.43	-0.52	0.42	-0.50	0.14
ACP					**0.82****	-0.56	**-0.59***	0.29	**-0.63***	0.38
ALP						-0.36	-0.36	0.08	-0.38	0.56
pH							**0.72****	**-0.79****	**0.74****	**-0.56***
Ca								-0.49	**0.81****	-0.26
K									**-0.71****	0.42
Mg										-0.35

The results of Mann-Whitney U test confirmed that sampling localities significantly differ in the content of ACP, ALP and Na ($p<0.05$*) (Table 4).Based the results we can conclude that the content of ACP, ALP and Na reached higher values at TP localities comparing PP.

Table 4. The results of Mann-Whitney U test expressing the differences in soil characteristics between different types of sampling sites.

soil characteristics		U	Z	p
Hg	Between different samplin g sites	13.0	0.46	0.50
pH		14.0	-0.56	0.57
FDA		9.00	1.36	0.17
BG		10.5	1.12	0.26

URE	9.00	1.36	0.16
ACP	7.00	1.68	**0.05***
ALP	6.00	1.84	**0.05***
Ca	17,00	-0.08	0.93
K	12.00	0.88	0.37
Mg	11.00	0.74	0.27
Na	6.00	1.84	**0.05***

*p<0.05

Contamination factor values ranged between 257 to 23.6 in the samples from tailing pond, and between 194 to 18.8 in the samples from the surrounding of processing plant area (Figure 1). Based the Hakanson (1980) classification, at all evaluated sampling sites very high contamination by mercury was determined (C_f>6). Fazekašová and Fazekaš (2020) used contamination factor for evaluation the soils sampled in urban area of Nižná Slaná. The results showed very high contamination by mercury.

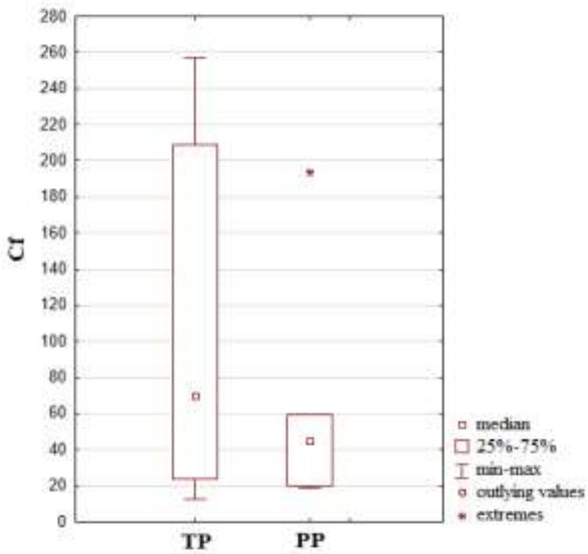

Figure 1 Boxplot expressing the mercury contamination factor values determined for two types of sampling area (TP – tailing pond, PP – processing plant area)

Conclusion

Environmental burdens in the form of old mining bodies are a threat to the surrounding environment due to insufficient security and almost no remediation interventions. The release of risk elements from them has a negative effect on the quality of the soil environment, its health, and fertility. The results showed, that the surrounding of taling pond and processing plant area in Nižná Slaná village is very high contaminated by mercury. No significant correlation between the content of mercury and the activity of soil enzymes, soil pH, and nutrients was found. Soil pH reached high values, which is not typical for such king of polluted area. Soil pH significantly positively influenced the content of Ca and Mg, and significantly negatively influenced the content of K and Na. Acid phosphatase, which was found as the most resistant to mercury pollution, significantly negatively correlated with Ca and Mg.

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References

Act No. 220/2004 on Protection and Agricultural Land Use. MPSR – Ministry for Land Management, 2004 (in Slovak).

Beckers, F., Rinklebe, J. (2017). Cycling of mercury in the environment: Sources, fate, and human health implications: A review, Critical Reviews in Environmental Science and Technology**,** 47, 693-794.

Čurlík J., Šefčík P. (1999). Geochemical Atlas of the Slovak Republic. Part V: Soils, Ministry of Environment, Bratislava (in Slovak).

Čurlík, J., Šefčík, P. (1999). Geochemical atlas of the Slovak Republic. Part V: Soils, Ministry of the Environment, Bratislava (in Slovak).

Demková, L., Árvay, J., Bobuľská, L., Hauptvogl, M., Michalko, M., Michalková, J., Jančo, I. (2020). Evaluation of Soil and Ambient Air Pollution Around Un-reclaimed Mining Bodies in Nižná Slaná (Slovakia) Post-Mining Area, Toxics, 8(4), 96.

Demková, L., Árvay, J., Bobuľská, L., Hauptvogl, M., Michalko, M. (2019). Activity of the soil enzymes and moss and lichen biomonitoring method used for the evaluation of soil and air pollution from tailing pond in Nižná Slaná (Slovakia), Journal of Environmental Science and Health, Part A, 54(6), 1–13.

Duan, Ch., Fang, L., Yang, C., Chen, W., Cui, Y., Li, S. (2018). Reveal the response of enzyme activities to heavy metals through in situ zymography, Ecotoxicology and Environmenmental Safe, 158, 106-111.

Dudek, M., Tajdus, K., Misa, R., Sroka, A. (2020). Predicting of land surface uplift caused by the flooding of underground coal mines—A case study, International Journal of Rock Mechanics and Mining Sciences, 132, 104377.

Eivazi, F., Tabatabai, M.A. (1988). Glucosidases and galactosidases in soils. Soil Biology and Biochemistry, 20, 601–606.

Enviroportal 2019. Environmental burden information system of Slovak republic, http://enviroportal.sk/environmentalne-zataze/, Accessed on 03/07/2021.

Fazekašová, D., Fazekaš, J. (2021). Soil Quality and Heavy Metal Pollution Assessment of Iron Ore Mines in Nizna Slana (Slovakia). Sustainability, 12, 2549.

Green, V.S., Stott, D.E., Diack, M. (2006). Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples, Soil Biology and Biochemistry, 38, 693–701.

Grejtovský, A. (1991). Influence of soil improvers on enzymatic activity of heavy alluvial soil. Plant, Soil and Environment, 37, 289–295.

Hakanson, L. (1980). An Ecological Risk Index for Aquatic Pollution Control. A Sedimentological Approach, Water Research, 14, 975–1001.

Higueras, P. et al. (2014). Mercury soil pollution in Spain: A Rewiew. In: Jiménez E., Cabañas B., Lefebvre G. (eds) Environment, Energy and Climate Change I. The Handbook of Environmental Chemistry, Springer.

Hohl, H., Varma, A. S. (2010). The living matrix. In: Soil Heavy Metals; Sherameti, I.; Varma, A., (eds) Soil Heavy Metals, New York: Springer, 1–19.

Khaziev, F.K. (1976). Soil enzyme activity. Nauka, Moscow.

Liu, S., Wang, X., Guo, G., Yan, Z. (2021). Status and environmental management of soil mercury pollution in China: A review, Journal of Environmental Management, 277, 111442.

Martinez, F., Cuevas, C., Walter, T., Iglesias, I. (2002). Urban organic wastes effects on soil chemical properties in degraded semiarid ecosystem. Seventeenth WCSS, Symposium No. 20, Thailand, 1–9.

R Core Team. R: A Language and Environment for Statistical Computing. R Found Stat Comput. 2016

Rybár, P., Sasvári, T., Hvizdák, L., Hvizdáková, J. (2006). Tracing the Slovak and German mining heritage in the Gelnica-Smolník area – mining tourism excursion project. Geotur Košice (5.-7. Október 2006), 209-214.

Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G. (2002). Comparison of Microbial Numbers and Enzymatic Activties in Surface Soils and Subsoils Using Various Techniques, Soil Biology and Biochemistry, 34(3), 387-401.

Wyszkowska, J., Kucharski, J., Waldowska, E. (2002). The Influence of Diesel Contamination on Soil Enzyme Activity, Rostlinná Výroba, 48, 58–62.

Zheng, L., Li, Y., Shang, W., Dong, X., Tang, Q., Cheng, H. (2019). The inhibitory effect of cadmium and/or mercury on soil enzyme activity, basal respiration, and microbial community structure in coal mine–affected agricultural soil. Annals of Microbiology, 69, 849–859.

ENVIRONMENTAL MANAGEMENT OF LIVESTOCK IN BOSNIA AND HERZEGOVINA ON THE EXAMPLE OF ENVIRONMENTAL PERMITS

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Abstract

Bosnia and Herzegovina has large areas of unused agricultural land that are very suitable for the development of livestock, which has recently become increasingly important in sustainable food production. Environmental management of livestock is imposed as one of the main challenges in politics of environmental protection. Entity legislation, in the field of environmental protection is a framework for environmental management in Bosnia and Herzegovina. Environmental management for large farms is a mandatory part of the environmental permit. The environmental permit is one of the main instruments of environmental policy at the local level in Bosnia and Herzegovina for agricultural business and food producers of eggs, meat, milk and other livestock products. The paper analyses the arguments for obtaining environmental permits for poultry farms in the municipality of Srbac. The research method is based on desk analysis. The subject of the analysis is 30 arguments obtained from local environmental permits, collected by authorised professional companies in the field of environmental protection from the entity of the Republic of Srpska. The collecting period of arguments was from 2011 to 2021 years. Desk analysis allowed identifying the main pollutions on poultry farms production from all elements of the environment and proposed the measures to reduce these contaminants respecting the regulations to national legislation. Finally, discussion and conclusion state the importance of the principles of circular economy in environmental management of livestock for transition to sustainable business of food production.

Keywords: *Bosnia and Herzegovina, sustainable food production, environmental management of livestock, environmental permits for poultry farms, circular economy.*

Introduction

Bosnia and Herzegovina (BiH) is a country with large areas of agricultural land suitable for livestock. Livestock in BiH has a long tradition and livestock products from this area are regionally known in the Southeast part of the European Union (EU). The production of food for the human population is the second goal of the Agenda 2030 for sustainable development.[9] Fassio and Tecco (2019) state that human nutrition depends on 5 animal species (primarily: meat, milk and eggs production). On the basis of this food production, commercial agricultural food production or agricultural business was created. The development of agricultural business has also contributed to countless environmental pollution. The assumption is that 1/5 of the total pollution on the planet earth is caused by agricultural business, i.e. commercial food production. Sustainable food production has become one of the key challenges of environmental

[9] Source: Agenda, 2018

management. Environmental management is directly related to three important principles viz. reduce, reuse, and recycle (RRR). These three principles are the basis of the circular economy (Andersen, 2006).
The concept of circular economy is a holistic framework and in addition to the positive economic effects of food production, it also takes into account the negative effects on the environment (Fogarassy and Finger, 2020). This growth concept is important because the opinion that the economy should grow primarily on natural resources without restrictions has been deviated from. The concept of circular economy is primarily implemented by entrepreneurship in order to make it sustainable, i.e. circular entrepreneurship or circular business (Abad-Segura et al., 2020). Paulik (2018) state that the implementation of the circular economy principles into agricultural business is only possible through environmental management. Environmental management of food business in BiH is a mandatory part of the environmental permit (EP) and its measures to prevent pollution. This paper presenting the current practice of obtaining EP and using the principles of circular economy in order to assess the contemporary state of sustainable agricultural business and food production.

Materials and Methods

The EP is mandatory for all large livestock farms that are in the system of food production for the human population on the territory of Republic of Srpska/BiH. In order to implement the standards of circular economy (RRR) through sustainable business - sustainable food production, it is necessary to identify the largest emissions - pollutants produced by livestock farms, its causes and the consequences if they stay or accumulate in the environment, to obtain a completed picture of economic gains and losses in one production cycle. The Law on Environmental Protection (Official Gazette of Republic of Srpska, No. 71/12, 79/15, 70/20) defines the issuance of EP and the Rulebook from the same Official Gazette defines all facilities that can be built and put into operation only if they have an EP.[10]Official Gazette of Republic of Srpska, No. 124/12, under item "dj" are defined facilities used in agriculture that require an EP. According to the Law on Environmental Protection (2012, 2015, 2020), it is obligatory to enclose argument for environmental protection (AEP) with the request for the issuance of an EP. AEP need to be prepared by an accredited professional institution for environmental protection registered on the territory of the Republic of Srpska/BiH.[11] The same AEP need to be submitted to the competent environmental protection institution for approval of EP issuance. The competent institution for issuing an EP may be a unit of local self-government (municipality or city) or the competent ministry of ecology[12] (if the production capacity is higher which requires an environmental impact assessment[13]).
AEP explains all significant farm pollutants that may affect environmental elements, all living things and human health. Monitoring is based on applicable laws and rulebook's for the territory of the Republic of Srpska (primarily the Law on Protection of Air[14] , Water[15] and Land[16], Waste

[10] Source: Rulebook on facilities that can be built and put into operation only if they have an environmental permit, 2012
[11] Source: Rulebook on conditions for performing activities in the field of environmental protection, 2013
[12] Source: Ministry of Spatial Planning, Construction and Ecology, 2021
[13] Source: Rulebook on projects for which an environmental impact study is conducted and criteria for deciding on the need for implementation and the scope of the environmental impact assessment, 2012
[14] Source: Law on the Air Protection, 2011, 2017
[15] Source: Law on the Waters, 2006, 2009, 2012, 2017
[16] Source: Law on the Agricultural Land, 2006, 2007, 2014, 2012, 2019

Managament[17], Nature Conservation[18], Animal Husbandry[19], Protection and Welfare of Animals[20], Veterinary Medicine[21], and relevants rulebook's[22,23,24,25,26,27,28,29,30,31]). On the other hand, measures to prevent or reduce pollutions are determined. Measures for environmental protection are proposed on the basis law, best available techniques and technologies[32,33]. In this way, ecological management on farms is established, as a precondition for the implementation of circular economy standards, i.e. the achievement of the sustainable development goals.
The method of work is a desk study on 30 subject AEP, collected from authorized professional companies[34], in the production of broilers in the municipality of Srbac, Republic of Srpska/BiH, with a capacity of 8000 to 50 000 broilers, for which the EP is issued by the local municipality.[35] Environmental management was analyzed through the content of the AEP. The content of the AEP is prescribed by the Law on Environmental Protection.[36]
Presented results (Tables 1, 2, 3) shows emissions/pollutants listed in all AEP or those that are most frequently repeated as major pollution points on farms. The examination involves professional description of the occurrence of these pollutants, a pollution professional measure or solution (respecting the law and greatest available techniques/technologies/practice) and monitoring (according to relevant rulebooks). The tables present environmental farm management system. In professional discussion, are presented the shortcomings and advantages of environmental management on farms in the Republic of Srpska/BiH, according to circular economy principles based on professional practice (EU practice) and science-policy evidence (international environmental agreements and national legislation). In the conclusions is given the final assessment of the system of environmental management of Republic of Srpska farms, as well as proposals for further development of the concept of circular economy for sustainable food business.

Results and Discussion

The most common emissions to air that are recognized on all farms are: gases from metabolic processes, odors from the decomposition of fertilizers, suspended particles during daily feeding and gases from boiler rooms (Table 1.). None of presented emissions are not subject to monitoring. Regular monitoring of gases from boiler rooms, according to the current legislation

[17] Source: Law on Waste Management 13, 15, 18, 20
[18] Source: Law on Nature Conservation, 14
[19] Source: Law on Animal Husbandry, 2006, 2011
[20] Source: Law on Protection and Welfare of Animals, 2008
[21] Source: Law on Veterinary Medicine, 2008, 2012
[22] Source: Decree on air quality values, 2012
[23] Source: Rulebook on air quality limit values, 2005
[24] Source: Rulebook on conditions for discharge of wastewater into surface waters, 2001
[25] Source: Rulebook on conditions for discharging wastewater into public sewers, 2001
[26] Source: Rulebook on treatment and drainage of wastewater for areas of cities and settlements where there is no public sewerage, 2001
[27] Source: Rulebook on permitted quantities of hazardous and harmful substances in agricultural land and water for irrigation and methods for their testing, 2016
[28] Source: Rulebook on categories, testing and classification of waste 15, 18
[29] Source: Red List of Protected Species of Flora and Fauna of the Republic of Srpska, 2012.
[30] Source: Rulebook on spatial and technical conditions for accommodation of farmed animals, facilities and equipment in animal husbandry, 2015
[31] Source: Rulebook on protection of animals for keeping and conditions that must be met by facilities for keeping animals, 2010
[32] Source: Rulebook on measures for prevention and reduction of air pollution and improvement of air quality and form of report on measurement of emissions of air pollutants, 2015
[33] Source: Rulebook on best available techniques for achieving environmental quality standards, 2008
[34] Source: Ekodozvola d.o.o., 2021; Institute for Protection and Ecology of the Republic of Srpska, 2021
[35] Source: Municipality of Srbac, 2021
[36] Source: Law on Environmental Protection, 2012, 2015, 2020

in the Republic of Srpska is done for boiler rooms over 250 kW.[37] Smaller farms have decreased boiler rooms. However, there is a large number of smaller farms with minor boiler rooms on the territory of the municipality of Srbac, and the law did not provide their monitoring if there is a large number of smaller boiler rooms in the same area. This is the first recognized lack of legislation for the territory of the Republic of Srpska/BiH.

In addition, there is no monitoring of gases from metabolic processes inside or near the object. Given that these gases are an integral part of the production process, and that they can negatively affect human health and the environment, it is necessary to set limit values within the facility and regular monitoring, similar to those in EU countries (such as Germany or Austria). This is the second recognized lack of legislation in the Republic of Srpska/BiH.

Also, odors from the decomposition of fertilizers have a very strong and adverse effect on human health and the environment. A situation of poor storage or non-existence of a lagoon for manure is very common. According to the EU Nitrates Directive[38], proper management of the permanent and construction of the lagoon is mandatory. It is necessary to implement EU legislation in Republic of Srpska/BiH. The third shortcoming of the legislation in RS was recognized there.

Finally, the problem of airborne particles was recognized during the daily feeding of broilers. The big problem here is the technology of food production, as well as the technology of automatic feeding. BiH legislation does not define the use of the best available technologies (eco-technologies) in the process of food production. This was recognized as the fourth shortcoming of the legislation in RS.

Table 1. Negative impact on the air

The emission *(negative impact on the air)*	1. Products of intensive metabolic processes in the form of a gaseous phase (during the ventilation of the poultry farm, carbon dioxide (CO^2) and water vapor, as well as dust and ammonia (NH_3) are emitted into the atmosphere).
	2. Decomposition of broilers manure creates unpleasant odors through various gases of ammonia (NH_3) and hydrogen sulfide (H_2S).
	3. Dust during mixing of animal feed or feeding, delivery and removal of growing broilers (down).
	4. Boiler chimney emissions up to 200 kW - flue gases and solid particles.
The professional explanation of the problem	1. Inadequate ventilation of object; Poor technical and technological solutions for air conditioning.
	2. Non-existence of lagoon or object for manure.
	3. Inadequate ventilation of the object.
	4. Poor heating management; Failure to maintain the chimney and boiler room system.
A professional measure to reduce or solve a problem *(techniques, technologies)*	1. Use technically correct devices and equipment to reduce emissions of pollutants into the air and prevent incidents; Ventilation of the installation of object should be reported in such a way that it satisfies the conditions prescribed by the broilers breeding technology, i.e. to

[37] Source: Rulebook on facilities that can be built and put into operation only if they have an environmental permit, 2012

[38] Source: Nitrates Directive, 1991

	satisfy the required quality and quantity of air exchange, as well as the condition of minimizing the spread of unpleasant odors outside the production object; Maintain the ventilation system in the farm's production premises in good condition in order to eliminate unpleasant odors in the environment; Sprinkle zeolite preparation on the floor of the farm in order to reduce the intensity of unpleasant odors (reduces the concentration of NH_3 and CO_2).
	2. After the cleaning of the object, broilers manure with a mat (fertilizer) should be temporarily disposed of in the designated area - lagoon within the subject plot (which needs to be built), protected from atmospheric influences and without the possibility of spreading unpleasant odors to the environment or export them directly to agricultural areas; In order to reduce the emission of unpleasant odors into the air, spread the manure on the ground during the cold weather without winds, and plow it immediately.
	3. Checking the correctness of the system for filling and emptying food silos; Checking the ventilation system periodically.
	4. Use environmentally - friendly fuel - wood, well dried; Clean the chimney regularly and maintain the boiler room.
Monitoring	1. NO.
	2. NO.
	3. NO.
	4. NO.

The most common emission to water recognized by all farms is improper storage of permanent (Table 2.). The constant has large amounts of nitrates and nitrogen that dissolve well with water. If the soil does not receive these substances adequately, groundwater pollution can occur. The big drawback is that the EU Nitrates Directive[39] has not been adopted in BiH. This problem of legislation is the same as the fourth recognized lack of legislation in the Republic of Srpska.

Table 2. Negative impact on the water

The emission *(negative impact on the water)*	Inadequate storage of fertilizers can contaminate the soil, and directly groundwater, primarily with nitrates (NO_3) or nitrogen (N).
The professional explanation of the problem	Non-existence of lagoon or adequate storage for litter; Nitrates dissolve well in water, and excessive amounts of nitrates in the soil cannot be used by agricultural crops, but they are leached from the soil and thus can cause groundwater pollution.

[39] Source: Nitrates Directive, 1991

A professional measure to reduce or solve a problem *(techniques, technologies)*	Construction of a lagoon which has implemented system for separation of urine and other aqueous impurities from feces.
Monitoring	NO.

The most common emissions to soil recognized by all farms are the discharge of wastewater after cleaning the facility and inadequate storage of dead broilers (Table 3.). It is mandatory to implement biosecurity measures on farms, which include regular disinfection, desinsection and deratisation of the object's on the end of each turn. Disinfection is often done unprofessionally and without clearly defined chemicals that are environmentally friendly for release into the environment. It is usually practice of farms, to do not meet the basic criteria of environmental protection for outfit, equipment and objects in animal husbandry in the Republic of Srpska/BiH. Legislation is not fully defined for environmental protection in this area. This is the fifth recognized lack of legislation in the Republic of Srpska/BiH.
In addition, inadequate storage of dead broilers can cause various types of diseases on the site and attract the attention of stray and wild animals. According to the valid veterinary legislation, proper storage of this type of waste is mandatory. In practice, this type of waste is resold to pet food manufacturers.

Table 3. Negative impact on the soil

The emission *(negative impact on the soil)*	1. Improper cleaning, i.e. washing and sanitation of the poultry object before the introduction of a new turns.
	2. Mortality of chickens, 2-4% of total production.
The professional explanation of the problem	1. Inadequate object without concrete channels - drains and collectors; Disinfectants with wastewater reach the surrounding soil.
	2. Inadequate storage of organic waste - dead broilers can cause disease and the arrival of stray animals at the site.
A professional measure to reduce or solve a problem *(techniques, technologies)*	1. Construction of new object or adaptation of existing object to the needs of production capacity with the installation of environmental wastewater collectors; Use of environmentally-friendly disinfectants.
	2. Adequate storage of this type of waste, freezing and further sale for processing of pet food or adequate recycling in production; Build a fence around the building and restrict access to stray or wild animals.
Monitoring	1. NO.
	2. NO.

Standards of circular economy, waste reduction, reuse and recycling are not present/or implemented prctice to a large extent on the surveyed farms. Waste reduction measures are not clearly defined according the Waste Framework Directive.[40] Also waste reuse measures, such as biomass for energy conversion or heating at the site in question, have not been recorded on any farm. In addition, none of the farms has the practice of collecting gases (through extraction and environmental technologies) that should be used as an energy source in the production of electricity or heating. However, it is a good practice to sell dead broilers for further use in the production of pet food. Recycling, i.e. sorting of recyclable waste is defined by measures of AEP.

Conclusion

Environmental management of livestock at the local level in the entity of the Republic of Srpska/BiH has major legal shortcomings in terms of defining pollution, measures and monitoring. Therefore, sustainable management of food production in accordance with the principles of the circular economy is not entirely possible at the local level. This could be a problem hampering the realization the second goal of Agenda 2030 for sustainable development viz. food security and nutrition and sustainable agriculture.

The link between sustainable development – circular economy – environmental farm management – sustainable agricultural business – sustainable food productions is neither recognized in BiH nor implemented in the legal and institutional framework. Therefore, it is necessary to improve the legal regulations in BiH on the example of environmental permits and environmental management of farms in the ares of local level, primarily the implementation of EU directives (such as Nitrates Directive) in the entity legislation to set Circular Economy (RRR) standards for sustainable agricultural production and sustainable food production.

References

Abad-Segura E., Batlles de la Fuente A., González-Zamar M.D., Belmonte-Ureña L.J., 2020. Effects of Circular Economy Policies on the Environment and Sustainable Growth: Worldwide Research. Sustainability, Vol. 12, No. 5792, pp. 1-27.

Agenda, 2018. The 2030 Agenda and the Sustainable Development Goals. United Nations.

Andersen M.S., 2006. An introductory note on the environmental economics of the circular economy. Sustainability Science, pp. 1-8.

Decree on air quality values, 2012. Official Gazette of the Republic of Srpska, No. 124/12. Republic of Srpska Law, Republic of Srpska.

Ekodozvola d.o.o., 2021. Ekodozvola d.o.o. authorized company for environmental protection in Republic of Srpska, official web site. Web page: http://ekodozvola.com/En/Default.aspx Accessed: 20.06.2021.

Fassio F.and Tecco N., 2019. Circular Economy for Food: A Systemic Interpretation of 40 Case Histories in the Food System in Their Relationships with SDGs. Systems, Vol. 7, No. 43, pages.

Fogarassy C. and Finger D., 2020. Theoretical and Practical Approaches of Circular Economy for Business Models and Technological Solutions. Resources, Vol. 9, No. 76, pp. 1-9.

[40] Source: Waste Framework Directive, 2008

Institute for Protection and Ecology of the Republic of Srpska, 2021. Public Scientific Research Institution, Institute for Protection and Ecology of the Republic of Srpska, official web site. Web page: https://www.institutzei.net/ Accessed: 20.06.2021.
Law on Agricultural Land, 2006. Official Gazette of the Republic of Srpska, No. 93/06. Republic of Srpska Law, Republic of Srpska.
Law on Agricultural Land, 2007. Official Gazette of the Republic of Srpska, No. 86/07 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Agricultural Land, 2010. Official Gazette of the Republic of Srpska, No. 14/10 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Agricultural Land, 2012. Official Gazette of the Republic of Srpska, No. 5/12 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Agricultural Land, 2019. Official Gazette of the Republic of Srpska, No. 58/19 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Animal Husbandry, 2006. Official Gazette of RS, No. 34/06. Republic of Srpska Law, Republic of Srpska.
Law on Animal Husbandry, 2011. Official Gazette of RS, No. 100/11 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Environmental Protection, 2012. Official Gazette of Republic of Srpska, No. 71/12. Republic of Srpska Law, Republic of Srpska.
Law on Environmental Protection, 2015. Official Gazette of Republic of Srpska, No. 79/15 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Environmental Protection, 2020. Official Gazette of Republic of Srpska, No. 70/20 Law on Animal Husbandry, 2011. Official Gazette of RS, No. 100/11 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Nature Conservation, 2014. Official Gazette of the Republic of Srpska, No. 20/14. Republic of Srpska Law, Republic of Srpska.
Law on Protection and Welfare of Animals, 2008. Official Gazette of RS, No. 111/08. Republic of Srpska Law, Republic of Srpska.
Law on the Air Protection, 2011. Official Gazette of the Republic of Srpska, No. 124/11. Republic of Srpska Law, Republic of Srpska.
Law on the Air Protection, 2017. Official Gazette of the Republic of Srpska, No. 46/17 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Veterinary Medicine in the Republic of Srpska, 2008. Official Gazette of the RS, No. 42/08. Republic of Srpska Law, Republic of Srpska.
Law on Veterinary Medicine in the Republic of Srpska, 2012. Official Gazette of the RS, No. 06/12 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Waste Management, 2013. Official Gazette of RS, No. 111/13. Republic of Srpska Law, Republic of Srpska.
Law on Waste Management, 2015. Official Gazette of RS, No. 106/15 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Waste Management, 2018. Official Gazette of RS, No. 16/18 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Waste Management, 2020. Official Gazette of RS, No. 70/20 (amendments). Republic of Srpska Law, Republic of Srpska.
Law on Waters, 2006. Official Gazette of the Republic of Srpska, No. 50/06. Republic of Srpska Law, Republic of Srpska.

Law on Waters, 2009. Official Gazette of the Republic of Srpska, No. 92/09 (amendments). Republic of Srpska Law, Republic of Srpska.

Law on Waters, 2012. Official Gazette of the Republic of Srpska, No. 121/12 (amendments). Republic of Srpska Law, Republic of Srpska.

Law on Waters, 2017. Official Gazette of the Republic of Srpska, No. 74/17 (amendments). Republic of Srpska Law, Republic of Srpska.

Ministry of Spatial Planning, Construction and Ecology, 2021. Ministry of Spatial Planning, Construction and Ecology Republic of Srpska, official web site. Web page: https://www.vladars.net/eng/vlada/ministries/MSPCEE/Pages/default.aspx Accessed: 20.06.2021.

Municipality of Srbac, 2021. Municipality of Srbac, Republic of Srpska, official web site. Web page: https://srbac-rs.com/ Accessed: 20.06.2021.

Nitrates Directive, 1991. Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC). European Union Law, European Union.

Paulik S. (2018). Critical appraisal of the circular economy standard BS 8001:2017 and a dashboard of quantitative system indicators for its implementation in organizations. Resources, Conservation & Recycling, No. 129, pp. 81–92.

Red List of Protected Species of Flora and Fauna of the Republic of Srpska, 2012. Official Gazette of the RS, No. 124/12. Republic of Srpska Law, Republic of Srpska.

Rulebook on air quality limit values, 2005. Official Gazette of RS, No. 39/05. Republic of Srpska Law, Republic of Srpska.

Rulebook on best available techniques for achieving environmental quality standards, 2008. Official Gazette of Republic of Srpska, No. 22/2008. Republic of Srpska Law, Republic of Srpska.

Rulebook on categories, testing and classification of waste, 2015. Official Gazette of RS, No. 19/15. Republic of Srpska Law, Republic of Srpska.

Rulebook on categories, testing and classification of waste, 2018. Official Gazette of the Republic of Srpska, No. 79/18 (amendments). Republic of Srpska Law, Republic of Srpska.

Rulebook on conditions for discharge of wastewater into surface waters, 2001. Official Gazette of the Republic of Srpska, No. 44/01. Republic of Srpska Law, Republic of Srpska.

Rulebook on conditions for discharging wastewater into public sewers, 2001. Official Gazette of RS, No. 44/01. Republic of Srpska Law, Republic of Srpska.

Rulebook on conditions for performing activities in the field of environmental protection, 2013. Official Gazette of the Republic of Srpska", No. 28/13. Republic of Srpska Law, Republic of Srpska.

Rulebook on facilities that can be built and put into operation only if they have an environmental permit, 2012. Official Gazette of the Republic of Srpska, No. 124/12. Republic of Srpska Law, Republic of Srpska.

Rulebook on measures for prevention and reduction of air pollution and improvement of air quality and form of report on measurement of emissions of air pollutants, 2015. Official Gazette of RS, No. 3/15. Republic of Srpska Law, Republic of Srpska.

Rulebook on permitted quantities of hazardous and harmful substances in agricultural land and water for irrigation and methods for their testing, 2016. Official Gazette of the Republic of Srpska, No. 56/16. Republic of Srpska Law, Republic of Srpska.

Rulebook on projects for which an environmental impact study is conducted and criteria for deciding on the need for implementation and the scope of the environmental impact assessment, 2012. Official Gazette of Republic of Srpska, No. 124/12. Republic of Srpska Law, Republic of Srpska.

Rulebook on protection of animals for keeping and conditions that must be met by facilities for keeping animals, 10. Official Gazette of RS, No. 136/10. Republic of Srpska Law, Republic of Srpska.

Rulebook on spatial and technical conditions for accommodation of farmed animals, facilities and equipment in animal husbandry, 2015. Official Gazette of RS, No. 100/15. Republic of Srpska Law, Republic of Srpska.

Rulebook on treatment and drainage of wastewater for areas of cities and settlements where there is no public sewerage, 2001. Official Gazette of RS, No. 68/01. Republic of Srpska Law, Republic of Srpska.

Waste Framework Directive, 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. European Union Law, European Union.

ASH CONTENT OF SOME OAK SPECIES

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Abstract

Oak, which is a member of the Fagaceae family, has a wide distribution in Turkey and there are 18 oak species naturally. They are mostly trees, some are tall shrubs, deciduous or evergreen plants in winter. The general area of oak forests in Turkey is 5.152.561.8 hectares in total. Oaks are divided into three groups as white oaks, red oaks and evergreen oaks according to the anatomical structure of their wood, leaf and bark characteristics and the ripening time of their fruits. Oak is valuable for Turkey in terms of species diversity, the area it covers, the use of wood and by-products. Oaks are used in areas such as furniture, plywood, carving, parquet, and building materials. Within the scope of this study, ash determination tests in oak woods were carried out from stem-woods. The ash amounts of sessile oak *(Quercus petraea)*, Hungarian oak *(Quercus frainetto)* and brash (Turkish) oak *(Quercus cerris)* were determined and the ash amounts were found to be respectively 0,41%, 0,51% and 0,57%. It was measured that the average ash amounts of oak species was 0,49%. When the average ash amounts of oak species we compared with broadleaves species ash amounts; it was determined that ash, poplar, maple, alder values we close to each other. Average ash of oak species, it is higher than coniferous species and higher than oak and beech, and lower than chestnut in the Fagaceae family. It is suggested that oak ash can be used in agriculture and soil nutrition for plants.

Keywords: *Wood ash, Fagaceae, Oak, Oak ash.*

Introduction

Quercus L. (Oak); it is a member of the Fagaceae family, which hosts 8 genera, including *Fagus* L. (beech) and *Castanea* L. (chestnut), and 620-750 species of these genera. Distributed in temperate and sub-tropical regions of the northern hemisphere. There are 18 species of oak naturally in Turkey. They are mostly trees, some are tall shrubs, deciduous or evergreen plants in winter. The general area of oak forests in Turkey is 5.152.561,8 hectares in total. Of this, approximately 2,105,937.4 hectares are plantation and the remaining 3,046,624.4 hectares are degraded, coppice and bush. Oaks are divided into three groups as white oaks (section: *Quercus*), red oaks (section: *Cerris*) and evergreen oaks (section: *Ilex*) according to the anatomical structure of their wood, leaf and bark characteristics and ripening time of their fruits (Akkemik 2018, Yaltirik 1984, URL 5).

Wood oak used building timber, soil, bridge construction, small ship construction, agricultural equipment, machine building, furniture, parquet, veneer timber, fence posts, traverse, wood charcoal, plywood, carving. Fruit and leaves are also used as animal feed (Ertas 1996, Yaltirik 1984, URL 5).

The oaks analysed within the scope of this study sessile oak (*Quercus petraea*), Hungarian oak (*Quercus frainetto*) and brash (Turkish) oak (*Quercus cerris*). These oaks were compared with

coniferous species (softwood), broadleaves species (hardwood) and Fagaceae family by ash determination.
Sessile Oak (*Quercus petrea*); It is the species with the widest distribution area among the oak species found in our country. It spreads naturally in most parts of Europe, the Caucasus, Iran and Turkey. It belongs to the group of white oak trees and is used in barrel production (Sahin 2016, Ozturk 2013).

(a) (b) (c)
Figure:1: Sessile oak (a) gallnut, (b) stem, (c) leaf (URL 2)

Hungarian Oak (*Quercus frainetto*); It spreads naturally in northwest Turkey, southeast and middle Europe, South Italy and the Balkans. It is one of the most preferred broadleaves species by the forest products industry. Belongs to the group of White oaks (Ozturk 2013, Sahin 2016).

(a) (b) (c)
Figure:2: Hungarian Oak (a) leaf, (b) stem (c) gallnut (URL 3)

Brash (Turkish) Oak (*Quercus cerris*); It spreads naturally in Western Turkey and most of Anatolia, in Central and Southeastern Europe. The feature that distinguishes it from other species is that the female flowers have hairy auricles. It is in the group of red oaks (Ozturk, 2013).

(a) (b) (c)
Figure:3: Brash (Turkish) (a) stem, (b) leaf, (c) gallnut (URL 4)

Wood is a chemically complex material and there are many chemical components in it. Most of the wood consists of organic (almost 99%) and a small amount of inorganic (about 1%) materials (Fengel and Wegener, 1989).

Inorganic materials are also in the ash formed as a result of the burning of wood. Ash content in temperate area is between 0,1-1%. The amount of ash in local tree species is 0,1-0,5% compared to the dry weight of the wood. While calcium (almost 70%), potassium and magnesium are present in the ash at a high rate; sodium, iron, sulphur, phosphorus, silicon, chlorine, aluminum and lithium are also found in small quantities. The mineral composition of wood undergo change depending on the habitat, climate, age, cutting and location of the trees (Fengel and Wegener 1989, Bozkurt and Erdin 2013).

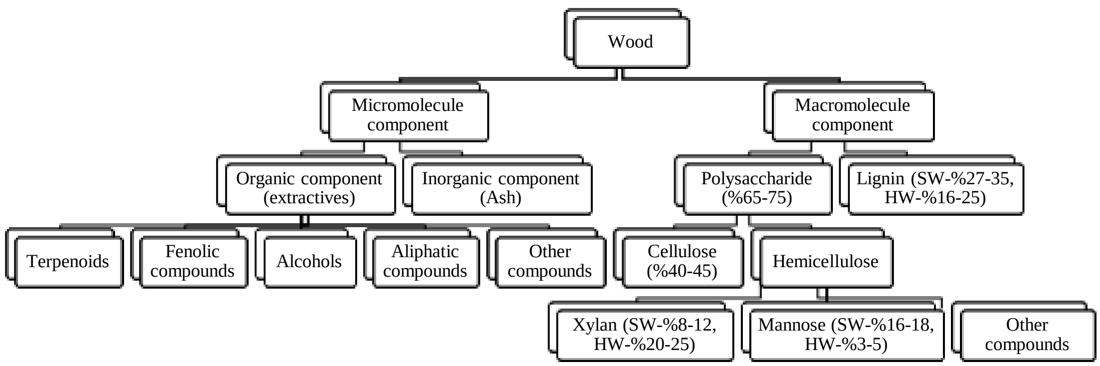

Figure:4: Chemical Component of Wood (Fengel and Wegener, 1989) (SW=Softwood (Coniferous species) HW=Hardwood (Broadleaves species))

The ash obtained as a result of wood burning is 1% of the wood weight. Wood species, humidity, specific gravity, ash and extractive materials are the parameters that affect the thermal values of wood. Since a certain part of the heat generated by combustion evaporates the water in the wood, the amount of moisture increases while the calorific value decreases. The calorific value also increases with the increase of extractive materials. As the amount of ash in the wood and bark increases, the calorie value decreases (OGM Bioenergy Report, 2019).

The stem-wood value of the oak is 4620 kcal/kg, and the branch-wood value is 4692 kcal/kg. While the average gross calorific value of stem-wood is 4664 kcal/kg, it is 4620 in branch-wood. The gross calorific value of oak is close to the stem-wood average (Erten and Onal, 1985). The thermal degradation of broadleaves species are lower than those of coniferous species, which is explained by the presence of heat-sensitive pentosans in broadleaves species (Atilgan and Peker, 2015).

Broadleaves species generally produce more ash than coniferous species (Fengel and Wegener, 1989). Elemental percentage in broadleaves species stem ash; potassium 20,4%, calcium 19%, phosphorus 4,2%, magnesium 3,6%, manganese 0,8%, iron 0,5%, zinc 0,4%, sulphur 2,1%, boron 0,05%, copper 0,04% (URL 1). Wood ash contains 5-30% carbon. The density of wood ash decreases with increasing carbon content. It depends on parameters such as chemical and physical properties, combustion temperature, species of wood (Siddique, 2008).

On average, the content of macrostructural materials in the ash of oak wood; total carbon 190,3 g/kg, total nitrogen 8,7 g/kg, phosphorus 22,9 g/kg, potassium 127,7 g/kg, calcium 268,8 g/kg, magnesium 34,9 g/kg and sulphur 24,3 g/kg. The content of the heavy metals in oak wood as an average; cadmium 10,4 g/kg, lead not found, zinc 592,2 g/kg, copper 197,6 g/kg, manganese 840,2 g/kg, nickel 32,8 g/kg and crom 27,3 g/kg (Becher *et al*., 2018).

The highest macrostructural material in oak wood ash is calcium, followed by total carbon. The lowest is total nitrogen. Manganese is the most in heavy metal content and followed by zinc. Lead has not been found, and cadmium is the lowest.

Macro elements in red oak ash *(Quercus rubra)*; aluminum 6,8 mg/kg, calcium 366 mg/kg, iron not measured, potassium 60,8 mg/kg, magnesium 52 mg/kg, manganese 14,9 mg/kg, sodium 0,8 mg/kg, phosphorus 15,6 mg/kg, sulphur 18 mg/kg, silicon not found. Macro elements in White oak; aluminum not determined, calcium 314 mg/kg, iron 0,9 mg/kg, potassium 102,5 mg/kg, magnesium 75,7 mg/kg, manganese 1,4 mg/kg, sodium not determined, phosphorus 5,6 mg/kg, sulphur 12,1 mg/kg, silicon 1,3 mg/kg (Pitman, 2006).

About 70% of the ash is considered garbage and is collected in the landfill. 20% is used for soil reinforcement, since it contains all essential minerals except nitrogen, it is a source of nutrients for plants, and when it is returned to the soil, it has a calcifying effect. It is used as fertilizer in agriculture. Two important properties of wood ash are its high pH and neutralizing capacity. Wood ash recycling is a possible way to prevent the increased acidity of the soil, and in forest ecosystems the addition of ash usually increases with dose. The remaining 10% of wood ash is used in various fields such as construction materials, metal recovery, pollution control, cleaning, public health, insulation, preparation of some foods (Karltun *et al.,* 2008, Sidduque 2012, Naylor and Schmidt 1986, Augusto *et al.,* 2008, Campbell 1990, Alptekin 2019).

Materials and Methods

Oak wood samples were obtained from Istanbul Fatih forest (Bahcekoy, Istanbul). Firstly, the bark parts of the samples were removed and wood cut into chips with a thickness of 2 or 3 mm. The screening process (40 mesh) was applied by grinding in a Wiley blade mill.

Dry matter determination of oak woods was carried out according to TAPPI T412 om-06 and ash determination according to TAPPI 211 om-85 standard.

Results and Discussion

The average dry matter ratio was found to be 90% in brash (Turkish) oak (*Quercus cerris*), 78% in sessile oak (*Quercus petraea*) and 90% in Hungarian oak (*Quercus frainetto*).

Sessile oak was the lowest, brash (Turkish) oak and Hungarian oak was the highest value. Percentages of ash determination in oak species; it was determined as 0.51% in Hungarian oak, 0.41% in sessile oak and 0.57% in brash (Turkish) oak. The average ash percentage is 0.49%. The highest was brash (Turkish) oak, the lowest was the sessile oak.

The comparison of oak species was made over the stem-wood values. Compared to coniferous species, the average ash of oak species was higher. As seen in Figure 5; oak species compared to broadleaves species; they have close values with ash, poplar, alder and maple.

Compared to the Fagaceae family (oak, chestnut and beech) given in Figure 5, the average ash of oak species is higher than that of beech, but lower than that of chestnut.

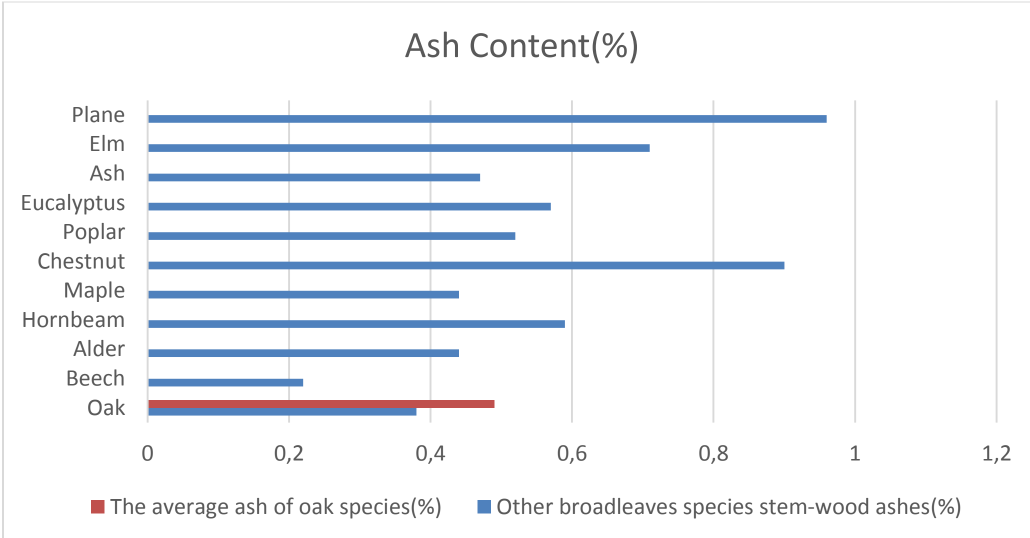

Figure:5: Compared with broadleaves species and ash average of oak species

Conclusions

The average ash of oak species; it is higher than coniferous species and close to some of the broadleaves species (ash, poplar, maple, alder), and higher than oak and beech, and lower than chestnut in the Fagaceae family. Oak wood ash can be used in agriculture and in soil for plant nutrition.

References

Akkemik, U. (Ed). (2018). Natural-Exotic Trees and Shrubs of Turkey, Publications of the General Directorate of Forestry, Ankara p.684.

Alptekin M. (2019). The Concept of Ash in Mersin-Silifke Juruks' Cultural Life, Asia Minor Studies journal, 7(1), p.34-45.

Augusto L., Bakker M. R., Meredieu C. (2008). Wood ash applications to temperate forest ecosystems—potential benefits and drawbacks, Review Article, Plant Soil, 306:181-198.

Atilgan A., Peker H. (2015). A Natural Energy Resources Wood: Combustion Property and Protection Methods, Review, Afyon Kocatepe University Journal of Science and Engineering, 15, 022201 (1-12).

Becher M., Jaremko D., Skwarek K., Symanowicz B. (2018). Possibilities for the Use of Wood Ashes in Agriculture, Journal of Ecological Engineering, Volume 19, Issue 3, May, pages 191–196.

Bozkurt A.Y., Erdin N. (2013). Wood Anatomy, Lecture notes, Istanbul University, Forest Faculty, Department of Forest Industry Engineering, Istanbul.

Campbell A. G. (1990). Recycling and disposing of wood ash, Tappi Journal Vol.73, No.9, pp.141-146 ref.26, 0734-1415.

Erten P., Onal S. (1985). Determination of Calorific Value of Native Species Woods and Barks, Forestry Research Institute Publications, Technical Bulletin Series, No:147.

Ertas A. (1996). Investigations on Silvicultural Characteristics of *Quercus hartwissiana* Steven, PhD Thesis, Istanbul University, Institute of science, November, Istanbul.

Fengel D. and Wegener G. (1989). Wood Chemistry, Ultrastructure, Reactions, Walter de Gruyter Berlin, New York.

Karltun E., Saarsalmi A., Ingerslev M., Mandre M., Andersson S., Gaitnieks T., Ozolinčius R., Varnagiryte-Kabasinskiene I. (2008). Chapter 4:Wood Ash Recycling – Possibilities And Risks, Sustainable Use of Forest Biomass for Energy: A synthesis with Focus on the Baltic and Nordic Region, March, Springer Science+Business Media B.V., p.79-108.

Naylor L M., Schmidt E J. (1986). Agricultural use of wood ash as a fertilizer and liming material, Tappi, 69(10), p.114-119.

OGM Bioenergy Report, (2009), May, Ankara.

Ozturk S. (2013). Turkey Oaks Diagnosis and Identification Guide, Publications of the General Directorate of Forestry, Department of Combating Forest Pests.

Pitman R. M. (2006). Wood ash use in forestry – a review of the environmental impacts, Oxford University Press, Forestry, Vol. 79, No. 5, November, England.

Siddique R. (2008). Chapter 9: Wood Ash, Waste Materials and By-Products in Concrete, Springer.

Siddique R. (2012). Utilization of wood ash in concrete manufacturing, Review, Resources, Conservation and Recycling, Elsevier, 67, p.27-33.

Sahin A. (2016). Spread of Oak Forests in Marmara Region, Operational Purposes and Planning, International Participation Oak Workshop Proceedings and Concluding Statement, Publications of the General Directorate of Forestry, Marmara Forestry Research Institute Directorate, 18-20 October, Igneada/Kirklareli.

TAPPI T 211 om-85 (2002). Ash in Wood, Pulp, Paper and Paperboard, Combustion at 525°C. TAPPI Press, Atlanta, Georgia, pp. 1-5.

TAPPI T 412 om-06 (2006). Moisture in Pulp, Paper and Paperboard, TAPPI Press, Atlanta, Georgia, pp. 1-3.

URL 1. https://www.forestresearch.gov.uk/documents/1032/use_of_ash_in_forestry.pdf Accessed on: 3/05/2021.

URL 2. https://www.konusanagac.com/konusan-agac/152/sapsiz-mese.html Accessed on: 12/072021.

URL 3. https://www.konusanagac.com/konusan-agac/42/macar-mesesi-quercus-frainetto.html Accessed on: 12/07/2021.

URL 4. https://www.konusanagac.com/konusan-agac/153/sacli-mese-quercus-cerris.html Accessed on: 12/07/2021.

URL 5. http://www.orkoop.org.tr/link/atlas.pdf Accessed on: 5/08/2021.

Yaltirik F. (1984). Turkey Oaks Diagnostic Guide, Publication of the General Directorate of the Ministry of Agriculture, Forestry and Rural Affairs, Istanbul, Book No:377.

THE QUALITY PARAMETERS OF RECYCLED PAPER

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Abstract

The use of waste paper as a raw material in the production of paper and cardboard is increasing day by day. Recycled paper is obtained by collecting and classifying waste paper and repulping it through the recycling process. The most commonly used waste paper types in recycling process are old corrugated cardboard, white office paper, old newspapers and magazine paper and waste paper mix consisting of a mixture of one or both of them. Non-fiber resources that vary according to the type of waste paper are removed in recycling processes. The heterogeneous waste paper raw material is desirable to form homogeneous pulp suitable for paper production. Recycling of waste paper and cardboard provide a new product with economic added value and appreciation. Increasing the production of recycled papers decreases the cutting of trees and this ensures the sustainability of forests and prevents the releases of hazardous chemicals to environment. Knowing the impact on quality parameters is important to analyze changes in fibers obtained from waste paper and cardboard recycling. Recycled fibers are rigid and more fragile than virgin fibers because they lose their flexibility. The bonding potential in the fibers is lesser and their surface areas are narrow and swelling properties of fibers are low. As they have undergone hornification, their breaking length and burst strength are low, while tear strength is high. Since the fine fibers are abounding, their opacity is high and their brightness is low. Ash content of recycled papers differs according to type of waste paper.

Keywords: *Waste papers, Recycled papers, Recycled fibers, Fiber morphology***.**

Introduction

Recycled paper is the paper produced as a new product by collecting, classifying (according to BS EN 643, 2014) of the waste papers and recycling them into pulp again. The most recycled waste paper sources are; mixed waste papers, old corrugated cardboard, white office papers, old newspaper and magazine papers. Those that are not recycled in terms of hygiene and cleaning; toilet papers, paper and towel wipes etc. are cleaning papers. In waste paper, depending on the type of waste paper, non-fiber resources; filler, starch, adhesives, staples, dye, coated materials, ink etc. are found. It is desirable to form the most suitable homogeneous pulp for paper production from the heterogeneous raw material coming from a wide variety of waste paper sources (Atik and Ok 2017, Akyıl 2018, Kırcı 2003).

The increase in consumption along with the growing population causes an increasing occurence of waste paper and cardboard, and a decrease in raw materials and natural resources. In order to ensure the sustainability of the raw material, the emergence of a new product with economic added value by applying the recycling process to paper and cardboard wastes gains importance.

The paper industry is one of the industries that widely uses the recycling process. According to the annual report of SKSV; while the total amount of recycled (domestic and imported) waste paper was 4,352,106 tons in 2019, it increased to 4,669,326 tons in 2020 and increased by 7,3%. Paper and cardboard industry recycling (total domestic waste paper amount/consumption) in Turkey in 2020 is 46,8%. The recycling rate for only packaging paper and cardboard is 59,5%. Total waste paper use is 3,627,052 tons in 2020 (SKSV, 2020).

Recycling Process

The efficiency is between 80-90% due to fiber losses in the recycling process and additives added to the fibers (Atik and Ok, 2017). The recycling processes of waste paper are respectively; pulping, screening and cleaning, dispersion, deinking and bleaching.

a)Pulping: It is the unit that enables the fibers of waste paper to be separated with the help of water and turned into pulp. Here, non-paper materials and ink and filler are removed (Kırcı 2003, Scott 2019).

b)Screening and Cleaning: It is the process of removing materials outside the paper, which are undesirable due to the size and magnitude of the pulp. In cleaning, very small solid materials are separated from the pulp (Kırcı 2003, Scott 2019).

c)Dispersion: It is the part where dirt and ink particles on the surface of the fiber are removed so that they are not visible in the final product and dispersed in the pulp suspension. Re-fibering of recycled paper by dispersion becomes easier (Kırcı 2003, Scott 2019).

d)Deinking: It is the section where the printing inks in the waste papers are removed. Parts of ink detached from the fiber surface are removed during screening, cleaning, flotation and washing of the waste pulp suspension. The effectiveness of ink removal varies with the size, shape and density of the ink particles (Kırcı 2003, Scott 2019).

e)Bleaching: The color of the pulp is lightened by the use of chemicals that decolorize the dye and colored lignin structures in the pulp. This process is preferred in the production of limited and high quality pulps (Kırcı 2003, Scott 2019).

Recycling effects less mechanical damage to the fibers during the pulping process. Chemical pulp causes fiber damage, changes in fiber morphology and chemical composition of the fiber. Mechanical pulping, on the other hand, is a harder process that often causes severe mechanical damage to the fibers, fiber shortening and the formation of fine fibers (Keranen and Retulainen, 2016).

Interactions between fiber properties and process variables

During the recycling process, the fiber dimensions of the waste pulp can be affected by the pulper (pulping), dispersion unit and refining processes. The fiber sizes mentioned here are; fiber dimensions, including the length of individual fibers and the cross-sectional areas of the fibers (Ellis and Sedlachek, 1993). As the recycling of papers is repeated, mechanical fragmentation occurs in the fibers, the debrises (fine fiber) in the pulp are removed during the screening and cleaning processes. The strength of the pulp decreases with each recycling process (Kırcı, 2003).

Advantages of Recycling

Since the raw material is waste paper, the dependence on the forest decreases, while the cutting of trees is prevented, the sustainability of the forests is ensured. Waste paper recycling processes are carried out easily, using less chemicals and less energy compared to wood pulp production. If secondary fibers are compared with primary fibers, they can be recycled 5 or 6 times. Filling and covering materials can easily adhere to the fibers. On account of the repetitive recycling of waste paper, water pollution, air pollution and water use are reduced. Recycling of waste will reduce the need for raw materials and contribute to the country's economy (Akyıl 2018, Bekiroglu and Mertoglu Elmas 2017, Kumar *et al.*, 2019).

Disadvantages of Recycling

Waste papers that are not classified well can cause problems when processing. During the recycling of waste paper, high fiber losses are experienced. As the number of recycling increases, the strength properties of the fibers decrease. Rising prices of chemicals, energy and waste paper can direct manufacturers to cheap, alternative fiber sources (Kırcı 2003, Sahin 2016).

Quality parameters of Recycled Paper

Waste paper is a used paper from virgin or secondary pulp. Therefore, the raw material of recycled paper quality has to be kept within certain limits, and this is often difficult. In order to ensure the desired production quality, the quality of recycled paper must be carefully determined and these quality variables must be kept under control. Important variables affecting quality parameters; fiber properties, strength properties, optical properties, ash content and contaminants.

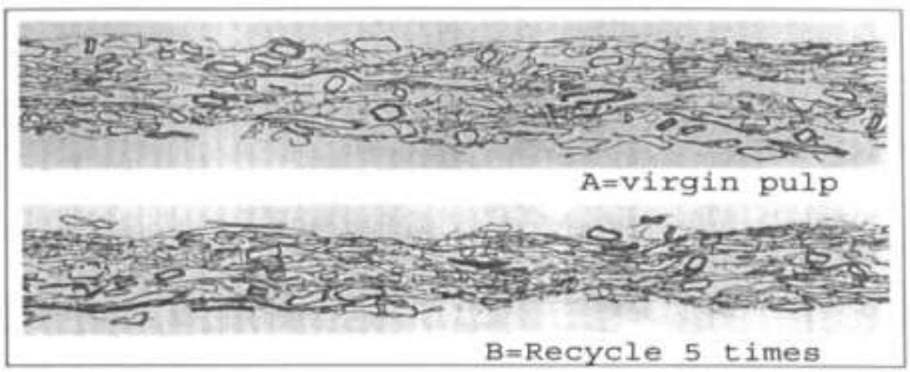

Figure:1: Primary fiber and 5 times recycled secondary fiber (Nazhad, 2005)

Secondary fibers are shorter than primary fibers, have a rigid structure, narrow surface areas and lower bonding potential. Fiber surfaces may be contaminated with resin and fatty acids. These materials reduce the surface energy of the fibers. At the same time, the swelling or plastification properties of the fibers are low, the fine fibers are high, and the individual fiber resistance is low. Theoretically, long fibers have higher strength properties than short fibers. The physical strength properties of papers produced from secondary fibers are lower than papers produced from primary fibers. The reason for this is the changes in the structure of cellulose as a result of drying (Nazhad 2005, Sahin 2014).

Cellulose fibers swell when treated with water, and shrink when water is removed. This situation is important for paper formation. In recycled papers, cellulose undergoes both a physical and chemical change. When dried fibers come together with water again, they lose their swelling properties in water. The condition in which this inhibited recurrent swelling occurs is called hornification (Minor 1994, Laivins and Scallan 1993). Hornified fibers are stiffer due to internal collapse and more resistant to fibrillation due to strong intrafiber bonding of polysaccharides at or near the fiber surface. The hydrophilic feature of hemicelluloses like cellulose in the building blocks of fibers allows the fibers to swell. If the hemicelluloses are removed from the cell wall, the swelling property of the fibers is negatively affected. Hornification reduces the tearing, bursting, folding resistance and density of the paper. There is a decrease in the resistance properties until the 4th recycling (Atalla and Minor 1992, Laivins and Scallan 1993, Pönni *et al.*, 2012, Sahin and Uner 2004).

Hubbe *et al.* (2007) explained the loss of swelling property of cellulose fibers during drying and rewetting, the decrease in flexibility of the fibers, the reduction of their surface area, and the fine fibers formed in the beaten paper. It has been mentioned that the changes in the strength properties of the paper due to the shortening of the fiber length, the fragilty of the recycled fibers increase and accordingly they are susceptible to breakage during refining.

Wistara *et al.* (1999) stated that the recycling process increases the amount of acid in the fibers and decreases the free surface energy. They explained that the formation of this situation may occur due to the deterioration of hemicelluloses, the increase of carboxylic groups on the surfaces or oxidation. According to AFM (atomic force microscope) and SEM (scanning electron microscopy) data, they claimed that fine fibers increase in recycling. In particular, they explained that the fine fibers increased the degree of crystallinity.

Diniz *et al.* (2004) reported that hornification, which causes a decrease in the hydrogen bonding potential of the fibers during the production of recycled papers, cannot be fully explained, but as a result of the studies, hydrogen bonding occurs after drying or water removal of cellulose fibers.

Ellis and Sedlachek (1993) studied the effect of recycled fibers on breaking length. They found that as the number of recycles increased, the breaking length decreased. In addition, they looked at burst resistance. They found that the burst resistance decreased (from 500 kPa to 300 kPa) with increasing recycling number. In addition, as the number of recycling increases; it has been reported that the tear strength increased by more than 20% and the stiffness property increased by more than 10%. At the same time, as the number of recycling increases; the apparent density (10%), elasticity-tensile (approximately 12%), breaking length (close to 20%), folding resistance (approximately 35%) and bursting resistance (approximately 40%) were also examined to decreases.

Wistara and Young (1999) reported that the strength properties and density of recycled papers decreased. While tearing resistance showed different properties from breaking and burst resistance, they explained that there was an increase in the first recycling result and a decrease in the second recycling. They explained that hemicelluloses are effective in regulating the physical strength properties of fibers in recycling.

Nazhad (2005) mentioned that the recycling effect on fiber properties depends on pulp making or papermaking background, but these effects disappear after the fifth cycle, even in the worst case scenario, recycled pulp loses 10-30% of its normal strength after the fifth cycle. He explained that breaking strength affects parameters such as fiber strength, fiber dimensions, and fine fibers density.

Atik (1999) used three types of paper as newsprint (recycle 6 times), white paper (recycle 6 times) and brown kraft (recycle 7 times) paper, applied recycling processes and performed the physical strength tests of the paper. He stated that there was not much change in the density of the paper after several recycles, and that recycling processes changed the physical strength properties of the paper. He explained that the tear strength started to decrease after the first recycling.

Sutjipto *et al.* (2008) found that as the recycling number of non-beaten softwood pulp increased, the tear, stress-breaking strength decreased, while the density first increased and then remained constant. In addition, as the number of recycling increases, the resistance properties in pulps of beaten softwoods increase; stress-breaking, apparent density decreased, and tearing increased.

Howard and Bichard (1992) found that the strength values (breaking length, burst index, tearing index) and density of bleached and beaten sulfate pulp were higher than those of sulphide pulp.
Gloss is one of the important optical properties. The gloss value is required to control the processes inside the factory such as fiber composition, ink content or deinking efficiency of the waste paper and to determine the performance of the machines. The gloss of recycled pulp controls not only the amount of ink but also the distribution of particle size. Ink particles smaller than 50 μm cannot be resolved individually by the human eye, but their presence reduces gloss and gives the pulp a gray tint. Ink particles over 50 μm appear as speckles. It has been stated that the opacity of the recycled papers from waste papers has increased (Bajpai 2014, Sahin 2014).
Waste paper as recycled paper raw material contains fillers and other additives besides fiber. Ash is important for the determination of the amount of inorganic materials originating from the filler in the pulp. The amount of ash varies according to the quality of the waste paper. Mixed office paper waste (15-35%), coated papers (25-35%) have the highest ash rates. Fillers and coating pigments with mineral content are used extensively in such papers (Kırcı 2003, Karıncalıoglu 2010).
Particles with a lower specific gravity and above 0.04 mm^2 are defined as dirt (Levlin and Söderhjelm 1999, Sahin 2014). One of the characteristics that can be used to distinguish the different grades of recycled paper is the nature of the additives and the fiber-free materials used in their manufacture. These materials should be treated as contaminants during recycling because they interfere with the recycling process and are often difficult to remove. Generally, non-cellulosic materials consistute 1-50% of the total weight of the recycled paper. Primary non-cellulosic contaminants include (Balos and Patterson, 1993):

- Fillers: kaolin, titanium dioxide, calsium carbonat and talk
- Resins, natural resin and wax
- Starch and gum
- Dyes and pigments
- Inks
- Hot melts, plastic veener and asphalt
- Styrofoam (solid foam) and treated tapes
- Heavy materials

Recycled paper materials, products made from secondary fiber, can be characterized by examining the relationship between the original sources of the fiber, and the recycled paper is usually recycled to a grade of similar or lower quality to the original (Balos and Patterson, 1993).

Conclusions

The quality parameters of recycled paper obtained from different sources of waste paper and cardboard were investigated. The quality parameters of recycled paper and cardboard are; fiber, strength and optical properties, ash content and contaminants. Quality parameters varied according to the class of waste paper and cardboard. Fiber loses swelling property in water and undergoes hornification, loses its stretching properties and becomes plastic, its bonding potential decreases, and individual fiber resistance is low. While the burst and breaking resistance of recycled papers is low, they have high tear resistance. Due to the ink content and the excess of fine fibers, the changes in the optical properties occur in the form of an increase in opacity, while the brightness and whiteness decrease. Since ash contains fillers and additives other than fiber, the amount of ash is high.

References

Akyıl, L. (2018). Recycling Policy of Waste Paper in the European Union, 6.International Techinologie Symposium Für Druck, Istanbul University-Cerrahpasa, 01-03 November, p.643-654.

Atalla R. H., Minor J. L. (1992). Strength Loss in Recycled Fibers and Methods of Restoration, Material Research Society Symp. Proc. Vol. 266.

Atik C. (1999). Recycling Effects on the Cellulose Fibers, PhD Thesis, IstanbulUniversity, Institute of science, Department of Forest Industry Engineer, 83148.

Atik C., Ok K. (2017). Paper Production and Sustainable Development in Turkey, Working Paper.

Bajpai P. (2014). Chapter 6-Effect of Recycling on Pulp Quality, Recycling and Deinking of Recovery Paper, Elsevier Inc, 978-0-12-416998-2, Pages 101-120.

Balos B. M, and Patterson J. V. (1993). Chapter 9-Recovered Paper Contaminants, Secondary Fiber Recycling, TAPPI Pres, ISBN:0898522676.

Bekiroglu S., Mertoglu Elmas G. (2017). Contribution to National Economy of Waste Paper Recycling (Example of Turkey's Hotels), International Journal of Environmental Sciences & Natural Resources, Research Article, Volume 2, Issue 1- March, ISSN: 2572-1119.

BS EN 643 Standard (2014). Paper and board- European list of standard grades of paper and board for recycling.

Diniz J.M.B.F., Gil M.H., Castro J.A.A.M. (2004). Hornification—its origin and interpretation in wood pulps, Wood Sci Technol 37, p.489–494.

Ellis R. L., Sedlachek K. (1993). Recycled vs. virgin fiber characteristics: a comparison, Recycled Fiber Strength, Vol. 76, No. 2, Tappi Journal.

Howard R.C., Bichard W. (1992). The Basic Effects of Recycling on Pulp Properties, Journal of Pulp And Paper Scıence, Vol. 18, No. 4, July.

Hubbe A. M., Rojas O. J., Venditti R. A. (2007). What happens to cellulosic fibers during papermaking and recycling? A review, BioResources, 2(4), p.739-788.

Karıncalıoglu M. (2010). Paper and Cardboard Production, Second Volume A- Introduction to Cellulose, Paper Factory Series 7, December.

Keranen J. T., Retulainen E. (2016). Changing Quality of Recycled Fiber Material. Part 1. Factors Affecting the Quality and an Approach for Characterisation of the Strength Potential, Recycling Pt. 1, BioResources, 11(4), 10404-10418.

Kırcı H. (2003). Paper Pulp Industry lecture notes, improved 2nd edition, Karadeniz Technical University Forest Faculty, Publishing No:72, Trabzon.

Kumar V., Kalra J.S., Verma D., Gupta S. (2019). Process and Environmental Benefit of Recycling of Waste Papers, International Journal of Recent Technology and Engineering ISSN: 2277-3878, Volume-8 Issue-2S12, September.

Laivins G.V., Scallan A.M. (1993).The Mechanism of Hornification of Wood Pulps, Products of Papermaking, Transaction of the Xth Fund. Res. Symp. Oxford, (C.F. Baker, ed.), pp 1235–1260.

Levlin J. E., Söderhjelm L. (Eds.). (1999). Pulp and Paper Testing, Papermaking Science and Technology, Book 17, ISBN 952-5216-17-9.

Minor J. L. (1994). Hornification-Its Origin and Meaning, Progress in Paper Recycling, Vol:3, No:2, February.

Nazhad M. M. (2005). Recycled Fiber Quality-A Review, J. Ind. Eng. Chem., Vol.11, No.3, p.314-329.

Pönni, R., Vuorinen, T., Kontturi, E. (2012). Proposed Nano-Scale Coalescence Of Cellulose In Chemical Pulp Fibers During Technical Treatments, BioResources, 7(4), 6077-6108.
SKSV(Cellulose Paper and Industry Foundation), (2020). Annual Report.
Sutjipto, E. R., Li, K., Pongpattanasuegsa, S., & Nazhad, M. M. (2008). Effect of recycling on paper properties, TAPPSA (technical articles).
Sahin H. T., Uner B. (2004). Wet Press and Drying Effects on Fiber Properties During Recycling Journal of SDU of Forestry Series: A, No: 1, ISSN: 1302-7085, Page:145-158.
Sahin H. T. (2014). A study on recovered secondary fiber for reuse, Journal of SDU of Forestry, Review article, 15: p.183-188.
Sahin H. T. (2016). Basic Principles on Paper Recycling and Deinking Process, Review article, European Journal of Science and Technology, Volume 4, No:7, p.31-37, June.
Scott G. M. (2019).Chapter 14-Recovered Paper, Waste (Second Edition)-A Handbook for Management, Academic Press, Pages 291-305.
Wistara N., Young R. A. (1999). Properties and treatments of pulps from recycled paper. Part I. Physical and chemical properties of pulps, Cellulose 6: p.291–324.
Wistara N., Young R. A., Zhang X. (1999). Properties and treatments of pulps from recycled paper. Part II. Surface properties and crystallinity of fibers and fines, Cellulose 6: 325–348.

FIBER PROPERTIES OF *RUMEKS CRISPUS* L. ROOT

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Abstract

A global shortage of conventional pulpable raw material is seen there were attempt in this century. Search for new fiber annual wood sources against hardwood has been underway during the last few decades. On the other hand, there were attempt to find and develop new fiber supplies such as wood particularly annual plants for paper and pulp industries. *Rumex crispus* L. plant is widely available in Turkey. Its extracts are known to have antimicrobial properties. It is thought that *Rumex* crispus will be used in the production of special papers due to its antimicrobial properties and also to obtain dyes. The anatomical image was taken in cross-section from the tubers of *Rumex crispus* L. and its relation to the fiber structure was investigated. Depending on the fiber properties obtained, the potential of these types to be used as raw material in paper production has been evaluated and discussed. Therefore, according to Shultze method were macerated *Rumex crispus* L. root samples and prepared microscopic slides for measuring fiber dimensions. In tests, fiber length, fiber width (diameter), lumen diameter and cell wall thickness were measured. When the fiber lengths of *Rumex crispus* L. annual plant are compared with annual plants such as hemp, barley straw, oat straw, rye straw, which are still in use, the shorter fiber dimensions show that it will allow its use in pulp like these plants. Also, it can be said that the pulp obtained from *Rumex cripus* L. which has a yellow pigment feature, can be used in the production of artistic special papers.

Key words: *Fiber properties, Rumex crispus* L., *annual plants, pulp, paper.*

Introduction

Using non-wood fiber for pulp and paper industry has seen huge demand in the world as a raw material. Also, the use of non-wood fibers is an increasing trend due to shortage of wood fibers and environmental considerations. Generally the commonly used non-wood pulp raw material sources are residues of agricultural plants such as bagasse, (straw and the silical associated problems), flax hemp kenaf (its inherently low lignin content and therefore higher cellulose content), jute (to produce specialty paper).
The trend towards alternative non-wood fiber sources is increasing in order to be an alternative to substitute wood sources such as decreasing broadleaves hardwoods and softwood a shortage of wood fibers and to find solutions to environmental concerns by evaluating the use of agricultural residues. Although until recently the use of non-wood fibers for pulp and papermaking was concentrated in countries where there are limited supplies of wood, even countries that no longer have sufficient wood supply due to environmental concerns shows a growing trend. This can be expected to grow further and the future said with confidence. Non-wood plant fibers appear to have as potential as traditionally produced pulp and papermaking raw material.

Rumex crispus (Labada) is a perennial vegetative cycle plant that can grow up to 150 cm tall. Most of the lower leaves are structured as substitute-like, reverse-spare-like and with widths greater than 3 times longer. The leaf stem is above, corrugated and has the largest plant cluster. (Chandra, 1998).

The plants can be found in non-cultivated, empty agricultural fields, in swampy areas and along road sides. It flowers during the period, May to August and can extensively be found in the following provinces of Turkey: Çanakkale, Istanbul, Bolu, Kastamonu; Coruh, Kars, Izmir, Ankara, Tunceli, Diyarbakır, Konya and Gaziantep (Davis,1965-1985; Yaltirik and Efe,1989; Mertoglu-Elmas *et al.* 2012)

Plant's roots and leaves are boiled for tea making. Leaves are also consumed in various ways. It grows extensively in Turkey.

A yellow colored pigment which is an antraquinone derivative, 1,5-dihydroxy-3-methoxy-7-methyl-antraquinone, can be extracted from *Rumex crispus* L. lumped root (Başkan *et al*., 2006). It has been proposed that the naturally colored special paper for using in the artistic field and in the packaging industry. This is very important specially using natural dye instead of synthetic dye (Mertoglu-Elmas *et al.* 2012).

The fibers of broadleaves woods (hardwoods) are shorter than those from coniferus (softwoods). Some annual's fibers are longer than hardwoods and softwoods. These fibers impart excellent optical properties to paper, however blending with softwood pulp is usually demanded to develop physical properties of paper.

The aim of this research was to determined the fiber dimensions of *Rumeks crispus* root and as well as fiber their suitability properties for pulping as an alternative fibre supply.

Materials and Methods

Naturally grown *Rumex crispus L.*samples were supplied from Atatürk Arboretum Bahçekoy, Istanbul, Turkey. As seen in Table 1 the specimens had average diameters approximately 4.0cm and 1.50m in heigth. Its roots were dried and chipped to prepare for fibering process. Fibering process of samples was performed according to Shultze's macerattion method (Mahesh *et al.* 2015; Chamberlain, 1915). After the fibers were thoroughly washed, they were mixed in the mixer for 3 minutes. The resulting fiber suspension was filtered in a Buchner funnel using a fine mesh strainer. Then, the fibers remaining on the strainer were placed in small-sized tubes for protection by adding glycerine to prepare microscope slides. While the mixture consisting of fiber and glycerin spreads homogeneously, it is dropped on the slide, a coverslip is placed on it and stabilized with varnish. When the suitability of raw materials for papermaking is evaluated, fiber dimensions such as fiber length, width, wall thickness and lumen width and provide important clues about the possibilities offered by the raw material for pulp production. In the measurements; fiber length, fiber width (diameter), lumen diameter and cell wall thickness were determined with an optical microscope (Leica DM2500, Istanbul, Turkey) in according to with TAPPI T232 cm-85 (1985) standarts.

Results and Discussions

Considering the fiber properties of *Rumex crispus* L., the possibilities using it as a raw material in the pulp and paper industry have been estimated through an analysis of results obtained from preliminary evaluation study of fiber morphology consist of fiber dimensions. *Rumex crispus* L.

root anatomic image was shown in Figure 1. As seen in Figure 1 tracheas were in the form of homogeneously undistributed clusters and their rays show a thick structure.

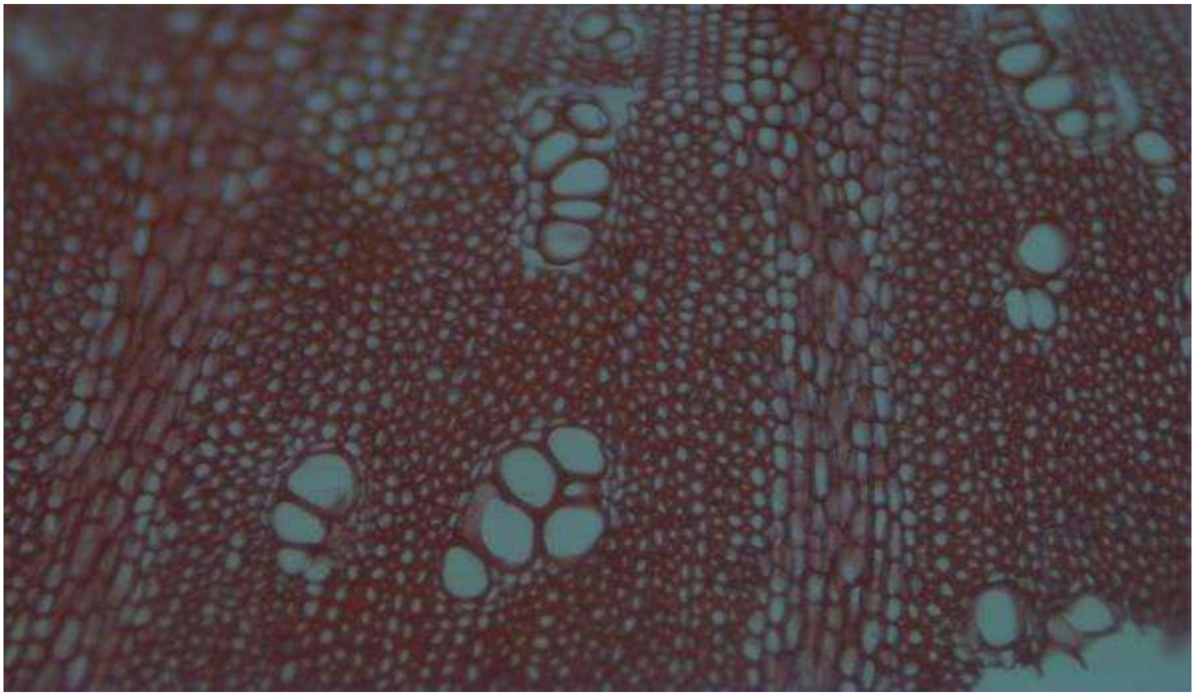

Figure:1: Image of a cross-section of the root of *Rumex crispus* L.

Images length of Rumex crispus L. was given in Figure 2.

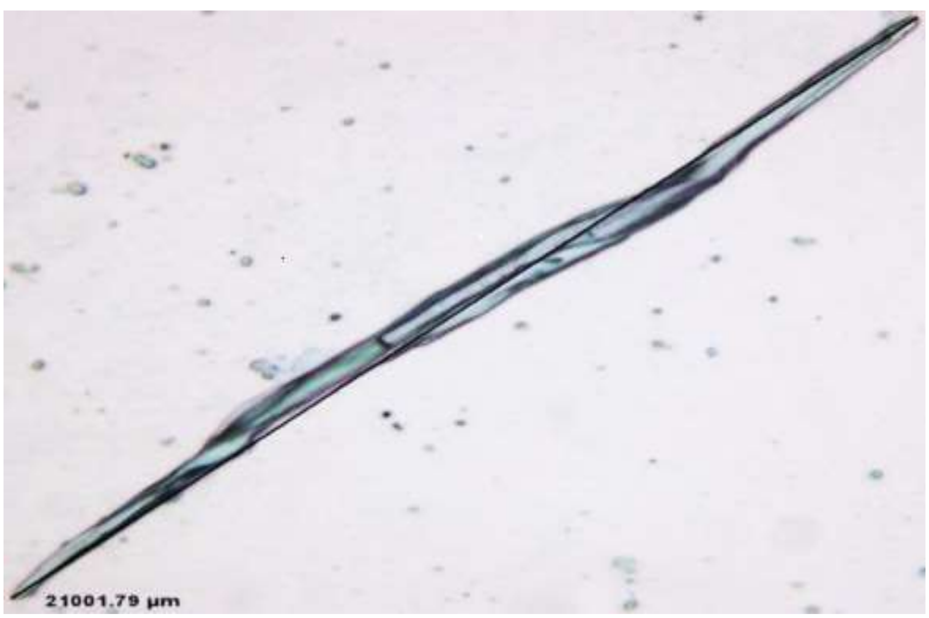

Figure: 2: Length of fibre of *Rumex crispus* L.

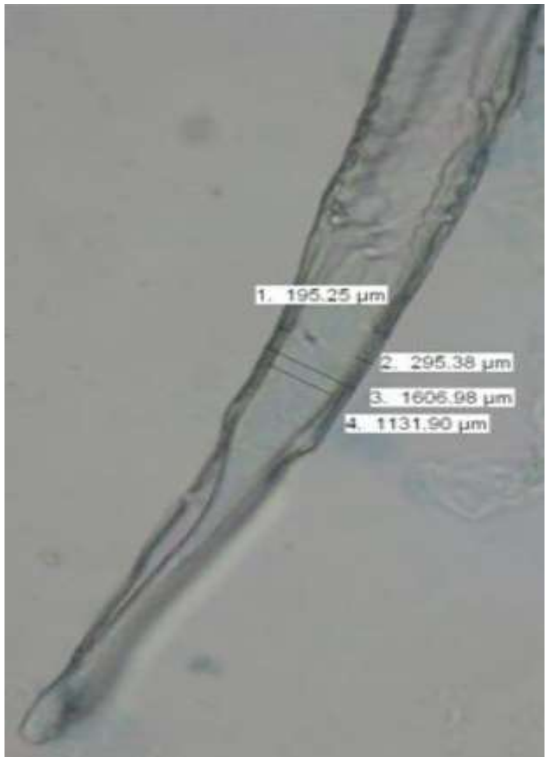

Figure: 3: Width (diameter) and cell wall thickness of fibre of *Rumex crispus* L.

Figures 2 and 3 show the measured dimensions of fiber such as fiber length, fiber width (diameter) and cell wall thickness. Morphological and dimensions fiber values were given in the Table 1.

Table 1. Morphological and fiber dimentions of *Rumex crispus* L.

Height (m)	1.50
Diameter DBH (cm)	4.00
Mean Fiber length (mm)	21.61
Fiber width (μm)	8.11
Wall thickness (μm)	1.75
Lumene diameter (μm)	4.61

DBH: Diameter at breast height

Fiber structure

Morphological fiber dimensions are length, width, cell wall, and lümen radius. Fiber dimensions act as indicators providing essential preliminary information about suıtability for pulp production and usability as wood raw material (Dinwoodie 1965; Bostancı 1987).

Suitability of wood and annual plant used as a raw material for paper production from pulp is evaluated over the resistance characteristics of pulp thorough the fiber length and fiber width. Fiber length affects paper strength properties positively. Cell wall thickness influences the

strength of individual fibers. The tearing resistance of paper made from very thin-walled fibers is quite low. Paper provides extremely thick-walled fibers exhibit low resistance and bulk due to insufficie flattening during paper formation (Elmas *et al*. 2018).
It has been observed that the average fiber length of the annual plants *Rumex crispus* plant (21.6mm), longer than the fiber length of cotton linter (7mm), close to the fiber length of hemp (bast) (25mm), oil flax (30mm). However, it was found that oat straw (44mm), barley stalk (47mm), rye straw (50mm) and hemp husk fibers had shorter fiber lengths (Atchison 1993; Atchison, 1997; Mengeloglu and Alma 1999 and Zomers *et al*,1995). The fiber length of *Rumex crispus* L. was typically in the middle class of long fiber sequence of annual plant species.
For the production of delignified pulp of hemp, organosolv processes can offer economical and environmental advantages, "hemp core and bast fibers can be readily pulped" (Zomers *et al*. 1995).
Comparing the fiber lengths of the *Rumex crispus* L. annual plant still in use with oil hemp, barley stalk, oat stalk, rye stalk have shorter fiber sizes, which indicates that it will allow their use in fiber pulp like these plants.

Conclusions

Rumex crispus L. has the potential as a sustainable raw material in paper production as a non-wood annual plant. Suitability of annual plant used as a raw material for paper production from pulp was evaluated over the dimensions of fiber thorough the fiber length and fiber width. Fiber length affects paper strength properties positively. The fiber length of *Rumex crispus* was typically in the middle class of long fiber sequence of annual plant species such as oat straw barley stalk, rye straw, which indicates that it will allow their use in fiber pulp like these plants.

References

Atchison, J.E., 1987. Data on non-wood plant fibers. In: The Secondary Fibers and Non-
Wood Pulping: Third edition (Ed: Hamilton, F.), TAPPI Press, Atlanta, USA.
Atchison, J.E., 1993. Data on non-wood plant fibers. In: Pulp and Paper Manufacture:
Secondary Fibers and Nonwood Pulping (Ed: Kocurek, M.J.), TAPPI Press, Atlanta, vol 3, pp. 4-13.
Chamberlain, C. J. (1915): Plant cell physiology: Schultze's macerattion method. – In: Chamberlain, C. J. (ed.) Methods in Plant Histology. University of Chicago press, Chicago.
Chandra M. (1998). "Use of Nonwood Plant Fıbers for Pulp and Paper Industry In
Asia: Potentıal in China The Faculty of Virginia Polytechnic Institute And State University on Partial Fulfillment of the Requirements for the Degree of Master of Forestry in Wood Science And Forest Products, Blacksburg, Virginia.
Başkan, S., Ozdemir, A.D., Gunaydın, K., and Erim, F. B. (2006). Analysis of antraquiones in Rumex crispus by micellar electrokinetic chromatography. Science Direct, Talanta.71, 747-750.
Bostancı, S. (1987). Kagıt Hamuru Üretimi ve Ağartma Teknolojisi,(Pulp production and Bleaching Technology) Karadeniz Technical University, Publishing Number:114, Trabzon, Turkey. pp.516.
Davis PH (Ed.) (1965–1985) Flora of Turkey and the East Aegean Islands,Vol..1–9.
Edinburgh University Press, Edinburgh.

Dinwoodie J.M.(1965).Growth stresses in timber-a review of European investigations. In: IUFRO Section 41Melbourne Avustralia.

Elmas, G.M., Gurboy, B.and Eray, I.N. (2018).Willow clones for pulp. Biores.13(4), 8555-8568.

Frinhani. P.E and De Oliveira R.C. (2006).The applicability of natural colorants in papermaking. Tappi J. 5(7), 3-7.

Mahesh, S., Kumar, P., Ansar, S. A. (2015). A rapid and economical method for the maceration of wood fibers in Boswellia serrata Roxb. –Wood Sci. and Techn. Tropical plant Research 2(2): 108-111.

Mertoglu-Elmas, G., Gunaydın, K. and Ozden O. (2012). Environmental friendly alkaline sulfite anthraquinone-methanol (ASAM) pulping with *Rumex crispus* plant extract of woody materials J. Environ. Biol. 33, 941-944.

Mengeloglu F.M. and Alma, H. (1999). In Composite Board Production of Wheat Stalks Using. KSU. J. Science and Engineering 5(2), 37.

TAPPI T232 cm-85 (1985)."Fiber length of pulp by projection."TAPPI Press. Atlanta, GA.

Yaltirik, F. and Efe, A. (1989). "Otsu Bitkiler Sistematiği (Herbaceous plants systematics)" Istanbul University Science Books, Istanbul.

Zomers, F.H.A., Gosselink R.J.A., van Dam, J.E.G., Tjeerdsma, B.F. (1995). Organosolv pulping and test paper characterization of fiber hemp. Tappi Journal, 78 (5), 149-155.

ASSESSEMENT OF WATER AND ECONOMIC PRODUCTIVITY IN IRRIGATION MANAGEMENT

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Abstract

Irrigation is extremely important for food security and reducing the risk of drought. Approximately 70% of water is used in irrigation at a global level. The quantity and quality of water resources are decreasing due to climate change, increasing population and frequent droughts. This requires the reduction and efficient use of water used in agricultural irrigation. For this reason, the crop yield to be obtained by additional irrigation water applied considering the irrigation and operating costs and net margin per unit land area-NM (US\$ ha^{-1}) as well as the irrigation water productivity-WP_I (kg m^{-3}) and net economical water productivity- $NEWP_I$ (US\$ m^{-3}) are also important components for assessment of irrigation schemes. Because, the farmers and irrigation schemes are an economic enterprise that aim at higher profit by increasing the yield to be obtained from the unit area. In addition, WP_I is also an indicator of whether the irrigation water is used effectively. However, the water productivity begins to decline while irrigation water yields increase. Connecting this approach, the right of the intersection of the irrigation water-yield curve and the irrigation water-water productivity curve shows unnecessary excess irrigation water application. Thus, it can be decided at which point the irrigation can be stopped. This can give more optimal irrigation strategy. In this article, the relationships between WP_I, $NEWP_I$ and NM are evaluated and discussed using some experimental results in semi-arid regions.

Key words: *water productivity, water economic productivity, irrigation management.*

Introduction

Water use in agriculture plays a key role for food security in the world. Water saving technologies and efficient use of water, prevention of water loss have become important for water user sectors. Agriculture is the sector that uses the most water, and modernization of irrigation systems and appropriate irrigation management are required. This is achieved by accurately determining the crop water requirement and knowing the water productivity and economic return of irrigation.

Efficient and sustainable use of water resources has become inevitable due to some main reasons such as global climate change, drought, increasing population, increase in irrigated lands, excessive use of water in agriculture using traditional irrigation in all over the world. In addition, water demands have increased because of the growing population and developing industry. Consequently, more efficient use of water in agriculture has become significant because of the largest water user sector.

Engineers and irrigation scientists have used water/irrigation use efficiency to describe how effectively the water supplied to plants is used and how much water is wasted in the field, farm, command area or irrigation scheme level. Thus it is defined as "the ratio of irrigation water used by the crops in a farm or irrigation district area during plant growth period to the water diverted from a river or other reservuar.
Improvement of agricultural water productivity and irrigation efficiency is a critical response for increasing water scarcity and the sustainability of ecosystems and natural life, to meet demands of indutries and cities (Sharma et al., 2015). Thus, the analysis of water productivity and/or irrigation efficiency which is critically important to food production and food security (Perry 2011, Çetin and Kara 2019).
There are many different and evaluation criteria for irrigation and/or irrigation schemes depending on the study purpose due to irrigation is a very complex and has many components in practice. In this article, "irrigation efficiency-IE" (%), "irrigation water productivity-WP_I" (kg ha^{-1}), "net economical water productivity-$NEWP_I$" (\$ m^{-3}) and "net margin per land area" (\$ ha^{-1}) are discussed for the assessment and analysing of agricultural irrigation. Because, these assessment criteria are more usefull for the evaluations that directly affect agricultural irrigation management which are important in terms of guiding technical and practical issues and decision makers.

Basic Definitions on Assessment of Irrigation Management

Previously, in most publications, "water use efficiency-WUE" was used for both the water use rate and the crop yield per volumetric unit water (water productivity-WP). However, in recent years, WUE or irrigation efficiency (IE) and WP have been used as two different concepts with different meanings (Pereira et al., 2012). Thus, IE is an indicator how much the water diverted from the source is used by the plants and its unit is %. In other words, it is the ratio of the irrigation water used by the plants to the amount of water diverted from the reservuar. Water prodctivity (WP_{ETc}) is production of biomass and/or crop yield per crop evapotranspiration. Irrigation water productivity (WP_I) is defined similarly biomass and/or crop yield per amount of irrigation applied and the unit is kg m^{-3}. The considerations and assessments in this article are based on irrigation water productivity (WP_I) because the amount of irrigation water used in a farm and/or irrigation scheme is more important for farmers and irrigation authority. IE interests mainly the water districts or management agencies, while WP interests more farmers and research community. WP better explains to perspectives linking water usage with production levels and economic benefit. IE is an indicator of how effectively or beneficially the water supplied to the system is used, while WP is the total amount of production obtained against unit water (Levidow et al., 2014).

Irrigation efficiency

In an irrigation event, there will be some water losses due to evaporation, channel seepage and in-field farming practices. The important thing is to convey and use water with minimum water loss. "Irrigation efficiency" defined and used herein associated water conveyance and field water application efficiency. The most ideal irrigation efficiency is about 70% in surface irrigation, 80-85% in sprinkler and 90-95% in drip irrigation (Irmak et al., 2011). The concept of irrigation efficiency used here can also be defined total irrigation efficiency. This definition and its numerical results reflects about the efficiency of irrigation infrastructures, an irrigation network and/or irrigation in a farmer's field and irrigation management. Irrigation efficiency can be, thus, calculated the formula given below.

$$IE = \frac{Qa}{Qd} \times 100 \qquad (1)$$

Where, IE is irrigation efficiency (%), Qa is amount of irrigation water applied into the plant root zone by plants (m^3 or $m^3\ h^{-1}$), Qd is amount of water diverted from the reservuar (m^3 or $m^3\ h^{-1}$).

Irrigation water productivity

Irrigation water productivity (WP_I) in agriculture is defined as the ratio between the actual crop yield achieved (Ya) and the water use, expressed in kg m^{-3} (Pereria et al., 2016; Cetin and Kara, 2019; Fernandez et al., 2020).

$$WP_I = \frac{Ya}{IWA} \qquad (2)$$

Where WP_I is irrigation water productivity (kg m^{-3}), Ya is crop yield (kg ha^{-1}) IWA is irrigation water applied ($m^3\ ha^{-1}$)

Net economical water productivity

Net economic water productivity ($NEWP_I$) is the economic return to a unit of water. $NEWP_I$ is, thus, the ratio of net margin per irrigated area to amount of irrigation water applied per irrigated area (Fernandez et al., 2020; Uygan et al., 2021). To determine it is important for the basin and obtain the costs and process of the products. This is more important to the farmer than the biophysical water productivity as it means income but the cost of tradeoff negates (Oweis et al., 2017). $NEWP_I$ together with WP_I are useful and important for deficit irrigation and/or water scarcity.

$$NEWP_I = \frac{NM}{IWA} \qquad (3)$$

$NEWP_I$ is net economical water productivity (US$ m^{-3}), NM is net margin per unit land area (US$ ha^{-1}), IWA is irrigation water applied ($m^3\ ha^{-1}$).

Net margin per land area

The net margin is more important for farmers. They must consider this as well as irrigation water productivity and/or net economical water productivity. The net margin is depended mainly on the irrigation water applied in arid and semi-arid regions even if other inputs affect also. Net margin per land area was computed the simple formula given below (Fernandez et al., 2020; Uygan et al., 2021)

$$NM = R\text{-}C \qquad (4)$$

Where, NM is net margin per unit land area (US$ ha^{-1}), R: Revenue or gross income (US$ ha^{-1}), C is total fix and variable costs (US$ ha^{-1})

The Role of Water Productivity on Water Saving

Crop water productivity varies with location, depending on such factors as cropping pattern, agronomy, climatic conditions, irrigation technology, field water management and infrastructure, and on the labour, fertilizer and machinery inputs (Kijne et al., 2003). However, use of drip irrigation systems that provide very high irrigation water savings is much more important than the others. According to the results of many researches, drip irrigation can save between 20-50% of irrigation water compared to surface irrigation (Shah, 2011; Çetin and Akalp, 2019).

According to the results of the study carried out by Cetin and Bilgel (2002), if surface irrigation (furrow) is used for cotton, approximately amount of irrigation water of 950 mm (9500 $m^3\ ha^{-1}$)

is required whereas if surface drip and subsurface drip irrigation is used, irrigation water of 600 mm (6000 m^3 ha^{-1}) and 550 mm (5500 m^3 ha^{-1}) are sufficient for optimum cotton yield, respectively (Çetin et al., 2021). Considering the results of this study, surface and subsurface drip irrigation can save irrigation water of 37% and 42% compared to surface (furrow) irrigation, respectively. In addition, the cotton seed yield increased in drip irrigation systems although the use of irrigation water was decreased significantly lower.

Depending on the developing technologies, it has become important to use surface and subsurface drip irrigation systems, especially in field crops such as cotton and corn planted in rows as well as horticultural crops (Çetin and Bilgel, 2002; Shah, 2011; Uygan et al., 2021). Because, it is possible to reduce the evaporation from the soil using drip irrigation, especially in the early development periods of the plants (the percentage of canopy cover is not yet fully developed) because of the whole land surface is not irrigated (not wetted). This provides the significant irrigation water savings compared to traditional irrigations (surface irrigation).

The irrigation water productivity was higher at lower irrigation water levels and/or deficit irrigation. The increase in WP_I decreases relatively as long as the irrigation water increases, and thus, it decreases considerably in over-irrigation. Accordingly, WP_I decreases on the one hand while the crop yield increases on the other hand. For an effective and sustainable water management, the right side of the intersection of the irrigation water-yield curve and the irrigation water-WP_I curve can be considered as non-beneficial irrigation water applications (Figure 1). However, this approach might not be valid for all circumtances. In this situation, the economic return and water saving conditions should be taken into account together.

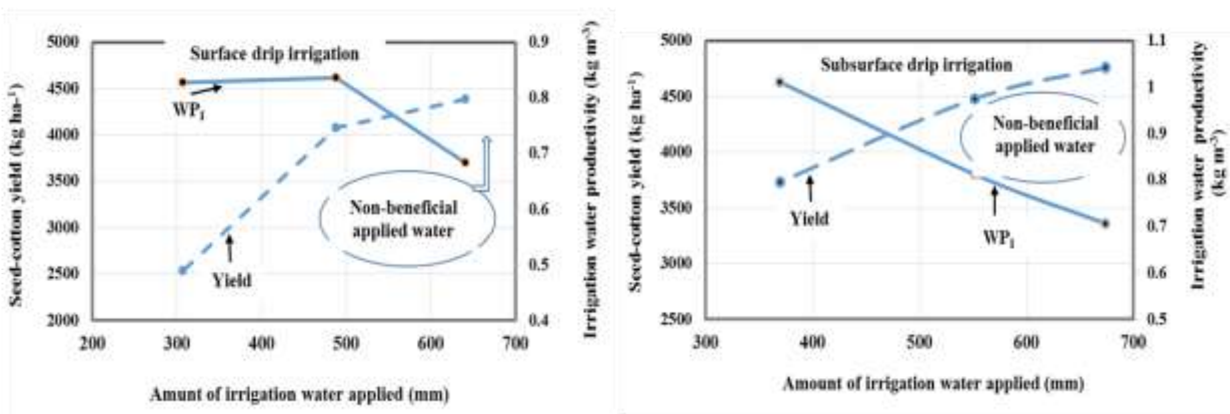

Figure 1. The relationships between irrigation water producrivity and seed cotton yield depending on the amount of irrigation water applied in surface and subsurface irrigtion drip (Cetin and Kara, 2019).

Evaluating drip irrigation systems in terms of irrigation water productivity and net economic water productivity, both surface and subsurface drip irrigation provided significantly higher values than those in surface irrigation. Connecting these results, the values of WP_I were 0.70 and 0.80 kg m^{-3} in surface and subsurface drip irrigation, respectively, while WP_I was 0.37 kg m^{-3} in furrow irrigation (Figure 2). Similarly, it is reported that subsurface drip irrigation increased both cotton yield and IWP compared to the other irrigation methods (Phene et al., 1992; Martinez and Reca, 2014; Jayakumar et al., 2015).

Use of Economic Water Productivity on Irrigation Management

According to the study carried out by Cetin et al. (2021), the net economic water producutivty ($NEWP_I$) using the surface and subsurface drip irrigation systems for cotton was 0.13 and 0.20 US\$ m^{-3}, respectively. These results were also significantly higher than 0.07 US\$ m^{-3} in the surface irrigation as WP_I (Figure 2). From this, it could be considered how important drip irrigation systems were important in terms of both water productivity and economic water productivity. Accordingly, the net income per unit of land, which is especially important for farmers, is higher in drip irrigation systems. All these data and evaluations are important especially in the creation of decision mechanisms in the management and sustainable use of water resources, and in terms of water saving and economic income. On the other hand, the net margin is depended mainly on the irrigation water applied. In other words, the main factor affecting the net income is irrigation. Because the increased level of irrigation water increased the yield (Perry, 2011). As a result, use of subsurface drip irrigation can provide very important advantages in terms of the total irrigation water used, water prodcutivity and total net incomes. The results showed that use of drip irrigation systems (especially subsurface drip) will be very important in terms of possible water shortage, decreasing water resources, farmers, irrigation schemes, regional and national income. However, all the advantages of drip irrigation systems can be realized/operated appropriately with the necessary implementation of correct engineering installation, suitable irrigation management and operation, taking into account the soil, crop and climate characteristics of the irrigated lands. In addition, all these systems need more expensive cost and experienced and trained users.

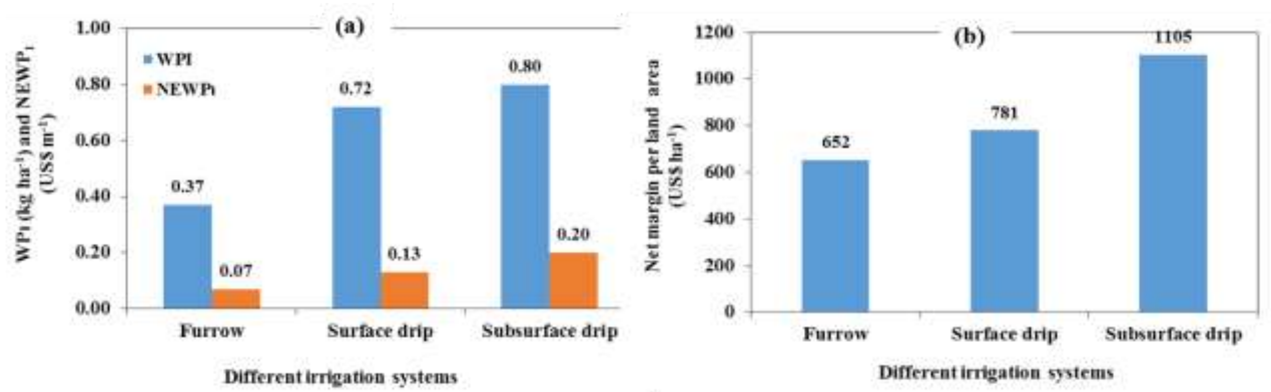

Figure 2. The effects of different irrigation systems on irrigation water productivity (WP_I) , net economic water productivity ($NEWP_I$) (a) and net income per irrigated land (b) (Çetin et al., 2021).

Conclusion

An appropriate irrigation management is extremely important in terms of the efficiency of irrigation investments and providing the necessary economic contribution and the sustainable use of soil and water resources. Water productivity, WP, (kg m^{-3}), net economic water productivity, ($NEWP_I$) (\$ m^{-3}) and land economic return for irrigated agriculture will be important for assessment of irrigation schemes and/or and decision makers. These data and assessments should be, thus, considered by farmers, irrigation operators, irrigation management authorities and decision makers at the regional, national and also international levels considering transboundary water.

References

Çetin Ö., Bilgel L. (2002). Effects of different irrigation methods on shedding and yield of cotton. Agricultural Water Management, Volume 54 (1): 1-15.

Çetin Ö., Kara A. (2019). Assesment of water productivity using different drip irrigation systems for cotton. Agricultural Water Management, 223: 105693

Çetin Ö., Üzen N., Temiz M.G., Altunten H. (2021). Improving cotton yield, water use and net income in different drip irrigation systems using real-time crop evapotranspiration. Polish Journal of Environmental Studies, 30: 5.

Çetin, Ö., Akalp, E. (2019). Efficient use of water and fertilizers in irrigated agriculture:Drip irrigation and fertigation. Acta Horticulturae et Regiotecturae 2: 97–102.

Fernández J.E., Alcon F., Diaz-Espejo A., Hernandez-Santana V., Cuevas M.V. (2020). Water use indicators and economic analysis for on-farm irrigation decision:A case study of a super high density olive tree orchard. Agricultural Water Management 237: 106074.

Irmak, S., Odhiambo, L.O., Kranz, W.L., Eisenhauer, D.E. (2011). Irrigation efficiency and uniformity, and crop water use efficiency. Biological Systems Engineering: Papers and Publications. 451. University of Nebraska – Lincoln, USA.

Jayakumar, M., Surendran, U., Manickasundaram, P. (2015). Drip fertigation programon growth, crop productivity, water, and fertilizer-use efficiency of Bt cotton in semi-arid tropical region of India. Communications in Soil Science and Plant Analysis, 46, 293.

Kijne J.W., Barker R., Molden D. (2003). Improving Water Productivity in Agriculture: Editors' overview. In Kijne, J.W., Barker, R. & Molden D. (eds.) "Water Productivity in Agriculture: Limits and opportunities for Improvement". International Water Management Institute, Colombo, Sri Lanka

Levidow L., Zaccari D., Maia R., Vivas E., Todorovic M., Scardigno A. (2014). Improving water-efficient irrigation: Prospects and difficulties of innovative practices. Agricultural Water Management 146: 84–94.

Martínez, J., Reca, J. (2014). Water use efficiency of surface drip irrigation versus an alternative subsurface drip irrigation method. Journal of Irrigation and Drainage Engineering, 140 (10), 04014030.

Oweis T., Biradar C., Haddad M. (2017). Supporting coordination and cooperation in water management in the Euphrates and Tigris Area. CPET, Agricultural water productivity, task force (TF-Ag). Final Report. International Center for Agricultural Research in the Dry Areas (ICARDA). Beirut, Lebanon.

Pereira L.S., Cordery I., Iacovides I. (2012). Improved indicators of water use performance and productivity for sustainable water conservation and saving. Agricultural Water Management, 108: 39–51.

Perry, C. (2011). Accounting for water use: terminology and implications for saving water and increasing production. Agricultural Water Management. 98: 1840–1846.

Phene, C.J., Hutmacher, R.B., Davis, K.R., 1992. Subsurface drip irrigation, cotton does not need to be a high water user. Proceedings of Cotton Engineering Systems Conference, 489.

Shah, S.K. (2011). Towards adopting nanotechnology in irrigation. Micro irrigation systems. India Water Portal: Karnataka, India.

Sharma B.R., Molden D., Cook, S. (2015). Water use efficiency in agriculture: measurement, current situation and trends. In. Drechsel, P, Heffer, P., Magan, H., Mikkelsen, R. &

Wichlens, D. (eds.) "Managing water and fertiliser for sustainable intensification". Paris, France: International Fertiliser Association. pp. 39-64

Uygan D., Cetin O., Alveroglu V., Sofuoglu A. (2021). Improvement of water saving and economic productivity based on quotation with sugar content of sugar beet using linear move sprinkler irrigation. Agricultural Water Management, 255: 106989.

ANTIMICROBIAL PROPERTIS OF ESSENCIAL OILS AGAINST ESCHERICHIA COLI

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Abstract

Overuse and overprescribing of antimicrobial drugs and disinfectants both in veterinary and human medicine have resulted in bacterial resistance leading to the need for new antimicrobial drugs. One of the possible alternatives is the use of essential oils - aromatic liquids of oil consistency, which by different methods are extracted from almost all parts of plants. It has been proven that essential oils, to varying degrees have antibacterial activity, that depends on the type of bacteria and the type and chemical composition of the oil used. The aim of this work is to examine the antibacterial activity of essential oil of St. John's wort, mint, cinnamon, clover, rosemary, thyme, garlic, fennel, lemon and reference antimicrobial drugs to the growth of the bacteria Escherichia coli. Agar diffusion method was used to determine the antibacterial effect of essential oils and different amounts of essential oils were prepared by dissolving with 96% ethanol alcohol (1:1 and 2:1). The largest activity was demonstrated by essential oils of St. John's wort, mint, cinnamon and cloves while essential oils of rosemary, thyme, garlic and fennel showed slightly weaker inhibition depending on whether they were used as pure oils or combined with alcohol. Also, the type of action of essential oils is determined.

Keywords: *antimicrobial drugs, antimicrobial activity, Escherichia coli, essential oils.*

Introduction

Etheric or essential oils are mixtures of highly volatile, lipophilic substances isolated from plants or plant parts. It is estimated that 3000 different essential oils are known, of which about 300 have commercial importance (Van de Braak et al.,1999). Essential oils are used in the food industry as aromas, in the pharmaceutical industry due to their pharmacological properties and in the perfume industry (Bauer et al., 1985, Bakkali et al., 2008.). Essential oils as natural, biologically active substances have insecticidal and antimicrobial activity and they are very important in the food industry for preserving food and preventing the development of pathogenic microorganisms. Spice plants or their essential oils, in addition to food, are used to achieve appropriate sensory characteristics, but which at the same time can provide both the appropriate stability and food safety (Kalaba et al., 2020.). Antimicrobial activity of spicy and medicinal plants has been known since ancient times. More than 1340 plants are known as a potential source of antimicrobial components, but a smaller number have been studied in detail (Wilkins and Board,1989). Flavonoids, saponins, tannins and alkaloids as natural ingredients of plants are known to have certain antimicrobial activity. It is also known that antimicrobial activity of plants such as thyme, rosemary, orange, cloves, etc., comes from essential oils (Radulovic et al., 2013). The amount of essential oils in plants varies widely and in some plants they are present in very small quantities (0.05 -0.1%) while in other plants the amount of essential oil can be up to 20%, like in cloves (Popović and Đurđević Milošević, 2008). In many studies it has been proven that some of the active components present in essential oil (carvacrol) can prevent the spread of *Salmonella spp*. in meat as well as to reduce the number of aerobic bacteria in meat and meat products and to act antibacterial on Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia, Mycoplasma gallisepticum, Clostridium perfingens, Salmonella enterica (Feizi al., 2013). Food products can be carriers of various pathogenic bacteria that enter food through raw materials, during the production process or subsequent contamination. Their presence or toxins can cause poisoning of people, sometimes with a lethal outcome. Escherichia coli is one of the most well-known microorganisms in general. This bacterium is a normal resident in the intestines of humans and many animals, but certain strains can cause urinary and intestinal infections and lead to very severe forms of the disease with a possible fatal outcome (WHO, 2015). Also, Escherichia coli is a very resistant bacterium that easily adapts to different conditions in the external environment. It is very often a contaminant of various foodstuffs, and its presence is an indicator of fecal contamination of food. E. coli infections are most associated with poultry meat, minced meat, milk and dairy products, ready meals, as well as in fresh fruits and vegetables and their products. (Buchanan and Doyle 1997). The most common mode of transmission is from human to food and from food to human because healthy people, but also patients, excrete these bacteria and they are regularly found on the hands and clothes of patients. Diseases caused by Escherichia coli are always associated with poor hygienic living conditions, as well as food preparation (Kalaba, 1999). Due to the resistance of bacteria to many antimicrobial drugs, but also the ability of plants to synthesize biologically active substances, the use of preparations of plant origin in the control and suppression of bacteria has become increasingly important. In the European Union, there are several preservatives based on essential oils that are commercially available and contain the essential oil of rosemary, sage and citrus and glycerol (Mendoza et al.1997.). The aim of this study was to determine the influence of essential oils of different plant species used in the food and pharmaceutical industry as well as the antimicrobial activity of various antimicrobial drugs on the growth of Escherichia coli and to determine the type of action.

Material and methods

Material

Commercially available essential oils were used: St. John's wort, mint, cinnamon, clover, rosemary, thyme, garlic, fennel and lemon.

Test microorganisms

As a test microorganism in this study was Escherichia coli, a clinical isolate from the collection of the Laboratory for Microbiology of Animal Food, Feed and Water. The culture of Escherichia coli is sown in the nutrient broth (N- broth) and incubate for 18 hours at 37^0C. Petri plates with the appropriate base (M-H agar) are inoculated with 0.1 ml bacterial suspension with a concentration of 10^5CFU/ml.

Method

A disk diffusion method was used to examine the effect of essential oils on the growth inhibition of Escherichia coli (Kirby-Bauer., 1996). Sterile cylinders with a diameter of 9 mm were placed on the surface of the prepared petri plate, under sterile conditions. 10 µl of the appropriate essential oil was added dropwise into the cylinders by micropipette and the petri dishes were incubated for 24 h at 37°C. The essential oil was used as pure, mixed with 96% alcohol in a ratio of 1:1 and in a ratio of 2:1. As a control, 10µl of 96% alcohol was added to the cylinder. Three repetitions were performed for each essential oil. After incubation for 24 h the results were read by determining the diameter of the inhibition zone and the mean value for each type of essential oil was calculated.

Antimicrobial drugs

An antibiogram method was used to examine the effect of antibiotics on the inhibition of Escherichia coli growth. Antibiotic susceptibility was researched by the disc diffusion technique according to the Bauer-Kirby method (1996), which is in line with the CLSI laboratory recommendations (Clinical and Laboratory Standards Institute - 2013). In this part of the work disc papers for antibiogram with appropriate antibiotic were used ("Liofilchem" s.r.i Italy): gentamicin 10 µg, ciprofloxacin 5 µg, cefotaxime 30µg, ofloxacin 5 µg, chloramphenicol 30 µg, streptomycin 10 µg, ampicillin 10 µg.

Medium

Nutrient broth and Müeller-Hinton agar (Laboratorios CONDA S.A Spain) were used in this study.

Type of action

Also, the type of action of essential oil was determined. To see if essential oil has a bactericidal or bacteriostatic effect, a small piece of agar was taken from the inhibition zones and added to the nutrient broth. Incubation was performed 24 hours at 37ºC. If after incubation there was a turbidity of broth it is considered that the presence of essential oil of this plant is bacteriostatic. If, after incubation the broth remained clear the origin of the essential oil of that plant was bactericidal.

Results and discussion

The results obtained by examining the influence of different essential oils on the growth of *Escherichia coli* are shown in Table 1.

Table 1. The inhibition zone (mm) of etheric oils for the growth of Escherichia coli

Plant	The inhibition zone (mm) Etheric oil		
	Etheric oil	Etheric oil : Alcohol (1:1)	Etheric oil : Alcohol (2:1)
Hypericum perforatum	38.66±0.94	27.66±2.06	24.00±4.24
Menthe piperita	33.33±2.36	30.33±0.47	30.00±0.00
Cinnamomum Zeylonicum	33.66±2.87	32.33±2.05	30.00± 0.00
Eugenija aromatica	29.00±0.82	2.33±1.25	27.00±5.10
Rosmarinus officinalis	27.00±1.41	28.33±0.47	24.33±0.47
Thymus vulgaris	24.66±0.48	24.33±0.94	21.33±1.25
Allium sativum	21.33±1.25	21.00±0.00	25.00±0.00
Foeniculum vulgare	15.66±1.25	13.33±0.47	13.33±0.47
Citrus limon	0	0	0

There is a notable difference in the activities of pure essential oils and alcoholic solutions of essential oils. Pure oil of St. John's wort, mint, cinnamon, cloves and fennel showed the greatest activity according to E. coli. Rosemary oil applied in proportion to oil: alcohol 1:1 showed greater activity than applied pure rosemary oil. Garlic oil applied in a ratio of 2:1 with alcohol showed a stronger effect than pure oil and of the combination oil: alcohol in a ratio of 1:1. Mint and cinnamon essential oil showed strong action in all combinations. The obtained results are in

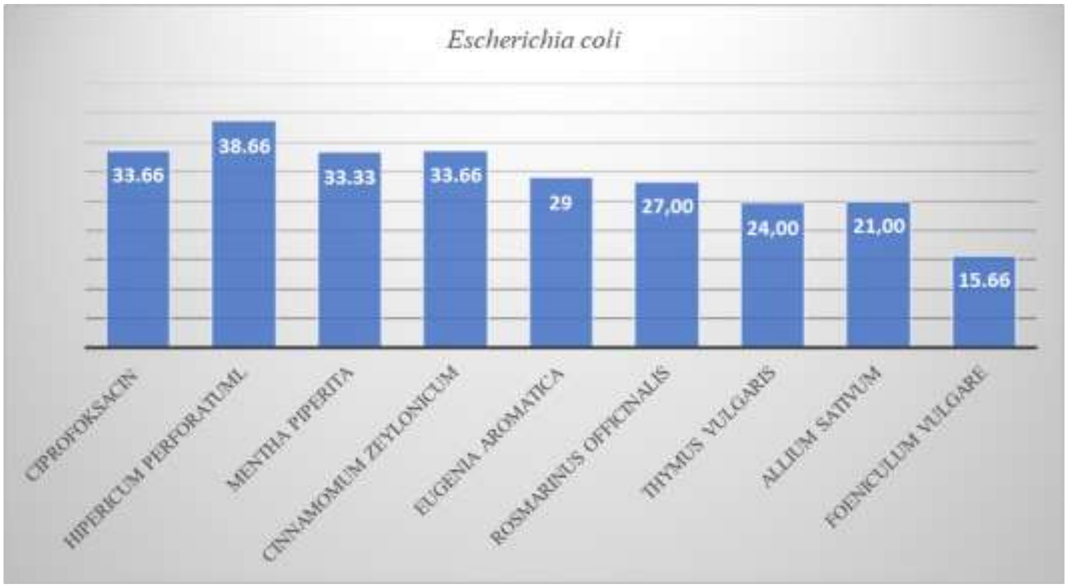
accordance with the works of other authors (Thompson et al. 2013, Burt and Reinders, 2003; Dorman and Deans, 2000; Emiroğlu et al. 2010, Kalaba et al. 2020, Kalaba et al., 2016a Kalaba et al., 2016b, Kalaba et al. 2019, Al-Nabusi I et al.2020, Lopez-Romero et al., 2015).
Lemon essential oils did not show antibacterial activity against E. coli in any combination which is contrary to the research of other researchers (Fisher and Phillips, 2006).
Studies have shown that essential oils in a concentration of 0.1 to 10 µl /g was very effective in preventing food spoilage. Oregano oil at concentrations of 7 to 21 µl/ g has an inhibitory effect on E. coli O157: H7 and leads to a decrease of bacteria in the eggplant compared to untreated control samples (Burt, 2004). Thyme essential oil in a concentration of 0.6% in combination with nisin (500 or 1000 IU / g) has an enhanced antibacterial effect against E. coli O157: H7 in minced beef. In the same study it was found that thyme essential oil in a concentration of 0.9% adversely affects the organoleptic properties of meat (Solomakos et al., 2008). Table 2 shows the zones of E. coli growth inhibition with antimicrobial drugs.

Table 2. Zones of growth inhibition of Escherichia coli achieved with different antibiotics.

Antimicrobial drugs	Zones of inhibition
Gentamicin	24,66±0,48
Ciprofloxacin	33,66±1,25
Cefotaxime	27,33±0,47
Ofloxacin	30,66±1,70
Chloramphenicol	30,00±0,81
Streptomycin	16,33±0,47
Ampicillin	0

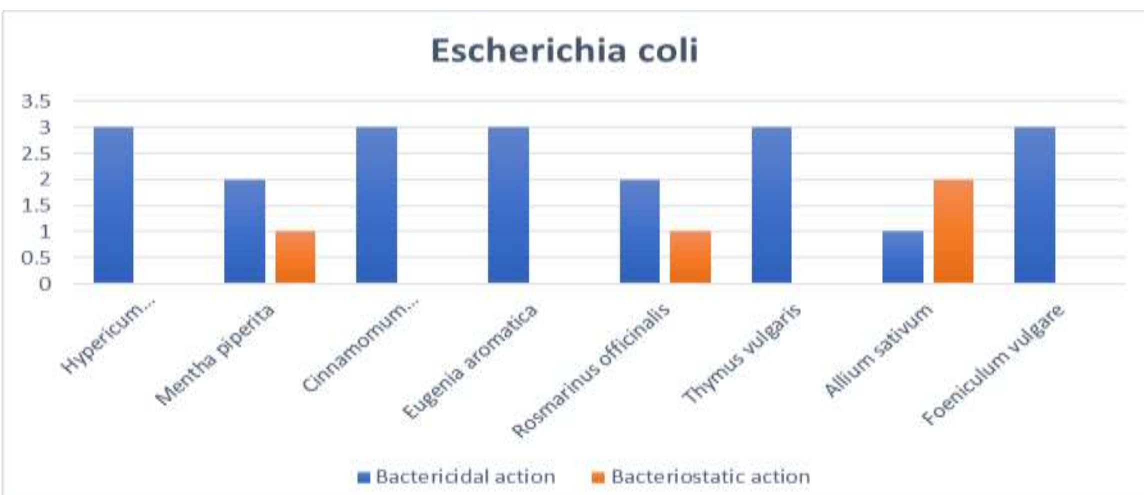
* Displayed values are given in mm and represent the mean value of inhibition zones for three measurements.

If the maximum inhibition zones of individual essential oils are presented in pure form and compared with the inhibition zone of the most effective antibiotic ciprofloxacin then the results are as in Picture 1., St. John's wort has more pronounced antibacterial activity against E. coli than the most effective antibiotic. Equally, it is noticeable that cinnamon has the same effect, and mint has the same effect as ciprofloxacin. Due to such characteristics, the essential oils of St. John's wort, cinnamon and mint stand out in relation to the other tested ones and are recommended for further research. The results agree with the results of other researchers who studied the effects of antimicrobial drugs on the growth of E. coli (Abu-Darwish et al. 2012, Sagdic O et al., 2009; Küçükbay et al., 2014; Behbahani et al., 2013).



Picture 1. Comparison of maximum zones of inhibition of essential oils and antibiotics ciprofloxacin in mm.

The antibacterial activity of essential oils and their components can vary from partial to complete inhibition of bacterial growth, so that essential oils exhibit bacteriostatic or bactericidal activity. (Abhay et al., 2017). When examining the type of effect of essential oils on the inhibition of E. coli growth it was found that all tested oils showed a bactericidal effect, except for mint and rosemary oils in one and garlic in two replicates when these oils had a bacteriostatic effect. Picture 2 shows the type of action of essential oils on Escherichia coli.



Picture 2. Bactericidal and bacteriostatic action of essential oils on Escherichia coli.

As can be seen from the picture all tested oils had a bactericidal effect, except for mint and rosemary oils in one and garlic in two replicates when they had a bacteriostatic effect. Based on the obtained results, essential oils of St. John's wort, mint, cinnamon, cloves, rosemary, thyme, garlic and fennel showed a good inhibitory effect on the growth of Escherichia coli and can be recommended for use in the food industry to prevent the development of Escherichia coli and disease which it can cause. Also, it can be concluded that a careful selection of essential oils in food production is required, which will also correspond to the organoleptic properties of the food and its composition (Gutierrez et al., 2009; Fabian et al. 2007; Beatriz Nunes Silva et al., 2020).

Conclusion

All tested essential oils, except lemon essential oil, show antimicrobial activity against Escherichia coli. A difference in the antimicrobial activity of essential oils was observed depending on the type of plant and the amount of oil. The essential oils of St. John's wort, mint, cinnamon and cloves showed the greatest activity, while the essential oils of rosemary, thyme, garlic and fennel showed slightly weaker inhibition, depending on whether they were used as pure oils or in combination with alcohol.

All tested oils had a bactericidal effect, except for peppermint and rosemary oils in one and garlic in two replicates when they had a bacteriostatic effect.

Individually or by combining vegetable essential oils, an effective mixture for inactivating Escherichia coli can be provided thus achieving adequate storage and preservation of food. Essential oils are a very topical alternative to synthetic antimicrobial drugs. They are increasingly used for industrial purposes as natural preservatives and preservatives in the food, cosmetics industry and as active ingredients in medical preparations. However, both their possible toxicity and safe doses for use in food, cosmetic or pharmaceutical products should be considered and therefore detailed studies on the toxicity, stability and safety of the tested essential oils are required.

References

Abhay K. Pandey, Pradeep Kumar, Pooja Singh, Nijendra N. Tripathi,Vivek K. Bajpai (2017); Essential Oils: Sources of Antimicrobials and Food Preservatives Front Microbiol. 2016; 7: 2161.Published online 2017 Jan 16. doi: 10.3389/fmicb.2016.02161

Abu-Darwish MS, Al-Ramamneh EAD, Kyslychenko VS, Karpiuk UV. (2012):The antimicrobial activity of essential oils and extracts of some medicinal plants grown in Ash-shoubak region – South of Jordan. Pak J Pharm Sci 2012; 25(1):239-246.

AL-Nabulsi, A, T. Osaili, A. Olaimat, W. Almasri M. Al-Holy, Z. Jaradat, M. Ayyash ,S. Awaishe, R. Holley (2020): Inhibitory effect of thyme and cinnamon essential oils against *E. coli* O157:H7 in Tahini, Food Sci. Technol vol. 40 no.4

Bauer K, Garbe D. (2002): Common Fragrance and Flavor Materials. Preparation, Properties and Uses. VCH Verlagsgesellschaft; 1985. 4. Packiyasothy EV, Kyle S. Antimicrobial properties of some herb essential oils. Food Aust 2002; 54 (9): 384–387.

Bakkali F, Averbeck S, Averbeck D, Idaomar M(2008):Biological effects of essential oils - A review. Food and Chemical Toxicology 46:446-475.

Behbahani MH, Ghasemi Y, Khoshnoud MJ, Faridi P, Moradli G, Najafabady NM. (2013):Volatile oil composition and antimicrobial activity of two Thymus species. Phcog J; 5:77-79.

Beatriz Nunes Silva, Vasco CadavezJosé António Teixeira, Ursula Gonzales-Barron (2020):, Effects of Essential Oils on *Escherichia coli* Inactivation in Cheese as Described by Meta-Regression Modelling *Foods 9*(6), 716; https://doi.org/10.3390/foods9060716

Buchanan, R.L. i Doyle, M.P.(1997): Foodborne disease significance of *Escherichia Coli* O157:H7 and other enterohemorrhagic *E.coli*, *Food Technology* 51, 69-76.

Burt S (2004): Essential oils: their antibacterial properties and potential applications in foods - a review. International Journal of Food Microbiology 94:223-253,

Burt, S.A., i Reinders, R.D.(2003): Antibacterial activity of selected plant essential Oils against *Escherichia coli*O157:H7, Letters in Applied Microbiology, 36, 162-167

CLSI M100-S23 Performance Standards for Antimicrobial SusceptibilityTesting, 2013.

Dorman, H:J:D. i Deans, S.G.(2000):Antimicrobila agents from plants antibacterial activity of plant volatile oils, Journal of applied microbiology 88, 308-316..

Emiroğlu KZ, Yemiş GP, Coşkun BK, Candoğan K. (2010): Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Science 86:283-288

Fabian Dusan, Marián Sabol, Katarína Domaracká, Dobroslava Bujnáková (2006): Essential oils--their antimicrobial activity against *Escherichia coli* and effect on intestinal cell viability; Toxicol In Vitro 2007 Apr;21(3):534-45.doi: 10.1016/j.tiv.2006.06.012. Epub 2006 Jul 14.

Fisher K , ds (2006):The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus in vitro and in food systems, J Appl Microbiol.;101(6):1232-40. doi: 10.1111/j.1365-2672.2006. 03035.x

Feizi, A-, Bijanzad,P.,Kabali, K.(2013): Effects of thyme volatile oils on performance of broiler chiockens.European Journal of Experimental Biology, 3:250-254

Fortino Solorzano- Santos, Maria Guadalupe Miranda- Novales (2012): Esential oils from aromatic herbs as antimicrobial agents , Food biotechnology-Plant biotechnology,Vol. 23 Issue 2; 136-141

Kalaba V., Sladojević Ž., Stanojević, Lj. (2019). Antibacterial activity of essential oil *Mentha piperita* L, VI International Congress "Engineering, Environment and Materials in Processing Industry", Jahorina, 2019 ;660-666 , *https://eem.tfzv.ues.rs.ba/*

Kalaba V., Gojković, V., Marjanović Balaban, Ž., Kalaba, D. (2016a). Timus vulgaris- Antimicrobial drug from nature, Book of Proceedings 2, VII International Scientific Agriculture Symposium, Agrofor International Journal,1456-1463

Kalaba Vesna, Dragana Kalaba, Milka Stijepić (2016b): Investigation of antimicrobial activity of essential oils *Rosmarinus Officinalis* и *Alium sativum* L, XI Conference of Chemists, Technologists and Ecologists of the Republic of Srpska , 381-388 Teslić

Kalaba V., Marjanović-Balaban Ž., Kalaba D., Lazić D., Gojković Cvjetković V. (2020): Antibacterial activity of essential oil of Juniperus communis L. Qualiti of Life 11 (1-2):18-24 DOI: 10.7251/QOL2001018K

Kalaba V. (1999): Comparative research of biochemical properties of Escherichia coli isolated from foodstuffs of animal origin, Faculty of Technology, University of Banja Luka,

Kirby-Bauer A (1996): Antimicrobial sensitivity testing by agar diffusion method J.Clin Pathol., 44; 493

Küçükbay FZ, Kuyumcu E, Çelen S, Azaz AD, Arabaci T. (2014): Chemical Composition of the Essential Oils of Three Thymus Taxa from Turkey with Antimicrobial and Antioxidant Activities. Rec Nat Prod; 8(2):110-120.

Lopez-RomeroJ.C., H. González-Ríos, Anabela Borges, M. Simoes (2015): Antibacterial Effects and Mode of Action of Selected Essential Oils Components against *Escherichia* *coli* and *Staphylococcus aureus* Volume, 2015. Evidence-Based Complementary and Alternative Medicine / | https://doi.org/10.1155/2015/795435

Mendoza-Yepes MJ, Sanchez-Hidalg LE, Maertens G, Marin-Iniesta F. (1997):Inhibition of Listeria monocytogenes and other bacteria by a plant essential oil (DMC) en Spanish soft cheese. J Food Saf.;47– 55

Popović G., Đurđević Milošević D. (2008): Influence of essential oils on the growth of Escherichia coli. Scientific-professional conference "Ecological Truth" with international participation, Sokobanja, Proceedings, pp 612-615

Radulović N.S., Blagojević P.D., Stojanović-Radić Z.Z., Stojanović N.M. (2013): Antimicrobial plant metabolites: Structural diversity and mechanism of action. Curr Med Chem; 20: 932-952

Sagdic O, Ozkan G, Aksoy A, Yetim H.(2009): Bioactivities of essential oil and extract of Thymus argaeus, Turkish endemic wild thyme. J Sci Food Agric; 89:791-795.

Solomakos N, Govaris A, Koidis P, Botsoglou N. (2008): The antimicrobial effect of thyme essential oil, nisin and their combination against Escherichia coli O157:H7 in minced beef during refrigerated storage. Meat Science 80:159-166

Thompson Aiysha, Dilruba Meah, Nadia Ahmed,Rebecca Conniff-Jenkins, Emma Chileshe, Chris O Phillips, Tim C Claypole, Dan W Forman Paula E Row (2013):Comparison of the antibacterial activity of essential oils and extracts of medicinal and culinary herbs to investigate potential new treatments for irritable bowel syndrome; BMC Complementary Medicine and Therapies 13, Article number: 338

Van de Braak SAAJ, Leijten GCJJ. (1999): Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries;, str. 116.

World Health Organisation (WHO)(2015): Antimicrobial resistance, Global Report on Surveillance. Available at:http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1. Acessed February 25, 2015.

Wilkins, K.M, Board, R. G.(1989): Natural antimicrobial systems. In: GouldG. W. ed. Mechanisms of action of food preservation procedures, London: Elsevie 2 chapter

GEOGRAPHICAL ORIGIN OF ALIEN INVASIVE FISH SPECIES IN SERBIA

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Abstract

Aquatic ecosystems around the world have been dramatically altered as fish are shifted around, whether for commercial fishing stock or the aquarium trade. Hydroecological conditions in Serbia have caused a high diversity of its ichthyofauna including about 94 permanent or occasional fish species recorded in Serbian watercourses. However, among them are numerous non-native fish species, which often show an invasive character. According to one of the lists of invasive species in Serbia, 29 species of fish are marked as invasive. In order to prevent further introductions of potentially invasive alien fish species, it is of great importance to know the ecology, but also the biogeography of those species that are currently considered invasive in Serbia. In general, among invasive fish species in Serbia, those originating from the Old World are more common than those coming from the New World. Palearctic origin has 14 invasive fish species, of which 8 have a primary geographic range limited to Europe, while 6 of them are known to have a primary geographic range in both Europe and Asia. There are 6 species of Nearctic origin, 5 species of Sino-Tibetan origin and 1 species of Neotropical origin, while the primary range of some species occupies more than one biogeographical region. However, in contrast to these species with very large primary geographic ranges, some of the species that have been marked as invasive in Serbia are even endemic to the Balkan or Apennine Peninsula, while some are classified as vulnerable according to the IUCN categorization.

Keywords: *Geographical origin, Invasive fish species, Serbia.*

Introduction

Freshwater organisms have lower dispersal abilities than terrestrial vertebrates and are geographically isolated in drainage basins that usually flow to the oceans (Leroy, *et al.* 2019). Fishes are subject to several biogeographical constraints that are unique to the aquatic world (Olden *et al.*, 2010). Their ability to move in response to environmental change is constrained by the dendritic arrangement of riverine ecosystems, as well as a variety of physiographic barriers (Fausch *et al.*, 2002). Drainage basins can be observed as "island-like" systems for strictly freshwater organisms (Dias *et al.*, 2014; Hugueny *et al.*, 2010; Rahel, 2007; Tedesco *et al.*, 2012). As a result of this, most freshwater fishes occupy only a fraction of the localities where they might otherwise thrive (Darlington, 1948).
Leroy *et al.* (2019) divided the world of freshwater fishes into two biogeographical supercontinental regions: the New World (America) and the Old World (Eurasia, Africa and Australia). At the second level, they found six major regions (named after Morrone, 2015), that are spatially nested within two supercontinental regions. The Old World supercontitental region include: Ethiopian region (covering the entire African continent and including areas north from the Sahara and a few basins in the Arabic Peninsula), Sino-Oriental region (including south-eastern Asia from India to Borneo, most of China and Mongolia, Korea and Japan), Palearctic

region (covering Europe, Central Asia and Siberia), Australian region (covering Australia, Tasmania and Papua – New Guinea). Madagascan has been identified as a distinct minor cluster of the Old World, due to the large number of endemic species and genera. The New World supercontitental region include: Neotropical region (covering the whole of South America and Mesoamerica up to Southern Mexico) and Nearctic region (covering North America and northern Mexico).

The Balkan Peninsula is considered one of the hotspots of European freshwater fish diversity (Oikonomou *et al.*, 2014). Hydroecological conditions in Serbia have caused a high diversity of its ichthyofauna, including 94 permanent or occasional fish species of 26 families recorded in Serbian watercourses (Lenhardt *et al.*, 2011). Area of Serbia belongs to the Palearctic region (Leroy, *et al.* 2019). The largest part of Serbia belongs to the Pontic province (Black Sea basin), while only a small part of it (western Kosovo and Metohija; Oikonomou *et al.*, 2014).

Increasing colonisation by alien organisms was observed in Serbian waters during the last few decades (Rat *et al.*, 2016). Earlier introductions of allochthonous species into Serbian waters were made primarily in order to increase ichthyoproduction for purposes of weed control and sport fishing, and also incidentally (Maletin *et al.*, 1997). The main route of fish introductions into Serbia was via the Danube and Tisza rivers from Romania and Hungary (Lenhardt *et al.*, 2011). According to ESENIAS country report (Rat *et al.*, 2016), 29 species of fish are marked as invasive in Serbia, while according to Lenhardt *et al.* (2011), there are 22 invasive fish species (IFSs) in Serbia.

Biological invasions are considered to be the second most important cause of species extinction after habitat destruction (Casal, 2006). Fish invasions are so widespread that they are now considered a significant component of global environmental change, and are recognized as a leading threat to native biodiversity (Dudgeon *et al.*, 2006). By dissolving physical barriers to movement and connecting formerly isolated regions of the world, human-mediated species introductions have dramatically reshuffled the present-day biogeography of freshwater fishes (Olden *et al.*, 2008). Data on the negative effect of non-native fish species on native species are reported in many papers dealing with allochthonous species in Serbia, but a detailed study is still lacking (Lenhardt *et al.*, 2011).

In order to prevent further introductions of potentially invasive alien fish species it is of great importance to know their ecology, but also their biogeography. The objective of the present paper is to provide an overview of geographical origin of those species that are currently considered invasive in Serbia.

Material and Methods

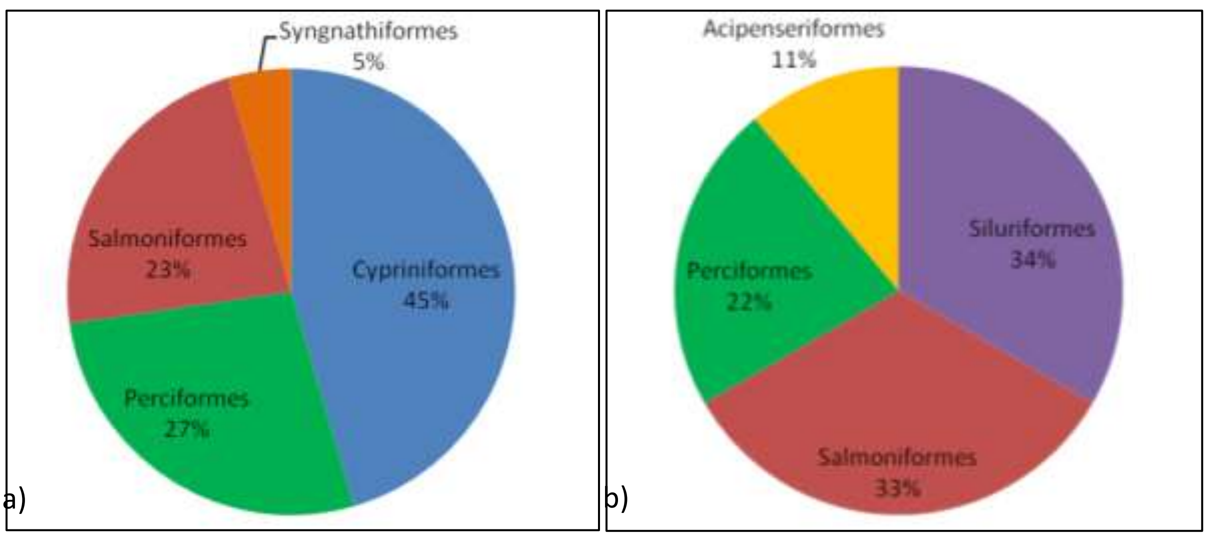
For the purposes of this paper, a list of IFSs according to Rat *et al.* (2016) was used. This list is part of Country report for Serbia, published by East and South European Network for Invasive Alien Species (ESENIAS). According to the report, 29 IFS can be found in Serbia. Rat *et al*. (2016) actually list 30 IFSs in Serbia. However, in addition to the species *Ponticola kessleri* (Gunther, 1861), they also list *Neogobius kessleri* (Gunther, 1861), which is the synonym of *Ponticola kessleri* (Gunther, 1861) according to FishBase (2021). For that reason, for the purposes of this paper, 29 instead of 30 IFSs were taken into account.

IFSs in Serbia are associated with data on their geographical origin, ie. with data on geographical area occupied by their primary range, according to FishBase (2021). Based on these data, it was determined to which biogeographical supercontinental region and to which biogeographical major region (according to Leroy *et al.* 2019) each of the species belongs. In this way, groups of invasive species in Serbia were created based on their geographical origin. For each group, a percentage share in the total number of IFSs was calculated.

Results and Discussion

All IFSs in Serbia can be classified into two large groups: those that come from the Old World (Europe, Asia, Africa) and those that come from the New World (North and South America). As many as 76% of invasive fish in Serbia originate from the Old World, while 31% originate from the New World. The total percentages are greater than 100, because the two species have a primary range that occupies parts of both the Old and New World.

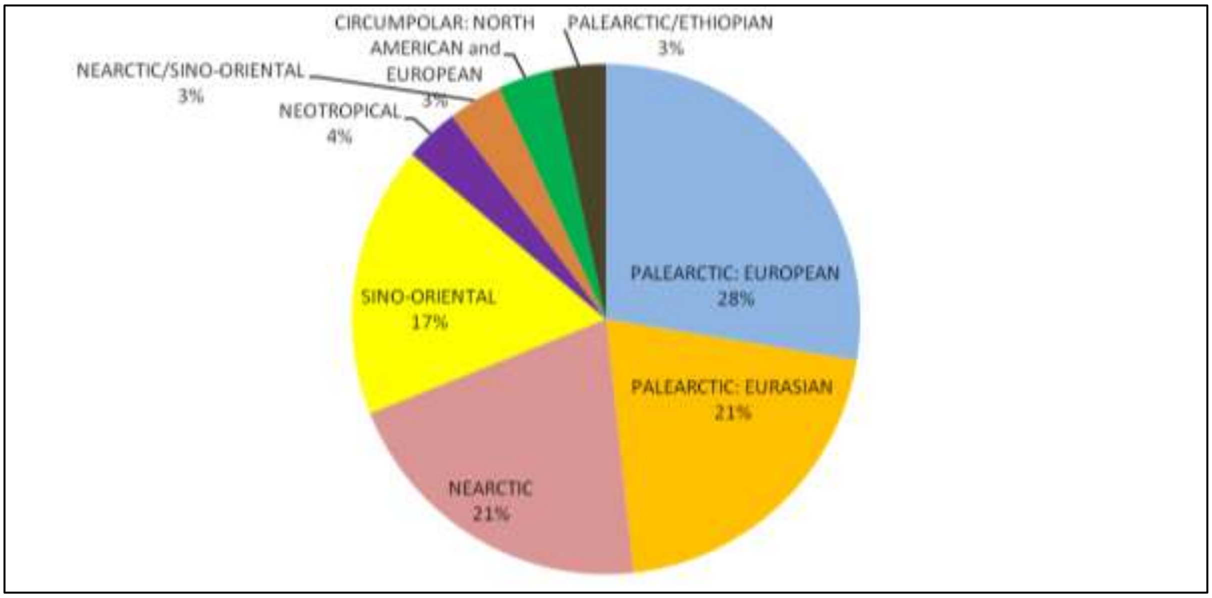
IFSs in Serbia belong to the following orders: Cypriniformes (10 species), Perciformes (8), Salmoniformes (6), Siluriformes (3), Acipenseriformes (1) and Syngnathiformes (1). Fish species from the orders Perciformes and Salmoniformes are represented in both groups – in the group of fish of the Old World, and in the group of fish of the New World. In contrast, IFSs from the orders Cypriniformes and Syngnathiformes are characteristic only for the group of fish from the Old World, while representatives of the orders Siluriformes and Acipenseriformes are characteristic only for the group of fish from the New World (Graphic 1).



Graphic 1. Percentage representation of representatives of different fish orders among IFSs in Serbia: a) fishes originating from the Old World; b) fishes originating from the New World

A large number of species from the order Cypriniformes among invasive fish in Serbia is expected, given the following facts: (1) Cypriniformes is the most diverse order of freshwater fishes (Tan and Ambruster, 2018); (2) cypriniforms occur throughout North America, Africa, Europe and Asia; (3) cypriniforms are dominant members of a range of freshwater habitats (Nelson, 2006); (4) many cypriniforms are important food and recreational fishes and they are popular in the global ornamental pet trade (Tan and Ambruster, 2018). Although members of this order can also be found in North America, all invasive cypriniforms are of Palearctic origin. The reason for this may be the following: (1) a higher degree of deliberate introduction of fish from Asia to Serbia, and/or (2) a greater diversity of Asian cyprinids, given that cypriniforms are

thought to have originated in South-east Asia, where the most diversity of this group is found today (Briggs, 2005).
Depending on the biogeographical region (according to Leroy *et al.* 2019) in which their primary range is located, IFSs in Serbia can be divided into Palearctic, Nearctic, Sino-Oriental and Neotropical. Palearctic species can be further divided into those whose primary range is limited to Europe (European) and those whose primary range occupies both Europe and Asia (Eurasian). Among the IFSs in Serbia are also those whose primary range includes more than one biogeographical region: Nearctic and Sino-Oriental, Nearctic and Palearctic (Circumpolar: North American and Euoropean), Palearctic and Ethiopian (Graphic 2).



Graphic 2. Percentage representation of IFSs in Serbia according to their geographical origin

Palearctic species are predominant among IFSs in Serbia. As many as 14 IFSs in Serbia (49% of total number) have a primary range that is entirely located in the Palearctic biogeographical region. Among them are 8 European (28% of total number) and 6 Eurasian (21 % of total number).
Palearctic European IFSs in Serbia are: *Salmo letnica* (S. Karaman, 1924), *Salmo macedonicus* (S. Karaman, 1924), *Proterorhinus semilunaris* (Heckel, 1837), *Alburnus albidus* (Costa, 1838), *Alburnus scoranza* (Heckel & Kner, 1858), *Pachychilon macedonicum* (Steindachner, 1892), *Rutilus basak* (Heckel, 1843), *Scardinius knezevici* (Biando & Kottelat, 2005). It is interesting that among these species there are as many as 6 whose primary range is limited to the Balkan Peninsula (rivers and lakes of Albania, Croatia, Greece, Montenegro and North Macedonia), and 1 whose primary range is limited to the Apennine Peninsula (FishBase 2021). This species, *Alburnus albidus*, is even on The IUCN Red List of Threatened Species (2021), in the category "Vulnerable" at global level, due to the next reasons: decline in the number of mature individuals, small area of occupancy, ongoing range reduction, severe fragmentation of population and decline in habitat quality.
Palearctic Eurasian IFSs in Serbia are: *Coregonus peled* (Gmelin, 1788), *Neogobius fluviatilis* (Pallas, 1814), *Neogobius gymnotrachelus* (Kessler, 1857), *Neogobius melanostomus* (Pallas, 1814), *Ponticola kessleri* (Gunther, 1861), *Carassius gibelio* (Bloch, 1783). These species

originate mainly from rivers and lakes of Sea of Azov, Black Sea and Caspian basins, whereas *Carassius gibelio* is usually considered as native from central Europe to Siberia (FishBase 2021). Some of these species have expanded their range due to the construction of the Rhine-Main-Danube Canal (Van Kessel *et al.*, 2009).

Nearctic IFSs in Serbia are: *Ameiurus nebulosus* (Le Sueur, 1819), *Ameiurus melas* (Rafinesque, 1820), *Polyodon spathula* (Walbaum, 1792), *Salvelinus fontinalis* (Mitchill, 1815), *Lepomis gibbosus* (Linnaeus, 1758), *Micropterus salmoides* (Lacepede, 1802). These species originate mainly from Great Lakes, Mississippi River basins, Gulf Slope drainages etc. All of the listed species are known as invasive worldwide and at least one country reports adverse ecological impact after their introduction (FishBase 2021). Despite that, *Polyodon spathula* is on The IUCN Red List of Threatened Species (2021), in the category "Vulnerable" at global level, due to exploitation and the effects of introduced taxa, pollutants, competitors or parasites.

Sino-Oriental IFSs in Serbia are: *Ctenopharyngodon idella* (Valenciennes, 1844), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Hypophthalmichthys nobilis* (Richardson, 1845), *Pseudorasbora parva* (Temminck and Schlegel, 1842). These species originate from the northern part of the Sino-Oriental biogeographical region, mainly from the Amur River system or some other Pacific dainages of East Asia. All of them are known as invasive worldwide and at least one country reports adverse ecological impact after their introduction (FishBase 2021). These species were mostly deliberately introduced into the waters of Serbia, in order to "improve" the fish stock in fishponds.

Neotropical IFS in Serbia is *Pterygoplichthys pardalis* (Castellnnau, 1855). This South American species primarily inhabits rivers of lower, middle and upper Amazon River basin, but it is introduced to countries outside its native range (FishBase 2021). There is evidence that in its introduced range this species disrupt aquatic food chains, decrease the abundance of native aquatic species and degrade aquatic plants or banks of waterbodies through burrowing and tunneling (Hoover *et al.*, 2004).

Nearctic/Sino-Oriental IFS in Serbia is *Oncorhynchus mykiss* (Walbaum, 1792), a marine, brackish and freshwater fish. This species is native for two biogeographical regions, since its primary range includes the Pacific slope of North America (from Alaska to California), but also the eastern Pacific coast (Kamchatka, Commander Islands, Sea of Okhotsk). Several countries reported adverse ecological impact after introduction of this species (FishBase 2021).

Circumpolar (North American and European) IFS in Serbia is *Salvelinus alpinus* (Linnaeus, 1758). This species inhabits sea water, freshwater and brackish water. It is native in Iceland, Scandinavia, northern Russia, but also in Alaska, Canada, Greenland and USA, in Atlantic, Arctic and Pacific coastal areas.

Palearctic/Ethiopian IFS in Serbia is *Syngnathus abaster* (A. Risso, 1826). Range of this marine, brackish and freshwater fish species occupies Eastern Atlantic and the Mediterranean and Black seas, but also the coasts of North Africa.

Conclusions

In order to prevent further introductions of potentially invasive alien fish species it is of great importance to know their ecology, but also their biogeography. IFSs in Serbia originate from all biogeographical regions exept Australian and Madagascan. Despite being considered invasive in some parts of the world, in some others some of these species are considered threatened.

Therefore, it is not enough just to have a list of species that are considered invasive, but it is necessary to investigate the degree of their invasiveness.

References

Briggs J.C. (2005). The biogeography of otophysan fishes (Ostariophysi: Otophysi): a new appraisal. Journal of Biogeography 32, 287–294.

Casal C.M.V. (2006). Global documentation of fish introductions: the growing crisis and recommendations for action. Biological Invasions 8, 3–11.

Darlington P.J. (1948). The geographical distribution of coldblooded vertebrates. Quarterly Review of Biology 23, 1–26.

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., Naiman R.J., Prieur-Richard A.-H., Soto D., Stiassny M.L.J., Sullivan C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81, 163–182.

Dias M.S., Oberdorff T., Hugueny B., Leprieur F., Jézéquel C., Cornu J. ‐ F., Tedesco P.A. (2014). Global imprint of historical connectivity on freshwater fish biodiversity. Ecology Letters 17(9), 1130–1140.

Fausch K.D., Torgersen C.E., Baxter C.V., Li H.W. (2002). Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. BioScience 52, 483–498.

Hoover J., Killgore K., Cofrancesco A. (2004). Suckermouth Catfishes: Threats to Aquatic Ecosystems of the United States? Aquatic Nuisance Species Research Program Bulletin 4(1), 1–9.

Hugueny B., Oberdorff T., Tedesco P.A. (2010). Community ecology of river fishes: A large ‐ scale perspective. In K. B. Gido & D. A. Jackson (eds.), "Community ecology of stream fishes: Concepts, approaches, and techniques", American Fisheries Society Symposium 73, Bethesda MD; pp. 29–62.

Lenhardt M., Marković G., Hegediš A., Maletin S., Ćirković M., Marković Z. (2011). Non-native and translocated fish species in Serbia and their impact on the native ichthyofauna. Reviews in Fish Biology and Fisheries 21, 407–421.

Leroy B., Dias M.S., Giraud E., Hugueny B., Jézéquel C., Leprieur F., Oberdorff T., Tedesco P.A. (2019). Global biogeographical regions of freshwater fish species. Journal of Biogeography DOI: 10.1111/jbi.13674

Maletin S., Đukić N., Miljanović B., Ivanc B. (1997). Status of allochthonous ichthyofauna of Pannonian basin in Yugoslavia. Ekologija 32, 87–98.

Nelson J.S. (2006). Fishes of the World, 4th edition. John Wiley & Sons, New York.

Oikonomou A., Leprieur F., Leonardos I.D. (2014).Biogeography of freshwater fishes of the Balkan Peninsula. Hydrobiologia DOI 10.1007/s10750-014-1930-5

Olden J.D., Kennard M.J., Leprieur F., Tedesco P.A., Winemiller K.O., Berthou E.A. (2010). Conservation biogeography of freshwater fishes: recent progress and future challenges. Diversity and Distributions 16, 496–513.

Olden J.D., Kennard M.J., Pusey, B.J. (2008). Species invasions and the changing biogeography of Australian freshwater fishes. Global Ecology and Biogeography 17, 25–37.

Rahel F.J. (2007). Biogeographic barriers, connectivity and homogenization of freshwater faunas: It's a small world after all. Freshwater Biology 52(4), 696–710.

Rat M., Simonović P., Glavendekić M., Paunović M., Stojanović V., Karaman M., Radišić D., Anačkov G. (2016). Overview of the invasive alien species in Serbia. In: ESENIAS Scientific Reports 1. State of the Art of Alien Species in South-Eastern Europe. Rat M., Trichkova T., Scalera R., Tomov R., Uludag A. University of Novi Sad, Novi Sad, Serbia, IBER-BAS Bulgaria, ESENIAS pp. 92–114.

Tedesco P.A., Leprieur F., Hugueny B., Brosse S., Dürr H.H., Beauchard O., Oberdorff T. (2012). Patterns and processes of global riverine fish endemism. Global Ecology and Biogeography 21(10), 977–987.

Tan M., Ambruster J.W. (2018). Phylogenetic classification of extant genera of fishes of the order Cypriniformes. Zootaxa 4476(1), 6–39.

Van Kessel N., Dorenbosch M., Spikmans F. (2009). First record of Pontian monkey goby, *Neogobius fluviatilis* (Pallas, 1814), in the Dutch Rhine. Aquatic Invasions 4(2), 421–424.

https://www.iucnredlist.org/ Accessed on 10/07/2021.

https://www.fishbase.de/search.php Accessed on 10/07/2021.

DENSITY AND HABITAT PREFERENCE OF AN INVASIVE SPECIES (*Diadema setosum*, Leske, 1778) IN THE MEDITERRANEAN SEA

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Abstract

Sea urchins are important members of benthic communities by impacting algal production and therefore the other members of the communities. A member of these organisms, *Diadema setosum* Leske, 1778, was introduced to Mediterranean Sea in 2006 and it has extended its distribution to various localities. However, previous studies on this invasive species have only focused on its occurrences in different parts of the Mediterranean Sea. Here, we conducted an observational study on the density of this sea urchin at six sites along the Turkish coast of Aegean Sea differing in the habitat types. Additionally, we determined the density of a native sea urchin, *Arbacia lixula* Linnaeus 1758, at the same sites to reveal the potential interactions between these two species. We found that *D. setosum* reached a notable density on the Turkish coast of Aegean Sea and this density was higher at the sites with hard bottoms. We further found an inverse relationship between the densities of *D. setosum* and of *A. lixula*. Overall, this study emphasizes the need of an urgent management/conservation plan since *D. setosum* has already reached the previously reported threshold density at which it is harmful to benthic communities in the Mediterranean Sea.

Keywords: *Arbacia lixula, competition, Diadema setosum, Mediterranean Sea, sea urchin.*

Introduction

Sea urchins are important members of benthic communities by exerting a top-down influence on macroalgae (Aronson & Precht, 2000). Their herbivorous diet with specific grazing preferences (Coppard & Campbell, 2005; Luza & Malay, 2019) directly impacts the macroalgal production and thus the benthic community structure (Qiu *et al*., 2014). These impacts may vary with bioerosion (Qiu *et al*. 2014), competitive exclusion of other benthic organisms (*reviewed in* Muthiga & McClanahan 2007), reductions in algal growth (Ishikawa *et al*., 2016) and the reductions in species composition that depends on algal production. Furthermore, they can favor coastal fish by providing shelter (Bilecenoğlu *et al*., 2019).

Diadema setosum Leske 1778, is an Indo-Pacific sea urchin (Muthiga & McClanahan, 2007) that has introduced to Mediterranean Sea. The first individuals of *D. setosum* were reported in 2006 from Kaş Peninsula (Yokes & Galil, 2006). Since the first introduction of the species, it has extended its distribution to the coasts of Lebanon (Nader & Indary, 2011) and Israel (Bronstein & Kroh, 2018), to Rhodes (Kondylatos & Corsini-Foka, 2015) and Simi (Galanos & Kritikos, 2019) islands, to the southeastern coastline of Turkey (Turan *et al*., 2011; Yapıcı *et al*., 2014), and to the Sea of Marmara (Artüz & Artüz, 2019). *D. setosum* has two genetic clades and a recently published distribution model predicted that these clades have different expected range of expansions due to habitat suitability in the Mediterranean Sea (Bronstein *et al*., 2017). However, *D. setosum* clade b has exceeded its predicted range to the Sea of Marmara (Artüz & Artüz, 2019), suggesting that this species can be harmful for a larger area in the Mediterranean Sea than it has been predicted.

Previous studies conducted on *D. setosum* in the Mediterranean Sea has only reported new localities where this species has spread since its first introduction in 2006. However, understanding the impacts of the introduced species and development of useful conservation and management plans to control for the impacts of introduced species require further knowledge such as density (Yokomizo *et al*., 2009) and the interactions with native species (Sax et al., 2005 and chapters therein). Therefore, here we investigate the abundance of this introduced species and its interaction with the native sea urchin (*Arbacia lixula*); they both utilize similar hard substrate habitats; across sites that differ in substrate type to understand whether *D. setosum* follows similar distribution pattern with its native habitats in the Mediterranean Sea.

Materials and Methods

We examined the density of the invasive, *Diadema setosum* and native, *Arbacia lixula* sea urchins at six coastal sites between 9th and 12th October 2019 on the Turkish Coast (Fig. 1). Similar to this, studies conducted on *D. setosum* in its native range showed its strong hard bottom preference (*reviewed in* Muthiga & McClanahan, 2007). Therefore, we selected our study sites based on differences in the bottom types, which have either soft bottom (sand) or hard bottom (rock) in order to understand if the *D. setosum* follows the same habitat preference in its invasive region (e.g., Mediterranean Sea) with the native one. We conducted our density observations at three sites with soft bottom and three sites with hard bottom. We scanned an area of approximately 100 m^2 at each site. We counted individuals of the native and the invasive sea urchins from coast to 15 m depth by SCUBA diving.

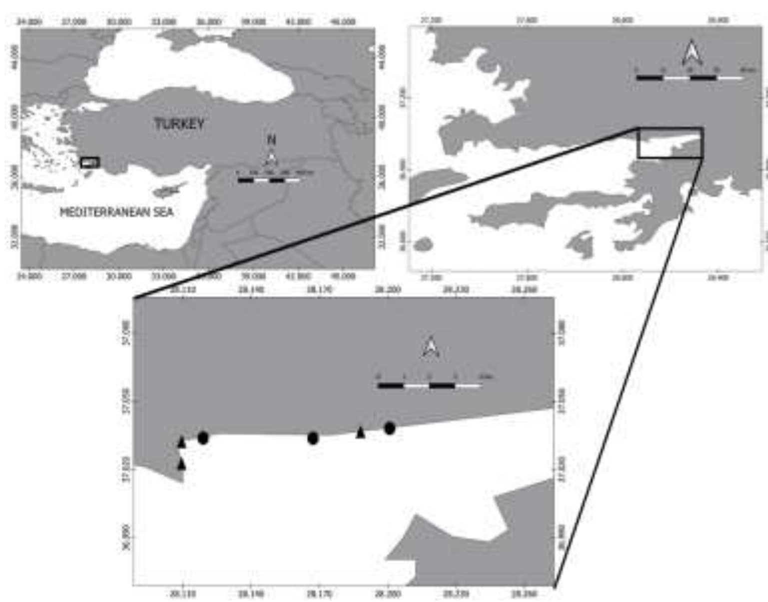

Figure 1. Study sites. In the map, circles indicate the sampling sites with hard bottom and triangles represent the sampling sites with soft bottom. The map was created with QGIS v3.10 software.

To understand if the densities of the invasive and of the native sea urchins varied between sites with different bottom types, we conducted two separate Linear Mixed-Effects Models (GLMMs) with a Poisson distribution. The bottom type (soft vs hard) was treated as categorical fixed factor. To control for spatial differences, site was added as random factor to these analyses. We further applied another GLMM with a Poisson distribution to assess whether the densities of the native sea urchin, *Arbacia lixula*, were related to the densities of the invasive sea urchin, *D. setosum*. We treated the density of *D. setosum* and the bottom types as fixed factors. We, again, treated the site as a random factor to control for the spatial variation between sites. All statistical analyses were conducted using the statistical software R version 3.6.2 (R Core Team, 2020).

Results and Discussion

Density of the invasive sea urchins, *D. setosum*, at the sites with soft bottom (average = 1.053 ind./m^2, range = 0.96- 1.2 ind./m^2) was significantly lower compared to the density at the sites with hard bottom (average = 3.166 ind./m^2, range = 1.5- 4.2 ind./m^2, GLMM, Z = -3.684, p < 0.001, Fig. 1, 2). In contrast, the density of the native sea urchins, *A. lixula*, at the sites with soft bottom (average = 0.053 ind./m^2, range = 0.02 - 0.08 ind./m^2) and density at the sites with hard bottom (average = 0.096 ind./m^2, range = 0.05 - 0.17 ind./m^2) was similar (GLMM, Z = -1.863, p = 0.062, Fig. 2). Further, the density of *D. setosum* was significantly higher compared to native sea urchin, *A. lixula*, at the sites with hard bottoms (GLMM, Z = 18.52, p < 0.001, Fig. 2) and soft bottoms (GLMM, Z = 11.64, p < 0.001, Fig. 2).

The differences in densities of the invasive *D. setosum* between sites with hard and soft bottoms is likely to reflect the natural habitat preference of the species. Similar results for the habitat choices of *D. setosum* have been reported by several authors from both experiments and observations in the wild from the species' native habitats (Dumas et al., 2007; Puspita et al., 2012). This habitat preference with hard substrate may be the result of the food choices of *D. setosum* (Muthiga & McClanahan, 2007). Alternatively, *D. setosum* may prefer hard substrates in order to avoid its potential predators by hiding between rocks and small crevices (Coppard & Campbell, 2005; Dumas et al., 2007; reviewed in Muthiga & McClanahan, 2007); however, no predator for this echinoid species in the Mediterranean Sea has been reported. Furthermore, various feeding preferences of *D. setosum* have previously been reported mostly from its native habitats (Coppard & Campbell, 2005; Luza & Malay, 2019); however, echinoid species can consume a variety of diet items (Coppard & Campbell, 2005), which is a common dietary trait for the successful invasive species (Slatyer et al., 2013; Machovsky-Capuska et al., 2016). Moreover, *Diadema* species consume an important portion of sand in order to obtain biofilm and particulate organic matter accumulate on the sand surface (Muthiga & McClanahan, 2007). These may explain the presence of *D. setosum* with a moderate density on sandy bottoms as shown in this study.

D. setosum can lead to dramatic shifts on the benthic communities by implying grazing pressure on macroalgae (Aronson & Precht, 2000). However, the degree of the pressure applied by this species is density-dependent (Ishikawa et al., 2016). Ishikawa et al. (2016) reported a threshold of 2 ind./m^2 at which the influence by *D. setosum* becomes drastic on the community composition. If a similar threshold also exists in the Mediterranean Sea, the density of *D. setosum* we currently report from the Turkish coasts is expected to have detrimental effects on algal proliferation. We further found that the densities of *D. setosum* in our study sites are close to the 2 ind./m^2 threshold also on soft (e.g., sand) bottom habitats. However, this threshold may

vary based on the habitat characteristics and biodiversity, therefore further studies should focus on the algal production and the community composition of the benthic communities that are under the influence of *D. setosum* in the Mediterranean Sea.
The density of the native sea urchin, *A. lixula*, was inversely related to the density of the invasive sea urchin, *D. setosum* (GLMM, $Z = -2.794$, $p = 0.0052$, Fig. 2); however, this relationship was not influenced by the bottom type (interaction of habitat type:density of invasive sea urchin, GLMM, $Z = 1.371$, $p = 0.17$).
This inverse relationship between the densities of native and invasive sea urchins could be the consequence of potential competitive interaction between *D. setosum* and *A. lixula*. Competitive interactions between *D. setosum* and other sea urchins in the range of their native habitats are common (*reviewed in* Muthiga & McClanahan, 2007). The consequences of these inter-specific interactions are based on the size of individuals with some exceptions (i.e., *Echinometra mathaei* outcompete other sea urchins with larger body sizes) (McClanahan, 1988). However, the magnitude of this potential competitive interaction was not the objective of the current study, therefore future studies should focus on this and other relationships with native species and the potential consequences of the latter for the community structure in the Mediterranean Sea.

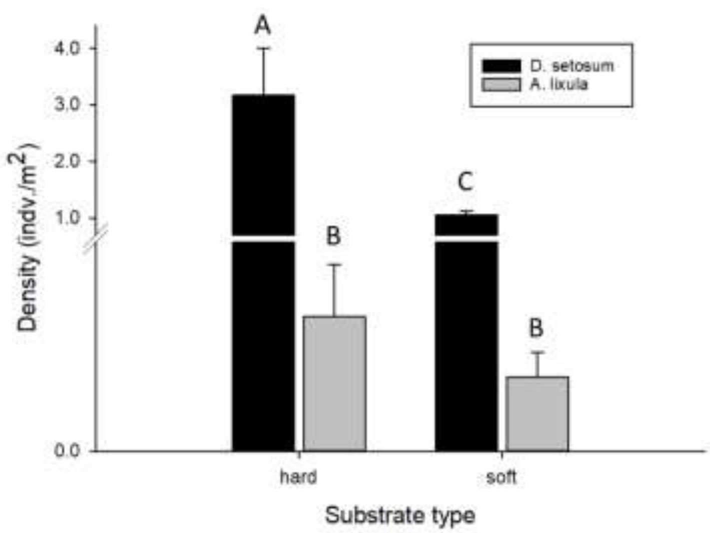

Figure 2. Variations in the density (average/m2 ± S.E.) of invasive, *Diadema setosum*, and native, *Arbacia lixula*, sea urchins between sites with hard and soft bottoms. Letters above bars indicate the significant differences.

Conclusion

Results of this study show that the invasive sea urchin, *D. setosum*, has reached to potential critical densities at several sites in the Mediterranean Sea which can harm benthic communities. Furthermore, their populations are larger compared to the native sea urchins within hard bottom habitats. However, the influence of this density increases on the community structure remains to be tested. Understanding the interactions with the native species will contribute to the management and conservation plans, which are urgently needed in the Mediterranean Sea.

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References

Aronson R.B., Prech W.F. (2000). Herbivory and algal dynamics on the coral reef at Discovery Bay, Jamaica. Limnology and Oceanography 45(1), 251- 255.

Artüz M.L., Artüz O.B. (2019). First and northernmost record of *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) in the Sea of Marmara. Thalassas 35, 375- 379.

Bilecenoğlu M., Yokeş M.B., Draman M. (2019). The invasive sea urchin *Diadema setosum* provides shelter for coastal fish- first observations from the Mediterranean Sea. Zoology in the Middle East 65(2), 183- 185.

Bronstein O., Kroh A. (2018). Needle in a haystack—genetic evidence confirms the expansion of the alien echinoid *Diadema setosum* (Echinoidea: Diadematidae) to the Mediterranean coast of Israel. Zootaxa 4497(4), 593- 599.

Bronstein O., Georgopoulou E., Kroh A. (2017). On the distribution of the invasive long-spined echinoid *Diadema setosum* and its expansion in the Mediterranean Sea. Marine Ecology Progress Series 583, 163- 178.

Coppard S.E., Campbell A.A. (2005). Distribution and abundance of regular sea urchins on two coral reefs in Fiji. Micronesica 37(2), 249- 269

Dumas P., Kulbicki M., Chifflet S., Fichez R., Ferraris J. (2007). Environmental factors influencing urchin spatial distributions on disturbed coral reefs (New Caledonia, South Pacific). Journal of Experimental Marine Biology and Ecology 344, 88- 100.

Galanos C.J., Kritikos S. (2019). *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae), first record from Simi Island, Hellas, Eastern Mediterranean. Parnassiana Arc 7, 15- 19

Ishikawa T., Maegawa M., Kurashima A. (2016). Effect of sea urchin (*Diadema setosum*) density of algal composition and biomass in cage experiments. Plank and Benthos Research 11(4), 112- 119

Kondylatos G., Corsini-Foka M. (2015). *Diadema setosum* moving west to the Hellenic Seas. In: Crocetta, F. et al., 2015. New Mediterranean Biodiversity Records. Mediterranean Marine Science 16(3), 682-702

Luza J.C.S., Malay M.C.M.D. (2019). Feeding preferences of the sea urchin *Diadema setosum* (Leske, 1778) in Taklong Island National Marine Reserve, Guimaras, Philippines. PeerJ Preprints 7, e27733v1.

Machovsky-Capuska G.E., Senior A.M., Simpson S.J., Raubenheimer D. (2016). The multidimensional nutritional niche. Trends in Ecology Evolution 31(5), 355- 365.

McClanahan T.R. (1988). Coexistence in a sea urchin guild and its implications to coral reef diversity and degradation. Oecologia 77, 210–218.

Muthiga N.A., McClanahan T.R. (2007). Ecology of *Diadema*. In: "*Edible sea urchins: biology and ecology"*, Lawrence JM (Eds). Elsevier, Amsterdam, pp 205- 225

Nader M.R., Indary S.E. (2011). First record of *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) from Lebanon, Eastern Mediterranean. Aquatic Invasions 6(1), 23- 25.

Puspita C., Moehammadi N., Irawan B. (2012). Study on the habitat preference of *Diadema setosum* in Bama Coast Baluran National Park. Berkala Penelitian Hayati 18, 19- 23.

Qiu J., Lau D.C.C., Cheang C., Chow W. (2014). Community-level destruction of hard corals by the sea urchin *Diadema setosum.* Marine Pollution Bulletin 85, 783- 788.

R Core Team. (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sax D.F., Stachowicz J.J., Gaines S.D. (Ed) (2005). Species invasions: insights into ecology, evolution and biogeography. Sinauer Press, Sunderland, MA.

Slatyer R.A., Hirst M., Sexton J.P. (2013). Niche breadth predicts geographical range size: a general ecological pattern. Ecology Letters 16(8), 1104- 1114.

Turan C., Erguden D., Uygur N. (2011). On the occurrence of *Diadema setosum* (Leske, 1778) in Antakya Bay, Eastern Mediterranean Sea. Journal of Black Sea/Mediterranean Environment 17(1), 78- 82.

Yapıcı S., Acar Ü., Türker A. (2014). First record of the alien sea urchin *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) from the Aegean Sea. Mediterranean Marine Science 15(3), 682

Yokes B., Galil B.S. (2006). The first record of the needle-spined urchin *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) from the Mediterranean Sea. Aquatic Invasions 1(3), 188- 190.

Yokomizo H., Possingham H.P., Thomas M.B., Buckley Y.M. (2009). Managing the impact of invasive species: the value of knowing the density-impact curve. Ecological Applications 19(2), 376- 386.

EFFECT OF NITROGEN FERTILIZATION ON TRITICALE GROWN FOR DUAL PURPOSE OF FORAGE PLUS GRAIN IN SEMI-ARID ENVIRONMENT

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Abstract

In Tunisia, lack of forage is one of the most important problems of livestock feeding during the autumn and winter seasons. One of the solutions is the practice of dual-purpose cereals; Cereals are grazed or cut at a young stage (tillering) and then allowed to re-grow up to grain production. Effect of application of three Nitrogen fertilization rates (0, 75 and 175 kg/ha) on Triticale variety Tcl 83 grown for grain production only and for the dual purpose of forage plus grain was studied in semi-arid region in Tunisia during 2018-2019 cropping season. Removal of forage for the dual purpose treatment was done at the pseudo stem erect stage (C30). Variance analysis showed that Tillers number/plant, spike number/m^2 and grain yield were significantly ($P<0.01$) influenced by the combined effect N fertilization and cutting at (C30) stage. This experiment has also shown that dual-purpose cultivation affected positively the grain protein content and nitrogen regime has a significant effect on thousand-kernel weight of the triticale produced at maturity stage. As per 2018-2019, when climatic conditions were favourable. The dual-purpose use of triticale could constitute a solution for the winter feed gap in the livestock cycle provided that adaptations of the traditional crop management to dual purpose, such as the sufficient nitrogen fertilization are undertaken.

Keywords: *Triticale, Dual purpose, Nitrogen, Semi-arid.*

Introduction

Under semi-arid conditions, Triticale can be used as forage and as grain during same cropping season. It is grazed or cut in a young stage (tillering) and then allowed to re-growth up to grain production. This will provide forage during winter season which is known as a forage deficit period. Researches about cereals dual-purpose for seeds and forage have given widely varying results according to the climate, to the specie, to the fertilization and to the cut or grazing stage. Under poor weather and fertility conditions, grain yield losses can be expected if cereals are grazed during the vegetative phase (Kilcher, 1982). Reduction in triticale grain yield was between of 9.6 to 50.8% for Nachit (1983) and 22% for Beji (2015). Decreases in grain yield after clipping have been attributed to a reduced number of spikes/m2 at harvest (Royo and al., 1993) and also to a reduced grain number (Bonachela and al., 1995) and kernel weight (Royo and al., 1994). Nevertheless, there have been observations of grain yield increases after forage removal, which were explained by a lower incidence of lodging (Droushiotis, 1984). Other results showed also that a properly managed grazing does not reduce grain yield in the dual-purpose system. It shows that the stocking pressure and number of cuts have been shown as important factors that influence the subsequent grain yield (Arzadun and al., 2003). Epplin and al. (2000) and Hossain and al. (2003) also suggested that an optimal choice of planting date and density is crucial if cereal is to produce high forage and grain yields. In the other hand, Nitrogen

fertilization, appears as one of the most limiting factors in the production of grain and forage. Appropriate fertilization (timing and quantity) is determining factor for a dual purpose use of cereals (Zagonel and al., 2002).
Other studies dealt with the influence of winter defoliation on quality traits but did not find detrimental effects of dual-purpose management on wheat grain protein or on dough strength parameters (Beji, 2016 ; Royo and al., 1994; Royo and Tribo, 1997; Khalil and al., 2002 ; Royo and al., 1997). Given the interest in cereals as dual purpose crop in northwest of Tunisia, this study was conducted to determine the impact of nitrogen fertilization on grain yield and quality for triticale grown for dual purpose.

Material and Methods

The present study was performed in el Kef region, in the experimental field of the Higher Institute of Agriculture Kef (36° 11' 9'' N, Longitude 8° 42' 59'' E; Altitude 652 m) during 2018-2019 cropping season. Trial was carried out in a clay-sandy-loamy soil with organic matter content around 1.54%. The climate is Mediterranean, with rainfall concentrated in in the autumn and winter. The average annual rainfall is 425 mm on the basis of 50 years. Climate temperature and rainfall from sowing to harvest are presented in Table 1.

Table 1. Mean temperature (°C) and rainfall (mm) of experimental field of the higher Institute of Agriculture Kef from sowing to harvest (2019-2019).

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rainfall (mm)	33	13	18	93	91	123	41	66	5
Temp Max	25	20	17	12	14	18	25	31	41
Temp Min	11	8	3	0	5	10	12	17	18

Triticale Variety used is the Tcl 83, the most known cultivar, used for sillage production and grain production as well. The experimental design was a Split plot one with 4 replications and each plot measured 10 m^2 in which the main factor was the crop use (grain use only or forage + grain use) and the secondary treatment is nitrogen fertilization rate. The two treatments within main factor were: (i) control plots which were only clipped at seeds maturity in order to estimate the grain yield and (ii) dual purposes plots which were clipped first time and harvested as forage at the stem erect stage (C30) and then let regrowth up to seeds maturity. For secondary factor three fertilization rates were tested ; (i) : control plots, unfertilized (N0), (ii) 75 kg/ha N applied (N75) and (iii) 150 kg/ha N applied (N150). Fertilization done with Ammonitrate (33%) and distributed according to cutting regime. For uncut plots (only grain use) 50% of total Nitrogen amount was applied at 3 leaf stage and 50% at end of tillering stage. For dual purpose plots, fertilization was applied 3 leaf three (50% of total amount) and 50% same day of forage cutting. Sowing was carried out early October at a density of 300 viable seeds/m^2. Forage harvest was done on 26 February 2019. Plants were cut about 8 cm above ground level. Grain harvest was made at seeds maturity on 28 June 2019. Weeds and diseases were chemically controlled.
Grain yield (GY), number of spikes/m^2 (SN/ m^2), number of grains per spike (NG/Sp), thousand-kernel weight (TKW) were determined for each plot at seed maturity, tillers number per plant (TN/Pt) measured at end of tillering period and grain crude protein (GCP) content was evaluated by means of the standards micro-kjeldahl procedure.

Analyses of variance (ANOVA) were performed using SAS (1985) and Means were separated using Duncan Test.

Results and Discussion

The results of the analysis of variance for grain yield and its components are shown in Table 2. Cutting at the green stage (C30) did not cause significant reduction of grain yield for dual-purpose use compared to grain use only. However, disparencies were noted and the grain yield reduction was more accentuated (-25%) under Fertilization regime 150N compared to unfertilized plots (-9%). The highest grain yield was obtained under regime 150N and when triticale was used only for grain (4.33 T/ha). Regrowth and capacity to produce high grain yield after defoliation was more important when N fertilization was higher. These results corroborate those of Hastenpflug and al. (2011) Zagonel and al. (2002) who found a positive interaction between nitrogen fertilization level and grain yield for wheat used under dual purpose management.
Analysis of variance has also showed that tillers number per plant (TN/Pt), number of spikes/m^2 (SN/ m^2) and thousand-kernel weight (TKW) were significantly affected by treatments studied. TN/Pt was significantly affected ($P<0.05$) by the interaction between management system and fertilization level. The dual purpose management and augmenting of nitrogen fertilization have increased the component TN/Pt. Higher tiller number (8) was measured on triticale used as forage and grain and fertilized with 150 kg/ha N.
SN/m^2 increased from 218 spikes /m^2 when triticale was not fertilized to 350 spikes /m^2 for triticale which has received 150 kgN/ha. Defoliation at stage (C30) has increased significantly ($P<0.05$) this parameter when fertilization was at (150N) regime and did not has effect for the two other fertilization regimes (0N and 75N). This is explained by the removal of the apical domination during final stage of the tillering period. In fact, with defoliation the predominant apex is eliminated and then tiller production restarts again and could drive to a higher number of productive tillers per plant (Briske and Richards, 1994). Bonachela and al. (1995) have also found that forage use during winter makes the tillering period longer and then capacity to make fertile tiller and spike greater. Compared with cereals species grown in temperate regions, triticale and barley cultivars adapted to Mediterranean climates have relatively short life cycles, especially in the phases before terminal spikelet (Kirby, 1991), Thus, a longer life cycle before the stage of maximum spikes number could increase tillering potential and, thereby, spikes number.
In the other hand, TKW was significantly affected only by fertilization regime ($P<0.05$) the higher TKW was measured on triticale fertilized with 150kg/ha N independently from management system. The decrease grain weight in the clipped treatment can be explained by a reduction of remobilisation of reserves and carbohydrates due to a shorter heading and flowering stages (C50-60) (Beji, 2016).
The third yield component, Number of grains/spike (NG/Sp), was a very stable variable, being similar for both treatments and the three fertilizations regimes.
This experiment has also shown that the fertilization regime has affected positively the grain protein content while the management system did not has effect on this parameter. This result is in agreement with Lestingi and al. (2010) who found a positive correlation between Nitrogen fertilization applied and grain crude protein. The higher grain protein content in this experiment has reached 102.5 g/kg DM which is considered high and similar to level indicated as adequate by Royo and al. (1997).

Table2. Yield components, grain yield and grain crude protein measured in different management system (MS) and different nitrogen regime (NR)

	TN/Pt	GY (T/ha)	SN/m²	NG/Sp	TGW (g)	GCP (G/KgDM)
Dual Purpose Unfertilized (0N)	6ab	1.03 c	207 d	23 a	38.4 b	99.3 b
Grain use only Unfertilized (0N)	4 c	1.21 c	229 d	26 a	38.2 b	99.8 b
Dual Purpose (N75)	7ab	2.98 b	308 c	31 a	39.5 ab	100.7 b
Grain use only (N75)	4 c	3.25 ab	318 bc	29 a	40.8 a	99.1 b
Dual Purpose (N150)	8 a	3.23 ab	368 a	28 a	39.3 ab	102.5 a
Grain use only (150N)	5bc	4.33 a	333 b	30 a	40.5 a	100.9 b
CV (%)	7.23	9.65	8.56	10.56	11.64	6.8
Manag. System	*	**	*	NS	NS	*
Nitrogen Regime	*	*	*	NS	*	NS
Manag System x Nitrogen Regime	*	*	*	NS	NS	NS

Distinct letters in the row indicate significant differences according to Duncan test (*$P \leq 0.05$, ** $P \leq 0.01$, NS: not significant).

Conclusion

Combined the two factor (Nitrogen and Dual purpose Management), we conclude that optimum grain yield was produced under grain use only and application of 150 kg N /ha. Capacity of regrowth increases with the quantity of Nitrogen applied.

Triticale could constitute a solution for animal breeders to fill winter deficit period as it provides green forage and could regrowth without a significant reduction of grain yield.

Further research which study the economic impact of dual purpose management of triticale compared to only grain use may aid to make the choice for the dual or single use of triticale

References

Arzadun M. J., Arroquy J. l., Laborde H. E., Brevedan R. E. (2003). Grazing pressure on beef and grain production of dual-purpose wheat in Argentina. Agronomy Journal. 95, 1157-1162.

Beji S. (2016). Yield and quality of dual-purpose barley and triticale in a semi-arid environment in Tunisia. African Journal of Agricultural Research. Vol. 11(30), pp. 2730-2735, 28 July, 2016.

Bonachela S., Orgaz F., Fereres E. (1995). Winter cereals grown for grain and for the dual-purpose of forage plus grain. I. Production. Field crop Research. 44:1-11.

Briske D. D., Richards J. H. (1994). Physiological responses of individual plants to grazing: Current status and ecological significance. Ecological implications of livestock herbivory in the west society. Range Management, Denver, CO, pp. 147-176.

Droushiotis D. N. (1984). The effect of variety and harvesting stage on forge production of barley in a low rainfall environment. The *Journal of Agricultural Science* Cambridge 102, 287-293.

Epplin F. M., Hossain I., Krenzer E. G. Jr (2000). Winter wheat automn-winter forage yield and grain yield response to planting date in a dual-purpose system. Agricultural System. 63, 161-173.

Lestingi A., Bovera F., De Giorgio D., Ventrella D., Tateo A. (2010). Effects of tillage and nitrogen fertilization on triticale grain yield, chemical composition and nutritive value *Journal* of the *Science* of *Food* and *Agriculture.* 2010 Nov; 90(14): 2440-6

Hastenpflug M., Martin T. N., Braida J. A., Barbosa D. K., Zielinski R. P., Refatti R. (2011). Grain yield of dual-purpose wheat cultivars as affected by nitrogen and cuttings. Bragantia, Campinas, vol. 70, n. 4, Pp.819-824

Hossain I., Epplin F. M., Krenzer Jr E. G. (2003). Planting date influence on dual-purpose winter wheat forage yield, grain yield, and test weight. Agronomy Journal 95, 1179–1188.

Khalil H.I., Carver B.F., Krenzer E. G., MacKown C.T., Horn G. W., Rayas- Duarte P. (2002). Genetic trends in winter wheat grain quality with dual-purpose and grain only management systems. Crop Science. 42:1112-1116.

Kilcher, M. R. (1982). Effect of cattle grazing on subsequent grain yield of fall rye (Secale cereale L.) in southwestern Saskatchewan. Canadian. Journal of Plant Science., 62: 795-796.

Kirby E. J. M. (1991). Cereal development in Mediterranean developments. In: Acevedo E, Fereres E, Gimenez C., Srivastava J. P. (Eds.). Improvement and management of winter cereals under temperature, Drought and Salinity Stresses. Proceedings Of a ICARDA-INIA Symposium, 1987 at Cordoba, Spain, pp. 66-84.

Nachit, M. (1983). The effect of clipping, during the tillering stage, on Triticale. Rachis, 2:11-12.

Royo C., Montesinos E., Molina-Cano J. L., Serra J. (1993). Triticale and other small grain cereals for forage and grain in Mediterranean conditions. Grass Forage Science. 48:11-17.

Royo C., Insa J.A., Boujenna A, Ramos JM, Montesinos E, Garcia Del ML (1994). Yield and quality of spring triticale used for forage and grain as influenced by sowing date and cutting stage. Field Crop Research.37:161-168.

Royo C., Tribó F. (1997). Triticale and barley for grain and dual-purpose (forage + grain) in Mediterranean-type environment. II. Yield, yield components, and quality. Australian Journal of Agricultural Research. 48:423-432.

Royo C., Lopez A., Serra J., Tribó F. (1997). Effect of sowing date and cutting stage on yield and quality of irrigated barley and triticale used for forage and grain. Journal of Agronomy and Crop Science. 179:227-234.

Zagonel J., Venâncio W. S., Kunz R. P., Tanamati H. (2002). Nitrogen doses and plant densities with and without a growth regulator affecting wheat, cultivar OR-1 Ciênc. Rural 32:25-29. http://dx.doi.org/10.1590/S0103-84782002000100005

CHARACTERIZATION OF PHOTOSYNTHETIC ACTIVE RADIATION IN BURGOS

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Abstract

Photosynthetically Active Radiation (PAR) is the fraction of the solar radiation spectrum that produces biochemical processes and influences in vegetation growth. PAR wavelength range is between 400 and 700 nm, being part of the visible light spectrum band (400-780 nm). This solar energy can be converted into biomass by photosynthesis in plants. Therefore, vegetation growth is directly related with solar radiation intensity and can be predicted with physical models. Two different models are normally implemented: one is based on photosynthetic photon flux density, Q_p (μmol·s^{-1}·m^{-2}) and the other is based on PAR (W·m^{-2}).
Q_p is not often measured in regular meteorological stations, consequently the main objective of this study is to stablish a methodology to estimate Q_p from solar irradiation measurements, R_s (W/m^2). The experimental. Specifically, the average value of the ratio Q_p/R_s was calculated every 10 minutes, from experimental data measured between April 2019 and January 2020 in Burgos, obtaining a global mean value of 1.93±0.15 μmol· J^{-1} .
The relationship between the ratio Q_p/R_s and the typology of the sky: clear, intermediate and overcast skies, accordingly to the CIE standard, is also analysed in this study. On the one hand, results show that there are statistically significant differences in the values of Q_p/R_s for each CIE standard sky type. On the other hand, aligned with other works in the bibliography, the overcast sky type shows the highest values of the ratio Q_p/R_s, contrary to clear and intermediate skies that show the lowest ratio.

Key words: *photosynthetic active radiation (PAR), vegetation growth, solar energy, sky types.*

Introduction

Photosynthetically Active Radiation (PAR) is the component of solar radiation that has the highest influence in plants growth. Its wavelength is between 400 and 700 nm, including photosynthetic photon flux (Q_p, (μmol·s^{-1}·m^{-2})) and energy (PAR (W·m^{-2})) (McCree, 1973). Q_p is usually measured with photon sensors and then can be converted into energy units through the McCree conversion factor, 4.57 $\mu mol \cdot J^{-1}$ $\pm 3\%$ according to climatic factors (Akitsu et al., 2015).
In most weather stations, PAR sensors are not included, so its value is usually obtained from solar radiation measurements, R_s. Although there is no single value for the ratio Q_p/R_s in bibliography, the differences between the values proposed by the different authors are small: 45-50% (Tsubo and Walker, 2005), 44-45% (Moon, 1940), close to 50% (Monteith and Reifsnyder, 1974). Additionally, authors also concluded that this ratio is not affected by the location, season, or daylight effects (Proutsos, Liakatas, and Alexandris, 2019).

The effect of sky conditions on the Q_p/R_s ratio has been studied in other works, concluding that, under overcast sky condition, Q_p/R_s ratio shows higher values (Blackburn and Proctor, 1983; Stigter and Musabilha, 1982).
The main objective of this study is to establish Q_p/R_s ratio in Burgos (Spain) and to analyse its dependence on the sky type. Available experimental data of horizontal solar global irradiation, R_s, photon photosynthetic flux density, Q_p, and CIE standard classification for homogeneous skies, determined through experimental sky scanner measurements, are used for this work. Figure 1 shows the main characteristics of each CIE standard sky type. The results obtained under different sky conditions are analysed over 10 months of real data.

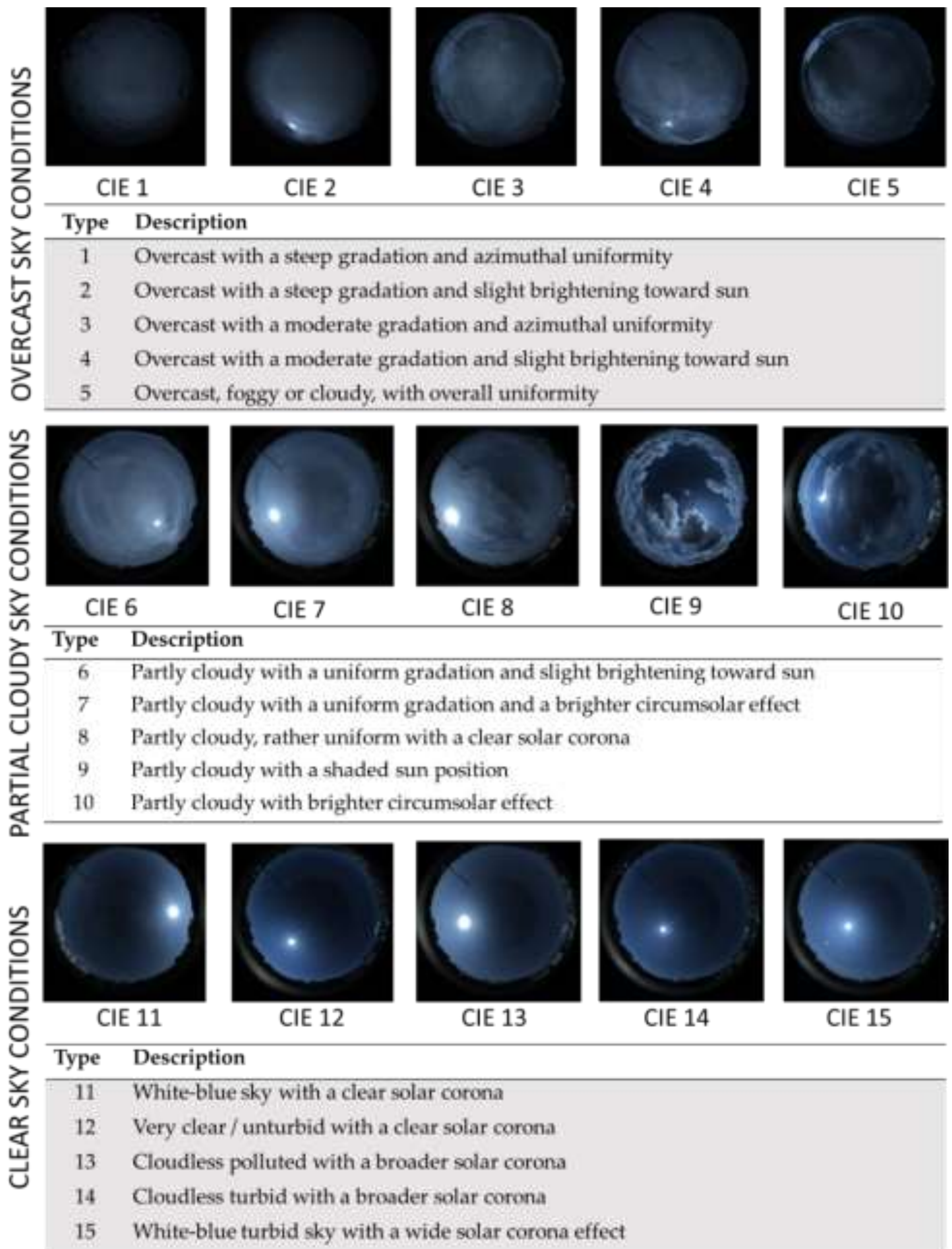

Figure 1. CIE Standard sky conditions in Burgos (Spain). Sky Camera SONA201D.

Material and methods

Global horizontal irradiation, R_s, and photosynthetic photon flux, Q_p, data are measured by a pyranometer (Hulseflux, model SR11) and by a photon meter (EKO instruments, model ML-020P) respectively. Additionally, the Sky luminance/radiance distribution is obtained from a commercial MS-321LR sky scanner from EKO. All parameters are registered every 10 minutes and Q_p and R_s data are filtered accordingly conventional quality criteria (Gueymard and Ruiz-Arias, 2016). When any measure is rejected, all other parameters registered simultaneously are rejects too. The experimental campaign of this study took place from April 1st, 2019 to January 31st, 2020.

Figure 2 shows the location of the weather station, which was described in a previous work (García-Rodríguez et al., 2020). It is placed at University of Burgos on the flat roof of the building, with no external obstructions or reflections from other surfaces.

Figure 2. Location of the experimental equipment on the roof of the Higher Polytechnic School building at University of Burgos, Spain.

Results and discussion

CIE Standard Sky Classification in Burgos from April, 1st, 2019 to January 31st, 2020

The normalization ratio (NR), introduced by Littlefair (Littlefair, 1994) in the original Standard Sky Luminance Distribution method (SSLD), deeply described in a previous work (Proutsos, Liakatas, and Alexandris, 2019), was used to determine the sky type in Burgos through the experimental campaign. Its frequency of occurrence (FOC) is plotted in Figure 3. As it can be seen, sky types 11, 12, and 13, which corresponds to clear sky, was predominant in Burgos, with a FOC value between 10% and 14.5%. On the other hand, sky types 5, 9, and 10 was less frequent with a FOC value of lower than 3%.

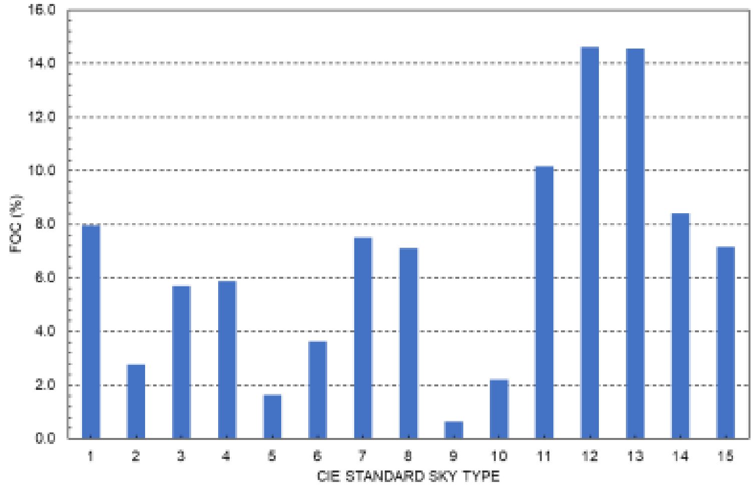

Figure 3: Frequency of occurrence (FOC, %) of CIE standard sky types in Burgos, Spain, between April 2019 and January 2020.

Align with it, when the study include only three categories of the sky (clear, partial, and overcast), as shown in Figure 4, the clear sky is the most frequent, with a FOC value close to 62%. On the other hand, partial and overcast skies have a FOC value of 23.96% and 22.92%, respectively.

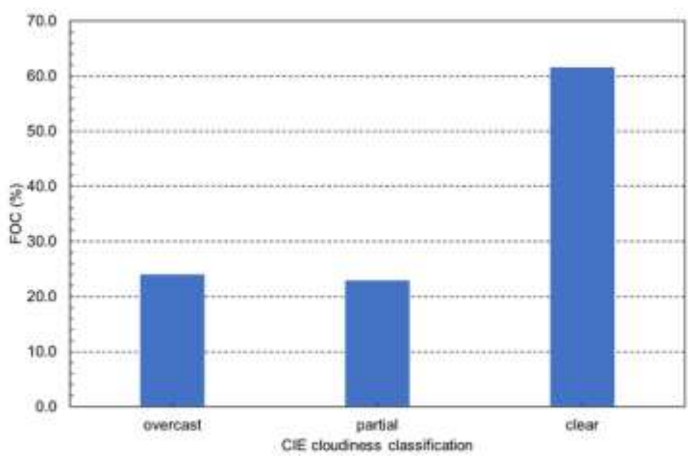

Figure 4: Frequency of occurrence (FOC, %) of CIE Cloudiness classification in Burgos, Spain, between April 2019 and January 2020.

Sensitivity of the ratio Q_p/R_s to the data timing interval

In addition to the Sky classification study, a preliminary study for the seasonal characteristics of the ratio Q_p/R_s was carried out with a data processing interval of ten-minutes, hourly, daily and monthly. Most relevant results are obtained when using ten-minutes data interval, where a strong relation is obtained between both variables (R^2=0.992), as it can be seen in Figure 5. The slope is calculated in 1.893±0.001 μmol·J^{-1} being close to the mean value of 1.93±0.15 μmol·J^{-1} with a standard deviation of ±0.15 μmol·J^{-1}. The results of the Q_p/R_s ratio agreed with those proposed by other authors, which should be in the range of 1.21 and 2.84 μmol·J^{-1} (Foyo-Moreno, Alados, and Alados-Arboledas, 2017)

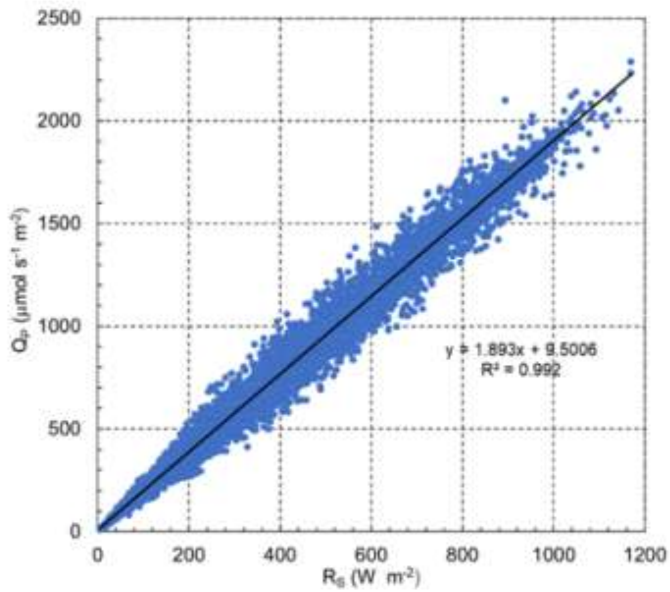

Figure 5. Relationship between the photosynthetic photon flux density, Q_p (μmol·s^{-1}·m^{-2}), and broadband solar irradiance, R_s (W·m^{-2}), measured in Burgos, from April 2019 to January 2020.

Variability of the ratio Q_p/R_s with the CIE Standard Sky types

Last study calculate Q_p/R_s ratio for each sky type when ten-minutes data interval is used. This results are represented in Figure 6. Although many outliers results were showed, the mean value of the Q_p/R_s ratio was obtained for the clear sky types 12,13,14 and 15 which show small standard deviation and interquartile range, between 0.06 to 0.11 μmol·J^{-1} and between 0.05 to 0.09 μmol·J^{-1} respectively. For partial and overcast skies, the dispersion of the data was almost similar. The highest Q_p/R_s ratio is obtained when sky is types 1,2,3 and 4, which belong to the overcast sky category. In addition, when the 15 types of skies are reduced to 3 categories, Q_p/R_s ratio increased while the cloudiness of the sky decreased, as shown in Figure 7.

In both Figures 6 and 7, crosses and horizontal lines inside the box, indicate the mean and the median respectively. The limits of the boxes give the 1st and 3rd quartiles, while the outer whiskers are the minimum and the maximum points. Circles represent outliers.

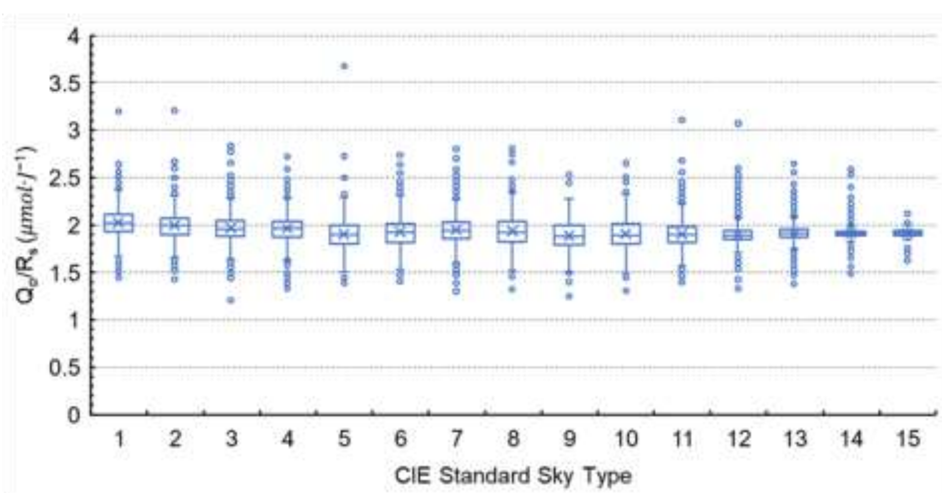

Figure 6. Box-plot ratio of photosynthetic photon flux density to broadband solar irradiance, Q_p/R_s, for each CIE standard sky type.

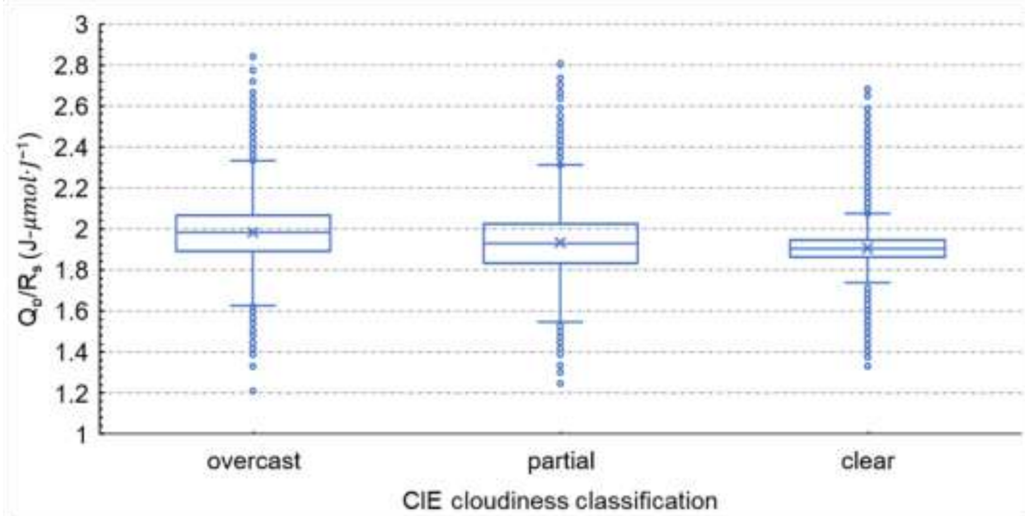

Figure 7: Box-plot of the of the ratio of photosynthetic photon flux density to broadband solar irradiance, Q_p/R_s , for CIE cloudiness sky classification.

Conclusion

The analysis of photosynthetic photon flux density to broadband solar radiation ratios of the data recorded between April 2019 and January 2020 in Burgos at 10-minute intervals, showed a dependence with the type of sky, classified according to the CIE standard,

The highest data of the Q_p/R_s ratio was obtained for overcast skies, while for clear skies, more scattered and smaller values were obtained. The global mean value is 1.93±0.15 $\mu mol \cdot J^{-1}$. In Burgos, the skies with the highest frequency of occurrence are clear skies, with a FOC of 62%, while overcast and partial skies have a FOC of 23.96% and 22.92%, respectively. As has been shown in this and other works, the factor that most influences the Q_p/R_s ratio is the presence of overcast conditions. Although other authors have used different climatological parameters for sky classification, the CIE sky classification provides a good general framework for representing real sky conditions, covering the entire likely spectrum of skies.

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References

Akitsu T et al. (2015). On the Stability of Radiometric Ratios of Photosynthetically Active Radiation to Global Solar Radiation in Tsukuba, Japan. Agricultural and Forest Meteorology vol. 209–210 pp.59–68.

Blackburn William J., and Proctor John T.A. (1983). Estimating Photosynthetically Active Radiation from Measured Solar Irradiance. Solar Energy vol. 31(2) pp.233–34.

Foyo-Moreno I., Alados I., and Alados-Arboledas L. (2017). A New Conventional Regression Model to Estimate Hourly Photosynthetic Photon Flux Density under All Sky Conditions. International Journal of Climatology vol. 37(March) pp.1067–75.

García-Rodríguez A., García-Rodríguez S., Díez-Mediavilla M., and Alonso-Tristán C. (2020). Photosynthetic Active Radiation, Solar Irradiance and the Cie Standard Sky Classification. Applied Sciences (Switzerland) vol. 10(22) pp.1–14.

Gueymard CA, and Ruiz-Arias JA. (2016). Extensive Worldwide Validation and Climate Sensitivity Analysis of Direct Irradiance Predictions from 1-Min Global Irradiance. Solar Energy vol. 128 pp.1–30.

Littlefair Paul J. (1994). A Comparison of Sky Luminance Models with Measured Data from Garston, United Kingdom. Solar Energy vol. 53 pp.315–22.

McCree K. J. (1973). The Measurement of Photosynthetically Active Radiation. Solar Energy vol. 15 pp.83–87.

Monteith J. L., and Reifsnyder William E. (1974). Principles of Environmental Physics. Physics Today vol. 27 pp.51–52.

Moon Parry. (1940). Proposed Standard Solar-Radiation Curves for Engineering Use. Journal of the Franklin Institute vol. 230 pp.583–617.

Proutsos Nikolaos, Liakatas Aristotle, and Alexandris Stavros. (2019). Ratio of Photosynthetically Active to Total Incoming Radiation above a Mediterranean Deciduous Oak Forest. Theoretical and Applied Climatology vol. 137(3–4) pp.2927–39.

Stigter C. J., and Musabilha V. M. M. (1982). The Conservative Ratio of Photosynthetically Active to Total Radiation in the Tropics. The Journal of Applied Ecology vol. 19 pp.853–58.

Tsubo M., and Walker S. (2005). Relationships between Photosynthetically Active Radiation and Clearness Index at Bloemfontein, South Africa. Theoretical and Applied Climatology vol. 80 pp.17–25.

THE RELATIONSHIP OF GLOBAL CLIMATE CHANGE WITH AGRICULTURE AND ENVIRONMENT

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Abstract

The demand for needs increases with the world population. The whole world is affected directly or indirectly while meeting the needs. The composition of atmosphere deteriorates as a result of human activities. This situation is an important problem that confronts us as global climate change. The aim of study is to examine the sectoral relationship of global climate change in Turkey. In addition, it is to reveal the effects on agriculture and environment. Excessive and unconscious use of fossil fuels and industrialization have an impact on climate change. Climate change has serious impacts such as drought, desertification, erosion and disruption of natural balance all over the world. It indirectly threatens sustainability in agriculture. It endangers the production and nutrition necessary for life. People unknowingly cause greater problems. Climate change affects socio-economic sectors and ecological balance. Its effects such as excessive rainfall, depletion of water resources, insufficiency of plant nutrients adversely affect agricultural activities. However, it is thought that the negative effects will be reduced with different methods used in agriculture such as organic farming, drip irrigation, land consolidation and use of renewable energy. Climate change, which is a global problem, can only be reduced by global measures.

Keywords: *Climate change, ecology, agriculture, environment, sustainability.*

Introduction

Global climate change is a mechanism that seriously affects humans, animals and all living things. Due to the nature of each living thing, its sensitivity to environmental and weather conditions is also different. Climate change has increased in recent years. This situation creates direct and indirect effects on human and environmental health. Özdağ (2011) stated in study that environmental destruction and health problems are on the agenda of climate change.

In the last 100 years, the global climate has warmed by about 0.5°C due to greenhouse gas emissions from anthropogenic activities. This warming process continues due to today's intense economic activities and increases in greenhouse gases released into the atmosphere. The British scientist Stern's claim suggested that even if no greenhouse gas emissions are released into the atmosphere from today, the global temperature will increase. He said that it will continue to increase between 0.5°C and 1°C in the coming decades (Stern, 2007). This report, known as the Stern report, includes climate models. It is predicted that the world will warm by 1.4°C to 5.8°C in the next century unless measures are taken to significantly reduce greenhouse gas emissions (Akalın, 2014).

Climate change also has negative effects on water resources. It is one of the important consequences of climate change, perhaps the most important. Water resources are the main source of life in the world (Anonymous, 2020). The first stage of human and environmental relations goes back to hunting and gathering periods. This stage has passed with the recognition of the environment and the effort to adapt to the environment. In the second stage, man began to dominate nature with the use of iron and the help of tools. In this period, people left behind the culture of living in harmony with nature for thousands of years. It has adopted the approach of unlimited exploitation against nature and beings in nature. Nature and the environment are preserved only as long as they are beneficial to humans. On the other hand, it has been used indefinitely. This situation emerged in the second period of the 20th century and started the third phase. Environmental problems, awareness of the environment and the need to take precautions have emerged. Thus, it was possible to take precautions against environmental problems. Afterwards, it has been understood that it is necessary to protect the environment for our own health. In the fourth stage, the environment began to be perceived as a cost problem. However, the inability to price environmental resources (such as the inability to price air, water and land) has emerged as an important problem. It has been suggested that with the last stage of consciousness, human beings are integrated with natural resources and other living things in the environment (Ertan, 2004).

Climate change is increasing its impact due to anthropogenic processes as well as natural processes. These effects of climate change are expected to emerge at global and regional scales. It can directly or indirectly affect agriculture, forest and vegetation, clean water resources, sea level, energy, human health and biodiversity in various ways. In addition, climate change puts pressure on agriculture socially and economically (Akalın, 2014). Therefore, climate change is a serious problem. In this context, there are a number of regulations regarding climate change at the global level. These are the United Nations Framework Convention on Climate Change, the Kyoto Protocol and the Paris Peace Agreement. When the regulations applied in the world are examined in terms of environmental ethics approaches, it is understood that they are anthropocentric. Although the Paris Agreement adopted in 2015 seems to be "more biocentric and ecocentric", it does not contain provisions to support these issues. The emphasis of the Paris Agreement on gender equality is not sufficient to comply with the principles of new ethical approaches (İğci and Çobanoğlu, 2019).

The aim of study is to evaluate the possible effects of climate change on agriculture and environment.

Material and methods

Studies on climate change and the environment were used in the research. It has been prepared by examining national and international studies within the scope of subject. In addition, the data of the Ministry of Agriculture and Forestry were researched.The effects of climate change have been mentioned all over the world. The impact of climate change has been addressed on agriculture, farming and environment.

Results and Discussion

Climate change is an important issue among the sustainable development goals. This issue concerns the whole world. While the priority targets are realized at the national level, global solutions should not be forgotten.

There are different activities according to different regions all over the world. Just as the industrial sector has some effects on climate change, the agricultural sector also has an effect. At the same time, since the agricultural sector is dependent on natural conditions, it is adversely affected by climate change. While agriculture affects climate change, it is also negatively affected by this situation. For this reason, climate change and the environment are intertwined within the scope of sustainability.

1. Climate change and agriculture

As is known, agricultural activities are directly related to the climate. Depending on climate change, changes occur in activities. Agricultural activities also cause climate change. Excessive irrigation, stubble burning, excessive and unconscious use of pesticides and fertilizers are some of them. Climate change also has negative effects on agriculture. Drought due to temperature increase affects crop yield and product quality. It is necessary to provide land management and sustainable agriculture with the awareness of globalization. Good agricultural practices and nature-friendly activities such as organic farming are among the agricultural activities aimed at preventing climate change.

The effects of climate change on agricultural activities and farm are examined in Table 1. Although these effects are considered directly on agriculture, they are also important in economic terms. Therefore, climate change also affects the development and development levels of countries. In addition, it is thought that the world's increasing food need and the problem of hunger will continue to increase.

Table 1. Effects of climate change

Agricultural effects	**Problems caused by farm**
Warmer and less rainy climatic conditions	Planting
Increase in extreme meteorological events	Harvest-threshing
Reduction in water resources	Tillage
Increase in drought severity	Fertilization
Deterioration of water and soil quality	Spraying
Degradation of the ecosystem and reduction of biodiversity	Cultural operations (hoeing, pruning, etc.)
Change in ecological fields	Yield
Decrease in agricultural production and quality	Quality
Increase in pests and diseases	Supply of water and irrigation water
Fertilizing and struggle problems	Plant diversity problems
Sustainable food security issues	Increasing CO_2 concentrations increase plant growth

Source: Anonymous, 2020 (www.tarimorman.gov.tr)

Due to climate change, soil and water regimes are changing. For this reason, agricultural production is decreasing and food security is in danger. Climate change in the long term; puts stress on water and other resources. It is also expected to degrade soils, cause desertification, increase pests and diseases, and destroy coastal ecosystems by raising the sea level (Akalın, 2014).

Climate change primarily makes the living conditions of people who live off the land more difficult. Then, it can threaten the health of all people by putting food safety at risk. Food security is defined by the World Agriculture Organization as the physical and economic access of all people to healthy, safe and nutritious food so that they can lead an active and healthy life. A possible food crisis and the endangerment of food security will cause bigger problems in the economic, social and political fields on a global scale. Coping with these situations is possible by reducing the negative effects of climate change on agriculture. In addition, it will be possible to

adapt to these negative situations (Kurukulasuriya and Rosenthal, 2003). Climate change is transforming the forms of agricultural production. Also, it causes stress on the amount of world food supply by reducing the productivity of agricultural products (Bindi and Olesen, 2000; McMicmael and Githeko, 2007).
Agricultural activities are responsible for about 20% of the increasing greenhouse gases in the World (Pathak and Wassmann, 2007). As a result of agricultural activities (energy consumption, production, animal breeding, fertilization, medicine, etc.), greenhouse gases such as CO_2, CH_4 and N_2O are released. Therefore, agricultural production is among the causes of climate change (Houghton, 2003).

2. Climate change and environment

The climate crisis has important effects on the environment, as in all areas. It causes many negative effects on the environment such as floods, strong winds, and reduction of biodiversity. Climate change has a direct impact on natural disasters and environment. Environmental problems arise with the consumption-oriented of individuals and urbanization. It is very costly to restore the broken system. Sometimes it is not even possible.
Climate change is a concern all over the world. It is a problem independent of the political and socioeconomic conditions of society. Environmental pollution should not be tolerated in the world. It also draws attention to climate change within the scope of economic development. In addition, it is necessary to invest in agriculture and renewable energy against climate change. Clean energies such as solar and wind are cheaper. With the increasing environmental awareness in recent years, the number of people who demand a cleaner country is increasing. There are 85% of people who think that the environment cannot be polluted even at the expense of economic development. This is an indication that the dirty investments to be made in the country from now on may attract more reactions (Doğru et al., 2020).
There are also some chain effects of global climate change in social and economic life (Doğan and Tüzer, 2011). Human-induced climate change is not appropriate and sufficient on its own in terms of directing thoughts about global environmental problems. In this context, higher moral standards and norms are required to solve a serious problem such as intergenerational justice in climate change (İğci ve Çobanoğlu, 2019). In this framework, ecological problems are seen as a policy problem to the extent of the negativities, threats and dangers they create for human beings. With the increasing environmental problems, the anthropocentric approach falls short of explaining the human-animal-nature relations (İğci ve Çobanoğlu, 2019).
The place of environmental problem on the agenda of humanity is not very old. Environmental problems are not only a problem that threatens non-human creatures, but also threatens the existence of human beings (Eren, 2015).

Conclusion and Recommendations

Agriculture and the environment cannot be separated from each other. All individuals have a duty to combat climate change. In this context, individuals should be educated on environmental and future awareness. Control of our needs and wants is important for the whole world. Individuals need to understand the importance of environmental problems. It is important to be conscious of taking measures to eliminate these problems. Recently, national solutions have started to be created on climate change with the renewable energy and recycling practices of local governments. The search for solutions by countries has accelerated as a result of environmental, economic and social pressures. In terms of climate change, it is important to prefer new technologies in agriculture. The support given to economical irrigation methods should be increased. In addition, it is necessary to focus on practices that reduce the risk situation. For this reason, agricultural insurance is important. Environmental health can also be protected by disseminating renewable energy in agriculture. In addition, high energy costs are reduced. Today, in order to eliminate these negativities due to climate change, first of all, it is necessary to determine the situation with climate change scenarios. Afterwards, these effects should be minimized by implementing adaptation and mitigation strategies effectively. National and international projects on sustainable agriculture and environmental studies should be supported. As a result; We must change ourselves, not the climate.

References

Akalın, M. (2014). "The Climate Change Impacts on Agriculture: Adaptation and Mitigation Strategies for these Impacts", Hitit University Journal of Social Sciences Institute, Year 7, Issue 2, pp. 351-377.

Anonymous (2020). "İklim Değişikliği ve Tarım", (Climate Change and Agriculture) Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Reform, Department of Agricultural Environment and Natural Resources Protection, Ankara.

Bindi, M., Olesen, J.E. (2000). "Agriculture", Assessment of Potential Effects and Adaptations for Climate Change in Europe: The Europe ACACIA Project (Ed).M. L. Parry. Norwich, United Kingdom: Jackson Environment Institute, University of East Anglia.

Doğan S. and Tüzer M. (2011). Global Climate Change and Its Potential Effects, C.U. Journal of Economics and Administrative Sciences, Vol 12, Number1, p: 25.

Doğru, B, Bagatır, B., Pultar, E. (2020). Türkiye'de İklim Değişikliği ve Çevre Sorunları Algısı (Perception of Climate Change and Environmental Problems in Turkey), Konda Research and Consultancy/ In collaboration with Climate News.

Eren, M. (2015). Responsibility Ethics to Cure the Environmental Issues,13:1, 439-452.

Ertan, B. (2004). 2000'li Yıllarda Çevre Etiği Yaklaşımları ve Türkiye (Environmental Ethics Approaches and Turkey in the 2000s). Journal of Administration Sciences (1:3) 2004 Journal of Administration Sciences. 93-108.

Houghton, R.A. (2003). "Why Are Estimates of The Terrestrial Carbon Balance So Different?", Global Change Biology, Vol.9, pp.500-509.

İğci, T., Çobanoğlu, N (2019). An Assesment of Climate Change and Global Agreements from the Perspective of Environmental Ethics, Ankara University Journal of Environmental Sciences, 7(2), 130-146.

Kurukulasuriya, P., Shane, R. (2003) Climate Change and Agriculture A Review of Impacts and Adaptations, The World Bank Envıronment Department June.

McMicmael A., Githeko, A. (2007) Human Health, Executive Summary, Working Group II: Impacts, Adaptation and Vulnerability, IPCC, 2007, pp.473.

Pathak, H., Wassmann, R. (2007) "Introducing Greenhouse Gas Mitigation as A Development Objective in Rice-Based Agriculture: I. Generation of Technical Coefficients", Agricultural Systems, Vol.94, pp.807–825.

Stern, N (2007) The Economics of Climate Change, The Stern Review, Cambridge.

Özdağ, U. (2011) Fifty Vocal Years After Silent Spring: Women, Environment, and Health. Journal of Faculty of Letters, Vol 28, Number 2, 179-199.

ANIMAL HUSBANDRY

THE HEALTH STATUS OF FOOT AND UDDER AT THE LEVEL OF DAIRY FARMS IN THE COMMUNE OF SIDI MHAMED BENALI

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Abstract

Among the dairy cattle production problems, mastitis and lameness rank first in terms of socio-economic impact. Our aim is assessment of the state of well-being of the udder and the foot in dairy cows and its relationship with milk production. A sample of 42 Prim Holstein and Montbeliard dairy cows in second lactation was used for milk analysis by pH paper test and for locomotion index determination. The results obtained show that the average quantity of milk produced by healthy cows is greater than that produced by cows infected at the rate of (14.90; 10.5) L/d respectively with $p < 0.05$. The rate of lame cows observed was 21.42%, being above the recommended alert threshold (it must be less than 15%). Lame cows presented a greater score for hoof growth and angle plumb than non-lame cows to a value of (1.11; 0.22) and (1.61; 0.83), respectively with $p < 0.05$. A mastitis prevalence of 47.61% was observed. The teat lesion score was higher in cows with mastitis than in healthy cows to a value of (0.81, 0) with $p \leq 0.05$ and the score 5 of the line mammary was determined as the best position score of the mammary gland to avoid the appearance of teat lesions. The score for the hygienic condition of the udder was higher in sick cows than in healthy cows to a value of (3.11, 2) with $p \leq 0.05$. In conclusion, the evaluation of the well-being of the foot and the udder reveals that the conditions for raising dairy cows are not satisfactory and there is no respect of the animal's welfare.

Keywords: *Animal welfare, dairy cattle, audit, lameness, mastitis.*

Introduction

Milk occupies an important place in the diet of the Algerian population. According to Srairi *et al.*, 2019, the milk crisis in Algeria is caused by a lack of production, associated with an increase in annual consumption. Several support programs have been adopted since independence to encourage the dairy sector and despite these efforts, powdered milk is still imported to fill a production deficit estimated at over 60% (Kaouche-Adjlane, 2019; Kebbal *et al.*, 2020). Many factors affect milk production, including the health of cows. Mastitis is considered to be the most important and costly pathologies affecting the dairy sector, as it results in a significant decrease in milk production (Davies *et al.*, 2009; Vikou and Gbangboche, 2019). The problems of mastitis in dairy cattle farms outground remain difficult to control in the Algerian context. Usually mammary gland dysfunction is due to the conditions of breeding and milking (Haj Mbarek *et al.*, 2013). The environment of the milking parlor (air, food used during milking, teats, quality of the litter and its degree of soiling) (Haj Mbarek *et al.*, 2013), contamination of the milk during its passage through the milking machine by the biofilm developed on its surface (Mtaallah *et al.*, 2002), the cleaning practices of the milking machine associated with other practices at the level of general hygiene and teats (Michel et al., 2006), the composition and the degree of maintenance of the litter can be one of the major points of the variability of the microbial load (Haj Mbarek *et al.*, 2013), the cleanliness of the cows as well as the lesional state of the udder have consequences on the one hand on the well-being of animals and on the other hand on milk quality (Ruegg, 2006; Kaouche-Adjlane, 2019). In dairy cattle breeding, lameness is also an important pathological dominant after parturition. she is the third disease behind mastitis and reproductive disorders. In addition to their economic repercussions, lameness is considered to be an impairment of well-being through the pain and discomfort caused (Endres, 2017). In order to participate effectively in improving the productivity of dairy cattle and the quality of the milk produced, we have deemed it necessary to assess the state of well-being of the udder and foot in dairy cows because this will allow us to:

- assess the quantitative losses of milk linked to mastitis and lameness.
- research the relationship between certain practices in breeding, mastitis and lameness.

Material and Methods

Our study was carried out from June until November 2020 at a dairy farm located in the region of sidi Mhamed Benali wilaya of Relizane (Algeria) on a sample of 42 cows of Montbéliarde and Prim_Holstein breeds in 2nd lactation and after the parturition. The herd is maintained, in the majority of cases, in zero grazing with a diet based mainly on oat hay, corn silage and concentrate. The building is designed of bricks, the floor is made of concrete, damaged and not mulched. Milking is carried out twice a day, in the morning and in the evening, mechanically in the barn using a milking cart. During milking, teat cleaning is carried out with water rarely with disinfectant. In general, the hygienic practices of milking and equipment were poorly applied. Experimental monitoring is based on recording the following parameters:

- Individual measurement of the quantity of milk produced during morning and evening milking. This measurement was calculated using a very resistant and light food-grade plastic container, with a capacity of 20L.
- The locomotion index assessment widely used scoring system is that described by Sprecher *et al*., 1997.
- The evaluation of the degree of cleanliness of the foot, is based on the grid described by Guatteo *et al.*, 2013.
- Assensment of plumb angle and of the growths is based on the diagnosis the hind limbs, because foot lesions most often concern the hind legs according to Solano *et al*., 2016, it is based on the observation of the animal in a resting situation based on a rating scale established by Bareille *et al*., 2014.
- A hygienic and sanitary score of udder were carried out jointly before milking, based on a scoring grid for lesion of the teat established by Gourreau *et al.*, 1995 and the scoring grid for udder hygiene according to the model of Lensink et Leruste, 2012, Regarding the location of the udder, we based ourselves on scale established by Lajudi *et al*., 2014.
- Analysis of the milk was determined at the time of milking with pH indicator paper (bromothymol paper). This test consists of placing a few drops of milk on the indicator paper, after removing the first jets. After 15 to 20 seconds of contact, the color change from yellow to green, or blue was considered to be from an infected udder (positive sample).

Statistical analysis was carried out by Statistica software using the Student test comparison of two means.

Results and Discussion

Total milk production is estimated at 253L / d with an average of 12.7 L / d, maximum and minimum production per cow is estimated at (19, 10) L / d respectively. This result is insufficient according to Kadi *et al.* 2007, for farms which breed the two breeds Prim Holstein and Montbéliarde.

1. The Lameness

According to statistical analysis of data of degree of cleanliness of foot of healthy and lame cows, we have found that the average of degree of cleanliness of two types of cow was identical to a value of (2 - 1.69) respectively, while we noted a significant difference in the outgrowth of hoof and plumb angle between non-lame cows and lame cows to a value of (0.22, 1.11) and (0.83, 1.61) with p = (0.0025, 0.001) respectively. We have also observed that the average quantity of milk produced by healthy cows is greater than that produced by lame cows to a value of (14.90; 10.5) L / d respectively with p = 0, 0001 (table 1).

Table 1. Comparison of outgrowth, angle, the cleanliness of the foot and the amount of milk between lame and non-lame cows

Cows	lame	Non-lame	
Outgrowth	X= 1.11 Std= 0.33	X= 0.22 Std= 0.66	*P*= 0.0025
Angle	X= 1.61 Std= 0.50	X= 0.83 Std= 0.57	*P*= 0.001
Cleanliness	X= 1.69 Std= 0.63	X=2 Std= 0.42	*P*= 0.16
Amount of milk (l/d)	X= 10.5 Std= 0.68	X= 14.90 Std= 2.72	*P*=0.0001

p<0,05, M=mean, Std= standard deviation

A loss of 31.49% of average milk production was noted . This coincides with the results found by Bouraoui *et al.,* (2014 a) who report that a cow with severe foot and limb problems can lose up to 35% of her milk production. This can be explained by Stress harmful reactions threaten animal welfare and production, because they consume energy and weaken the body at several levels. Environmental and hygrometric faults, the type of sleeping arrangements, as well as unfavorable social interactions (such as, for example, overdensity) (Wang, 2016 ; Solano *et al.*, 2016 ; Ito *et al.*, 2014 ; Endres, 2017) can place cows under repeated stress, with a potential impact on the quality of immune defenses, thus leading to pathologies. The rate of lame cows observed is 21.42%, which is above the recommended alert threshold which must be less than 15%. This hight frequency is due to the quality of housing, indeed the lack of litter associated with damaged concrete floors allowed the water to stagnate causing very high humidity. According to Borderas *et al.,* (2004), the tissues that make up the hoofs of cows absorb water quickly thus reducing their hardness which makes the foot vulnerable to bacterial attack. the hoof growths associated with a lack of plumbness can be explained by the fact that animals uncomfortable on their hind feet or who present in pain will tend to spread their limbs, that is to say modify its plumbs to rest the affected foot from any pressure (Bareille *et al.*, 2014) , An excess of growth of hooves makes it possible to highlight a disruption of balance between (push / wear) of horn which is linked to a lack of abrasiveness of soil, therefore the absence of grooving of surface concrete is a risk factor for the appearance of growths thus leading to problems with locomotion. (Fox *et al.*, 1991).

2. Mastitis

A mastitis prevalence of 47.61% was observed. The physical condition of teats is an indicator of quality of environment, milking procedures and the milking system used. It also helps define the risk of intra-mammary infections. We have noted in Table 2 that the teat lesion score is higher for udders with subclinical and clinical mastitis by repport to the healthy udder to a value of (0.77, 0.81, 0) respectively with p = (0.000032, 0.00040), The evaluation of cleanliness of udder is also important because it has consequences on the one hand on the comfort of the animal and on the other hand in hygienic quality of milk. We observe in Table 2 that the score of degree of cleanliness of udder affected by clinical and subclinical mastitis is greater than that of healthy udder to a value of (3.11, 2.72, 2) respectively, with p = (0.000097, 0.0014).

Table 2. Comparison of udder cleanliness and teat lesions between healthy cows and cows with mastitis

<table>
<tr><td>udder</td><td>Subclinical mastitis</td><td>healthy udder</td><td>Clinical mastitis</td></tr>
<tr><td rowspan="3">Teat lesion</td><td>M=0.77
Std=0.44</td><td>M=0
Std=0.19</td><td>M=0.81
Std=0.60</td></tr>
<tr><td colspan="2">P= 0.000032</td><td></td></tr>
<tr><td></td><td colspan="2">P =0.00040</td></tr>
<tr><td rowspan="3">Udder cleanliness</td><td>M=2.72
Std=0.92</td><td>M=2
Std=0.27</td><td>M=3.11
Std=0.68</td></tr>
<tr><td colspan="2">P= 0.0014</td><td></td></tr>
<tr><td></td><td colspan="2">P =0.000097</td></tr>
</table>

It is observed in Table 3 that the best position for having a teat without lesion is the rating 5 of mammary line with p = (0.003, 0.00005) for a high and low position of the udder respectively.

Table 3. Comparison of teat lesions between different positions of the mammary gland

<table>
<tr><td>Position of the mammary gland</td><td>(1-3)</td><td>5</td><td>(7-9)</td></tr>
<tr><td>Lesion</td><td>X=0.64
Std= 0.74</td><td>X=0
Std=0.00</td><td>X=0.85
Std= 0.66</td></tr>
<tr><td rowspan="2"></td><td colspan="2">P= 0.003</td><td></td></tr>
<tr><td></td><td colspan="2">P= 0.00005</td></tr>
</table>

So the risk factors for the appearance of mastitis are linked to the method of breeding and anatomical position of udder by report to the hock. The total absence of litter within the breeding is considered a risk factor, essential for mastitis. The work carried out by Brouillet, have shown that the incidence of mastitis is strongly linked to the quality and quantity of litter (Brouillet, 1990), because a failure of litter will promote the accumulation of dirt and increase the proliferation of germs in the environment, indeed the udder cleanliness results showed that the infected udders were very dirty moreover presence of teat lesion is considered one factor predisposing colonization of germ and thus leading to the appearance of mastitis, the work of Fox LK *et al.*,(1991), have showed than The appearance of teat lesion are due either to an incorrect adjustment of the milking machine or because lack of flexibility of the teat sleeves (Federici, 2004) or to defects in the conception of housing or of its use, thus exposing cows to various udder trauma (Bouraoui *et al.,* 21014b ; Kebbal *et al*., 2020). We noticed in the field that breeders did not change the sleeves every year, according to Federic-Mathieu *et al.,* (2002). Changing the sleeves every year prevents the appearance of mastitis. (Bouraoui *et al*., 21014b) Scoring the mammary line by report to the hock is important in order to avoid teat injury, 33.33% of cows with a mammary line score of 5 did not show any injury. Neijenhuis *et al*., (2002), believe that a defective anatomical location of udder and its teats exposes it to trauma during: difficult lifting, an lying on rough ground, an trampling, slipping, pushing and scratching this increasing the risk of subclinical mastitis (Ficher *et al*., 2003). Indeed 66.66% of cows with mastitis (subclinical and clinical) presented lesions at the level of the teats because their udders were in an inadequate position.

Conclusion

According to the results of this study we found that the problem of lameness and mastitis is linked to the animal's environment (the quantity of litter, the quality of the soil, the maintenance of the milking machine, the building hygiene , ………), thus significantly influencing milk production. It is possible to consider the use of animal welfare assessment technique on the farm to identify cows affected earlyer in order to ensure good prevention. These techniques are within the reach of breeders, including those with low technical skills, because, in addition to their low cost, they are easy to perform, read and interpret.

References

Bareille N., Delacroix M., Ennuyer M., Geollot S., Prod'homme J., Roussel P., Seegers H., Toczé C. (2014). Maitrise des boiteries dans les troupeaux laitiers (Control of lameness in dairy herds). Version 2, UMT Maitrise de la santé des troupeaux bovin, page 17-77.

Bouraoui R., Jemmali B., M'hamdi N., Ben Mehrez C., Rekik. (2014 a). Etude de l'incidence des boiteries et de leurs impacts sur la production laitière des vaches laitières dans le subhumide tunisien (Study of the incidence of lameness and their impact on the milk production of dairy cows in the Tunisian subhumid). Journal of New Sciences, 9(2), 01 : 7-17.

Bouraoui R., Selmi H., Mekni A., Chebbi I., Rouissi H. (2014 b). Impact des conditions de logement et des pratiques de traite sur la santé mammaire et la qualité du lait de la vache laitière en Tunisie (Impact of lodging conditions and milking practices on mammary health and milk quality of dairy cows in Tunisia). Livestock Research for Rural Development, 26 (3).

Borderas T F., Pawluczuk B., De Passillé A M., Rushen J. (2004). Claw hardness of dairy cows: relationship to water content and claw lesions. J Dairy Sci, 87:2085-2093.

Brouillet P. (1990). Logement et environnement des vaches laitières et qualité du lait (Housing and environment of dairy cows and milk quality). Bull. G.T.V., 4, B, 357 : 13-33.

Davies G., Genini S., Bishop S.CGiuffra E. (2009). An Assessment of Opportunities to Dissect Host Genetic Variation in Resistance to Infectious Diseases in Livestock. Animal, 3 (03): 415- 436.

Endres M.I. (2017). The relationship of cow confort and flooring to lameness disorders in dairy cattle. Veterinary Clinics of North America. Food Animal Practice, 33 (2) : 227-233.

Federici C. (2004). Logement et flambée de mammites cliniques (Housing and outbreak of clinical mastitis). Journée Nationale des G.T.V, Tours: 781-787.

Federic-Mathieu C., Godin M. (2002). La machine à traire: fonctionnement, incidence sur la santé des mamelles (The milking machine: operation, impact on udder health). Journée nationales des G.T.V Tours, 369-394.

Ficher R., Sutter-Lutz B., Berger L. (2003). Contrôler les mammites à *Staphylococcus aureus (Controlling mastitis at Staphylococcus aureus)*. Le Point Vétérinaire, 33(228) : 50-54.

Fox LK., Nagy JA., Hillers JK., Cronrath JD., Ratkowsky DA. (1991). Effects of post-milking teat treatment on the colonization of Staphylococcus aureus on chapped teat skin. Am. J. vet. Res., 52 (6) : 799-802.

Gourreau JM., Arfi L., Brouillet P., Coussi G., Fieni F., Lacombe JF., Paulizzi L., Simonin F., Radigue PE. (1995). Accidents et maladies du trayon (Teat accidents and diseases). Ed. France Agricole, Paris, 287 pp.

Guatteo R., Arnoult A., Menard J.L., Bareille N. (2013). Elaboration d'une grille de notation spécifique de la propreté des pieds des bovins laitiers et investigation des facteurs de risque en période hivernale (Elaboration of a scoring grid to assess feet cleanliness in dairy cattle and identification of risk factors in the winter period) . Renc. Rech. Ruminants, 20 : 379-382.

Haj Mbarek R., M'Sadak Y., Kraiem K. (2013). Effets des caractéristiques mammaires et de la propreté des vaches sur les comptages cellulaires individuels du lait en élevage hors sol dans la région de Sousse (Tunisie) (Effects of the cow's mammary characteristics and cleanliness on somatic individual cell count in battery farming cattle in the region of Sousse (Tunisia)) . Renc. Rech. Ruminants. 20 : 398.

Ito K., Chapinal N., Weary D.M., Von Keyserlingk M.A.G. (2014). Associations between herd-level factors and lying behavior of freestall-housed dairy cows. Journal of Dairy Science, 97 (4) : 2081-2089.

Kadi S.A., Djellal F., Berchiche M. (2007). Caractérisation de la conduite alimentaire des vaches laitières dans la région de Tizi-Ouzou, Algérie (Characterization of feeding practices of the dairy cows in Tizi-Ouzou area, Algeria). Livestock Research for Rural Development, 19 (4): https://www.researchgate.net/publication/259008731

Kebbal S., Baazize-Ammi D., Gharni I., Hanzen C., Guetarni D. (2020). Étude descriptive des facteurs de risque des mammites et caractéristiques managériales des exploitations laitières de la wilaya de Blida (Descriptives study of mastitis risck factors and managerial characteristics of dairy farms in the wilaya of BLIDA). *Revue Agrobiologia*, 10(1): 1975 - 85

Kaouche-Adjlane S., (2019). Facteurs de variation qualitative et quantitative de la production laitière Revue Bibliographique (Factors of qualitative and quantitative variations in dairy production. Bibliographic review), Revue Agriculture, 10(1) : 43 – 54

Lajudie P., Berrechet P., Robergeot F., Deconchat P., Tournardre O., Roy M., Gout P., Roussie C., Hanriot J.C., Laloe D. (2014). Guide pratique du pointage des bovins de race à viande, du sevrage à l'âge adulte (Practical guide to pointing beef cattle, from weaning to adulthood). Institut d'élevage, p 5-71.

Lensink J. et Leruste H. (2012). L'observation du troupeau bovin (Observation of cattle herd). Editions France agricole, 99-106.

Michel V., Hauwuy A., Chamba J.F. (2006). Gestion de la flore microbienne des laits crus par les pratiques des producteurs (Raw milk microbial composition: differences in links with microbial practices). Renc.Rech.Rum., 13 : 309-312.

Mtaallah B., Oubey Z. et Hammami H. (2002). Estimation des pertes de production en lait et des facteurs de risque des mammites subcliniques à partir des numérations cellulaires de lait de tank en élevage bovin laitier (Assessment of milk yield losses and subclinical mastitis risk factors using bulk milk somatic cell counts in dairy herds). Revue Méd. Vét., 153, 4 : 251-260

Neijenhuis F., Barkeman H.W., Hogeveen H., Noordhuizen J.P. (2002). Classification and longitudinal examination of callused teat ends in dairy cows. Journal of Dairy Science, 83 : 2795-2804.

Ruegg PL. (2006). The role of hygiene in efficient milking. WCDS. Advances in Dairy Technology, 18 : 285-293.

Solano L., Barkema H.W., Mason S., Pajor E.A., Leblanc S.J., Orsel K. (2016). Prevalence and distribution of foot lesions in dairy cattle in AQlberta, Canada. Journal of Dairy Science, 99 (8) : 6828-6841.

Sprecher D J., Hostetler D E., Kaneene J B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology, 47 :1179-1187.

Srairi M T. , Chatellier V. , Corniaux C. , Faye B. , Aubron C. , Hostiou N. , Safa A. , Bouhallab S. , Lortal S. (2019). Réflexions sur le développement du secteur laitier et sa durabilité dans différentes parties du monde (Thoughts on Development of the dairy sector and its durability in different parts of the world). INRA Prod. Anim. , 32 (3) : 339-358.

Vikou R., Gbangboche A. B. (2019). Les Mammites Infectieuses, Obstacles à L'amélioration de la Santé Animale et à la Production de Lait et du Fromage (Infectious Mastitis Obstacles to Improving Animal Health and Production of Milk and Cheese). European Scientific Journal, 15, 6 : 343-363.

Wang S. (2016). Effects of stocking density on behavior, productivity, and confort indices of lactating dairy cows. Journal of Dairy Science, 99 (5) : 3709-3717.

DAILY BEHAVIOR OF LOCAL BREED CATTLE AT THE AULNAIE OF AIN KHIAR DURING THE SUMMER

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Abstract

The alder grove is a characteristic environment of the wetland of El Tarf. This ecosystem is now exposed mainly to free grazing by local cattle. The aim of this work is the determination of the diurnal behavior of cows of local breed "Brown Atlas" at the level in the alder grove of Ain Khiar. For this purpose, 5 cows were daily followed for 10 days to determine their feeding activities and their behavior during the summer. During the summer, the animals are locked up at night in the sheepfold and go out to the pasture at dawn. They roam all the plots chosen by the shepherd for a total of 14 hours per day. However, their feeding activities are restricted to 8 hours of feeding (57% of total time). In addition, during the hottest hours, the animals are parked in a make shift pen in the middle of the forest, and the rest of the time, the animals spend resting, which is 37% of the total time. As for the choice of food, the animals are restricted to single species of *Typha latifolia* (L, 1753). The body condition score of the animals decreases considerably during the summer and can go down to 2 or 1,5.

Keywords: *Alder grove, cattle, feeding Behaviour, Paturage.*

Introduction

FAO analytical study on livestock in Africa highlights the insufficient productivity of pastoral systems and their negative environmental impacts on soil and atmosphere (Steinfeld *et al*., 2006). Yet extensive livestock farming remains a keystone of rural populations' activities in Africa due to its economic, ecological and social functions (Chirat *et al*., 2008). However, because of their considerable impact on the structure and dynamics of plant communities, herbivores play a key role in the maintenance and enhancement of grasslands, and conversely, the abundance, distribution and nutritional value of these plant resources largely affect the acquisition of nutrients by these mammals, and consequently their performance (Van Langeveld and Prins, 2008). Therefore, understanding the interaction between herbivores and plant resources remains a major challenge for ecosystem management. The Aïn Khiar alder grove is a characteristic environment of the National Park of El-Kala, particularly rare in Algeria. Indeed, Eastern Numidia is the only African area with alder groves. Such ecosystems probably have high biological and scientific value in the Maghreb. Junqua (1954) already stated that "it is only in the circle of the Calle that we find the known North African stands of the glutinous alder (Alnus glutinosa)". It is therefore a sensitive site which fully justifies its Ramsar classification, although the area is not supposed to be accessible to livestock.

which is characterised by a marked seasonality in which the value of the food supply fluctuates with the season. Forage availability peaks in spring, decreases rapidly in summer, then picks up again in autumn depending on the rainfall regime before decreasing again in winter (Mebirouk-Boudechiche *et al.,* 2011), The objective of this study is to gain a better understanding of the activities and feeding behaviour of local cows of the "Brune de l'Atlas" breed in the Ain Khiar alder grove, one of the rangelands of northeastern Algeria, and determine its impact on this fragile area.

Material and Methods

The Ain Khiar alder grove is located between the coastal dunes and the agricultural plain of El Tarf in the extreme North East of Algeria (36°48'09"N 8°19'04"E). It covers an area of 170 ha and enjoys a Mediterranean climate with hot, dry summers and mild, wet winters, where average annual rainfall is around 800 to 1200 mm (Chaker-Houd *et al*., 2017). The monitoring of the activity rhythm of four-year-old non-pregnant adult cows of the local breed "Brune de l'Atlas" with an average BCS of 2.5 was carried out daily in the morning and in the afternoon by means of 8-minute visual observations at 40-minute intervals for the duration of a 10-day trial, during the summer season of 2019. The amount of dry matter consumed was evaluated by taking samples simulating the bite of the animals, i.e. the "hand plucking" method. The breed studied is known for its hardiness, its resistance to cold and humidity, and its adaptation to the food resources of wooded and well-watered mountains (Chellig, 1992). In addition to its resistance to certain diseases (Yakhlef, 1989), the breed has good walking abilities on difficult, even rugged terrain. Samples of the vegetation consumed by our females were also taken, dried in an oven set at 50°C for 48 hours and then ground. For the analysis of primary chemical according to AOAC (1990) procedures.

Results and Discussion

During this summer season and following the survey carried out with the farmers and the daily follow-up of the cows selected on the alder grove, we were able to determine a single free grazing station, totally open herbaceous composed exclusively of *Typha latifolia* (L, 1753), a species that grows on waterlogged or even flooded ground. During the hottest hours of the day, the females are parked with the rest of the herd in shaded fenced areas on bare ground.

Chemical composition of the single species ration of the cows during the summer season

The food bowl of our females during the summer season is composed exclusively of *Typha Latifolia* leaves, an herbaceous vegetation in the form of a cattail belonging to the Typhaceae family, the rate of coverage of which is 100% due to the availability of water in the alder grove itself. Table 1 reports the chemical composition of *Typha latifolia*. Our species can be qualified as a mineral-rich species with a good total nitrogen content, the dry matter content is around 21%, while the fibre content is over 34%. The fact that the animals are only interested in one plant species reassures us about their impact on the tree layer.

Table 1: Chemical composition of the simulated ration (*Typha latifolia*) during the summer season

Designation	*Typha latifolia*
Dry matter (%)	20.3
Mineral matter (% DM)	16.69
Carbohydrates (%DM)	83.3
Crude protein (%DM)	10.2
Crude fibre (%DM)	34.4

Circadian rhythm and feeding activities of cows on the alder grove

During the summer, cows leave the barn at dawn and spend an average of 14 hours on the alder grove. The daily monitoring shows that grazing is the main daily activity, with local cows spending 480 min or 57% of the total grazing time. While 330 minutes are spent resting and just 30 minutes moving, which represents 37% and 4% of the total grazing time respectively, 10 minutes are spent on average for watering per day. Observations made in the Sahelian environment show that grazing occupies 59% of the animal's activity at pasture (Sanon *et al.*, 2006). These activities would be linked to the season effect where the time spent grazing can increase from 54% in the rainy season to 84% in the dry season (Ouedraogo-Koné *et al.*, 2006). The diurnal ingestion times, rest and movement that we observed are much higher than the results obtained on cattle grazing on natural grassland (Mebirouk-Boudechiche *et al.,* 2011) and Borgou bulls grazing fallow land (Babatounde' *et al.*, 2009). Moreover, according to Balent and Gibon (1986), the duration of grazing is strongly linked to the length of the day and to weather conditions, which can greatly reduce it by delaying the animals' exit time (frost) or by disturbing their activity rhythm.

Table 2. Different activities of cows on the range during the summer season

Activity	FT	RT	TT	WT
Time (mn)	480	330	30	10
%	57	37	4	2

FT: feeding time, RT: resting time, TT: travel time, WT: watering time

Feeding behaviour parameters

The circuit and the time in free grazing of the local breed cows during the heat season seem to be determined by the temperatures and the orientations of the shepherds,These results confirm the work of CHIRAT, 2008 in the hot season due to the high level of insolation. Resting times are significantly longer in the hot season ($p < 0.001$). They also noted that there was no ruminating during the day of divagation. This suggests that the consistent tethering time (at least 12 hours per day) and the importance of movement to ensure a certain level of intake limits the time spent on rumination. As it is not possible to dissociate grazing from movement, we describe this system as 'walking'. The amount of dry matter ingested by our cows during this summer period is 7548 g of DM/animal/day with a total number of tines of 296 NTS/observation, and a tine weight of 3.4 g of DM. While at the end of spring and the beginning of summer the cows still seem to be in good health due to the availability of fodder, as the summer progresses their BCS decreases considerably from 2.5 ±0.78 to 2 ±0.57. This can be explained by the high heat which causes stress and probably leads to a decrease in appetite in addition to the decrease in feed availability. Mebirouk-Boudechiche *et al*. (2011) reported an NTS of 41 Bite/min but the TSW was lower than ours, i.e. 2.5 g DM, for local breed cows grazing on natural grassland dominated by legumes. Thus, animals try to compensate for the low amount taken per mouthful by increasing the frequency of mouthfuls and/or grazing time in order to maintain their feed intake rate (Penning, 1986; Allden and Whittaker, 1970).

Table 3. Main parameters assessed during observation of cows on pasture

Parameter	CT	TSF	NTS	TSW	IQ	BCS	
value	48	37	296	3.4	7548	beginning	end
						2.5 ±0.78	2 ±0.57

CT: Counting time in minutes, TSF: Teeth strike frequency NTS: Total number of teeth strikes on the vegetation during the 8 minute teeth strike counting period, TSW: Average teeth strike weight (in g DM), IQ: Intake quantity (in g DM/animal/day), BCS: Body condition score,

Conclusion

At the end of this study, we can conclude that the Ain Khiar alder grove, in spite of its floristic richness, turns into a hostile terrain with limited food availability during the hot season. The monitoring of local cows of the breed "Brune de l'Atlas" on the alder grove of Ain Khiar shows that grazing is the main daily activity compared to moving and resting. The movements are determined firstly by the position of the obligatory crossing points (pens and water points). Secondly, it is as if the intake is determined by the vegetation units crossed, and finally by the herder. The cows graze in the morning and afternoon and rest at midday, spending more than 14 hours on the grove. The composition of the feed bowl, the estimation of the ingested quantities and the weights of the teeth strokes give a picture on the adaptive and selective behaviour of our cows during the summer season.

References

Adler PB., Raff DA., Lauenroth WK. (2001). The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128, 465-479.

Babatounde S., Sidi H., Houinato M., Mensah G.A., Sinsin. A.B. (2009). Study of eating behaviour of young Borgou bulls in Benin's north-soudanienne fallow field;. Renc. Rech. Ruminants, 16.

Balent G., Gibon A. (1986) The grazing behaviour of sheep in the Central Pyrenees during winter. Reproduction, Nutrition, Development., 26 (1 B), 267-268.

Chaker-Houd K., Mebirouk-Boudechiche L., Maatallah S., El-Hamza T. (2017). Nutritional value of Sulla coronaria L. sampled from natural populations in northeastern Algeria. Fourrages, 232 : 347-352.

Chaker-Houd K., Boudechiche L., Maatalah S., Bouras R., Oudini E.H. (2020). Activities, quantities ingested and feeding behavior of crossbred goats« Arbia » at the Ain Khiar aulnaie (Ageria) .RENC. RECH. RUMINANTS, 25, pp 588.

Chirat G., Ickowicz A., Diaf H., Bocquier F. (2008). Study of key factors of spatial and feeding behaviour of free grazing cattle herds on an agrosylvopastoral tropical territory. Renc. Rech. Ruminants, 15, 327- 330.

Distel R.A., Laca E.A., Griggs T.C., Demment M.W. (1995). Patch selection by cattle: maximisation of intake rate in horizontally heterogeneous pastures, Applied Animal Behaviour Science, 45, 11-21.

Junqua C. (1954). About Hydrocirius columbiae S., and the biogeographical interest of La Calle. Bull. Sté. Hist. Nat. Af. Du N., t. 45, 7-8, p. 318-322.

Mebirouk-Boudechiche L., Boudechiche L., Maatallah S., Chemmam M., Menasri G. (2011). Mebirouk-Boudechiche L., Boudechiche L., Maatallah S., Chemmam M., Menasri G. (2011). Feeding behaviour of locally-bred cows on plain grassland in the north-east of Algeria, 205. 53-59.

Ouedraogo-Kone, S., Kabore-Zoungrana, C. Y. et Ledin, I. (2006). Behaviour of goats; sheep and cattle on natural pasture in the sub-humid zone of West Africa. Livest. Sci. 105, 244-252.

Penning, P. D., Parsons, A. J., Orr, R. J., Harvey, A., Champion, R.A. (1995). Intake and behaviour responses by sheep, in different physiological states, when grazing monocultures of grass or white clover. Applied animal behaviour science, 45(1-2), 63-78.

Sanon A (2006). Displacement of an herbaceous plant species community by mycorrhizal and non-mycorrhizal Gmelina arborea, an exotic tree, grown in a microcosm experiment In Mycorrhiza, 16(2),125-32.

Steinfeld H., Gerber P., Wassenaar T., Castel V., Rosales M., Dehaan C. (2006). Livestock's long shadow, environmental issues and option. rome: fao, lead initiative,390.

Van Langeveld F ., Prins H.H.T. (2008). Introduction on resource ecology. in: resource ecology: spatial and temporal dynamics of foraging (ed. by h.h.t. prins et f.van langeveld), pp. 1-6 Springer.

Yekhlef H. (1989). Extensive milk production in Algeria", Options Méditerranéennes - Série Séminaires, 6, 135 -139.

EFFECT OF INCORPORATION OF THE PLANT EXTRACTS IN NATURAL CASING ON CONTENT OF FATTY ACIDS IN FERMENTED SAUSAGES

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Abstract

In this study, the effect of natural casing treated with ethanol extract of *Aronia melanocarpa* and *Cornus mas* on the fatty acid content in fermented sausages "sucuk" type was evaluated. The sausages were produced in industrial conditions, stuffed into the pretreated natural casings, vacuum packaged, and stored at 4°C for six months. Five groups of sausages were tested, C1 (natural casing without treatment), C2 (treated with 6% (v/v) ethanol), C3 (treated with ascorbic acid), A (treated with ethanol extract of *A. melanocarpa*), and D (treated with ethanol extract of *C. mas*). The qualitative and quantitative composition of fatty acids in the tested samples was determined by the GC-FID analytical technique. Palmitic (C16:0) and stearic (C18:0) acids were predominant of the saturated fatty acids (SFA) and their quantity ranged from 15.93% to 27.50%, and 13.81% to 24.74%, respectively. Oleic acid (C18:1) in the range from 19.02% to 31.87% was the most represented unsaturated fatty acid (USFA). Based on the obtained results, it can be concluded that treated casings had a statistically significant effect ($p<0.05$) on the content of the identified fatty acids in tested samples. During six months of storage, it was noticed a decrease in SFA content and an increase in USFA content, especially C22:1, C24:1, and C22:6. The ratio SFA/USFA ranged from 1.04% to 1.50% after production and from 0.53% to 0.69% after six months of storage. The ratio of polyunsaturated fatty acids (PUFA) n-6/n-3 ranged from 0.27% to 5.14% after production and from 0.23% to 1.65% at the end of the testing period.

Keywords: *fermented sausage, extract, natural casing, fatty acids.*

Introduction

Consumers, especially in developed countries, are increasingly interested in the elements of nutrition food values and the impact of food ingredients on health. The content of fatty acids in food is of great importance for a healthy human diet. All around the world, meat traditionally occupies a central position. The fat content and fatty acid composition of meat are significant properties that affect the quality of meat and meat products. Special importance is attached to the use of fats in human nutrition, especially omega 3 and omega 6 fatty acids and their mutual relationship (Pleadin *et al*., 2016; Ahmad *et al*., 2018).

The production of dry fermented sausages has a very long tradition. Fermented sausages are made of minced meat and fatty tissue of various animals, with the addition of salt, nitrates/ nitrites, sugar, ascorbic acid and different spices. The mixture is filled into natural and/or artificial permeable casings of various diameters and then subjected to fermentation and ripening in ambient or controlled conditions for several weeks or months (Stajić *et al*., 2012).

Sucuk is a fermented sausage originating from Turkey, popular in most the Middle East and European countries. Manufacturing of sucuk varies regionally, but in general, recipes consist of

beef and beef fat and/or sheep tail fat. The mixture is filled into a beef small intestine and tied with rope (Yıldız-Turp and Serdaroglu, 2008).
The use of edible coatings and films as a barrier to the transit of oxygen and water to preserve food quality has intensified recently (Hromiš *et al*., 2013; Ozvural *et al*., 2016). These coatings act, thereby slowing oxidation reactions and retaining moisture (Tyburcy *et al*., 2010; Shon *et al*., 2011). This is the main mechanism by which coatings enhance quality and prolong storage life. Furthermore, adding plant extracts and some natural bio preservatives gives the coatings antimicrobial and antioxidant properties (Raesi *et al*., 2016; Król *et al*., 2017). Plant extracts are generally added to prevent oxidation of fats and proteins, and/or inhibit the growth and development of bacteria, yeasts and molds, or prevent spoilage of the product (Botsaris *et al*., 2015). In recent decades, many researchers have tested plants and plant extracts, such as oregano, sage, rosemary, grape seed (Man and Jaswir, 2000), thyme, white, red and mountain peony, angelica, sedge, marjoram, wild marjoram, cumin, ginger, plum, aloe vera (Nuñez *et al*., 2008). This study aimed to investigate the effect of submerging natural casing in plant extracts (*A. melanocarpa* and *C. mas*) on the fatty acid content in fermented sausages.

Materials and methods

For the preparation of extracts, fruits were washed and the petioles and the seeds removed. For ethanol extract, 40g of fruits were homogenized with 160 mL 80% (v/v) ethanol, first on a Polytron PT 3100 homogenizer, 10 minutes at 8000 rpm, then in an ultrasonic bath for 30 minutes, and 30 minutes on a magnetic stirrer. The mixture was filtered and the obtained filtrate was evaporated to a dry residue, first on a vacuum evaporator and then in a dry sterilizer at 50°C. The dry extracts prepared in this way were stored in a dark place until the moment of use. The prepared dry extract of *A. melanocarpa* was diluted to 45 mg/mL and extract of *C. mas* was diluted to 22.5 mg/mL in 6% (v/v) ethanol and used for casing treatment. Duration of submersion was 24h for all casings.
Domestic fermented sausages, sucuk, were produced traditionally under industrial conditions. The emulsion consisted of beef meat 80%, beef fat 16%, nitrite salt 2.5%, spices: black pepper 0.3%, white pepper 0.15%, garlic granules 0.35%, additive (mixture of dextrose, salt, sucrose, ascorbic acid, preservative E252 (2.5%) 0.6%, starter culture 0.01%. After grinding and mixing in the cutter, the sausage emulsion was filled into natural beef casings with the addition of all components. For this study, five samples of domestic fermented sausages: C1-the control (natural casing without treatment); C2 (natural casing treated with 6% (v/v) ethanol), C3 (natural casing treated with ascorbic acid), A (natural casing treated with ethanol extract of *A. melanocarpa*), and D (natural casing treated with ethanol extract of *C. mas*) were made.
The sausages were first tempered (18-20°C) for 8h, then smoked (beech wood) for three days (20-23°C, 92-95% RH), and finally left for fermentation (ripening) at 16-18°C for eight days.
After production, sausages were vacuum-packed and stored in a cooling chamber at 4°C until sampling (usual storage conditions for this kind of product). Analyses of sausages were carried out after production and after six months of storage.
The total fat extraction was performed by the reference method BAS ISO 1443:2007, the principle of determination is based on the hydrolysis of the sample with dilute hydrochloric acid.
Fatty acid methyl esters of the tested samples were prepared by dissolving samples in hexane and direct esterification with saturated KOH/methanol solution (David *et al*., 2005). The equipment used to determine fatty acids was Clarus 680 Perkin Elmer gas chromatograph with FID detector,

Elite-wax L 60 m column, ID 0.32, DF 0.5, with the standard: Sigma Aldrich Supelco 37 components FAME MIX. Injector and detector temperature 250ºC, sample volume 1µL, temperature regime: 60ºC for 2 min, 10ºC/min up to 200ºC, 5ºC/min up to 240ºC, and 30 min at 240ºC, flow 1.5 mL/min, nitrogen gas (carrier). The total duration of analysis was 54 min. The composition of fatty acids is shown as a percentage of individual fatty acids in the total amount of identified fatty acids (g/100 g of total fatty acids).
Statistical processing of the obtained results was performed using the Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 (Armonk, NY, United States). Results were presented as mean values of three individual measurements ± standard deviations. The significance of differences between arithmetic means was determined and expressed with 95% probability (Tukey's test).

Results and discussion

The fatty acid composition of analyzed fermented sausages is given in Table 1. The most common SFA presented in fermented sausages were palmitic acid (C16:0) ranged 20.96-27.50% after production (samples D and C2, respectively) and 15.93-18.07% (samples C3 and C1 respectively) after storage, stearic (C18:0) ranged from 22.98-24.74% after production (samples A and C3, respectively) and 13.81-16.00% after storage (samples A and C1, respectively) and myristic (C14:0) ranged from 3.22-5.72% after production (samples D and C3, respectively) and 2.09-2.74% after storage (samples C3 and C2 respectively). Literature data confirm results from this study (Wójciak *et al*., 2015; Pleadin *et al*., 2016). Other identified SFA were found in much smaller amounts in the analyzed samples.
Oleic acid (C18:1c+t) with content from 25.58% (sample D) to 31.87% (sample A) after production, and from 19.02% (sample C3) to 25.37% (sample C1) after storage, was predominant among MUFAs, which coincides with the literature data for similar products (Barbir *et al*., 2014; Lešić *et al*., 2017). Oleic fatty acid is the most important fatty acid of all kinds of meat, and in beef, it makes up 33% of total fatty acids (Barbir *et al*., 2014). It was an evident increase of MUFA n-9 (C22:1) and C24:1+C22:6 in all samples during storage, especially in sample C3.
Linoleic acid (C18:2c+t) was the dominating PUFA with values around 3.34-6.65% after production, which is in accordance with literature data (Liguori *et al*., 2015, Alabiso *et al*., 2021). During storage, content of the linoleic acid decrease (1.72-2.70%). It can be noticed that statistically significant differences ($p<0.05$) exist between analyzed samples.
Changes that occur in fats and fatty acids in meat can be controlled using various strategies, such as animal dietary supplements, the addition of antioxidants, processing, and the use of special packaging (Amaral *et. al*., 2018).
The results of the total content of SFA and USFA as well as their mutual ratio in the tested samples are given in Table 2. Based on the obtained results, it can be noticed that the total content of SFA was the highest after ripening (from 51.08% in sample D, to 60.04% in sample C1), and decreases during storage (from 34.83% in sample A, to 40.80% in sample C1). In the case of MUFA, the situation is reversed, content increases during storage. The content of PUFA slightly increased during storage. For most traditional cured meat products from Croatia and Slovenia, the proportion of fatty acids was MUFA>SFA>PUFA, except 'budjola' and technologically similar products, for which the order was SFA>MUFA>PUFA (Pleadin *et al*., 2016).

The PUFA/SFA ratio could be a good indicator of the nutritional quality of sausages (Wójciak *et al*., 2015). WHO/FAO experts have reported guidelines for a "balanced diet" in which the suggested ratio of PUFA/SFA is above 0.4, but a recommendation by the British Department of Health for PUFA/SFA is more than 0.45 (Teixeira *et al*., 2020), to prevent an excess of SFA and PUFA with a negative effect human health (Parunović *et al*., 2017). The PUFA/SFA was within the recommended value, ranged from 0.11 to 0.34 for all samples, except sample A after storage (0.56). Another indicator of the quality is n6/n3 PUFA ratio, and according to Teixeira *et al*. (2020), this ratio should be less than 4. Samples C3 and A after production had higher values (4.07 and 5.14, respectively) than recommended. Based on results, MUFA n-9 and PUFA n-3 content in samples treated with vitamin C and both fruit extracts were rather high compared to control C1 and sample C2. On the protection of unsaturated fatty acids from oxidation greatly affect substances with strong antioxidant activity (Hromiš, 2015).

Table 1. Fatty acid composition (means±standard deviations) of model fermented sausage after ripening (0) and after six months (6) of chilling storage (% of total fatty acids)

		M	C1	C2	C3	A	D
SFA	C10:0	0	**0.07**^{ab}±0.02	n.d	**0.07**^{ab}±0.01	**0.07**^{ab}±0.01	**0.09**^{b}±0.01
		6	**0.60**±0.02	n.d	n.d	n.d	n.d
	C12:0	0	**0.33**^{a}±0.04	**0.19**^{b}±0.02	**0.21**^{b}±0.04	**0.22**^{ab}±0.04	**0.20**^{b}±0.02
		6	n.d.	**0.15**±0.00	n.d	n.d	n.d
	C14:0	0	**5.67**^{a}±0.24	**3.33**^{b}±0.06	**5.72**^{a}±0.16	**4.58**^{ab}±0.86	**3.22**^{b}±0.06
		6	**2.49**^{ab}±0.04	**2.74**^{c}±0.06	**2.09**^{d}±0.00	**2.33**^{a}±0.12	**2.55**^{b}±0.05
	C15:0	0	**1.08**^{a}±0.18	**0.45**^{bA}±0.05	**0.68**^{b}±0.13	**0.70**^{b}±0.13	**0.52**^{b}±0.03
		6	**0.58**^{a}±0.02	**1.30**^{bB}±0.18	**0.72**^{a}±0.03	**0.50**^{a}±0.09	**0.52**^{a}±0.03
	C16:0	0	**26.04**^{a} ±0.55	**27.50**^{b}±0.36	**25.80**^{a}±0.42	**26.59**^{ab}±0.32	**20.46**^{c}±0.17
		6	**18.07**^{a} ±0.16	**16.87**^{ab}±0.48	**15.93**^{b}±0.61	**16.06**^{b}±0.43	**16.68**^{ab}±0.48
	C17:0	0	**2.11**^{a}±0.03	**1.49**^{bc}±0.09	**1.85**^{ab}±0.11	**1.64**^{bc}±0.30	**1.09**^{c}±0.13
		6	**0.84**±0.01	**0.80**±0.26	**0.95**±0.13	**0.80**±0.11	**1.00**±0.04
	C18:0	0	**24.14**^{a} ±0.47	**24.61**^{a}±0.13	**24.74**^{a} ±0.15	**22.98**^{b}±0.17	**22.28**^{b}±0.23
		6	**16.00**^{a} ±0.12	**15.19**^{abc}±0.24	**14.27**^{bc}±0.80	**13.81**^{c}±0.86	**15.63**^{ab}±0.46
	C20:0	0	**0.42**^{a}±0.05	**1.49**^{b}±0.06	**0.20**^{c}±0.03	**0.22**^{c}±0.04	**0.24**^{d}±0.02
		6	**2.22**^{a}±0.09	**1.85**^{ab}±0.15	**1.48**^{bc}±0.14	**1.33**^{c}±0.05	**1.55**^{bc}±0.31
MUF	C14:1	0	**0.40**±0.08	**0.32**±0.09	**0.41**±0.05	**0.40**±0.07	**0.48**±0.08

		6	**2.41**^{a}±0.01	**3.22**^{b}±0.19	**1.63**^{c}±0.04	**0.36**^{d}±0.00	**0.78**^{e}±0.05
	C15:1	**0**	**0.44**±0.06	**0.35**±0.02	**0.32**±0.07	**0.32**±0.06	**0.49** ±0.02
		6	**0.45**^{a}±0.07	**1.22**^{b}±0.15	**0.38**^{a}±0.03	**0.56**^{a}±0.04	**0.51**^{a}±0.11
	C16:1	**0**	**3.29**^{a}±0.12	**2.50**^{b}±0.01	**2.28**^{c}±0.08	**2.31**^{bc}±0.10	**2.35**^{bc}±0.03
		6	**1.73**^{ab}±0.02	**0.97**^{c}±0.06	**1.61**^{ab}±0.31	**1.28**^{bc}±0.11	**1.86**^{a}±0.05
	C17:1	**0**	**0.59**^{a}±0.03	**0.50**^{a}±0.02	**1.00**^{b}±0.06	**0.75**^{a}±0.14	**1.22**^{b} ±0.05
		6	**1.75**±0.04	n.d	n.d	n.d	n.d
	C18:1	**0**	**26.11**^{a}±0.26	**26.27**^{a}±0.10	**25.65**^{a}±0.24	**31.87**^{b}±2.73	**25.58**^{a}±0.34
	c+t	**6**	**25.37**^{a}±0.07	**22.82**^{b}±0.25	**19.02**^{c}±0.08	**19.42**^{c}±0.10	**24.94**^{a}±0.54
	C20:1	**0**	**0.21**^{a}±0.04	**0.36**^{b}±0.03	**0.13**^{c}±0.02	n.d	**1.67**^{d}±0.02
		6	**3.54**^{a}±0.01	**2.84**^{b}±0.18	**2.11**^{c}±0.19	**1.68**^{cd}±0.07	**1.33**^{d}±0.32
	C22:1	**0**	**0.52**^{a}±0.17	**1.44**^{b}±0.15	**1.43**^{b}±0.07	**0.76**^{a}±0.06	**1.56**^{b}±0.04
		6	**9.89**^{a}±0.38	**13.44**^{b}±0.26	**18.58**^{c}±0.82	**14.98**^{d}±0.30	**14.20**^{bd}±0.10
	C24:1	**0**	**1.10**^{a}±0.12	**1.61**^{b}± 0.02	**1.57**^{b}± 0.02	**0.86**^{c}±0.01	**7.65**^{d}±0.06
	+C22:6	**6**	**11.47**^{a}±0.01	**14.95**^{b}±0.56	**18.68**^{c}±1.02	**14.70**^{b}±0.58	**14.73**^{b}±0.53
PUFA	**C18:2c+t**	**0**	**5.24**^{a}±0.01	**5.92**^{ab}±0.04	**6.65**^{b}±0.12	**5.04**^{a}±0.81	**3.34**^{c}±0.04
		6	**2.61**^{a}±0.10	**1.72**^{b}±0.02	**2.53**^{a}±0.26	**2.37**^{a}±0.10	**2.70**^{a}±0.01
	C18:3n6	**0**	**0.17**^{a}±0.03	n.d	**0.11**^{b}±0.00	**0.10**^{b}±0.01	n.d
		6	n.d	n.d	n.d	n.d	n.d
	C18:3n3	**0**	**0.59**^{a}±0.07	**0.37**^{b}±0.03	**0.44**^{ab}±0.09	**0.44**^{ab}±0.08	**0.57**^{a}±0.03
		6	n.d	n.d	n.d	n.d	n.d
	C20:2	**0**	**0.21**±0.05	n.d	n.d	n.d	n.d
		6		n.d	n.d	n.d	n.d
	C20:3n3	**0**	**0.38**^{a}±0.02	n.d	**0.17**^{b}±0.03	**0.13**^{b}±0.02	n.d
		6	n.d	n.d	n.d	n.d	n.d
	C22:2	**0**	**0.53**±0.05	n.d	n.d	n.d	n.d
		6	n.d	n.d	n.d	**9.83**±0.83	n.d
	C20:5+C21:0	**0**	**0.14**^{a}±0.01	**1.32**^{b}±0.01	**0.56**^{c}±0.05	n.d	**7.99**^{d}±0.10

	6	n.d	n.d	n.d	n.d	n.d
C20:3n6		**0.22**±0.05	n.d	n.d	n.d	n.d
	6	n.d	n.d	n.d	n.d	n.d

[a-d] mean values with different letters in the same raw differ statically significantly with 95% probability (p <0.05)

Table 2. The total content of SFA and USFA and their ratio of model fermented sausages after ripening (0) and after six months (6) of chilling storage (% of total fatty acids)

	C1		**C2**		**C3**		**A**		**D**	
	0	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**
∑SFA	60.04	40.80	59.72	38.90	59.55	35.44	57.00	34.83	51.08	38.95
∑MUFA	32.10	50.88	32.55	51.95	32.02	52.67	36.84	45.61	37.22	50.97
∑PUFA	7.86	8.32	7.73	9.15	8.43	11.89	6.16	19.56	11.70	10.08
SFA/USFA	1.50	0.69	1.48	0.64	1.47	0.55	1.33	0.53	1.04	0.64
PUFA/SFA	0.13	0.20	0.13	0.24	0.14	0.34	0.11	0.56	0.23	0.26
MUFA/SFA	0.53	1.25	0.55	1.34	0.54	1.49	0.65	1.31	0.73	1.31
MUFA/PUFA	4.08	6.12	4.21	5.68	3.80	4.43	5.98	2.33	3.18	5.06
MUFA n-9	26.53	35.26	27.71	36.26	27.08	37.60	32.63	34.26	26.74	39.14
PUFA n-3	1.59	5.72	1.83	7.47	1.66	9.34	1.01	7.38	8.36	7.37
PUFA n-6	6.28	2.61	5.92	1.72	6.76	2.53	5.19	12.20	3.34	2.70
n-6/n-3	3.93	0.46	3.23	0.23	4.07	0.27	5.14	1.65	0.40	0.37

SFA- saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; USFA- unsaturated fatty acids.

Conclusion

Palmitic (C16:0) and stearic (C18:0) acids were predominant of the saturated fatty acids (SFA) and oleic acid (C18:1) was the most represented unsaturated fatty acid (USFA) in all analyzed sample sausages. During six months of storage, it was noticed a decrease in SFA content and an increase in USFA content. The PUFA/SFA was within the recommended value, except in sample A after storage. Ratio n6/n3 PUFA also was within the recommended value after storage. Based on the obtained results, it can be concluded that incorporation of the plant extracts in natural casing had a statistically significant effect ($p<0.05$) on the content of the identified fatty acids in tested samples.

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References

Ahmad R.S., Imran A., Hussain M.B. (2018). Nutritional composition of meat. Meat Science and Nutrition. 61:77045.

Alabiso M., Maniaci G., Giosuè C., Di Grigoli A., Bonanno A. (2021). Fatty acid composition of salami made by meat from different commercial categories of indigenous dairy cattle. Animals, 11, 1060.

Amaral A.B., da Silva M.V., Lannes S.C (2018). Lipid oxidation in meat: mechanisms and protective factors – a review. Food Science and Technology, p.p. 1-15.

Barbir T., Vulić A., Pleadin J. (2014). Masti i masne kiseline u hrani životinjskog podrijetla (Fats and fatty acids in food of animal origin). Veterinarska Stanica 45 (2), p.p. 97-110.

BAS ISO 1443:2007. Meat and meat products - Determination of total fat content

Botsaris G., Orphanides A., Yiannakou E., Gekas V., Goulas V. (2015). Antioxidant and antimicrobial effects of *Pistacia lentiscus* L. extracts in pork sausages, Food Technology and Biotechnology. 53(4), p.p. 472– 478.

David F., Sandra P., Vickers A.K. (2005). Column selection for the analysis of fatty acids methyl esters, Agilent Technologies Palo Alto, CA, USA, p.p. 1-12.

Hromiš N., 2015., Razvoj biorazgradivog aktivnog ambalažnog materijala na bazi hitozana: sinteza, optimizacija svojstava, karakterizacija i primena (Development of biodegradable active packaging material based on chitosan: synthesis, optimization of properties, characterization and application). Doktorska disertacija, Tehnološki fakultet, Novi Sad

Hromiš, N., Šojić, B., Škaljac, S., Lazić, V., Džinić, N., Šuput D., Popović S. (2013). Effect of chitosan-caraway coating on color stability, lipid oxidation of traditional dry fermented sausage. Acta periodica technologica, 44, p.p. 57-65.

Król Ż., Kulig D., Marycz K., Zimoch-Korzycka A., Jarmoluk, A. (2017). The effects of using sodium alginate hydrosols treated with direct electric current as coatings for sausages. Polymers, 9(11), 602.

Lešić T., Krešič G., Kravar S.K., Pleadin J. (2017). Nutritional quality of fat in industrial sausages. MESO: Prvi hrvatski časopis o mesu, XIX (6), p.p. 496-503.

Liguori A., Belsito E.L., Di Gioia M.L., Leggio A., Malagrinò F., Romio E., Siciliano C., Tagarelli A. (2015). GC/MS analysis of fatty acids in Italian dry fermented sausages, The Open Food Science Journal 9(1), p.p. 5-13.

Man Y., Jaswir I. (2000). Effect of rosemary and sage extracts on frying performance of refind, bleached and deodorized palm olein during deep-fat frying. Food Chemistry, Volume 69, Issue 3, p.p. 301-307.

Nuñez de Gonzalez M.T., Hafley B.S., Boleman R. M., Miller R. K., Rhee K. S., Keeton J. T. (2008). Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation. Meat Science, 80(4), p.p. 997–1004.

Ozvural E.B., Huang Q., Chikindas, M.L. (2016). The comparison of quality and microbiological characteristic of hamburger patties enriched with green tea extract using three techniques:

Direct addition, edible coating and encapsulation. LWT - Food Science and Technology, Volume 68, p.p. 385-390.
Parunović N., Radović Č., Savić R. (2017). Sensory properties and fatty acids profiles of fermented dry sausages made of pork meat from various breeds. IOP Conference Series: Earth and Environmental Science, 85, 012014.
Pleadin, J., Vulić A., Lešić T., Demšar L., Polak T., Kovačević D. (2016). Sastav masnih kiselina tradicionalnih hrvatskih i slovenskih suhomesnatih proizvoda (Fatty acid composition of traditional Croatian and Slovenian cured meat products). Meso. vol. XVIII, br. 1, p.p. 44-52.
Raesi M., Tabaraei A., Hashemi M., Behnampour N. (2016). Effect of sodium alginate coating incorporated with nisin, *Cinamonum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of *Listeria monocytogenes* during refrigeration. International journal of food microbiology, 238, p.p. 139–145.
Shon J., Eo, J. H., Choi J. H. (2011). Gelatin coating on quality attributes of sausage during refrigerated storage. Korean Journal for Food Science of Animal Resources, 31(6), p.p. 834–842.
Stajić, S., Perunović, M., Stanišić, N., Žujović, M., Živković, D. (2013), Sucuk (turkish-style dry-fermented sausage) quality as an influence of recipe formulation and inoculation of starter cultures. Journal of Food Processing and Preservation, 37, p.p. 870-880.
Teixeira A., Fernandes A., Pereira E. (2020). Fat content reduction and lipid profile improvement in Portuguese fermented sausages alheira, Heliyon, Volume 6, Issue 10.
Tyburcy A., Wasiak P., Cegie A. (2010). Application of composite protective coatings on the surface of sausages with different water content, Acta Scientiarum Polonorum: Technologia Alimentaria 9 (2), p.p. 151-159.
Wójciak K. M., Karwowska M., Dolatowski Z. J. (2015). Fatty acid profile, color and lipid oxidation of organic fermented sausage during chilling storage as influenced by acid whey and probiotic strains addition. Scientia Agricola, 72(2), p.p. 124-131.
Yıldız-Turp G., Serdaroğlu M. (2008). Effect of replacing beef fat with hazelnut oil on quality characteristics of sucuk – A Turkish fermented sausage. Meat Science, 78(4), p.p. 447–454.

EFFECT OF THAWING METHODS ON THE PHYSICO-CHEMICAL PROPERTIES OF TURKEY MEAT

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Abstract

Freezing meat, as a method of canning, aims to maximize the storage period, while preserving the quality and nutritional value of the product after thawing. The aim of this paper was to examine the effect of thawing methods on the physico-chemical properties of turkey meat. Freezing of the meat samples was performed at a temperature of -20ºC. Prior to analysis, the samples were thawed in one of the following ways: 10 hours at 4°C (in a refrigerator), 2 hours at 20°C (at room temperature) and in a microwave oven (2450 MHz, 700 W), until a temperature of 4°C in the center of a meat piece. The following analyzes were performed: determination of weight loss, determination of moisture, ash, fats and proteins content, measurement of water binding capacity, instrumental measurement of color and texture, measurement of a_w value and pH value. Based on the results obtained in this paper, it can be concluded that thawing methods have a statistically significant effect ($p<0.05$) on the physico-chemical properties of turkey meat. The samples of turkey meat thawed in the refrigerator had the lowest weight loss (1.79%), the highest water content (75.34%) and the highest water binding capacity (2.65 cm^2), compared to the samples thawed in the microwave and at room temperature. It can be concluded that by slow thawing of turkey meat (at the refrigerator temperature), water has enough time to rebind to proteins, and in that case there will be less separated liquids.

Keywords: *Turkey meat, Thawing, Physico-chemical properties.*

Introduction

Freezing is frequently used for preserving food products in the food industry. Freezing is the process of canning food products at low temperatures. Due to heat dissipation, the temperature of the product drops below the freezing point (cryoscopic point), and water in the food is converted into a solid state (ice) (Nesvadba, 2008). That, practically, leads to the termination of chemical, biochemical and microbiological processes in food. The transition of water from a liquid state to ice, with a simultaneous increase in the concentration of dissolved substances, affects the reduction of water activity in food (Delgado and Sun, 2007). By reducing the activity of water below 0.7, the metabolic activity of most microorganisms is significantly decreased or completely stopped (Nesvadba, 2008). The more solid water becomes, the greater the stability of the frozen product (Nesvadba, 2008; Sablani *et al*., 2010). Freezing is an anabiotic canning procedure. That does not mean that the microorganisms are destroyed during the freezing of food. Instead, there are no conditions for their further activity in the frozen product (Pham, 2008; Sánchez-Alonso *et al*., 2012; Espinoza Rodezno *et al*., 2013; Sman, 2013). When the food is thawed, the microorganisms are reactivated. Refrigerated and frozen storage is widely used to

increase the shelf life of meat and meat products (Custódio *et al*., 2018; Rajkumar *et al*., 2020). However, the formation of ice during freezing causes physical and chemical changes in the product (Berry *et al*., 2008; Sučić *et al*., 2010; Gambuteanu *et al*., 2014; Li *et al.*, 2018). These changes are the result of ice formation and lead to changes in product quality after thawing. The intensity of the changes depends mainly on the freezing rate, storage conditions and thawing method (Fellows, 2000; Muela *et al*., 2012; Zequan *et al*., 2019).
After freezing and storage in the frozen state, most foods must be thawed before use. Thawing is the process opposite to freezing (Nesvadba, 2008). To melt the ice crystals, energy is brought into the food. In addition to the freezing rate and storage conditions, the quality of thawed food is also affected by the thawing rate. Frozen meat can be thawed in different ways. The most frequently used conventional methods are thawing in a refrigerator, in cold water, in a microwave oven, and at room temperature. New ways of thawing include high-pressure thawing, ohmic thawing, and acoustic thawing (Akhtar *et al*., 2013).
This paper aims to examine the influence of different thawing procedures (in the refrigerator at 4°C, at room temperature i.e., 20°C and in the microwave oven) on the physico-chemical properties of turkey meat (*Pectoralis major*).

Materials and methods

Analyses were performed on a sample of fresh turkey meat from the slaughterhouse. The pieces were cut into 2.0 cm thick slices, packed in polythene bags and, after marking, frozen at a temperature of -20ºC. After freezing the samples were kept at -20 °C for 24 h, until the analysis. All analyses were performed on fresh unfrozen samples after freezing and after certain defrosting treatments of the samples. This allowed a comparison of the measured parameters before freezing and after thawing. Before the analysis, the samples were thawed in one of the following ways: 10 hours at 4°C (in a refrigerator), 2 hours at 20°C (at room temperature) and thawing in a microwave oven (2450 MHz, 700 W) to the temperature of 4°C in the centre of the piece of meat.
The freezing/thawing weight loss was determined by measuring the weight of fresh meat samples before freezing and after thawing. Differences in weight were expressed as a percentage of the mass of fresh meat measured immediately before freezing.
The moisture content in the meat samples was determined using the drying method (BAS ISO 1442:2007).The total content of ash was determined gravimetrically after incineration at 550°C (BAS ISO 936:2007). Determining the content of fat in meat samples was performed by the extraction method (BAS ISO 1443:2007). The protein content was determined based on the total nitrogen content measured using the Kjeldahl method and multiplying by a 6.25 factor (BAS ISO 937:2007).
The compression method was used to measure the binding capacity of the turkey meat. Determining the water-binding capacity (the squeezed meat juice) was based on measuring the released water (juice) when pressure was exerted onto muscle tissue (Grau and Hamm, 1953). The difference between the surface, determined by the digital planimeter (Placom KP-92N, Koizumi, JAPAN), below the meat films and the total surface area (juice-moistened surface filter) was taken as a measure of the squeezed juice (cm^2).
Water activity (a_w) was measured by direct readings in Aw Meter (Novasina LabMaster-AW 1119971). The pH value was measured by inserting a pH meter (Hanna instruments, HI 2211)

into aqueous meat extract. The calibration of the pH meter was performed using standard buffer solutions before and during the reading of the values obtained.

Instrumental colour measurement was performed using a spectrophotometer CM-2600d (Konica Minolta Sensing Inc., Japan), with 8 mm port size, illuminant D65 and a 10° standard observer and after the standardization of the instrument concerning the white calibration plate. Colour parameters, expressed as CIE L*, a* and b* values, were determined as respective indicators of lightness, redness, and yellowness.

Texture was determined mechanically, using a Texture Analyzer TA.XT plus (Stable Micro Systems), which measures the shear force needed to cut the sample. Warner-Blatzler shear force was used with the HDP/BSK knife cutting blade. The load cell was 25 kg, the rate was 4.00 mm s^{-1}, and the distance was 20.00 mm. The test samples were prepared by cutting rectangular shapes of the meat (1×1 cm, length 5 cm) on which the measurement was performed. The instrument measured the force (kg) needed to move the knife cell a certain distance (mm) into the meat sample. Prior to measurement, the meat samples were heat-treated (in an airtight bag, in a water bath) until a temperature of 75°C was reached in the center of the product (Mandić et al., 2013).

All analyses were performed with three repetitions except for instrumental colour and texture measurement, which was performed with 10 repetitions.

Statistical processing of the obtained results was performed using the Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 computer program for Windows (Armonk, NY, United States). The results obtained in this paper are presented as mean values ± standard deviation (SD). The significance of differences between arithmetic means was determined by analysing the variance with one independent variable (One way ANOVA) and multiple interval tests (Dankan Post-hoc test) and expressed with 95% probability ($p<0.05$).

Results and discussion

As can be seen from the results presented in Table 1, the thawing method has a statistically significant influence ($p<0.05$) on the weight loss and chemical composition of turkey meat. The analysis showed that significant weight loss was observed in the meat thawed in the microwave (4.04%). This result differs significantly from the results obtained for meat thawed at room temperature (2.59%) and refrigerator temperature (1.79%). The formation of ice in the product during freezing causes physical and chemical modifications of the product. The main physical changes in frozen products are mechanical damage, water migration, ice recrystallization and water release during thawing (Sučić *et al.*, 2010; Savanović *et al.*, 2017). Since the specific volume of ice is higher than the specific volume of water, freezing can cause mechanical damage inside the product. The spread of ice crystals in the product causes tissue damage, and therefore food releases a certain amount of water after thawing.

The chemical composition of meat depends on the type of animal, race, sex, age, breeding and feeding method, nutritional status, and the part of the carcass from which the meat is taken. According to the share in the overall volume, water may be considered the basic meat component (Rede and Petrović, 1997). During thawing a part of the water comes out of the food after the transition from the solid to the liquid state. Fluid leakage is the result of the crystals formed by freezing and the inability of muscles to absorb the fluid during thawing (Delgado and Sun, 2007; Sučić *et al*., 2010; Savanović *et al*., 2019).

In addition to the freezing rate and storage conditions, the quality of thawed food can be partially affected by the thawing rate. Slow thawing (especially in meat) is believed to allow water more time to re-bind to proteins, which reduces the release of liquid. If the food is slowly thawed, the ambient temperature is not high, which has a somewhat inhibitory effect on microorganisms. Accordingly, slow thawing has a less detrimental impact on food quality (Vereš, 2004). Fresh samples of turkey meat had an average water content of 75.61% before freezing. After thawing, the highest water content was in those thawed in the refrigerator (75.34%). That was followed by the samples thawed at room temperature (74.78%), while the lowest water content was found in the samples thawed in the microwave oven (73.84%). An analysis of the basic chemical composition of the tested samples showed that, in parallel with the decrease in the water content of the thawed turkey meat, there was a statistically significant ($p<0.05$) increase in the content of other ingredients (proteins, fats, ashes). That is shown in Table 1.

Table 1. Weight loss and chemical composition of tested turkey meat samples

Parameter	Fresh meat	Thawing method		
		In refrigerator	At room temperature	In microwave oven
Weight loss (%)		1.79±0.09[a]	2.59±0.08[b]	4.04±0.20[c]
Water content (%)	75.61±0.13[c]	75.34±0.26[c]	74.78±0.18[b]	73.84±0.07[a]
Protein content (%)	22.30±0.18[a]	22.33±0.06[a]	23.13±0.07[b]	23.44±0.10[c]
Fat content (%)	0.52±0.04[a]	1.09±0.06[b,c]	0.82±0.04[b]	1.35±0.03[c]
Ash content (%)	1.16±0.04[a]	1.18±0.02[a]	1.20±0.06[a]	1.29±0.03[b]

[a-c] Mean values with a different letter in the same row are statistically significantly different from 95% probability ($p<0.05$)

The property of meat to retain water and water added under certain conditions, even when exposed to an external force such as pressure or heating, is known as the water-binding capacity (WBC) or water-holding capacity. The major water-binding carrier in muscles is myofibrillar proteins; this is due to their specific chemical structure (Rede and Petrović, 1997). Freezing of meat mainly results in a reduced water-binding capacity and an increased amount of liquid phase released during thawing. That is explained by changes in the protein state caused by the formation of ice crystals (Savanović and Grujić, 2017; Li *et al*., 2018). The fluid released during meat defrosting is affected by various changes taking place during the storage and freezing of meat. These changes can be freezing-related tissue damage (Li *et al*., 2018), protein oxidation (Estévez, 2011; Utrera *et al*., 2014) or changes in the isoelectric point of the protein (Estévez, 2011). The water-binding capacity is an indirect measure of protein denaturation (Savanović and Grujić, 2017). As can be seen from the results presented in this study, the thawing methods had a statistically significant influence ($p<0.05$) on the water-binding capacity of turkey meat after thawing (Table 2). The compression method showed statistically significantly higher ($p<0.05$) amounts of fluid released from meat samples thawed at room temperature (3.41 cm^2) and in a microwave oven (4.74 cm^2) compared with those thawed in refrigerator (2.65 cm^2) and with unfrozen (fresh) meat (2.31 cm^2). Therefore, the meat thawed using slower thawing methods had a greater water-retaining capacity and released less fluid during compression than meat thawed using faster thawing methods. In the released cellular fluid, water-soluble substances, minerals, vitamins and proteins are lost during defrosting of the product, which reduces its nutritional value (Liu *et al*., 2013). Dehydration of frozen products is a significant factor as it causes a decrease in weight and quality of the product, like changes in colour, appearance, texture etc. (Li *et al.*, 2018).The obtained results showed that thawing methods had no statistically significant effect ($p>0.05$) on the change in a_w and pH value of turkey meat (Table 2).

Table 2. Influence of thawing methods on water-binding capacity (WBC), aw and pH value of turkey meat

Parameter	Fresh meat	Thawing method		
		In refrigerator	At room temperature	In microwave oven
WBC (cm^2)	2.31 ± 0.23^a	2.65 ± 0.12^a	3.41 ± 0.36^b	4.74 ± 0.18^b
a_w	0.966±0.00	0.965±0.00	0.967±0.00	0.966±0.00
pH value	5.73±0.06	5.81±0.06	5.77±0.10	5.77±0.09

$^{a\text{-}b}$ Mean values with a different letter in the same row are statistically significantly different from 95% probability ($p<0.05$)

Table 3. shows the values of the instrumentally measured colour and texture parameters of the tested samples of turkey meat. From the obtained results, it was observed that thawing procedures had a statistically significant effect ($p<0.05$) on the colour and texture of turkey meat. On the surface of meat samples thawed in the refrigerator and those thawed at room temperature, a statistically significantly higher ($p<0.05$) value for illuminance (L* value) was measured than on fresh meat. The L* value on the surface of the samples thawed in the microwave oven was statistically significantly lower ($p<0.05$). In all three thawing methods, there was an increase ($p<0.05$) in the share of red colour (a* value) and yellow colour (b* value) compared to those measured on fresh meat.

Due to structural changes in the tissues, the texture of thawed meat is softer (as shown by the analysis results presented in Table 3). Fresh meat had the highest hardness (1.57 kg), and the meat thawed using other methods had lower hardness (it was softer).

Table 3. Instrumentally measured color and texture parameters of fresh and thawed turkey meat

Parameter	Fresh meat	Thawing method		
		In refrigerator	At room temperature	In microwave oven
L*	56.40 ± 1.80^b	58.41 ± 1.69^c	59.95 ± 1.65^c	53.21 ± 2.03^a
a*	-0.33 ± 0.23^a	1.24 ± 0.20^c	0.49 ± 0.13^b	1.10 ± 0.33^c
b*	7.79 ± 0.78^a	10.28 ± 0.87^c	9.96 ± 0.68^c	9.14 ± 0.88^b
Texture (kg)	1.57 ± 0.16^c	$1.08\pm0.14^{a,b}$	0.99 ± 0.07^a	1.13 ± 0.17^b

a-c Mean values with a different letter in the same row are statistically significantly different from 95% probability ($p<0.05$)

Conclusion

The results in this paper indicate that the thawing procedures affect ($p<0.05$) the physic-chemical properties of turkey meat. Turkey meat samples thawed in the refrigerator had the highest water content and the highest water-binding capacity compared to those thawed in the microwave and at room temperature. Based on the obtained results, it can be concluded that slow thawing of turkey meat (at the refrigerator temperature) allows water to re-bind to proteins, which results in a lower amount of separated liquids. If the food is slowly thawed, the ambient temperature is not high, which could have an inhibitory effect on microorganisms. In addition to changes in the basic chemical composition, different thawing procedures result in the change in colour and texture of thawed turkey meat.

References

Akhtar S., Khan M. I., & Faiz F. (2013). Effect of Thawing on Frozen Meat Quality: A comprehensive Review, Pakistan Journal of Food Sciences, 23(4), 198-211.

BAS ISO 1442:2007, Meat and meat products - Determination of moisture content

BAS ISO 1443:2007, Meat and meat products - Determination of total fat content

BAS ISO 936:2007, Meat and meat products - Determination of total ash

BAS ISO 937:2007, Meat and meat products - Determination of nitrogen content

Berry M., Fletcher J., McClure P., Wilkinson J. (2008). Effects of Freezing on Nutritional and Microbiological Properties of Foods. In Frozen Food Science and Technology; Evans, J. A.,Ed.; Blackwell Publishing Ltd: Oxford, UK, pp 26-50.

Custódio F.B., Vasconcelos-Neto M.C., Theodoro K.H., Chisté R.C., Gloria M.B.A. (2018). Assessment of the quality of refrigerated and frozen pork by multivariate exploratory techniques, Meat Science, 139, 7-14.

Delgado A.E., Sun D. (2007). Influence of surface water activity on freezing/thawing times and weight loss prediction, Journal of Food Engineering, 83(1), 23–30.

Espinoza Rodezno L.A., Sundararajan S., Solval K.M., Chotiko A.,Li J., Zhang J., Alfaro L., Bankston J.D., Sathivel S. (2013). Cryogenic and air blast freezing techniques and their effect on thequality of catfish fillets, LWT-Food Science and Technology, 54(2), 377-382.

Estévez M. (2011). Protein carbonyls in meat systems: A review, Meat Science, 89, 259–279.

Fellows P. (2000). Food Processing Technology – Principles and Practice, 2nd edition. Woodhead Publishing, Cambridge, UK, pp 418-440.

Gambuteanu C., Patraşcu L., Alexe P. (2014). Effect of freezing-thawing process on some quality aspects of pork Longissimus dorsi muscle, Romanian Biotechnological Letters, 19(1), 8916-8924.

Grau R., Hamm, G. (1953). Eine Einfache Methode zur Bestimmung der Wasserbindung in Muskel (A simple method for determining water retention in muscles), Naturwissenschaften, 40, 277-259.

Li D., Zhu Z., Sun D.-W. (2018). Effects of freezing on cell structure of fresh cellular food materials:a review, Trends in Food Science & Technology, 75, 46-55.

Liu D., Liang L., Xia W., Regenstein J. M., Zhou, P. (2013). Biochemical and physical changes of grass carp (Ctenopharyngodon idella) fillets stored at -3 and 0 °C, Food Chemistry, 140, 105–114.

Mandić, S., Vučić, G., Stojković, S. (2013). Kontrola kvaliteta mesa i proizvoda od mesa (Quality control of meat and meat products). Tehnološki fakultet, Univerzitet u Banjaluci.

Muela E., Sañudo C., Campo M.M., Medel I., Beltrán J.A. (2012). Effect of freezing method and frozen storage duration on lamb sensory quality, Meat Science, 90, 209-215.

Nesvadba P. (2008). Thermal Properties and Ice Crystal Development in Frozen Foods. In: Evans J. (ed). Frozen Food Science and Technology, Blackwell Publishing Ltd.

Pham Q.T. (2008). Advances in Food Freezing/Thawing/Freeze Concentration Modelling and Techniques, Japan Journal of Food Engineering, 9(1), 21–32.

Rajkumar R., Irshad A., Pandiyan C. (2020). Influence of Different Thawing Methods on Physico-chemical and Microbiological Characteristics of Broiler Chicken Meat, International Journal of Current Microbiology and Applied Sciences, 9,11, 1213-1220.

Rede R., Petrović Lj. (1997).Tehnologija mesa i nauka o mesu (Meat technology and meat science). Tehnološki fakultet Novi Sad, Srbija, pp 378-383.

Sablani S.S., Syamaladevi R.M., Swanson B.G. (2010). A Review of Methods, Data and Applications of State Diagrams of Food Systems, Food Engineering Reviews, 2(3), 168-203.

Sánchez-Alonso I., Martinez I., Sánchez-Valencia J., Careche M. (2012). Estimation of freezing storage time and quality changes in hake (Merlucciusmerluccius, L.) by low field NMR, Food Chemistry, 135(3), 1626–1634.

Savanović D., Grujić R. (2017). Protein changes during the freezing and storage of meat and meat products, Veterinary Journal of Republic of Srpska, Vol.XVII, No.1, 105–118.

Savanović D., Grujić R., Savanović J. (2019). The influence of the freezing rate on the physico-chemical properties of pork meat (*M. Longissimus dorsi*), Acta Periodica Technologica, 50, 228-235.

Savanović D., Grujić R., Mandić S., Savanović J. (2017). Uticaj smrzavanja na kvalitet prehrambenih proizvoda (Effect of freezing on food products quality), Proceedings V International Congress "Engineering, Environment and Materials in Processing Industry", March 15th-17th, Jahorina, Republic of Srpska, Bosnia and Hercegovina, 565-582.

Sman R.G.M., Voda A., van Dalen G., Duijster A. (2013). Ice crystal interspacing in frozen foods, Journal of Food Engineering, 116(2), 622–626.

Sučić R., Cvrtila Ž., Njari B., Kozačinski L. (2010). Senzorne, kemijske i mikrobiološke promjene u smrznutom mesu peradi (Organoleptic, chemical and microbiological changes of frozen poultry meat), Meso, 7(6), 342-351.

Utrera, M., Parra, V., Estévez, M. (2014). Protein oxidation during frozen storage and subsequent processing of different beef muscles, Meat Science, 96, 812–820.

Vereš M. (2004). Principi konzervisanja namirnica (Principles of food preservation), Poljoprivredni fakultet, Beograd.

Zequan X., Zirong W., Jiankun L., Xin M., Hopkins D.L., Holman B.W.B., Bekhit A.E.A. (2019). The effect of freezing time on the quality of normal and pale, soft and exudative (PSE)-like pork, Meat Science, 152, 1-7.

SENSORY PROPERTIES OF CHEESES OBTAINED BY DIFFERENT PROCESSES HEAT-ACID COAGULATION OF MILK

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Abstract

The sensory properties of food product have an influence on overall quality and consumers' product acceptance. Different factors during processing have an impact on quality characteristics of the final product. As a very important foodstuff in human nutrition, cheese can be produced in different ways and have different composition and sensory properties. The aim of this study was to examine the influence of coagulation conditions (type of coagulant and coagulation temperature) on the sensory properties of six produced cheese samples. Milk coagulation was performed at 85°C (samples 1,3,5) and 95°C (samples 2,4,6), and citric acid (samples 1 and 2), tartaric acid (samples 3 and 4) and acetic acid (samples 5 and 6)were used as coagulants. A team of 10 trained evaluators has performed sensory analysis of tested cheese samples by ranking samples according to acceptability and using a 5-point scoring system (1=very considerable deviation from expected quality to 5=no deviation from expected quality) to assess appearance (external and cross-sectional), odour, flavour and consistency. Based on the obtained results, it can be concluded that coagulation conditions have a statistically significant effect ($p<0.05$) on the appearance, flavour and consistency of produced cheeses. Sample five had the highest score (5.00) for external appearance, and the cross-sectional appearance was the best in sample four (score 4.80). The odour of all samples was excellent (score 5.00), while the samples five and six had slightly lower scores for flavour and consistency. Sample four was most acceptable by ranking according to overall acceptability.

Keywords: *Milk, cheese, sensory properties.*

Introduction

The organisation and implementation of product and process quality control are prerequisites for a successful business. In addition to specific instrumental and analytical methods, the modern food industry uses the results of the sensory analysis as a set of additional information on the quality of tested products. The sensory properties of the products are of great importance for the overall acceptability of food products (Radovanović and Popov-Raljić, 2000; Jimenez-Maroto *et al*., 2016; Lipkowitz *et al*., 2018; Kandasamy *et al*., 2020). For a product to become competitive on the market and be able to meet consumer expectations, special attention should be paid to sensory properties during the development and improvement of product quality.

Sensory assessment of food quality includes techniques that can be used to accurately measure food sensations while controlling and eliminating the impact of data on product origin and the trademark impact of a well-known competing producer and the impact of other factors on the acquired impressions about the product quality (Lawless and Heymann, 1999; Budimir *et al*., 2004). To be offered on the market, food products must meet the prescribed minimum quality

and safety requirements. Different sensory analyses of selected quality indicators are performed to examine the impact of various factors on dairy products quality (Papetti and Carelli, 2013; Lipkowitz *et al*., 2018; Vanbergue *et al*., 2018; Brighenti et al., 2020; Manzocchi *et al*., 2021).
The sensory properties of milk and dairy products are specific to each type or group of products if they are of appropriate quality, designed and shaped to be attractive and pleasant to consume. Cheese is the crown of dairy production, which is the oldest and safest form of concentrating and preserving the nutritional value of milk. Cheese is not only a valuable dairy product but has recently become a favourite food to enjoy, especially in countries with a high standard of living, where its consumption is also growing (Popović-Vranješ, 2015). A very important foodstuff in human nutrition, cheese can be produced in different ways, which results in different composition and sensory properties.
This study aimed to examine the influence of coagulation conditions (type of coagulant and coagulation temperature) on the sensory properties of cheeses obtained by heat-acid coagulation of milk.

Material and Methods

Cheese production was carried out at the milk processing plant (mini cheese factory) of the Secondary Agricultural School in Banja Luka. Six models of cheese samples were produced for this experiment. Pasteurized cow's milk containing 3.2% fat (produced by Pađeni Dairy, Bileća, Republika Srpska, Bosnia and Herzegovina) bought in a local supermarket was used to make cheese. The milk was stored in a refrigerator at +4°C before it was processed into cheese. Fresh pasteurized milk was heated to the appropriate temperature (85°C and 95°C), and citric, tartaric, and acetic acids were used to coagulate the milk (Table 1). After the addition of acid, the mixture was blended well and rested for 10 minutes to obtain curd. The resulting curd was filtered through gauze into a strainer. The hot drained curd was weighed and 1.5% of salt was added to it. After salting and mixing, the curd was placed in a mould for pressing. The pressing took one hour, and the load was 2 kg per one kilo of cheese. The finished cheese was cooled in a refrigerator to +4°C and stored at the same temperature until the analysis. The analysis was performed 24 hours after production at the Faculty of Technology, University of Banja Luka.

Table 1. Type of coagulant and coagulation temperature used for production of cheeses

Sample	1	2	3	4	5	6
Type of coagulant	Citric acid	Citric acid	Tartaric acid	Tartaric acid	Acetic acid (9%)	Acetic acid (9%)
Amount of coagulant (%)	0.30	0.30	0.30	0.30	1.50	1.50
Coagulation temperature (°C)	85	95	85	95	85	95

Sensory evaluation of the selected cheese quality indicators was performed by a group of ten trained evaluators. The assessed sensory properties were the external appearance, cross-sectional appearance, odour, flavour, and consistency. The assessment was performed using a 5-point scoring system (from 1 = very considerable deviation from expected quality to 5 = no deviation at all) (BAS ISO 22935-2:2011; BAS ISO 22935-3:2011), with an explanation of the given assessment.

To compare the overall sensory quality of the tested cheeses, the samples were ranked according to overall acceptability – from the most acceptable one (1st place) to the least acceptable one (6th place). The evaluators entered the sample codes in appropriate sections of the evaluation sheet. A sum of ranks was determined for each sample. The value of the sum of ranks was compared with the range of tabular values corresponding to the number of repetitions (n=10) and the number of treatments (6 ranked samples), which for the level of statistical significance $p<0.05$ was 25-45 (Radovanović and Popov-Raljić, 2000). If the sum of the ranks is less than the minimum numerical value of the table range, such product is designated as "superior". If the sum is higher than the maximum numerical value of the table range, the product is marked as "inferior". If the sum of the ranks is equal to the limit numerical values of the corresponding table range or is within it, the product is of "standard" quality.
Statistical processing of the results obtained by scoring method was performed using the Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 computer program for Windows (Armonk, NY, United States). The results obtained by scoring method are presented as mean values of the individual results ± standard deviation (SD). The significance of differences between arithmetic means was determined by analysing the variance with one independent variable (One way ANOVA) and multiple interval test (Dankan Post-hoc test) and expressed with 95% probability ($p<0.05$).

Results and discussion

The results of sensory analysis obtained by the scoring method are shown in Table 2. Following the results, it was found that coagulation conditions (type of coagulant and coagulation temperature) had a statistically significant effect ($p<0.05$) on external appearance, cross-sectional appearance, flavour, and consistency of produced cheeses. On the other hand, the statistically significant influence of coagulant type and temperature coagulation on cheese odour was not established ($p>0.05$). The sensory analysis showed that all cheese samples produced by heat-acid coagulation of milk were of high sensory quality.

Table 2. Results of sensory analysis obtained by scoring method

Sensory property	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
External appearance	4.50 ± 0.33^{b}	4.00 ± 0.24^{a}	4.00 ± 0.24^{a}	4.50 ± 0.33^{b}	5.00 ± 0.00^{c}	4.50 ± 0.24^{b}
Cross-sectional appearance	4.50 ± 0.33^{b}	4.00 ± 0.24^{a}	4.50 ± 0.24^{b}	4.80 ± 0.26^{c}	4.50 ± 0.33^{b}	4.00 ± 0.00^{a}
Odour	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Flavour	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}	4.50 ± 0.33^{a}	4.40 ± 0.21^{a}
Consistency	5.00 ± 0.00^{d}	5.00 ± 0.00^{d}	4.50 ± 0.24^{c}	5.00 ± 0.00^{d}	4.20 ± 0.26^{b}	4.00 ± 0.41^{a}

$^{a\text{-}d}$ mean values with different letters in the same row differ statically significantly with 95% probability ($p<0.05$)

The appearance of the cheese is assessed visually, usually before the cheese is consumed or when the cheese is prepared for consumption by cutting or spreading. The visual properties include the colour, presence of large lumps and visual texture. These create sensory expectations of what the cheese should taste. Since the appearance may prevail over other modalities, the visual aspects of cheese can often have a considerable impact on the experience of the other characteristics (Budimir *et al*., 2004). The colour of the cheese is another important indicator of quality. During the sensory analysis of the cheese appearance, the colour (that should be white and uniform, in correspondence to the quality of the given product) was evaluated. During the technological process, some ingredients may react with an undesirable substance that accidentally gets into the product, which shows deviations or problems during the process. In extreme cases, this can lead to errors that make the product unsuitable for use (Mandić and Perl, 2006).

In examined samples, the scores for the external appearance ranged from 4.00 (samples 2 and 3) to 5.00 (sample 5). Sample 5 had a smooth surface, shiny, uniform colour on the surface, while the other samples were slightly rough and uneven with noticeable yellowish transitions, which was scored 4.50 (samples 1, 4 and 6) and 4.00 (samples 2 and 3).

Sample 1, which was produced with citric acid at 85°C, had a yellowish, slightly uneven surface. Sample 2, which was produced with citric acid at 95°C, also had a yellowish colour and a somewhat rougher surface than sample 1. Sample 3, which was produced with tartaric acid at 85°C, had a noticeable uneven surface colour but the surface itself was smoother than in samples 1 and 2. Sample 4, which was produced with tartaric acid at 95°C, had a rough surface and uneven colour. Sample 5, which was produced with acetic acid at 85°C, had a uniform white colour and a smooth surface. Sample 6, which was produced with acetic acid at 95°C, had noticeable surface roughness.

The cross-section appearance of examined samples was evaluated with average scores from 4.00 to 4.80. The samples 2 and 6 had a mean value for the cross-section appearance of 4.00 because slight inhomogeneities, irregularities, and depressions were observed in the cross-section. The mean value of the cross-section appearance of samples 1, 3 and 5 was slightly higher and amounted to 4.50, while sample 4 earned the statistically highest ($p<0.05$) mean score of the cross-section appearance (4.80).

The odour of food products should provide intense pleasure, and it is analysed retro nasally – by inhaling and identifying volatile ingredients released in the mouth by evaporation during chewing, chopping, heating, and mixing with saliva. A smell uncharacteristic of the product may be a result of a mistake during production, which may render the product unusable for human consumption. The odour of the cheese depends on its type. Fresh, unripened cheese should have a pleasant odour, neither too strong nor too weak, characteristic of milk, discreet and without unpleasant odours. According to statistically processed evaluation data of the tested samples odour, it is noticeable that there is no statistically significant difference between the samples ($p>0.05$). This is because all tested samples received the maximum score for the odour (5.00).

The flavour analysis of the produced cheese and the palate impression examine the properties like salinity and acidity. The acidity should be slightly pronounced to create a refreshing sensation during chewing. Otherwise, the experience of flavour begins before consumption, when the consumer smells the cheese, but the main experience is gained during consumption (Budimir *et al*., 2004). The flavour of cheese depends on its type. Like the odour, the flavour of unripened cheese is also mild. The flavour should be clearly expressed, characteristic of the product and without uncharacteristic flavours. It should have a moderate flavour and salinity. The mean flavour scores of the tested cheeses are shown in Table 2. Based on the processed data, it can be stated that the mean values of the flavour scores of samples 1, 2, 3 and 4 did not differ statistically significantly ($p>0.05$). The mean flavour score of these samples was 5.00. Samples 5 and 6, produced with acetic acid, had lower ($p<0.05$) flavour scores (score 4.50 for sample 5 and score 4.40 for sample 6) than other samples.

Texture or consistency can be defined as a property of cheese that results from a combination of physical properties, including size, shape, number, nature, and conformation of structural elements experienced through a combination of the sense of touch (tactile texture), appearance (visual texture) and hearing (auditory texture). For example, the softness of fresh cheese can be assessed after cutting. Cheese texture characteristics often include elasticity, firmness, crumbliness, graininess, cohesiveness, and adhesion in the description (Mandić and Perl, 2006). The consistency of cheese depends on its water content. Based on that, cheese was classified into types, with each type having its characteristic consistency parameters. Table 2. shows the mean values of the consistency scores of the tested samples. The consistency of samples 1, 2 and 4 did not differ significantly ($p>0.05$); the samples had optimal hardness (neither too hard nor too soft) and were compact. The cheese was not sticky, and the consistency of the samples was scored 5.00. For sample 3, the consistency score was 4.50 because sample 3 was slightly softer than other samples. The consistency of sample 5 earned the mean score of 4.20 because the sample crumbled in the mouth and was slightly drier than other samples. Similar was sample 6, with the consistency score of 4.00 because it crumbled in the mouth and was noticeably drier than other samples.

Table 3. shows the results obtained by ranking the samples according to the overall acceptability of product quality. Based on the performed ranking, i.e., the sum of the ranks, sample 4 stood out compared to other cheese samples. Sample 4, produced with tartaric acid at 95°C, had a minimum sum of ranks (24), which was less than the minimum tabular value of 25. Therefore, this sample can be described as a "superior quality product". Other samples had sums of ranks within the tabular range (25-45). They were classified as "standard quality products".

Table 3. Ranking results according to the overall acceptability of product quality

Sample	Number of repetitions						Sum of ranks*	Cheese quality
	Place 1	Place 2	Place 3	Place 4	Place 5	Place 6		
1	3	1	2	2	2	0	29	Standard
2	2	0	2	2	2	2	38	Standard
3	0	2	2	2	2	2	40	Standard
4	2	4	2	2	0	0	24	Superior
5	2	1	2	0	2	3	38	Standard
6	1	2	0	2	2	3	41	Standard

*The range of tabular values corresponding to the number of repetitions (n=10) and the number of treatments (6 ranked samples), for the level of statistical significance $p<0.05$ is 25-45 (Radovanović and Popov-Raljić, 2000).

Conclusions

In food quality control procedures, sensory analysis methods play an indispensable role. Quality control and precise definition of different types of cheese can be performed by applying sensory methods of analysis. Based on the results obtained in this paper, it can be concluded that heat-acid coagulation of milk can produce cheeses of high sensory quality. The appearance, flavour and consistency of the produced cheeses depend on the type of coagulant and coagulation temperature. By ranking the samples according to the total acceptability, sample 4, produced with tartaric acid at a coagulation temperature of 95°C, was the most acceptable.

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References

BAS ISO 22935-2:2011. Milk and milk products - Sensory analysis - Part 2: Recommended methods for sensory evaluation.

BAS ISO 22935-3:2011. Milk and milk products - Sensory analysis - Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring.

Brighenti M., Govindasamy-Lucey S., Jaeggi J. J., Johnson M. E., Lucey J. A. (2020). Behavior of stabilizers in acidified solutions and their effect on the textural, rheological, and sensory properties of cream cheese. *Journal* of *Dairy Science*, 103, 2065–2076.

Budimir J., Marić S., Kubiček R., Spaho N. (2004). Senzorska analiza (Sensory analysis). Tehnološki fakultet Univerziteta u Tuzli, Tuzla.

Jimenez-Maroto L. A., Lopez-Hernandez A., Borneman D. L., Rankin S. A. (2016). A comparison of fresh, *pasta filata*, and aged Hispanic cheeses using sensory, chemical, functional, and microbiological assessments. Journal of Dairy Science, 99, 2680–2693.

Kandasamy S., Yoo J., Yun J., Byul Kang H., Seol K.-H., Ham J.-S. (2020). 1H HRMAS-NMR based metabolic fingerprints for discrimination of cheeses based on sensory qualities. Saudi Journal of Biological Sciences 27, 1446–1461.

Lawless T. H., Heymann H. (1999). Sensory evaluation of Food, Principles and Practices. A Chapman & Hall Science Book, Gaithersburg, Maryland.

Lipkowitz J. B., Ross C. F., Diako C., Smith D. M. (2018). Discriminating aging and protein-to-fat ratio in Cheddar cheese using sensory analysis and a potentiometric electronic tongue. *Journal* of *Dairy Science,* 101, 1990–2004.

Mandić M. L., Perl A. (2006). Osnove senzorske procjene hrane (Basics of sensory evaluation of food). Prehrambeno - tehnološki fakultet, Osijek.

Manzocchi E., Martin B., Bord C., Verdier-Metz I., Bouchon M., De Marchi M., Constant I., Giller K., Kreuzer M., Berard J., Musci M., Coppa M. (2021). Feeding cows with hay, silage, or fresh herbage on pasture or indoors affects sensory properties and chemical composition of milk and cheese. Journal of Dairy Science, 104, 5, 5285-5302.

Papetti P., Carelli A. (2013). Composition and Sensory Analysis for Quality Evaluation of a Typical Italian Cheese: Influence of Ripening Period. Czech Journal of Food Sciences, 31, 438–444.

Popović – Vranješ A. (2015). Specijalno sirarstvo (Special cheese making). Poljoprivredni fakultet Univerziteta u Novom Sadu, Novi Sad.

Radovanović R., Popov-Raljić J. (2000). Senzorna analiza prehrambenih proizvoda (Sensory analysis of food products). Poljoprivredni fakultet Beograd, Tehnološki fakultet Novi Sad, Beograd-Novi Sad.

Vanbergue E., Hurtaud C., Peyraud J.-L., Beuvier E., Duboz G., Buchin S. (2018). Effects of n-3 fatty acid sources on butter and hard cooked cheese; technological properties and sensory quality. International Dairy Journal, 82, 35-44.

NUTRITIONAL VALUE OF TRAPPIST CHEESE

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Abstract

The objective of this paper is to present research findings, obtained for nutritional values of Trappist cheese, produced by the monks of the Banja Luka "Maria Zvijezda" monastery, in cooperation with Agricultural Cooperative Livac. Cheese is an important food and is a part of people's daily nutrition. New trends that are promoting a balanced diet are also setting new requirements for the familiarity with nutritional value of cheeses. Until now there has been no opportunity to analyse nutritional value of Trappist cheese due to the specificity of its production. Some analyses might have existed, but the library was destroyed back in 1945, and possibly written traces if there had been any. Some of the characteristics of Trappist cheese are that it is in the shape of a ring, it weighs between 1,6 kg and 2.0 kg, and it has a natural rind, which is yellowish, thin and smooth. The conditions for its ripening and the manner of its ripening are specific and they give this cheese a special aroma. The ripening period is minimum 90 days, under specific air humidity and temperature conditions. The cheese matures on wooden shelves, being regularly turned around and wiped by hand. The consistency of cheese body is soft, elastic, tender and it is easy to cut. The cut is smooth, without or with just a few holes, while the colour of cheese body is yellowish. It is characterized by clean, milk-specific aroma, and is moderately salty with quite meltable texture. The milk fat content is 34,5 grams, saturated fatty acids 24,7 grams, carbohydrates 0.8 grams, proteins 27.5 grams and the content of NaCl amounts to 2,05 g/100 grams of cheese. The energy value of Trappist cheese is 424 kcal or 1758 kJ, Water content is 41.6 g/100 grams, while the dry matter amounts to 56,8 g/100 g.

Key words: *nutritional values, monastery Maria Zvijezda, cheese Trappist.*

Introduction

At the present time, as a result of fast-paced lifestyle and stress, people are aware of the need to pay more attention to proper nutrition. A balanced diet has become an integral part of our lives and is an unavoidable aspect of finished products' labelling.

Milk and dairy products have always been a part of the diet, having a beneficial effect on humans. Nowadays we are striving for newly set goals, which is to prevent certain diseases, in other words to protect our health by means of food (German et al. 2003). We may find on the market a wide choice of milk and dairy products, which base their functional properties on their natural, high-value nutritional composition (Tudor and Havranek, 2009).

Cheese is a type of food having high nutritional value and it takes a significant place in a healthy and well-balanced diet (Gregurek, 2015). The nutritional value of cheese is affected by numerous parameters, of which the most important one is milk, used for the cheese production, followed by the cheese making technological process and finally the cheese ripening period. As such, cheese represents a very good source of proteins and milk fat. On the other hand, it is a good source of fat, which is vitamin-meltable, and in case of mature cheeses (with longer aging time) there is a slightly lower lactose content, which makes them suitable for the diet of consumers who are lactose-intolerant.

Table 1. Composition of some types of cheese (Thomas et al. 2000)

Type of cheese	Water g/100g	Protein g/100 g	Fat g/100g	Carbohydrates g/100g	Cholesterol mg/100g	Energy value	
						kcal	kJ
Brie	48,6	19,2	26,9	Traces	100	319	1323
Camembert	50,7	20,9	23,7	Traces	75	297	1232
Cheddar	36,0	25,5	34,4	0,1	100	412	1708
Cheddar with less fat	47,1	31,5	15,0	Traces	43	261	1091
Cottage sir	79,1	13,8	3,9	2,1	13	98	413
Edam	79,1	13,8	3,9	2,1	80	338	1382
Emmentaler	35,7	28,7	29,7	Traces	90	382	1587
Feta	56,5	15,6	20,2	1,5	70	250	1037
Gauda	40,1	24,0	31,0	Traces	100	375	1555
Gryère	35,0	27,2	33,3	Traces	100	409	1695
Mozzarela	49,8	25,1	21,0	Traces	65	289	1204
Parmesan	18,4	39,4	32,7	Traces	100	452	1880
Ricotta	72,1	9,4	11,0	2,0	50	144	599
Roquefort	41,3	19,7	32,9	Traces	90	375	1552

Milk fat in cheese affects its firmness, cohesiveness of cheese body, fullness of taste and cheese aroma. It directly influences nutritional values of cheese, and as we can see in the Table 1, some cheeses contain large amount of fat (Cheddar, Gryère). Given that the total fat intake is being limited nowadays, while a large number of consumers are trying to endorse recommendations of nutritionists, the modern food industry is producing more and more cheeses with a lower proportion of milk fat. The cholesterol content in cheese varies from 13 -100 mg/100g of cheese, depending on the type of cheese. A good number of people display certain changes in the blood cholesterol if their intake of cholesterol through food is 250-800 mg/day (McNamara, 1987).

The protein percentage in cheese ranges from 3-40% depending on the type of cheese. Of the proteins in cheese, the most represented one is casein, given that the largest quantity of whey protein is separated from curd into whey (Gregurek, 2015).

Salt (NaCl) plays an important role in the cheese production process, while its concentration in cheese depends on the technology itself. The increased intake of salt can be the cause of hypertension, which is a risk factor for coronary and heart diseases (Gregurek, 2015).

In Bosnia and Herzegovina, there are several high-value cheeses with the long production tradition. They are of different qualitative properties and energy values (Dozet et al. 1979). All this represents a significant heritage of this region.

Table 2. Qualitative and energy values of autochthonous cheeses in BiH (Dozet et al. 1979).

Type	Fat %	Protein %	Energy value	
			kcal	kJ
Travnik cheese	25,39	19,807	326,44	1366,5
The Travnik cheese type	24,1	20,414	307,83	1288,42
Feta	21,0	22,634	288,1	1205,84
Livno cheese	32,03	27,357	410,01	1716,22
Sirac	29,3	29,612	393,9	1648,6
Low-fat cheese	9,25	33,354	222,78	932,44
Fat cheese	20,67	15,875	256,67	1074,3
Low-fat cheese	4,27	21,696	128,42	537,48
Dry cheese	39,5	31,172	495,2	2072,48

Table 2. depicts qualitative and energy value of some autochthonous cheeses, being produced in Bosnia and Herzegovina either traditionally or industrially (Dozet et al. 1979).

History of Trappist cheese

The very start of cheese production in the Maria Zvijezda monastery was in 1872, in a small cheese factory built by Abbot Franz, who named his cheese "Swiss cheese". However, the operation of this cheese factory did not last for too long due to a cattle disease which caused a lack of milk. The production of "real" Trappist cheese began in 1882, when brother Ignatius, from the French monastery „Port-du-Salut", came to the Banja Luka monastery. He spent half a year training brother Luka in cheese making. Initially, the cheese was being made only for the monastery community, but later on it was produced for the market of Austria and Hungary, but also for the entire Europe. There was a very high demand for it, while this cheese won numerous awards at different European fairs. At first, the monks were processing milk from their own property, but later on they started purchasing it from the local farmers (Bozinovic 2009).

Maria Zvijezda monastery kept the auxiliary cheese-making facility, but the real cheese factory was built in Josipovac (nowadays Bosanski Aleksandrovac), where a branch of the Maria Zvijezda monastery was opened in 1887.

However, not everything went smoothly there either, especially in the first year. Basically, due to a lack of experienced cheese makers, they initially faced problems with the cheese quality. That is why Abbot Bonaventure I sent brother Dositej to France in 1888, where he spent one year in the French monasteries and finally in Port-du-Salut learning how to make cheese. Upon his return, he started training his brothers on cheese making process, who had to take an oath to keep the cheese production process a secret. This secret was passed down from one brother to another, or they would take a secret with them to the grave. Kirin wrote about this: Although Trappist cheese was predominantly represented in the cheese making activities for an entire century, there is very little information about the cheese making technological procedure in the professional literature. Due to its secrecy, there is no official description of the production process and there is no original Trappist cheese, therefore we can only assume what it was like (Budimir and Bagaric 2015).

A conclusion may be drawn from this secrecy: "The quality of cheese and the art of its making rested for the most part in the method of its production. Basically, a dozen of specialists partook in the cheese production. Each operation was exclusively performed by one of the cheese makers. Each cheese maker mastered his task to the perfection, while the work of others remained a secret to him." A success of the Josipovac branch prompted Abbot Bonaventura I to found another branch. The colonists were marking a significant economic development in the Windthrst colony (nowadays Nova Topola). The Trappist monks purchased the land from one of the colonists in 1893. and founded Marienburg branch – Marijin Dvor (Nova Topola) there. The cornerstone was laid on 18 March 1893. Apart from other economy-oriented facilities, they also opened a cheese factory. The locals would bring them milk which they processed to make cheese and butter (Bozinovic, 2009).

Cheese production in both branches was successful. Everyday they would buy 2000-3000 litres of milk. With the expansion of this cheese factory, the purchase of milk reached as much as 8000 litres. There was 100-200 tons of cheese produced every year. The surplus of purchased milk was pasteurized and transported to Banja Luka, where it was offered for sale. The milk was appreciated both for its quality and its price. It was cheaper than the milk from other retailers. The cheese was packed in 4.8 kg package and was mailed, i.e. it was shipped by rail to the customers throughout the Monarchy, but also beyond its borders. The monks were also official supplier of cheese for the Royal Palace in Belgrade (Bozinovic, 2009).

So far, there have been no reliable data on the organoleptic properties of the original Trappist cheese, while the secrecy of its making does not allow us to get an insight into the production technology. Various authors assume that the technology was identical to today's Saint Paulin cheese, which is a successor to Port-du Salut i Port-Salut (Kirin 2003). It was mainly chemical composition, quality and organoleptic properties of semi-hard cheeses, produced in the former communist dairies that were analysed, (Budimir and Bagaric, 2015).

The cheese production was revived again in 2008 when Father Tomislav went to France in the monastery of Mont-des-Cats and learned the technique of cheese making and brought back the recipe for cheese production (Budimir, 2012).

Materials and methods

The analysis and sampling was carried out at the ZZ Livac farm, located in Aleksandrovac, the municipality of Laktasi, Bosnia and Herzegovina. This agricultural cooperative is engaged in raw milk production. Since 2008, the Maria Zvijezda Trappist cheese has been produced in cooperation with the Trappist monks in the newly built cheese-making plant, according to the recipe owned by the Maria Zvijezda monastery. Currently, about 2.5 tons of Trappist cheese is being produced on a monthly basis. The cheese factory has HACCP and ISO 2008:2009 certificates and is a subject to regular veterinary inspection control. The cheese factory has an export license for the European Union, and as such it abides by all applicable hygiene and health regulations.

The sampling was performed in 2020, where 5 cheese samples were taken in accordance with the Republic of Srpska regulations for food analysis, currently in force.

Physicochemical analysis was conducted by the Public Health Institute, the Republic of Srpska Public Health Institute, the Department for Sanitary Chemistry, Food Testing Laboratory, being an accredited laboratory for food analysis in Bosnia and Herzegovina.

The tests were performed in order to establish: the content of milk fat according to UMH 158 method; of saturated fatty acids content, UMH 466: of carbohydrates as per UMH 356; and of sugar, UMH 159. As for the protein content, the tests were carried out based on the Bosnia and Herzegovina Official Gazette, No. 82/13, Annex II, Section V and UMH 076 method was applied in order to determine NaCl content.

Results and discussion

Trappist cheese belongs to a group of semi-hard cheeses, easy to cut. It differs from other products present on the market under the same name which are either too soft or too hard to cut. Unfortunately, given that in the past there were no restrictions in using this original cheese name, different variants of Trappist cheese can be found on the market.

The softness and easy cutting are a result of the special method of preparation and definitely come from special conditions under which it matures. It is important to highlight here that the original Trappist cheese ripens under specific conditions, where adequate everyday procedures of turning it around and wiping it are being applied. In Bosnia and Herzegovina, the technological production of Trappist cheese takes place in the facilities where the cheese is "dried" for fifteen days and is then delivered to stores. For the original Trappist cheese to mature it takes between 75 and 90 days, which ensures its special consistency and taste.

It is in a shape of a ring, with 980 cm diameter and the size of 7-9 cm (Budimir D. 2012).

Table 3. Analysis of the composition of the Marie Zvijezda Trappist cheese

Parameter	g/100 g
Fat	34,5
Saturated fatty acids	24,7
Carbohydrates	0,8
Sugar	0,0
Protein	27,5
NaCl	2,05
Energy value in 100 g: 424 kcal o 1758 kJ	

As mentioned earlier, no previous analysis of the Trappist cheese exists, therefore in the further discussion we will compare it with some of the above listed cheeses.

Table 3 shows that there is 34,5 grams of fat in Maria Zvijezda Trappist cheese. Given that this cheese is made from full-fat milk, and has somewhat longer ripening time, we are to expect such a slightly higher fat content. The fat content is approximate, i.e. it is a bit higher than in Cheddar or Gryère. If we are to compare it with the BaH cheeses, the fat content is somewhat higher than in the local Livno cheese/Livanjski sir (made from sheep milk) but lower than in the autochthonous "dry" cheese from Bosnia and Herzegovina.

The content of saturated fatty acids in Trappist cheese amounts to 24.7 grams in 100 grams of cheese. The obtained result is relatively high, but due to an increased fat content such a result is in correlation with the previous parameter. In the tested samples, the content of carbohydrates was 0.8 grams in 100 grams of cheese. This result represents a mean value in comparison with earlier analysed cheeses. It is somewhat lower when compared with Ricotta, Edam or Feta, but is higher than in Cheddar, Gryère. The protein content observed in the analysis was 27.5 grams. This is similar to Gryère and Emmentaler, and Livno cheese, but is slightly lower than is the case with Sirac. On the other hand, it is much lower than the protein content in the cheese in a sac or Parmesan.

The salt content is 2.05 grams in 100 grams of cheese, and no sugar was found in the samples.

The energy value of 100g of Maria Stern Trappist cheese is 424 kcal, or 1758 kJ. These values are close to the energy content of Cheddar or Gryère, and when compared with autochthonous Bosnian cheeses, it is identical to Livno cheese, but slightly lower than in dry cheeses.

All of the above results indicate that it is a full-fat, high-energy product.

Conclusion

It was the 160th anniversary of the Trappists' arrival to Banja Luka in 2019. With their arrival they contributed to the improvement of both economic and cultural development of this region, but unfortunately very little is known about this. What the Cooperative is trying to achieve is to bring the activities of the monks, or at least a part of their craft history, closer to the public. This community was affected by difficult moments several times in the past, especially in the end of the II World War. Just like their community was striving to retain the population in this area and provide them with the employment opportunities, we are trying to achieve the same in the present time. We hope that we will manage to employ an additional number of people and pursue the production of the Maria Zvijezda Trappist cheese, as well as the manufacturing of other products nourishing the tradition of the monks' community. The original Trappist cheese belongs to a group of semi-hard, full-fat cheeses with high energy value, and it can be consumed on a daily basis, either as a side dish or an ingredient in the meal preparation. This paper provides the grounds for the further analysis of Trappist cheese and the possibility for the growth of small cheese producers on the territory of Bosnia and Herzegovina, through their adequate protection.

References

Božinović, I. (2009): Series on the occasion of the 140th anniversary of the arrival of the Trappists, Nezavisne novine, Banja Luka.

Budimir, D. (2012): Organoleptic properties of Trappist cheese, 40th Croatian Symposium of Dairy Experts with International Participation, Lovran, Croatia, Proceedings, 43-44.

Budimir, D., Stipić Bagarić, M (2015): „History of trappist chesse" Technologica Acta, Vol. 9. Number 2., page 11-16.

Dozet, N., Stanišić, M., Bijeljac, S. (1979): Quality value of milk and dairy products in the hilly and mountainous area of BiH, Mlijekarstvo 29/1, 2 - 6.

German, J.B., Morgan,C.J., Ward,R.E. (2003): Milk: a model for nutritions in the 21 st century. The Australian Journal of Dairy Tehnology, vol. 58, No 2, 49-54.

Gregurek Lj., (2015): Cheese production - theory and practice, Probiotik d.o.o, Zagreb

Kirin, S. (2003): 120 years of Trappist cheese, Mljekarstvo, 53 (1), 51-64, Zagreb.

McNamara, D.J.(1987): Effects of fat-modified diets on cholesterol and lipoprotein metaabolisam, Annual Review of Nutritions,7, 273- 290

Thomas, P., O'Connor, T.P., O'Brien, N.M (2000): Nutritional aspects of cheese, Fundamentals of Cheese Science, An Aspen Publications , Maryland,504 – 514.

Tudor M., Havranek J., (2009): Nutritional and health value of fermented milk. Croatian Journal of Food Technology, Biotechnology and Nutrition 4 (3-4), 85-91.

INFLUENCE OF THE AGE OF LAYING HENS ON EGG PRODUCTION INTENSITY OF DIFFERENT EGG WEIGHT GROUPS

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Abstract

The aim of this paper is to examine the egg production of commercial flock of light line hybrids Lohmann Brown, as well as the influence of laying hen age on egg production intensity of different egg weight groups, housed in conventional cage system. The examinations were performed in the production conditions on the farm "Agrovet" in Foca, Bosnia and Herzegovina. Since it is established that commercial flocks in egg production are kept until the end of 72nd week of age (WA) as the final instance in production, the aim of this paper is to examine what happens to production performance, ie laying intensity, after the established 72nd week of laying hen age. In this paper, it is the period of seven weeks, until the 79th week of age of the laying hen of the Lohmann Brown hybrid, where the production period lasted for 61 weeks, ie 427 days.
Based on the results of examination, it was concluded that egg production, as well as laying intensity gradually increase to 27th week of age, when the maximum laying intensity was reached, followed by gradual decline in egg production until the end of the flock exploitation period, with occasional variations.
Analized by the weight groups, during 61 weeks of production, the class M was the most represented with a laying intensity of 47.47%, followed by the class L (31.64%), class XL (3.12%) and then class S with 1.79%.
A positive coefficient of phenotypic correlation was determined between the age of laying hen and laying intensity in all egg weight groups. It was statistically very highly significant ($p<0.001$) until 36th week of age, highly significant between 36th and 42nd week of age ($p<0.01$), and significant from 42nd to 52nd week of age ($p<0.05$).

Key words: *Lohmann Brown, Age, Egg production intensity, Egg weight groups.*

Introduction

Today, intensive egg production is based on exploiting of light-line hybrids of lying hen, which are characterized by great production opportunities (Mahmutovic, 2011). The hybrids for egg production are divided in hybrids for production of eggs with coloured shell and eggs with white shell. The hybrids for production of eggs with coloured (brown) shell are larger, heavier, with stronger constitution, calmer temperament, and more resistant to deseases than hybrids for production of eggs with white shell. The most famous hybrids in our country are Isa Brown

(SSL), Hisex Brown, Lohmann Brown, Tetra SL, Harco (Bogosavljevic-Boskovic and Mitrovic, 2005). Line hybrids for the production of coloured eggs, due to higher demand, are more represented in the world, and in our country as well. Our research is conducted on coloured eggs hybrid Lohmann Brown. Laying intensity is the most important feature of laying hen, representing the number of eggs laid in a certain period of time per one laying hen, expressed as a percentage. Rakonjac (2016) believes that the success in the production of eggs for consumption largely depends on the choice of the most productive and most suitable hybrid of laying hen, which would give the best results in appropriate farm conditions. Neglecting any of these factors will have a negative impact on the health of laying hen, consequently on the production and cost-effectiveness of keeping the flock. Based of the results of research by Rakonjac (2016) conducted on light-line hybrid Isa Brown, the average laying intensity was 77.88%, and the maximum intensity was achieved at 28^{th} week of age. Also, in the last week of the experiment, the Isa Brown hybrid achieve the laying intensity of 72.40%.

Examining the production performance of laying hen hybrid for brown egg shell Hy-Line Brown, Vukic-Vranjes et al. (2009) found the maximum laying intensity of 92.10% at 28^{th} week of age. Based on the examination of production characteristics of the Lohmann Brown hybrid laying hen, in the period from 20^{th} to 72^{nd} week of age, Pandurevic (2011) came to the following conclusions: The laying intensity in the 20^{th} week of age was 18.29%; the maximum laying intensity was reached in 28^{th} week of age and was 95.71%; in the middle of production cycle (48^{th} week of age) the laying intensity was 89.57%, while at the end of production cycle (72^{nd} week of age) 4.91 eggs were produced, ie intensity of laying was 70.14%.

Viewed by classes, the class L has the largest share in total number of eggs (49.09%), followed by class M with 38.57%, while classes XL and S has significantly less share with 7.68% and 4.66% respectively.

Examining the production of eggs for consumption of hybrid Isa Brown, Pandurevic et al. (2015) found that the highest average egg production was in third month of production, at 93.60%. After that, the intensity of producing eggs decrease to 69.90% when the flock was excluded of production cycle. Examining the egg production of Hisex Brown hybrid, Gjorgovska et al. (2016) found the average intensity of egg production was around 82%.

The average intensity of producing eggs of Lohmann Brown hybrid, kept in conventional cage from 20^{th} to 60^{th} week of age, obtained in research by Dikmen et al. (2017) amounted 87.10%.

Since it is established that commercial flocks in egg production are kept until the end of 72^{nd} week of age (WA) as the final instance in production, the aim of this paper is to examine what happens to production performance, ie egg laying intensity, after the established 72^{nd} week of laying hen age. In this paper, it is the period of seven weeks, until the 79^{th} week of age of the laying hen of the Lohmann Brown hybrid, where the production period lasted for 61 weeks, ie 427 days.

Material and Methods

As the initial material for the research, we have used hens of light line hybrids Lohmann Brown, housed on the farm "Agrovet" Foča, with at least 18 weeks of age, i.e. the experiment started in the 19^{th} week of age of laying hens. Production cycle lasted for 61 weeks (79 weeks old hens).

In the course of raising (exploitation) of the commercial flocks, a technology proposed by the selector of laying hens hybrid was used. All technological phases (feeding, power, temperature, lighting, ventilation, drainage system and collecting the eggs) are automatically regulated.

The eggs were sorted daily, into four weight groups, according to EU standards adopted in 2012, as follows: XL – very large (egg weight >73 g), L – large (63-73 g), M – medium (53-63 g), S – small (egg weight <53 g).

According to technological norms, the commercial flock of light line hybrid starts delivering eggs in the 19th week of age (production intensity around 5.50%), maximum intensity reaches between 26th and 28th week of age (around 94%), then gradually decreases until the end of flock exploitation in 72nd week of age, when the intensity was around 72%. On average, for 53 weeks of egg production, the laying intensity is about 85% and about 316 eggs per settled laying hen.

Based on everyday records of egg production during the experiment, the intensity of egg production was defined by weight groups (S, M, L, XL), by weeks of production (%) and for the entire production cycle (61 weeks, from 19th to 79th week of age), for all egs and by different weight groups. The average laying intensity within production week was calculated by dividing the total number of delivered eggs during the week by the number of hens and then dividing by seven. The obtained results are expressed as a percentage for each week of production and in total for the entire period of production.

Based on the obtained results, a phenotypic correlation was determined between the age of commercial flock and the intensity of producing eggs (from the 14th week of exploitation to the end of the production cycle).

The coefficient of phenotypic correlation is calculated according to pattern (Latinovic, 1996):

$$r_{xy}=\frac{\sum xy-\frac{\left(\sum x\right)\left(\sum y\right)}{n}}{\sqrt{\left[\sum x^2-\frac{\left(\sum x\right)^2}{n}\right]\left[\sum y^2-\frac{\left(\sum y\right)^2}{n}\right]}}$$

The strength of phenotypic correlation coherence is discussed on the basis of the Roemer-Orphal classification (Latinovic, 1996). The testing of significance of differences between various ages of laying hens, in terms of monitored characteristics, was performed using an appropriate t-test model with the same number of repetitions according to the following pattern:

$$t=\frac{\bar{x}_1-\bar{x}_2}{Sp\sqrt{1/n_1+1/n_2}}$$

$$S_p=\sqrt{\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}}$$

The software package SPSS - Statistical Package for Social Sciences was used for statistical analysis. As statistically significant were taken differences $p<0.05$.

Results and Discussion

Egg production is the most important feature of laying hen and it is important to produce as many eggs as possible during the production cycle, as the success of the production primarily depends on this parameter. At the same time, the intensity of production should be in accordance with biological and physiological abilities of the laying hen at a given age, as well as to achieve optimal share of various weight groups of eggs for a certain period of exploitation.

It should be noted that relatively small number of authors examined the production characteristics of laying hen after the usual 72 weeks of age.

The eggs on the farm "Agrovet" were collected daily and, according to EU standards, classified by weight into four groups: S (<53 g), M (53-63 g), L (63-73 g) and XL (>73 g).

Table 1 shows the values of egg laying intensity by weight groups (%).

Table 1. Egg laying intensity according to the weight groups (%)

Weeks of age/production	S (<53 g)	M (53-63 g)	L (63-73 g)	XL (>73 g)	All eggs
$WA_{19/1}$	27.00	1.48	0.07	0.13	28.68
$WA_{20/2}$	22.12	39.29	0.47	0.36	62.24
$WA_{21/3}$	19.70	61.88	0.94	0.61	83.16
$WA_{22/4}$	18.64	68.04	1.72	1.11	89.51
$WA_{23/5}$	10.70	76.82	4.90	1.63	94.05
$WA_{24/6}$	6.64	77.81	8.68	1.63	94.76
$WA_{25/7}$	4.28	76.83	12.30	1.30	94.71
$WA_{26/8}$	0.32	76.14	16.08	1.37	93.91
$WA_{27/9}$	**0**	**73.42**	**20.25**	**1.09**	**94.76**
$WA_{28/10}$	0	72.03	20.46	1.06	93.55
$WA_{29/11}$	0	70.69	21.63	0.86	93.18
$WA_{30/12}$	0	69.99	22.58	0.77	93.34
$WA_{31/13}$	0	68.12	24.20	0.81	93.13
$WA_{32/14}$	0	66.32	25.83	0.95	93.10
$WA_{33/15}$	0	64.53	27.29	0.97	92.79
$WA_{34/16}$	0	61.05	30.00	1.10	92.15
$WA_{35/17}$	0	56.06	34.11	1.58	91.75
$WA_{36/18}$	0	55.87	33.00	2.01	90.88
$WA_{37/19}$	0	60.81	28.72	0.93	90.46
$WA_{38/20}$	0	59.32	30.17	0.97	90.46
$WA_{39/21}$	0	58.50	30.47	1.29	90.26
$WA_{40/22}$	0	56.85	31.15	1.75	89.75
$WA_{41/23}$	0	54.96	32.84	1.37	89.17
$WA_{42/24}$	0	57.42	30.72	1.17	89.31
$WA_{43/25}$	0	53.20	32.97	2.03	88.20
$WA_{44/26}$	0	50.62	31.88	3.22	85.72
$WA_{45/27}$	0	36.57	45.31	3.50	85.38
$WA_{46/28}$	**0**	**36.54**	**49.52**	**2.22**	**88.28**

$WA_{47/29}$	0	34.21	51.91	2.07	88.19
$WA_{48/30}$	0	34.44	51.74	1.77	87.95
$WA_{49/31}$	0	41.96	43.39	2.19	87.54
$WA_{50/32}$	0	47.07	36.91	2.72	86.70
$WA_{51/33}$	0	51.67	32.41	2.57	86.65
$WA_{52/34}$	0	49.61	34.09	2.43	86.13
$WA_{53/35}$	0	47.88	36.02	2.89	86.79
$WA_{54/36}$	0	44.53	37.78	3.73	86.04
$WA_{55/37}$	0	43.40	38.15	4.43	85.98
$WA_{56/38}$	0	44.93	36.08	4.40	85.41
$WA_{57/39}$	0	48.97	32.64	3.73	85.34
$WA_{58/40}$	0	47.67	33.41	3.82	84.90
$WA_{59/41}$	0	45.83	34.12	3.64	83.59
$WA_{60/42}$	0	42.57	36.62	3.83	83.02
$WA_{61/43}$	0	42.62	36.28	4.36	83.26
$WA_{62/44}$	0	47.64	30.91	4.03	82.58
$WA_{63/45}$	0	38.88	38.50	4.06	81.44
$WA_{64/46}$	0	36.14	40.76	4.17	81.07
$WA_{65/47}$	0	34.09	41.81	5.04	80.94
$WA_{66/48}$	0	34.02	41.78	5.05	80.85
$WA_{67/49}$	0	33.50	41.44	5.06	80.00
$WA_{68/50}$	0	34.10	39.41	4.89	78.40
$WA_{69/51}$	0	33.15	40.51	4.98	78.64
$WA_{70/52}$	0	32.44	39.87	5.49	77.80
$WA_{71/53}$	0	30.95	40.18	5.82	76.95
$WA_{72/54}$	**0**	**28.47**	**41.32**	**6.00**	**75.79**
$WA_{73/55}$	0	25.53	42.53	6.56	74.62
$WA_{74/56}$	0	26.07	41.97	7.10	75.14
$WA_{75/57}$	0	28.70	39.10	6.26	74.06
$WA_{76/58}$	0	28.86	37.00	6.06	71.92
$WA_{77/59}$	0	26.22	37.07	7.17	70.46
$WA_{78/60}$	0	24.05	37.72	8.24	70.01
$WA_{79/61}$	**0**	**24.25**	**38.24**	**8.19**	**70.68**
Average	**1.79**	**47.47**	**31.64**	**3.12**	**84.02**

As it can be seen from the table 1, during 61 weeks of production, the class M has the highest intensity of 47.47%, followed by class L (31.64%), class XL (3.12%) and class S with 1.79%.
The mass of eggs increases along with the age of laying hen, so does the share of certain weight groups during the production cycle, which shows certain phenotypic correlation with the age of laying hen. The table 2 shows the correlation, significance of differences and the strength of correlation for two weight classes M and L, which combined make the largest part of all eggs produced during the entire production cycle.

Table 2. Phenotypic correlation between the age of laying hen and laying intensity (%) of different egg weight groups

Weeks of age/ production	All eggs		M (53-63 g)		L (63-73 g)	
	r_{xy}	Strength of correlation	r_{xy}	Strength of correlation	r_{xy}	Strength of correlation
32/14	0.639***	Strong	0.544*	Strong	0.979***	Complete
33/15	0.616***	Strong	0.493*	Medium	0.980***	Complete
34/16	0.592***	Strong	0.429*	Medium	0.982***	Complete
35/17	0.569***	Strong	0.347^{ns}	Weak	0.985***	Complete
36/18	0.543**	Strong	0.277^{ns}	Weak	0.985***	Complete
37/19	0.518**	Strong	0.244^{ns}	Very weak	0.974***	Complete
38/20	0.496**	Medium	0.207^{ns}	Very weak	0.966***	Complete
39/21	0.475**	Medium	0.172^{ns}	Very weak	0.958***	Complete
40/22	0.454**	Medium	0.132^{ns}	Very weak	0.952***	Complete
41/23	0.432**	Medium	0.090^{ns}	None	0.949***	Complete
42/24	0.412*	Medium	0.063^{ns}	None	0.940***	Complete
43/25	0.389*	Weak	0.021^{ns}	None	0.936***	Complete
44/26	0.356*	Weak	-0.026^{ns}	None	0.929***	Complete
45/27	0.325*	Weak	-0.122^{ns}	Very weak	0.936***	Complete
46/28	0.311*	Weak	-0.200^{ns}	Very weak	0.942***	Complete
47/29	0.297*	Weak	-0.271^{ns}	Weak	0.947***	Complete
48/30	0.283*	Weak	-0.330^{ns}	Weak	0.952***	Complete
49/31	0.268*	Weak	-0.361^{ns}	Weak	0.951***	Complete
50/32	0.251*	Weak	-0.374^{ns}	Weak	0.940***	Complete
51/33	0.235*	Very weak	-0.373^{ns}	Weak	0.917***	Complete
52/34	0.218^{ns}	Very weak	-0.378^{ns}	Weak	0.901***	Complete
53/35	0.205^{ns}	Very weak	-0.387^{ns}	Weak	0.890***	Very strong
54/36	0.190^{ns}	Very weak	-0.403*	Medium	0.883***	Very strong
55/37	0.176^{ns}	Very weak	-0.420*	Medium	0.877***	Very strong
56/38	0.161^{ns}	Very weak	-0.432*	Medium	0.866***	Very strong
57/39	0.146^{ns}	Very weak	-0.432*	Medium	0.848***	Very strong
58/40	0.131^{ns}	Very weak	-0.435*	Medium	0.832***	Very strong
59/41	0.111^{ns}	Very weak	-0.443*	Medium	0.819***	Very strong
60/42	0.091^{ns}	None	-0.456*	Medium	0.812***	Very strong
61/43	0.073^{ns}	None	-0.469*	Medium	0.805***	Very strong
62/44	0.054^{ns}	None	-0.469**	Medium	0.785***	Very strong
63/45	0.032^{ns}	None	-0.487**	Medium	0.783***	Very strong
64/46	0.010^{ns}	None	-0.507**	Strong	0.785***	Very strong
65/47	-0.011^{ns}	None	-0.529**	Strong	0.788***	Very strong
66/48	-0.031^{ns}	None	-0.549***	Strong	0.791***	Very strong
67/49	-0.052^{ns}	None	-0.567***	Strong	0.792***	Very strong
68/50	-0.078^{ns}	None	-0.583**	Strong	0.790***	Very strong
69/51	-0.100^{ns}	Very weak	-0.598***	Strong	0.789***	Very strong
70/52	-0.124^{ns}	Very weak	-0.613***	Strong	0.788***	Very strong
71/53	-0.148^{ns}	Very weak	-0.628**	Strong	0.786***	Very strong

72/54	-0.175^{ns}	Very weak	-0.644***	Strong	0.786***	Very strong
73/55	-0.202^{ns}	Very weak	-0.661***	Strong	0.788***	Very strong
74/56	-0.226^{ns}	Very weak	-0.676***	Strong	0.788***	Very strong
75/57	-0.251^{ns}	Weak	-0.688***	Strong	0.784***	Very strong
76/58	-0.280^{ns}	Weak	-0.698***	Strong	0.776**	Very strong
77/59	-0.309^{ns}	Weak	-0.709***	Strong	0.768**	Very strong
78/60	-0.337^{ns}	Weak	-0.721***	Strong	0.762**	Very strong
79/61	-0.361^{ns}	Weak	-0.731***	Strong	0.757**	Very strong

***p<0.001; **p<0.01; *p<0.05; ^{ns}p>0.05

The results in table 2 show that the eggs of class M during 14th, 15th and 16th weeks of production show a statistically significant (p<0.05), strong and medium strong positive correlation between the age of hen and the weight of eggs. From $WA_{35/17}$ to $WA_{53/35,}$ no statistically significant phenotypic correlation was noticed between the age of hen and the weight of eggs (p>0.05). From $WA_{54/36}$ to the end of the production cycle ($WA_{79/61}$), a statistically significant negative correlation was noticed between the age of hen and the weight of eggs, and from $WA_{66/48}$ to the end of production cycle, the phenotypic correlation was very highly significant (p<0.001), except for $WA_{68/50}$ and $WA_{71/53}$ when it was highly significant. The coefficient of phenotypic correlation at the end of production cycle was (r_{xy} = -0,731***).
Unlike the class M, the strength of correlation for eggs of class L was complete, ie very strong, positive in direction, and statistically very highly significant (p<0.001) until 76th week of age, and statistically highly significant from the 76th week of age (p<0.01). The coefficient of phenotypic correlation at the end of production cycle was (r_{xy} = 0,757**).
With the age of laying hens, the intensity of producing eggs gradually decreased, and positive coefficients of phenotypic correlations were determined between the age of hens and laying intensity of all weight groups, and it was statistically very highly significant until the 36th week of age. The coefficient of correlation was statistically very significant (p<0.01) between the 36th and 42nd week of age, while it was statistically significant (p<0.05) between 42nd and 52nd week of age. From $WA_{52/34}$ to the end of exploitation, the correlation coefficients were not statistically significant (p>0.05), with a coefficient of phenotypic correlation being negative from $WA_{65/47}$ to $WA_{79/61}$, and the correlation was weak, very weak or none (r_{xy} = -0.361^{ns}).
Similar results for correlation, significance of differences and strength of correlation between the age of hens and laying intensity (%) of various weigth groups of eggs during entire production cycle, in the same examined hybrid, were obtained by Pandurevic (2011). The coefficients of phenotypic correlations at the age of 72nd weeks were (r_{xy}= -0,718***) for class M, (r_{xy}=0,802***) for class L, and for class XL (r_{xy}= $-0,178^{ns}$).

Conclusion

Based on the examination of production performances of light-line hybrid Lohmann Brown, during experiment that lasted for 61 production week, the following conclusions can be drawn:
The egg production, as well as intensity of laying, gradually increased until the 27th week of age, when the maximum laying intensity was reached, followed by gradual decline in egg production until the end of flock exploitation, with occasional variations. At the age of 27th weeks, the egg laying intensity reached the maximum of 94.76%. The average egg laying intenstity for the entire production cycle (from WA_{19} to WA_{79}) was 84.02%.

The examined flock records slightly higher values of egg laying intensity by examined periods, as well as for stated period of expoitation in comparison to the technological norms.
Sorted by weight groups during 61 weeks of production, the class M has the highest intensity of 47.47%, followed by class L (31.64%), class XL (3.12%) and class S with 1.79%.

References

Bogosavljevic-Boskovic S., Mitrovic S. (2005): Gajenje različitih vrsta živine [Breeding different species of poultry]. Univerzitet u Kragujevcu, Agronomski fakultet Čačak, pg. 249,

Dikmen B. Y., Ipek A., Sahan U., Sozcu A., Can Baycan S. (2017): Impact of different housing systems and age of layers on egg quality characteristics. Turkish Journal of Veterinary and Animal Sciences (2017), 41., 77-84 p.

Gjorgovska N., Filev K., Levkov V., Gjorgjievski S., Kostov V., Nastova R. (2016): The effect of feed rich in dha on egg production, egg components and dha content in yolk. Bulgarian Journal of Agricultural Science 22 (Supplement 1), 10-14 r.

Latinovic D. (1996): Populaciona genetika i oplemenjivanje domaćih zivotinja [Population genetics and breeding of domestic animals]. Praktikum, Univerzitet u Beogradu Poljoprivredni fakultet Beograd.

Mahmutovic H. (2011): Uticaj vrste hibrida koka nosilja i mase jaja na omjer bjelance/žumance i kvalitet konzumnih jaja [Influence of the type of laying hen hybrid and egg weight on egg white/yolk ratio and quality of table eggs]. agroTECH, VI Savjetovanje o proizvodnji i preradi hrane sa međunarodnim učešćem, 15-16 str., Gradačac.

Pandurevic T. (2011): Uticaj starosti na proizvodnost, kvalitet i hemijski sastav jaja lakog linijskog hibrida kokoši [Influence of the age on productivity, quality and chemical composition on eggs of light-line hybrid hens]. Doktorska disertacija. Poljoprivredni fakutet Istočno Sarajevo.

Pandurevic T., Ristanovic B., Lalovic M. (2015): The Influence of Age Hens on the Intensity Load Capacity from Different Weight Groups Eggs. Animal Science and Biotechnologies 48, 294-297 p., Cacak, Serbia.

Rakonjac S. (2016): Proizvodnja i kvalitet proizvoda kokoši nosilja iz alternativnih sistema gajenja [Production and product quality of lying hens from alternative breeding systems]. Doktorska disertacija. Univerzitet u Kragujevcu Agronomski fakultet u Čačku.

Vukic-Vranjes M., Steiner T., Tolimir N., Vekic M. (2009): Uticaj fitogenih aditiva na proizvodne performanse kokoši nosilja [Influence of phytogenic additives on production performances of laying hens]. Poljoprivredne aktuelnosti, 3-4, 91-100 str., Beograd.

BEE COLONY SWARMING EVENT DETECTION IN PRECISION BEEKEEPING

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Abstract

Honey bee colony swarming event is a good example of an animal group performing a synchronised action with aim to leave the original hive and depart for a new location. Swarming has always been seen by the beekeepers as an extremely important event, which requires an immediate response. During the swarming majority of the bees leave the hive and this dramatically reduces the productivity potential of the colony and profitability of the beekeeper. Thus, remote detection of this event should be done by application of the bee colony monitoring system within the precision beekeeping approach. Remote monitoring of bee colonies helps to reduce the stress and unnecessary activities of bee colonies by minimising the number of visual inspections. There are several technological possibilities for remote detection of swarming. During the swarming event temperature in the colony increases, overall hive weight decreases and there is a specific sound emitted by the colony. Within this article various parameter dynamics are summarised and demonstrated for the successful identification of the swarming event. We summarised findings from various literature sources and also provide our own experimental results conducted for the swarming detection.

Keywords: *Precision beekeeping, Precision apiculture, bee colony swarming, remote swarming detection, bee colony monitoring.*

Introduction

Bees are very important pollinators - around 85% of flowers are pollinated by bees (Warnke, 2009), helping to preserve the wild biodiversity, as well. Besides this, honey bees (Apis mellifera) are the most economically important managed insects (Moritz & Southwick, 1992; Aizen & Harder, 2009).

To help the beekeepers to improve the understanding of the processes in the bee colony without looking inside the hive many sensing technologies are introduced. The technological inventions and their implementation for honey bee colony monitoring are summarized under the term precision beekeeping or precision apiculture.

Precision Beekeeping (PB) is a sub-branch of Precision Agriculture and is an apiary management strategy based on the remote monitoring of individual bee colonies with aim to minimise resource consumption and maximise the productivity of bees (Zacepins et al., 2015).

As well in recent years multiple stress factors have led to a decline of honey bee colonies (Klein et al., 2007) and this event has emphasized the significance of a continuous and remote monitoring to investigate factors that may negatively affect the life cycle of bees (Nolasco et al., 2019).

Remote monitoring of bee colonies minimizes the number of visual inspections, therefore helps to reduce the stress of the bee colonies and can increase the colony health (Stalidzans et al., 2017). The impact of physical inspections (e.g., manual weighing) is also stated by (Komasilovs et al., 2019). Currently the most feasible colony parameters available for constant monitoring are temperature, humidity, weight, acoustics and vibration. By measuring those parameters different states of the bee colony can be detected, like brood rearing, death, swarming, absconding etc. Inside hive temperature and humidity are the most commonly used metrics, when applying precision beekeeping (Zacepins & Karasha, 2013; Meikle & Holst, 2015; Zacepins et al., 2015; Meikle et al., 2017). Hive weight is also a useful metric for monitoring the productivity of a colony (Fitzgerald et al., 2015; Lecocq et al., 2015; Meikle et al., 2017). Several studies have underlined that some behaviors of the honey bees are strictly related to variation in produced sound (Hunt & Richard, 2013; Robles-Guerrero et al., 2017).

Focus of this paper lays at the bee colony swarming detection, as swarming has always been seen by beekeepers as an extremely important event, which requires an immediate response. We aim to review the knowledge related to the bee colony remote swarming detection and summarise the parameter values, which correspond to the swarming event. Basically, beekeeper's notification of a swarming event should be a default functionality for any precision beekeeping system.

Bee colony swarming is a natural way of proliferation of the bee colony. During the swarming the queen leaves the colony with a group of worker bees to establish a new colony in a different place. As a result, the original colony reproduces two or more colonies (Michener, 1974; Heinrich, 1981; Seeley, 1982; Winston, 1987). The main disadvantage of natural swarming for commercial beekeeping is its spontaneous character, as in some years there could be more swarms than in others (Simpson, 1958).

It is known that beekeepers can apply different bee colony management techniques to minimise the risk of potential swarming events. However, it is almost impossible to fully exclude swarming by beekeeping practice, especially with a large number of colonies kept on different apiaries in different geographical locations. Swarms can be detected on-site, as usually swarmed bee colony finds a tree near the apiary to prepare for a longer journey to a new colony place (Seeley & Buhrman, 1999). But the problem resides in the fact that bee colonies are placed far away from the beekeepers' living place and usually swarming is identified only post factum, when the swarm is already gone.

The automatic remote detection of swarming events would have a large impact on beekeeping, as when part of the bee colony leaves the hive, this dramatically reduces the productivity potential of the colony. But if the beekeeper is notified about the colony swarming he/ she still has the opportunity to catch the swarm and place it back into the old hive or a new one (Seeley, 2010).

Swarming detection by temperature

Bee colony temperature monitoring can be characterised by:

- the low cost of data collection as sensors are relatively cheap,
- the low volume of data, even if the measurements are very frequent,
- the easy processing of the data.

It has been discovered that swarming can be detected by temperature monitoring (Seeley et al., 2003; Ferrari et al., 2008; Kridi, de Carvalho & Gomes, 2014; Kviesis & Zacepins, 2016; Zacepins et al., 2016; Kviesis et al., 2020). In the period of warm-up for the final take-off, the temperature above the upper hive body rises by 1.5-3.4 C from normal brood rearing temperature 34-35°C to 37-38°C (Zacepins et al., 2016). A schematic representation of temperature dynamics during the bee colony swarming event is shown below (see Fig. 1):

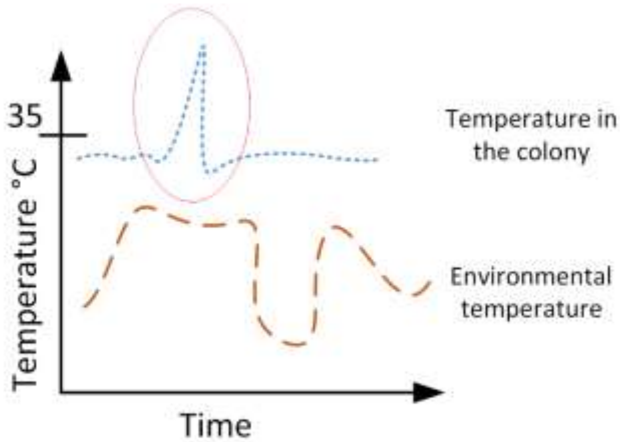

Figure 6. Temperature dynamics in general during swarming event

The chart below (see Fig. 2) clearly demonstrates the swarming moment, when the colony is leaving the original hive. This event is one example of bee colony swarming that was recorded in June 2015 in Jelgava, Latvia, when multiple Appis Mellifera colonies were constantly monitored. It is worth mentioning that detection of such an event is highly dependent on the temperature measurement interval - frequent measurements (with intervals in a range from one to five minutes) are required. It was observed that the spike in temperature only lasts for 20-30 minutes, therefore with less frequent measurement intervals (e.g., every 10-20 minutes) swarming cannot even be detected.

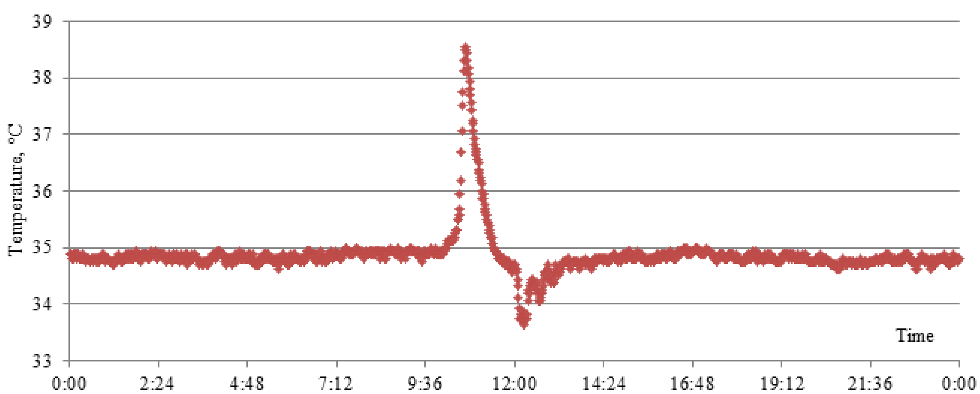

Figure 7. Temperature curve during swarming event in June 2015 in Jelgava, Latvia

To automatically detect swarming events from the temperature measurements several approaches can be used. It is possible to apply fuzzy logic for early abnormality detection in temperature patterns (Kviesis et al., 2020), k-means clustering (Kridi, 2014), simple condition-based mathematical algorithms (Zacepins et al., 2016) or other artificial intelligence methods (Kviesis & Zacepins, 2016).

Swarming detection by sound

Swarming bees generate a specific audio signal (Zgank, 2019), but the earliest electronic swarming detection system based on the sound analysis was "Apidictor," which was "in regular use" by Woods (Woods, 1959), indicating the age of the automatic swarming detection idea.

There are studies that demonstrate correlation between the amplitudes and frequencies of the bee colony sound and identify swarming (Dietlein, 1985; Ferrari et al., 2008; Qandour et al., 2014).

Most of the sounds produced by the bee colony have been characterised by a low fundamental frequency between 300 and 600Hz and their corresponding harmonics. Quandour et al., states that frequencies from 100 till 2000Hz with a single pulse sent by scout bees are triggering colony hissing and preparing for swarming.

Ferrari (Ferrari et al., 2008) proposed a method for predicting the swarming period, based on the labelling of sounds. Swarming is indicated by an increase in the power spectral density at approximately 110Hz. When the bees are beginning to swarm, the sound is augmented in amplitude and frequency to 300Hz and, occasionally, a rapid change occurs from 150Hz to 500Hz (Ferrari et al., 2008).

Bencsik (Bencsik et al., 2011) has identified patterns of vibration signals in colonies during swarming with specific sensors for detecting the frequency of vibrations.

Swarming detection by weight dynamics

During the swarming event part of the bees are leaving the hive within a short period of time. By knowing the size of the colony, it could be possible to evaluate the size and the weight of the swarm itself.

Usually during the swarming around 75% of worker bees are leaving the hive (Seeley & Rangel, 2012), so it can be concluded that the bigger the colony, the bigger the swarm itself. It is also possible to evaluate the potential weight of the swarm. The weight of one bee is approximately one tenth of a gram (https://www.reference.com/pets-animals/much-bee-weigh-2e57426f63f44979), so 10000 bees will weigh 1 kg. Even when the swarm is relatively small, a decrease in weight should be sufficient to be detected by a monitoring system.

In literature only few authors have mentioned the application of weight monitoring for swarming detection (Meikle et al., 2008; Linton, 2012).

A schematic representation of weight dynamics during the bee colony swarming is shown below (see Fig. 3.):

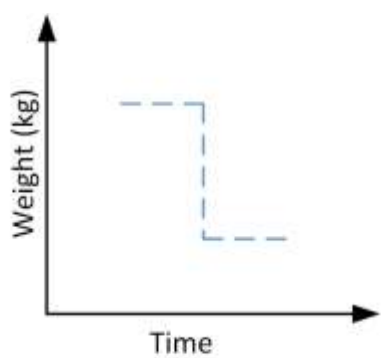

Figure 8. Weight dynamics in general during a swarming event

Figure 4 demonstrates a bee colony swarming event, detected by the temperature and weight measurements during a real-time bee colony monitoring in June 2020, in Witzenhausen, Germany. It can be observed that during the swarming, weight of the colony significantly decreases and is a good indicator for the beekeeper to react immediately. The weight drops as a large number of bees leave the hive in a short time.

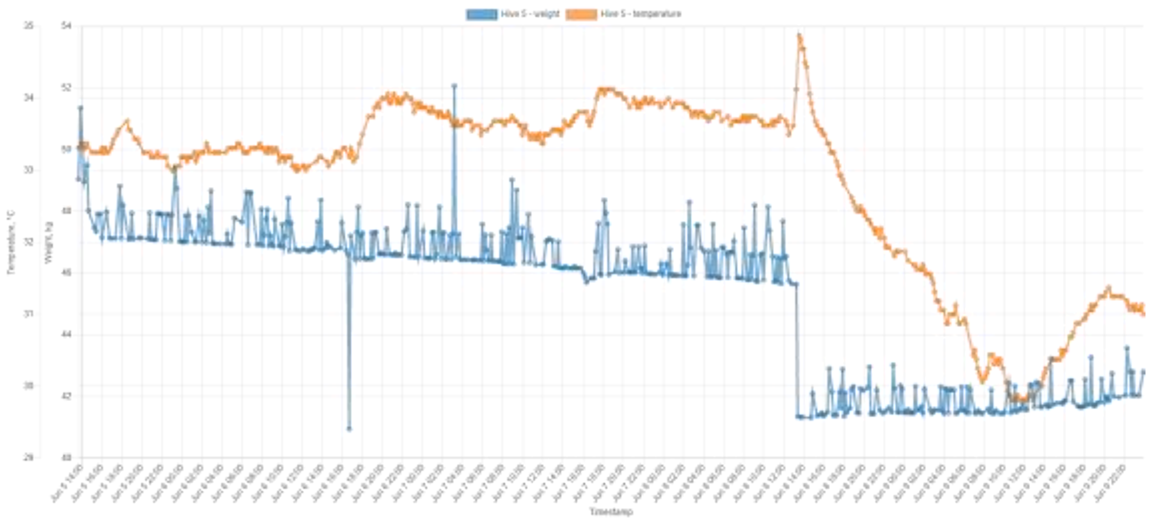

Figure 9. Changes in temperature (orange line) and weight (blue line) during a swarming event in June, 2020 in Witzenhausen, Germany. Image from the SAMS project (Wakjira et al., 2021) public report D4.4 (https://sams-project.eu/wp-content/uploads/2020/12/GA_780755_D4.4_Concept-of-the-DSS.pdf)

Available commercial solutions/products for swarming detection

Nowadays there are many precision beekeeping systems available which are focused on bee colony remote monitoring. Some of the solutions provide the functionality only to record the parameters without any deep data analysis and notification for beekeepers. But there are some commercial products which states that beekeepers will be informed if swarming of the colony will be predicted and/ or detected. For example, BuzzBox monitoring system from osbeehives.com states that artificial intelligence is applied to inspect hive's health and report updates to mobile app continually throughout the day. System can detect swarming, missing queen, healthy, sick, or collapsed hives in real-time. Another monitoring system from solutionbee (https://solutionbee.com/) reports alarms for intrusion, swarming or other colony issues.

Conclusions

Swarming is one of the bee states that, if not controlled, can significantly affect beekeepers' finances. Swarming is one of the events in the precision beekeeping that can be detected by individual parameter monitoring or can be combined with other parameters to increase the reliability. The most common parameters used for the swarming detection are temperature, sound and weight. If the temperature can be measured by relatively cheap sensors and data can be analysed with computationally light methods in order to detect swarming, sound monitoring, on the other hand, is more complex to analyze, but can provide more information, even predict the swarming event." The usage of weight data for swarming detection can be considered as an additional benefit (increases the reliability to correctly detect such an event), as weight primary is used for the foraging activity monitoring.

Acknowledgment

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References

Aizen, M. A. & Harder, L. D. 2009. The Global Stock of Domesticated Honey Bees is Growing Slower than Agricultural Demand for Pollination. Current Biology, 19(11), pp. 915–918. doi: 10.1016/j.cub.2009.03.071.

Bencsik, M., Bencsik, J., Baxter, M., Lucian, A., Romieu, J. & Millet, M. 2011. Identification of the honey bee swarming process by analysing the time course of hive vibrations. Computers and electronics in agriculture, 76(1), pp.44-50.

Dietlein, D. G. 1985. A method for remote monitoring of activity of honeybee colonies by sound analysis. Journal of Apicultural Research, 24(3), pp. 176–183.

Ferrari, S., Silva, M., Guarino, M. & Berckmans, D. 2008. Monitoring of swarming sounds in bee hives for early detection of the swarming period. Computers and electronics in agriculture, 64(1), pp.72-77.

Fitzgerald, D.W., Murphy, F.E., Wright, W.M., Whelan, P.M. & Popovici, E.M. 2015. Design and development of a smart weighing scale for beehive monitoring. In 2015 26th Irish Signals and Systems Conference (ISSC) (pp. 1-6). IEEE.

Heinrich, B. Y. B. 1981. The mechanisms and energetics of honeybee swarm temperature regulation. Journal of Experimental Biology, 91, pp. 25–55.

Hunt, J. H. & Richard, F.-J. 2013. Intracolony vibroacoustic communication in social insects. Insectes Sociaux, 60(4), pp. 403–417. doi: 10.1007/s00040-013-0311-9.

Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the royal society B: biological sciences, 274(1608), pp.303-313.

Kridi, D. S., de Carvalho, C. G. N. & Gomes, D. G. 2014. A predictive algorithm for mitigate swarming bees through proactive monitoring via wireless sensor networks. In Proceedings of the 11th ACM symposium on Performance evaluation of wireless ad hoc, sensor, & ubiquitous networks, pp. 41–47.

Kviesis, A., Komasilovs, V., Komasilova, O. & Zacepins, A.. 2020. Application of fuzzy logic for honey bee colony state detection based on temperature data. Biosystems Engineering, 193, pp.90-100.

Lecocq, A., Kryger, P., Vejsnæs, F. & Jensen, A.B. 2015. Weight watching and the effect of landscape on honeybee colony productivity: Investigating the value of colony weight monitoring for the beekeeping industry. PLoS One, 10(7), p.e0132473.

Linton, F. 2012. Hive Monitoring Technology: High Tech Hives. American Bee Journal, pp. 767–769.

Meikle, W.G., Rector, B.G., Mercadier, G. & Holst, N. 2008. Within-day variation in continuous hive weight data as a measure of honey bee colony activity. Apidologie, 39(6), pp.694-707.

Meikle, W.G., Weiss, M., Maes, P.W., Fitz, W., Snyder, L.A., Sheehan, T., Mott, B.M. & Anderson, K.E. 2017. Internal hive temperature as a means of monitoring honey bee colony health in a migratory beekeeping operation before and during winter. Apidologie, 48(5), pp.666-680.

Meikle, W. G. & Holst, N. 2015. Application of continuous monitoring of honeybee colonies. Apidologie, 46(1), pp. 10–22. doi: 10.1007/s13592-014-0298-x.

Michener, C. D. 1974. The social behavior of the bees. Cambridge: Harvard University Press. Available at: Biophy Library, Wits.

Nolasco, I., Terenzi, A., Cecchi, S., Orcioni, S., Bear, H.L. & Benetos, E. 2019. Audio-based identification of beehive states. In ICASSP 2019-2019 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP) (pp. 8256-8260). IEEE.

Qandour, A., Ahmad, I., Habibi, D. & Leppard, M. 2014. Remote beehive monitoring using acoustics signals. Acoustics Australia, 42(3), pp. 204–209. doi: 10.1007/s40857-015-0016-5.

Robles-Guerrero, A., Saucedo-Anaya, T., González-Ramérez, E. & Galván-Tejada, C.E. 2017. Frequency Analysis of Honey Bee Buzz for Automatic Recognition of Health Status: A Preliminary Study. Res. Comput. Sci., 142, pp.89-98.

Seeley, J. & Rangel, T. D. 2012. Colony fissioning in honey bees: size and significance of the swarm fraction. Insectes Sociaux. doi: 10.1007/s00040-012-0239-5.

Seeley, T. D. 1982. How Honeybees Find a Home. Scientific American, 247, pp. 158–168.

Seeley, T. D. 2010. Honeybee democracy. Princeton University Press.

Seeley, T. D. & Buhrman, S. C. 1999. Group decision making in swarms of honey bees. Behavioral Ecology and Sociobiology, 45(1), pp. 19–31. doi: 10.1007/s002650050536.

Simpson, J. 1958. The factors which cause colonies of Apis mellifera to swarm. Insectes Sociaux, 5(1), pp. 77–95. doi: 10.1007/BF02222430.

Southwick, E. E. & Southwick, J. L. 1992. Estimating the Economic Value of Honey Bees (Hymenoptera: Apidae) as Agricultural Pollinators in the United States. Journal of Economic Entomology, 85(3), pp. 621–633.

Wakjira, K., Negera, T., Zacepins, A., Kviesis, A., Komasilovs, V., Fiedler, S., Kirchner, S., Hensel, O., Purnomo, D., Nawawi, M., Paramita, A., Rachman O.F., Pratama A., Al Faizah N., Lemma M., Shaedlich S., Zur A., Sperl M., Proschek K., Gratzer K & Brodschneider R. 2021. Smart apiculture management services for developing countries—the case of SAMS project in Ethiopia and Indonesia. PeerJ Computer Science, 7, p.484.

Winston, M. L. 1987. The Biology of the Honey Bee. Cambridge: Harvard University Press. doi: 10.1002/9780470114735.hawley00624.

Woods, E. 1959. Electronic prediction of swarming in bees. Nature, 184(4690), pp. 842–844. doi: 10.1038/184842a0.

Zacepins, A., Brusbardis, V., Meitalovs, J. & Stalidzans, E. 2015. Challenges in the development of Precision Beekeeping. Biosystems Engineering, 130, pp. 60–71. doi: 10.1016/j.biosystemseng.2014.12.001.

Zacepins, A., Kviesis, A., Stalidzans, E., Liepniece, M. & Meitalovs, J. 2016. Remote detection of the swarming of honey bee colonies by single-point temperature monitoring', Biosystems Engineering. Elsevier Ltd, 148, pp. 76–80. doi: 10.1016/j.biosystemseng.2016.05.012.

Zacepins, A. & Karasha, T. 2013. Application of temperature measurements for the bee colony monitoring: a review. In Proceedings of the 12th International Scientific Conference 'Engineering for Rural Development'. Jelgava, Latvia, pp. 126–131.

Zgank, A. 2019. Bee Swarm Activity Acoustic Classification for an IoT-Based Farm Service. Sensors, 20(1), p. 21. doi: 10.3390/s20010021.

RESEARCH ON THE MANAGEMENT OF DAIRY BREEDING IN A FARM IN THE DORNELOR BASIN IN ROMANIA

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Abstract

The paper aims to highlight the management of dairy cattle breeding on a farm in the Dornelor Basin, Suceava County, with the strengths and problems encountered in this farm. The farm is located in Panaci commune, Coverca village, with 60 head of cattle for milk, comprised of the following breeds: Bălțată Românească, Brună, Bălțată cu Negru Românească and Pinzgau. The paper is based on data taken from the National Register of Holdings on 23.10.2020. Data were statistically processed in the following indicators: dairy cow heads, heifer heads, female and male youth heads, daily milk/cow head production, total/lactation production, percentage of milk fat and protein, number of mountain products benefiting from the optional status of "mountain product". From the processed data it appears that the problems of this farm are represented by the insurance of the feed base, the lack of qualified personnel and the main advantage is represented by the fact that the farmer capitalizes the milk production obtained from cattle on the farm.

Keywords: *cattle, farm, milk, mountain product, dairy products.*

Introduction

The area under study is represented by the Dornelor Basin, Romania, also called "Ţara Dornelor". The Dornelor Basin (figure 1) or "Ţara Dornelor", located on the territory of Suceava county is a tectono-erosive depression, which together with the Câmpulung depression delimits between the northern group and the central group of the Eastern Carpathians. Given the geographical position of Dornelor Country (Dornelor Basin), it can be attributed the definition of mountainous rural space. The Dornelor Basin (figure 1.1), a mountainous area, attracts the attention of specialists, researchers for studies and analyzes with a tourist, agrotourism potential, high through the varied gastronomy specific to the Bucovina area, the traditions, culture and hospitality of its inhabitants. The significant share in the economy of the localities in the Dornelor Basin is held by agriculture and especially the raising of cattle for milk and meat. This animal husbandry activity has its advantages and disadvantages for mountain farmers. The strategic guidelines present the main directions to be followed to ensure the increase of attractiveness and sustainable development of the disadvantaged mountain area, by valuing resources, stabilizing the population, maintaining cultural identity, increasing economic power at local level, while maintaining ecological balance and protecting the environment (Ciocan-Alupii and Maciuc, 2021, *Considerations regarding the activity of animal breeding in the mountain area in relation to the distribution of milk processing centers and units in the mountain area of Romania).*

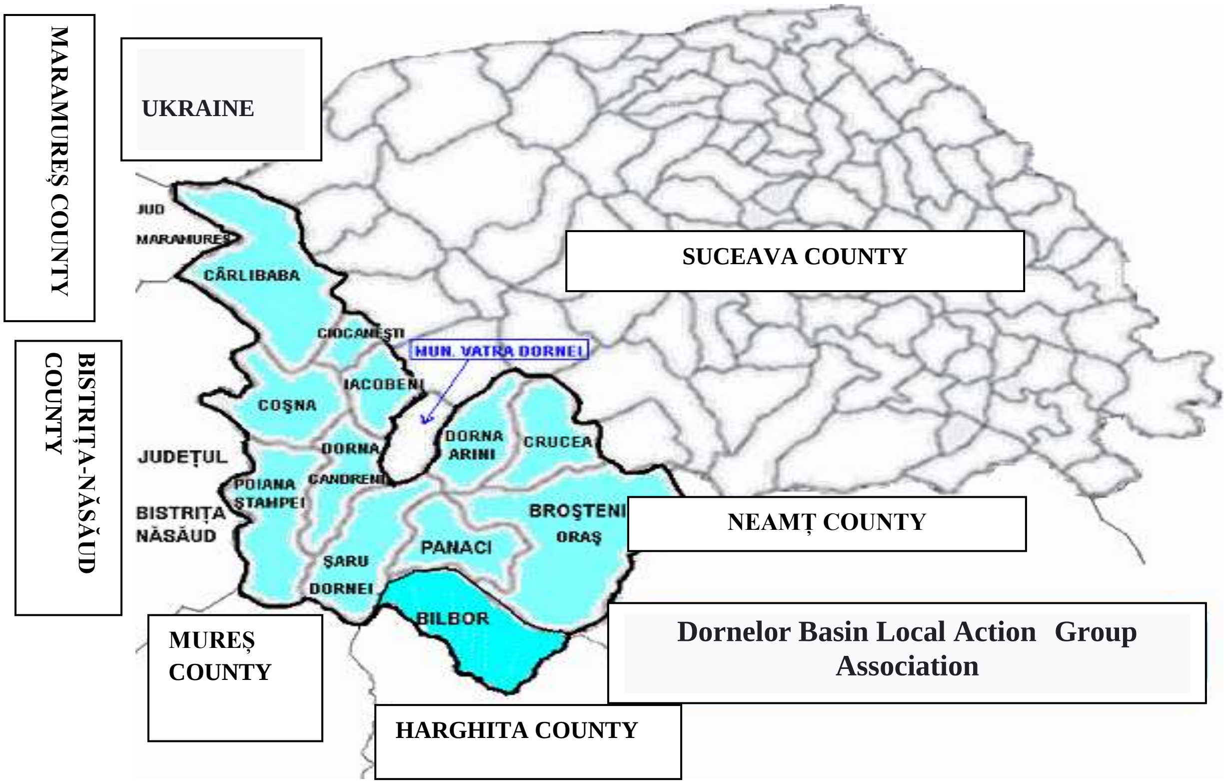


Figure 1. Map of the Dornelor Basin (http://galbazinuldornelor.ro, accessed on 14.01.2021)

Material and method

The research was carried out in the conditions of the household system of exploitation of dairy cows, in a farm from Dornelor Basin, Panaci commune, Coverca village. Dairy cows (60 heads) were studied, belonging to the Romanian Bălțată, Brună, Bălțată cu Negru Românească and Pinzgau breeds. The farmer owns a company whose object of activity is milk processing but also an agro-tourist boarding house. The primary data came from the National Register of Holdings on 23.10.2020. Several aspects were analyzed: dairy cow heads, heifer heads, female and male youth heads, daily milk / cow head production, total production / lactation, percentage of milk fat and protein, number of mountain products benefiting the statement of optional quality "mountain product". Once systematized, the data were processed and interpreted by methods specific to such research - arithmetic mean (), arithmetic mean error (± s) standard deviation (s), coefficient of variability (V%) and significance test p, using the program of statistics, analysis of variance and covariance (SAVC). The complexity of the aspects followed required the use of a diversified work methodology depending on the aspects pursued, using and respecting the investigation methodology recommended by the literature.

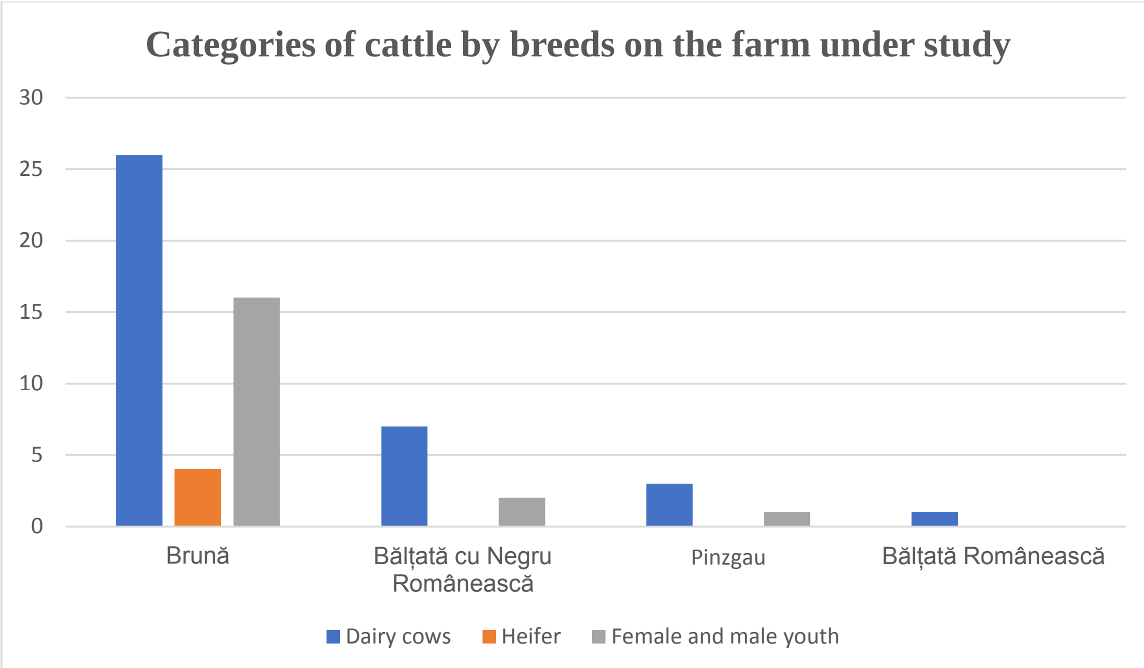
Results and discussions

The intensification of animal husbandry, the traditional occupation and the need for fast milk processing imposed the construction of the milk industrialization factory from Vatra Dornei (Dorna Lactate). Together with the Dorna Candrenilor section, the factory produced numerous varieties of cheese and butter. Unfortunately, at the moment this milk processing unit no longer works, being an opportunity for small producers to open milk processing points in the localities in the Dornelor Basin. In this sense, the farmer whose farm we are analyzing, owns a commercial company (S.C DANY LYLY SRL) that processes milk from the locality and from the surrounding localities. Along with the harmony of the typical mountain landscape, dominated by spruce forests that descend to the hearth of the depression, where they intertwine with rich meadows, these elements of anthropic capitalization of a generous potential, give Ţara Dornelor a strong personality, a particularly favorable brand image. for the development of agro-tourism (Ciocan-Alupii M, Maciuc V., 2021, *Considerations regarding the activity of animal breeding in the mountain area in relation to the distribution of milk processing centers and units in the mountain area of Romania*). The study undertaken in the Dornelor Basin was intended to be an x-ray of the situation of cattle breeding and the impact it has on the specific environment of mountain areas. The rather rational use of permanent meadows, with natural fertilization, without pesticides and with a good floristic composition, attributes special qualities to dairy products and meat. Operating technologies have remained largely traditional, but with the frequent use of livestock effluent storage facilities, so that greenhouse gases from these sources have not exceeded the permitted limits. Of course, there are still emissions from natural processes with a significant share (Toma C., Ciocan-Alupii M., Bocănici M., 2021, *Dairy Cattle Breeding Farm - trumps and restrictions on environmental impact*). The number of cattle on the holding under study is shown in Table 1.

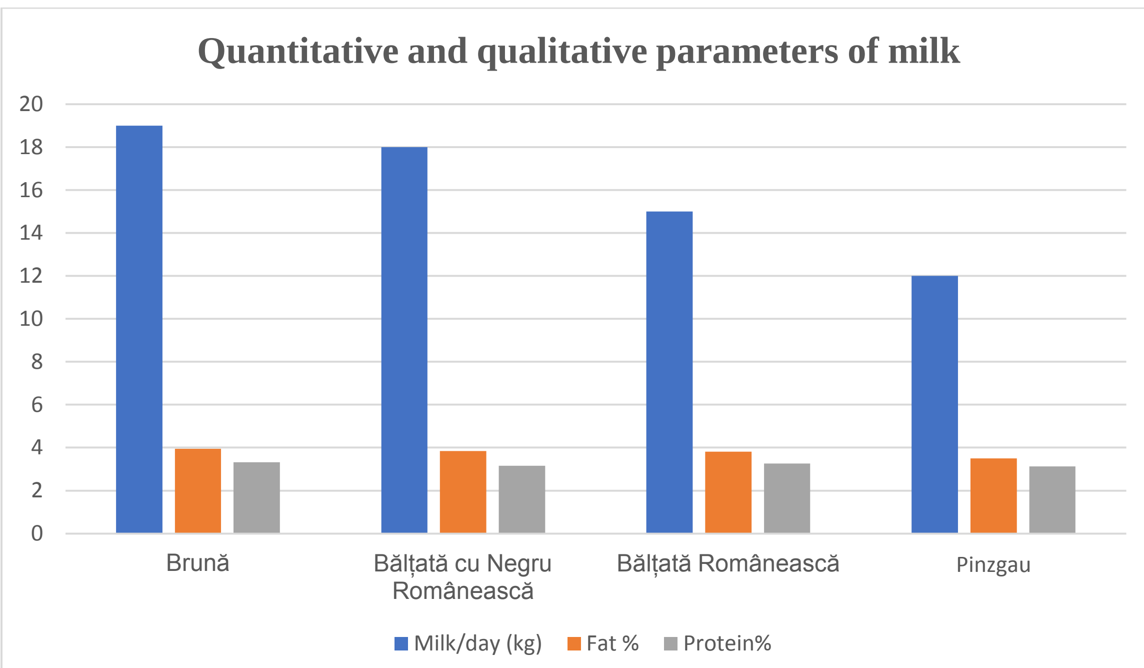
Table 1.Cattle herd by age categories and quantitative and qualitative parameters of milk

Current number	Race	Category			Total milk production/ head	Daily amount of milk / day / head	Fat %	Protein %
		Dairy cows	Heifer	Female and male youth				
1.	Brună	26	4	16	5795	19	3,94	3,32
2.	Bălțată cu Negru Românească	7	-	2	5490	18	3,85	3,16
3.	Pinzgau	3	-	1	3660	12	3,5	3,26
4.	Bălțată Românească	1	-	-	4575	15	3,82	3,12
Total		37	4	19	19520	16	3,77	3,21

(Source: Retrieval of data from the Genealogical Register)



Graph 1. Categories of cattle by breeds



Graph 2. Quantitative and qualitative parameters of milk

Table 1 shows that the breeds on the farm under study are Brown, Romanian Black Spotted, Romanian Spotted and Pinzgau. Out of a total of 60 head of cattle, 26 heads represent dairy cows of the Brown breed, 7 heads of dairy cows of the Bălțată cu Negru Românească breed, 1 of the Bălțată Românească breed, 7 heads are heifers of the Brown breed, 16 male and female youth heads of the breed Brown, 2 female and male youth heads from the Bălțată cu Negru Românească breed and 1 female youth head from the Pinzgau breed. Regarding the milk production, it can be observed that in the Brown breed a total milk production of 5795 liters was obtained, with 3.94% fat and 3.32% protein, in the Bălțată cu Negru Românească breed a total production of 5490 liters, with 3.85% fat and 3.16% protein, in the Pinzgau breed 3660 liters of milk, with 3.5% fat and 3.26% protein and in the Bălțată Românească breed a total production of 4575 liters of milk with 3.82% fat and 3.12% protein. We find that the best milk production, both quantitatively and qualitatively, the farmer obtained from the Brown breed, followed by the Romanian Black Spotted, Romanian Spotted and Pinzgau breeds. Hence the explanation for the larger herd belonging to the Brown breed, recognized for the quality of milk rich in kappa casein, but also for a good adaptability for the mountain area. Regarding the daily milk / head production, it can be observed (table. 1) that the lowest production was obtained from the Pinzgau breed, 12 liters of milk, followed by the Bălțață Românească breeds 15 liters of milk / head, Bălțată cu Negru Românească with a daily production of 18 liters of milk / head and the Brown breed with 19 liters of milk / head. The quantity of milk obtained from cows is capitalized by the Dany Lily Limited Liability Company, which belongs to the farmer, who has several varieties of cheese certified as a "mountain product" such as:

• "Călimani" svaițer (figure 2);
• Cheese „Călimani;
• "Călimani" smoked cheese;
• "Călimani" kneaded cheese;
• Telemea „Călimani."

The company processes 2000 liters of milk daily, obtained from its own farm but also from farmers in Șaru Dornei and neighboring localities. Dairy products are capitalized through their own stores but also through the agro-tourist boarding house that the company owns. The mountain products mentioned above are of superior quality, being tasty, obtained from the milk of cows that have grazed in the mountain area, where the pastures are rich in medicinal plants, giving a special taste to these dairy products. Of the 5 mountain products, some are fresh cheeses, others are matured cheeses. As impediments in the development of the activity within the farm, respectively within the milk processing unit, there is the lack of qualified personnel but also the provision of the animal feed base.

Following the analysis we can say that in the Dornelor Basin, as in the farm studied, farmers face various problems such as: that of feed areas and the possibilities to ensure diversified feed resources, skilled labor, the degree of endowment technical-material, with equipment and mechanization installations, of the biological material used in exploitation (cow breed) and last but not least of the way of capitalization of the production or even of the existing traditions. Regarding the welfare of cattle that is being talked about more and more in the U.E. and which ultimately determines the level and quality of production, farmers know and want to implement the five fundamental freedoms: freedom from discomfort - animals must have an adequate living environment, which includes a shelter and a comfortable area of rest; freedom to express their natural behavior - animals must be given sufficient space and the company of animals of the same species; freedom from hunger and thirst - animals must have unlimited access to fresh water and adequate food to maintain their health; freedom from fear and stress - animals must be treated in a way that does not cause them mental suffering; freedom from pain and disease - animals must be provided with a rapid diagnosis and appropriate treatment.

Figure 2. Svaițer ‘’ CĂLIMANI ”[https://taradornelor.ro/hai-in-tara-dornelor-locul-unde-se-produce-cel-mai-bun-svaiter/ accessed on 10.12.2020]

Conclusions

The breeds of cattle exploited on the farm are represented by Bruna which has the highest milk production, respectively 19 liters / head, Bălțată cu Negru Românescă with a production of 18 liters of milk / head, Bălțată Românească with 15 liters of milk / head and Pinzgau with a production of 12 liters of milk / head;

The farmer is faced with the lack of qualified staff to ensure the proper conduct of activities on the farm but also in the milk processing unit;

The milk on the farm is used in the processing unit owned by the farmer, resulting in high-quality dairy products, which benefit from the optional quality label 'mountain product', being both fresh and matured products.

For the mountain area, closed-flow farms, which also practice agro-tourism, are profitable.

References

Ciocan-Alupii M., Maciuc V., 22-23 october 2020, Scientific Symposium USAMV Iași, „The evolution of dairy products that benefited from the mention of optional quality "mountain product" in the mountain area of Romania, during the period 2017-2020", Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, vol. 74, pag 109

Ciocan-Alupii M., Maciuc V. (2021), Agriculture for Life, 02-05.06.2021, Bucharest „Study on animal breeding in the mountain area of Romania in relation to the distribution of collection centers and milk processing units", currently being published

Ciocan-Alupii M., Maciuc V. – 4-6 june 2020, Bucharest, Agriculture for life „Considerations regarding the mountainous area of Romania: present and perspectives in relation to the breeding activity of cattle", Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020 ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750, pag 301

Toma C., Ciocan-Alupii M., Bocănici M. (2021). International Scientific Session Agricultural Economics and Rural Development Research,"Dornelor Basin dairy farm - environmental impact and restriction"s, currently being published

Maciuc V., Doliş M. Ciocan-Alupii M., Nistor-Anton M., 22-23 octombrie 2020, Scientific Symposium USAMV Iași, „Research on meat production management in a cow farm" Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, vol. 74

Nistor-Anton M., Maciuc V., 22-23 octombrie 2020, Scientific Symposium USAMV Iaşi „Study of milk production indices for cattle breeds exploited in private farms in Neamt county", Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, vol. 74, pp 39-42 ref.25.

Nistor-Anton M, Maciuc V.,4-6 iunie 2020, Bucharest, Agriculture for life.„Study on the number of cattle and production obtained in Neamț county between 2010-2018", Scientific Papers: Series D, Animal Science - The International Session of Scientific Communications of the Faculty of Animal Science . 2020, ISSN 2285-5750, ISSN CD-ROM 2285-5769, ISSN-L 2285-5750, ISSN Online: 2393 – 2260, Vol. LXIII, No. 1, p338-342.

Popa D, Bozdog C., Silvaș E., Rusu M., (2007). „The valorisation with the bullfights of the pastoral fund in the mountain and sub-mountain areas", the Research-Development Institute for Cristian-Sibiu Montanology

http://galbazinuldornelor.ro, accessed on 14.01.2021)

https://taradornelor.ro/hai-in-tara-dornelor-locul-unde-se-produce-cel-mai-bun-svaiter/ accessed on 10.12.2020

STUDY OF MILK PRODUCTION INDICES IN MARAMUREŞ BROWN BREED EXPLOITED AT THE SECUIENI NEAMȚ AGRICULTURAL RESEARCH AND DEVELOPMENT STATION

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Abstract

In this scientific paper, the analysis of milk production indices was performed, on standard lactation (305 days) in maturity equivalent (MS), on successive lactations, in the Brown of Maramureş breed, exploited at the Secuieni - Neamț Agricultural Research and Development Station. This breed of cattle belongs to the mixed morphoproductive type of milk - meat and was formed following the crossing between cows from the primitive local breeds Barn de steppe and Mocănița with bulls of the Schwyz or Brown Alpina breed (originally from N-E Switzerland). Cattle of this breed have a high degree of adaptability to all areas of landforms, especially those of hill and mountain specific to the area of Neamț County. They are also characterised by high productive longevity - up to 12-13 years. For the analysis of milk production indices, data from accredited associations was used to perform the official control of milk production (COP): the Association of Animal Breeders "Operator I.A" Neamț, as well as data from the Genealogical Register. An average milk production of 6074.00 kg, was recorded on normal lactation at lactation III, with a minimum production of 4724.00 kg and a maximum production of 7684.00 kg. Regarding the milk fat and protein content, the results recorded were also satisfactory, respectively, the average fat percentage was 4.02%, with a maximum limit of 4.40% and a minimum of 3.21%, at the second lactation when it recorded the highest values. The percentage of registered protein had an average value of 3.48%, with a maximum limit of 3.77% and a minimum limit of 3.28%, also at the second lactation. Regarding the fat/protein ratio, it recorded values of 1.19, a value that is close to the normal limits of this breed (1.20). The results obtained for the bulls of the Brown from Maramureș breed in this farm demonstrate that at optimal maintenance and feeding conditions, this breed of cattle expresses its genetic potential very well.

Keywords: *cattle, milk production, fat percentage, protein percentage, COP.*

Introduction

Cattle are the main suppliers of milk, a food product of first necessity, "of social interest", for the vigor and health of the population (Acatincăi, St., 2010). On the other hand, cattle are the animals that most efficiently transform primary production into milk production, namely 25.8% of energy consumed and 33.6% of protein consumed, having the property of successfully using cellulose feeds using microflora. and rumen microfauna (Georgescu, Gh., Ujică, V., et al., 1988). The quantity and quality of milk obtained from each dairy cow, as well as the total increased herd, depend on a number of factors, among which the most important are: genetic induced potential, quantitative level, assortments and quality of feed used in feeding dairy cows, age of cows, frequency of milking within 24 hours, duration of breast rest, duration of lactation, technological comfort offered to cows, through the rearing and endowment systems adopted, the calendar season in which lactation occurs and the state of health of cows (Georgescu Gh., Stanciu G., Velea C., Ujică V., 1990). The genetic factor must be considered first, because the productive potential of the animal depends on it, while the other factors create the conditions for expressing this potential (Dronca D., 2003). Regarding the composition of cow's milk, it varies a lot, depending on a large number of factors including breed, season, stage of lactation, milking interval, animal health, and feeding level. Milk is a repeatable production, which is obtained as a consequence of the process of reproduction of animals and cows, respectively. In order to know the productive capacity of a cow, the quantitative control must cover as long a period of its life as possible. The perfect and complete knowledge of the productive capacity of a cow requires the daily registration of the milk produced, by measuring it, establishing the production on lactations and then on the whole productive life, by summing the productions on lactations (Grosu, H., Pascal, A., 2005). This daily recording is cumbersome due to the large amount of work involved. Therefore, a solution was reached based on a mathematical criterion, namely that the entire lactation of a cow be divided into periods of a certain period of time, at which controls are performed, thus determining the production the the day of said controls. This production multiplied by the days between controls, gives the approximate production per period and by summing the productions per periods, the production on lactation results (Maciuc V., 2006). In the Secuieni Agricultural Research and Development Station, the Brown de Maramureş breed is raised and exploited, which is a mixed breed of milk-meat. It is a local breed formed by the absorption cross between the Barn of Steppe and Shepherd breeds with the Schwyz type cattle, deriving in Brown of Maramureş starting with 1881. Between 1890 and 1910, they were continuously imported from Austria and Germany, and in 1904 from Switzerland, bringing annually 200-500 cows and heifers of the Schwyz breed (Podar, C., Oroian, I., 2003). In 1907, Maramureş type cattle were brought to Moldova, loc. Mălini. Milk production varies widely depending on operating conditions, averaging 3,500 kg with 3.9% fat. The potential is over 5000 kg on normal lactation (Virginia Z., Maciuc V., Nacu Gh., Zota D., 2003).

Materials and method

In this scientific paper, the analysis of milk production indices was performed, on standard lactation (305 days) in maturity equivalent (MS), in the Brown of Maramureș on a herd of 51 dairy cows, on 3 successive lactations (lactation I - III), on a total of 89 lactations bred and exploited at the Secuieni - Neamț Agricultural Research and Development Station. In order to analyze the performance for milk production in this breed of cattle, a series of indicators were studied, such as: quantity of milk on normal lactation, fat content, total amount of fat, protein content, total amount of protein. At the Secuieni - Neamț Agricultural Research and Development Station, the periodic control, both quantitative and qualitative, is applied with very good results. The official control of milk production in cows from this farm is performed by the Association of Animal Breeders "Operator I.A" Neamț at an interval of 28 days. The primary data was extracted from the records of this holding, but also from the records of the administrative offices. They have been systematized, statistically processed and interpreted by methods specific to such research. The statistics, respectively the parameters, which characterize a normal distribution, are on the one hand the average or median ($\overline{X}$), and on the other hand the dispersion indices represented by standard deviation (s), coefficient of variation (V%), Min and Max of the pursued character.

Results and discussions

If the data in Tables 1, 2 and 3 are analyzed, it is found that the quantity of milk during normal lactation has an average value of 5089.92 kg, with individual variability limits between 3193.00 kg and 5924.00 kg of milk. At first lactation, an average value of 5683.26 kg of milk was recorded, at second lactation a minimum limit of 4797.00 kg of milk and a maximum limit of 6722.00 kg was recorded, and at the third lactation an average value of production of milk was obtained at standard lactation of 6074.00 kg milk with variables ranging from 4724.00 kg of milk to 7684.00 kg of milk at standard breastfeeding. Regarding the lactation curve, in successive lactations, it shows an upward trend from 5089.92 kg for the first lactation to 6722.00 kg for the third lactation.

These results demonstrate the very good precocity of this breed, achieving very good productive performances, starting from the second lactation, which is reflected in the economy of this breed on the productive life (Georgesu Gh., 2000).

As such, this breed of cow is responsive, if it is provided with good conditions of maintenance and feeding, managing to express its genetic potential, which in the literature is 5000-5500 kg of milk per standard lactation.

The values of the standard deviation s = 676.48 kg of milk at lactation I, s = 556.304 kg of milk at lactation II, and at the third lactation s = 1279.357 kg of milk, highlighting the pronounced variability in this population of researched cattle, which offers possibilities of genetic selection and improvement by keeping more variants and multiplying, in the process of reproduction, valuable genotypes (Maciuc V., 2006).

The fat content of milk shows the same high variability, with an average of 3.88 ± 0.091% and individual limits between 3.40 and 4.40%. Depending on the sequence of lactations, the maximum fat content between the analyzed lactations is recorded in the second lactation (4.04%), after which it decreases at the third lactation to 2.98%.

The protein content was 3.48 ± 0.025%, with a large individual variability, the amplitude of the variability having limits between 3.31% and 3.64%, at the first lactation, continued with a slight decrease at the second lactation and at the lactation the third is the lowest value, respectively of 3.32% ± 0.071%, with limits between 3.17% and 3.64%. Corresponding to the quantity of milk and the fat and protein content, the amount of fat on the entire population was 197.00 ± 7,353 kg, respectively 176.85 ± 6,741 kg protein, which proves that the milk obtained from this breed is of very good quality (Stanciu, G., 2005).

Table 1. Mean values and variability of milk production indices at lactation I.

Character	**n**	$\overline{X}$	**±s**$_{\bar{x}}$	**s**	**V%**	**Minim**	**Maxim**
Duration of normal lactation days	38	296.15	6.828	24.620	8.313	216.00	305.00
Milk kg	38	5089.92	186.791	673.486	13.232	3193.00	5924.00
Fat %	38	3.88	0.091	0.329	8.475	3.40	4.40
Fat kg	38	197.00	7.353	26.511	13.457	132.00	230.00
Protein %	38	3.47	0.025	0.088	2.546	3.31	3.64
Protein kg	38	176.85	6.741	24.307	13.744	110.00	213.00

Table 2.Mean values and variability of milk production indices at lactation II

Character	**n**	$\overline{X}$	$\pm s_{\bar{x}}$	**s**	**V%**	**Minim**	**Maxim**
Duration of normal lactation days	38	303.71	1.237	7.623	2.510	258.00	305.00
Milk kg	38	5683.26	90.244	556.304	9.788	4797.00	6722.00
Fat %	38	4.04	0.049	0.304	7.519	3.21	4.44
Fat kg	38	227.95	3.924	24.188	10.611	172.00	270.00
Protein %	38	3.48	0.022	0.138	3.959	3.28	3.77
Protein kg	38	200.16	3.018	18.602	9.294	168.00	245.00

Table 3. Mean values and variability of milk production indices at lactation III

Character	**n**	$\overline{X}$	$\pm s_{\bar{x}}$	**s**	**V%**	**Minim**	**Maxim**
Duration of normal lactation days	13	305.00	0.000	0.000	0.000	305.00	305.00
Milk kg	13	6074.00	522.295	1279.357	21.063	4724.00	7684.00
Fat%	13	2.98	0.288	0.705	23.668	2.40	4.24
Fat kg	13	175.33	8.674	21.248	12.118	149.00	200.00
Protein %	13	3.32	0.071	0.173	5.221	3.17	3.64
Protein kg	13	200.33	13.928	34.115	17.029	172.00	244.00

Conclusion

The natural conditions, particularly favorable for the breeding of dairy cows from the Brown breed of Maramureș, materialize in:

Neamț County has large areas of natural pastures, a total area of meadows located outside the 83 Territorial Administrative Units of 98,255 ha. (Source: Directorate for Agriculture and Rural Development), which are very well exploited by this breed of cattle.

Regarding the breed Brown of Maramureș in Romania, we must specify that, although it has a large area of expansion, it entered a continuous decline after 1990, both in number and spread, Frisian and Holstein Frisian. Even if they have a more valuable genetic potential, they fail to adapt to the conditions in the hill and mountain areas specific to this area, on the one hand, and on the other hand, if optimal conditions of maintenance and feeding are not ensured, they fail to express the potential, obtaining results that are not satisfactory.

The Brown breed of Maramureș has a uniform breastfeeding, and the quality of the milk is also remarkable, among the best, due to the high content of proteins and fats, characteristic for a breed crossed with specialized breeds for milk production, but with a low percentage of protein and fat to significantly improve these characteristics.

In the improvement of Brown of Maramureș cattle from the SCDA Secuieni farm, the aim is to increase the herd, raising the genetic potential for milk by using valuable parents, to transmit to the offspring the traits they want through appropriate pairing. The genetic structure, especially in terms of its homogeneity, but also in terms of induced potential, currently provides a production of more than 6000 kg of milk at the third lactation, on average on the farm.

Within the population, there are more variants that have achieved maximum productions of 10024 kg of milk.

The normalization of the nutritional requirements and of the zootechnical and sanitary-veterinary conditions is indispensable for cattle raising and for the profitability of the farm.

Aknowledgements

Association of Animal Breeders "Operatorul I.A" Neamț, Agricultural Research and Development Station Secuieni Neamț.

References

Acatincăi, St., 2010 - Cattle breeding technology. Agropint Publishing House, Timișoara.

Alexoiu, A., Roșca, L., 1988 - Practical guide for selection and management of matings in cattle farms. Ceres Publishing House, Bucharest.

Dinescu, St., Săbădeanu P., 1966 - Feeding animals in small and medium farms. Ceres Publishing House, Bucharest.

Dinescu S., 2002, Milk production in Romania - Restrictions - Opportunities - Solutions, Ceres Publishing House Bucharest.

Dronca D., 2003, Animal breeding, Mitron Publishing House, Timişoara.

Georgescu, Gh., Ujică, V., et al., 1988 - Treaty for cattle breeding. Vol.I, Ceres Publishing House, Bucharest, pp. 53-55.

Georgescu Gh., Stanciu G., Velea C., Ujică V., 1990, The technology of cattle breeding. Didactic and Pedagogical Publishing House, Bucharest.

Georgesu Gh., 2000 - Milk and dairy products. Ceres Publishing House, Bucharest.

Georgescu Gh. Et al., 2007 - The book of the milk producer and processor, vol. II, Ceres Publishing House, Bucharest.

Grosu, H., et al., 1996 - Prediction of the improvement value in dairy cattle. Labor, I.C.P.C.B., Bucharest.

Grosu, H., Pascal, A., 2005 - Genetic improvement programs in animal husbandry. Ceres Publishing House Halga, P., et al., 2005 - Animal nutrition and nutrition, Alfa Publishing House, Iași.

Ivancia, Mihaela, 2007 - Animal breeding. Alfa Publishing House, Iași.

Maciuc V., Nacu G., Zaharia V., Zota D., 2003 - Animal breeding. Alfa Publishing House, Iași.

Maciuc V., Ujică V., Nistor I., 2003 - Practical guide for genetic improvement of cattle for milk production. Alfa Publishing House, Iași.

Maciuc V., 2006 - Cattle breeding management. Alfa Publishing House, Iași.

Neaţă, Gh., 2005 - Genetic breeding of dairy cows - a national priority. In: Journal of Animal Husbandry and Veterinary Medicine (Bucharest), № 1.

Onaciu, G., 1999, Aptitudes for milk production of a population of Romanian Bălţată cattle from central Transylvania. Symposium.USAMV Cluj-Napoca, 21-23 oct.

Onaciu, G., Velea, C., 2000 - Assessment and control of cattle production. Science Book Publishing House. Cluj Napoca.

Podar, C., Oroian, I., 2003 - Raising and exploitation of dairy cows in households. Editura Tipomar, Tg. Mures.

Stanciu, G., Acatincăi, St., Czister, L.T., 2005 - Cattle breeding technology. Eurostampa Publishing House, Timișoara.

Ujică V et al, 1998 - Characterization of a BNR cattle population from the eastern part of Romania according to some productive indices, Simp. Del. Nat. The rebirth of Romanian Animal Husbandry - a certainty of the second millennium, USA.

Usturoi, M.G., 2007 - Milk and milk technology. Alfa Publishing House, Iași.

Velea, C., Mărginean, Gh., 2004 - Production, reproduction and improvement of cattle. Vol. III, Bucharest Agrotechnical Publishing Hous.

Virginia Z., Maciuc V., Nacu Gh., Zota D., 2003 - Animal breeding, 255 pp. Alfa Publishing House.

Zalan, K., 2005 - Feeding, a factor influencing milk quality. Symposium "Cattle farm in the perspective of integration into the European Union". Second edition, September 9-10.

STUDY OF THE DYNAMICS OF BETA-HYDROXYBUTYRATE AND HEPATO-SPECIFIC MARKERS IN THE BLOOD OF NEWLY CALVED COWS WITH DIFFERENT BODY CONDITION

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Abstract

Most cows have an increase in ketone body production a few days before calving. After calving, the prerequisites for ketogenesis are even more enhanced in cows. Lack of glucoplastic substances in the diet leads to the mobilization of fats and the development of ketonemia and liver lipidosis. We carried out a study in newly calved cows in the conditions of livestock farming in the Leningrad region (Russia). Two groups of 12 cows were formed. The criterion for the formation of groups is fatness (BSC) at the beginning of the dry period (in the first group - over 3.75, in the second group - 3.0-3.5). Blood samples were taken three times - 3-5 days after calving, then twice more with an interval of two weeks. In the blood serum, the content of BHB, total bilirubin, bile acids, as well as the activity of the enzymes AST and GGT were investigated. Studies have shown that in cows in both groups, the dynamics of changes in ketones, bilirubin and bile acids is unidirectional, but in cows with increased body condition these indicators were higher. Immediately after calving, the difference between the groups for these parameters was 21.3, 36.6 and 10.8%, respectively. The maximum values were determined 2-3 weeks after calving, then their decrease was observed. One month after calving, in cows with increased body condition, the activity of AST and GGT was higher by 19.2 and 16.0% in comparison with the indicators of cows in the second group. The results obtained confirm the negative effect of obesity in cows at the end of pregnancy on metabolic processes after calving.

Keywords: *Cows, Blood serum, Biochemical parameters, Metabolism, Body condition*.

Introduction

Most cows have an increase in ketone body production a few days before calving (Senger, 2012). This is facilitated by increased absorption of glucose from the maternal circulatory system by the fetus (Korochkina *et al*., 2017, Kozitsyna *et al*., 2018). In the last two months of pregnancy, there is an intense increase in fetal weight. Therefore, with a deficiency of glucoplastic substances in the cow's body, ketogenesis is activated to provide energy for the growth of fetal tissues (Esposito *et al*., 2014, Leibova *et al*., 2018). After calving, the prerequisites for ketogenesis are even more enhanced in cows. If the ration of a fresh cow is not properly provided with easily digestible glucoplastic feed, then the mobilization of fats from the depot is sharply increased (Smith *et al*., 1997, LeBlanc, 2010, Solomakhin *et al*., 2019).

The subsequent cascade of events leads to the development of ketonemia and liver lipidosis (Moiseeva *et al*., 2019). In the blood, the concentration of non-esterified fatty acids increases sharply, which migrate from peripheral tissues to the liver, which is one of the most important organs of lipid metabolism (Cherepanov, Agafonov, 2009, Irons *et al*., 2014). Here, part of the

fatty acids undergoes beta-oxidation to ensure oxidative metabolism and ketogenesis; part is used for the resynthesis of triglycerides and the incorporation of the latter into very low density lipoproteins, which are subsequently exported to mamillar tissue to participate in the formation of milk fat. In highly nourished cows, tissue lipolysis occurs intensively and an excess of lipid molecules occurs in hepatocytes, which do not have time to oxidize or be incorporated into lipoproteins, which are the transport form of lipids in the systemic circulation (Plemyashov, Moiseenko, 2010, Omarov, Slesareva, 2017, Karpenko *et al*., 2018).
At the same time, an increase in tissue lipid mobilization suppresses the appetite of a newly calved cows, which subsequently leads to a decrease in feed intake and aggravation of energy starvation. There is evidence of the detrimental effect of obesity in cows on their health in the postpartum period. In connection with the above, we set the task to study the content of β-hydroxybutyrate and hepato-specific markers in the blood serum of fresh cows with different body condition.

Material and Methods

We carried out a study in newly calved cows in the conditions of the Osminskoye livestock farm in the Leningrad region (Russia). Two groups of newly calved cows, 12 heads each, were formed. The criterion for the formation of groups is fatness at the beginning of the dry period. The cows of the first group have fatness over 3.75 points, cows of the second group - 3.0 - 3.5 points on the BSC scale. Blood was taken three times - the first stage - 3-5 days after calving, the second stage - after 15-20 days and the third stage - 30-35 days after calving. In the blood serum, the content of β-hydroxybutyrate (BHB), total bilirubin, bile acids, as well as the activity of the aspartate aminotransferase (AST) and gammaglutamyltransferase (GGT) enzymes were investigated. The study was carried out in the winter-spring season: the beginning of the dry period in January-February, calving in cows - in March-April.

Results and Discussion

The results of the studies carried out are presented in tables 1, 2.

Table 1. Assessment of body condition of cows before and after calving

Study period:	Group 1	Group 2	Certaintly factor in intergroup comparison (P)
The beginning of the dry period	4.04±0.08	3.33±0.06	P<0.001
3-5 days after calving	3.63±0.05***	3.15±0.12	P<0.001
30-35 days after calving	2.96±0.07***	3.0±0.08***	P>0.05

* - P<0.05, ** - P<0.05, *** - P<0.01(when compared with the baseline within the group)

Studies have shown that in cows of the first group, body condition according to points for the dry period decreased by 10.2% (P <0.001), in cows of the second group - by 5.4%. During the first month of lactation in the first and second groups, there was a decrease in body condition by

18.5% and 4.8%, respectively. At the same time, in both groups, there is a significant decrease in the indicator in comparison with the initial value (P <0.001).

Table 2. Dynamics of biochemical parameters in cows in the first month after calving

Investigated indicators	Research stages	Group 1	Group 2	Certaintly factor in intergroup comparison (P)
BHB, mmol/l	1	1.36±0.10	1.07±0.06	P<0.01
	2	1.63±0.18	1.23±0.11	P>0.05
	3	1.44±0.11	1.01±0.07	P<0.001
Bilirubin, μmol/l	1	5.82±0.67	3.69±0.37	P<0.05
	2	7.29±0.56	5.18±0.54*	P<0.05
	3	6.11±0.62	3.17±0.39	P<0.001
Bile acids, μmol/l	1	50.56±2.3	45.11±2.61	P>0.05
	2	64.91±4.31**	48.85±5.24	P<0.05
	3	58.95±2.90*	44.06±5.00	P<0.05
AST, U/l	1	112.26±4.27	106.14±3.68	P>0.05
	2	119.03±7.07	103.6±5.98	P>0.05
	3	123.89±6.3	100.12±2.8	P<0.01
GGT, U/l	1	27.04±1.77	25.84±1.35	P>0.05
	2	25.3±1.85	26.88±1.31	P>0.05
	3	30.42±1.65	25.56±1.61	P<0.05

* - P<0.05, ** - P<0.05, *** - P<0.01 (when compared with the baseline indicator within the group for each indicator)

The BHB concentration in cows of the first and second groups immediately after calving was 1.36±0.10 and 1.07±0.06 mmol/l, after two weeks - 1.63±0.18 and 1.23±0.11 mmol/l. Thus, in both groups, an increase in ketogenesis is revealed - by 16.6% and 13.0%, respectively. A month after calving, the concentration of BHB in the blood decreases and is 1.44±0.11 mmol/l in cows of the first group, and 1.01±0.07 mmol/l in the second group (P <0.001 by intergroup comparison).

The post-calving bilirubin level was significantly higher in cows of the first group by 36.6% and amounted to 5.82±0.67 μmol/l. Two weeks later, in cows of the first and second groups, the indicator increased by 20.2% and 28.7%, respectively. A month later, there is a decrease in the level of bilirubin in both groups, however, in cows with optimal body condition, the indicator is 34.0% lower (P <0.001).

The level of bile acids in the blood serum is initially higher in the first group by 10.8%. In the future, there is an increase in the indicator in both groups, while the maximum values are determined within 15-20 days after calving. Further, at the end of the observation period, the concentration of bile acids decreases, but in the first group the indicator remains above the initial level, and in the second it returns to the initial value. When comparing between the groups at the second and third stages of the study, a significant increase in the indicator is determined by 24.7% and 25.3% (P<0.05).

When comparing the activity of blood serum aspartate aminotransferase, the progressive increase in the indicator in cows of the first group during the observation period from 112.26±4.27 to

123.89±6.3 U/L is determined. In cows of the second group, there is a slight downward trend - from 106.14±3.68 to 100.12±2.8 U/L.
With regard to the activity of GGT, there is a tendency to an increase in the indicator in animals of the first group at the end of the experiment, while in the second group, slight fluctuations of this indicator are revealed. In the period of 30-35 days after calving, the GGT activity is higher by 16.0% in cows of the first group ($P<0.05$).

Conclusions

Summing up, it can be argued that cows with increased body condition during the dry period during early lactation undergo adverse changes in comparison with animals with optimal body condition. There is progressive weight loss during the first month of lactation. Obese cows have increased production of ketones, bilirubin and bile acids compared to cows with optimal body condition. An increase in the activity of hepato-specific enzymes - AST and GGT is also determined. Studies have shown that cows with a body condition of 3.0 - 3.5 points, all other things being equal, cope better with the load during the transit period. It is important to control the fatness of lactating cows and prevent their obesity at the end of the lactation period.

References

Leibova, V.B., Shapiev, I.Sh., Nikitkina, E.V., Plemyashov, K.V., Anipchenko, P. (2018) Biochemical status of cows in the dry period in connection with reproductive performance and milk productivity. Reproduction in Domestic Animals. 53, S2, 157-158.

Cherepanov, G.G., Agafonov, V.I. (2009) Identification of substrate metabolic fluxes in lactating cows. Problems of the biology of productive animals. 2, 44-60.

Solomakhin, A.A., Mityashova, O.S., Rykov, R.A., Smekalova, A.A., Lebedeva, I.Yu. (2019) Dynamics of changes in indicators of protein-carbohydrate metabolism in the first trimester of lactation in black-and-white cows in connection with reproductive potential. Genetics and animal breeding. 4, 20-25.

Moiseeva K., Anipchenko P., Vasileva S. Karpenko L., Vasil'ev R., Pilaeva N., Bakhta A., Panova N., Trushkin V., Nikitin G., Nikitina A., Ershova O. (2019) Dynamics of cholesterol and triglycerides in the serum of cows with liver lipidosis. Journal of Animal Science. 97, S3, 208.

Esposito, G., Irons, P.C., Webb, E. C., Chapwanya, A. (2014) Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144, 3-4, 60-71.

Karpenko, L.Yu., Pilaeva, N.V., Vasiliev, R.M., Vasileva, S.V. (2018) Comparative assessment of the dynamics of the main indicators of metabolism in cows with different milk productivity. Issues of legal regulation in veterinary medicine. 3, 190-192.

Korochkina, E.A., Romanenko, L.V., Anipchenko, P.S. (2017) On the issue of maternity paresis of cows and measures of its prevention. Genetics and animal breeding. 3, 83-86.

Kozitsyna, A., Karpenko, L., Bakhta, A., Anipchenko, P., Balykina, A. (2018) Mycotoxin eliminator "Elitox" in lasttrimester pregnant cows application impact on immune blood profile of offspring. Reproduction in Domestic Animals. 53, S2, 153.

LeBlanc, S. (2010) Monitoring metabolic health of dairy cattle in the transition period. J. Reprod Dev. 56, 29–35.

Smith, T.R., Hippen, A.R., Beitz, D.C., Young, J.W. (1997) Metabolic characteristics of induced ketosis in normal and obese dairy cows. J. Dairy Sci. 80, 1569–1581.

Omarov, M.O., Slesareva, O.A. (2017) Prevention of metabolic disorders in cows. Collection of scientific papers of the North Caucasian Research Institute of Livestock. 6, 1, 229-235.

Plemyashov, K.V., Moiseenko, D.O. (2010) Reproductive function of highly productive dairy cows with metabolic disorders and its correction. Issues of legal regulation in veterinary medicine. 1, 37-40.

Senger, Ph. L. Pathways to Pregnancy and Parturition, 3rd Edition. Redmond, OR: Current Conceptions, [2012] ©2012, 390 p.

THE PHYTOBIOTIC EFFECT OF NETTLE (Urtica Simensis S.) ON THE GROWTH OF CHICKEN "COBB 500"

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Abstract

In this paper, the influence of ground nettle leaves as a phytogenic supplement in broiler nutrition on production results and growth parameters was examined. The diet test was performed according to the principle of group control and lasted 42 days. One-day-old Cobb 500 provenance chickens were used for the experiment, and tests were performed on chickens of both sexes with an average body weight of 48.67 ± 2.49 g. The experiment was performed on a total of 120 broilers divided into 4 groups of 30 chickens. That is, the chicken was divided into control group K (no phyton-nettle supplement), experimental O-I group (phytogen-nettle supplement, 1%), experimental O-II group (phytogen-nettle supplement, 1.5% in food) and experimental O-III group (with phytogen-nettle supplement, 2% in food). In fattened chickens the best result of body weight was found in chickens of experimental groups: O-III those consuming food with the addition of 2% nettle (2658.94 g), O-II those consuming food with the addition of 1.5% nettle (2450.00 g) and O-I those consuming food with the addition of 1% nettle (2330,20 g) relative to the control group K having the lowest body weight (2120.10 g).; Average food consumption was highest in the O-I trial (128.00 kg) compared to the control group consuming more food (12.70 kg) compared to the O-II (121.60 kg) and experimental groups O-III (112.23 kg). Obtained food conversion results per kilogram increment show that the experimental group O-III receiving nettle supplement of 2% had the best food conversion per 1 kg increment (growth 1.37 kg / kg) compared to the experimental groups receiving nettle supplement of 1.5% (1.66 kg) / kg growth), ie. nettle content in the mixture of 1% (growth of 1.77 kg / kg). The worst results were shown by the control group consuming food without adding nettle.

Key words: *broilers, phytogenic additives, nettle, conversion, growth.*

Introduction

Livestock production as a branch of animal husbandry has made remarkable progress in the last thirty years. The newly created hybrids for both meat production and egg production have contributed to the poultry production taking on the character of industrial production. The greatest importance of livestock production is reflected in the fact that products of high biological value for human consumption are obtained in a short time. Many years of genetic research in modern poultry production is based solely on the creation and utilization of line hybrids. The production of high quality hybrids is a very long and complex business. In fact, it comes down to the formation of mostly pure, and rarely synthetic, lines that give offspring with highly expressed production traits. Due to the specific structure and function of the poultry digestive system, the diet differs from other animals, so quality poultry concentrates are mostly used in poultry. Mercury cooks carbohydrates almost over 90%, and proteins and fats between 80 and 90%. Due to the high efficiency of food utilization, poultry growth is very intense. The main feature of modern poultry production today is the tendency to reduce the use of antibiotics in food, while exploring alternatives, to ensure effective disease control and high production (Pešić et al., 2011). Uses include probiotics, prebiotics, organic acids, enzymes, and increasingly, herbal additives, with some stimulating effect and antibacterial potential. Such herbal supplements are called phytobiotics and their use and research into their effects are becoming increasingly popular. They are attributed to effects such as: stimulation of food consumption, action against bacteria, coccidia, parasites, then anti-inflammatory and immunostimulating action.

Phytobiotics have great potential for use in broiler fattening because of their antioxidant properties, their effect on improving food quality, digestive functions, as well as their ability to improve the body's immune status. This is further compounded by their antibacterial properties, making phytobiotics a natural iantioxidant and a substitute for growth stimulants (Windisch et al., 2008). One such phytobiotic is nettle, it is extremely rich in various useful ingredients: protein, carbohydrates, fats, calcium, phosphorus, iron, vitamins C, A, B2 and K, carotene, pantothenic acid, etc. It also contains flavonoids, organic acids, nitrogenous substances and more. Nettle leaves (Urtica dioica) are rich in caffeic acid ethers and flavonoids, which are attributed to certain immunostimulating, anti-inflammatory effects, and are good growth stimulants and antioxidants. Safamehr et al. (2012), in their research, show the influence of the addition of different concentrations of nettle mixed in the fattening mixture on production results and selected biochemical parameters and found that the experimental groups showed a statistically significant weight gain on the 42nd day of fattening and Conversion rates were significantly lower, and Pilad also had significantly lower serum triglyceride and cholesterol concentrations than controls.

Al-Mashhadani et al. (2013) set up a similar experiment with the addition of different concentrations of chamomile oil in the fattening mixtures of chickens, conducted and concluded that the chickens of the experimental groups had statistically significantly higher final weight and gain as well as statistically significantly lower food conversion compared to the control group chickens. . They also found that the test group chicks had statistically significantly lower plasma cholesterol and glucose concentrations than the control group chicks. Similar results were obtained by Al-Kassei and Kalhel (2011) in the experiment of the effect of dandelion added at different concentrations in compound feedingstuffs for fattening chickens on the production results of fattening chickens. The aforementioned authors found that the chickens of the experimental groups had a statistically significantly higher final live weight and gain compared to the chickens of the control group.

The aim of the experiment was to examine the effect of the contents of nettle leaf on production results (body weight, weight gain, feed conversion) of chickens in fattening.

Material and Methods

In this paper, the influence of minced nettle as a phytogenic supplement in broiler nutrition on the production results and parameters of chicken growth was examined. After harvesting, the nettle leaves were dried in natural light, after which they were ground and added to the concentrate mixture. The grinding of nettle leaves exposes the influence of the main constituent of flavonaid leaves.

Investigations were performed with experimental chickens housed in the production facilities of the poultry farm "Pilepromet", in the period from March to April 2020. The diet test was performed according to the group-control principle and lasted for 42 days. One-day-old Cobb 500 provenance chicks were used for the experiment, and chicks of both sexes with an average body weight of 48.67 ± 2.49 g were used for testing.
The experiment was performed on a total of 120 chickens divided into 4 groups of 30 chickens. That is, chickens were divided into control group K (without phytogen-nettle supplement), experimental OI group (with phytogen-nettle supplement, 1%), experimental O-II group (with phytogen-nettle supplement, 1.5% in food)) and the experimental O-III group (with the addition of phytogen-nettle, 2% in food). The experiment lasted for 42 days and was divided into two phases. The first phase lasted 0-20. and the second phase of 21-42. of the day. In the first phase, chickens consumed food with 22% of CP, while in the second phase, they consumed food with 17% of CP.
At the beginning and end of each phase of the experiment, the body weight of each animal was individually measured and the food consumed, all with the aim of obtaining data that was used to calculate other production results. The statistical program SPSS Statistics 25 was used to process the results. Significance of differences between group mean values was checked by line method (GLM), analysis of variance (ANOVA), at significance level P <0.05 and P <0.01.

Results and Discussion

The tables show the results as mean and standard deviation. For the purpose of monitoring body weight as one of the four basic production indicators, the body weight of chickens was checked once a week, and the results obtained were statistically processed, shown in Table 1.

Table 1. Average weight of chickens per week, (g).

WEEK OF WEIGHT		CONTROL (K)	VIEW GROUP (O-1)	VIEW GROUP (O-2)	VIEW GROUP (O-3)	DIFFERENCES
The beginning of the first week	$\bar{X}$+Sd	48,43±1,89	48,11±1,66	48,40±1,57	48,40±1,71	
	p		0.579	0.090	0.574	do not exist
The end of the first week	$\bar{X}$+Sd	158,89±20,76	174,02±13,65	177,52±19,21	196,03±14,36	
	p		0.034*	0.174	0.939	since
The end of the second week	$\bar{X}$+Sd	321,20±36,23	335,20±24,22	336,25±20,20	411,23±16,78	
	p		0.740	0.116	0.008**	since
The end of the third week	$\bar{X}$+Sd	607,81±40,24	702,14±34,34	773,62±30,84	807,56±10,48	
	p		0.401	0.144	0.007**	since
The end of the fourth week	$\bar{X}$+Sd	1069,12±134,20	1196,23±110,23	1236,10±99,25	1307,53±86,23	
	p		0.701	0.644	0.019*	since
The end of the fifth week	$\bar{X}$+Sd	1685,31±208,23	1782,20±168,23	1798,69±179,36	1994,23±98,50	
	p		0.959	0.704	0.050*	since
The end of the sixth week	$\bar{X}$+Sd	2120,10±250,20	2330,20±245,20	2450,00±240,10	2658,94±126,30	
	p		0.696	0.434	0.032*	since

(K) -control group, (O-1) -concentrated mixture + 1% nettle flour, (O-2) - concentrated mixture + 1.5% nettle flour, (O-3) - concentrated mixture + 2%, $\bar{X}$- arithmetic mean, sd-standard deviation, p-statistical probability * p <0.01 ** p <0.05

Table 1 shows that the average body weights of the chicks at the beginning of the experiment were uniform, and as expected, there were no statistically significant differences (P> 0.05) between the groups. At the end of the first week, the average body weight of the control group chickens was greater than in the other three experimental groups, but the differences between the groups were small so that there was no statistically significant difference. The weight of the chickens in the experimental groups achieved by the addition of nettle is in line with the results obtained by Tabari et al., 2016; Keshavarz et al., 2014; Loetscher et al., 2013; Nasiri et al., 2010. Measurements in test group O-3 showed the highest body mass at the end of the second week, while the lowest values were recorded in control group K. The differences in average body masses between test group O-2 and group O-2 and O-3 were statistically significant. significant (P <0.05). In the following weeks, the trend of body mass movement by groups continued, so that at the end of week 3 and week 4, when the best values were achieved by the O-3 group of chickens with statistical significance (P <0.01), and the best body mass at at the end of week 5 and week 6 it was achieved in chickens of the O-3 experimental group with statistical significance (P <0.05). The highest final weight gain was achieved by chickens from O-3, (2658.94 g), slightly lower values were observed in O-3 and O-2, (2450.00 g; 2330.20 g). while the lowest was in control group K chickens (2120.10 g). The consumption of chicken feed in the experiment was monitored weekly by groups, and the results obtained in absolute and relative values are shown in Table 2.

Table 2. Table 2. Average food consumption by groups and weeks of fattening, (kg).

WEEK OF WEIGHT	CONTROL (K)		VIEW GROUP (O-1)		VIEW GROUP (O-2)		VIEW GROUP (O-3)	
	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%
first week	4,40	100,00	4,90	111,36	5,20	118,18	5,10	115,90
second week	9,60	100,00	9,10	94,79	9,40	97,91	8,40	89,36
third week	17,80	100,00	17,10	96,06	17,00	95,50	15,80	88,23
Total first-third 22% CP	31,80	100,00	31,10	97,79	31,60	99,37	29,30	92,13
fourth week	25,10	100,00	26,20	104,38	22,10	88,04	20,53	81,79
fifth week	32,40	100,00	32,60	100,61	30,40	93,82	28,10	86,72
sixth week	37,90	100,00	38,10	100,52	37,50	98,94	34,30	90,50
Total fourth-sixth 18% CP	95,40	100,00	96,90	101,57	90,00	94,33	82,93	86,92
TOTAL	**127,20**	**100,00**	**128,00**	**100,62**	**121,60**	**95,59**	**112,23**	**88,23**

(K) -control group, (O-1) -concentrated mixture + 1% nettle flour, (O-2) - concentrated mixture + 1.5% nettle flour, (O-3) - concentrated mixture + 2%, $\bar{X}$ - arithmetic mean,% -index

The previous table shows that the food consumption in the first week of the experiment was higher in the experimental groups compared to the control group, and the highest food consumption had the chickens of the experimental group O-2 (5.2 kg), which is in comparison with the control group. group (4.4 kg) by 18.18% more. Chicks in the O-1 and O-3 experimental groups (4.9 kg; 5.1 kg) consumed 11.13% and 15.90% more food, respectively, than the control group chicks. The result obtained with the addition of nettle (experimental groups 1,2,3) is in line with the results of the study by Tabari et al., 2016; Keshavarz et al., 2014; Loetscher et al., 2013; Nasiri et al., 2011. When observing the period of consumption of food in the second, third, fourth, fifth and sixth weeks of the experiment, the trend of food consumption in the test group chickens was changed relative to the control group of chickens. The observed difference is most clearly visible when looking at the three-week review, with the O-1, O-2, and O-3 experimental groups consuming less food by 2.21%, 0.63%, and 7.87%, respectively. control group.

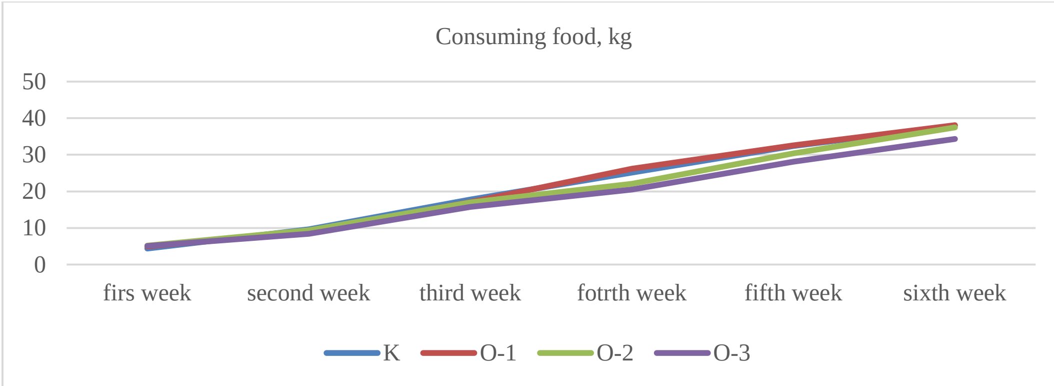

Figure 1. Average food consumption by groups and weeks of fattening, (kg).

This trend of food consumption by groups also continued in the next three-week period, that is, the fourth, fifth and sixth weeks of the trial. The results obtained show that during this period only the experimental group O-2 had higher food consumption in comparison with the control group by 1.57%, while the experimental groups O-1 and O-3 consumed less food compared to the control group by 5.67% and 13.08%, respectively. The significance of the obtained results was increased by their comparison with the results of scientists who published the results that examined different concentrations of nettle supplement in food, e.g. 1% (Safamehr et al., 2012) and 1.5% (Mansoub, 2011), while Kwiecien and Winiarska - Mieczan (2009) and Ghasemi et al. (2014) obtained such results by adding 2% nettle. On the basis of the known values of weekly fattening increments of chickens and food consumption, the conversion of food per kilogram of growth was calculated, which is calculated from the ratio of total food consumption and total growth. The obtained food conversion results are shown in Table 3.

Table 3. Average feed conversion per kg of growth (by groups and weeks of fattening), (kg).

WEEK OF WEIGHT	CONTROL (K)		VIEW GROUP (O-1)		VIEW GROUP (O-2)		VIEW GROUP (O-3)	
	kg/kg	%	kg/kg	%	kg/kg	%	kg/kg	%
first week	1,32	100,00	1,29	97,77	1,34	103,87	1,15	85,82
second week	1,77	100,00	1,86	105,08	1,97	105,91	1,30	65,99
third week	2,06	100,00	1,54	74,75	1,30	84,41	1,33	102,30
<u>Total first-third</u>	1,71	100,00	1,56	91,22	1,54	98,71	1,26	81,81
fourth week	1,81	100,00	1,76	97,23	1,59	90,34	1,37	86,16
fifth week	1,75	100,00	1,85	105,71	1,80	97,30	1,37	76,11
sixth week	2,90	100,00	2,31	73,44	1,92	83,11	1,72	89,58
<u>Total fourth-sixth</u>	2,10	100,00	1,97	93,80	1,77	89,84	1,48	83,61
TOTAL	**1,95**	**100,00**	**1,77**	**90,77**	**1,66**	**93,78**	**1,37**	**82,53**

(K) -control group, (O-1) -concentrated mixture + 1% nettle flour, (O-2) - concentrated mixture + 1.5% nettle flour, (O-3) - concentrated mixture + 2%, (kg / kg)¯- food conversion per kg. increment,% -index

Considering the results presented in Table 3. we can see that the best food consumption was achieved by the experimental groups in the whole period of the crane conversion, with the emphasis on the experimental group O-3, which in the total observed period of 42 days achieved lower food consumption compared to the control group by 17.47%, while the experimental groups O-2 and O-1 also performed better by 9.23% and 6.22%, respectively. Addition of nettle at a concentration of 2% in the concentrated mixture resulted in less food consumption. These results are consistent with the results obtained by several authors (Tabari et al., 2016; Loetscher et al., 2013; Nasiri et al., 2011; Safamehr et al., 2012; Ghasemi et al., 2014).

Conclusion

The effect of the nettle, and especially of the phytogens found in the nettle leaf, has shown an impact on the production results of COBB 500 fattening chicks; In the fattened chickens, the best results in body weight had the chickens of the experimental groups: O-3 who consumed food with the addition of 2% nettle (2658.94 g), O-2 who consumed food with the addition of 1.5% nettle (2450.00 g), O-1 who consumed food with the addition of 1% nettle (2330.20 g) relative to the control group having the lowest body weight (2120.10 g); Average food consumption was highest in the O-1 (128,00 kg) experimental group compared to the control group that consumed more food (127.20 kg) compared to the O-2 (121.60 kg) and O experimental groups. O-3 (112.23 kg); Obtained food conversion results per kilogram of increment show that the O-3 experimental group receiving 2% nettle supplement had the best conversion of food per kg increment (1.37 kg / kg increment) compared to the experimental groups receiving 1.5% nettle supplement (1.66 kg / kg increase), ie nettle content in the mixture of 1% (1.77 kg / kg increase). The worst results were shown by the control group who consumed food without the addition of nettle.

References

Al – Kaisse, G. A. M., Khalel, E. K. (2011). The potency of chamomille flowers (Matricaria chamomilla L.) as feed supplements (growth promoters) on productive performance and hematological parameters constituents of broiler. *International journal of poultry science,* 10: 726 – 729.

Al – Mashhadani, E. H., Al – Mashhadani, H., Al – Shamire, J. S. (2013). Effect of supplementing different levels of chamomille oil on broiler performance and some physiological traits. *International journal of poultry science* 12: 426 – 429.

Ghasemi, H. A., Taherpour, K., Hajkhodadani, I., Akhavan-Salamat, H. (2014). Comparative effects of nettle (Urtica dioica) and commercial feed additives on productive performance and blood lipid profile of broiler chickens. *Journal of animal science advances*, 4: 633 – 640.

Keshavarz, M., Rezaeipour, V., Asadzadeh, S. (2014). Growth performance, blood metabolites, antioxidant stability, carcass characteristics of broiler chickens fed diets containing nettle (Urtica dioica L.) powder or essential oil. *International journal of advanced biological and biomedical research*, 2: 2553 – 2561.

Kwiecien, M., Winiarska – Mieczan, A. (2009). Effect of addition of herbs on body weight and assesment of physical and chemical alterations in the tibia bones of broiler chickens. *Journal of elementology*, 14: 705 – 715.

Loetscher, Y., Kreuzer, M., Messikommer, R. E. (2013). Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. Poultry science, 92: 2938 – 2948.

Mansoub, N. H. (2011). Comparison of effects of using nettle (Urtica dioica) and probiotic on performance and serum composition of broiler chickens. Global veterinaria, 6: 247 – 250.

Nasiri, S., Nobakht, A., Safamehr, A. (2010). The effects of different levels of nettle Urtica dioica L. (Urticaceae) medicinal plant in starter and grower feeds on performance, carcass traits, blood biochemical and immunity parameters of broilers. *Iranian journal of applied animal science, 1: 177 – 181.*

Pešić, B., Milošević, B., Spasić, Z., Stolić, N., Milanović, V., (2011). Phytogenics as new sustainable approach in egg industry, International Conference ''Research people and actual task on multidisciplinary sciences'', Lozenec - Bulgaria, Volume 1, pp 116-120

Safamehr, A., Mirahmadi, M., Nobakht, A. (2012). Effect of nettle (Urtica dioica) medicinal plant on growth performance, immune responses, and serum biochemical parameters of broiler chickens. *International research journal of applied and basic sciences*, 3 (4): 721 – 728.

Tabari, M. A., Ghazvian, K. H., Irani, M., Molaei, R. (2016). Effects of dietary supplementation of nettle root extract and pumpkin seed oil on production traits and intestinal microflora in broiler chickens. *Bulgarian journal of veterinary medicine*, 2: 108 – 116.

Windisch, W., Schedle, K., Plitzner, C., Kroismayr, A., (2008). Use of phitogenic products as feed additives for swine and poultry. *Journal of Animal science* 86, 140- 148

THE HONEY AND ENVIRONMENT

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Abstract

The paper presents potential residues of polychlorine biphenyls (PCBs) and sulfonamides in honey. Investigation was performed analyzing 20 samples of acacia and meadow honey. The samples were collect from 4 different parts of Republic of Srpska (RS), Hercegovina, Romanija, Doboj and Karajina region. For determination of residual levels of polychlorine biphenyls, gas chromatography with electron-capture detection (GC/ECD) was used. Analysis of sulfonamides was done with ELISA test in honey (5-600 µg/kg). Results indicate that in the described beekeeping zones very small amount of investigated residues in honey were detected. Also the results are evaluated with current regulations: Commission Regulation (EU) n 37/2010; Official Gazette of BiH, No. 89/12, 92/17 and 21/19 and Official Gazette of the FRY No. 5/92 and 11/92". Honeybees are the main indicators of the environment. Where pollution with toxic gases is high, honey bees find it difficult to survive, which is also a sign that the environment is endangered. Therefore, by raising honey bees, people protects nature and themselves as someone who belongs to nature and its environment. The analysis in 4 regions of RS has determined the ecologically clean environment that provides an opportunity for the development of beekeeping in the other parts of the state.

Keywords: *Honey, polychlorine biphenyls, sulfonamides, residues, environment.*

Introduction

Honey bees play an extremely important role in the ecosystem and their importance should not only be measured by the benefits they give from the direct production of high quality food, they also have an irreplaceable role in pollination, which certainly results in a contribution of billions of dollars. Bees are the most dominant and most specialized pollinators, there are about 17,000 known species (Michener, 2000). The damage caused by the absence of their activity can be much greater and there is not only the question of lost earnings but the overall reduction of food of plant origin, which directly threatens our survival (Kićović, *et al.,* 2004).

Honey as the primary product of the honey bee is a natural product, variable not only in color, taste and smell but also in its chemical composition (Abadžić, 1982), and depends on the ecological environment and plant cover of honey plants. Having in mind the number of possible sources of honey pasture, it is understandable that there are no completely identical honeys from different localities (Ađić, 1932).

In the countries of the European Union (EU), the results of analyses of honey for the content of heavy metals and pesticide residues serve as an indicator of the degree of environmental pollution, ie the state of product quality in that area.

The presence of polychlorine biphenyls are the results of uncontrolled spillage and atmospheric deposition is associated with excessive use in the past (González Sagrario, *et al.* 2002).

Polychlorinated biphenyls are especially toxic because they decompose slightly during metabolic reactions in living organisms. They are also susceptible to biomagnification, ie they enter the food chain through accumulation in lower organisms and plants. All these properties of polychlorinated biphenyls make it a big problem in the field of environmental protection (Gavrančić and Skala, 2000).

The bioindicators of environmental pollution are honey bees and their products owing to their intense foraging activity in nearness to their hive (Santosa, *et al.,* 2021). During their collection of water, nectar, and pollen from flowers, bees can carry environmental contaminants in their hives, via inhalation, or ingestion, when being exposed to a large number of pollutants such as pesticides, in a radius ranging from 1.5 to 3 km around the hive, depending on food abundance (García-Valcárcel, *et al,*. 2016; Herrera López, *et al.,* 2016; Drummond, *et al.,* 2017).

The environment may be affected with organic pollutants, for example, PCBs, which are chemicals that bioaccumulate through the food chain and pose a risk of causing adverse effects to human health and the environment (Zhao, *et al,*. 2017). In past (also nowadays in some states) PCBs were primarily used in electrical equipment, such as transformers and capacitors, as insulators and they reached accumulated global production approximately 1.3 million tons (Zhao *et al.,* 2017). Due to inappropriate disposal of equipment containing PCBs environment is contaminated through the anthropogenic activities. Therefore, research has proven the presence of PCBs remains in water, soil, food, animals, and humans (Roszko, *et al.,* 2014; Mohr, *et al.,* 2015). Also those environmental pollutants is associated with adverse health effects, including cancer and diabetes, in addition to neurobehavioral and immunological changes in humans (Ali, *et al.,* 2016; Mustieles, *et al.,* 2017; Vafeiadi, *et al.,* 2017; Ziegler, *et al,*. 2017).

In Republic of Srbska prescribed value for PCBs in honey is not defined, only respecting the regulations Official Gazette of the FRY No. 5/92 and Official Gazette of the FRY No. 11/92, where milk and milk products, eggs, poultry, other animal meat, fish, shellfish, crustaceans and molluscs, the permitted concentrations of PCBs are indicated. EU and some other countries implemented Stockholm Convention on Persistent Organic Pollutants (POPs). States Parties to this Convention have an obligation to establish, prohibit or restrict the production, trade and use of POPs, as well as to reduce or eliminate emissions of 12 POPs chemicals (aldrin, chlordan, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, PCB, PCDD / PCDFs) into the environment.

The use of different antibiotics in honey and other honey bee products are usually treat in a veterinary setting, such as streptomycin, sulfonamide, and chloramphenicol. Generally, beekeepers has practice to use antibiotics (at relatively high doses) to treat infections, or as "growth promoters (Duarte, *et al.,* 2020). Residues that occur, as a result of unconscious use of antibiotics in order to prevent honey bee diseases cause food safety issues (Korkmaz, *et al.,* 2017) .

In terms of public health, antibiotic residues are also potentially dangerous (Bogdanov, 2006, Al-Waili, *et al.,* 2012). For the majority of food produced by animals treated with sulfonamides and tetracyclines, maximum residue limits (MRLs) have been established, but not for bee products such as honey. The honey is traded as international product for human's consumptions and most of countries accept standards set by the Codex Alimentarius (Tillotson and Theriault 2013).

In EU countries, the use of antibiotics in beekeeping is not legal and MRLs is not established for antibiotics in honey according to the regulations, which means that honey containing antibiotic residues is not permitted to be sold.

In Republic of Srbska Official Gazette of the FRY No. 5/92 and 11/92, is recognized, where milk and milk products, meat and meat products, eggs and egg products, freshwater fish, honey and other foodstuffs of animal origin may be placed on the market if they do not contain sulfonamides residues in quantities greater than 0.10 mg/kg.
The honey bees are the main indicators of the environment. Where pollution with poisonous gases is high, bees have a hard time surviving, which is also a sign that the environment is endangered. Therefore, by raising honey bees, man protects nature and himself as works of that nature;
This paper indicates presenting the possibilities of production honey in 4 regions of RS with emphasis on environmental quality of beekeeping areas.
Presented research should show the environment in which we are engaged in agriculture, ie the possibilities of sustainability of our products, through quality, on the world market.

Materials and Methods

Samples were collected at the end of the honey collection season. A total of 20 samples of different species of honey (acacia and meadow honey) were taken from 4 different parts: from Hercegovina region were collected 5 samples of meadow honey, the same was in Romanija region in Bosnia and Herzegovina, for both, Doboj and Krajina were taken 5 samples of acacia honey (Bilić-Šobot, 2020). After collection of samples they were sent at the Institute of meat hygiene and technology in Belgrade for future analysis. For determination of residue of polychlorine biphenyls it was used solid-phase extraction cleanup followed by gas chromatography with electron-capture and nitrogen-phosphorus detection (GC/ECD) detection limit mg/kg: 0.001 described by Herrera *et al.* (2005). The analysis of sulfonamides was done with ELISA test in honey (5-600 μg/kg). It was used documented method according to the manufacturer's instructions for diagnostic kit: Sulphonamides ELISA test kit Cat No 1056, (5-600 μg/kg) MaxSignal® Bioo Scientific Corporation, USA. Respectively, samples were prepared and analyses by described manufacture procedure "MaxSignalTM Chloramphenicol (CAP) ELISA Test Kit Manual Catalog #: 1013" available at http://www.medibena.at/media/bioo/produc
In this paper, the results were compared with the following legal regulations: Official Gazette of BiH No. 37/09 and 25/11 and 21/19 and Official Gazette of the FRY No. 5/92 and Official Gazette of the FRY No. 11/92.

Results and Discussion

Table 1 shows the results for the presence of polychlorinated biphenyls and sulfonamides. The values obtained for all 20 honey samples were 0.001 for biphenyls and 0.005 mg/kg for sulfonamides. The prescribed values are not defined by Official Gazette of the FRY No. 5/92 and Official Gazette FRY no. 11/92 for PCBs, while sulfonamides residues are under prescribed value max. 0.10 mg/kg.

Table 1. Results of laboratory test of honey samples for the presence of residues of polychlorine biphenyls and sulfonamides

	Meadow honey	Acacia honey	Prescribed value
Units	mg/kg		
Number of honeys	10	10	
Polychlorine biphenyls	< 0.001		not defined
Sulfonamides	< 0.005		max. 0.10

max.- maximum

The research of Erdoğrul *et al.*, (2007) examined 35 samples of honey for the presence of polychlorinated biphenyl residues, where 8 of 35 samples of residues were founded in ranged around 0.25 to 3.07 ng g−1. Recently published study by Santos *et al*,. (2021) shows the investigation of PCBs residues in honey on 90 samples of tasted commercial honey. Where 15 of 90 samples had PCBs residues, with most highly detected concentration 531 ng g^{-1}. Reported data were not in agreement with ours, where residues of polychlorinated biphenyls ether for meadow and acacia honey, was 0.001 mg/kg, which is far less according to the research of Erdoğrul *et al*., (2007). The same reports were published by Saitta *et al.*, (2017) with not detect any of the 18 PCBs analyses in Italian honey samples from the regions of Sicily and Calabria by GC-MS/MS. The presence of PCBs in the honey indicates that the honey bees were exposed to these pollutants, confirming the presence of environmental contamination, reported in some countries (Herrera, *et al*., 2005; Chiesa, *et al*. 2016, Erdoğrul, *et al.*, 2007, Santos, *et al*., 2021)
This contamination in different geographical origins and countries is probably associated with the micro-regions visited by the honey bees or with some global pollution. As most countries respect and implement the Stockholm Convention, monitoring of PCBs in the environment and in food is necessary to prevent human exposure, especially through food intake. Honey quality and safety are essential concerns of current society. The presence of antibiotic residue in food is particularly dangerous for human health, its monitoring (on residues in honey and food) is essential because of humans' health. Presented results indicated some residues of sulfonamides in the meadow and acacia honey but in regular values (prescribed by Official Gazette of the FRY No. 5/92 No. 11/92). Also some other authors reported presents of sulfonamides residues in honey (Duarte, et al., 2020; Mahmoudi, et al., 2014; Reybroeck, 2003) The results obtained from study Mahmoudi et al., (2014) indicate the differences between autumn and summer honey samples on sulfonamides residues, where from 135 honey samples, 20 (14.8%) were positive on sulfonamides residue in samples from autumn season. As indicate before, presents of antibiotic/sulfonamides primary depends of beekeeper himself and implemented biosecurity measures in the apiary. Ours results are encouraging, since reduced sulfonamide values were detected, but even that it is desirable to every beekeeper have monitoring of antibiotic residues as regulars biosecurity plan (once on year) in all beekeeping products, such as wax, bee venom, pollen, and royal jelly. Since mostly of these products are used either as dietary supplements or as medicinal remedies, especially for infants.

Conclusions

Quality and health testing is expensive, often inaccessible to honey producers, and some types of testing need to be covered by regular annual monitoring planned and funded by the state. It is necessary to complete the legal regulations governing this area and the areas of the laboratory that meet the requirements in order to give full legitimacy to the tests and so that legal measures can be implemented. Testing the quality and health of honey and other bee products results primarily in protecting human health from unsafe products, and thus protecting producers and markets, stimulating producers to raise product quality and safety, which would make way for the EU "flower gold" of the region .This would prevent the placement of unhealthy and low-quality honey, both on the domestic and foreign markets, would help producers to gain confidence in the quality of honey. Presented results indicated, that RS is convenient to production of all honey bee products in a clean living environment.

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References

Abadžić N. (1982). Pčele i zdravlje Nolit Beograd. [Bees and health, Neolit, Belgrade].

Ađić S. (1932). Odabir najmedonosnijih biljaka Jugoslavije, Beograd. [Selection of the most honey-bearing plants of Yugoslavia, Belgrade].

Ali N., Rajeh N., Wang W., Abualnaja K.O., Kumosani T.A., Albar H.M.S., Eqani S.A.M.A.S., Ismail I.M.I. (2016). Organohalogenated contaminants in type 2 diabetic serum from Jeddah, Saudi Arabian Environ Pollution 213 206–212. doi:10.1016/j.envpol.2016.01.087

Al-Waili N., Salom K., Al-Ghamdi A., Ansari M.J. (2012). Antibiotic, Pesticide, and Microbial Contaminants of Honey: Human Health Hazards. Science World Journal 1–9.

Bilić-Šobot D. (2020). Physicochemical characteristics of acacia and meadow honey from different regions of the Republic of Srpska/Bosnia and Herzegovina with an emphasis on the environment of beekeeping zones. Biotechnology in Animal Husbandry. 36 (1), 63-74. https://doi.org/10.2298/BAH2001063B.

Bogdanov, S. (2006). Contaminants of Bee Products. Apidologie. 37 1–18.

Chiesa L.M., Labella G.F., Giorgi A., Panseri S., Pavlovic R., Bonacci S., Arioli F. (2016). The occurrence of pesticides and persistent organic pollutants in Italian organic honeys from different productive areas in relation to potential environmental pollution. Chemosphere. 154 482–490.doi:10.1016/j.chemosphere.2016.04.004.

Drummond J., Williamson S.M., Fitchett A.E., Wright G.A., Judge SJ. (2017). Spontaneous honeybee behaviour is altered by persistent organic pollutants. Ecotoxicology. 26

Duarte K.M.R., Saturno F. D. P. (2020). Identification of sulfonamides in commercial honey Indian Journal of Animal Research 77, 2020 https://doi.org/10.17523/bia.2020.v77.e1476

Erdoğrul O. (2007). Levels of selected pesticides in honey samples from Kahramanmaras Turkey., Food Controlol, 18 (7) 866–871.

European Union. European Commission. (2009, December 22). Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (Commission Regulation (EU) n 37/2010). oOfficial Journal of the European Communities.

García-Valcárcel A.I, Molero E, Tadeo J.L, Hernando M.D. (2016). Determination of selected environmental contaminants in foraging honeybees. Talanta. 148 1–6. doi:10.1016/j.talanta.2015.10.064.

Gavrančić S., Skala D. (2000). Polihlorovani bifenili-osobine, primjena i tehnologije razgradnje. Hemjiska Industrija 54 (2), 53-63. [Polychlorinated biphenyls-properties, applications and decomposition technologies. Chemical Industry 54 (2), 53-63].

González Sagrario M., Miglioranza K.S.B, Aizpún de Moreno J.E., Moreno V.J., Escalante A.H., (2002). Polychlorinated biphenyls in different trophic levels from a shallow lake in Argentina. Chemosphere, 48 (10), 1113-1122.

Herrera A., Pérez-Arquillué C., Conchello P., Bayarri S., Lázaro R., Yagüe C., Ariño A. (2005). Determination of pesticides and PCBs in honey by solid-phase extraction cleanup followed by gas chromatography with electron-capture and nitrogen-phosphorus detection. Analytical and Bioanalytical Chemistry. 381 (3) 695–701. doi:10.1007/s00216-004-2924-3.

Herrera López S., Lozano A., Sosa A., Hernando M.D., Fernández-Alba A.R. (2016). Screening of pesticide residues in honeybee wax comb by LC-ESI-MS/MS. A pilot study. Chemosphere. 163 44–53. doi:10.1016/j.chemosphere.2016.07.008.

Kićović D. M., Vujanović D. L., Jakšić P. N. (2004). Osnove zaštite i unapredjenja životne sredine. Univerzitet u Prištini sa sjedištem u Kosovskoj Mitrovici. [Basics of environmental protection and improvement. University of Pristina based in Kosovska Mitrovica].

Korkmaz S.D, Kuplulu O., Cil G.I., Akyuz E. (2017). Detection of sulfonamide and tetracycline antibiotic residues in Turkish pine honey, International Journal of Food Properties, 20 (1), S50-S55, DOI: 10.1080/10942912.2017.1288135.

Saitta M., Di Bella G., Fede M.R., Lo Turco V., Potortì A.G., Rando R., Russo M.T., Dugo G. (2017). Gas chromatography-tandem mass spectrometry multi-residual analysis of contaminants in Italian honey samples. Food Additives & Contaminants Part A. 34 (5) 800–808.

Mahmoudi R., Norian R., Pajohi-Alamoti M. (2014). Antibiotic Residues in Iranian Honey by Elisa, International Journal of Food Properties. 17 (10) 2367-2373, DOI: 10.1080/10942912.2013.809539

Michener C. D (2000). The Bess of the World, Universyti Press. Baltimore and London.

Mohr S., Sifuentes Dos Santos J., Schwanz T.G., Wagner R., Mozzaquatro J.O., Lorenzoni A.S., Costabeber I.H. (2015). Polychlorinated biphenyls in umbilical cord serum of newborns from Rio Grande do Sul state, Brazil. Clin Chim Acta. 451 323–328. doi:10.1016/j.cca.2015.10.019.

Mustieles V., Fernández M.F., Martin-Olmedo P., GonzálezAlzaga B., Fontalba-Navas A., Hauser R., Olea N., Arrebola J.P. (2017). Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environment International 104 48–57. doi:10.1016/j.envint.2017.04.002.

Ordinance on quantities of pesticides, metals and metalloids and other toxic substances, chemotherapeutics. anabolics and other substances that may be present in foodstuffs "(Official Gazette of the FRY No. 5/92 and Official Gazette of the FRY No. 11/92).

Reybroeck W. (2003). Residues of antibiotics and sulfonamides in honey on the Belgian market. Apiacta. 38 23–30.

Roszko M., Szymczyk K., Jędrzejczak R. (2014). Influence of hen breeding type on PCDD/F, PCB & PBDE levels in eggs. Science of the Total Environment. 487 279–289. doi:10.1016/j.scitotenv.2014.04.033

Rulebook on honey and other bee products (Official Gazette of BiH No. 37/09 and 25/11).

Santos M.D, Vareli C.S., Janisch B.,. Pizzutti I.R, Fortes J., Sautter C.K., Costabeber I.H., (2021) Contamination of polychlorinated biphenyls in honey from the Brazilian state of Rio Grande do Sul, Food Additives & Contaminants: Part A, 38 (3), 452-463, DOI: 10.1080/19440049.2020.1865578.

Tillotson G.S., Theriault N. (2013). New and Alternative Approaches to Tackling Antibiotic Resistance. F100 prime Report. 5, 5. https://doi.org/10.12703/P5-51.

Vafeiadi M., Roumeliotaki T., Chalkiadaki G., Rantakokko P., Kiviranta H., Fthenou E., Kyrtopoulos S.A., Kogevinas M., Chatzi L. (2017). Persistent organic pollutants in early pregnancy and risk of gestational diabetes mellitus. Environment International 98 89–95. doi:10.1016/j.envint.2016.10.005.

Zhao S., Breivik K., Liu G., Zheng M., Jones K.C., Sweetman A.J. (2017). Long-term temporal trends of polychlorinated biphenyls and their controlling sources in China? Environ Science Technology 51 (5) 2838–2845. doi:10.1021/acs.est.6b05341.

Ziegler S., Schettgen T., Beier F., Wilop S., Quinete N., Esser A., Masouleh B.K., Ferreira M.S.V., Vankann L., Uciechowski P., Rink L., Kraus T., Brümmendorf T.H., Ziegle P. (2017). Accelerated telomere shortening in peripheral blood lymphocytes after occupational polychlorinated biphenyls exposure. Arch Toxicol 91(1) 289–300. doi:10.1007/ s00204-016-1725-8

CORRELATIONS BETWEEN THE QUANTITY OF FORAGED POLLEN, THE NUMBER OF FORAGERS AND THE MORPHOLOGICAL TRAITS OF THE HONEY BEES

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Abstract

In four-year monitoring of two generations of honey bee colonies (queen bees and their offspring), the correlation coefficients between morphological traits for the amount of foraged pollen, traits on which the foraging activity of honey bees depends. Considering morphometric traits, the tongue length, the length and width of the wings and basitarses were measured. In field conditions, scouts and forager bees were counted, the total amount of foraged pollen and the amount of foraged alfalfa pollen were measured. It was found that there is a very strong correlation between the amount of foraged pollen and the observed traits that determine the foraging activity of the honey bee, as well as the size of the basitarus at a statistically very significant level ($p < 0.01$). There was a strong correlation between the traits that determine the foraging activity with each other ($p < 0.01$). The size of the basitarsus (length and width) was strongly correlated with most traits. The wing length was in medium correlation with most traits ($r = 0.3$-0.6), while the wing width with all traits was weakly or negatively correlated. The length of the proboscis was in the medium strong correlation with most of the observed traits. It is weakly correlated with the width of the basitarus and the wing, and weakly to negatively correlated with the wing length.

Keywords: *honey bee, correlation coefficients, pollen foraging, morphological traits, number of foragers.*

Introduction

In addition to the amount of nectar, the amount of pollen that the bee colony collects during the year is just as important for the normal nutrition of the honey bee colony. Bees get the energy component of their diet (sugars) from nectar, and the building component (proteins, fats, vitamins, amino acids, etc.) from pollen. Farrar (1936) also proved that the strength of the colony in the spring (the amount of bees that survive the winter) is positively correlated with the pollen stocks in the fall. In addition, colonies with larger pollen reserves grew faster in the spring and foraged more honey during the grazing period. The amount of pollen collected by the colony has a very wide range considering a variety of authors, from 5.56 kg, as reported by Synge (1946) and Keller *et al*., (2005) to 221.79 kg per colony as noted by Eckert (1942). The amount of pollen in a colony is influenced by a large number of factors that can be positively or negatively correlated. Keller *et al*., (2005) stated that the amount of foraged pollen per colony is influenced by 10 or more variables (e.g. strength of the colony, i.e. population size, abundance of brood,

surrounding honey plants, weather conditions, etc.). In colonies with higher abundance of brood, pollen stocks are reduced because they are intensively spent on brood nutrition. The size of the colony affects the pollen foraging because it was found that smaller colonies forage significantly more pollen. It was also determined that the number of pollen foragers increases if empty honeycomb is added between the frames with the brood. The amount of pollen in the colony increases in proportion to the increase in the amount of brood (Georgijev *et al*., 2003). In the research of Jevtić *et al*., (2005), the strength of the colony (quantity of bees and brood area) had a great influence on pollen foraging and consumption. A two-year study by Georgijev and Plavša (2005) proved that there is a positive correlation between the amount of bees and the amount of brood with the productivity of colonies (honey yield). Mladenović and Radoš (2010) determined the positive correlation between the strength of the colony and pollen stocks per colony. The highest degree of interaction was found between the amount of bees and the amount of pollen per colony ($r = 0.48$), while the lowest interaction was recorded between the amount of pollen and the amount of honey per colony ($r = 0.13$). Bilaš and Krivcov (1991) claimed that certain morphological traits (proboscis length) are positively correlated with the productivity of the colony. However, the same trait is negatively correlated with queen fertility and resistance of bees to nosemosis.
The aim of this study was to determine the correlation coefficients between the total amount of foraged pollen, the amount of foraged alfalfa pollen, the number of scouts and foragers and morphological traits that affect pollen foraging.

Material and methods

The experiment lasted four years, and two generations of honey bee colonies sampled from different regions of Serbia (Rasina, Peshter, Moravia, Banat, Timok and Kopaonik) were studied. All colonies were in standard LR hives. The queens in the first generation were from the original regions, and in the second generation they were produced in apiaries from their own localities, so that the best queens from each region were used for the mothers, and the fathers were mixed (free mating).
Morphological traits were determined using binocular microscope of the "Olympus" brand with a magnification of 25 times, with a measuring thread on the eyepiece with which body parts of bees were measured. During the spring inspection, 50 bees were taken from each company, which were suffocated, and then their body parts (head, wing and leg) were separated, placed on a glass slide and measured using binocular microscope.
The traits on which the amount of foraged pollen per colony depends (number of scouts and number of foragers) were determined at the time of alfalfa flowering. In the first year, the colonies were located on an alfalfa plot of 30 ha in the vicinity of Smederevska Palanka. In other years, the colonies were stationed at the alfalfa site in Dobričevo near Ćuprija (area of 20 ha). The following traits were monitored:
- Number of scouts per colony: the total number of honey bees flying out of one colony in 3 minutes was recorded. Counting was performed four times during the day, twice in the morning (at 9^{00} and 11^{00}) and twice in the afternoon (15^{00} and 17^{00}).
- Number of forager bees per colony: in all experimental colonies in the specified time period (3 minutes) and terms (before and after noon) the number of bees returning to the colony with a load of pollen was recorded.

- Quantity of foraged pollen per colony: in order to determine the amount of foraged pollen, as well as the types of plants from which pollen was collected, pollen catchers were set up in all colonies. Pollen was taken from the catchers a total of 5 times during the experiment, then dried, measured and analyzed to determine which plant species it came from.
- Quantity of alfalfa pollen per colony: after drying and sorting, the amount of alfalfa pollen per colony was determined by measurement. Correlation coefficients were calculated for all monitored traits using STATISTICS 10.0 program.

The following scale was used to assess the strength of the correlations.

0.00 - 0.30 - weak correlation
0.31 - 0.60 - medium strong correlation
0.61 - 0.90 - strong correlation

Results and discussion

During the observation, the colonies foraged an average of 79.19 ± 20.8 g of pollen per measurement, a maximum of 108.63 g and a minimum of 55.25 g (Table 1).

Table 1. Statistical indicators for the amount of foraged pollen, number of collectors and morphological characteristics

Traits	Pollen total (g)	Pollen alfalfa (g)	No. of scouts before noon	No. of scouts after noon	No. of scouts before noon	No. of scouts after noon	Basitarsus length (mm)	Basitarsus with (mm)	Wing length (mm)	Wing width (mm)	Proboscis length (mm)
Average	79,19	18,69	275,13	251,56	86,68	42,26	2,07	1,10	9,61	3,21	6,41
Min	55.25	8.98	172.70	174.80	61.68	27.13	1.96	1.03	9.29	3.04	5.93
Max	108.63	28.45	332.10	304.45	123.90	57.78	2.14	1.15	9.86	3.48	6.78
St. dev.	20.80	8.55	42.98	38.51	19.35	10.27	0.07	0.05	0.20	0.12	0.25
Cv	0.26	0.46	0.16	0.15	0.22	0.24	0.03	0.04	0.02	0.04	0.04
N	18	18	18	18	18	18	18	18	18	18	18
Std.Err.	4.90	2.01	10.13	9.08	4.56	2.42	0.02	0.01	0.05	0.03	0.06
t-value	16.15	9.28	27.16	27.71	19.00	17.46	124.68	101.72	200.48	111.46	110.42

The total amount of foraged pollen was significantly correlated with most of the observed traits (Table 2). It had particularly high correlation coefficients with the amount of foraged alfalfa pollen (r = 0.96) and with the amount of foragers in the morning (r = 0.92). In addition to the above traits, the amount of foraged pollen was in strong correlations with the number of scouts, the number of pollen grains and the size of the basitarzus (length and width). The obtained results for these traits were statistically very significant (p <0.01). This trait was in a medium strong correlation with wing length and proboscis length. Wing width did not have a significant effect on pollen foraging, so the correlation coefficient was extremely low (r = 0.04). Lebedev (2001) reports that pollen intake is highly correlated (r = 0.82) with the strength of the colony and the abundance of brood per colony. Georgijev *et al*. (2003) found that the correlation coefficient between the amount of brood and the amount of pollen foraged in the spring is extremely high r = 0.93, while in the fall it is significantly lower r = 0.16. Jevtić *et al*., (2009) found that the amount of pollen at the spring inspection was negatively correlated with the amount of honey at the spring and autumn inspection (r = -0.30 and r = -0.23). Analyzing the studies of a large number of authors, Keller *et al*., (2005) found that all but one source (Mc Lellan, 1978) agree that there is a positive correlation between pollen stocks and brood surface in bee colonies. Wille *et al*., (1985) found that there is a negative correlation (r = -0.283) between annual pollen intake and worker bees lifespan. This negative correlation changes to positive but weak (r = 0.145) when pollen intake per bee is taken into account. In strong colonies, pollen foraging does not negatively affect the lifespan of worker bees.

Table 2. Correlation between the amount of foraged pollen, the number of foragers and morphological traits of honey bee colonies from six regions in Serbia

Trait	Pollen alfalfa	Scouts b.n.	Scouts a.n	Foragers b. n.	Foragers a. n.	Basitarsus length	Basitarsus width	Wing width	Wing length	Proboscis length
Pollen total	0.96**	0.75**	0.69**	0.92**	0.74**	0.79**	0.67**	0.43	0.04	0.45
Pollen alfalfa		0.75**	0.74**	0.85**	0.76**	0.80**	0.65**	0.41	0.14	0.54
Scouts b. n.			0.91**	0.80**	0.66**	0.74**	0.48*	0.16	0.20	0.47
Scouts a. n				0.69**	0.74**	0.72**	0.47	0.26	0.20	0.60**
Foragers b. n.					0.79**	0.73**	0.60**	0.36	-0.06	0.39
Foragers a. n.						0.73**	0.66**	0.42	0.02	0.40
Basitarsus length							0.73**	0.43	0.24	0.40
Basitarsus with								0.52*	0.16	0.21
Wing width									-0.35	-0.15
Wing length										0.27

* marked values are significant for p<0.05
** marked values are significant fo p<0.01

The amount of foraged alfalfa pollen averaged at 18.69 ± 8.55 g per measurement and ranged from 8.98 g to 28.45 g. This trait has a high coefficient of correlation with the number of scouts and the number of foragers (before and after noon) and the size of the basitarzus. Such high coefficients are quite understandable, bearing in mind that the monitoring of the amount of foraged pollen was performed at the time of alfalfa flowering. In addition, the activity of the honey bees in foraging pollen in this period (July) is significantly higher in the morning compared to the afternoon. It is important to point out that there is a medium strong (almost strong) degree of correlation (r = 0.54) between the length of the proboscis and the amount of foraged alfalfa pollen. This leads us to the conclusion that colonies that forage a larger amount of alfalfa pollen have a longer proboscis. It is known that alfalfa has a special mechanism of flower opening that is not adapted to the pllination by honey bees, so they usually avoid collecting pollen on this species. Considering the high degree of correlation with the size of basitarzus, we can conclude that colonies that have a longer proboscis and larger basitarzus forage more alfalfa pollen and thus better pollinate this important field crop. The traits on which the foraging activity of the honey bees depends (the number of scouts and foragers) were strongly correlated with each other. During the experiment, an average of 275.13 ± 42.98 bees were counted per colony in the morning count, and 251.56 ± 38.51 in the afternoon. More pollen was counted in the morning (86.68 ± 19.35) compared to the afternoon count (42.26 ± 10.27). The number of scouts in the morning had the highest correlation with the number of scouts in the afternoon (r = 0.91). Considering other traits, the correlation coefficients ranged from r = 0.66 to r = 0.80 (Table 2).

There are several studies that have observed the relationship between pollen stock and colony size (number of worker bees). Mc Lellan (1978) found no significant correlations between the number of bees and the amount of pollen retained in the catchers on any of the six days studied. Similarly, Wille (1981) found that pollen stock and colony strength do not change synchronously during the season (they are not correlated). Dreller *et al*., (1999) found that the number of pollen

foragers decrease in colonie with more capped brood compared to those with more open brood. Schmickl and Crailsheim (2004) found that the number of pollen foragers increases significantly if an empty honeycomb frame is placed between the frames with brood, i.e. if the empty space increases. The morphological traits obtained by measuring 50 bees per each colony had the following values: proboscis length 6.41 ± 0.25 mm, wing length 9.61 ± 0.20 mm, wing width 3.21 ± 0.12 mm, basitarsus length 2.07 ± 0.07 mm and basitarsus width 1.10 ± 0.05 mm.
The length and width of the basitarzus were strongly correlated with each other (r = 0.73), which was not the case for the length and width of the forewing, which were negatively correlated (r = -0.35). Negative but very low correlation (r = -0.15) was noted beween proboscis length and wing length so it can be concluded that colonies with a longer proboscis value have slightly shorter wings. It is interesting to note that the wing width has almost no correlation with both pollen foraging in general and the amount of foraged alfalfa pollen (Table 2).
In the research of Jevtić *et al*., (2011), most morphological traits showed moderately strong and strong correlation both with each other and with the productivity of colonies. Milne et al., (1986) measured 387 bee samples and determined that the size of the tibia is 1.54 - 2.28 mm^2. The same authors found that there is a high correlation between the size of the corbicula and the amount of foraged pollen. The honey bee lines that foraged the largest amount of pollen had the largest corbicula. Souza *et al*., (2002) found that there is a correlation between morphological traits and productivity of honey bee colonies. The highest degree of correlation with productivity was in the size of the corbicula (r = 0.58). In this study, all morphological traits were mutually in a mildly strong and strong correlation relationship. Zarate *et al*., (2008) found in an Africanized bee in Mexico that there is a high correlation between colony productivity and weight gain per society, size (volume) of honeycomb, foraging activity, and syrup intake rate. They also found a very low correlation coefficient between the productivity of the colony and the life expectancy of worker bees.

Conclusion

After four years of monitoring of honey bee colonies, it was determined that the amount of foraged pollen per colony is strongly influenced by the number of scouts and foragers(strong correlation r <0.61), the size of the basitarus (length and width) and the time when pollen was foraged. The traits on which the foraging activity of the honey bee depends (the number of scouts and foragers) are in strong correlations with each other, with the total amount of foraged pollen and the amount of foraged alfalfa pollen. The length of the proboscis and the length of the wing are in a medium strong correlation with the pollen foraging, both with the total amount and with the amount of alfalfa pollen. Wing width was very weakly correlated with the observed features, and it was also negatively correlated with wing length (r = -0.35).

References

Bilas G.D., Krivcov N.I. (1991): Bee selection. Agropromizdat. Moskva, pp. 1- 304.
Dreller C.R., Page E., Fondrk M.K. (1999) Regulation of pollen foraging in honeybee colonies: effects of young brood, stored pollen, and empty space. Behavioral Ecology andSociobiology 45: 227–233.
Eckert J.E. (1942): The pollen required by a colony of honeybees. Journal of Economic Entomology 35: 309–311.

Farrar C.L. (1936): Influence of pollen reserves on the surviving populations of over-wintered colonies. American Bee Journal 76: 52–454.

Georgijev A., Mladenović M., Nedić N. (2003): Experimental calculation of the correlation between the cell surface and the intake of nectar and pollen in bee colonies. XXXVIIIth Apimondia International Apicultural Congress. August 24-29. Ljubljana, Slovenia, p. 760.

Jevtić G., Mladenović M., Nedić N. (2005): The Influence of the Quantity of Honeybees and Honey Reserves on Wintering of Honeybee Colonies. 8th International Symposium Modern Trends In Livestock Production Belgrade Zemun, Serbia And Montenegro 5.-8.10. 315-321.

Jevtić G., Mladenović M., Anđelković B., Nedić N., Sokolović D., Štrbanović R. (2009): The correlation between colony strenght, food suply and honey yield in honey bee colonies. Proceedings 9th International Symposium Modern Trends in Livestock Production. Belgrade-Serbia 7-9 October, 1141-1149.

Jevtić G., Anđelković B., Lugić Z., Đokić D., Mladenović M., Nedić N. (2011): Correlation of morphologic and production traits of honey bee colonies from Serbia. Proceedings 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production" Belgrade, Republic of Serbia 5 – 7th October. 1761-1771.

Keller I., Fluri P., Imdorf A. (2005): Pollen nutrition and colony development in honey bees – Part II Bee World 86(2): 27-34

Lebedev V.I. (2001): Biological potential of bee semi for harvesting and harvesting perga. Material of the coordination meeting and conference. Moskva, 60-63.

Mc Lellan, A.R. (1978): Growth and decline of honeybee colonies and inter-relationships of adult bees, brood, honey and pollen. Journal of Applied Ecology 15: 155–161.

Milne C.P.Jr., Hellmich R.L., Pries K.J. (1986): Corbicular size in workers from honeybee lines selected for high or low pollen hoarding. Journal of A. Research 25: 50-52.

Mladenović M., Radoš R. (2010): Correlation between the strength of colony, the honey area and pollen area of the observed lines of yellow honey bee in Vojvodina. Biotechnol. & Biotechnol. EQ. 24/2010/SE Special edition. Second Balkan Conference on Biology 21-23 May 2010, Plovdiv, 423-426.

Schmickl T., Crailsheim K. (2004): Inner nest homeostasis in a changing environment with special emphasis on honey bee rood nursing. Apidologie 35: 249–263.

Souza D.C., Cruz C.D., De Ooliveira Campos L.A., Regazzi A. J. (2002): Correlation between honey production and some morphological traits in Africanized honey bees (*Apis melifera*). Ciência Rural, v. 32, n. 5, 2002.

Zarate O.C., De Araujo-Freitas, Medina A.L., Velasqez A.J. Quezada-Euan J.G. (2008): Phenotypic correlations of field and laboratory tests with honey production in Africanized honey bees (*Apis mellifera*). Apidologie 39, 523–530.

Wille H. (1981): Immigration and emigration/rhymes and inconsistencies (I). Northwest German beekeeping newspaper 33: 186–190; 214–218.

Wille H., Imdorf A., Buhlman G., Kilchenmann V., Wille M. (1985): Relationship between pollen input, brood rearing and mean life expectancy of workers in bee colonies (*Apis mellifica* L.). Information from the Swiss Entomological Society 58: 205–214.

A NEED FOR THE INTRODUCTION OF THE PIG CARCASS CLASSIFICATION SYSTEM IN THE REPUBLIC OF SERBIA

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Abstract

The Republic of Serbia has a long tradition in the pig production. Serbia is one of the few countries in the region with the notable pig production without the implemented system for categorization of carcasses based on the lean meat content. Some of the ex-Yugoslavian countries started with the implementation of this system in the late 1990-s while the challenges of implementation of carcass classification is still in front of Serbia. The purpose of every pig breeding program is in the function on the increase in animal performance to obtain as many as possible of fattening pigs in as short as possible period with the good characteristics of the carcasses. There is a characteristic situation in Serbia. There are two types of producers: 1) large scale farms with the huge production of fattening pigs per year and 2) relatively important number of small, family orientated farms with small capacity, especially in Vojvodina, the north part of Serbia. However, meat processing industry is very concentrated and five meat processing plants take about 80% of the all market. Producers who invested in the breeding program are not rewarded and do not have stimulation for the further improvement. Moreover, some meat processing plants started to implement the carcass classification system. However, it put the producers even in more depending position toward the meat industry. There is a need for the introduction of carcass classification system by the independent body in the Republic of Serbia.

Key words: *pig production, carcass classification system.*

Introduction

Pig production has long tradition in Serbia. The annual production is about 300 thousand of tones (Statistical Office of the Republic of Serbia, 2018). In comparison of the pig production with the neighboring countries, Serbia is still at the first place. In Croatia, for example, annual production is about 100 thousand of tones (Kralik et al., 2017). The producers in the pig sector roughly can be divided in two groups: huge number of small producers with small number of animals per farm, and about 400 commercial farms with huge number of animals per farms (Lierop et al., 2015). The economic power of small producers is modest as the use of the new technology and breeding programs. On the other side the meat processing industry is very concentrated; five companies take up to 80% of the market. They have huge economic power. The small producers have small accumulation of capital, the extreme climate condition as the drought or the excessive rain affects more this producers. More over for the small producers is more difficult to get bank loan necessary for improvement in equipment, breeding program.
One of the characteristics of pork market is cyclicality in the production volumes and prices (Jeremić et al., 2018). The characteristic of the cyclicality is imbalance of production and

demand which usually resulting in the price drop. It is easy to assume that small scale producers are more burdened by this cyclicality. The quality of meat carcass is very important trait for the producers and end consumers. Some European countries started developing the model to assess the quality in the early sixties (Petrovic et al., 2009). The early standard SEUROP was developed and started to be use in 1989. In Serbia the legislation regarding pork carcasses, still partially in use, is from 1985. The legislation has lot shortcomings, due to the fact that is outdated.

The purpose of every breeding program is to improve the growth and carcass traits of pigs. Heritability (h2) is a measure of the variation of some investigated trait in the population due to the genetics. Heritability for the quality of carcass is medium to high (Radovic et al. 2013). That means that that this trait can relatively be easy improved in population. Serbia, especially north part of Serbia, has good condition for the intensive farming, a lot of very fertile land good for crop production. Moreover Serbia is one of the main exporters of corn in the region.

Results and Discussion

The data from the Republic of Serbia indicates the huge possibilities for the improvement in the pig sector. Pig production has a good possibility for the further development and increase of the production. However, at the moment producers are not stimulated to produce the pig carcasses with the better carcass traits, all carcasses regardless of the quality are paid the same. The producers are not stimulated to produce pig's carcasses with the better quality and to invest in breeding program. All fattening pigs are paid on the base of the live weight regardless of carcass quality. The SEUROP system of classification in EU has been existed already for several decades. It was developed from the need to harmonize the system of carcass classification and that this system to be a base for a price for fattening pigs. By this system it is possible to compare the price of fattening pigs in the different countries EU members.

Almost all neighboring countries of Serbia started to use the SEUROP system of classification for pig carcasses. The system gives good results in the increase of quality of carcass traits and lean meat content. First ex Yugoslavian country which started with this system was Slovenia in 1996, before that in the use was the legislation from 1985 which is still in the use in Serbia. As the results of the use the SEUROP system in Slovenia in the period of eight years (1996-2004) the average lean meat content in the carcasses was increased from 51.9 to 55.9% in 2004. As the result of this the percent of the carcasses of S and E class has been almost tripled (from 21.3 to 58.2%) during this period (Čandek-Potokar et al., 2005). The Bulgaria started to use the SEUROP system of classification in 2005. It resulted in the significant increase of the carcasses in the S class and decrease of those in E and U class during the years of observation (Nakev et al. 2019). The similar situation was in Romania, the implementation of SEUROP standard generated the increase in lean meat content and carcass quality (Balan et al. 2010)

There is really not a lot of data regarding carcass classification and lean meat content of pigs slaughtered in Serbia. One of the reasons is that in the last 20 years lean meat content was not measured on the slaughter line in Serbia (Dokmanović et al. 2013). Occasionally, some data can be found as a part of the study regarding measuring lean meat content in the pigs carcasses, however the results are not very encouraging (Dokmanović et al. 2013; Vasilev et al., 2009; Kosovac et al., 2009). During 2018 the study was done regarding the average lean meat content in the carcasses of pigs in the eight slaughterhouses in the region of Vojvodina, Serbia. Lean meat content was measured in 65764 of carcasses. The majority of carcasses were in E and U class (from 50-60% of lean meat content). However only 3.15% of carcasses were in the S superior class with more than 60% of lean meat (Radović et al. 2021). In Slovenia, as a consequence of carcasses being graded, the carcasses in the classes E and S were almost tripled in the period from 1996-2004 (Čandek-Potokar et al., 2005) The experience from the neighboring countries which implemented SEUROP system is a very encouraging. Investment in better breeding program and nutrition gives good results in pig production and enhance competitiveness of this production. The positive effect of implementation of SEUROP classification will be visible and will have good effect on the basic pig production as soon as system starts to be implemented. Previously there were some attempts in this direction, however it was never brought to the end. The most expenses in the live pig production go to animal nutrition about 70-80%. The protein deposition necessary for the lean growth is energy more efficient that the fat growth expressed in the needed metabolic energy. Fat deposition requires about 16% more energy per unit of gain. However the water deposition is big part of lean meat gain which increases advantages of lean: fat growth to the 4:1 ratio (Patience et al., 2015). Profitability of production is on the thin line between the price of the fattening pigs and the expenses needed for the production. For one kilogram of meat it is needed 1.25 kg of feed, while for one kilogram of fat it is needed 4kg of feed Ministry of Agriculture, Food and Rural Affairs). The lean meat growth is much more efficient than the fat. It is very clear that production of pigs with the higher lean meat content is economically more purposeful. The average annual production is about 300 thousand of tons. Average weight of fattening pigs is about 100kg that means 3 millions of pigs. The estimated carcass yield of pigs is about 75% which means 225.000t of carcasses. Average lean meat content of pig carcasses in Serbia is 54%. Hence the annual production of lean meat is about 121.500 t. With the increases of lean meat content in only 1% the meat production can be increase for about 3 thousand of tones with the same production. Meat is more expensive and production costs are less, while fat is less expensive and production costs are much higher. Therefore, producers and meat industry will easily recognize the need to put more effort to improve selection work needed to increase lean meat content.

Conclusion

Serbia is one of the rare countries in Europe without SEUROP system of classification with relatively developed pig production. The data from the Republic of Serbia indicates the huge possibilities for the improvement in the pig sector. Pig production has good possibilities for the further development and increase of the production. However, at the moment producers are not stimulated to produce the pig carcasses with the better carcass traits because all the produced carcasses regardless of the quality are paid the same. It is needed to make system which will be adequate in the present condition in Serbia. On one side we have very dichotomous group of producers who are too much burdened with the fluctuation on the market. On the other side there is Serbian meat industry, which is very concentrated, about 80% of this industry is owned by only five companies. Their interest is only profit regardless of pig production. So it is not so rare import of pig meat or pig meat products. Serbia is a huge exporter of corn. It would be much better and economically justified to incorporate that corn in animal production and then export the animal products as meat and meat products.

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Reference

Balan, Ioana Mihaela, Cornelia Petroman, Ioan Petroman, Diana Marin, Ramona Ciolac, Loredana Heber, and Dora Manuela Orboi. 2010. "Romanian Quality Pork Carcasses in 2009." Animal Science and Biotechnologies 43 (2): 389–90.

Čandek-Potokar, Marjeta, Kovač Milena, Malovrh Špela. 2005. "Slovenian Experience in Pig Carcass Classification According to Seurop During the Years 1996 To 2004." Journal of Central European Agriculture 5 (4): 323–30.

Petrović Lj, Tomović V., Džinić Natalija, Tasić Tatjana, and Ikonić P. 2009. "PARAMETERS AND CRITERIA FOR QUALITY EVALUATION OF PORK CARCASS HALVES." *Tehnologija Mesa / Meat Technology* 50: 121–39.

Jeremić, M., Lovre, K., Matkovski, B. (2018). "Serbian Pork Market Analysis." Ekonomika Poljoprivrede 65 (4): 1449–60.

Kosovac, O. (2009.) "Kvalitet Svinjskih Trupova Na Liniji Klanja, Prema.Pdf" 25: 791–801.

Kralik, I., Tolušić, Z., Jelić, S. (2017). "Proizvodnja Svinjskog Mesa u Republici Hrvatskoj i u Zemljama Europske Unije."

Lierop, A. van Bussel – van, C. van Wagenberg, and D. Somers. 2015. "Serbian Pig Sector: An Overview Serbian Pig Sector : An Overview." Livestock Research Report 843.

Dokmanovic, M., Tesic, M., Teodorovic, V., Karabasil N., Markovic, R, Todorovic, M, Djuric, J. 2013. "Assessment of Pig Carcass Meatiness in Serbia." Veterinarski Glasnik 67 (3–4): 227–36.

Ministry of Agriculture, Food and Rural Affairs. 2020. "Comparing Carcass Value." 2020. http://www.omafra.gov.on.ca/english/crops/facts/00-067.htm.

Nakev, Jivko, Teodora Popova, Jivko Nakev, and Teodora Popova. (2019). "Results of the Application of SEUROP for Pig Carcass Classification in Bulgaria." Bulgarian Journal of Agricultural Science 25: 17–22.

Patience, John F., Mariana C. Rossoni-Serão, and Néstor A. Gutiérrez. 2015. "A Review of Feed Efficiency in Swine: Biology and Application." *Journal of Animal Science and Biotechnology* 6 (1): 1–9. https://doi.org/10.1186/s40104-015-0031-2.

Radovic, C., Petrovic, M., Zivkovic, B., Radojkovic, D., Parunovic, N., Brkic, N.. Delic N. (2013). "Heritability, Phenotypic and Genetic Correlations of the Growth Intensity and Meat Yield of Pigs." Biotechnology in Animal Husbandry, 29 (1): 75–82.

Radović, I., Polovinski-Horvatović, M., Mirkov, M., Malešević, S. (2021). "Lean Meat Content of Pig'S Carcasses in the Eight Slaughterhouses in the Region of Vojvodina, Serbia." Food and Feed Research 48 (1), 23-28

Statistical Office of the Republic of Serbia (2018). Statistical Yearbook of the Republic of Serbia 2018. Beograd: Statistical Office of the Republic of Serbia.

Vasilev, D. (2009). "Mesnatost Trupova Svinja Poreklom Iz Farmskog Uzgoja i Individualnih Gazdinstava Zavisno Od Mase Polutki i Klasi Fi Kacije Prema SEUROP Standardu," 85–89.

INFLUENCE OF REARING SYSTEM ON THE CARCASS TRAITS AND CARCASS CONFORMATION OF FATTENING CHICKENS

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Abstract

The aim of the research is to determine the slaughter characteristics and carcass conformation of chickens reared in the conventional production system or in organic production. Random samples of 6 carcasses from each production system were taken for the determination of basic slaughter traits: carcass weight, yield and percentage of individual carcass parts and weight and share of abdominal fat. The following characteristics of carcass conformation were measured: abdominal wall thickness, breast depth, keel length, breast angle, breast girth and drumstick girth. Also, pH values of breast and drumstick meat were determined. The collected data were analyzed by ANOVA, using Fisher LSD post hoc test, in the program Statistica, TIBCO, version 14. The results showed that the system of meat production had a significant effect on most of the examined parameters. Chickens raised in conventional system had significantly higher carcass weight and consequently higher weight of the all carcass parts. The share of breast was higher in birds from conventional system, while the share of the wings, thighs and back was higher in organic chickens. The content of abdominal fat was significantly higher in organic chickens. Regarding the carcass conformation organic chickens had significantly lower values compared to conventional ones for all examined traits. The results confirmed that there is a significant difference in carcass quality and yiled of different carcass parts between organic and conventional chickens which could have a strong influence on consumers preferences.

Key words: *Chickens, Carcass, conformation, Organic production.*

Introduction

Consumer interest in organic products is constantly growing in recent decades (Castellini *et al.*, 2008). They are especially interested in chicken meat from the organic system because the intensive poultra meat production has led to the deterioration of poultry welfare and meat quality (Mikulski *et al.,* 2011; Perić *et al.*, 2018). Many consumers assume that the meat of free-range chickens is tastier and healthier than that of birds kept in a poultry house (Fanatico *et al.*, 2007; Li *et al.*, 2017). Also, the meat yield and the carcass conformation are different in chickens from organic production compared to broiler chickens raised in intensive systems (Perić *et al.*, 2016). Conformation means the general structure and appearance of the animal, with the focus on development of certain parts of the body. From the point of view of the slaughter industry and consumer interests, it is very important that chickens have good meat yields, desirable conformation, optimal fat distribution and appropriate skin color (Bogosavljević-Bošković *et al.*, 2006).

In Serbia, organic production is still at the beginning, although the demand for organic products is growing (Rodić *et al.*, 2006). Bogosavljević-Bošković *et al.* (2011) pointed out that reasons for

the development of extensive systems in poultry production are to improve rearing conditions, reduce environmental pollution and enhance meat quality.
The key factor which affects the production and slaughter traits of broilers is the genotype (Berri *et al., 2005*). According to Castellini *et al.* (2008) and Araújo (2011) meat quality is the result of the interactive effects of many factors like the strain of birds (fast- or slow- growing), stocking density, age of the birds, access to pasture, environmental temperature, physical activity and feed. All these factors significantly differ in intensive broiler production compared to alternative systems.
The aim of this work was to determine and to compare the slaughter characteristics of chickens raised in two different production systems: conventional and organic system.

Materials and methods

Research has been conducted at the University of Novi Sad, Faculty of Agriculture. Chickens were smpled from commercial farms. Organic chickens were raised on the certified organic farm in Vojvodina. Birds of slow growing strain Red Ranger were used for fattening which lasted for 81 days. Birds were housed and managed according to the principles of organic production. Birds were fed with four different mixtures which were based on organically grown triticale (70% of the diet), corn and pasture.
The second group of birds was raised on the conventional farm for 6 weeks. ROSS 308 broilers were raised inside the barn, on a deep litter, with stocking density of 17 birds/m^2. They were fed with standard mixtures (starter, grower and finisher) and the feed was provided *ad libitum*.
At the end of the fattening periods, random samples of 6 carcasses from each production system were taken for analyses. Analyses had been done at the Laboratory of chicken meat and egg quality, Department of Animal Science, Faculty of Agriculture, Novi Sad. The dressed cold carcasses were dissected into primal cuts (breast, drumstick, thigh, wing and back) following the method established by the Regulation on Poultry Meat Quality (Rašeta and Dakić,1984). Carcass conformation was measured by calliper and tape measure. Breast angle was measured with special device created for that purpose. The pH value was determined using a pH meter device (TESTO 205) with a glass electrode (Mettler Toledo, Greifensee, Switzerland) for direct determination of the pH value in meat. The pH meter was calibrated with standard phosphate buffers (calibration buffer pH was 7.02 and 4.00 at 20^0 C) and adjusted to the measured muscle temperature.
Statistical analyses were done in program STATISTICA 14 (TIBCO, 2020). All data were analyzed by ANOVA and significant differences among treatments were determined using Fisher LSD post hoc test. Significance levels were set at $p < 0.05$ and $p<0.01$

Results and discussion

Rearing systems had a significant effect on weight and share of carcass cuts (Table 1). Birds from conventional systems had significantly higher weight of whole carcass and breast ($P<0.01$) compared to organic chickens. The weight of drumstick, thigh and back was also higher in conventional birds, but the differences were not statistically significant ($P>0.05$). Only weights of wings were slightly higher in organic birds.

Table 1. Effect of production system on weight and proportion of carcass cuts (mean ±SD)

Carcass parts	Weight of the carcass parts,g		Share of the carcass parts,%	
	Conventional	Organic	Conventional	Organic
Whole carcass	2179 ± 140.6^{A}	1696 ± 275.2^{B}	100.00	100.00
Wings	217.33 ± 11.99	219.17 ± 53.19	9.98 ± 0.42^{B}	12.80 ± 1.10^{A}
Drumsticks	307.17 ± 29.52	267.51 ± 74.04	14.09 ± 0.92	15.57 ± 1.55
Thighs	310.67 ± 51.59	271.67 ± 31.39	14.22 ± 1.78^{b}	16.11 ± 0.96^{a}
Breast	851.00 ± 67.62^{A}	468.83 ± 67.72^{B}	39.06 ± 1.94^{A}	27.75 ± 1.63^{B}
Back	449.67 ± 58.59	402.67 ± 69.80	20.66 ± 2.67^{b}	23.71 ± 0.75^{a}
Abdominal fat	43.67 ± 9.42^{B}	66.83 ± 11.16^{A}	2.00 ± 0.38^{B}	4.05 ± 0.98^{A}

$^{A\text{-}B}$ Values in the same raw with different superscripts are significantly different (P<0.01)
$^{a\text{-}b}$ Values in the same raw with different superscripts are significantly different (P<0.05)

Reagrding the share of carcass parts it can be seen that organic birds had higher share of wings, drumstick, thighs and backs, while the conventional birds had significantly higher share of breast in the carcass. Similar results were reported by Grashorn and Serini (2006) and Perić *et al*. (2016) who stated that broiler chickens have a genetic predisposition for high breast meat yield compared to pure or slow growing breeds. Comert *et al.* (2016) suggested that for the yield and share of carcass parts the genetic strain might be more significant than system of rearing.
It is interesting that weight and share of abdominal fat pad was significantly higher in organic birds which indicate that organic birds had higher content of fat in the body. On the contrary, Perić *et al.* (2016) found lower content of abdominal fat in organic chickens compared to conventional ones. Castellini *et al.* (2002) reported significantly higher content of abdominal fat in birds with 81 days of age compared to the birds raised for 51 days. It seems that age of the birds has a significant influence on the content of the abdominal fat. Blagojević *et al.* (2011) reported a higher yield of abdominal fat in fast growing chickens compared to slow growing strains but both strains were raised for 81 days.
The measured parameters clearly showed a highly significant difference (P<0.01) in carcass conformation between chickens from organic and conventional production (Table 2). It can be noticed that all measured parameters are higher in conventional chickens, which is mainly a consequence of the genotype. Fast-growing hybrids have been selected for higher meat yield, which means that they have greater widths, depths and girths of those parts of the body where the highest quality portions of meat are located. This is confirmed by the size of the breast angle, which is significantly higher in broiler chickens, which indicates better breast development and higher white meat yield.

Table 2. Effect of production system on carcass conformation (mean ±SD)

Parameters	Conventional production	Organic production
Carcass weight, g	2179 ± 0.16^{A}	1696 ± 0.27^{B}
Abdominal wall depth, cm	0.9 ± 0.12^{B}	1.17 ± 0.12^{A}
Breast depth, cm	14.67 ± 0.81^{A}	10.83 ± 0.75^{B}
Drumstick girth, cm	18.25 ± 0.82^{A}	15.17 ± 1.08^{B}
Breast girth, cm	37.92 ± 1.06^{A}	31.75 ± 2.11^{B}
Keel length, cm	17.5 ± 0.83^{A}	11.25 ± 0.75^{B}
Breast angle, ^{o}	145 ± 10.48^{A}	101 ± 8.75^{B}

$^{A-B}$ Values in the same raw with different superscripts are significantly different (P<0.01)

The only parameter that was higher in organic chickens was the thickness of the abdominal wall, which is primarily a consequence of the older age of the chickens. Hopić *et al.* (2000) and Pavlovski *et al.* (2007) established that main factors which affect the carcass conformation are genotype and sex of the birds. Blagojević (2011), reported significantly better conformation measures with better development of breasts in Hubbard Classic genotype compared to slow growing strains (Master Gris and Farm Q). Results published by Strugar (2018) who found better conformation of the birds of hybrid ROSS 308 compared to pure breed White Rock, confirmed highly significant effect of genotype on the measures of carcass conformation.
The pH value is a reliable parameter to obtain information about the meat quality. The pH value of muscle tissue is related to the biochemical state of the muscles at the time of slaughter. It directly or indirectly affects other properties of meat such as: ability to bind water, color, softness, taste, durability and more. (Vučić G., 2014). In our research, there were no significant differences in pH value of meat neither in thigh nor in breast meat (Table 3).

Table 3. pH value of drumstick and breast meat

Type of meat	Conventional production	Organic production
Thigh	6.13 ± 0.13	6.27 ± 0.12
Breast	5.84 ± 0.10	5.97 ± 0.15

It is considered that the pH value of chicken breasts ranges from 5.8 to 6.3 for normal meat quality (Ristić and Damme, 2013) which is in line with our research. Castellini *et al.* (2002) reported that pH of breast meat in organic chickens ranged from 5.75 to 5.80.

Conclusions

From the results of this work it can be concluded that the production system had a significant effect on carcass characteristics of chickens. Chickens raised in conventional system had significantly higher carcass weight and consequently higher weight all of the carcass parts. The share of breast was higher in birds from conventional system, while the share of wings, thigh and back was higher in organic chickens. The content of abdominal fat was significantly higher in organic chickens. Regarding the carcass conformation organic chickens had significantly lower values compared to conventional ones for all examined traits. The results confirmed that there is a significant difference in carcass quality between organic and conventional chickens which could have a strong influence on consumers preferences.

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References

Araújo W.A.G., Luiz F. T. Albino L.F.T, Sakomura N.K. and PaulinoP.V., Campos A.M. (2011). Meat quality in "in door" and "out door" production systems of poultry and swine. Open Journal of Animal Sciences vol. 1, pp. 75-88.

Berri C., LeBihan-Duval E., Baéza E., Chatrin P. Picgiraad L., Jehl N., Pickard M., Quentin M., Duclos M.J. (2005). Further processing characteristics of breast and leg meat from fast, medium and slow growing commercial chickens. Animal Research, vol.54, pp. 123-134.

Blagojević M. (2011): Uticaj genotipa na intezitet porasta i kvalitet trupa i mesa brojlerskih pilića u ekstenzivnom sistemu gajenja. Doktorska disertacija, Poljoprivredni fakultet, Novi Sad.

Bogosavljević Bošković S., Kurčubić V., Petrović M., Radović V. (2006). The effect of sex and rearing system on carcass composition and cut yields of broiler chickens. Czech Journal of Animal Science vol.51, pp. 31-38.

Bogosavljević-Bošković S., Dosković V., Mitrović S., Rakonjac S., Petrović M.D. (2011). Broiler meat quality: The effect of rearing system and lenght of fattening period, Biotechnology in Animal Husbandry, vol.27 (4), pp. 1635-1642.

Castellini, C., Mugnai, C., Dal Bosco, A. (2002). Effect of organic production system on broiler carcass and meat quality. Meat Science vol. 60, pp. 219-225.

Castellini, C., Berri, C., Le Bihan-Duval, E., Martino, G. (2008). Qualitative attributes and consumer perception of organic and free-range Worlds Poultry Science Journal vol.64, pp. 500-512.

Cömert M., Şayan Y., Kırkpınar F., Bayraktar ÖH., Mert S. (2016). Comparison of carcass characteristics, meat quality and blood parameters of slow and fast grown female Broiler chickens raised in organic or conventional production system. Asian-Australasian Journal of Animal Science vol. 29, pp. 987-97.

Grashorn M.A., Serini C. (2006). Quality of chicken meat from conventional and organic production. XII European Conference, Verona, Italy. World's Poultry Science Journal. Book of abstracts, 62, 268.

Fanatico A.C., Pillai P.B., Emmert J.L., Gbur E.E., Meullenet J.F., Owens C.M. (2007). Sensory attributes of slow-and fastgrowing chicken genotypes raised indoors or with outdoor access, Poultry Science, vol. 86, pp. 2441-2449.

Hopić S., Vidović V., Milošević N., Supić B., Pavlovski Z. (2000). Uticaj genotipa i godine na klanične osobine tovnih pilića. Biotehnologija u stočarstvu, vol. 17 (5-6) pp. 47-53.

Li Y., Luo Ch., Wang J., Guo F (2017). Effects of different raising systems on growth performance, carcass, and meat quality of medium-growing chickens. Journal of Applied Animal Research, vol. 45, (1) pp. 326-330.

Mikulski D., Celej J., Jankowski J., Majewska T., Mikulska M. (2011). Grow performance, carcass traits and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access. Asian-Australian Journal Animal Science, vol. 24, pp. 1407–1416.

Rašeta J. and Dakić M. (1984). Higijena mesa (Higijena mesa živine i jaja). Veterinarski fakultet Beograd.

Pavlovski Z., Škrbić Z., Cmiljanić R., Lukić M., Tomašević D. (2007). Uticaj sistema gajenja i bioloških faktora na konformaciju trupa i klanične osobine pilića u tovu, Biotechnology in Animal Husbandry, vol. 23, br. 3-4, str. 59-66.

Perić L., Đukić-Stojčić M., Bjedov S. (2016). Slaughter traits of chickens raised in organic or conventional production systems. International Symposium on Animal Science, 24-25.11. Belgrade, Serbia, pp. 472-477.

Perić L., Strugar V., Bogosavljević Bošković S., Đukić Stojčić M. (2018). Effect of genotype and sex on carcass characteristics of chickens raised in extensive system. Biotechnology in Animal Husbandry, vol. 34 (4) pp. 405-417.

Ristić M., Damme, K. (2013). Significance of pH-value for meat quality of broilers: Influence of breed lines. Veterinarski glasnik, vol. 67 (1-2) pp. 67-73.

Rodić V., Perić L., Vukelić N., Milošević N. (2006). Consumer's attitudes towards chicken meat produced in extensive systems. World's Poultry Science Journal, XII European Poultry Conference, Book of Abstracts, vol. 62, pp. 186-187.

Strugar V. (2018). Rezultati tova pilića rase White Rock i hibrida Ross 308 u uslovima slobodnog ispusta. Magistarska teza, Poljoprivredni fakultet, Novi Sad.

Vučić G., (2014). Doktorska disertacija: Uticaj tehnoloških postupaka na boju i kvalitet salamurenih proizvoda, Tehnološki fakultet, Banja Luka

THE IMPACT OF ORGANIC PRODUCTION OF GEESE ON THE ENVIRONMENT THROUGH THE PRISM OF EU LEGISLATION

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Abstract

The authors present the specifics of organic production and its impact on the environment when it comes to poultry production - geese. Geese are characterized by modesty in terms of breeding conditions and they are a type of poultry that enters breeding relatively late, characterized by modest egg production during the season, while the results suggest a general conclusion that the age of geese has a significant impact on egg fertilization and laying goose, as well as the mass of eggs, and thus the final product. Having in mind the characteristics of geese that significantly distinguish them from other poultry, it is important to point out the minimized negative impact on the environment of this type of production, which was carried out according to the standards of the Common Agricultural Policy of the EU. Organic production provides high value products certified as organic food, which increases the price of a unit product, reduces consumer health risks and enables the entrance on the global market.

Key words: e*nvironmental protection, European Union, Geese production, Organic production, Poultry production.*

Introduction

The methods of organic agriculture are ecological forms of production which enable the protection of agro-ecosystems if biological and ecological findings about the environment are added to the existing knowledge of agronomy. This type of production is based on the principles of agroecology and has been applied in the European Union (EU) member states since 1991 (Regulation (EEC) 2092/91), whereas today it is a key objective of the Common Agricultural Policy (1992) of the EU whose main goal is the preservation and protection of the environment and rural areas (Regulation (EU) 2020). It is precisely for this reason that organic production is a controlled way of producing 'from field to table', which prevents a potential harm on the ecosystem and, consequently, human health.

In order to achieve these goals, the EU through positive regulations requires agricultural producers to take care of the effects of their agricultural activities and their impact on the environment. In this way, the environmental protection unites not only producers with consumers, but policy creators, too (Kaljević Zimonja et al., 2012).

Food and Agricultural Organization (FAO, 1961) defines organic agriculture as a process of sustainable development of rural areas in accordance with available resources, tradition and biodegradable potential of habitats, which comprises finalized crop and livestock production, the preservation and renovation of natural resources and return to the traditional values and knowledge. Organic agriculture can be best understood as an attempt to identify methods for production of organic food with the minimization of negative effects of intensive agriculture on the environment. Finally, organic agricultural production is a completely controlled production cycle, according to the binding provisions of the International Federation of Organic Agricultural Movements (IFOAM, 2016).

The problem that organic production faces is the impossibility of functioning in more developed countries in which contemporary agriculture with its aggressive application of all disposable chemicals has caused the deterioration of water, air and soil quality. Organic products make only about 1% of the total world food market today, although there is a quite high global demand for them (Vehapi, 2019).

Our country fulfills almost all conditions required for the organized development of certified organic food of high quality which meets the needs of consumers and increased export and, in accordance with this, the making of profit that does not harm the environment.

Finally, contemporary scientific findings and technological achievements, with an undeniable legal support, make the implementation of organic production in all forms of agriculture possible. It should not be forgotten that one of the crucial characteristics of organic production is the principle of unity of crop and livestock production (Written Questions E-0079/99 and E-0197/99). The general principle in organic livestock production is the emphasis on selecting animal breeds according to their adaptability to local conditions, their vitality and resistance to diseases; organic livestock production needs species and breeds resistant to contagious diseases and not susceptible to health problems. The advantage should be given to the autochthonous species and breeds (Hirstov i Relić, 2005).

This paper shows the results of organic production of goose, meat and eggs, in adequate ecological conditions, since geese are mostly bred in semi-extensive conditions in our country (Republic of Serbia) and in the region. More specifically, the contemporary tendencies of developing self-sustainable agriculture and self-sustainable poultry breeding, especially within the framework of producing natural ecologically-friendly food, reaffirm using the diverse breeds of poultry, primarily geese, and ducks (Mitrovic and Djekic, 2013). Geese typically require modest breeding conditions, especially regarding food and housing. Modern goose farms are rare not only in our country and region, but in the whole world (Milošević and Perić, 2011).

Material and methods

The experimental part of research has been done in the private farm 'Anser' (Triješnica, Bijeljina, Republic of Srpska, Bosna and Herzegovina). where the breeder flock of Italian white goose is bred in a semi-intensive keeping system. The farm produces eggs for setting and one-day old goslings. The farm has an adequate number of adapted facilities in which geese dwell during night and poor weather conditions. Geese spend the rest of the time in a free space – pasture which is abudnant in this farm. As a result, they mostly only need a modest additional meal (grain seeds). It is desirable to keep geese in the fields after the harvest of cereals and legumes, feed them with kale, carrots, turnip heads or whole turnip split into halves, fresh turnip porridge and boiled potatoes, because the digestive tract of geese is capable of digesting cellulose from most nutrients. For this reason, in summer geese can graze and additionally have a slight meal, whereas in winter this is not possible (Labatut, 2002).

Italian white goose is both an egg-laying and lean goose of an extraordinary quality. Rosiński (2000) points out that Italian white goose is a good egg-layer (more than 65 eggs), that fertilization of eggs is around 81.4% and hatchability of goslings out of the total number of fertilized eggs is around 80.8% and that the quality of meat is good.

A random sampling method was used during the season of laying: 1200 breeding eggs were incubated successively from February to July (6 times – each month) in four production cycles i.e. during four years (2014 – 2017). During the season of egg production (February- July) for each production cycle 50 eggs were incubated each month (50 eggs x 6 months = 300 eggs) and during the four year long period of using the breeder flock of geese the total of 1200 breeding eggs were incubated (300 eggs x 4 production cycles = 1200 eggs).

During the incubation periods, the nubmer and percent of fertilized eggs (fertilization value) were determined as well as the number and percent of hatched goslings out of incubated and fertilized eggs. Automatically, the number and percent of unfertilized eggs were determined as well as the number and percent of fertilized eggs with dead embryos.

Before being placed into the incubator, eggs were individually measured (their weight, width and length), their shells were marked with a grafite pencil and disinfected. All eggs (fertilized, unfertilized, eggs with dead embryos) were individually measured on the 25^{th} day of incubation.

The elementary data processing was done by applying standard varioational statistical methods (descriptive statistics). For the majority of characteristics of incubated eggs, or hatched goslings, the following parameters were calculated: the arithmetic mean (x), the standard deviation of the arithmetic mean ($S\bar{x}$), the standard deviation (S) and coefficient of variation (C.V.).

Results and discussion

It should not be forgotten that geese and ducks have significantly lower reproductive potential in comparison to hens, since the production of eggs is seasonal. For this reason, a significantly lower number of eggs is produced during a production cycle, with embryo mortality during egg incubation being siginificantly higher than in hens (Rosiński and Bednarczyk, 1997).

Similarly to the situation in the majority of the EU member states, domestic autochtonous breeds such as the Sombor Kaporka (Sombor crested), the black Svrljig Hen, the Naked Neck and the Banat Naked Neck are bred in the Republic of Serbia in solid nubmers in rural farms in semi-extensive or extensive systems and also in a certain way in the production of organic eggs and even meat. The foreign pure breeds bred in Serbia are the New Hampshire, Neked Neck, White Leghorn and Rode Island Red.

Based on conducted experiments and obtained results, Mitrović et al. (2014) gave a detailed review of the comparative study of production and reproduction characteristics of two breeds (the New Hempshire and Sombor Kaporka) , two breeds of hens widely bred in the Republic of Serbia. Both breeds are bred in the semi-extensive system of keeping. The authors examined the intensity of laying capacity (egg production) in a certain time period (60 days), egg mass, external indicators of egg quality (length, width and the egg shape index), the mass of chicken hatched in a natural traditional way and a relative proportion of chicken in the egg mass. On top of that, they determined the coefficient of phenotype correlation and the strength of the relationship between the majority of measured indicators. On the basis of obtained and displayed results, the authors argue that breeds the New Hampshire and the Sombor Kaporka showed satisfactory production and reproduction characteristics in the semi-extensive system of breeding and that both breeds can be successfully used in the organic poultry production. In addition, the authors emphasize that the natural way of lying (under the brood hen) in comparison to the artificial incubation of eggs give better incubation results, and especially the percentage of hatchability from fertilized eggs and relative share of chicken in the egg mass.

Đermanović et al. (2013), Đermanović and Mitrović (2013) determined phenotype variability of external indicators of the quality of breeding stock eggs (egg mass, length, width and egg shape index) of the Partridge-Coloured Italian and Leghorn breed raised in the semi-intensive system. The eggs of the Partridge-Coloured Italian compared to the eggs of the Leghorn had greater mass (62.25 g - 61.01 g), greater length (55.72 mm - 55.19 mm), whereas the egg shape index was insignificantly higher in the Leghorn eggs (76.08% - 75.65%), but the differences were not statistically confirmed (P>0,05).

Similarly to the egg production, a great number of domestic and foreign authors in different time period examined the possibility of using pure, autochthonous breeds and their hybrids (slow-growing and fast-growing) bred in different systems of keeping in the production of organic meat, and all this respecting the binding provisions of the EU legislation that regulates organic poultry production.

When incubational values of eggs for setting are mentioned, people usually think of the percent of fertilized, and the percent of hatched goslings, out of the total nubmer of incubated and fertilized eggs, dynamics of embryo growth (the loss of egg mass during the embryo development), egg characteristics and hatched goslings, as well as their mutual connections. In order to show results in a clearer way, all the mentioned indicators will be presented separately, although it should be taken into account that all these characteristics are more or less interrelated – they condition each other.

During the four production cycles, the total of 1200 eggs were incubated, out of which 1066 were fertilized, i.e. 88.83% (Table 1). The greatest fertilization of eggs was realized in the second year (272 eggs – 90.67%), and the least in the fourth year (262 eggs – 87.33%), although the egg fertilization was on a satisfactory level each year.

Table 1. – Fertilization and hatchability of goose eggs for four production cycles (1 - 4 years of age of geese; I, I I, I I I, IV) and by phases (months) of the production cycle (February – July; F - J)

Indicators/years	**I**	**II**	**III**	**IV**	**F**	**M**	**A**	**M**	**J**	**J**	Total
No. of eggs incubated	300	300	300	300	200	200	200	200	200	200	1200
Fertilized eggs % of fertilized eggs	268 89.33	272 90.67	264 88.00	262 87.33	181 90.50	187 93.50	179 89.50	176 88.00	174 87.00	169 84.50	1.066 88.83
Unfertilised eggs % of unfertilised eggs	32 10.67	28 9.33	36 12.00	38 12.67	19 9.50	13 6.50	21 10.50	24 12.00	26 13.00	31 15.50	134 11.17
Eggs with dead embryos (A) % of eggs with dead embryos	32 10.67	36 12.00	27 9.00	28 9.33	23 11.50	18 9.00	19 9.50	19 9.50	22 11.00	22 11.00	123 10.25

Eggs with dead embryos (B) % of eggs with dead embryos	32 11.94	36 13.23	27 10.23	28 10.69	23 12.71	18 9.63	19 10.61	19 10.80	22 12.64	22 13.02	123 11.54
Goosling hatched (A) % of goosling hatched	236 78.67	236 78.67	237 79.00	234 78.00	158 79.00	169 84.50	160 80.00	157 78.50	152 76.00	147 73.50	943 78.58
Goosling hatched (A) % of goosling hatched	236 88.06	236 86.76	237 89.77	234 89.31	158 87.29	169 90.37	160 89.39	157 89.20	152 87.36	147 86.98	943 88.46

(A) – of incubated eggs; (B) – of fertilized eggs.

The hatchability of goose eggs, observed by production cycles, was also on a satisfactory level, the hatchability out of the nubmer of incubated eggs being the highest in the third year (79.00%), and out of the nubmer of fertilized eggs in the second year of the production cycle (86.76%). For the total of four production cycles, the hatchability out of the number of incubated eggs was 78.58%, and out of fertililzed eggs was 88.46% (Table 1).

For the whole analyzed period, it is noticeable that the phase (month) during the season had impact on the observed indicators (Table 1). The best fertilization was achieved in March (187 eggs - 93.5%), and the poorest in July (169 eggs – 84.5%). The hatchability of goslings out of incubated eggs was between 84.5% (March) and 73.5% (July), and out of fertilized eggs between 90.37% (March) and 86.98% (July).

The results presented in the table show that the total fertilization of eggs was on average 88.83% (11.17% of unfertilized eggs), 10.25% of eggs with dead embryo out of the total number of incubated eggs, i.e. 11.54% out of the number of fertilized eggs, 78.58% of hatched goslings out of the nubmer of incubated eggs and 88.46% out of the number of fertilized eggs.

The presented results bring us to the conclusion that the age of geese and phase (month) during season (February-July) have an impact on fertilization and hatchability. This is confirmed by research done by Kent and Murphy (2003) who determined that egg production and hatchability gradually increase with age starting from age one until a certain limit, depending on the breed and type of geese. Bednarczyk and Rosiński (1999) discovered that in both types of Italian white goose (WD1 and WD3) the greatest hatchability out of fertilized eggs happened at the beginning of the season of laying 84.2% (WD1) abd 80.00% (WD3), which gradually declined until July, when it was 60.5% and 56.9%. The growth trend regarding the percent of hatchability during the season of egg laying, that the mentioned authors observed, is quite similar to our results. The difference is in the fact that the percent of hatchabililty out of fertilized eggs in our research was significantly more favourable (for each of the four production cycles (years) and for the total of four seasons 88.46%, Table 1).

Depending on the egg treatment (pre-heating of eggs before incubation) Kucharska-Gaca et al. (2016a) in the White Koluda goose eggs in the second and third season of laying found the egg fertilization that was between 79.3% (the control group eggs) and 82.8% (JI group- 6h pre-heated eggs), and the hatchability out of incubated and fertilized eggs was respectively between 66.8% and 80% and between 60.1% and 75%, which is significantly less in comparison to our results for the egg fertilization and hatchability in the Italian White goose. Even lower production of goslings out of fertilized eggs (62.2% - 61.1%) was found by KRD-IG (2013), Pakulska et al. (2003), and the poorest hatchability was found by Kucharska-Gaca et al. (2016b) in various weight groups, more specifically in the weight group of eggs which weighed between 181g and

200g (48.2% - out of incubated eggs; 73.7% - out of fertilized eggs). Kucharska-Gaca et al. (2016b), Bobko and Svetlik (2002), regardless of the weight group, came to the significantly higher embryo mortality than in invested i.e. fertilized eggs during the incubation period (11.3% - 20.9%) in comparison to our results (10.25% and 11.54% Table 1). Similarly, Rachwal (2008) finds that the egg mass has an impact on the embryo mortality and argues that eggs of smaller mass have lower embryo mortality and vice versa, which to a certain extent differs from our results. In addition, Pécsi et al. (2010) point out that the hatchability does not go over 70% because goose eggs are very difficult for hatching since they are quite big, have very hard shell and need regular cooling and bedewing. The similar fertilization of the Italian White Goose, hatchability and embryo mortality was found by Mitrovic et al. (2016), Mitrović et al. (2018), and Von Luttitz (2003). They emphasize that a phase during the production (season) of egg laying has an impact on the mentioned indicators and the average egg mass, i.e. the mass of hatched goslings.

Mazanowski and Chelmonska (2000), Mazanowski and Adamski (2002), Mazanowski and Bernacki (2006), Đermanović et al. (2008) examined the reproductive characteristics of goose crossbreeds that were created by the reciprocal crossing of typically three goose breeds, one of which was the White Italian (White Italian x Slovakian x Graylag), or two breeds (Chinese Knob Goose and domestic goose). Their results were similar or slightly worse than ours concerning the fertilization and hatchability. Golze (1991), Shalev et al. (1991), Vognivenko and Debrov (1997), Shalev (1998), Kirmizibayrak and Altinel (2001), Von Luttitz (2003), Shi et al. (2008), Scripnic and Modvala (2010) confirm that, in addition to the mentioned factors, genotype, the system of keeping, photoperiod and egg bedewing also have a significant effect on the incubation values (fertilization, hatchability and embryo mortality) and egg and gosling characteristics (egg mass, gosling mass on the first day, etc.).

A great number of authors did research on reproductive characteristics of other poultry species, mostly hens and turkeys, in a somewhat larger volume and they came to the similar conclusion that genetics and paragenetics have effect on their reproductive characteristics (fertilization, hatchability, chicken and turkey egg mass, egg length, egg width and egg shape index), to a certain extent similarly to geese (goose eggs).

Conclusion

Egg fertilization and hatchability are the most significant factors in the reproduction of all poultry species, including geese, since geese produce significantly lower number of eggs during the production cycle. The best egg fertilization (90.67%) was achieved in the second production cycle, then in the first cycle (89.33%) and the third (88%), while the worst was in the fourth cycle (87.33%). The fertilization for the four years in total was on average 88.83%. Therefore, the goose age had an impact on the number and percentage of fertilized eggs, the highest fertilization being achieved in the second and first cycle, then slightly decreasing in the third and fourth production cycle.

The egg hatchability, observed by production cycles, was also on a satisfactory level, the highest hatchability out of incubated eggs happening in the third production cycle (79%) and out of fertilized eggs in the second production cycle (86.76%). The hatchability for the total of production cycles was 78.58% for incubated eggs and 88.46% for fertilized eggs.

In all production cycles, it was in March that the best fertilization was achieved and it was 92% in the first cycle (year), and 94% in the second, third and fourth cycle. At the end of the season

(July), the fertilization was the lowest, going between 86% (first and second year) and 82% (fourth year).

In all production cycles (years), the most favourable hatchability in fertilized eggs happened in February and March (around 90% and more). Observing by months, the fertilization and hatchability during each production cycle, it can be noted the phase (month) during the season of egg production from February to July had effect on the measured indicators.

In the four-year period, the total of 943 goslings were hatched out of 1 200 incubated eggs. This egg category (the eggs out of which goslings came) was particularly examined because it is the most important link in the goose reproduction chain and goose meat production. This also goes for all other poultry.

The conclusion can be drawn that organic livestock production is not a typical extensive production (although it does have certain characteristics of that system). It is intensive with the acceptance of limitations set by the habitat. It limits the pollution in the habitat itself, which results in the preservation of the whole biological community, the satisfactory income and possibilities for the development of additional activities such as tourism (Council Directive 98/58/EC). In addition, organic production provides high value products certified as organic food, which increases the price of a unit product, reduces consumer health risks and enables the entrance on the global market. Of course, this production requires more work force, which is considered as very positive in the contemporary global trend of rural communities depopulation.

Finally, these activities prove that the standards of organic agriculture prescribed by the Common Agricultural Policy of the EU can be very successfully implemented in our region and that it is very desirable to apply them to the states who are not members or signatory states of the EU, such as our country, due to the goals achieved by this way of production, both concerning the production results and environmental protection.

References

Bednarczyk M., Rosiński A. (1999): Comparation of Egg Hatchability and In Vitro Survival of Goose Embryos of varios Origins. Poult. Sci., 78: 579-585.

Bobko M., Svetlik I.S. (2002): Weight losses of geese hatching eggs during incubation. Proceedings of the International Scientific Conference Rearing of poultry and small livestock in the 3rd millennium, 17-18 September, Nitra, Slovakia, 115-119.

Common Agricultural Policy established in 1962, according to Article 39 of Treaty on the Functioning of the European Union, OJ C 326, 26.10.2012, p. 47–390.

Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes, OJ L 221, 8.8.1998, p. 23–27.

Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91, OJ L 189, 20.7.2007, p. 1–23.

Council Regulation (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs, OJ L 198, 22.7.1991, p. 1–15.

Đermanović V., Anđelić-Buzadžić, G., Rajević M., Purić V., Mitrović S. (2008): Analysis of the result of incubation of goose eggs of two – breed goose crossbreads. XXII Aronomist, Veterinarians and Technologist Advising, Vol. 14, 3-4, 135–142. Agroekonomik PKB, Belgrade.

Đermanović V., Mitrović S. (2013): Phenotype vatiability of external quality traits of fertile eggs of partridge Italian bread in semi extensive breeding system. Biotehnology in Animal Husbandry, 29 (3), 477 – 482.

Đermanović V., Mitrović S., Đekić V., Mitrović M. (2013): Quality assessment of breeding eggs of leghorn breed hens reared in a semi – extensive system. Collection of Scientific Papers, Vol. 19, 3 – 4, 173 – 178.

FAO, *http://www.fao.org*

Golze M. (1991): Four years of use and the right time of hatching results in more hatching eggs and gosling for laying geesse. Tierzucht, 45: 524-526.

International Federation of Organic Agriculture Movements (IFOAM) (2016) Organic in Europe: Prospects and Developments 2016, available ats: http://www.ifoam-eu.org/sites/default/files/ifoameu_organic_in_europe_2016.pdf

Kaljević Zimonja, I., Petrović, N., Vukadinović, J. (2012): Organic production in Eureopan union and Serbia. Foreign Legal Life 1/2012:228 – 238.

Kent J.P., Murphy K.J. (2003): Synchronized egg laying in flocks of domestic geese (Anser anser). Appl. Anim. Behav. Sci., 82: 219-228.

Kirmizibayrak T., Altinel A. (2001): Some of Parameters related to most yield Characteristic of Japanese quail (Coturnix coturnix Japonica). Istanbul Univ. Vet. Fak. Derg., 27: 309-328.

Krayowa Rada Drobiarstwa – Izba Gospodarcza w Warszawie (2013): Wyniki Oceny Wartości Uzutkowey Drobiu w 2011 roku (The National Poultry Council – Chamber of Commerce in Warsaw., 2013. Assessment Results of Poultry Value in Use in 2011 year). Wiad. Drob., 149-185 (in Polish)

Kucharska-Gaca Joanna, Adamski M., Joanna Kuźniacka, Emilia Kowalska (2016a): Goose eggs hatching technique improvement with the use of preincubation. Acta. Sci. Pol. Zootechnica, 15(2): 37-46.

Kucharska-Gaca Joanna, Adamski M., Joanna Kuźniacka, Emilia Kowalska (2016b): Influence of the weight of hatching eggs on the hatchability indices and on the body weight of geese in rearing and after fattening with oats. Acta. Sci. Pol. Zootechnica, 15(3): 67-82.

Labatut, M. C. (2002): Goose production in Chile and South America, in: Buckland, R. & Guy G. (Eds) Goose Production, FAO Animal Production and Health paper No.154, Part 2, pp. 93 – 110. (Rome, Italy Publishing Management Service, FAO)

Mazanowski A., Adamski M. (2002): Evaluation of reproductive traits and egg quality in Astra G geese during the first reproductive period. Ann.Amim. Sci., 2 (2): 67-78.

Mazanowski A., Bernacki Z. (2006): Characteristics of reproductive traits and egg traits in Graylag goose (Anser anser L.) crossbreds. Arch. Geflügelk., 70 (2), 56-63.

Mazanowski A., Chelmonska B. (2000): The effects of reciprocal crossing of White Koluda and Greylag crossbred geese with Slovakian geese. Annals of Animal Science - Roczniki Naukowe Zootechniki 27(4), 85-103.

Milošević, N., Perić, L. (2011): Poultry Production Technology. Faculty of Agriculture, University of Novi Sad, Novi Sad.

Mitrović S., Đermanović V., Đekić V., Milivojević V., Simić D. (2014): Comparative studies on the reproductive and produktive traits of New Hampshire and Sombor Crested chicken breeds reared in seni – extensivi production system. Proceedings of the International Symposium on Animal Science, Beograd – Zemun, Srbija, 61-67.

Mitrović S., Pandurević T., Milojević M., Jokić Ž., Đermanovoić V., Mičić S., Vlačić J. (2016): Effects of egg weight and shape index on incubation results of the White Italian Goose. VII

Internationalni Scientific Agriculture Symposium, „Agrosym 2016", 6-9 October 2016, Jahorina, Bosnia and Herzegovina. Proceedings 2016: 2534-2540.
Mitrović, S., Đekić, V. (2013): Organic poultry production (monographic work). Faculty of Agriculture Zemun, University in Belgrade, Belgrade.
Mitrović, S., Milojević, M., Đukić Stojčić, M. (2018): Phenotype correlation of external and incubation traits of Italian White Goose eggs and goslings after hatching. Indian J.Anim. Res., 52(4): 497-501.
Pakulska Elzbieta., Badowski J., Bielińska H., Bednarczyk M. (2003): Wplyw wieku na cechy fizyczne jaj i wylęgowść pisklat gęsi Bialych Koludzkich (Age of effects of physical traits of eggs and hatchability of White Koluda goslings). Zesz. Nauk. Prz. Hod., 68(4): 71-78 (in Polish).
Pécsi A., Kozák J., Nikodémusz E. (2010): A Photographic Guide to Goose Embryo Development. Poultry Industrialism.
Rachwal A. (2008): Wplyw masy jaja na wylęgowość, wielkość pisklęcia, jego tempo wzrostu oraz wykorzystanie paszy (The influence of egg weight on hatchability, size of chick, its growth rate and utilization of pesture). Pol. Drob., 10: 13-16 (in Polish).
Regulation (EU) 2020/2220 of the European Parliament and of the Council of 23 December 2020 laying down certain transitional provisions for support from the European Agricultural Fund for Rural Development (EAFRD) and from the European Agricultural Guarantee Fund (EAGF) in the years 2021 and 2022 and amending Regulations (EU) No 1305/2013, (EU) No 1306/2013 and (EU) No 1307/2013 as regards resources and application in the years 2021 and 2022 and Regulation (EU) No 1308/2013 as regards resources and the distribution of such support in respect of the years 2021 and 2022, OJ L 437, 28.12.2020, p. 1–29.
Rosiński, A. (2000): Analysis of direct and correlated effectsof selection in two goose strains (in Polish). Rocz.AR Poznań 309:5-107.
Rosiński, A., Bednarczyk, M. (1997): Influence of genotype on goose egg hatchability. Archiv für Geflügelkunde 61(1): 33 – 39.
Scripnic, E., Modvala, S. (2010): Process of Increasing the Incubation Indices of Geese Eggs. Bulletin UASVM J. Anim. Sci. Biotechnol., 67(1-2): 334-338.
Shalev B.A. (1998): Growth rate of geese of various breeds. The 36th Annual Convention. Zichron Yaacov, 65-66.
Shalev B.A., Dvorin A., Herman R., Katz Z., Bornstein S. (1991): Long-term goose meat enterprise. Brit. Poult. Sci. J., 40: 221-226.
Shi Z.D., Tian Y.B., Wu W., Wang Z.Y. (2008): Controlling reproductive seasonality in the geese. World's Poultry Sci. J., 63(3): 343-355.
Vognivenko L., Debrov V. (1997): Assessment of reproductive and productive ability of geese with different methods of breeding. Visnik Agrarnoi Nauki., 4: 63-65.
Von Luttitz H. (2003): Crescãtoria de rate şi gâşte. Editura M.A.S.T.
Written Question No. 197/99 by Anita Pollack - Production of foie gras, Official Journal C 320, 06/11/1999, P. 0125.

EFFECTIVE POPULATION SIZE AND INBREEDING RATE OF HOLSTEIN POPULATION IN VOJVODINA AUTONOMOUS PROVINCE (SERBIA)

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Abstract

Holstein populations around the world are facing an increasing inbreeding rate. Despite being the most numerous dairy breed worldwide, Holstein effective population sizes are quite small. This is a result of intensive use of artificial insemination and high selection pressure. Recent data show that after the introduction of genomic selection, the inbreeding rate started increasing even faster. Results of inbreeding are well described in dairy cattle. Inbred cows have lower milk yield, worse reproduction parameters, and shorter survival in production. The objective of this study was to examine the inbreeding rate and population size of the Holstein population registered with the cattle breeding organization in Vojvodina province, Serbia. The pedigree file was formed for 36,733 animals born between 2013 and 2017. Inbreeding coefficients and effective population sizes were calculated using PopRep software (FLI). The average inbreeding coefficient steadily increased from 0.51% to 1.08% during the period observed. Effective population size was stabile when calculated according to a number of parents, with an average of 4,643 animals. Effective population size, obtained by the method of computing the relationship matrix with uncertain parentage, which was method proposed by software, was small and decreasing, ranging from 120 to just 65 in the period observed.

Key words: *Effective population size, inbreeding, Holstein cattle.*

Introduction

Inbreeding is mating of animals that are related by ancestry, i.e., animals that have a known common ancestor. Inbreeding increases homozygosity. One consequence of that is the occurrence of rare genetic disorders in progeny due to increased chance of detrimental recessive alleles to meet and be expressed (VanRaden and Miller, 2006). Another consequence is the overall reduction of birth size, growth rate, reproductive performance etc. This effect is known as inbreeding depression. Inbreeding depression is also probably mainly caused by recessive mutations (dominance theory) and recessive mutations at closely positioned loci on the same chromosome (pseudo-overdominance), while heterozygote advantage (overdominance theory) and epistasis playing a lesser role (Charlesworth and Willis, 2009).
Despite being the most numerous dairy breed worldwide, Holstein effective population sizes are small (Brotherstone and Goddard, 2005). This is a result of a combination of high selection pressure and artificial insemination (AI) that made possible for selected bulls to have a huge number of offspring. This combination resulted in an unprecedented spread of genetic material from small number of superior animals, and complete removal of all others from breeding population. More than 99% of USA AI bulls are direct descendants, by male lineage, of just two bulls born in 1950s (Yue *et al.*, 2015). Two additional male lineages were reconstructed at Penn

State University in 2017. by using stored semen of bulls belonging to lost lines, and modernized by mating with oocytes from today's elite females (Dechow *et al*., 2020), but question remains whether this lineages will find commercial interest and have any chance to bring diversity to current Holstein population.

Inbreeding is traditionally calculated based on pedigree data and expressed as a coefficient of inbreeding (F), as proposed by Wright (1922). It is equal to a chance that two alleles in a given animal are of the same origin, assuming an equal probability of every allele to be passed from one generation to another. When genomic data become available, new measures of inbreeding emerged. Bjelland *et al*. (2013) have used three measures to express inbreeding using single nucleotide polymorphism markers (SNP): percent homozygosity, runs of homozygosity (ROH) and genomic relationship matrix. These measures have advantage of not being dependent on pedigree data and not relying on the theoretical probability of alleles passing. Consequently, it can be expected that genomic measures of inbreeding will be better predictors of inbreeding depression. This is proven by simulation (Kardos *et al*., 2015). However, Nietlisbach *et al*. (2017) found, studying wild bird population, than genomic measure they used - homozygosity measured at microsatellites, is less well correlated with phenotype fitness than pedigree-based inbreeding coefficient. Pryce *et al*. (2014) have found low to moderate (0.26-0.53) correlations between pedigree-based inbreeding coefficient and genomic measures of inbreeding in Holstein and Jersey cows, but much stronger correlations (0.67-0.87) in research on Holstein bulls (Pryce *et al*., 2012). Authors explained this with worse pedigree recording for females than for males (Pryce *et al*., 2014).

The average inbreeding coefficient for Holstein breed increased from 0.4% in 1970 to 9% in 2021 in USA Holstein population (CDCB, 2021). It was estimated to be 3.5% in Australian Holstein population in 2007 (Pryce *et al*., 2014) and 5% in Dutch-Flemish Holstein-Friesian population in 2012 to 2016 (Doekes *et al*., 2018). It increased from 4% in 1990 to more than 9% in 2018 in Canada (Makanjuola *et al*., 2020). Inbreeding is also rising in small, importing Holstein populations like in Luxemburg and Tunisia, reaching 2-3% in 2000 (Hammami *et al*., 2007). It was predicted that genomic selection can reduce inbreeding (Daetwyler *et al*., 2007), but contrary to that, after introduction of genomic selection, average inbreeding coefficient started increasing even faster than before. During the last decade, average growth of the inbreeding coefficient in USA Holstein population was 0.3% per year, compared to 0.1% per year a decade before (CDCB, 2021). Same is true in Dutch-Flemish Holstein-Friesian population (Doekes *et al*., 2018), and Canadian Holstein population (Makanjuola *et al*., 2020).

The objective of this study was to examine the inbreeding rate and population size of the Holstein population registered with the cattle breeding organization in Vojvodina province, Serbia.

Materials and methods

The pedigree file was formed for 36,733 animals born between 2013 and 2017, registered as purebred Holstein with the cattle breeding organization in Vojvodina province, Serbia. Together with the ancestors, data file included 82,342 animals, and reached as far back as data was available. Pedigree completeness for animals observed, as calculated according to MacCluer *et al*. (1983), was fair, being 90% for 2 generations deep, 74% for 4 generations deep and 62% for six generations deep. It was somewhat lower for the first years of period observed and increased toward the end (Table 1).

Coefficients of inbreeding were calculated according to Wright (1922), while effective population sizes were calculated using six different methods: N_e-Cens (Wright, 1923), N_e-Ln (Pérez-Enciso, 1995), N_e-Ecg (Gutiérrez *et al*., 2009), N_e-ΔFp, N_e-ΔFg and N_e-Coan (Falconer and Mackay, 1996). All calculations were done using PopRep software of Friedrich Loeffler Institute (Groeneveld *et al*., 2009), and best method for estimating effective population size was chosen according to algorithm proposed by the same software.

Table 1. Pedigree completeness (%) for animals born between 2013 and 2017.

Year	Number of animals	Gen. 1	Gen. 2	Gen. 3	Gen. 4	Gen. 5	Gen. 6
2013	8430	99.4	82.2	70.4	62.3	55.7	49.9
2014	7954	99.8	87.6	77.6	70.5	64.3	58.5
2015	8872	99.9	91.8	83.5	77.1	71.2	65.5
2016	7632	100.0	94.6	87.7	82.0	76.4	70.9
2017	3845	100.0	96.1	90.4	85.2	79.9	74.5
Total	36733	99.8	89.7	80.8	74.1	68.1	62.5

Results and discussion

The average inbreeding coefficient increased steadily from 0.51% to 1.08% during the period observed (Table 2). It increased at the similar rate like in USA Holstein population before introduction of genomic selection (CDCB, 2021). In the same time, percentage of inbred animals also increased steadily, reaching 87% in 2017. This is probably a result, in part, of better pedigree completeness. Cassell *et al*. (2005) have shown that average inbreeding coefficient was 0.04 for Holstein animals with pedigree completeness less than 31% up to 5 generations, 1.65 for animals with pedigree completeness between 31 and 70%, and 2.06 for animals with pedigree completeness greater than 70%. However, even when taking imperfect completeness of pedigree data into account, observed inbreeding level of Holstein population in Vojvodina province during the period observed was very low. Reported inbreeding coefficients for other Holstein populations are usually much higher, ranging from 2-4% in Luxemburg, Tunisia and Australia (Hammami *et al*., 2007, Pryce *et al*., 2014), 5% in Denmark and Belgium (Doekes *et al*., 2018) and 9% in Canada and USA (Makanjuola *et al*., 2020, CDCB, 2021). This can be explained with the implementation of the breeding plan for Holsten population in Vojvodina province (BOTV, 2019), that culls out animals with high inbreeding coefficient. This measure reduces the number of animals that are suitable for the breeding population, but results in a lower inbreeding level in the population. Contrary to the average inbreeding, maximum levels of inbreeding decreased during the period observed (Table 2), probably as a result of culling animals with high inbreeding coefficient from breeding population.

Table 2. Number of inbred animals and inbreeding coefficients for animals born between 2013 and 2017.

Year	Number of animal		Inbred, % of total	Inbreeding coefficient		
	Total	Inbred		Average	SD	Maximum
2013	8430	4038	48%	0.0051	0.013	0.2581
2014	7954	4881	61%	0.0065	0.012	0.2586

2015	8872	6544	74%	0.0081	0.010	0.1333
2016	7632	6290	82%	0.0096	0.011	0.1262
2017	3845	3353	87%	0.0108	0.011	0.1366

Effective population size was stabile when calculated according to a number of parents (N_e-Cens), with an average of 4,643 animals. Out of six methods used for estimation of effective population size, N_e-Ln was chosen using the algorithm of PopRep software. In this method, effective population size is estimated by computing the relationship matrix with uncertain parentage (Perez-Enciso, 1995). When estimated by this method, effective population of Holstein breed in Vojvodina province was small and decreasing, ranging from 120 to just 65 in the period observed. This is similar to the estimate for the Spanish Holstein population of 66 to 79 (Rodríguez-Ramilo *et al*., 2015), 69 to 102 for Dutch-Flemish Holstein-Friesian population (Doekes *et al*., 2018) and 43 to 66 for Canadian Holstein population (Makanjuola *et al*., 2020). These populations, despite beeing numericaly much larger, also have simmilary small effective population size due to high and increasing inbreeding rate.

Table 3. Effective population size of Holstein breed in Vojvodina province as estimated using 6 different methods.

Year	N_e-Cens	N_e-ΔFp	N_e-ΔFg	N_e-Coan	**N_e-Ln**	N_e-Ecg
2013	4605	-122	273	134	**120**	420
2014	4645	-140	191	128	**104**	396
2015	4724	-159	139	109	**84**	363
2016	4785	-195	117	107	**72**	336
2017	4456	-225	109	102	**65**	323

Holstein populations around the world are a good example how intensive selection, especially genomic, and reproductive technologies, primarily AI, are drastically reducing effective population size and unavoidably increasing the inbreeding rate. Even when measures to control inbreeding temporarily increased the effective population size (Stachowicz *et al*., 2011), subsequent introduction of new technologies have reduced it back rapidly (Makanjuola *et al*., 2020). Inbreeding coefficients are even higher for AI bulls compared to the rest of population (CDCB, 2021). Question remains whether breeding plans should balance a need for intensive selection with measures to keep the inbreeding level low. However, Doekes *et al.* (2019) shows, on Dutch Holstein-Friesian population, that only the new inbreeding results in inbreeding depression, while ancient inbreeding even can have slightly favorable effects. This can be explained with purging effect (Gulisija and Crow, 2007, Mc Parland *et al*., 2009). Also, Doekes *et al.* (2019) argues that increase of inbreeding level that occurred in Dutch Holstein-Friesian population resulted in milk reduction of just 150 kg per lactation, while the realized genetic progress resulted in milk increase of 2,200 kg per lactation.

Conclusion

It can be concluded that Holstein population of Vojvodina province has low, but increasing inbreeding rate, that is currently around 1%, and small effective population size that is less than 100. It can be expected that the rate at which inbreeding level is rising will increase in the future, since similar trends are observed in worlds leading Holstein populations, especially with widespread use of the genomic selection. This will have to be addressed in new breeding plans.

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References

Bjelland D. W., Weigel K. A., Vukasinovic N., Nkrumah J. D. (2013). Evaluation of inbreeding depression in Holstein cattle using whole-genome SNP markers and alternative measures of genomic inbreeding. Journal of dairy science, 96(7), 4697-4706.

Brotherstone S., Goddard M. (2005). Artificial selection and maintenance of genetic variance in the global dairy cow population. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1459), 1479-1488.

BOTV, Breeding organization for the territory of AP Vojvodina (2019). Main breeding plan for Holsten population in Vojvodina province. Faculty of Agriculture, Novi Sad.

Cassell B. G., Adamec V., Pearson R. E. (2003). Effect of incomplete pedigrees on estimates of inbreeding and inbreeding depression for days to first service and summit milk yield in Holsteins and Jerseys. Journal of Dairy Science, 86(9), 2967-2976.

Council on Dairy Cattle Breeding, CDCB (2021). Inbreeding information – Holstein or Red and White. https://queries.uscdcb.com/eval/summary/inbrd.cfm Accessed on 30/06/2021.

Charlesworth D., Willis J. H. (2009). The genetics of inbreeding depression. Nature reviews genetics, 10(11), 783-796.

Daetwyler H. D., Villanueva B., Bijma P., Woolliams J. A. (2007). Inbreeding in genome-wide selection. Journal of Animal Breeding and Genetics, 124(6), 369-376.

Dechow C. D., Liu W. S., Specht L. W., Blackburn H. (2020). Reconstitution and modernization of lost Holstein male lineages using samples from a gene bank. Journal of dairy science, 103(5), 4510-4516.

Doekes H. P., Veerkamp R. F., Bijma P., Hiemstra S. J., Windig J. J. (2018). Trends in genome-wide and region-specific genetic diversity in the Dutch-Flemish Holstein–Friesian breeding program from 1986 to 2015. Genetics Selection Evolution, 50(1), 1-16.

Doekes H. P., Veerkamp R. F., Bijma P., de Jong G., Hiemstra S. J., Windig J. J. (2019). Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. Genetics Selection Evolution, 51(1), 1-16.

Falconer D. S., Mackay T. F. C. (1996). Introduction to quantitative genetics. Essex. UK: Longman Group.

Groeneveld E., Westhuizen B. D., Maiwashe A., Voordewind F., Ferraz J. B. S. (2009). POPREP: a generic report for population management. Genetics and molecular research, 8(3), 1158-1178.

Gulisija D., Crow J. F. (2007). Inferring purging from pedigree data. Evolution: International Journal of Organic Evolution, *61*(5), 1043-1051.

Gutiérrez J. P., Cervantes I., Goyache F. (2009). Improving the estimation of realized effective population sizes in farm animals. Journal of Animal Breeding and Genetics, 126(4), 327-332.

Hammami H., Croquet C., Stoll J., Rekik B., Gengler N. (2007). Genetic diversity and joint-pedigree analysis of two importing Holstein populations. Journal of dairy science, 90(7), 3530-3541.
Kardos M., Luikart G., Allendorf F. W. (2015). Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. Heredity, 115(1), 63-72.
MacCluer J. W., Boyce A. J., Dyke B., Weitkamp L. R., Pfenning D. W., Parsons C. J. (1983). Inbreeding and pedigree structure in Standardbred horses. Journal of Heredity, 74(6), 394-399.
Makanjuola B. O., Miglior F., Abdalla E. A., Maltecca C., Schenkel F. S., Baes C. F. (2020). Effect of genomic selection on rate of inbreeding and coancestry and effective population size of Holstein and Jersey cattle populations. Journal of dairy science, 103(6), 5183-5199.
Mc Parland S., Kearney F., Berry D. P. (2009). Purging of inbreeding depression within the Irish Holstein-Friesian population. Genetics Selection Evolution, 41(1), 1-8.
Nietlisbach P., Keller L. F., Camenisch G., Guillaume F., Arcese P., Reid J. M., Postma E. (2017). Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. Proceedings of the Royal Society B: Biological Sciences, 284(1850), 20162763.
Pérez-Enciso M. (1995). Use of the uncertain relationship matrix to compute effective population size. Journal of Animal Breeding and Genetics, 112(1-6), 327-332.
Pryce J. E., Hayes B. J., Goddard M. E. (2012). Novel strategies to minimize progeny inbreeding while maximizing genetic gain using genomic information. Journal of dairy science, 95(1), 377-388.
Pryce J. E., Haile-Mariam M., Goddard M. E., Hayes B. J. (2014). Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. Genetics Selection Evolution, 46(1), 1-14.
Rodríguez-Ramilo S. T., Fernández J., Toro M. A., Hernández D., Villanueva B. (2015). Genome-wide estimates of coancestry, inbreeding and effective population size in the Spanish Holstein population. PLoS One, 10(4), e0124157.
Stachowicz K., Sargolzaei M., Miglior F., Schenkel F. S. (2011). Rates of inbreeding and genetic diversity in Canadian Holstein and Jersey cattle. Journal of dairy science, 94(10), 5160-5175.
VanRaden P. M., Miller R. H. (2006). Effects of nonadditive genetic interactions, inbreeding, and recessive defects on embryo and fetal loss by seventy days. Journal of dairy science, 89(7), 2716-2721.
Wright S. (1922). Coefficients of inbreeding and relationship. The American Naturalist, 56(645), 330-338.
Wright S. (1923). Mendelian analysis of the pure breeds of livestock: I. The measurement of inbreeding and relationship. Journal of heredity, 14(8), 339-348.
Yue X. P., Dechow C., Liu W. S. (2015). A limited number of Y chromosome lineages is present in North American Holsteins. Journal of dairy science, 98(4), 2738-2745.

THE HEAVY METALS IN THE LIVER OF FATTENING PIGS AND MANGULICA IN THE REGION OF VOJVODINA, SERBIA

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Abstract

Meat and meat products are an important part of daily nutrition of human population. They are an important source of minerals and nutrients essential for human nutrition. Heavy metals are pollutants that, in some cases, can be found in meat and meat products. Lead (Pb) and cadmium (Cd) are heavy metals without any functions in animal organism. Their concentration is regulated through the legislation. The edible offal, like liver, is at the higher risk of the increased amount of Pb and Cd. The allowed concentration for Pb and Cd in liver is 0.5 mg/kg in the Republic of Serbia. The aim of the study was to determine the concentration of Pb and Cd in the liver of fattening pigs (22) and Mangulica (6) from different locations in Autonomous Province Vojvodina, Serbia. Fattening pigs (three breed hybrids) were reared in intensive condition on the formulated feed on sixteen different farms in Vojvodina. They were slaughtered at the age of six months, approximately at 105-110 kg of live weight. Mangulica were from two different locations, reared extensively without any formulated feed. They were slaughtered at the age of two years and at about 100 kg live weight. In all analyzed liver samples, the concentration of cadmium was in the allowed range. However, Pb concentrations in 10 liver samples of fattening pigs and in 4 liver samples of Mangulica exceed the allowed concentration.

Key words: *Lead (Pb), Cadmium (Cd), Fattening pigs, Mangulica pigs.*

Introduction

The pork is one of the most commonly consumed meats in the Republic of Serbia. The annual production is about 300 thousands of tones (Statistical Yearbook, Republic of Serbia 2018). The quality of meat and meat products (as edible offals) are from the great importance for the end consumers. The presence of some of the heavy metals in modern condition of production is almost infeasible. Lead (Pb) and Cadmium (Cd) are trace elements without any biological function in the body of the animals or humans. The elevated level of heavy metals such as lead and cadmium in the environment and food can be a serious problem so the levels of these heavy metals are regulated through the legislation. Usually the elevated levels of trace elements are in the proximity of industrial areas.

The fattening period in the condition on the commercial farms in Serbia usually take from 3-4 months, until the pigs reach 105-110 kg of the end live weight. During this period pigs are fed with formulated feed for the starting, growing and finishing period. Mangulica is an autochthonous breed in Serbia, the life span of the pigs is much longer than the life span of the fattening pigs. Mangulica are pigs reared in free range condition; their nutrition is not based on the concentrated food with added premix.

Lead can be naturally found in the nature however, due to the anthropogenic impact it can be pollutant and enter the human food chain. The human population is exposed to heavy metals via food, water air and soil. However, the food is the major source of exposure (EFSA, 2012). The primary place of accumulation of lead in animals are skeleton, the half- live for lead is about 30 days in blood, and in bones from 10-30 years. The maximum residue level for the lead (Pb) in the meat of bovine, pigs, sheep and poultry is 0.1mg/kg, and for edible offal is 0.5 mg/kg by the EU regulation (Andrée et al., 2010). The Serbian regulation is in accordance with the EU regulation (RS Pravilnik 2011). In the last decades the concentration of lead in food in the majority of EU countries had been reduced due to the effort to reduce emission of lead in the environment and ban of gasoline with lead (Bilandžić et al., 2010; EFSA, 2010; Andrée et al., 2010). However, there is not an abundance of data regarding the occurrence of lead in the meat and meat products in the Republic of Serbia. Some studies appoint that the increased level of lead in local environment is due to the vicinity of lead battery smelters (Mandić-Rajčević et al. 2018). In 4 years long study about heavy metal occurrence in the locally produced vegetables in the Vojvodina in some cases more than 40% of samples had the elevated level of lead (Pajević et al. 2018). Higher concentrations then the recommended by the International Dairy Federation of heavy metals including lead have been found in the milk in Serbia (Davidov et al., 2019). However, the analyzed samples of edible offal of fattening pigs in three different studies done in Serbia did not showed any indications of the increased lead concentration (Milićević et al., 2010; Mitić et al., 2012; Nikolic et al., 2017).
Cadmium does not have any biological function in animal organism. The main source of possible contamination of cadmium is food in non smoking population (Andrée et al. 2010). The allowed concentration of cadmium in liver of animal is 0.5 mg/kg, while in the kidneys is 1 mg/kg (RS Pravilnik 2011). Cadmium have very long half life from 10 to 30 years (Andrée et al. 2010). Some data suggested that in extensively reared pigs cadmium can have higher values than allowed by legislation of the Republic of Serbia (Nikolić, 2018). In the another study the levels of cadmium in the fattening pigs livers were in the allowed range but in the higher concentration than reported in some developed countries (Tomović et al., 2011).
The purpose of this research was to estimate the occurrence of heavy metals in the liver of two type pigs reared in the Autonomous Province Vojvodina, Serbia. Fattening pigs produced in the intensive condition on the commercial farms with the nutrition based on the formulated feed. Mangulice are extensive breed pigs with the nutrition which are not based on the formulated feed and with much longer life span

Material and method

Fattening pigs

Samples of liver were taken from random choice fattening pigs (three breed hybrids) on the slaughter line of three different slaughterhouses in the 2019-2020. The fattening pigs were approximately six months old, weighing from 100-110 kg and originating from sixteen different commercial pig farms located in Autonomous Province Vojvodina, Serbia. The fattening pigs' diet was based on the locally produced corn and soybean meals and was formulated to meet the animals' nutritional needs. The fattening of the pigs was divided in three phases: the starting, growing and finishing periods.

Mangulica
Six samples of liver of Mangulica were taken from two locations in Vojvodina. The Mangulica were reared in free range condition. The pigs were slaughter at the weighing 100-105 kg at the age of almost two years old.

Sampling preparation
Upon homogenization, all the samples were frozen at -20 °C until further analysis. Analytical methods lead and cadmium concentrations were determined after the wet sample digestion in concentrated nitric and hydrochloric acid (3:1, v/v) using a Reacti-ThermTM Heating/Stirring module Thermo Scientific (Rockford, USA). The analysis was performed using a Perkin-Elmer PinAAcle 900T THGA/FL atomic absorption spectrometer, a longitudinal Zeeman-effect background correction system, equipped with a transversely heated graphite atomizer (THGA) and an AS900 auto sampler. Hollow cathode lamps (HCL) were used for lead and cadmium detection. The analysis was carried out according to the method described by Beuković et al. (2015). The basic descriptive statistical data were done by processing the obtain data in the program Statistica 16.

Results and discussion

The results of the occurrence of lead (Pb) and cadmium (Cd) in the liver of the fattening pigs and Mangulica in mg/kg are showed in the Table 1. The average concentration of cadmium (Cd) in the liver of fattening pigs was 0.108 mg/kg, while in the liver in Mangulica was 0.209 mg/kg. Albeit the concentration of cadmium in the liver of Mangulica was higher than in the liver of the fattening pigs, it was in the allowed concentration of 0.5 mg/kg. However, the concentration of lead (Pb) in the liver in the both types of pigs was higher in more than several samples than it is allowed by current legislation in the Republic of Serbia. In ten (45.45%) out of 22 samples of the liver of the fattening pigs the found concentration of lead was higher than allowed. Moreover, in four out of six samples of the liver of Mangulica pigs the concentration of lead was also higher (66.67%) than allowed. The maximum found value for lead in the liver of fattening pigs was 1.890 mg/kg, while for the liver of Mangulica was 1.955mg/kg.

Tabela 1. Lead (Pb) and Cadmium (Cd) concentration (mg/kg) in the liver of fattening pigs and Manuglica from the region of Autonomous Province Vojvodina, Serbia

	Lead (Pb)		Cadmium (Cd)	
	Fattening pigs	Mangulica	Fattening pigs	Mangulica
average	0.703	1.142	0.106	0.209
N	22	6	22	6
SD	0.511	0.745	0.088	0.050
Min	0.212	0.379	0.060	0.137
Max	1.890	1.955	0.483	0.262
Permitted value	0.500	0.500	0.500	0.500
> 0.500	45.45	66.67	0	0

The concentration of cadmium in liver of fattening pigs in our study was in accordance with some other studies done in Serbia, where the average found concentration of cadmium in was 0.13 mg/kg (Tomović et al. 2011). In another study done in Serbia the concentration of cadmium was somewhat lower than in our and was in the range from 0.005-0.085 mg/kg for the period of five years (Nikolic et al. 2019). The cadmium concentration was higher in the liver of Mangulica than in the liver of fattening pigs. Most possible because of the almost four times longer life span of Mangulica and longer possible period of accumulation. The similar observation was found in another study where the concentration of cadmium was higher in Mangulica than in commercially produced pig. However, concentration was much higher with the average value of 1,612±0,229 mg/kg (Nikolić et al., 2018). The concentration of the lead found in the liver of fattening pigs and Mangulica in our study are rather surprising. Even the average concentrations of lead in fattening pigs and Mangulica 0.703 mg/kg and 1.142 mg/kg are above the legislation limit, with the range from 0.212-1.890 and 0.379-1.955 mg/kg in fattening pigs and Mangulica respectively. It is very hard to explain our results. In previous studies there were no any indications regarding the increased level of lead in edible tissue of pigs (Milićević et al. 2010; Nikolic et al. 2017b). Samples of liver of fattening pigs are from sixteen different farms and by that from the sixteen different locations in Vojvodina. Each farm has its own management and on each farm there were numerous possibilities for the source of contamination through food, water or soil. However, food is very different for the Mangulica and fattening pigs. Fattening pigs are fed with the formulated feed in which is incorporated premix. Premix and some feed additives can in some cases be the source of contamination. In the liver of fattening pigs 2008 in Australia the lead as high as 3.3 mg/kg for fresh weight was detected. Trace back investigation revealed the source. Zinc oxide feed additive which is used early in post weaning period, had contained in excess 8% of lead (Maclachlan et al., 2015). However, mangulice are fed only with the basics without the formulated food and premix in it. There are data which are indicating that the occurrences of lead in the environment in the region of Serbia are sometimes increased which resulted in increased level in the milk (Davidov et al. 2019) or the vegetables (Pajević et al. 2018). However, in the s observation done in period between 2007- 2017 in the two suburban areas of Belgrade heavy metals did not pose any risk in animal feed (Mitrovic et al. 2019). Lead in the environment is the effect of the androgenic influence. Our samples are from the sixteen different locations in Vojvodina and possible that the increased level of lead is the effect of environmental pollutant and local industry. Majority of farms previously state owned were built in the 1970s and 1980s and then privatized. Some of them are renovated and some of them are still with the old objects and infrastructure, so there are many possible sources of lead.

Conclusion

From the results of our work it seems that the occurrence of cadmium in the liver of the fattening pigs and Mangulica are in the allowed range and do not pose any health risks. However, the occurrence of lead in the liver of the both fattening pigs and Mangulica are increased and pose a possible health risk. There is a need for the further investigation of the origin of the lead exposure. The Mangulica and fattening pigs are reared completely different and in the different condition, with the different life span and yet in the liver of the both type the occurrence of lead was increased in some samples (45.45% for the fattening pigs, 66.67% for mangulice). It is possible that there is multiple source of lead.

Reference

Andrée, Sabine, W. Jira, K. H. Schwind, H. Wagner, and F. Schwägele. 2010. "Chemical Safety of Meat and Meat Products." *Meat Science* 86 (1): 38–48.

Bilandžić, N., Marija Sedak, Maja Crossed D Signokić, and Branimir Šimić. 2010. "Wild Boar Tissue Levels of Cadmium, Lead and Mercury in Seven Regions of Continental Croatia." *Bulletin of Environmental Contamination and Toxicology* 84 (6): 738–43.

Davidov, Ivana, Zorana Kovacevic, Dragica Stojanovic, Mira Pucarevic, Miodrag Radinovic, Natasa Stojic, and Mihajlo Erdeljan. 2019. "Contamination of Cow Milk by Heavy Metals in Serbia." *Acta Scientiae Veterinariae* 47 (1): 2–5. EFSA. 2010. "Scientific Opinion on Lead in Food." *EFSA Journal* 8 (4): 1–151.

Food, European, and Safety Authority. 2012. "Lead Dietary Exposure in the European Population." *EFSA Journal* 10 (7): 1–59. Maclachlan, Dj, F. Dixon, E. Klim, J. Lutze, M. Matisons, D. Moir, and G. Smith. 2015. "An Investigation into Exposure of Pigs to Lead from Contaminated Zinc Oxide in 2007-2008." *Australian Veterinary Journal* 93 (3): 72–78. Mandić-Rajčević, Stefan, Zorica Bulat, Vesna Matović, Martin Popević, Milan Lepić, Bojana Mandić, Mića Jovanović, Vincent Haufroid, Miloš Žarković, and Petar Bulat. 2018. "Environmental and Take-Home Lead Exposure in Children Living in the Vicinity of a Lead Battery Smelter in Serbia." *Environmental Research* 167 (July): 725–34.

Milićević, Dragan R., Milijan Jovanović, Verica B. Jurić, Zoran I. Petrović, and Dubravka Ž Vuković. 2010. "Assessment of Toxic Elements' Content in Swine Kidneys: Pathomorphological Analysis." *Archive of Oncology* 18 (1–2): 17–22. Mitić, Snežana S., Milan B. Stojković, Aleksandra N. Pavlović, Snežana B. Tošić, and Milan N. Mitić. 2012. "Heavy Metal Content in Different Types of Smoked Meat in Serbia." *Food Additives and Contaminants: Part B Surveillance* 5 (4): 241–45.

Mitrovic, Branislava, Borjana Vranjes, Olga Kostic, Veljko Perovic, Miroslava Mitrovic, and Pavle Pavlovic. 2019. "Presence of Radionuclides and Toxic Elements in Feedstuffs and Food of Animal Origin." *Veterinarski Glasnik* 73 (1): 30–39.

Nikolic, D., S. Jankovic, J. Djinovic-Stojanovic, T. Radicevic, S. Stefanovic, D. Spiric, and S. Simunovic. 2019. "Cadmium Level Trend in Liver and Kidney of Pigs from Serbia during 2014-2018." *IOP Conference Series: Earth and Environmental Science* 333 (1).

Nikolic, Dragica, Jasna Djinovic-Stojanovic, Sasa Jankovic, Nikola Stanisic, Cedomir Radovic, Lato Pezo, and Mila Lausevic. 2017. "Mineral Composition and Toxic Element Levels of Muscle, Liver and Kidney of Intensive (Swedish Landrace) and Extensive (Mangulica) Pigs from Serbia." *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* 34 (6): 962–71. Nikolić, M Dragica. 2018. "mineral composition of muscle, liver and kidney of intensively and extensively grown pigs in serbia." University of belgrade faculty, faculty of technology and metallurgy.

Pajević, Slobodanka, Danijela Arsenov, Nataša Nikolić, Milan Borišev, Dejan Orčić, Milan Župunski, and Neda Mimica-Dukić. 2018. "Heavy Metal Accumulation in Vegetable Species and Health Risk Assessment in Serbia." *Environmental Monitoring and Assessment* 190 (8).

RS Pravilnik. 2011. *Pravilnik o količinama pesticida, metala i metaloida i drugih otrovnih supstancija, hemioterapeutika, anabolika i drugih supstancija koje se mogu nalaziti u namirnicama*, no. Sl. list SRJ", br. 5/92, 11/92-ispr. i 32/2002 i "Sl. glasnik RS," br. 25/2010-dr. pravilnik i 28/2011-pravilnik: 198.

Tomović, V. M., L. J.S. Petrović, M. S. Tomović, Z. S. Kevrešanc, M. R. Jokanović, N. R. Džinić, and A. R. Despotović. 2011. "Cadmium Concentrations in the Liver of 10 Different Pig Genetic Lines from Vojvodina, Serbia." *Food Additives and Contaminants: Part B Surveillance* 4 (3).

THE INFLUENCE OF FORAGE AND CONCETRATE RATIO IN THE DIET ON ESSENTIAL FATTY ACID CONTENT IN COWS MILK FAT

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Abstract

The overview of domestic and foreign investigations was given in the paper about the influence of forage and concentrate ratio in the diet on production and chemical composition of milk, and on the polyunsaturated fatty acid (PUFA) content in cows milk fat. Special attention was given to conjugated linoleic acid (CLA) because of its proved antidiabetic and anticarcinogenic properties. Green forages like pasture has positive effect on PUFA content, but significantly decreases milk synthesis. Increase in milk yield can be achieved with the increase in quality forage and/or with the increase of concentrates in the diet. There are differences between various forages, so that grass and legume silages have more favorable effects on milk fat synthesis and fatty acid profile compared to maize silage. The increase of energy in the diet with the aim to increase milk yield can be achieved with the use of carbohydrate (starch) and feeds high in oil. High starch content in the diet provides lower production of volatile fatty acids (acetic and β-hydroxibutyric), which are used for *de novo* synthesis of fatty acids (<16:0) in the udder. The result of such feeding regime is decrease of PUFA and CLA content in milk fat. The use of some high oil feeds in the concentrates increased PUFA content significantly. Among the investigated species (soybeans, sunflower, linseed, cotton see, peanuts) the highest increase in CLA content (by 60%) was obtained with heat processed soybeans, compared to control ($p<0.01$).

Key words: *feeding, cows, milk, fatty acids.*

Introduction

Unlike other animals ruminants largely use structural carbohydrates to satisfy their energy needs (due to activity of cellulolytic mikroflora in the rumen), which decreases the cost of their production. However, the use of large quantities of forage feeds (fresh or conserved) is limiting the meat or milk production. That is the reason why it is very important to produce high quality forages (Đorđević et al., 2010, 2011) and to supplement the diet with concentrates (Grubić and Đorđević, 2005; Đorđević et al., 2014; Khan et al., 2012; Rego et al., 2016). Low quality of forages is decreasing their intake which is forcing the increase in concentrates in the diet. Byström et al. (2002) conclude that due to low quality of forages (late cut, low energy and protein, high cellulose content) the voluntary intake of forages was lower (1,6 kg SM/100 kg TM) than expected (2-2,5 kg SM/100 kg TM). Intensive dairy production demands maximal use of concentrates in the amount of up to 60% of dry matter in the diet. With the decrease in milk fat content, the fatty acid ratio is changed so that there is an increase i saturated fatty acid (SFA) content, which are considered responsible for cardiovascular problems. Contrary to that, the amount of polyunsaturated fatty acids (PUFA) is decreased which are considered as healthy for humans (Đorđević et al., 2003; 2010; 2016; 2020b). Some of the PUFA are precursors for biologically active eicosanoids, which are important in immunological and inflammatory response (McDonald et al., 2011). Especially important is conjugated linoleic acid (CLA) because it has anticarcinogenic effect, it is also helpful against diabetes and has anti-inflammatory effects (Collomb et al., 2008).

Ruminant milk and meat are the most important source of CLA in human nutrition since they provide about 70% of total CLA requirements and that is the reason why that area is investigated in recent times, with the aim to modify content and ratio of PUFA (Đorđević et al.,2020a,b). Many genetic and nongenetic factors contribute to variations in amount of produced milk fat and fatty acid (FA) content in milk. Amount the nongenetic factors the most important is nutrition, through used feeds and forage to concentrate ratio (Radonjic et al., 2019; Đorđević et al., 2021).

The influence of concentration and type of diet on milk fat content

Milk fat is traditionally highly priced product in animal production, because it is obtained from living animals fed with forages. The milk price was until recently formed based on fat content. In recent years the consumers changed their regard for milk fat considering the health problems that it may induce. However, those effect are result of the presence of some FA. Modern investigations showed that human nutrition with fats rich in lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) increase incidence of obesity and coronary conditions (Arnould and Soyeurt, 2009). In spite of that the goal of every milk producer is to obtain product with maximal fat content.

Milk fat is the most variable ingredient, depending on numerous factors, of which feeding is especially important. According to Bauman and Griinari (2001) there are two main theories to explain milk fat depression (MFD): 1. Lack of precursors for milk fat synthesis in the udder and 2. Problems in milk fat synthesis. One of the most mentioned reasons is insufficient amount of acetic acid (the main precursor for milk fat synthesis) that is supplied to udder. Acetic acid is the product of microbial degradation of fiber in the rumen. When high concentrate diets are used the amount of produced acetic acid in decreased and the amount of propionic acid is increased. Similar problem occurs if the forage feeds are too finely ground (silage, total mixed ration – TMR). Bauman and Griinari (2003) showed that in cows fed standard and high concentrate diets similar amount of acetic acid is produced but the amount of propionic is increased, which is changing their relative ratio. However, the absolute amount of acetic acid that is reaching the udder is little changed. Some researchers showed that there is no significant change in milk fat content with the increased amounts of concentrates. Machado et al. (2014) fed cows with various concentrate to forage ratio (35:65, 45:55 i 55:45% in dietary dry matter) and they found that with the increase of concentrates there is linear response in higher production of fat corrected milk (FCM) with the non-significant decrease in milk fat percent and lactose. Mendosa et al. (2016) fed cows with TMR in control group and in experimental groups I and II there were fed with cut green mass 4 or 8 hours per day. They found that with the increase of green mass intake there is significant decrease in milk yield (from 34.4 to 32.7 kg/day; $p<0.05$), FCM (from 37,6 to 35.1 kg/day; $p<0.05$)) and milk fat (from 1,40 to 1,29 kg/day; $p<0.10$), while decrease in milk fat percent (from 4.10 to 3.99 %) was not significant. Their conclusion was that with the increase in concentrates in the diet there was increase in milk and milk fat yield (absolute value) and the decrease in milk fat percentage (relative value).

If low quality forage is used the intake is decreased but the time for digestion is increased which as a result has lower supply of acetic acid to the udder and the MFD occurs. This is why it is very important to pay maximal attention to the quality of forage feeds. One of the ways to produce quality forages is to choose the optimal time for harvesting (Božičković et al., 2016), but also the appropriate manipulations when forages are conserved (Đorđević et al., 2012a,b). Aside from that, the use of high quality forages is enabling the lower use of concentrates, which is reducing the feeding cost and has good effects on health and reproduction.
The significant problem with the use of larger quantities of concentrates is the decrease in ruminal pH value, acidosis, which has negative effects on cellulolytic bacteria in the rumen and acetic acid synthesis (Mendoza et al., 2016). That is why the buffering compounds are added to high concentrate diets and special attention is paid to effective fibre. The correct amount of effective fibre is enabling optimal rumination time and acid neutralization in the rumen (Stojanović et al., 2012). By preserving the optimal pH value on the rumen the maximal activity of cellulolytic bacteria in the rumen is achieved, which as a result has appropriate supply of acetic acid to the udder.

The influence of concentration and composition of the diet on fatty acid content

Milk fat consists of triglycerides (about 98%), with about 400 different FA (Jensen, 2002). FA with C chain of 4-14 C atoms and part of C16 is created in de novo synthesis in the udder using acetic and β-hidroxybutyric acid (products of microbial digestion in the rumen). The rest of C16 and longer chain FA are taken from the blood after being transferred from the intestines (Kęsek et al., 2014), or came from body reserves (Parodi, 2004). The content of fatty acid that are produced from fatty reserves varies from about 5% in well fed animals to ore that 20% in early lactation when cows are in negative energy balance (Lake et al., 2005). Bacterial flora in the rumen is producing also odd numbered FA (C 15:0 and C 17:0) (German and Dillard, 2006). Conversion of FA C10:0-C18:0 into monounsaturated fatty acids (MUFA) is also happening in the udder. Animal cells cannot synthesize FA with several double bonds (linoleic acid with two and linolenic acid with three double bonds), which is why they are essential and must be supplied with food. Linoleic and linolenic acids from feeds and with incomplete biohydrogenisation in the rumen are main precursors for CLA. Bacterial biohydrogenisation of linoleic acid the CLA is formed but it is rapidly converted into trans-11 oleic acid (18:1), which is why concentration of cis-9 trans-11 CLA isomer is very low in the rumen and milk. CLA is also formed endogenously in tissues. Biohydrogenisation has large physiological significance since unsaturated FA ate toxic for ruminal microorganisms. Since the lipid degradation intensity in the rumen is larger than the speed of biohydrogenisation it is very important that ruminant diets do not contain larger amounts of fats (up to 7% of dry matter). The higher amount can have negative effects on cellulolytic bacteria and decreased production of acetates, which can depress milk fat. (Grubić et al., 2007). The use of high oil feeds in dairy cow nutrition can produce such problem but also have positive effects on PUFA and CLA content. Several experiments showed that heat processed soybeans have best effects on PUFA and CLA content compared to other high oil feeds. According to Liu et al. (2008), the use of whole soybean grain increased CLA content by 60% ($p<0.01$) compared to control group.
Among all genetic and nongenetic factors, the key role in fat modification is played by nutrition and specially with fresh green forage like pasture or cut green mass (Đorđević et al., 2017), through botanical composition and development phase (Radonjić et al., 2019). Dhiman et al.

(1999) showed that pasture fed cows had 5.7 times more CLA in their milk compared with those fed with conserved feeds (hay, silage). During drying (for hay) or wilting (for silage) there are significant losses in FA. On the other side, feeding mostly with forages do not provide enough dry matter intake for maximal milk yield. The optimal dry matter intake is achieved with the use of concentrates or TMR. However, the large amounts of concentrates influences biohydrogenisation of linoleic acid to trans-10, cis-12 CLA isomer which has strong inhibitory influence on milk fat synthesis (Grubić et al., 2007). It is shown that addition of trans-10, cis-12 CLA decreases milk fat content, which is not happening with cis-9, trans-11 CLA (Bauman, 2001). Since many countries have limited pasture areas the most likely solution is the use of TMR, combined with the green forages produced as crops. Mendosa et al. (2016) in mentioned example, while feeding cows with TMR (T_0) and cut green forage (*Lolium multiflorum*) fed *ad lib* for 4 (T_4) or 8 (T_8) hours determined significant increase of cis-9, trans-11 CLA and MUFA, and decrease in SFA. They also observed linear but not significant increase in PUFA content (table 1).

Table 1. Milk yield and fetty acid profile (Mendosa et al., 2016)

Parameters	T_0	T_4	T_8	P - value
Milk, kg/d	34.4^{a}	34.9^{a}	32.7^{b}	0.027
3,5% FCM, kg/d	37.6^{a}	37.3^{a}	35.1^{b}	0.012
Fat, kg/d	1.40^{x}	1.37^{xy}	1.29^{y}	0.068
Fat, %	4.10	3.95	3.99	0.604
Cis-9, trans-11 CLA	0.49^{a}	0.65^{ab}	0.77^{b}	0.062
SFA	72.16^{a}	70.90^{ab}	68.71^{b}	0.020
MUFA	24.39^{a}	25.36^{ab}	27.29^{b}	0.025
PUFA	3.46	3.74	3.90	0.133
n6:n3 ratio	8.03^{a}	6.77^{ab}	5.64^{b}	0.035

a,b - Within a row, means with different superscripts are different ($P < 0.05$).
x,y - Within a row, means with different superscripts are different ($P < 0.10$).

Conclusion (and suggestions)

Nutrition is one of the most important factors that influence production and quality of milk. In order to obtain maximal milk yield, with the highest percent of fats and PUFA it is necessary to: provide the best possible quality of forage feeds; use hay in all diets (minimal amount 2.5 kg per cow in long form); optimally balance diet using latest recommendations, normative and models; provide appropriate physical effectivity of diet; mix properly TMR; carefully use feeds with high oil content and provide optimal pH value in the rumen.

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References

Arnould, V.M.R., Soyeurt, H. (2009): Genetic variability of milk fatty acids. Journal of Applied Genetics, 50: 29-39.

Bauman, D. E., Peterson, D. G., Corl, B. A., Baumgard, L. H., Perfield II. J. W. (2001): Update on conjugated linoleic acids (CLA). Proc. Coprnell Nutr. Conf. Pp. 170-178. Ithaca, New York.

Bauman, D.E., Griinari, M. (2001): Nutritional regulation of milk fat syntthesys. Ann. Rev. Nutr., 23: 203-227.

Bauman, D.E., Griinari, M. (2003): Nutritional regulation of milk fat synthesis. Annual Review of Nutrition, 23:203-227.

Božičković, A., Simić, A., Grubić, G., Znidarsic, T., Djordjević, N., Stojanović, B. (2016): Testing of a modified methodology for determination of mean stage of development in alfalfa. Crop Science, 56: 891-898.

Byström, S., Jonsson, S., Martinsson, K. (2002): Organic versus conventional dairy farming – studies from the Öjebyn Project. UK organic Research 2002: Proceedings of the COR Conference, 26-28th March 2002, Aberyswyth, pp. 179-184.

Collomb, M., Bisig, W., Butikofer, U., Sieber, R., Bregy, M., Etter, L. (2008): Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming system. International Dairy Journal, 18:976-982.

Dhiman, T. R., Anand, G. R., Satter, L. D., Pariza, M. W. (1999): Conjugated linoleic acid content of milk from cows fed different diets. Journal of Dairy Scinece, 82: 2146-2156.

Đorđević, N., Grubić, G., Stojanović, B. (2007): The influence of feedstuff type and diet composition on amount and quality of milk produced. Contemporary agriculture, 56, 5: 12-17.

Đorđević, N., Grubić, G., Dinić, B., Lević, J., Stojanović, B., Božičković, A. (2010): Animal feed quality – past and present. XII international Symposium on Forage Crops of Republika of Serbia - Forage Crops Basis of the Sustainable Animal Husbandry Development. Biotechnology in animal husbandri, 26, book 1, 249-260.

Đorđević, N., Grubić, G., Dinić, B., Stojanović, B., Božičković, A. (2011): Forage quality as a part of a modern concept of ruminant nutrition. International Scientific Symposium of Agriculture „Agrosym Jahorina 2011“, Jahorina, 10-12. November. Proceedings, 218-225.

Đorđević, N., Grubić, G., Stojanović, B., Dinić, B., Božičković, A. (2012a): Contemporary aspects of lucerne use in animal nutrition. 6th Central European Congress on Food, CEFood2012, 23-26.06.2012, Novi Sad, Serbia. Proceedings, 1514-1519.

Đorđević, N., Dubljević, R., Damjanović, M., Mitrović, D., Milenković, N. (2012b): The contemporary methods in the production of maize silage. The First International Symposium on Animal Science, Faculty of Agriculture, University of Belgrade, Serbia, 08-10. November, 2012. Proceedings, 480-487.

Đorđević, N., Stojanović, B., Grubić, G., Božičković, A. (2014): Forages in organic animal production systems. Proceedings of Research Papers PKB INI Agroekonomik, 1-4: 175-186.

Đorđević, N., Grubić, G., Stojanović, B., Božičković, A., Radonjić, D. (2017): The influence of feeding on fatty acid content in milk fat. Proceedings of Research Papers PKB INI Agroekonomik, 23, 3-4: 21-30.

Đorđević N., Radonjić D., Grubić G., Stojanović B., Božičković A. (2020a). Influence of pasture on fatty accid content in cow's milk. XXV Symposium on biotechnology with international participation, 13-14. marth 2020. Proceedings 1: 257-264.

Đorđević N., Grubić G., Stojanović B., Božičković A., Stojković B., Radonjić D. (2020b). The influence of silage diets on the fatty acid content in milk fat. XI International Agricultural Symposium «AGROSYM 2020 ». Virtual conference, 8-9 Octobar 2020, Bosnia and Herzegovina. Book of proceedings, 807-812.

Đorđević N., Radonjić D., Grubić G., Stojanović B., Božičković A., Stojković B. (2021): Effect of dietary fat on the content of essential fatty acids in milk fat at ruminants, XXVI Symposium on biotechnology with international participation, 12-13. marth 2021. Proceedings 1: 219-226.

Grubić, G., Đorđević, N. (2005): Concentrates in dairy cows nutrition. XI International feed technology simposium "Quality Assurance. Vrnjačka Banja, May 30th – June 3rd 2005. Proceedings, 233-241.

Grubić, G., Đorđević, N., Stojanović, B. (2007): The influence of the diet on milk fat depression. Proceedings of Research Papers PKB INI Agroekonomik, 3-4: 21-32.

German, J.B., Dillard, C.J. (2006). Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Critical Reviews in Food Science and Nutrition, 46:57-92.

Jensen R.G. (2002). The Composition of bovine milk lipids: January 1995 to December 2000. Journal of Dairy Science, 85: 295-350.

Kęsek M., Szulc T., Zielak-Steciwko A. (2014). Genetic, physiological and nutritive factors affecting the fatty acid profile in cows' milk – a review. Animal Science Papers and Reports, 32:95-105.

Lake, S.L., Weston, T.R., Scholljegerdes, E.J., Murrieta, C.M., Alexander, B.M., Rule, D.C., Moss, G.E., Hess, B.W. (2007): Effects of postpartum dietary fat and body condition score at parturition on plasma, adipose tissue, and milk fatty acid composition of lactating beef cows. Journal of Animal Science, 85: 717-730.

Liu, Z.I., Yang, D.P., Chen, P., Lin, S.B., Jiang, X.Y., Zhao, W.S., Li, J. M., Dong, W.X. (2008). Effect of dietary sources of roasted oilseeds on blood parameters and milk fatty acid composition. Czech Journal of Animal Science, 53: 219-226.

Mashado, S.C., McManus, C.M., Stumpf, M.T., Fisher, V. (2014): Concentrate:forage ratio in the diet of dairy cows does not alter milk physical attributes. Tropical Animal Helath and Production, 46:855-859.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A., Wilkinson R.G. (2011). Animal nutrition, seventh edition. Prentice Hall.

Mendoza, A., Cajarville, C., Repetto, J. L. (2016). Short communication: Intake, milk production, and milk fatty acid profile of dairy cows fed diets combining fresh forage with a total mixed ration. Journal of Dairy Science 99:1938–1944.

Parodi P.W. (2004). Milk fat in human nutrition. The Australian Journal of Dairy Technology, 59, 1: 3-59.

Radonjic, D., Djordjevic, N., Markovic, B., Markovic, M., Stesevic, D., Dajic-Stevanovic, Z. (2019): The effect of phenological phase of dry grazing pasture on fetty acid composition of the cows milk. Chilean Journal of Agricultural Research, 2: 278-287.

Rego, O.A., Cabrita, A.R.J., Rosa, H.J.D., Alves, S.P., Duarte, V., Fonseca, A.J.M., Vouzela, C.F.M., Pires, F.R., Bessa, R.J.B. (2016): Changes in milk production and milk fatty acid composition of cows switched from pasture to a total mixed ration diet and back to pasture. Italian Journal of Animal Science, 15:1, 76-86.

Shingfield, K.J., Reynolds, C.K., Lupoli, B., Toivonen, V., Yurawecz, M.P., Delmonte, P., Griinari, J.M., Grandison, A.S., Beever, D.E. (2006): Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. Animal Science, 80: 225-238.

Stojanovic, B., Grubic, G., Djordjevic, N., Glamocic, D., Bozickovic, A., Ivetic, A. (2012): Effects of different levels of physically effective fibers in diets for cows in early lactation. Spanish Journal of Agricultural Research, 10, 1: 99-107.

EFFECT OF SELENIUM FROM FOOD ON PHEASANT MEAT QUALITY

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Abstract

In this paper we examined the effects of usage of organic selenium in the nutrition of pheasants on breast meat quality in the experiment that lasted for two months. Forty five pheasants were divided into three groups of 15 pheasants, and were fed with mixtures of organic selenium supplementation in concentrations of 0.2 mg/kg (I group) and 0.3 mg/kg (II group), while the food of the control group was without selenium supplementation. The obtained results showed that the introduction of organic selenium into pheasant mixtures did not have statistically significant effect ($p>0.05$) on the average breast meat weight and chemical composition of meat: water, fat, protein, pH and ash content. Organic selenium in a concentration of 0.3 mg/kg of the mixture had a positive effect on increasing the water holding capacity of the breast meat and on sensory traits compared to meat of the control group and the I group which was fed with 0.2 mg/kg of organic selenium in the mixture. The ability of water retention in breast meat of the II group of pheasants was higher than in control group by 0.75% i.e. 0.58% in comparison to I group. Sensory traits of breast meat of the II group of pheasants were for 15 points better than sensory traits of meat of the control group, that is for 7 points better than the I group of pheasants. Statistically significant differences were determined among the average values of selenium concentration in white breast meat of II group pheasants ($p<0.05$) and the control group of the pheasants which are fed without selenium supplementation.

Keywords: *organic selenium, meat quality, pheasant.*

Introduction

The common pheasant is one of the most widespread birds on the planet earth. Thanks to artificial breeding in pheasantries the number of this feathered game, which is a favorite prey for hunting, as well as an alternative to poultry farming in the production of quality meat, has increased. During the last few years, pheasant farms have been developed around the world as a new branch of agriculture for commercial meat production.

Organically bound selenium in the form of selenomethionine manifests a strong antioxidant effect in poultry organism, directly affecting the increase in the concentration of the enzymes of glutathione peroxidase (GPx) in the liver and the reduction in the concentration of lipid peroxidase and hydrogen peroxide produced during normal metabolic activity, thus gaining meat of exceptional quality high in selenium content. Selenium deficiency and loss of GPx enzyme activity cause cell membrane damage, free radical accumulation, and cell decay. The antioxidant effect of selenium protects poultry from atherosclerosis, degenerative processes of the pancreas and kidneys, reproductive disorders, tumorigenesis and at the same time selenium also has an immunostimulatory effect (Dukes, 1993; Kang *et al*., 2000; Dlouha *et al*., 2008).Oral intake of selenium by food results in increased content of its concentration in tissues and eggs (Edens, 1997; Surai *et al*., 2002; Edens *et al*., 2004). Selenium deficiency in the organism of poultry causes numerous pathological changes and diseases such as: atrophy of the pancreas, kidney damage, exudative diathesis, reduced fertilization and immunity. Selenium is also an activator of thyroid hormones, which among other things, participate in the thermogenesis of poultry, so for that reason, the concentration of selenium in the organism of birds under heat stress can be significantly reduced. (Silva *et al*., 2002).

The poultry and birds' production depends on numerous stress factors (overpopulation, heat stress, diseases), which is why selenium, as an integral part of many selenoproteins, actively participates in their prevention by activating antioxidant mechanisms. The accumulation of selenium in the organism of poultry is the highest in the kidneys, followed by the liver and pancreas, while the lowest concentrations are in adipose tissue and feathers. In the case of surplus, selenium is predominantly deposited in the liver and muscles (Surai, 2000; Surai *et al*., 2002).

Meat quality is the sum of the traits and characteristics of a product based on its ability to satisfy the consumer. Meat quality primarily depends on its nutritive value i.e. chemical composition and sensory traits. Poultry and bird meat are of great importance due to high content of biologically valuable proteins, fats, vitamins, minerals and essential fatty acids (Franco *et al*., 2013). The breast meat of poultry and birds is high in protein, low on fat and cholesterol compared to meat of other domestic animals which makes it a dietary product (Barroeta, 2006).

There is a significant number of papers in the literature engaged in the nutritional effect of selenium on poultry, but a very small number of available researches explain the effect of organic selenium on the quality of pheasant breast meat. The aim of this paper was to determine the effect of adding organic selenium in pheasant feed on the quality of breast meat, its chemical composition, sensory traits meat acceptability and selenium content.

Material and Methods

The experiment was conducted with 45 common pheasants, both male and female, of average body weight of 385 ± 75 g and age of 42 days. They were divided into three groups with 15 pheasants in each group. The experiment lasted for 60 days and during that period accomodation and nutrition of pheasants were adjusted to the floor way of raising.

We used a pelleted mixture for pheasant nutrition and it was formulated according to the nutritional needs of pheasants (NRC, 1994). The organic selenium contained in *Alkosel®* preparation (Lallemand, Fra) was added to the premixes, and after appropriate mixing, the premix with selenium was applied to the complete mixtures and mixed again with the complete mixture. The control group was fed with food without selenium supplementation, while the food of experimental I group contained organic selenium in a concentration of 0.2 mg/kg, and the food of experimental II group contained organic selenium in a concentration of 0.3 mg/kg. The raw material and chemical composition of pheasant food is shown in Table 1.

Throughout the entire duration of the experiment, the consumption of food and water was at will. The average sample of complete mixtures for pheasant nutrition was analyzed for the basic chemical composition at the beginning of the experiment (content of air dry matter, ash, crude proteins, fats and cellulose), by applying standard analytical methods of chemical food examination (AOAC, 1990). Calcium and phosphorus content was determined by the colorimetric method on the Tecator system (SRPS ISO 6490-1/2001; SRPS ISO 6491/2002),

while the content of metabolic energy and amino acids was obtained by calculation based on their content in nutrients (INRA-AFZ, 2004).
Control measurements of pheasant body weight were performed on an electronic scale with an accuracy of ± 0.5 grams, both at the beginning and the end of the experiment, based on which the average body weight was calculated. Throughout the experiment, the health condition of pheasants and their mortality were monitored. By the method of random sampling at the end of 60 days experiment, seven pheasants were taken from each group, they were individually measured before slaughter, and after primary slaughter processing and water cooling, chilled carcasses were cut into basic pieces in a manner regulated by Ordinance on the quality of poultry meat (Official Gazette SFRY No. 1/81 and 51/88). During the cutting the brest muscle was separated and measured on an automatic scale with an accuracy of ± 0.5 grams.

Table 1. Ingredients and nutrient content of complete feed mixture for pheasants

Component (%)	Group Control (K), I and II
Maize	40
Wheat bran	3
Soybean meal	24
Sunflower meal 33%	4.3
Alfalfa meal	3
Yeast	3.5
Soybean grits	12
Sunflower meal 42%	5
Lysine	0.1
Methionine	0.2
Limestone	1.6
Mono-Ca-phosphate	1.5
Salt	0.3
Additive Peletin	0.5
Premix	1

The chemical feed composition of all three groups (%): Proteins 24.25; Cellulose 6.05; Fats 4.63 Ash 7.35; Dry matter 88.20; Calcium 1.02; Total phosphorus 0.82; ME 12.75 MJ/kg; Lysine 1.35; Methionine + Cystine 0.90. *Composition of premix in 1kg of complete mixture:*Vitamin A (IU/kg) 15000;Vitamin D_3 (IU/kg) 3000; Vitamin E (mg/kg) 32; Biotin (mg/kg) 0.20; Vitamin C (mg/kg) 15; Folicacid (mg/kg) 1.20; Niacin (mg/kg) 30; Pantothenicacid (mg/kg) 15; Vitamin B_6 (mg/kg) 3.20; Vitamin B_2 (mg/kg) 7; Vitamin B_1 (mg/kg) 2.10; Vitamin B_{12} (mg/kg) 0.03; Cholinechloride (mg/kg) 500; Fe (mg/kg) 40; Mn (mg/kg) 80; Cu (mg/kg) 8; Zn (mg/kg) 60; J (mg/kg) 0.80; Co (mg/kg) 0.45; Antioxidant BHT (mg/kg) 100.

The chemical composition of breast meat was analyzed by using standard methods. The total water content was determined by drying the samples to constant weight (SRPS ISO 1442/1998), the total ash by burning and annealing the sample at a temperature of 500°C to 600°C (SRPS ISO 936/1999), pH of the meat with a pH meter (SRPS ISO 2917 : 2004), total proteins by the Kjedahl method based on nitrogen content (SRPS ISO 937/1992), total fat by Soxlett extraction with pre-drying of the sample (SRPS ISO 1443/1992). The content of selenium in white breast

meat of pheasants was determined by atomic absorption spectrophotometry with the hydride technique (SRPS EN 16159 : 2012).
The ability to retain water in the breast meat was specified by measuring the content of total moisture in it, after 24 and 48 hours from the time of cooling the meat to 4°C. The quality of sensory traits of meat was examined by the method of determining the difference in meat acceptability – by Rank test, assessed by 7 evaluators (Baltić, 1993; Baltić *et al*., 1997; SRPS EN ISO 8587/2006) The evaluators assessed the characteristics of the meat that can be registered by the senses (smell, taste, juiciness, softness and appearance), whereby the obtained differences in the acceptability of the meat represent differences in the overall impression of all evaluators. Before starting the examination, all the samples were thermally processed on the grill for about 15 minutes and after that the evaluators rated the samples with the grades the most acceptable, less acceptable and the least acceptable. Within the statistical analysis of the obtained results, the analysis of variance was performed by a standard procedure with testing of the statistical significance of the differences between certain groups and examined parameters by applying LSD test.

Results and Discussion

The chemical composition of complete feed mixtures for pheasants' nutrition completely met the nutritional needs of pheasants in fattening and was in accordance with the recommended norms of the NRC (1994). There were no health issues or pheasant deaths throughout the duration of this experiment.

Table 2. Chemical composition of the breast muscle and selenium content in meat

Chemical composition	Control group	I group	II group
Mass (g)	221.50±28	224.60 ± 18	229.42±32
pH meat	6.10±16	6.02±32	6.19±42
Moisture (%)	72.63±23	72.38 ± 52	72.49±55
Fat (%)	1.08±56	1.10 ± 23	1.11±36
Total protein (%)	25.14±14	25.15 ± 11	25.11±87
Ash (%)	1.18±41	1.20 ± 09	1.20±23
Selenium (mg/kg)	0.121±13	0.129±03	0.135±33*

*p<0.05 (Control group and II group pheasants)

By the reviewing of the obtained results of the chemical composition of breast meat (Table 2), it can be concluded that the presence of organic selenium in pheasant feeding mixtures caused relative raising of higher values of breast muscle mass, electrochemical reaction of meat, water and fat content but without determined statistically significant differences between treatments (p>0.05). In the analysis of the obtained results of the breast weight we noticed that its weight was the lowest in pheasants of the control group with 221.50 grams, and the highest was in pheasants of II group with 229.32 grams. The relative uniformity of the chemical composition of breast meat in terms of the content of the analyzed parameters between the examined groups of pheasants is in accordance with the research of Cvrtila *et al*., (2007). In our experiments, no statistically significant differences were determined between the treatments (p>0.05) of examined basic chemical parameters of meat. Tucak *et al*., (2008) state that the breast weight of

adult pheasants raised in an aviary ranged from 248 to 295 grams. In comparison to our results, the authors determined higher values of proteins, fat and ash in pheasant breast meat. In our experiment, the protein and fat concentration in pheasant meat were uniform, whereas the protein content was higher than the fat content. The explanation for this phenomenon is the age of the pheasants, because in our research we dealt with young, not adult pheasants.
Results of selenium content in breast meat samples shown in Table 2, indicate that the average selenium content was the lowest in the control group 0.121 mg/kg, slightly higher was in the I group 0.129 mg/kg and the highest was in the II group of pheasants 0.135 mg/kg. Statistically data processing on selenium content in breast meat we determined a statistically significant difference ($p<0.05$) among control group that did not receive selenium in food and the II group of pheasants that received organic selenium with food in concentracion of 0.3 mg/kg. Several researchers have confirmed, in their experiments, that the positive effect of increasing the concentration of selenium in the musculature of broilers whose food was treated with organic selenium compared to the control group without supplement or with inorganic selenium supplementation in food (Edens *et al*., 2004; Payne *et al*., 2005; Dlouha *et al*., 2008).

Table 3. Water loss in breast meat of pheasant

Water loss in meat	Control group		I group		II group	
	g	%	g	%	g	%
Water loss after 24 h	1.30	1.79	1.10	1.52	0.85	1.17
Water loss after 48 h	1.95	2.68	1.70	2.10	1.40	1.93
Difference, %	-	-	-	0.58	-	0.75

The results of white breast meat moister loss are shown in Table 3. The best relative values of water retention in white breast meat was achieved by II group both after 24h (1.17%) and after 48h (1.93%) from the time of meat cooling, so in comparison to control group it had better efficiency by 0.75%. Similar results were achieved by I group, which in comparison to control group had better water retention in meat by 0.58%. The worst results were recorded in the control group which had relative value of water retention of 1.79% after 24h, and 2.68% after 48h. Our research confirmed the positive antioxidant effect of selenium on the musculature of the breast meat because I group and II group of pheasants achieved less water loss during storage. Meat quality can successfully be maintained during storage by adding selenium antioxidants to the feeding of domestic animals, because selenium is deposited in musculature cells, which improves the ability of the cells to retain water (Surai, 2000). According to Edens (2001), selenium in the muscle tissue reduces fat peroxidation during meat storage and thus affects the preservation of meat quality. Organic selenium in form of selenomethionine has a positive effect on meat quality because it increases the activity of glutathione peroxidase (GSH-Pk) and maintains a low level of lipid peroxidase which results in cell membrane stability and the ability to bind water (Edens *et al*., 2000; Edens *et al*., 2004).

Table 4. The assessment of the overall acceptability of broiler breast meat samples

Groups	Groups		
	Control group	I group	II group
The sum of ranks	47	39	32
Control group	-	8	15
I group	-	-	7
II group	-	-	-

A very important indicator of meat quality, besides chemical composition, is its sensory traits which were examined by the Rank test method in this experiment, or meat acceptability by consumers, whereby lower sum of ranks represent better meat acceptability. In Table 4 we displayed the differences in assessment of the breast meat acceptability of the examined broilers. By ranking of the breast meat samples, the samples of II group (32 points) were evaluated as the most acceptable, which at the same time had the highest difference in the sum of ranks of 15 points, then I group of pheasants (39 points) with the difference in the sum of ranks of 7 points in comparison to the control group, and as the least acceptable were the breast meat samples of the control group (47 points). Based on the Rang test results obtained in these researches, we can conclude that the appliance of organic selenium in food had a positive effect on acceptability of meat of examined pheasants. According to many authors, the highest effect on food acceptability has compounds that are carriers of smell and taste. (Baltić, 1993: Ivanović *et al*., 2012). The appliance of organic selenium in food has a positive effect on the appearance, juiciness, smell and colour of meat (Surai *et al*., 2002), while the increased juiciness is the result of better water retention in meat (Džinić *et al*., 2006).

Conclusions

Based on the results obtained in the conducted experiments, it can be concluded that the addition of organically bound selenium to pheasant food in a concentration of 0.3 mg/kg had a stimulating effect on selenium content in breast meat (p>0.05). Organic selenium added in the food of II group in a concentration of 0.3 mg/kg had a positive effect on sensory traits of breast meat by 0.75% and the ability to retain water in breast meat by 15 points compared to control group which was fed without selenium supplementation. The general conclusion is that the appliance of selenium in increased concentrations has its significance in production of pheasant meat with high content of selenium for human consumption.

References

AOAC, Association of Official Analytical Chemists (1990): Official Methods of Analysis, Washington, DC: 15th ed, by Kenneth Helrich. AOAC, Arlington, Virginia, USA: 1-246.

Baltić, M., Teodorović, B.V. (1997): Higijena mesa riba, rakova i školjki.Veterinarski fakultet Beograd: 1-250. (Baltić, M., Teodorović, B.V. 1997: Hygiene of fish, crab and shellfish, Faculty of Veterinary Medicine, Belgrade: 1-250).

Baltić, Ž.M. (1993): Kontrola namirnica. Institut za higijenu i tehnologiju mesa, Beograd. (Baltić, Ž.M**.** 1993: Food control, Institute of Meat Hygiene and Technology, Belgrade).

Barroeta A.C. (2006): Nutritive value of poultry meat: Relationship between vitamin E and PUFA. Worlds Poult. Sci. J. 2006; 63: 277-284.

Cvrtila, Ž., Hadžiosmanović, M., Kozačinski, L., Zdolec, N., Filipović, I., Severin, K., Mašek, T. (2007): Kemijski sastav mesa fazanskih kopuna. Meso, Zagreb. Vol. IX, br 3: 148-151.

Dlouha, G., Ševčikova, S., Dokoupilova, A., Zita, L., Heindl, J., Skrivan, M. (2008): Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers. Czech J. Anim. Sci., 53, 2008 (6): 265-269.

Dukes H.H. (1993): Physiology of Domestic Animals. 11th Edition. Editor: Swenson J. Melvin, Reece O. William, Ithaca, USA: 1-962.

Džinić, N., Tomović, V., Petrović, Lj., Perić L. (2006): Uticaj dodataka selena različitog porekla u hranu za piliće na kvalitet m. pectoralis.Tehnologija mesa. 47, 5-6: 199-203.

Edens, F.W. (1997): Potentional for organic selenium to replace, selenite, in poultry diets. Zootecnica International 20: 28-31. https://zootecnicainternational.com/online-magazine.

Edens, F.W., T.A. Carter, C.R. Parkhurst and A.E. Sefton. (2000): Effect of selenium source and litter type on broiler feathering. Journal of Applied Poultry. 9: 407-413.

Edens F.W. (2001): Involvement of Sel-Plex in physiological stability andperformance of broiler chickens. In:Biotechnology in the Feed Industry. Proceedings of Altech's 12th Annual Symposium (LyonT.P.and Jacques K.A., Eds.), 2001, 419 pages. Nottingham University Press, Nottingham, UK: 349-376.

Edens F.W. and Kymberly M. G. (2004): Selenium sources and selenoproteins in practical poultry production. Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's Twentieth Annual Symposium. Edited by TP Lyons and KA Jacques, Typeset by Nottingham University Press, Nottingham, England.: 35-56.

Franco, D., and Lorenzo, J. M. (2013): Meat quality and nutritional composition of pheasants (Phasianus colchicus) reared in an extensive system. British Poultry Science, 54: 594–602.

INRA-AFZ (2004): http://www.feedtables.com

Ivanović, S., Teodorović, V., Baltić, Ž. M. (2012): Kvalitet mesa (biološke i hemijske opasnosti). Naučni institut za veterinarstvo Srbije, Beograd: 356. (Ivanović, S., Teodorović, V., Baltić, Ž. M. 2012: Meat quality (biological and chemical hazards), Scientific Veterinary Institute of Serbia, Belgrade: 356).

Kang, B.P.S., Mehta. U., Bansal, M.P. (2000): Hyperlipidemia and Type-1-5'-monodeiodinase activity: Regulation by selenium supplementation in rabbits. Biological Trace Element Research. volume 77: 231-239.

National Research Council NRC. (1994): Nutrient Requirements of Poultry. 9th edition. The National Academies Press, Washington, DC: 1-176.

Payne, R.L., and Southern L.L. (2005): Comparison of inorganic and organic selenium sources for broilers. Poultry Science, 84: 898-902.

Pravilnik o kvalitetu mesa pernate živine. 1981: Službeni list SFRJ, broj 1. (Regulation on the quality of poultry meat, 1981: Official Gazette of SFRY, number 1).

Pravilnik o kvalitetu mesa pernate živine. 1988: Službeni list SFRJ, broj 51. (Regulation on the quality of poultry meat, 1988: Official Gazette of SFRY, number 51).

Silva, C.M.G., Gloria, M.B.A. (2002): Bioactive amines in chicken breast and thigh after slaughter and during storage at +4^{o}C and in chicken-based meat product. Food Chemistry. Volume 78, Issue 2: 241-248.

SRPS EN 16159:2012. Hrana za životinje. Određivanje selena atomskom apsorpcionom spektrometrijom sa hidridnom tehnikom (HGAAS) posle mikrotalasne digestije (digestija sa 65 % azotnom kiselinom i 30 % vodonik peroksidom). (SRPS EN 16159:2012.Feedstuff. Determination of selenium by hydride atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide).

SRPS EN ISO 8587/2006 Senzorna analiza - Metodologija – Rangiranje. (SRPS EN ISO 8587/2006. Sensory analysis – Methodology – Ranking).

SRPS ISO 1442/1998. Meso i proizvodi od mesa. Određivanje sadržaja vlage. (SRPS ISO 1442/1998. Meat and meat products. Determination of moisture content).

SRPS ISO 1443/1992. Meso i proizvodi od mesa. Određivanje sadržaja ukupne masti. (SRPS ISO 1443/1992. Meat and meat products, Determination of total fat content).

SRPS ISO 2917:2004. Meso i proizvodi od mesa. Merenje pH vrednosti (referentna metoda). (SRPS ISO 2917:2004. Meat and meat products. Measuring of pH values (reference method).

SRPS ISO 6490-1:2001. Hrana za životinje. Određivanje sadržaja kalcijuma, volumetrijska metoda. (SRPS ISO 6490-1:2001. Feedstuff. Determination of calcium content, volumetric method).

SRPS ISO 6491:2002. Hrana za životinje. Određivanje sadržaja fosfora, spektrometrijska metoda. (SRPS ISO 6491:2002. Feedstuff. Determination of phosphorus content,spectrometric method).

SRPS ISO 936/1999. Meso i proizvodi od mesa. Određivanje ukupnog pepela. (SRPS ISO 936/1999. Meat and meat products. Determination of total ash).

SRPS ISO 937/1992. Meso i proizvodi od mesa. Određivanje sadržaja azota (referentna metoda). (SRPS ISO 937/1992. Meat and meat products. Determination of nitrogen content (reference method).

Surai, P.F. (2000): Organic selenium: benefits to animals and humans, a biochemist's view. In: Biotechnology in the Feed Industry: Proceedings of Alltech's 16th Annual Symposium (T. P. Lyons and K. A. Jacques, eds), Nottingham University Press, Nottingham, UK: 205-260.

Surai, P. F. and Dvorska, J. E. (2002): Effect of selenium and vitamin E content of the diet on lipid peroxidation in breast muscle tissue of broiler breeder hens during storage. Proceedings of Australian Poultry Science Symposium. Editors: R.A.Pym, Publisher: World Poultry Science Association, Volume 14: 187-192.

Tucak, Z., Skrivanko, M., Posavcevic, S., Periskić, M., Bošković, I., Jumić V. (2008): Influence of keeping pheasants in captivity vs. nature on the biological value of meat and its use in human nutrition. Coll. Antropol. 32 (2008) 3: 959–962.

QUALITY AND SENSORY PROPERTIES OF WHITE-BRINED GOAT CHEESE

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Abstract

The aim of this study was to examine the influence of different starter cultures on the composition and sensory characteristics of white-brined goat cheeses. Four varieties of cheese (A, B, C, D) were produced according to the same technological procedure. Quality parameters, with special emphasis on the dynamics of ripening, were determined after production, on the 10th, 20th and 30th day of ripening. Since the cheeses were produced from full-fat goat's milk, after the ripening period, they belonged to the group of full-fat cheeses (with a fat content in the dry matter higher than 45%). As a result of different starter cultures, in variants A and B intensive proteolysis occurred after 20 days of ripening, while in variant C increase of Ripening Index was observed after 10 days. The most intense proteolytic changes were found in variant A at the end of ripening period, where the soluble nitrogen (SN) content in 12% TCA was 154.9 mg%, while the lowest intensity of proteolytic changes was determined in variant B with the average SN content in 12% TCA of 116.3 mg%. The best sensory evaluations were given to cheese variants B and C, which were evaluated with excellent quality (4.92 and 4.56), while cheese variants A and D had very good quality (4.47 and 4.36).

Key words: *goat milk, cheese in brine, sensory quality.*

Introduction

Production and processing of goat's milk is characteristic for the Mediterranean and the Balkan Peninsula, and highly developed and organized goat breeding is specific for France. The processing of goat's milk into cheese has a very long tradition, especially in some countries such as France, where there are over 90 different types of goat's cheese (Alichanidis and Abd El-Salam, 2004; Alichanidis and Polychroniadou, 2008). In the last two decades, the upward trend in production and processing of goat milk into various milk products, especially white cheeses in brine, was observed in Serbia. A shorter period of lactation is the reason why goat milk products have seasonal character. Therefore, freezing of milk or curd could be the way to overcome this problem (Kljajevic et al., 2017). Technological characteristics of goat milk are significantly different compared to cow's milk, resulting in a softer curd and a smaller cheese yield. Also, the sensory characteristics are different, given the specific composition of milk fat in goat's milk due to the presence of capric, caprylic and caproic fatty acids (Dozet et al., 2000, 2004, 2005). The aim of this work was to investigate the influence of different starter cultures on the composition and sensory characteristics of white-brined goat cheeses.

Material and method

All studies were conducted in the Milk Technology laboratory at Faculty of Agriculture, Belgrade, during the months of June and July. Goat's milk from the farm "Beocarpa" Kukujevci, Serbia, was used for the production of white cheese in brine. Depending on the various strains of microorganisms and their share in the starter culture 4 variants of white-brined goat cheese were produced:

- A: *Lb. plantarum* BGVLS2-18 - 33%, *Lc. lactis* subsp. *lactis* BGVL3-8 - 33%, *Leu. pseudomesenteroides* BGVL3-29 - 17%, *Leu. pseudomesenteroides* BGVL3-63 - 17%;
- B: *Lb. plantarum* BGVLS2-18 - 50%, *Lc. lactis* subsp. *lactis* BGVL3-8 - 50%;
- C: *Lb. plantarum* BGVLS2-18 - 33%, *Lc. lactis subsp. lactis* BGVL3-8 - 33%, *Leu. pseudomesenteroides* BGVL3-29 - 34%;
- D: *Lb. plantarum* BGVLS2-18 - 33%, *Lc. lactis subsp. lactis* BGVL3-8 - 33%, *Leu. pseudomesenteroides* BGVL3-63 - 34%.

The average content of fat and protein of the raw goat milk used was 2.50±0.71 and 2.62±0.36, respectively and the pH was 6.24±0.47. Raw goat milk was pasteurized (65°C/30 min), cooled to 33°C, and starter cultures and $CaCl_2$ were added. After 30 min, rennet (Caglificio, Clarici Italy) was added in concentration of 0.0325 g/L. Coagulation took place within 40 min at 32-33°C. The curd was cut crossways in cubes of 1 cm^3 and left for 5 min. Then, it was gently agitated for another 5 min to facilitate whey expulsion and carefully transferred into the mold. After that, the curd was left for 30 min to self-press, and then pressed for 20 min at 2 kg/kg, followed by pressing at 4 kg/kg during 40-60 min. After that, the cheese curd was cut into pieces of 10×10×4 cm, dry salted with 3.0% NaCl and left overnight. The next day, cheese slices were placed into plastic containers and covered with 6% NaCl brine. Ripening was conducted at 14°C during 30 days. The cheeses were sampled at 1, 10, 20 and 30 days of ripening.

The chemical composition of cheese samples was determined using standard methods: dry matter (IDF 4A:1982), fat (IDF 5B:1986), total nitrogen (IDF 20B:1993) and NaCl content (IDF 88A:1988).The protein content was calculated as the nitrogen content multiplied by 6.38. Using these parameters fat in dry matter (FDM) and moisture in non-fat solids (MNFS) were calculated. The pH of cheeses was measured using a pH meter (Consort, Belgium). Each analysis was done in triplicate. Proteolysis was monitored by measuring the contents of water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN) according to Kuchroo and Fox (1982). After 30 days of ripening, the overall sensory quality was determined by point rating scale method (Radovanović and Popov-Raljić, 2001) by trained judges.

Data were subjected to one-way analysis of variance (ANOVA) with Statistica 6.0 software (Stat Soft. Inc., Tulsa, USA), and comparisons of means was done by t-test at $p<0.05$.

Results and discussion

Physico-chemical characteristics of four cheese variants are presented in Table 1. According to the fat in dry matter content (FDM) and the Codex standard 208 (1999), after the ripening period, all cheeses belonged to the group of full-fat cheeses. Variant D had significantly lower ($p<0.05$) FDM. Cheese B was the only one having similar FDM content as goat cheeses reported by Barać et al. (2016) and traditional Serbian white-brined cheeses produced from cow and sheep milk (Barać et al., 2019). Regarding moisture in nonfat solids (MNFS), all cheese variants were classified as soft cheeses. After 30 days of ripening no significant differences ($p>0.05$) in salt content and pH values were found.

Table 1. Physico-chemical characteristics of cheeses

Cheese variant	Time of ripening (days)	Parameter					
		DM (%)	FDM (%)	MNFS (%)	Proteins (%)	NaCl (%)	pH
A	1	42.76±2.36^{d}	38.30±2.26^{e}	68.45±3.25^{c}	17.48±1.59^{bc}	3.77±0.19^{d}	5.16±0.07^{a}
	10	43.44±5.76^{cd}	49.22±7.61^{bc}	71.94±7.48^{bc}	17.84±0.61^{bc}	3.85±0.16^{d}	4.66±0.38^{bc}
	20	45.55±1.24^{abc}	51.88±3.36^{ab}	71.30±2.42^{bc}	17.86±1.22^{bc}	3.99±0.43^{cd}	4.33±0.34^{cd}
	30	45.93±2.57^{abc}	53.63±2.26^{ab}	71.74±0.92^{bc}	18.56±0.87^{ab}	4.50±0.01^{a}	4.17±0.64^{de}
B	1	36.43±3.58^{f}	43.14±5.05^{de}	76.94±2.77^{a}	13.46±1.67^{d}	4.23±0.56^{abc}	5.45±0.14^{a}
	10	41.51±0.80^{d}	44.88±3.01^{cd}	71.64±2.49^{bc}	16.33±0.52^{cd}	4.25±0.22^{abc}	4.56±0.17^{bcd}
	20	45.35±2.45^{bcd}	56.78±0.87^{a}	73.60±1.75^{ab}	17.90±0.50^{bc}	4.39±0.66^{ab}	4.19±0.02^{de}
	30	46.90±1.25^{ab}	55.97±1.82^{a}	72.00±1.53^{bc}	18.32±0.79^{abc}	4.49±0.16^{a}	4.16±0.12^{de}
C	1	40.80±3.14^{de}	43.38±2.01^{de}	71.84±3.8^{bc}	14.27±0.41^{d}	3.89±0.06^{cd}	5.27±0.13^{a}
	10	42.21±1.06^{d}	50.17±1.48^{bc}	73.78±0.59^{ab}	16.45±1.30^{cd}	4.05±0.26^{bc}	4.83±0.25^{b}
	20	45.52±2.19^{abc}	51.86±3.75^{ab}	71.39±5.01^{bc}	17.67±0.29^{bc}	4.49±0.18^{a}	4.07±0.03^{de}
	30	48.06±0.39^{a}	51.67±0.19^{ab}	69.09±0.25^{c}	18.72±1.22^{abc}	4.38±0.08^{ab}	4.21±0.09^{de}
D	1	39.78±0.59^{e}	41.04±2.33^{de}	71.83±0.59^{bc}	16.30±0.90^{cd}	4.23±0.25^{abc}	5.11±0.04^{ab}
	10	42.29±2.21^{d}	48.87±1.57^{bc}	72.23±1.20^{ab}	19.12±1.75^{abc}	4.32±0.61^{ab}	4.11±0.19^{d}
	20	46.93±0.52^{ab}	46.89±0.60^{cd}	68.04±0.32^{c}	20.38±0.27^{ab}	4.56±0.03^{a}	3.90±0.06^{de}
	30	47.61±1.93^{ab}	47.65±3.52^{cd}	67.76±2.66^{c}	21.32±0.46^{a}	4.54±0.19^{a}	3.86±0.03^{e}

DM - dry matter, FDM - fat in dry matter, MNFS - moisture in nonfat solid. Data within the same column marked with different lowercase letters are significantly different at $p<0.05$

Proteolytic activity is measured by the water soluble nitrogen per total nitrogen (WSN/TN) ratio known as the Ripening Index (RI). The higher RI values suggest more intensive initial proteolysis in brined cheese. The WSN fraction mainly consists of the whey proteins, proteose-peptone, low molecular weight peptides derived from casein hydrolysis, free amino acids and weakly bounded caseins (Mallatou et al., 2004). According to Terzić-Vidojević et al. (2013) *Lc. lactis* ssp. *lactis* BGVL2-8 has good acidification ability and antimicrobial activity, while *Lb. plantarum* BGVL2a-18 has good proteolytic activity and ability to form exopolysaccharides.

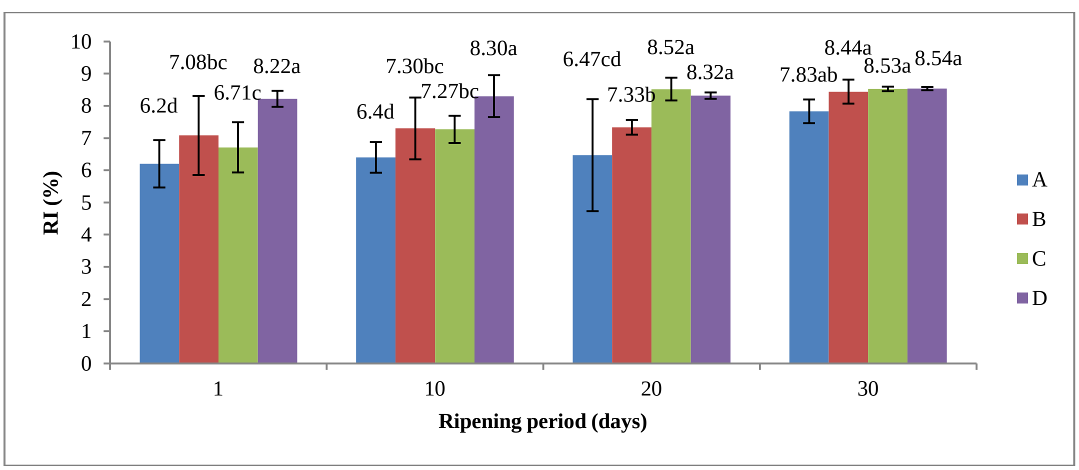

Figure 1. The change of ripening index

* Data marked with different lowercase letters are significantly different at $p<0.05$

As shown in Fig. 1. as a result of different starter cultures, in variants A and B intensive proteolysis occurred after 20 days of ripening, while in variant C increase of RI was observed after 10 days. The highest RI was found in variant D and did not change significantly ($p>0.05$) throughout whole ripening period. High levels of RI in cheese D at the beginning of ripening are probably the result of the proteolysis during curd processing. Significant reduce of pH value (Table 1) during the first 10 days of ripening may have contributed to bacterial activity which explains the absence of RI changes during ripening. Nevertheless, obtained results are in agreement with data presented by Barać et al. (2013) for 30-days-old white-brined goat cheese.

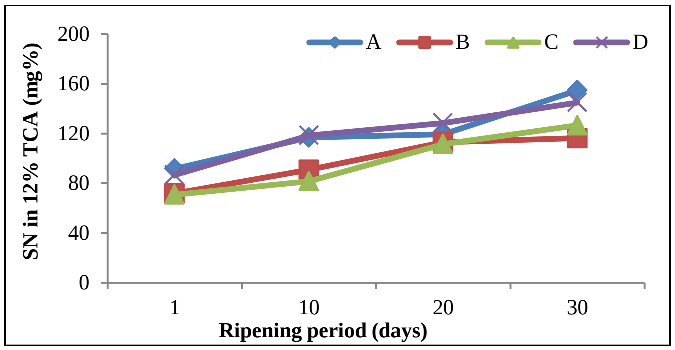

Figure 2. The change of TCA-SN during ripening

TCA-soluble nitrogen is traditionally considered as an index of "ripening depth" since it indicates progress of secondary proteolysis and the degree of formation of low-molecular-mass peptides (<15 000 Da) (Ardö et al., 1999; Tejada et al., 2008). As presented in Fig. 2. the level of TCA-SN increased during ripening. After 30 days values of TCA-SN were in the range 116.3-154.9 mg% and significantly higher ($p<0.05$) comparing to the first day of ripening in all cheese variants (70.9-91.7 mg%). However, those values were much lower than values reported for traditional Serbian white cheeses in brine (Barać et al., 2019). Variants B and C had less pronounced secondary proteolysis which can indicate slightly slower bacterial activity of those starter cultures. In the overall quality of food products, in addition to hygienic-toxicological, technological and nutritional factors, a significant place belongs to the sensory characteristics of the product. Sensory quality parameters usually include: appearance, color, flavor, taste, softness, structure, consistency and texture, where each group of products has specific sensory elements of quality, and thus specific sensory properties of quality (Radovanović and et al., 2001). The results of sensory analysis of white-brined goat cheese after 30 days of ripening are shown in Fig. 3. Taste is one of the most important sensory attributes in overall sensory quality of dairy products, including cheeses. Since cheese variant B had flavor, taste and consistency typical for goat white cheeses in brine, it was evaluated with the highest scores for those parameters. Furthermore, those scores were significantly higher ($p<0.05$) for variant B comparing to the other investigated cheese variants. Slices of cheese B also had uniform white color, unlike other cheese variants, especially variant D, in which slightly darker and more uneven color was observed. However, differences in color of cheese slices were not significant ($p>0.05$). Regarding taste, significant difference ($p<0.05$) was also established between variant A and variants C and D since they were slightly more acidic than the other cheese variants. Furthermore, cheese D had undefined and unexpressed taste. As a result pondered mean value of variant D was the lowest (4.36).

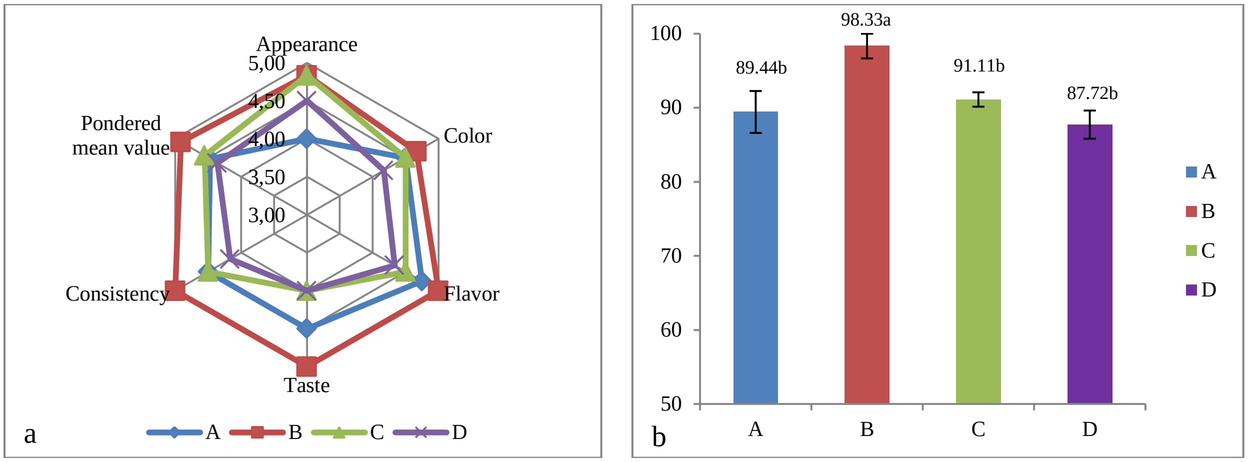

Figure 3. Sensory evaluation of white-brined goat cheese (a – Sensory quality parameters; b – Percentage of maximum quality)

* Data marked with different lowercase letters are significantly different at $p<0.05$

Percentage of maximum quality for investigated cheese varieties was in the range 87.72-98.33% (Fig. 3b). Due to the highest scores for all investigated sensory parameters, cheese variant B had significantly higher ($p<0.05$) percentage of maximum quality and was evaluated as the best among investigated variants of white-brined goat cheese.

Conclusion

Four variants of goat white-brined cheese were produced according to the same technological procedure using different starter cultures (A, B, C, D). All variants were classified as full-fat and soft cheeses. As a result of different starter cultures, different trends and rates of proteolysis were observed. According to sensory evaluation, both variants B and C were rated as excellent with the average pondered mean values 4.92 and 4.56, respectively. Since variants A and D had slightly lower overall scores (4.47 and 4.36), their quality was evaluated as very good.

Acknowledgement

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References

Alichanidis, E., Abd El-Salam, M.H. (2004). Cheese Varieties Ripened in Brine. in Cheese: Chemistry, Physics and Microbiology. Elsevier Applied Science, London.

Alichanidis, E., Polychroniadou, A. (2008). Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: a review. Dairy Science and Technology, 88 (4-5), 495-510.

Ardö, Y, Polychroniadou A. (1999). European Cooperation in the Field of Scientific and Technical Research (Organization), COST Action 95. Laboratory manual for chemical analysis of cheese, Office for Official Publications of the European Communities, Luxembourg.

Barać, M., Smiljanić, M., Pešić, M., Stanojević, S., Jovanović, S., Maćej, O. (2013). Primary proteolysis of white brined goat cheese monitored by high molarity Tris buffer SDS PAGE system. Mljekarstvo, 63 (3), 122-131.

Barac, M., Pesic, M., Zilic, S., Smiljanic, M., Stanojevic, S., Vasic, M., Despotovic, S., Vucic, T., Kostic, A. (2016). Protein profiles and total antioxidant capacity of water-soluble and water-insoluble fractions of white brined goat cheese at different stages of ripening. International Journal of Food Science and Technology, 51 (5), 1140-1149.

Barac M., Vucic T., Zilic S., Pesic M., Sokovic M., Petrovic J., Kostic A., Sredovic- Ignjatovic I., Milincic, D. (2019). The Effect of In Vitro Digestion on Antioxidant, ACE-Inhibitory and Antimicrobial Potentials of Traditional Serbian White-Brined Cheeses. Foods, 8 (3), 94; doi:10.3390/foods8030094

Codex Alimentarius (1999). Codex Group Standard for Cheeses in Brine (Codex Standard 208-1999); Joint FAO/WHO Food Standards Program; FAO: Rome, Italy

Dozet, N., Maćej, O., Jovanović, S. (2000). The white cheese in the brine from goat's milk. Journal of Scientific Agricultural Research, 63 (1-2), 343-356.

Dozet, N., Maćej, O., Jovanović, S. (2004). Goat milk and goat cheese-basis for quality food production. Biotechnology in Animal Husbandry, 20 (5-6), 147-156.

Dozet, N., Maćej, O., Jovanović, S. (2005). Condition and production of cheeses made of ewe and goat milk. Biotechnology in Animal Husbandry, 21 (1-2), 133-146.

IDF 4A:1982: Determination of the Total Solid Content (Cheese and Processed Cheese).
Brussels, Belgium.

IDF 5B:1986: Determination of Fat Content (Cheese and Processed Cheese Products). Brussels, Belgium.

IDF 20B:1993: Determination of nitrogen content. Brussels, Belgium.

IDF Standard 88A:1988: Determination of Chloride Content (Cheese and Processed Cheese). Brussels, Belgium.

Kljajevic, N., Miloradovic, Z., Miocinovic, J., Jovanovic, S. (2017). Textural and physico-chemical characteristics of white brined goat cheeses made from frozen milk and curd. The use of square I - distance statistics. Mljekarstvo, 67 (2), 130-137.

Kuchroo C.N., Fox P.F. (1982). Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. Milchwissenschaft, 37, 331–35.

Mallatou, H, Pappa, E, Boumba, VA. (2004). Proteolysis in teleme cheese made from ewes', goats' or a mixture of ewes' and goats' milk. International Dairy Journal, 14, 977–87.

Radovanović, R., Popov-Raljić, J. (2001). Sensory analysis of food products. Faculty of Agriculture, Belgrade and Faculty of Technology, Novi Sad.

Tejada, L., Abellána, A., Cayuelaa, J.M., Martınez- Cachaa, A., Fernandez-Salguero, J. (2008). Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. International Dairy Journal, 18, 139-146.

Terzić-Vidojević, A. Tolinački, M., Nikolić, M., Veljović, K., Jovanović, S., Maćej, O., Topisirović, LJ. (2013). Artisanal Vlasina Raw Goat's Milk Cheese: Evaluation and Selection of Autochthonous Lactic Acid Bacteria as Starter Cultures. Food Technology and Biotechnology, 51 (4) 554–563.

CURRENT SITUATION OF ANATOLIAN BUFFALO HUSBANDRY AND BREEDING POSSIBILITIES IN VAN PROVINCE OF TURKEY

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Abstract

Livestock sector in Van province of Eastern Anatolia is an important source of income for the local people. The objective of this survey is to present the current situation and the potential of buffalo husbandry and breeding opportunities in Van province of Eastern Anatolia in Turkey. However, it is possible to say that water buffalo breeding has decreased considerably for last years. Even though this is the case, especially buffalo husbandry is indispensable for indigenous people. Van province is one of the most prominent cities of this area in which its public sustain themselves by animal production, because there are many wetlands in Van province. For this reason, buffalo husbandry should be expanded in Van province. According to the results of research, the dilemma as such as low yield in buffalo, low number of animals raised, limited marketing opportunity, insufficient reproductive cycle of the animal, insufficient technical knowledge, inadequate cooperatives, inadequate and expensive veterinary service are most problems to create efficient breeding programs. According to the data of 2020, there are a total of 1001 head buffalo in Van province. In Van province of Eastern Anatolia, buffalo husbandry participates with 0.71% in general livestock production in Turkey with very low share. Anatolian buffalo breed is more common in this region. Anatolian buffalo, among Mediterranean water buffalo which are subgroup of river buffalos, is raised in Turkey. Milk yield, lactation period, birth weight of calves, and daily live weight gain during feeding in Anatolian buffaloes are 800-1000 kg, 200-250 days, 30 kg, and 550-600 g, respectively. The buffalo milk from this breed is consumed by the household as raw milk. As a result, the preservation and the development of Anatolian buffalo breed as a genetic source is very important. Water buffalo breeding needs to be developed in Van province.

Keywords: *Buffalo milk, Husbandry, Improvement, Van province, Water buffalo.*

Introduction

Water buffaloes in Turkey are believed to have originated from Mediterranean water buffaloes, which are a sub-group of river water buffaloes, and are called Anatolian water buffaloes (Soysal, 2006). Water buffalo farming has advantages related to the resistance of water buffaloes to natural conditions and diseases, their ability to benefit from feed and to turn poor feed into meat and milk, and finally, their low cost compared with cows (Canbolat, 2012). The number of animals in Turkey has decreased significantly in the last thirty years. The decrease in the number of buffaloes is more serious. In particular, the support given to livestock in recent years has led to an increase in the number of animals in Turkey. This increase was also reflected in buffalo breeding.

Water buffalo husbandry in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications. With the inquiry works, results are determined as yield of buffalo is low, the number of animals raised are very small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry.

Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). The first practices that should be done to increase the profitability in livestock enterprises should be directed to environmental regulations. As the environmental factors improve, the genotype needs to be improved accordingly (Öztürk, 2009).

Muş province was chosen as the research area in this study as one of the important provinces for water buffalo farming in the East Anatolia region in Turkey. Few studies of water buffalo farming have been found as a result of a literature review. There are no studies on water buffalo farming in Muş province. Agriculture is the major source of income in the province, and the pasture and meadow areas suitable for livestock have a high potential for new investments. The number of water buffaloes was 13749 head in 1991, and declined sharply to 3296 head in 2009. After this year, it started to increase with animal husbandry support by the government (Işık and Gül, 2016).

The most common diseases in buffalo husbandry enterprises of Van province are foot and mouth disease, brucellosis, mastitis. Farmers are generally evaluating their milk themselves. Mostly, farmers are milking by hand.

Livestock activities in Van province of Eastern Anatolia are an important source of income for the local people. Van province is one of the most prominent cities of this area in which its public sustain themselves by animal production. For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the water buffalo stock in this province. The aim of this survey is to put forward the current situation and the potential of water buffalo husbandry and breeding opportunities in Van province of Eastern Anatolia in Turkey.

Demographic Structure of Van Province

Van province is located in Lake Van in Eastern Anatolia Region Chapter of the Upper Murat-Van in a closed basin on Turkey. The border with the Iranian State lies to the east of Van. Van is surrounded by Van Lake from west. It is also surrounded by cities such as Ağrı from north and Bitlis from west, Siirt, Şırnak, Hakkari from south and Iran from east. Van city is nineteenth the most crowded city in Turkey. As of the end of 2019, its population is 1.136.757 people. Van is the fifth largest province in terms of surface area in Turkey. Its area is 21.334 km^2. Although Van Lake and its valleys are covered with rich vegetation, the mountains are generally barren (Anonymous, 2021). Economy of Van province is an economy mainly based on agriculture and animal husbandry, aquaculture, and tourism. The livestock sector in Van province has been developing and gaining importance day by day.

As Van province is in the lower status in terms of socio-economic development (in the 5th level), its unemployment rate high and employment rate low, and more than half of the working population in different sectors is concentrated on the agriculture sector significant migrations occurred in the province (Elmastaş and Yılmaz, 2015). The educational status of the buffalo farmers and families of Van province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country.

The Importance of Water Buffalo Husbandry in Van Province

Buffalo production is an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Buffalo production is indispensable and an important source of income for farmers in Van province. Van province is suitable for both the small ruminant breeding and the cattle and the water buffalo husbandry in terms of large pasture areas, water resources, and climate characteristics.

The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001).

It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. However, small ruminant husbandry is a major industrial sector in Van province in the Eastern Anatolia of Turkey (İnan and Aygün, 2018). Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level.

It is important to understand the socio-economic characteristics of water buffalo farmers. This was done with the hope of identifying those characteristics that may impact and also help to explain the farming activities of the area. The characteristics considered were age, educational attainment, household size, land acquisition type, farming experience, agricultural land, and farm output sizes. Lack of information on benefits of water buffalo dairy products creates an important problem in the marketing of these products for farmers. Therefore, infrastructures required for promotion of water buffalo dairy products should be prepared and policies related to promotional advertisement should be implemented. To reach the desired level of water buffalo farming, incentives on water buffalo should be maintained, policies should be improved by using input subsidies, producers should be trained on water buffalo farming, and improvements in policies of promotion and benefits of water buffalo dairy products may be useful for establishing a water buffalo culture in Turkey. Maintaining traditional methods in water buffalo farming prevents targeted efficiency expectations. Therefore, transferring new modern husbandry techniques to producers is an important issue for policy makers. (Işık and Gül, 2016).

Number of Buffalo in Van Province

Characteristics of buffalo husbandry in Van province are small farms with 2 to 3 cows per farm (80-85% of total number of farms). The most common of buffalo breeds in Turkey is the Anatolian water buffalo breed. It is very important to define the condition, potential and

problems of breeding sector especially with respect to the water buffalo, the cattle, sheep, and goat stock in this province. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. The most important of these problems is roughage.
Inventory studies constitute the basis of all kinds of studies that are planned to be carried out at national or regional level. Therefore, important suggestions for the Van region will be presented with this study. Number of buffalo and cattle is presented in Table 1 in Turkey and in Table 2 in Van province.

Table 1. Number of buffalo and cattle in Turkey (head) (TOB, 2021).

Year	Buffalo	Cattle	Total
2015	133766	13994071	14127837
2016	142073	14080155	14222228
2017	161439	15943586	16105025
2018	178397	17042506	17220903
2019	180826	18070500	18251326
2020	188771	18626219	18614990

Table 2. Number of buffalo and cattle in Van province (head) (TOB, 2021).

Year	Buffalo	Cattle	Total
2015	264	166944	167208
2016	308	162172	162480
2017	394	185349	329688
2018	536	176438	176974
2019	490	179713	180203
2020	485	185240	185725

The numbers of indigenous water buffalo are low and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Van province of Eastern Anatolia in Turkey, the indigenous water buffaloes are found to be small in numbers. Therefore, the milk productivity of the indigenous buffalo is very low. Improvement in productive and reproductive performance is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers.
Since the farms in the region are small and the prices of inputs are high, water buffalo farming has not developed enough to reach the level of market-oriented production. Therefore, policies should be made according to the size of farms in the region and more economical production can be achieved by solving market-related problems. The milk productivity of water buffaloes in the region is low and it is more obvious especially on small farms. Thus, milk yield could be increased by animal improvements. In this way, milk yield could reach a desired level (Işık and Gül, 2016).

Buffalo Products in Van Province

It is considered that the size of the establishment has no effect on cattle and buffalo breeding practices (Özyürek et al., 2014). Unfortunately, in most of the enterprises studied in the region, there are no suitable environments for animal husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to the country's economy. Meat and milk products from buffalo in Turkey are presented in Table 3. In Turkey, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of animal farms. In large-scale researches conducted in 2016, it is observed that the bodies of buffaloes and cows in milk processing are unacceptably dirty (up to 70%), thus causing frequent foot, breast and reproductive health and milking hygiene problems (TOB, 2021).

Table 3. Meat and milk products from buffaloes in Turkey (tons) (TOB, 2021).

Year	Meat	Milk
2015	326	62751
2016	351	63085
2017	1339	69401
2018	402	75742
2019	73	70341
2020	250	75300

It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.
Zoonotic diseases are one of the most important problems of workers in animal husbandry. Workers and animals must be vaccinated against various zoonotic diseases. The rules of order and hygiene must be taken into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of workers, and a separate toilet in each household to facilitate personal hygiene. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.

Conclusion

Like the Asian countries, buffalo breeding in Turkey has been performed by traditional methods and production per cow is low in comparison with intensive buffalo production systems such as Italy, but little is known about variation of production traits. All scientific research so far has shown that the water buffalo husbandry in this region is also done in the traditional production system. In addition we think that databases for Anatolian buffaloes have under developed, showing that they have a wider range of data because of problems in herd management system. Especially in some seasons, buffaloes have been hold at night outside, and therefore in these regions it is difficult to develop and pursue the effective data collect processes. Van province has ecological conditions suitable for animal production. In Van province of Eastern Anatolia, buffalo production participates with 0.71% in general livestock production in Turkey with very low share. The most common of buffalo breeds in Van province is the Anatolian buffalo breed. The milk from this buffalo breed is either consumed by the household as raw milk or in making cream and yoghurt. As a result, preservation and development of native buffalo breed as a genetic source is very important. If the current potential especially in the Eastern Anatolian Region and in Turkey is evaluated, it can become very important in this region. It can be said that Van province has an important place in terms of its features for buffalo presence and animal production.

References

Anonymous (2021). Van geographical location (Van coğrafi konumu). Van İl Kültür ve Turizm Müdürlüğü. https://van.ktb.gov.tr/TR-90248/cografi-konumu.html.

Canbolat Ö. (2012). Buffalo breeding and current situation in Turkey. Journal of Tarım Türk 30: 176-180.

Elmastaş N., Yılmaz S. (2015). Migrations in the province of Van (Van ilinde göçler). Turkish Studies, International Periodical for the Languages, Literature and History of Turkish or Turkic, 10(10): 403-428.

Işık M., Gül M. (2016). Economic and social structures of water buffalo farming in Muş province of Turkey. R. Bras. Zootec., 45(7): 400-408.

İnan İ., Aygün T. (2019). The body weight after shearing and the greasy wool yield of Red Karaman ewes at different raising conditions in Turkey. Journal of Advanced Agricultural Technologies, 6(2): 139-143.

Mutaf S., Aklan S., Seber N. (2001). Design principles of animal shelters and shelter types suitable for GAP region (Hayvan Barınaklarının Projelendirme İlkeleri ve GAP Yöresi için Uygun Barınak Tipleri). TMMOB Makine Mühendisler Odası, II. GAP ve Sanayi Kongresi, 29 - 30 Eylül, Diyarbakır.

Öztürk N. (2009). Structural features of dairy farms in Mardin province (Mardin İlindeki süt sığırcılığı isletmelerinin yapısal özellikleri). M.Sc. Thesis, Department of Animal Science, Selçuk University, Institute of Natural & Applied Sciences, Van, Turkey, 74pp.

Özyürek S., Koçyiğit R., Tüzemen N. (2014). Structural features of dairy farmers in the Erzincan: The example of Çayırlı district. Journal of Tekirdag Agricultural Faculty, 11(3): 19-26.

Soysal İ. (2006). Production of water buffalo and products. Tekirdağ University, Agriculture Faculty Department of Animal Science, Lecture note, Tekirdağ.

Şeker İ., Köseman A. (2015). Cattle, sheep and goat breeding activity in Elazığ (Elazığ ilinde büyükbaş ve küçükbaş hayvancılık faaliyetleri). Harran Üniv. Vet. Fak. Derg., 4(1): 36-44.

TOB (2021). Ministry of Agriculture and Forestry: Livestock Breeding: Cattle Husbandry (Tarım ve Orman Bakanlığı: Büyükbaş Hayvancılık: Sığırcılık). https://www.tarimorman.gov.tr/HAYGEM/Belgeler/Hayvanc%C4%B1l%C4%B1k/B%C3%BCy%C3%BCkba%C5%9F%20Hayvanc%C4%B1l%C4%B1k/2019%20Y%C4%B1l%C4%B1/Buyukbas_Hayvan_Yetistiriciligi.pdf.

OCCUPATIONAL HEALTH AND SAFETY CULTURE IN ANIMAL PRODUCTION IN TURKEY

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Abstract

Animal production has an important place in agricultural activities. The aim of this review is to provide sensitivity about occupational health and safety, accidents, diseases, and musculoskeletal disorders in animal production sector in Turkey. It is also aimed to provide suggestions for occupational health and safety that increase production in animal breeding. The livestock activities in Turkey have been characterized by the different regional applications. Animal production is associated with a variety of occupational illnesses and injuries. The issue of occupational health and safety in animal production is very important as it is in many other areas. In practical work related to crop and livestock production to ensure safety and to prevent accidents at work, it is important to take necessary precautions. In such an environment and in a certain direction, employees are exposed to occupational accidents. Farm workers can often experience unavoidable health problems and even death may occur. In addition, there is also a risk of contracting an occupational disease that may occur in the future in livestock farming. The most common hazards at the animal production in Turkey are the zoonotic diseases, the ergonomics, the noise, the air conditioning, the chemicals, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for the workers at the livestock enterprises, the field, and the factories dealing with the feed, skin, and meat. In Turkey, preventive measures have started to be taken on occupational health and safety in livestock production. This information has been prepared based on the personal observations and the experiences directly in the local area.

Keywords: *Animal production, Occupational disease, OHS culture, Zoonotic disease.*

Introduction

The Occupational Health and Safety (OHS) has a vital importance in livestock production. It has also significant economic implication particularly in terms of medical costs and economic productivity losses. Occupational safety and accident risk factors of revealing awareness of breeders are not yet fully come to avoid. The livestock activities in Turkey have been characterized by the different regional applications. Animal production has an important place in agricultural activities. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of occupational health and safety in animal production is very important as it is in many other areas (Aygün et al., 2014; 2019a).

Occupational diseases and accidents that can be encountered by workers at business have caused the losses of very serious economic and the qualify person in animal production. In addition, the sustainability of production is negatively affected. The most common hazards at the animal production in Turkey are the zoonotic diseases, the ergonomics, the noise, the air conditioning, the chemicals, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases etc. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for the workers at the livestock enterprises, the field, and the factories such as the feed, the skin, and the meat (Aygün et al., 2019b).
The risk factors that the livestock workers faced vary according to the sector. In sheep-goat husbandry, the most important task in the care-feeding and management of animals falls into workers. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses.
Sheep and goat breeding in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications (Aygün, 2017). It is true that more occupational health and safety intervention research focusing on preventing illness and injury needs to be conducted. Conducting this type of research is difficult and time-consuming; however, without increasing the number and methodological rigor of these studies, it will be difficult to identify effective intervention methods and confidently encourage their use (Goldenhar and Schulte, 1996). Animal production is associated with a variety of occupational illnesses and injuries.
The object of this review is to provide sensitivity about occupational health and safety, accidents, diseases, and musculoskeletal disorders in animal production sector. It is also aimed to provide suggestions for occupational health and safety that increase production in animal breeding. This information has been prepared based on the personal observations and the experiences directly in the local area.

The Place and Importance of Animal Production in Turkey

The livestock activities in Turkey have been characterized by the different regional applications. The activities of animal breeding sector are especially an important source of income for the indigenous people in Turkey.
Commonly, it was aimed to be offered some information about living culture and stockbreeding activities of nomadic tribes that they come from the south to east because of weather warming up with. Studies made by some researchers in order to achieve their political goals on political and ideological studies relating to a multi-ethnic structure and stockbreeding activities that is the main source of income of the politicized tribes were the main theme of this study. One of the most important issues to be taken into account for sustainable animal breeding sector in Turkey is no doubt farming culture. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds (Aygün et al., 2013; İnan and Aygün, 2019).
In Turkey, livestock sector has a considerable potential and is an important part of agricultural sector and economy. The livestock products, including meat, milk, eggs, honey, wool, and hides, play a significant role in the Turkish economy. In general, animal production constituted approximately 25 % of agricultural production value. The sector's contribution to farm income is substantial, and activities related to livestock production and marketing are important to the

economic development of rural areas in Turkey.. In parts of country where agriculture and farming are limited because of land shape or limited land and high number of population, households make their living with animals, especially in Eastern and Southeastern Anatolia. However, in these regions, mostly traditional techniques are used, and the results are not much satisfactory when compared to developed countries.

Livestock products are an important source of household income for many farmers and households in rural areas. For small farmers, livestock products such as cattle, sheep and goat generate income and ensure food security for these households because an important amount of their incomes comes from the sales of animal and milk.

The Most Common Occupational Accidents in Animal Husbandry

The hazard is anything that has the potential to harm. Hazard can affect the person, the material and the process. Also, hazards can cause accidents, diseases, loss of product, and machine damage etc. The occupational risk refers to the combination of the likelihood and severity of an injury or illness resulting from exposure to a hazard.

Since livestock workers spend a great deal of time outdoors, they are at risk for physical stress from excessively cold and excessively hot environments. The magnitude of heat and cold stress problems in agriculture is not well documented. Tolerance to such environments varies among individuals and may be difficult to predict. Livestock workers should be provided the means to compensate for extremes of temperature. For example, adequate water supplies while working outdoors in hot climates are essential.

In animal husbandry sector, the most important task in the care-feeding and management of animals falls into workers.

Workers who are away from social habitats and who work in the hills may be exposed to allergies or poisoning caused by the attack of various wild animals, such as bee or insect bites, as well as plants grown in the spring, pollen of fungi or various flowers. Employees are camels exposed to the sun because the work area is mostly open space. Therefore, excessive exposure to sunlight can cause dermatological problems.

Since livestock workers spend a great deal of time outdoors, they are at risk for physical stress from excessively cold and excessively hot environments. The magnitude of heat and cold stress problems in agriculture is not well documented. Tolerance to such environments varies among individuals and may be difficult to predict. Livestock workers should be provided the means to compensate for extremes of temperature. For example, adequate water supplies while working outdoors in hot climates are essential.

Workers in animal husbandry have exposure to other hazards that may increase their risk of health problems: climate-dependent problems, such as heat stroke or cold shock, and occupationally caused infections such as *anthrax, ascariasis, encephalitis, leptospirosis, rabies, salmonellosis, tetanus, and coccidioidomycosis.* Sensory problems are common: eye problems, caused by irritation, infection, or injury from the wind, sun, dust or soil, agricultural chemicals, debris ejected from farm machinery, and allergic reactions to plants, and hearing problems due to noise from farm machinery and cannery work.

The Most Common Occupational Diseases in Animal Production

An "occupational disease" is any disease or disorder contracted primarily as a result of an exposure to risk factors arising from work activity. "Work-related diseases" have multiple causes, where factors in the work environment may play a role, together with other risk factors, in the development of such diseases. The World Health Organization emphasizes the following: Carrying our estimates of the global burden of disease from major occupational risks, such as injuries, airborne exposures, carcinogens, ergonomic stressors, noise and other specific risks. Working with ILO to develop diagnostic and exposure criteria for occupational diseases and to enable primary and secondary health care providers to detect and report such diseases (WHO, 2020).

The most common risk factors at animal production sector in Turkey are the ergonomics, the noise, the air conditioning, the chemicals, the occupational diseases, the zoonotic diseases, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress and, the skin-borne diseases etc. Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field (Aygün, 2017).

Zoonotic disease is naturally called vertebrate animals to humans, and humans to animals to diseases or infections. Zoonoses are infections that are spread from animals to humans. The World Health Organization (WHO) defines zoonotic diseases as 'any diseases or infections that are naturally transmitted between vertebrate animals and humans.' Agents causing zoonotic diseases may be bacteria, fungi, viruses, parasites or any other communicable agents, for example prions. Currently there are over 200 recognized zoonoses, some of which have a worldwide distribution and others which are localized to specific regions. The situation is not static, and emerging zoonotic diseases are continually being recognized, both animal diseases which have spread to humans for the first time and existing zoonoses spreading to new geographical areas. Occupational zoonotic diseases are most common where there is close contact between animals and humans at work, for example in animal husbandry and agricultural occupations, although workers in a wide range of other occupations may also be exposed to zoonotic agents, including those employed in the outdoor leisure industry or the waste water industry and laboratory workers. There are many occupational zoonotic diseases in the world, many of which occur very rarely, although some do pose a significant health risk for workers in certain occupations. While the incidence of specific zoonoses varies from country to country, there are many occupational zoonoses that occur across Europe, although not every disease is present in every country. (Cook and Farrant, 2020)

Other health and safety risks include skin problems, hearing loss, stress, and mental well-being issues particular to farming and the rural way of life. Occupational skin disorders are common in livestock workers. The effects of sun exposure are an important cause of morbidity in animal breeders group. Farmers' lung is one of many forms of *hypersensitivity pneumonitis*. This problem is becoming rare, which is likely due to the reduction of exposure to organic dust from the increasing mechanization of agriculture and the effect of livestock health and safety programs (Von Essen and McCurdy, 1998). Another danger for farmers is the waste of animals. Animal wastes are frequently stored underground and are a source of toxic gases. Entering confined spaces used for manure storage can lead to fatalities, which are often caused by hydrogen sulfide exposures (Von Essen and McCurdy, 1998).

Some Suggestions and Possible Preventions

It is extremely important that the breeders and the organizations engaged in animal production have knowledge of occupational health and safety. The nature of animal production requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.
Zoonotic diseases are one of the most important problems of farmers in animal production. Workers and animals must be vaccinated against various zoonotic diseases.
With regard to the control of occupational zoonoses, there are some general control measures which reduce the risk of infection for a wide range of zoonoses. These include the following:

- good personal hygiene practices, especially washing with soap and warm water;
- covering cuts and scratches with waterproof dressings;
- wearing of appropriate PPE, for example gloves, overalls, respiratory protection – this must provide relevant protection, while also being suitable for carrying out the required task;
- good hygiene practices for animal husbandry; and
- use of an appropriate disinfectant to clean potentially contaminated areas.

For certain zoonoses, there is an effective vaccine available and it may be appropriate to administer this to individuals in high risk occupations, for example laboratory workers handling infected animals. In many cases there are effective prophylaxis and treatments available. For these to be used to maximum advantage, it is necessary for workers to be aware of any diseases they may be at risk from and to be able to recognise early symptoms of these diseases. For certain occupations it may be required for workers to inform their employer if they have a weakened immune system (Cook and Farrant, 2020).
The rules of order and hygiene must be taken into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of women workers, and a separate toilet in each household to facilitate personal hygiene.
These approaches are necessary to obtain the cooperation of nomadic workers and their employers so that occupational exposures and protection as well as health consequences are accurately and completely ascertained. In addition, information about health effects should be obtained in a way that is not only culturally sensitive but also meaningful to study participants and yet comparable to that obtained through standardized instruments. Undertaking studies of occupational health risks in this population with these considerations will not only contribute to the understanding of such risks but can also further preventive efforts and lead to better health in this high-risk population. Effective prevention can reduce suffering and death and contribute to enhanced productivity in the workplace. In this way, both the employers and the employees gain (Aygün and Demir, 2015).
Taking precautions for occupational health and safety are very difficult, costly and time consuming. Among the difficulties is the varied nature of agriculture, the many ethnic groups engaged in the activities, the traditionalist view of farming families, and rapidly changing technology. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.
There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of

occupational health and safety in animal production is very important as it is in many other areas. Occupational diseases and accidents that can be encountered by livestock workers at business have caused the losses of very serious economic and the qualify person in animal production (Aygün, 2015).

As a result, the work-related accidents encountered workers in the plateau have also been emphasized. This information has been prepared based on the personal observations and the experiences directly in the local area. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness.

The issue of occupational health and safety in animal production is very important as it is in many other areas. In addition, the sustainability of production is negatively affected. Occupational accidents and diseases that can be encountered by workers have caused the losses of very serious economic and the qualify persons in animal husbandry sector. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field. There are a number of characteristics of Turkish agriculture that need to be acknowledged for an effective occupational health and safety response to the farm injury or illness problem. In Turkey, preventive measures have started to be taken on occupational health and safety in livestock production.

References

Aygün T., Karakuş K., Tuncer S.S., Keskin S., Çelen M.F. (2013). Nomadic culture and sheep husbandry in Eastern Anatolia. VI. Balkan Animal Conference, Balnimalcon 3-5 October 2013, Tekirdağ, Türkiye, s: 187.

Aygün T., Erkan C., Karakuş F. (2014). Occupational health and safety culture in animal husbandry, problems and solutions. International Mesopotamia Agriculture Congress, 22-25 September 2014, Diyarbakır, Turkey, p: 937-937.

Aygün T. (2015). Occupational health and safety culture in animal industry (Hayvancılık sektöründe işçi sağlığı ve iş güvenliği kültürü ve önemi). 9. Ulusal Zootekni Bilim Kongresi, 3-5 Eylül 2015, Selçuk Üniv. Ziraat Fakültesi Zootekni Bölümü, Konya.

Aygün T. (2017). The occupational health and safety for berivans at zoma life in Hakkari Province (Hakkâri ilindeki zoma yaşamında berivanların sağlığı ve iş güvenliği). Uluslararası Katılımlı 1. Tarım ve Gıda Etiği Kongresi, 10-11 Mart 2017, 163-164, Ankara Üniv. Ziraat Fakültesi, Ankara.

Aygün T., Erkan C., Karakuş K., Çelikyürek H. (2018). The occupational health and safety in animal production. I. International Agricultural Science Congress, 9-12 May 2018, vol.1, no.1, pp.198-198, Van.

Aygün T., Erkan C., Çelikyürek H. (2019a). Occupational health and safety culture for berivans and shepherds in small ruminant husbandry. International Agricultural Congress of Muş Plain Proceedings Book, Cilt ISBN: 9786055137052, 24-27 September 2019, Muş, Turkey, pp: 491-497.

Aygün T., Erkan C., Karakuş K., Alarslan E. (2019b). The occupational accidents and preventions at sheep husbandry in Muş province. 11th International Animal Science Conference, 20-22 October 2019, Cappadocia, Nevşehir, Turkey, pp.127-130.

Cook B., Farrant J. (2020). Occupational zoonoses. Health & Safety Laboratory, UK. https://oshwiki.eu/wiki/Occupational_zoonoses

Goldenhar L.M., Schulte P.A. (1996). Methodological issues for intervention research in occupational health and safety. American Journal of Industrial Medicine, 29: 289-294.

İnan İ., Aygün T. (2019). The body weight after shearing and the greasy wool yield of Red Karaman ewes at different raising conditions in Turkey. Journal of Advanced Agricultural Technologies, 6(2): 139-143.

Von Essen S.G., McCurdy S.A. (1998). Health and safety risks in production agriculture. West J Med, 169: 214-220.

WHO. (2020). Occupational health: Occupational and work-related diseases https://www.who.int/occupational_health/activities/occupational_work_diseases/en/

NOMADIC ACTIVITIES OF SMALL RUMINANT HUSBANDRY IN MUŞ PROVINCE OF EASTERN ANATOLIA IN TURKEY

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Abstract

In this survey, the nomadic life culture and the nomadic small ruminant husbandry in Muş province of the Eastern Anatolia Region in Turkey have been discussed. Also, detailed information about nomadic families in Muş province dealing with stockbreeding activities has been given. This information has been prepared based on the personal observations and the experiences directly in local area. Stockbreeding activities of nomadic and semi-nomadic tribes have been examined. The migrants are mostly from Şırnak, Batman, Mardin, and Siirt provinces to the Muş highlands. These migrants make as pedestrian their arrival and return to the plateau. Shepherds in their family are responsible for maintenance and feeding of animals on the plateau. According to the data of 2020, there are a total of 1,235,552 head goats and sheep in Muş province. In the highland small ruminant husbandry towards the end of the spring, animal herds are taken to the plateaus which are cool and grassy as opposed to the drying of pastures and the start of heat. For a period of 3-5 months, sheep remain in control by shepherds in highland. After the weather cools down, highland breeders and sheep return to villages or to their settlements. Sheep herds usually consist of 300 to 500 heads. One of the most important issues to be taken into account for sustainable small ruminant husbandry in this region is no doubt the nomadic life culture. In order to get more abundant products such as milk, cheese, wool and so on, the people of the region have to go to highlands with the arrival of spring to find better grazing and water areas for animals. The results indicate that solving the problems of nomadic and semi-nomadic families is very important to sustain stockbreeding of sheep and goats, and to benefit Muş province economically.

Keywords: *Goat husbandry, Muş province, Nomadic life, Sheep husbandry Transhumance.*

Introduction

Sheep and goat production is an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat, milk, and wool. Eastern Anatolia Region of Turkey has a different character than other regions in terms of climate conditions. In the winter months, when there are severe storms in this region, extreme cold occurs. Muş is one of the most prominent cities of this area in which its public sustain themselves by animal production. The socio-economic and cultural structure, climate, and topographic features of the region have played a role in gaining importance of sheep breeding. Sheep breeding has a special place and importance in animal production in Muş province. According to the investigations in the region, sheep breeding pasture-based are dominant (Aygün et al., 2019; İnan and Aygün, 2019). For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the small ruminant husbandry in this city.

It can be said that the fact that sheep breeding is carried out in herd rather than management level at the enterprise level has an important effect on the spread of plateau and nomadic sheep farming with the effect of structural difference. This situation is more evident in the Eastern and Southeastern Anatolia Region in Turkey. In this sense, since sheep farming is mostly done by nomadic families or tribes, it is important to compile information about this section and to determine the general characteristics of the nomadic families (Aşkan and Aygün, 2020).

Animal production is one of the most important economic sectors of Turkey. Sheep production systems in Turkey depend on factors such as the natural and socio-economic conditions of the regions, the availability of feed resources, the connection to plant production and, the consumption habits of the population. These are systems of the stock breeding, the highland sheep husbandry, and the nomadic livestock breeding (Kaymakçı, 2010).

Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015).

This review concludes that the nomadic activities and the production habits of breeders in sheep and goat have been discussed. This information has been prepared based on the personal observations and the experiences directly in local area.

Demographic Structure of Muş Province

Muş province is a province in eastern Turkey. It is 8,196 km^2 in area and has a population of 406,886 according to a 2010 estimate, down from 453,654 in 2000. The provincial capital is the city of Muş. It is surrounded by cities such as Erzurum from north, Bingöl from west, Siirt and Diyarbakır from south and Van from east (Anonymous, 2021). Economy of Muş province is an economy mainly based on agriculture and animal production. Livestock sector in Muş province is developing day by day and gaining importance.

The educational status of breeders and families of Muş province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal production and the economy of the country.

The Importance of Small Ruminant Production in Muş Province of Eastern Anatolia Region in Turkey

Sheep production is an industrial sector that they transforms the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat, milk and, wool. There are breeds such as White Karaman, Red Karaman, Awassi, Dağlıç, Kıvırcık, and Karayaka among local sheep breeds of Turkey (İnan and Aygün, 2019). Small ruminant production is indispensable and an important source of income for farmers in Muş province. Muş province is suitable for small ruminant breeding in terms of large pasture areas, water resources, and climate

characteristics. It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops.
Sheep and goat in this region has been adapted to regional differences, and has been characterized by the prominence of different applications. In the highland sheep production, sheep flocks are removed to the highlands with cool and plenty of grassy plains by pressing hot and dry towards the end of spring.
Sheep and goat production systems in Turkey depend on factors such as the natural and socio-economic conditions of the regions, the availability of feed resources, the connection to plant production and, the consumption habits of the population. These are systems of the stock breeding, the highland sheep husbandry, and the nomadic livestock breeding.
For a period of 3-5 months, sheep remain in control by shepherds in the highland. After the weather cools down, sheep go back to the villages or the farms in the plain. Sheep herds usually consist of 300 to 500 heads. Each sheep is composed of lots of different people with a lot of expenses, depending on the number of animals contributes. Sheep herds were formed by gathering business owners with a different number of animals. Sheep herds are taken to the highlands by grazing or by road transport.

Nomadic Activities in Small Ruminant Production in Muş Province of Eastern Anatolia Region in Turkey

One of the most important examples of livestock farming is highland small ruminant husbandry in Muş province. In order to get more abundant the products such as milk, cheese, wool and so on, the people of the region have to go to the highlands with the arrival of spring animals to find better grazing and water areas. With the arrival of spring to the first zone in the region is exited. With the start of the cold days of autumn again return to the settlements. In this province, it is recommended to keep records in order to obtain a sustainable income source from small ruminant production. Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level.
The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001). The numbers of indigenous sheep and goat are high and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Muş province of Eastern Anatolia in Turkey, the indigenous sheep and goat were found more in numbers. But the milk productivity of the indigenous sheep and goat breeds is very low. Therefore, improvement in productive and reproductive performance of sheep and goat is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers. The population of sheep and goat in its native tract is decreasing steadily and there is no information on its present status in its home tract. Pure animals are still found in and around Muş province. The sheep-goat breeders in this region are forced to migrate from the home tract to the cities along without their flocks and herds, due to economic and social problems of the region. Migration realised during the 20 years. In this process, they sold their

animals. During the course of migration, the number of sheep and goat in the region decreased steadily. In the current time they are returning back to their dwellings systematically.

The Importance of Berivans and Shepherds in Small Ruminant Production

Berivan and shepherd workers are inseparable parts of each other. They are the most important workers of highland and nomadic animal production. Their ages may range from 10 to 70. Berivan is called as women milking the ewe and the nannie. Milking in migrant small ruminant breeding systems is done by berivans. Berivans are not the only ones who are responsible for sheep milking. They are laboring at all stages of the processing of the obtained plumbing. Berivans and shepherds have many challenges for in nomadic small ruminant husbandry (Aygün, 2017). Berivans and shepherds are one of the most underserved and understudied populations in the Turkey. One characteristic of the traditional nomadic lifestyle group is that berivans and shepherds themselves are certain fixed tents that they find themselves under dangerous conditions and for long hours.

Overview of the General Problems of Small Ruminant Breeders in Highland

The most of sheep production in Turkey are carried out in extensive or semi-intensive systems. The most of sheep breeds reared in Turkey are characterized as rough-mixed wool genotypes and their wools are usually used to carpet, blanket, quilt and weaving socks. Turkey has an important place in the production of fleece with carpet type in the world. However, the wool industry and production in Turkey have not developed and fluctuations of the wool prices negatively affected the wool of production. Recently, there has been an increase for sheep production in Turkey and for use of rough-mixed wool in the world (Öztürk and Odabaşı, 2011).

One of the most important issues to be taken into account for sustainable small ruminant breeding in the region is no doubt nomadic living culture of tribes that they presented this culture and breeding system to nowadays. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds.

Unfortunately, in most of the farms studied in the region, there are no suitable environments and management conditions for small ruminant husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of breeders will increase with the livestock breeding in the region and will contribute to the country's economy.

In this region, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of small ruminant farms. In large-scale researches conducted in last years it is observed that the bodies of animal in milking and shearing processing are unacceptably dirty, thus causing frequent foot, breast and reproductive health, shearing, and milking hygiene problems (TOB, 2021).

It is extremely important that the breeders and the organizations engaged in animal production have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by

determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.
Zoonotic diseases are one of the most important problems of workers in livestock sector. Workers and animals must be vaccinated against various zoonotic diseases. The rules of order and hygiene must be taken into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of workers, and a separate toilet in each household to facilitate personal hygiene. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.
Sevinç (1972) reported in her study in this region that collected the solutions of the problems of nomadic ovine livestock in 5 items in general and said that it is necessary;
-To protect the pasture and meadow vegetation in the winter lands with the highland and highland slopes and to take continuous measures to ensure their development,
-To identify and secure migration routes in the most convenient way,
-To provide a continuous and secure market order for migratory sheep breeders,
-To improve and increase the efficiency of the nomadic ovine animals with effective measures compatible with the lives of migratory ovine livestock families,
-To bring order to a structure that will modernize ovine livestock within a certain period of time for migratory sheep breeders. Unfortunately, it can be said that these problems are still not resolved today.
One of the most important problems of the immigrants in the region is undoubtedly related to the education of our children, especially girls, which is emphasized in every platform that is important in the future of the country. Children can always start their education life one step behind. Girls do not even have such a chance, in this way of life.

Conclusion

In this paper, the nomadic activities and the production habits of breeders in sheep and goat production have been discussed in Muş province of Turkey. This information has been prepared based on the personal observations and the experiences directly in local area. Muş province has ecological conditions suitable for sheep and goat presence and animal production. If the current potential especially in Muş province is evaluated, it can become very important in Eastern Anatolia Region of Turkey. It is hoped that it has now been understood by the state that migratory breeders, which have been neglected in many ways for many years, are people engaged in animal production in the Eastern Anatolia Region of Turkey. The results indicate that solving the problems of nomadic and semi-nomadic families is very important to sustain stockbreeding of sheep and goats, and to benefit Muş province economically.

References

Anonymous (2021). Muş geographical location (Muş coğrafi konumu). https://en.wikipedia.org/wiki/Mu%C5%9F_Province.

Aşkan R., Aygün T. (2020). Determination of milk yield traits and some components of milk in Akkaraman crossbred ewes maintained at rural conditions. Journal of Animal Production, 61(1): 23-31.

Aygün T. (2017). The Occupational Health and Safety for Berivans at Zoma Life in Hakkâri Province (Hakkâri ilindeki zoma yaşamında berivanların sağlığı ve iş güvenliği). Uluslararası Katılımlı 1. Tarım ve Gıda Etiği Kongresi, 10-11 Mart 2017, 163-164, Ankara Üniv. Ziraat Fakültesi, Ankara.

Aygün T., Erkan C., Çelikyürek H. (2019). Occupational health and safety culture for berivans and shepherds in small ruminant husbandry. International Agricultural Congress of Muş Plain Proceedings Book, Cilt ISBN: 9786055137052, 24-27 September 2019, Muş, Turkey, s: 491-497.

İnan İ., Aygün T. (2019). The body weight after shearing and the greasy wool yield of Red Karaman ewes at different raising conditions in Turkey. Journal of Advanced Agricultural Technologies, 6(2): 139-143.

Kaymakçı M. (2010). *Advanced Sheep Husbandry" "İleri Koyun Yetiştiriciliği,"* Ege University Press, Third Edition, Bornova, İzmir, Turkey.

Mutaf S., Aklan S., Seber N. (2001). Hayvan Barınaklarının Projelendirme İlkeleri ve GAP Yöresi için Uygun Barınak Tipleri (Design principles of animal shelters and shelter types suitable for GAP region). TMMOB Makine Mühendisler Odası, II. GAP ve Sanayi Kongresi, 29 - 30 Eylül, Diyarbakır.

Öztürk Y., Odabaşı F. (2011). The investigation of yield and morphological characteristics of Hamdani sheep in Van area: I. Various yield characteristics of sheep (Van ve yöresinde Hamdani koyunlarının verimleri ve morfolojik özelliklerinin araştırılması: I. Koyunların çeşitli verim özellikleri). Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi, 2(2): 75-80.

Sevinç A. (1972). The main problems and solutions of nomadic animal husbandry (Göçer hayvancılığın başlıca sorunları ve çözüm yolları). Verimlilik Dergisi, 1(4):740-748.

Şeker İ., Köseman A. (2015). Cattle, sheep and goat breeding activity in Elazığ (Elazığ ilinde büyükbaş ve küçükbaş hayvancılık faaliyetleri). Harran Üniv. Vet. Fak. Derg., 4(1): 36-44.

TOB (2021). Ministry of Agriculture and Forestry: Livestock Breeding (Cattle Husbandry) (Tarım ve Orman Bakanlığı: Büyükbaş Hayvancılık) (Sığırcılık). https://www.tarimorman.gov.tr/HAYGEM/Belgeler/Hayvanc%C4%B1l%C4%B1k/B%C3%BCy%C3%BCkba%C5%9F%20Hayvanc%C4%B1l%C4%B1k/2019%20Y%C4%B1l%C4%B1/Buyukbas_Hayvan_Yetistiriciligi.pdf.

COMPARISON OF EGGS FROM DIFFERENT EGG PRODUCTION SYSTEMS IN TERMS OF CAMPYLOBACTER

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Abstract

One of the most important food-related pathogens worldwide is Campylobacter. The aim of this study was to compare eggs obtained from different egg production systems in terms of *Campylobacter*. In the study, four different egg production systems were used: Conventional cage system, enriched cage system, barn system and free-range system. In the conventional system, the stocking density is set to 600 cm^2/hen. The enriched cages contain that the hens must have at least 750 cm^2 of floor space per hen, a nest, perches, and litter. In the free-range system, seven animals per square meter were housed in the indoor area. In outdoor, an area of 4 m^2 is set for each animal. In the free system, there are walking areas where animals can stay in the open area for at least 8 hours. Housing hens in non-cage alternative systems, such as free-range, floor systems, and aviary systems, allow them to express their natural behaviors with more freedom of movement. The hens were fed with 18% HP and 2800 kcal ME/kg during the experiment. In the study, there were four experimental groups, and each experimental group were formed from four replications. Twenty chickens in each replication and eighty chickens in each group were used. ATAK-S, one of our domestic hybrids, was used as animal material. *Campylobacter spp*. enumeration were made in 5 randomly taken eggs from each group for a total of 2 times, at the 34^{th} and 36^{th} weeks of the laying period. *Campylobacter* was not detected in any of the eggs obtained from all egg production systems in both periods. As a result, it is clear that there will be no problems for humans in different egg production systems in terms of *Campylobacter*.

Keywords: *Conventional cage, enriched cage, barn systems, free-range system, Campylobacter.*

Introduction

In many countries of the world, the cage rearing system is dominant in the rearing of layer hens and this situation will probably continue for a long time. The reason for this is that raising chickens in cages is the most economical mode of production, the price of eggs is low and the product reaches all segments of the society (Rakonjac *et al*., 2017). Conventional cage egg production has been banned in European Union countries since 2012 and egg production has been suggested to be done in alternative systems (EU, 1999). One of these production systems is the free-range system.

Campylobacteriosis is the most frequent zoonotic disease in humans worldwide (Ghareeb *et al*., 2013). The case of human campylobacteriosis, which is characterized by watery and bloody diarrhea in humans, occurs in approximately 2-5 million cases in humans in the United States (Friedman *et al*., 2000).

Cases of campliobacteriosis in humans can generally occur with consumption of raw poultry meat, contact with pets, consumption of raw milk and meat, and water (Friedman *et al*. 2000; Jacobs-Reitsma 2000; Corry and Atabay 2001; Park 2002). While Jones and Musgrove (2007) found thermotolerant Campylobacter infection in 1.1% of eggshells, Sato and Sashihara (2010) reported that 27.9-36% of pasteurized liquid eggs were positive for Campylobacter.
The aim of this study is to compare eggs obtained from different egg production systems in terms of *Campylobacter strains*.

Material and Methods

This study was carried out at Selcuk University, Agriculture Faculty, Department of Animal Science. In the study, four different egg production systems were used: Conventional cage system, enriched cage system, barn system and free-range system. In the study, there were four experimental groups, and each experimental group were formed from four replications. *Campylobacter* spp. was counted in 5 eggs randomly taken from each group 2 times, in the 34^{th} and 36^{th} weeks of the egg production period. A total of 40 eggs were used in microbiological analyses.All egg samples kept for pre-enrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 8 hours at 35 °C was diluted serially in pre-sterilized 9 ml ringer solutions. 0.1 mL of inoculum of each dilution was spread onto the petri dishes containing Campylobacter Selective Agar LAB112 supplemented with X112 (LABM, UK) and Horse Blood Lysed (Liofilmchem, Italy). Petri dishes were incubated at 37 °C for 48 hours using the microaerophilic media provided by Anaerocult C (Merck KGaA, Darmstadt, Germany). The biochemical verification tests were applied to white and beige colonies at the end of the incubation and the results were given.
Analysis of variance was considered if parametric test assumptions were met in terms of *Campylobacter spp*. numbers detected in the experimental groups.

Result and Discussion

Campylobacter spp. load of eggs produced in different egg production systems is given in Table 1. Campylobacter spp. was not detected in any of the eggs obtained from the production systems. Normally, it is stated that the microbial load of the shell of eggs produced in the free-range system, which is one of the alternative egg production systems, is higher than in other systems. But according to the results of our study, it is important that Campylobacter, a pathogenic microorganism for humans, is not detected in the eggshell. Sahin et al. (2003) did not detect Campylobacter in any of the broiler breeder egg samples produced at different ages. But they found Campylobacter spp. in the feces of animals of the same age. Park (2002) stated that Campylobacter has a high sensitivity to dry environment and oxygen and that it is quite difficult for it to stay alive in an oxygenated environment. The absence of campylobacter in our study may probably be due to the ovulation of eggs into the follicles and the environment is high in oxygen.

Table 1. *Campylobacter spp* burden of eggs produced in different egg production systems.

Groups	Period 1	Period 2
G	TE	TE
Z	TE	TE
K	TE	TE
S	TE	TE
SE	-	-
P-Value	-	-

G: Conventional cage system, Z: enriched cage system, K: barn system, S: free-range system

Baker *et al.* (1987) reported that no *Campylobacter* spp. was found from eggs obtained from 23 farms. Similarly, it is consistent with studies indicating that no *Campylobacter* was detected in the shell of eggs obtained from farms (Acuff *et al*., 1986; Izat and Gardner, 1988). On the other hand, Doyle (1984) found *Campylobacter* in only 2 eggs of 226 eggs. He also stated that he did not detect *Campylobacter* in feed and drinking water, and that feed and water could not be the source of *Campylobacter* spp.

Conclusion

According to the results of our study, no *Campylobacter* was detected in eggs produced in the free filling system. In this case, there is no transmission in terms of campylobacter, which can be a problem for humans in the eggs produced in this system.

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References

Acuff G. Vanderzant C., Hanna M., Ehlers J; Gardner, F. (1986). Effects of handling and preparation of turkey products on the survival of Campylobacter jejuni, Journal of Food Protection, 49/8, 627-631.

Baker RC., Dulce M., Paredes C; Qureshi, RA. (1987). Prevalence of Campylobacter jejuni in eggs and poultry meat in New York State, Poultry Science, 66/11, 1766-1770.

Corry J.E., Atabay H.I. (2001) Poultry as a source of Campylobacter and related organisms. Journal of Applied Microbiology 90, 96S–114S.

Doyle MP. (1984). Association of Campylobacter jejuni with laying hens and eggs, Applied and Environmental Microbiology, 47/3, 533-536.

Friedman C. R., Neimann J., Wegener H.C, Tauxe R.V. (2000) Epidemiology of C. jejuni infections in the United States and other industrialized nations. In Campylobacter, 2nd edn. ed. Nachamkin, I. and Blaser, M.J. pp. 121–1m38. Washington, DC: ASM Press.

Ghareeb K., Awad W. A., Mohnl M., Schatzmayr G., Boehm J. (2013). Control strategies for Campylobacter infection in poultry production. World's Poultry Science Journal, 69/1, 57-76.

Izat, A, Gardner, F. (1988). Incidence of Campylobacter jejuni in processed egg products, Poultry science, 67/10, 1431-1435.

Jacobs-Reitsma, W. (2000) Campylobacter in the food supply. In Campylobacter, 2nd edn. ed. Nachamkin, I. and Blaser, M.J. pp. 467–481. Washington, DC: ASM Press.

Jones, D, Musgrove, M. (2007). Pathogen prevalence and microbial levels associated with restricted shell eggs, Journal of food protection, 70/9, 2004-2007.

Park S.F. (2002) The physiology of Campylobacter species and its relevance to their role as foodborne pathogens. International Journal of Food Microbiology 74, 177–188.

Sahin O., Kobalka P; Zhang Q. (2003). Detection and survival of Campylobacter in chicken eggs, Journal of Applied Microbiology, 95/5, 1070-1079.

Sato, M, Sashihara, N. (2010). Occurrence of Campylobacter in commercially broken liquid egg in Japan, Journal of food protection, 73/3, 412-417.

MODELING OF GROWTH IN JAPANESE QUAILS CATEGORIZED BY EGG WEIGHT

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Abstract

Egg weight shows great variation in poultry, and it is known that there is a positive and strong relationship between egg weight and live weight. In this study, it was aimed to compare the hatching egg weight groups in terms of weekly body weights and Gompertz growth curve parameters in Japanese quails. A total of four egg weight groups were formed in the study. Accordingly, the eggs obtained from the breeding flock were classified according to their weight as being less than 8 g (TRT1), 8-10 g (TRT2), 10-12 g (TRT3) and heavier than 12 g (TRT4). In the study, it was determined that the weight of Japanese quails increased throughout the week, including hatching, in parallel with the increase in hatching egg weight, and the differences between the group averages in terms of egg weight were significant. Similar to weekly body weight values, differences were observed between the groups in terms of asymptotic body weight parameter of the Gompertz growth curve model and weight averages of the curve inflection point ($P<0.05$ for both traits). In terms of β_1 and β_2 parameters, the difference was found to be insignificant. As a result, it is possible to obtain faster growing and heavier birds from high weight hatching eggs.

Keywords: *Hatching eggs, Gompertz, Growth curve, Nonlinear regression, Quail.*

Introduction

Meat and eggs obtained from poultry are the cheapest products that meet the basic nutritional needs of people. These products are consumed intensively because they have enough ingredients needed for public health and contain essential nutrients in a good way. With this supply, poultry production has increased significantly throughout the world in the last 30 years. This has led to an increase in the production not only of chicken species, but also of species such as goose, duck, partridge, ostrich, and quail. The Japanese quail (*Coturnix coturnix japonica*), which is among these species, is used as a model animal in both commercial production and scientific studies. Japanese quails are disease resistant birds that can be easily raised in small areas, are inexpensive to house and feed. Japanese quails reach sexual maturity in about 6 weeks and are usually used for full egg production at 50 days of age. The growth rate in Japanese quails is also quite high (Haqani et al., 2021; Yavuz et al., 2019; Üçkardeş and Narinç 2014; Karaman et al., 2013; Karadavut et al., 2017). With proper breeding, 250 eggs can be obtained from female quails in a year. If quails have not been subjected to any selection to change their body weight, adult male quails weigh about 100-140 g, while females weigh 120-160 g. With domestication, cage adaptation and intensive production, these weight values are higher today. Japanese quails raised for commercial meat production are usually slaughtered at the age of 5-6 weeks and their carcass yield is between 65-70% (Narinç et al., 2014). As in many countries, quails raised for meat purposes in Turkey are priced as pieces in the market. As a natural consequence of this marketing system, producers want to slaughter at an earlier age to increase profitability, while consumers prefer larger carcasses. Extending the fattening period to obtain heavier carcasses from low body weight genotypes leads to undesirable results such as an increase in inedible parts and excessive fattening due to the development of genital organs (Minvielle, 2009). This situation means economic loss for the producer and poor-quality product for the consumer (Narinç and Aksoy, 2012). It is known that chick hatching weight increases due to the increase in hatching egg weight in poultry of different species (Yannakopoulos and Tserveni-Gousi, 1987; Pinchasov, 1991). In various studies with quails, positive and strong relationships were found between hatching egg weight and hatching weight (Skewes et al. 1988; Uddin et al. 1994; Yıldırım and Yetişir, 1998). In this case, if the production starts with chicks obtained from high-weight eggs, high slaughter weight can be obtained in a short fattening period. In this study, it was aimed to compare the hatching egg weight groups in terms of weekly body weights and Gompertz growth curve parameters in Japanese quails.

Material and Methods

The experiment was carried out in quail coops located in the Animal Husbandry Facilities of Akdeniz University, Faculty of Agriculture, Department of Animal Science (Antalya, Turkey). The animal material of the study consisted of 475 randomly mated quail chicks obtained from a total of 600 eggs collected simultaneously from a previously unselected parent flock. A total of four egg weight groups were formed in the study. Accordingly, the eggs obtained from the breeding flock were classified according to their weight as being less than 8 g (TRT1), 8-10 g (TRT2), 10-12 g (TRT3) and heavier than 12 g (TRT4). Each treatment group was divided according to egg weights, and one-day-old chicks were assigned a wing number, and all yield records were followed individually until the end of the experiment. Individual live weights of quails were weighed weekly. One-day-old chicks were housed in brooder cages in the chick rearing room with a placement frequency of 75 cm^2/quail from hatching to sex determination at the third week. The chicks were housed at 32 °C for the first three days and lowered by 1 °C every three days to provide the in-house temperature of 27 °C at the end of the second week. The quails were fed ad libitum powder compound feed containing 24% HP and 2900 kcal/kg ME throughout the experiment. After the third week, the quails were transferred to 5-storey colony cages with five compartments on each floor, and they were housed in these cages with a placement frequency of 220 cm^2/quail until the end of the experiment.

The Gompertz growth model was used in the nonlinear analysis of the growth pattern of quails (Akbaş and Oğuz, 1998; Alkan et al., 2009). The Gompertz growth curve model is $y=\beta_0\exp(-\beta_1\exp(-\beta_2 t))$. In the model, "t" represents time and "y" represents live weight. Among the model parameters, β_0, adult (asymptotic) weight; β_1 is the integration constant; β_2 represents the instantaneous growth rate (Narinç et al. 2010). The inflection point weight ($BNA=\beta_0/e$) and the inflection point age ($BNY=\ln(\beta_1)/\beta_2$) of the model were calculated. The estimation of the model parameters was carried out by the Levenberg-Marquardt iteration method using the NLIN procedure of the SAS 9.3 program (Ricklefs, 1985; SAS Ins, 2011).

Results and Discussion

In the study, weekly live weight averages and statistical analysis results of quails in the experimental groups are presented in Table 1. According to this, it was determined that egg weight categories and weekly live weights increased in parallel throughout all weeks starting from hatching weight. These results are in agreement with the results of Tserveni-Gousi and Yannakopoulos (1990), Tufft and Jensen (1991), Yannakopoulos and Tserveni-Gousi (1987) in broiler chickens; Tserveni-Gousi (1987), Uddin et al. (1994); Yannakopoulos and Tserveni-Gousi (1987), Skewes et al. (1988) in quail. These results contradict with the reports of Laskey and Edens (1985), Pinchasov (1991), Yıldırım and Yetisir (1998) that the heavy chicks at hatching could not maintain these advantages.

Table 1. The average values of weekly body weights of treatment groups and results of statistical analyses

Treatment	BW0	BW7	BW14	BW21	BW28	BW35	BW42
TRT1	5.26^{d}	17.82^{c}	31.19^{c}	48.80^{c}	77.24^{c}	108.11^{c}	131.12^{d}
TRT2	6.65^{c}	20.31^{b}	37.81^{b}	59.57^{b}	95.78^{b}	127.61^{b}	155.01^{c}
TRT3	7.90^{b}	19.51^{b}	37.12^{b}	55.65^{b}	96.61^{b}	132.57^{b}	165.58^{b}
TRT4	8.94^{a}	22.68^{a}	44.94^{a}	66.50^{a}	110.19^{a}	148.73^{a}	178.78^{a}
SE	0.06	0.39	0.80	1.04	1.40	1.62	1.62
P Value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TRT: Treatment, BW: Body weight, SE: Standard error

Table 2. The average values of parameters and inflection points of Gompertz growth curve model and results of statistical analyses

Treatment	β_0	β_1	β_2	IPW	IPT
TRT1	202.45^{d}	3.87	0.051	74.70^{d}	26.62
TRT2	212.91^{c}	3.93	0.058	78.56^{c}	23.77
TRT3	256.28^{b}	4.11	0.052	94.57^{b}	27.34
TRT4	269.63^{a}	3.84	0.052	99.50^{a}	25.75
SE	4.12	0.21	0.001	1.56	0.57
P Value	0.000	0.245	0.365	0.000	0.124

TRT: Treatment, β: Gompertz model parameter, SE: Standard error; IPW: Weight of inflection point, IPT: Time of inflection point

The average values of parameters and inflection points of Gompertz growth curve model and results of statistical analyses are shown in Table 1. The growth curves drawn according to the experimental groups are also presented in Figure 1. When the results were examined, differences were observed between the groups in terms of the weight averages of the asymptotic body weight parameter (β_0) of the Gompertz growth curve model and weight averages of the inflection point of the curve, like the weekly body weight values ($P<0.05$ for both characteristics). In terms of β_1 and β_2 parameters, the difference was found to be insignificant. There is no study in the literature in which the growth of quails obtained from different egg weight classes are modeled.

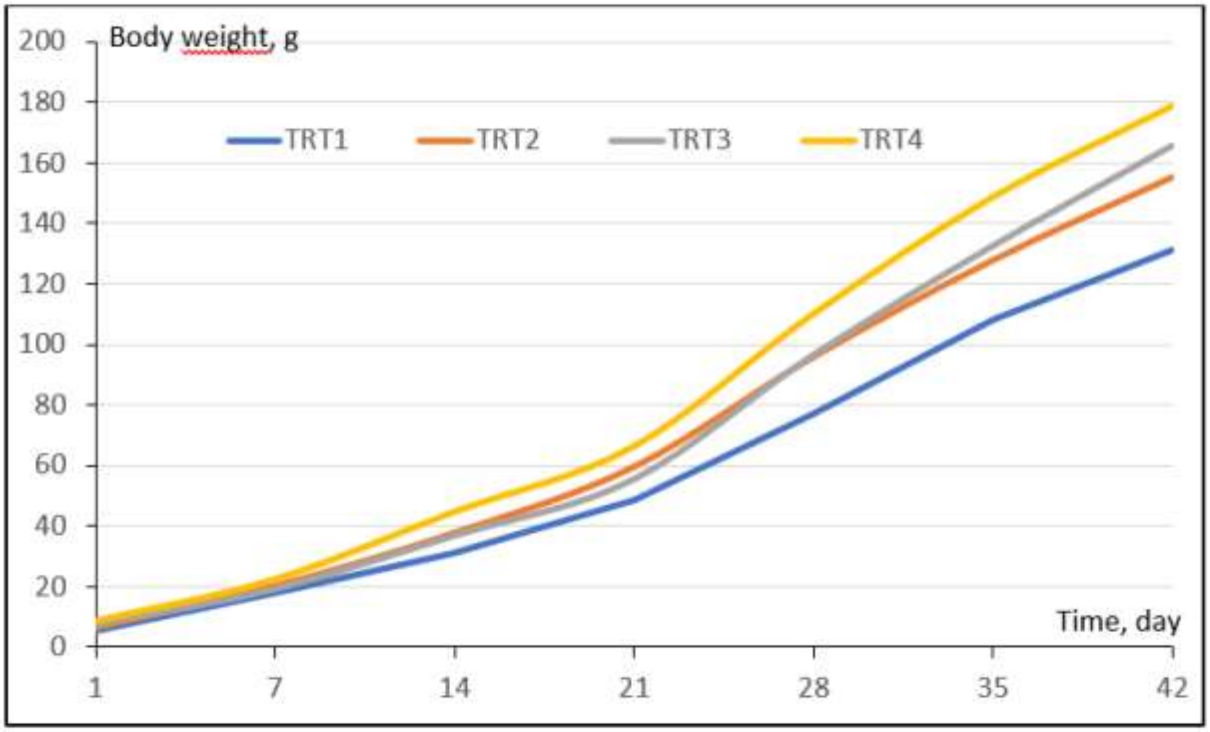

Figure 1. The growth curves of Japanese quails divided into different egg weight categories

Conclusion

As a result of this study, it was determined that hatching egg weight was effective on growth performance in Japanese quails. Considering the weekly live weights of Japanese quails, it was determined that quails hatched from eggs over 12 g reached higher body weight levels than quails hatched from smaller eggs. As a result, it is thought that it will be better to raise chicks to be obtained from high weight eggs.

References

Akbas, Y., & Oguz, I. (1998). Growth curve parameters of lines of Japanese quail (Coturnix coturnix japonica), unselected and selected for four-week body weight. Archiv fuer Gefluegelkunde (Germany).

Haqani, M. I., Kawamura, K., Takenouchi, A., Kabir, M. H., Nakamura, Y., Ishikawa, A., & Tsudzuki, M. (2021). A Growth Performance and Nonlinear Growth Curve Functions of Large-and Normal-Sized Japanese Quail (Coturnix japonica). The Journal of Poultry Science, 58(2), 88-96.

Karadavut, U., Taskin, A., & Genc, S. (2017). Comparison of growth curve models in Japanese quail raised in cages enriched with different colored lights. Revista Brasileira de Zootecnia, 46, 839-846.

Karaman, E., Narinc, D., Firat, M. Z., & Aksoy, T. (2013). Nonlinear mixed effects modeling of growth in Japanese quail. Poultry Science, 92(7), 1942-1948.

Laskey, JW. & Edens, FW. (1985): Hatch Weight Selection: Effect on Post-Hatch Growth in The Japanese Quail (*Coturnix coturnix japonica*), Comp. Biochem. Physol, A. 82 (1): 101-104.

Minvielle, F. (2009). What are quail good for in a chicken-focused world? World's Poultry Science Journal, 65(4), 601-608.

Narinç, D., & Aksoy, T. (2012). Effects of mass selection based on phenotype and early feed restriction on the performance and carcass characteristics in Japanese quails. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 18(3), 425-430.

Narinc, D., Karaman, E., & Aksoy, T. (2014). Effects of slaughter age and mass selection on slaughter and carcass characteristics in 2 lines of Japanese quail. Poultry Science, 93(3), 762-769.

Narinc, D., Karaman, E., Firat, M. Z., & Aksoy, T. (2010). Comparison of non-linear growth models to describe the growth in Japanese quail. Journal of Animal and Veterinary Advances, 9(14), 1961-1966.

Pinchasov, Y. (1991). Relationship between the weight of hatching eggs and subsequent early performance of broiler chicks. British Poultry Science, 32(1), 109-115.

Ricklefs, R. E. (1985). Modification of growth and development of muscles of poultry. Poultry Science, 64(8), 1563-1576.

Skewes, P. A., Wilson, H. R., & Mather, F. B. (1988). Correlations among egg weight, chick weight, and yolk sac weight in Bobwhite quail (*Colinus virginianus*). Florida Scientist, 159-162.

Tserveni-Gousi, AS. (1987): Relationship Between Parental Age, Egg Weight and Hatching Weight of Japanese Quail, Br. Poult. Sci. 1987 Dec; 28 (4): 749-752.

Tserveni-Gousi, AS. ve Yannakopoulos, AL. (1990): Quality characteristics of pheasant eggs and effect of egg weight and shell quality on chick weight, Arch Gefluegelkd, 54, 2, 54-56, 1990.

Tufft, LS. ve Jensen, LS. (1991): Effect of age of hen, egg weight, and sex on chick performance and lipid retention, Poult. Sci., 1991 Dec; 70 (12):2411-2418.

Uckardes, F., & Narinç, D. (2014). An application of modified Logistic and Gompertz growth models in Japanese quail. Indian Journal of Animal Sciences, 84(8), 903-907.

Uddin, M. S., Paul, D. C., & Huque, Q. M. E. (1994). Effect of egg weight and pre-incubation holding periods on hatchability of the Japanese quail eggs in different seasons. Asian-Australasian Journal of Animal Sciences, 7(4), 499-503.

Yannakopoulos, A. L., & Tserveni-Gousi, A. S. (1987). Research note: Effect of breeder quail age and egg weight on chick weight. Poultry Science, 66(9), 1558-1560.

Yannakopoulos, AL. ve Tserveni-Gousi, AS. (1987): Effect of Breeder Quail Age and Egg Weight on Chick Weight, Poult. Sci. 1987 Sep; 66 (9): 1558-1560.

Yannakopoulos, AL. ve Tserveni-Gousi, AS. (1987): Relationship of parents' age, hatching egg weight, and shell quality to day-old chick weight as influenced by oviposition time, Poult. Sci. 66 (5): 829-833.

Yavuz, E., Önem, A. B., Fahrettin, K., Çanga, D., & Şahin, M. (2019). Modeling of Individual Growth Curves in Japanese Quails. Black Sea Journal of Engineering and Science, 2 (1), 11-15.

Yildirim, İ., & Yetişir, R. (1998). Effects of hatching egg weight and parental age on the hatching weight and 6th week live weight in Japanese quail (*Coturnix coturnix japonica*). Turkish Journal of Veterinary and Animal Sciences, 22(4), 315-320.

THE EFFECT OF DIFFERENT PLANT VEGETATION IN FREE-RANGE SYSTEM ON EGGSHELL MICROBIAL LOAD

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Abstract

The aim of this study is to investigate the effect of different plants in the free-range system on eggshell microbiology. In the study, 3 different forage plants [(Trifolium repens+ lolium perenne (A), Lotus Corniculatus+ lolium perenne (G) and Medicago sativa + lolium perenne (Y)] were planted in the out-door area. In the study, a total of 12 pens (1.5 * 2m) were made and 20 chickens were randomly placed in each pen. ATAK-S layer hybrid was used as animal material. Total aerobic mesophilic bacteria (TAMB), total coliform, *E. coli* and *Salmonella* loads were determined. A total of 24 eggs (8 egg / group) were used in the study. TAMB values were found as 5.38 log cfu / g eggs, 5.18 log cfu / g eggs and 5.35 log cfu / g eggs in the A, G, and Y groups, respectively (P>0.05). Total coliform values were found as 3.47 log cfu / g eggs, 1.48 log cfu / g eggs and 4.16 log cfu / g eggs in the A, G, and Y groups, respectively (P>0.05). *E. coli* values were found 2.47 log cfu / g eggs, 1.41 log cfu / g eggs and 2.89 log cfu / g eggs in the A, G, and Y groups, respectively (P>0.05). According to the results obtained from our study, it was seen that different plant vegetation in the out-door area did not have a significant effect on the eggshell microbial load.

Keywords: *Coliform, E. Coli, Free-range, Out-door area, Plant vegetation, TAMB.*

Introduction

In many countries of the world, the cage rearing system is dominant in the rearing of layer hens and this situation will probably continue for a long time. The reason for this is that raising chickens in cages is the most economical mode of production, the price of eggs is low and the product reaches all segments of the society (Rakonjac et al., 2017). Conventional cage egg production has been banned in European Union countries since 2012 and egg production has been suggested to be done in alternative systems. One of these production systems is the free-range system. There are studies that show that the eggshell microbial load of eggs produced in the alternative system is greater than that produced in conventional cage systems. (De Reu et al., 2008; Parisi et al., 2015). Parisi et al. (2015) stated that they found an average of 1.0 log CFU/mL more in eggs produced in the Free-range system than caged eggs. Jones et al. (2011) stated that Free-range eggs had the greater levels of shell emulsion coliforms, as well as yeast and mold, compared with Conventional cage eggs. Similarly, Galvao et al. (2018) reported that the average enterobacteria count (log $CFUmL^{-1}$) was 0.09 for conventional and 1.73 for free-range systems. Although there is no requirement for vegetation in the out-door area in the free-range system, it can be advantageous for animals to perform their natural behavior. In the free-range system, animals need to have green plants in the out-door area in order to continue their well-being and healthy lives. In this study, it was aimed to compare different plant varieties in the out-door area in terms of eggshell microbial load.

Material and Methods

The research carried out at the Faculty of Agriculture, Selcuk University. In the study, 3 different forage plants [(*Trifolium repens+ lolium perenne* (A), *Lotus Corniculatus+ lolium perenne* (G) and *Medicago sativa + lolium perenne* (Y)] were planted in the out-door area. Each group is formed from 4 subgroups. Egg samples were taken when the animals were thirty-two weeks old. Total aerobic mesophilic bacteria, coliform, coli and salmonella were analyzed in 8 eggs taken from each. Egg samples were transferred aseptically to sterile sampling bags (LP Italiana SPA, Milano, Italy) then this contents that will be subjected to microbial analysis were kept about 30 min. with 50 mL of sterile phosphate buffer saline (PBS) (Merck KGaA, Darmstadt, Germany). Ten-fold serial dilutions of the samples were prepared using sterile PW (pH 7.0 ± 0.2, Merck KGaA, Darmstadt, Germany). Total mesophilic aerobic bacteria were enumerated by using Compact Dry TC (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) and petri dishes were incubated at 30°C for 24-48 hours. Total coliform bacteria and *Escherichia coli* number were counted by using Compact Dry EC (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) and petri dishes were anaerobically incubated at 37°C for 24-48 hours by means of Anaerocult A (Merck KGaA, Darmstadt, Germany). The results were calculated as the means of log colony forming units per g sample (log CFU/g sample) of the eggshells. For *Salmonella* spp. detection, Buffered Peptone Water (BPW) (Merck KGaA, Darmstadt, Germany) was used as pre-enrichment broth and appropriate dilutions from this media were pippetted on Compact Dry SL (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan), then these petri dishes were incubated at 37ºC for 24-48 h. Parametric test assumptions were met to compare the numbers of different microorganisms detected in the eggs obtained from the A, G and Y experimental groups, and then variance analyzes were performed.

Result and Discussion

The effect of different forage plant in the out-door area on the TAMB, coliform, *E. coli* and *Salmonella* spp. load of the eggshell is given in Table 1. In terms of TAMB counts, the differences between groups were not statistically significant. TAMB counts were determined as 5.38 log cfu/g eggs in A group, 5.18 log cfu/g eggs in G group and 5.35 log cfu/g eggs in Y group. TAMB numbers were 5.38 log kob/g eggs in group A, 5.18 log kob/g eggs in group G and 5.35 log kob/g eggs in Y group. Samiullah et al. (2014) determined the TMAB in eggs produced in the free-range system between 2.50 and 4.39 log cfu/egg.

Table 1. The effect of different forage plant in the out-door area on the eggshell microbial activity (Log Cfu/g egg; $\bar{X}$)

Group	TMAB	Coliform	E. coli	Salmonella
A	5.38	3.47	2.37	-
G	5.18	1.48	1.41	-
Y	5.35	4.16	2.89	1/8
SEM	0.242	0.882	0.851	-
P-value	0.833	0.138	0.485	-

A: *Trifolium repens+ lolium perenne*, G: *Lotus Corniculatus+ lolium perenne*, Y: *Medicago sativa + lolium perenne*

Total coliform numbers were determined as 3.47 log cfu/g eggs in A group, 1.48 log cfu/g eggs in G group and 4.16 log cfu/g eggs in Y group Y, and the differences between the groups were statistically insignificant. Jones et al. (2011) found that the number of coliforms in eggs produced in a free-range system was 4.31 to 5.36 log cfu/g eggs.

E. coli counts were determined as 2.37 log cfu/g eggs in A group, 1.41 log cfu/g eggs in G group and 2.89 log cfu/g eggs in Y group, and the differences between the groups were statistically insignificant. *E. coli* is one of the main food pathogens and can be transmitted to people due to the cross contamination when food products are insufficient and during the meal preparation (Adeboye et al., 2020). Samiullah et al. (2014) found the total number of *Enterobacteriaceae* in the free-range system between 0.84 and 2.10 log cfu/egg. Tomar et al., (2021) detected coliform in 21% of eggs obtained from chickens raised in the free-range system.

Only *Salmonella* has been identified in one of the eight eggs analyzed in the Y group. In this regard, Tomar et al. (2021) detected *Salmonella* in 20% of eggs produced in the free-circulation system. Samely, Chousalkar et al. (2016) stated that eggs produced in the free-range system were contaminated with *Salmonella* between 5 and 7.5%.

Conclusion

According to the results obtained from our study, it was seen that different plant vegetation in the out-door area did not have a significant effect on the eggshell microbial load.

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References

Adeboye, O. A., Kwofie, M. K., & Bukari, N. (2020). Campylobacter, Salmonella and Escherichia coli Food Contamination Risk in Free-Range Poultry Production System. Advances in Microbiology, 10(10), 525-542.

Chousalkar, K., Gole, V., Caraguel, C., & Rault, J. L. (2016). Chasing Salmonella Typhimurium in free range egg production system. Veterinary microbiology, 192, 67-72.

De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T. B., Uyttendaele, M., & Herman, L. (2008). Bacterial contamination of table eggs and the influence of housing systems. World's poultry science journal, 64(1), 5-19.

Galvão, J. A., Biondo, A. W., Possebon, F. S., Spina, T. L. B., Correia, L. B. N., Zuim, C. V., & Pinto, J. P. D. A. N. (2018). Microbiological vulnerability of eggs and environmental conditions in conventional and free-range housing systems. Semina: Ciências Agrárias, 39(1), 133-141.

Jones, D. R., Anderson, K. E., & Musgrove, M. T. (2011). Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management. Poultry science, 90(9), 2063-2068.

Parisi, M. A., Northcutt, J. K., Smith, D. P., Steinberg, E. L., & Dawson, P. L. (2015). Microbiological contamination of shell eggs produced in conventional and free-range housing systems. Food Control, 47, 161-165.

Rakonjac, S., Bogosavljević-Bošković, S., Škrbić, Z., Perić, L., Dosković, V., Petrović, M. D., & Petričević, V. (2017). The effect of the rearing system, genotype and laying hens age on the egg weight and share of main parts of eggs. Acta Agriculturae Serbica, 22(44), 185-192.

Samiullah, S., Omar, A. S., Roberts, J. R., & Chousalkar, K. (2014). Effect of production system and flock age on egg quality. In Proceedings of the 27th Annual Australian Poultry Science Symposium, Sydney, New South Wales (pp. 133-136).

Tomar, O., Akarca, G., Istek, Ö., & Ataseven, M. (2021). Investigation of quality properties of chicken eggs collected periodically from different poultry systems depending on the season. Journal of Food Processing and Preservation, 45(3), e15225.

AVIAN HERPESVIRUSES AND THEIR POTENTIAL IMPACTS ON POULTRY HEALTH

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Abstract

Herpesviruses can infect all animals and poultry. 19.8 billion eggs and 2.2 million tons of poultry meat were produced in Turkey in 2020. Due to high production capacity, avian herpesviruses (AHVs) have a significant economic and ecological impact potential on poultry health in Turkey and around the world. AHVs are group of pathogens that affect most species of domesticated and wild birds and belongs to Alphafaherpesvirinae. Gallid alphaherpesvirus-1 (GaHV-1) causes infectious laryngotracheitis (ILT). It is potentially fatal and widely recognized as one of the most contagious chicken disease. Transmission occurs through respiratory tract. ILT can affect all birds, including poultry, broilers, free-range, commercial laying hens and wild birds. ILT tends to occur more frequently in areas with heavy broiler production. Marek's disease virus (MDV) agent Gallid alphaherpesvirus-2 (GaHV-2) is highly contagious, characterized by tumors, unilateral leg paralysis and visual disturbances in poultry. Despite having a very protective vaccine, it can cause significant economic losses in laying hens. Infection transmit by respiration. Psittacid herpesvirus-1 (PsHV-1) is the agent of Pacheco's disease. It is highly contagious and affecting exotic species, particularly in parrots of high economic value. Due to high mortality rate, it is a concern of health for domestic bird markets and breeders. Most of the herpes virus infections of poultry are persistent in individuals and are ubiquitous in populations, and the viruses can be scattered for long periods after infection. Prevention and control of AHV infections mainly include improved biosecurity measures, healthy and young poultry production, coordinated rapid diagnostics, and vaccination.

Keywords: *Avian herpesviruses, Gallid alphaherpesvirus, Infectious laryngotracheitis, Mareck Disease, Pacheco Disease.*

Introduction

Avian herpesviruses (AHVs) are group of pathogens that affect most species of domesticated, captive and wild birds. The herpesviridae virus family consists of alpha, beta and gammaherpesvirinae subfamilies. A large number of avian herpesviruses from eight orders have been identified to date. Six of these have importance in veterinary medicine; infectious laryngotracheitis virus (ILT), Marek's disease virus (MDV), Pacheco's parrot disease virus (PsHV-1), Columbid herpesvirus-1 or Pigeon herpesvirus (PIHV) and duck plague virus or duck viral enteritis (DEV) causes infections in avian. Herpesvirus of turkeys (HVT) is nonpathogenic in Turkeys and chickens, however, it is known to be effective in the formation of protective immunity against MDV1 and hence it is used in vaccine studies (Biggs, 1982; Kaleta, 1990; Towazewski *et al*., 2001; Witter, 1972). The Committee on Taxonomy of Viruses (ICTV) has classified them all taxonomically as members of the Alphaherpesvirinae subfamily. Most herpes viruses from wild, captive and domesticated birds are tentatively classified as betaherpesvirinae and Avian beta- or gamma herpesviruses have not been identified precisely yet (Alfonso *et al.*, 2001; Biggs, 1982; Kaleta, 1990; Towazewski *et al*., 2001; Trapp and Osterreider, 2008). AHVs are DNA viruses and belonging to the herpesvirus family. Herpesviruses share a common structure; they contain a relatively large, monopartite, double-stranded, linear DNA genome encoding 100-200 genes, surrounded by the capsid, which is an icosahedral protein structure, viral mRNAs, and a bilayer lipid envelope (Biggs, 1982; Kaleta, 1990; Murphy *et al.,* 1999; Pirbright, 2021; Towazewski *et al*., 2001; Trapp and Osterreider, 2008). Gallid alphaherpesvirus-1 (GaHV-1) was first described in 1926 and causes infectious laryngotracheitis (ILT) (May and Tittsler 1925).

It is potentially fatal and widely recognized as one of the most contagious diseases of chickens. Marek's disease virus (MDV) agent Gallid alphaherpesvirus-2 (GaHV-2) is highly contagious, characterized by tumors, unilateral leg paralysis and visual disturbances in poultry. Psittacid herpesvirus-1 (PsHV-1) is the agent of Pacheco's disease. It is highly contagious and affecting exotic species, particularly in parrots of high economic value. Columbid herpesvirus-1 or Pigeon herpesvirus (PIHV), cause infections in pigeons (Tomaszewski *et al.,* 2001). Herpesviruses known to cause inclusion body hepatitis and splenitis in wild birds such as falcons and owls were reported to be identical to Columbid herpesvirus-1. Owls, like other raptors, are abnormal hosts for this alphaherpesvirus, and the infection is usually courses in a subclinical form. It is known that wild birds fed with infected pigeons are getting the disease in this way (Beaufrère and Laniesse, 2016; WHA, 2012). Duck enteritis virus (DEV) causes duck viral enteritis (DVE) or duck plague and DVE is one of the main contagious and deadly diseases of ducks, geese and swans (Dhama et al., 2017). Herpesvirus of turkeys (HVT) is a non-pathogenic virus of domestic turkeys and can be found in all poultry environments. It is antigenically and genetically classified as the third serotype within the Marek's disease virus (MDV) group of avian herpes viruses (Alfonso *et al.,* 2001; Callnek and Writer, 1991; Witter, 1972).

Poultry meat production amongst the world became the most produced in 2015. Poultry meat and egg production reaches to 132 and 90 million tons respectively at 2019 and represented 39% percent of global meat production. According to the forecasts of FAO, while the total meat amount will reach 358.9 million tons in 2025, the amount of poultry meat will maintain its position as the most produced meat with a share of 37.8 percent with 135.8 million tons (FAO, 2021; TUIK, 2021; Türktarım, 2021; ZMO, 2021). It was reported that 19.8 billion eggs and 2.2 million tons of poultry meat were produced in Turkey in 2020 and ranked 8th in production worldwide. Due to high production capacity, avian herpesviruses (AHVs) have a significant economic and ecological impact potential on poultry health in Turkey and around the world (TUIK, 2021; Türktarım, 2021; ZMO, 2021). This review focuses on avian herpes viruses, especially the infectious laryngotracheitis virus and Marek's disease virus, which are significantly important for poultry health and poultry production.

Infectious Laryngotracheitis (ILT)

Infectious laryngotracheitis (ILT) is a highly contagious avian herpes virus infection caused by Gallid alpha herpesvirus type 1 (GaHV-1), commonly known as infectious laryngotracheitis virus (ILTV) and is economically important for poultry health and poultry production worldwide. In studies conducted after it was first discovered in 1925, it has been reported that the disease is occasionally seen in some poultry enterprises in Turkey (Bagust *et al.,* 2000; MSD, 2021; Kahn, 2005; Kaleta, 1990; May and Tittsler 1925; Murphy *et al.,* 1999; Trapp and Osterreider, 2008). Infectious laryngotracheitis agent, ILT belongs to Alphaherpesvirinae subfamily and representing all the morphological characteristics of alphaherpesviruses. The disease is mostly observed in adult animals and laying hens of any age can be infected, commonly progressing in acute, subacute and chronic forms, characterized by respiratory system inflammation, cough, respiratory distress and conjunctivitis. Roosters and heavy breeds are known as more susceptible to the disease. The mortality rate varying between 10-30%. Morphologically electron microscopy of chicken embryo cell cultures infected with ILT virus represented that the virus resembled the Herpes simplex virus structure. The virus structure is cuboidal and the viral particles are in icosahedral formation. Virions are in hexagonal structure and about 80-100 mm in diameter. The whole virus particle has an irregular envelope membrane surrounding the nucleocapsid and is 95-250 mm in diameter (Bagust *et al.,* 2000; Callnek and Writer, 1991; Murphy *et al.,* 1999; Trapp and Osterreider, 2008; Yumuşak, 1988). Infection begins when the virus is adsorbed on the cell surface. Viral entry occurs with pinocytosis in the cell. Adsorption varies with the cells and the amount of inoculum. ILT virus particles are sensitive to lipolytic agents, heat and some disinfectants. It can survive for a longer period in lyophilization at -20 and -60°C and destroyed in 10-15 minutes at 55 °C (Yumuşak, 1988). The virus is mostly transmitted by respiratory tract; sick animals spread the virus around with nasal discharge and coughing, especially in the air as droplets. Transmission through the conjunctiva is possible. The crowded, neglected and unhygienic conditions of the henhouses, the intensity of stress factors and the winter months are effective in the emergence and spread of the infection in the henhouses. In such cases, mortality varies between 10-30%, and may increase up to 50% with the accompanying of secondary infections. Virus can be isolated from trachea up to 2 months after patients recover. There is no vertical transmission were reported of the virus to the egg (Bagust *et al.,* 2000; Biggs, 1982; Kahn, 2005, Kaleta, 1990; MSD, 2021; Yumuşak, 1988).

There are commonly variable non-specific clinical signs are observed mostly including respiratory distress, ocular lesions, enteritis and liver involvement. Clinical signs and respiratory symptoms are not pathognomonic for the disease. Infected birds are inactive and no appetite. The precursor symptoms are ocular discharges. If the disease progresses, the ocular discharge increases, conjunctivitis may associated and the eyelids swell and stick together, the nasal discharge increases and the animal's face starts to swell, they extend their heads upwards to breathe, wheezing sounds are made during breathing, the virus is spread around with coughing and sneezing at around 5-12 days after exposure. Occlusion of the trachea and especially the larynx with mucoid secretion or plugging fibrin tissue causes an increase in deaths from asphyxia. In cases where secondary infections complications and stress factors are high, deaths can reach up to 50%. Animals exposed to the virus do not always become clinically ill and may appear healthy. The incidence of the disease may vary depending on the pathogenicity of the virus strain, host health condition and stress factors. Low virulence virus strains cause few or no mortality, with mild respiratory infection and can lead to a slight decrease in egg production. Latent infections are possible after recovery of the diseased birds which of those remain an infection source as a lifelong carrier of the infection. The role of latent infected animals is quite high in the continuation of the virus shedding, its spread and the emergence of the disease. In addition, it is known that if a preventive vaccination is made in poultry houses with such animals that do not show any clear clinical signs, the disease may become active in animals with latent infection and animals can easily get sick and die. Disease symptoms in infected animals begin to subside after 2 weeks, and recovery is usually observed within 4-8 weeks, but some animals are

known to show signs of illness for a longer period and low productivity in laying animals can reach 50%. There is no effective etiological medication and treatment of the disease. Secondary bacterial infections in severe cases may control with choices of antibiotics. Lowering the dust level and keeping the birds in quiet hen houses with maintaining a clean water system may avoid transmission between flock members. Transmission can be possible with infected birds and fomites. Birds that die from the disease should be destroyed immediately. Transportation of infected birds, and spread of contaminated litter facilitates wit lack off biosecurity spread of the ILT virus (Bagust *et al.,* 2000; Biggs, 1982; MSD, 2021; Kahn, 2005; Kaleta, 1990; Murphy *et al.,* 1999; Pirbright, 2021; Trapp and Osterreider, 2008, Yumuşak; 1988).
Diagnosis is based on virus isolation, serological tests, and laboratory animal tests, PCR based molecular diagnosis and histopathology of trachea and conjunctiva of the suspected animals. The key principles of disease control include rapid diagnosis, improved biosecurity, and coordinated efforts for vaccination. Biosecurity measures are a leading barrier of protection in ILT, as in other viral diseases. Preventive vaccinations success depends on a good determination of the vaccine administration strategy. Live attenuated vaccines and viral vector vaccines are in choices for the development of adequate immunity (Bagust *et al.,* 2000; Biggs, 1982; Kahn, 2005, Kaleta, 1990; MSD, 2021; Yumuşak, 1988).

Marek's Disease

It is a viral, infectious, neural, tumorous, lethal and lymphoproliferative disease. Marek's disease virus (MDV) etiological agent Gallid alphaherpesvirus-2 (GaHV-2) is highly contagious, characterized by tumors, unilateral leg paralysis and visual disturbances in poultry. MDV represented all the characteristic morphological features of the alphaherpesviruses. There are three serotypes of MDV; Serotype-I is virulent and oncogenic, while serotype-II in chicken and serotype-III in turkey are non-oncogenic (Witter, 1972). Chickens are the main species affected by Marek's disease (MD or fowl paralysis). MD can be seen commonly worldwide and affects both commercial and backyard poultry and can cause death or severe productivity loss and is rarely seen in some other bird species. There are two forms of MD of those are nervous and visceral forms. Nervous form occurs typically with a progressive paralysis, visceral form occurs as tumors in internal organs, and most diseased birds usually die (Alfonso, 2001; Biggs, 1982; Calnek abd Witter, 1991; Biosecurity Tasmania, 2021; Kahn, 2005, Kaleta, 1990; Murphy *et al.*, 1999; Pirbright, 2021; Trapp and Osterreider, 2008). The disease develops as a result of accumulation and reproduction of mononuclear lymphocytes in the liver, kidney, spleen, gonads, pancreas, iris, lung, muscle and skin, and causes tumors and pathological changes in most of the nerves and internal organs. In the neural form of the disease, the affected peripheral nerves cause paralysis, while the locomotor nerves cause incoordination, shaky gait and unable to stand. Ballerina posture (characterized by extending one leg forward and the other leg back) is very characteristic for Marek's disease. When the wing and neck nerves are affected, the wings drop and the head falls down. As the vagus is affected, the crop enlarges, droops, and breathing difficulties are observed. In some cases, ocular forms may be seen with blindness. In the visceral form, tumors in internal organs accompanied with depression, loss of appetite and weight, greenish diarrhea, dehydration, pallor, and exhaustion (Biggs, 1982; Biosecurity Tasmania, 2021; Kahn, 2005, Kaleta, 1990; MSD, 2021; Trapp and Osterreider, 2008).
The diagnosis of Marek's disease can be made with a detailed veterinary examination. Evaluation of the clinical findings together with the post-mortem examination will not only help in the

correct diagnosis, but will also enable other possible diseases to be ruled out. Browning and thickening of the sciatic nerves and plexus brachialis, which is one of the characteristic findings of Marek's disease observed in the postmortem examination, and the presence of gray color and depigmentation in the eyes are among the findings confirming the diagnosis of the disease. In addition, lymphoid tumors of various sizes are encountered in the internal organs, depending on the severity and course of the infection. Tumors are localized in ovary, spleen, liver, kidney, testis, intestines, glandular stomach, muscles, skin, bursa fabricius and heart. Marek's disease virus is highly contagious, the main route of transmission is respiratory and can be widely found wherever chickens are raised. When the virus introduced newly to a flock, it spreads rapidly among unvaccinated birds, and most chickens in an unvaccinated flock become infected. Infected chickens carry the virus for life and shed it for long periods, whether they are infected or not (Biggs, 1982; Biosecurity Tasmania, 2021; Kahn, 2005, Kaleta, 1990; Murphy *et al.,* 1999; MSD, 2021; Trapp and Osterreider, 2008). The virus sheds from feather roots and spreads easily in feathers and dust, the susceptible bird ingesting the virus when inhaling infected dust particles. The virus can also easily transmit to other farms and susceptible flocks through people with access to poultry facilities, vectors (rodents, birds, insects, etc.) and contaminated equipment. The virus is quite resistant to the environment and room temperature and can survive for several months. It is known that there is no hen-to-chicken transmission through eggs. There is no effective etiological medication and treatment of the Marek's disease and symptomatic birds usually dies. Diseased birds should be immediately removed from the flock and humanely euthanized. Other birds in the flock are also likely to be infected at this stage, so close monitoring of all birds is important. Virus isolation, PCR-based molecular, serological, histopathological and cytotoxic tests are performed for the laboratory diagnosis of the disease. ELISA, fluorescent antibody assay, agar gel precipitation and neutralization tests are widely used in serological tests. A high level of biosecurity measures are the first measure in disease prevention. A biosafety checklist should be established across the farm and for bird keepers and its compliance monitored. A detailed deep disinfection and cleaning process should be experienced before the introduction of new birds in the flocks of an enterprise with Marek's disease in the previous period. Chicks should be reared separately to be free of infected feathers and dust from older birds. Standard hygiene measures are also important, including thorough cleaning and disinfection of kennels and equipment between groups of chicks with a disinfectant effective against viruses. Good nutrition and protection from other diseases and parasites are also very important. These practices will help maintain flock health and ensure that birds have optimum resistance to Marek's disease infection (Biggs, 1982; Biosecurity Tasmania, 2021; Kahn, 2005, Kaleta, 1990; Murphy *et al.,* 1999; MSD, 2021; Trapp and Osterreider, 2008). The second important issue is vaccination. Although vaccines are widely used in commercial poultry farming, it is often not possible to purchase a small number of doses for use in small family businesses and backyard chickens, as they are commercially available in volumes of 1000 doses and above. For these free-range birds, the best protection against Marek's disease can be obtained by purchasing correctly vaccinated birds from a trusted breeder. Vaccination alone will not prevent Marek's disease. Especially for commercial flocks, it is important to have good biosecurity to allow vaccinated chicks to develop immunity before being exposed to a serious virus threat. Currently, the generally accepted vaccination protocol is the use of a combination of frozen live vaccines containing chicken herpesvirus (CVI-Rispens strain, Serotype 1) and turkey herpesvirus (HVT - serotype-3). It is applied to day-old chicks and it is aimed to ensure the development of immunoglobulins before entering the hen with the immunity effect of the chicks.

However, the more effective method recommended is to vaccinate the chicks before hatching (in-ovo). This allows the chicks to gain a period of three to four days for the development of immunity. In addition, the HVT strain is used as vector virus in vector vaccine combinations to protect against some other viruses (Alfonso *et al.*, 2001; Biggs, 1982; Biosecurity Tasmania, 2021; Kahn, 2005, Kaleta, 1990; Murphy *et al.*, 1999; MSD, 2021; Trapp and Osterreider, 2008).

Conclusions

Poultry meat production, which has been increasing worldwide, became the most produced meat in 2015. FAO reported that poultry is raised by approximately 80% of rural households in developing countries. There are approximately 12 thousand poultry enterprises throughout Turkey and the sector grows by an average of 8% every year. In the poultry industry, the control of diseases that adversely affect animal health and productivity, especially avian herpesvirus infections, is of great importance for healthy food consumption.

References

Alfonso C.L., Tulman E.R., Zsak Z., Lu L., Rock D.L., Kutish G.F. (2001). The Genome of Turkey Herpesvirus. J Virol. Jan; 75(2): 971–978.

Avian Herpesviruses. https://www.pirbright.ac.uk/publications/avian-herpesviruses Accessed 10.07.2021.

Bagust T.J., Jones R.C., Guy J. S. (2000). Avian infectious laryngotracheitis. Rev. Sci. Technol. 19:483–492.

Beaufrère H., Laniesse D. (2016). Medicine of strigiformes. Current Therapy in Avian Medicine and Surgery, First Edition, St. Louis, Elsevier.

Biggs P.M. (1982). The epidemiology of avian herpesviruses in veterinary medicine. Dev Biol Stand. 52:3-11.

Calnek B.W., Witter R.L. (1991). Marek's disease. Ninth Edition, Ames, Iowa, Iowa State University Press.

FAO (2021). Meat market review. http://www.fao.org/3/cb3700en/cb3700en.pdf.

FAO (2021). Poultry production. http://www.fao.org/poultry-production-products/production

Dhama K., Kumar N., Saminathan M., Tiwari R., Karthik K., Kumar A., Palanivelu M., Shabbir M.Z., Malik Y.S., Singh RK. (2017). Duck virus enteritis (duck plague) - a comprehensive update. Vet Q. 37(1):57-80.

Kahn C.M. (2005). The Merck Veterinary Manual. Nineth Edition, Merck, Merial Publications.

Kaleta E.F. (1990). Herpesviruses of birds--a review. Avian Pathol. 19(2):193-21.

Infectious Laryngotracheitis. https://www.msdvetmanual.com/poultry/infectious-laryngotracheitis/infectious-laryngotracheitis-in-poultry Accessed on 10.07.2021.

Mareks disease. Biosecurity Tasmania. https://dpipwe.tas.gov.au/biosecurity-tasmania/animal-biosecurity/animal-health/poultry-and-pigeons/mareks-disease Accessed on 10.07.2021

May H.G., Tittsler R.P. (1925). Tracheo-laryngitis in poultry. J. Am. Vet. Med. Assoc. 67:229–231.

Murphy A.F., Gibbs E.P.J., Horzinek M.C., Studdert M.J. (1999). Veterinary Virology, Third Edition. Boston New York Sydney Tokyo Toronto, Elsevier.

Poultry meat statistics. https://www.statista.com/statistics/237597/leading-10-countries-worldwide-in-poultry-meat-production-in-2007/Accessed 10.07.2021.

Tomaszewski E., Wilson V.G., Wigle W.L., Phalen D.N. (2001). Detection and Heterogeneity of Herpesviruses Causing Pacheco's Disease in Parrots. Journal of Clinical Microbiology. 39(2): 533–538.
Trapp S., Osterrieder N. (2008). Encyclopedia of Virology (Third Edition), Academic Press.
Turkish White Meat Production (Türkiye Beyaz Et Sektörü). https://www.zmo.org.tr/resimler/ekler/d24d7c78bb96fc2_ek.pdf Accessed 10.07.2021
Poultry Sector in Turkey (Türkiye'de kanatlı sektörü). http://www.turktarim.gov.tr/Haber/401/turkiyede-kanatli-sektoru-her-yil-yuzde-8-buyuyor Accessed 10.07.2021
Turkish Statistical Agency Report on Poultry (Türkiye İstatistik Kurumu Raporu). https://data.tuik.gov.tr/Search/Search?text=tavuk Accessed 10.07.2021
Yumuşak M. (1988). Infectious Laryngotracheitis. Etlik Vet. Mikrob. Derg. 6 (3).
WHA Fact sheet: Herpesviruses and Australian wild birds | June 2012; 2-6.
Witter R.L. (1972). Turkey herpesvirus: lack of oncogenicity for turkeys. Avian Dis.16; 666-70.

COW MILK INSULIN LIKE GROWTH FACTOR-1: RISK OR BENEFIT FOR HUMAN HEALTH?

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Abstract

Cow milk is widely consumed by human children and adults due to its nutritional value. Besides nutrients, milk contains naturally occurring hormones, including insulin like growth factor-1 (IGF-1), that may alter their blood levels in the consumers. At the same time, the impact of IGF-1 on human health is still unclear and controversial. The aim of the study was to determine IGF-1 concentrations in cow colostrum and milk and to discuss them from the aspect of human health. Twenty Holstein cows were enrolled in the study and subjected to colostrum, milk and venous blood sampling in order to determine the IGF-1 concentration by the radioimmunoassay (RIA). Colostrum was sampled at 2, 14 and 26 hours (1^{st}, 2^{nd} and 3^{rd} colostrum) after calving, while milk and venous blood were taken 10 days after calving. The concentration of IGF-1 was the highest in the 1^{st} colostrum and decreased significantly with each subsequent sampling, so the lowest concentrations were detected in milk sampled 10 days after calving. High concentrations of IGF-1 observed in colostrum, especially in the 1^{st} colostrum, reflect its potential for eventually use in the treatment of various intestinal diseases. However, further research should determine the conditions and limitations of its application. On the other hand, additional research is needed to determine whether IGF-1 in milk has harmful effects on human health and whether it is necessary to limit the permitted presence of this hormone in cow milk.

Keywords: *cow milk, insulin like growth factor-1 (IGF-1), human health.*

Introduction

Cow milk is considered as nutritionally valuable food in the human diet, which is consumed from birth to old age (Marangoni et al. 2019). It contains proteins of high biological value, fats, carbohydrates, minerals and vitamins, that provide beneficial effects on the overall growth of children and qualify the use of milk in the prevention and treatment of them malnutrition (Agostoni and Turck 2011; Turck 2013). In addition to nutrients, cow's milk also contains biologically active substances, including immunoglobulins, lactoferrin, enzymes, cytokines, growth factors and naturally occurring hormones. The presence of biologically active substances in cow milk is essential for the newborn (Kirovski 2015). They support the growth of the newborn calves and the development and activity of their gastrointestinal and immune functions (Kirovski et al. 2002; Georgiev 2008; Park and Nam 2015). However, when consumers ingest biologically active substances, they may also cause a wide range of biological effects in the body. For instance, milk hormones could affect hormones levels in the blood and may affect physiological and pathological processes in the organism of consumers (Malekinejad and Rezabakhsh 2015). Therefore, it has been proven that milk estrogens have harmful effects on human health (Snoj and Majdič 2018), while the impact of insulin-like growth factor-1 (IGF-1) from cow's milk has not been elucidated and attracts attention from researchers and consumers also.

Bovine IGF-1 is a polypeptide composed of 70 amino acids predominantly synthesized in the liver but can also be synthesized in other tissues and exhibit endocrine, paracrine, and autocrine effects (Fruchtman et al. 2002). This hormone is known as a strong mitogen factor, promoting cell growth and proliferation and inhibiting apoptosis (Clatici et al. 2018). In all biological fluids, IGF-1 is almost entirely (95-99%) bound to one of six structurally similar IGF binding proteins (IGFBP-1 to -6) (Forbes et al. 2012). Consequently, less than 1 % of IGF-1 circulates freely – unbound to IGFBPs (Clemmons 1993; Kirovski et al. 2008; Melnik 2009). The concentration of IGF-1 in cow milk varies widely, from approximately 1 ng/mL to 150 ng/mL, depending on the stage of lactation, milk composition, and other environmental factors (Malekinejad and Rezabakhsh 2015). Thus, the highest concentrations of IGF-1 occur immediately after parturition in colostrum and then gradually decrease until it falls to the level of 1 to 5 ng/mL (Šamanc et al. 2009; Castigliego et al. 2011).

There are several reasons why the presence of IGF-1 in cow milk is a source of human health issues. IGF-1 has a highly conserved sequence across species because bovine IGF-1 can bind with identical affinity to IGF receptors on human tissues, thus achieving physiological effects (Renaville et al. 2002; Melnik 2009). Furthermore, there are data on both beneficial and harmful effects of IGF-1 on human health in scientific literature. Ungvari and Csiszar (2012) report that low levels of circulating IGF-1 increase the risk of cardiovascular and cerebrovascular diseases in humans. At the same time, a low concentration of IGF-1 in the early phase of acute myocardial infarction predicts a worse prognosis (Conti et al. 2001). The protective effect of IGF-1 on Alzheimer's disease (O'Neill et al. 2012) and all forms of dementia, including Parkinson's disease dementia (Westwood et al. 2014), has also been documented. Typically, there are numerous estimates that IGF-1 has a positive effect on bone health; therefore, it is considered that clinical determination of IGF-1 may be useful in assessing the risk of bone fractures (Lombardi et al. 2005; Locatelli and Bianchi 2014).

On the other hand, numerous epidemiological studies have linked high levels of circulating IGF-1 to an increased risk of several cancers in humans. In this context, breast, prostate, lung and colon-rectal cancers are most commonly reported in the literature (Sutariya et al. 2018). This is important because the digestion of IGF-1 ingested through cow's milk is prevented by milk proteins, which potentially enables its intestinal absorption. Thus, higher milk consumption leads to an increase in circulating IGF-1 levels by 20-30% in children and 10-20% in adults (Melnik 2009). At the same time, homogenization and pasteurization do not significantly decrease the content and activity of IGF-1 in cow milk (Collier et al. 1991). Therefore, Tate and coworkers (2011) suggest that patients with prostate or breast cancer should be informed about the possible promotional effects of dairy products.

The study aimed to determine the concentrations of IGF-1 in cow colostrum and milk, and analyze them in the context of human health.

Materials and Methods

Animals and Housing

The experiment was conducted at the commercial dairy farm in Belgrade, Serbia. Twenty Holstein cows were assigned for the study. Cows received diets as total mixed ration (TMR) to allow ad libitum feed intake throughout the study. After calving, cows were assigned to the lactation diet (crude protein = 161 g/kg DM; crude fat = 21 g/kg DM; NEL = 7.3 MJ/kg DM; ADF = 221 g/kg DM; NDF = 358 g/kg DM). Diet was formulated to either meet or exceed the NRC (2001) requirements. The feed was offered in two equal portions at 0700 and 1600 h, and cows had free access to water. Cows were maintained under a loose housing system.

Colostrum and Blood Sampling Procedures

The primary (1^{st}), secondary (2^{nd}) and tertiary (3^{rd}) colostrum were sampled from each cow 2, 14, and 26 h after calving, respectively. The milk sample was taken 10 days after calving. The samples were collected in sterile cups and were stored at – 20 °C for the further analyses. Blood was sampled by *v. jugularis* puncture at day 10 day after calving at 1000 h. The samples were collected into serum clot activator tubes.

Sample Preparation and IGF-1 Measurement

Colostrum and Milk Samples. Thawed colostrum and milk were centrifuged (2000×*g*, 20 min) and the fatty layer was carefully removed through vacuum aspiration. Casein was precipitated by adding 175 µL of rennet to 10 mL of centrifuged colostrum supernatant. After 30 min of incubation at 37 °C, samples were centrifuged for 15 min at 3000×g, and whey (supernatant) was aliquoted and stored at − 20 °C until analysis. IGF-1 concentration in colostral and milk whey was determined using radioimmunoassay (RIA-IGF-I, INEP, Belgrade, Serbia) (Nikolić et al. 1996).

Blood Samples. On the day of sampling, blood in the serum clot activator tubes was kept at room temperature and was allowed to clot. Serum was decanted, centrifuged within 30 min at 3000×g, portioned into aliquots of 1.5 ml, and stored in polypropylene microtubes at − 20 °C until analyses. Concentrations of IGF-1 in blood were measured by radioimmunoassay (RIA-IGF-I, INEP, Belgrade, Serbia) (Nikolić et al. 1996).

Statistical analysis

The statistical analysis was performed by determination of the mean value and standard error of the mean (SEM) for each sample. The statistically significant difference between the samples was evaluated by the Student's t-test and *p* values less than 0.05 were considered to be statistically significant. The analyses were performed using Excel 2019 software (Microsoft, Redmond, WA, USA) (Vega et al. 1991).

Results and Discussion

Preliminary results of our study demonstrate that the highest concentrations of IGF-1 are present in the 1st colostrum (59.23±3.32 nmol/L) (Figure 1). At the same time, these values were statistically significantly higher compared to the 2nd colostrum (37.91±2.49 nmol/L; $p<0.001$), 3rd colostrum (22.03±1.44 nmol/L; $p<0.001$) and milk (19.16±0.77 nmol/L; $p<0.001$). Moreover, Figure 1 reveals that the concentration of IGF-1 at each subsequent colostrum sampling period, including milk sampling on the 10 days after calving, was significantly lower ($p<0.05$, respectively) than in the previous one. Consequently, the lowest concentrations of IGF-

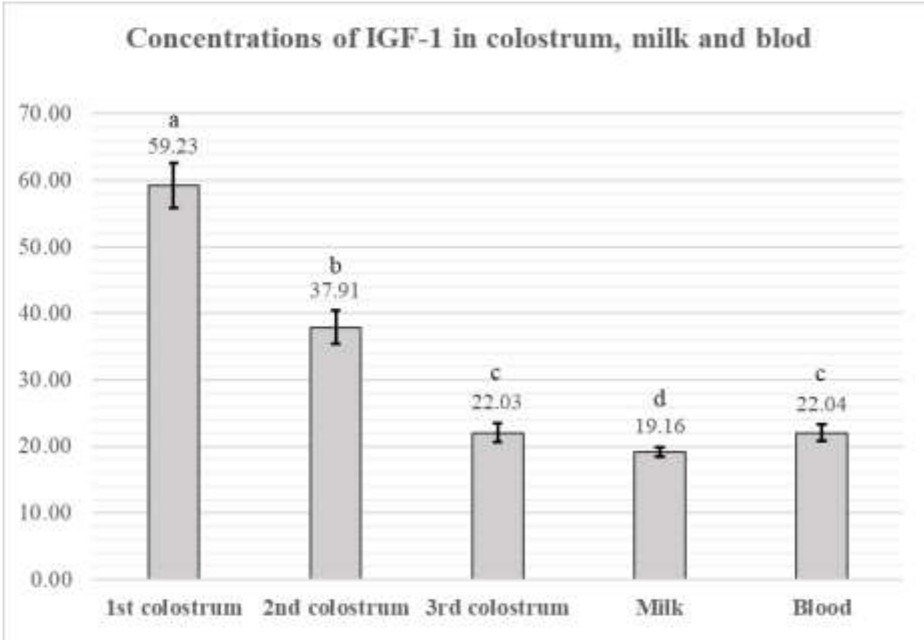

Figure 10. Mean IGF-1 concentrations (± SEM) in cow colostrum, milk and blood. 1st colostrum – sampled 2 hours after calving; 2nd colostrum – sampled 14 hours after calving; 3rd colostrum – sampled 26 hours after calving; Milk and Blood – sampled 10 days after calving. Different lowercase letters above the bars indicate a statistically significant difference ($p < 0.05$).

1 were found in milk sampled 10 days after calving, which were also significantly lower ($p<0.001$) than those determined in the blood serum (22.04±1.27 nmol/L) on the same day.

The obtained results suggest that the concentration of IGF-1 in colostrum decreases rapidly within the first day after calving, but a similar trend remains until the tenth day after calving. Analogous findings were obtained by Elfstrand and colleagues (2002), who reported that the highest concentrations of IGF-1 were present in colostrum in the first six hours after calving. Mentioned researchers also documented a rapid decrease in the concentration of IGF-1 in colostrum by 44% after 11-20 hours. This is relatively consistent with our results reflecting a 30% and 63% reduction in IGF-1 colostrum concentration after 14 and 26 hours after calving. Additionally, the further decline in IGF-1 concentration in cow's milk found in our study confirms the previously published results of Daxenberger et al. (1998), who described that IGF-1 concentrations in milk decrease gradually until 7 weeks after calving and remain low until 33 weeks of lactation. Finally, the biological importance of high IGF-1 levels in colostrum can be explained by its beneficial effect on the morphological and functional development of newborn calves (Blum and Hammon 2000; Kirovski et al. 2009).

Since colostrum is not a standard part of the human diet, the high level of IGF-1 in this mammary secretion cannot be considered a health risk for consumers. Moreover, previous literature data indicate that cow colostrum can be viewed as a functional food with health benefits for consumers. This is based on the fact that cow colostrum is a consummate source of biologically active proteins that can modulate the immunological function and improve intestinal peristalsis of consumers. Accordingly, a whole range of bovine colostrum-based products, such as various dietary supplements, drinks, and chewing gums, can be found in the markets of certain countries (Dzik et al. 2017; Mir et al. 2021). In addition, there is a growing scientific interest in the field of beneficial effects of bovine colostrum related to the physiological effects of IGF-1.

Mir and coworkers (2021) report that IGF-1 from bovine colostrum repairs intestinal morphology and function, stimulating the proliferation of intestinal crypt cells. IGF-1 also exhibits anti-inflammatory effects by several described mechanisms (Zatorski et al. 2016). Consequently, there are recommendations for using bovine colostrum or purified IGF-1 to treat inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis as the most common forms (Yadav et al. 2016; Zatorski et al. 2016). This perspective in the use of colostrum is very important because the number of diagnosed cases of IBD has increased dramatically worldwide in the last 50 years. Noteworthy, the number of new cases is growing in the population of children (Molodecky et al. 2012; Gasparetto and Guariso 2013). Furthermore, Crohn's disease and ulcerative colitis are accompanied by decreased intestinal production and circulating levels of IGF-1, which leads to other health complications. Indeed, patients with Crohn's disease or ulcerative colitis are at increased risk of developing osteopenia, osteoporosis, and osteoporotic bone fractures (Koutroubakis et al. 2011). In this context, the large amounts of IGF-1 in cow colostrum, shown by results of our previous (Kirovski et al. 2012) and presented study, only support the recommendations for the use of colostrum in the treatment of various intestinal diseases. However, subsequent research should examine the limitations of its use, because IGF-1 may have a promotional effect in patients with premalignancy conditions (Zatorski et al. 2016).

In contrast to colostrum, cow's milk and dairy products are widely used in human nutrition in our cultural sphere; therefore, consumer's interest in cow's milk safety is far greater compared to colostrum. Although it has been confirmed in various animal models (mice and rats) and cell cultures that IGF-1 promotes the growth of cancer, especially prostate (Tate et al. 2011; Cao et al. 2015) and breast cancer (Mawson et al. 2005), it is not entirely clear whether milk-borne IGF-1 can achieve a similar effect in humans. Namely, the proven increase in circulating IGF-1 in humans after milk consumption (Melnik 2009; Ventura et al. 2020) may not be the only consequence of intestinal resorption of IGF-1. There is a theory that dairy proteins are responsible for increasing IGF-1 levels after milk consumption (Giovannucci et al. 2003) because they are a source of essential amino acids that up-regulate hepatic IGF-1 gene expression (Thissen et al. 1994). If this theory is confirmed by future research, milk will be incriminated as a food independent of IGF-1 concentrations.

On the other hand, there is a theory that milk proteins, such as casein, protect IGF-1 from digestion, therefore the IGF-1 remains active in the serum after milk consumption (Melnik 2009). Accordingly, Philipps et al. (2000) suggested that radiolabeled IGF-1 could be found in the portal circulation at low concentrations. However, it is difficult to extrapolate these results to humans. Therefore, it is necessary to determine the essence of the increase in circulating IGF-1 in humans after milk consumption, as well as the critical levels of IGF-1 in cow's milk.

Conclusions

In the presented investigation, we found that IGF-1 concentrations are highest immediately after calving (first two hours) and decrease rapidly during the following 24 hours. After that, IGF-1 concentration gradually decreases, which is indicated by the measured values of IGF-1 in milk sampled on the tenth day after calving. Future research should examine the potential benefits of high concentrations of IGF-1 in colostrum, or the possible adverse effects of cow milk IGF-1 on human health.

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References

Agostoni C, Turck D (2011). Is cow's milk harmful to a child's health? Journal of pediatric gastroenterology and nutrition, 53(6), 594-600.

Blum JW, Hammon H (2000). Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves, Livestock Production Science, 66(2), 151-159.

Cao Y, Nimptsch K, Shui IM, Platz EA, Wu K, Pollak MN, Giovannucci EL (2015). Prediagnostic plasma IGFBP-1, IGF-1 and risk of prostate cancer, International journal of cancer, 136(10), 2418-2426.

Castigliego L, Li X, Armani A, Mazzi M, Guidi A (2011). An immunoenzymatic assay to measure insulin-like growth factor 1 (IGF-1) in buffalo milk with an IGF binding protein blocking pre-treatment of the sample, International dairy journal, 21, 421-6.

Clatici VG, Voicu C, Voaides C, Roseanu A, Icriverzi M, Jurcoane S (2018). Diseases of Civilization–Cancer, Diabetes, Obesity and Acne–the Implication of Milk, IGF-1 and mTORC1. Mædica, 13(4), 273-281.

Clemmons DR (1993). IGF binding proteins, and their functions. Molecular Reproduction and Development, 35, 368 –375.

Collier RJ, Miller MA, Hildebrandt JR, Torkelson AR, White TC, Madsen KS, ... Lanza GM (1991). Factors affecting insulin-like growth factor-I concentration in bovine milk, Journal of dairy science, 74(9), 2905-2911.

Conti E, Andreotti F, Sciahbasi A, Riccardi P, Marra G, Menini E, ... Maseri A (2001). Markedly reduced insulin-like growth factor-1 in the acute phase of myocardial infarction, Journal of the American College of Cardiology, 38(1), 26-32.

Daxenberger A, Sauerwein H, Breier BH (1998). Increased milk levels of insulin-like growth factor 1 (IGF-1) for the identification of bovine somatotropin (bST) treated cows, Analyst, 123(12), 2429-2435.

Dzik S, Miciński B, Aitzhanova I, Miciński J, Pogorzelska J, Beisenov A, Kowalski IM (2017). Properties of bovine colostrum and the possibilities of use, Polish Annals of Medicine, 24(2), 295-299.

Elfstrand L, Lindmark-Månsson H, Paulsson M, Nyberg L, Åkesson B (2002). Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing, International Dairy Journal, 12(11), 879-887.

Forbes B, McCarthy P, Norton R (2012). Insulin-like growth factor binding proteins: a structural perspective, Frontiers in endocrinology, 3, 38.

Fruchtman S, McVey DC, Borski RJ (2002). Characterization of pituitary IGF-I receptors: modulation of prolactin and growth hormone. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 283(2), 468-476.

Gasparetto M, Guariso G (2013). Highlights in IBD epidemiology and its natural history in the paediatric age, Gastroenterol Res Pract, 1-13.

Georgiev IP (2008). Differences in chemical composition between cow colostrum and milk. Bulgarian Journal of Veterinary Medicine, 11(1), 3-12.

Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, Rimm EB, Willett WC (2003). Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men, Cancer Epidemiology and Prevention Biomarkers, 12(2), 84-89.

Kirovski D, Nikolić JA, Stojić V (2002). Serum levels of inuslin-like growth factor I and total protein in newborn calves offered different amounts of colostrum, Acta Veterinaria Beograd, 52 (5-6), 285-298

Kirovski D, Lazarević M, Baričević-Jones Ivona, Nedić O, Masnikosa R, Nikolić Judith A (2008). Effects of peroral insulin and glucose on circulating Insulin-like growth factor-I, its binding proteins and thyroid hormones in neonatal calves, Canadian Journal of Veterinary Research, 72, 253-288.

Kirovski D, Šamanc H, Fratrić N, Gvozdić D, Hristov S, Sladojević Ž, Mircu C, Tulcan C, (2009). Koncentracija kortizola, insulinu sličnog faktora rasta – I i imunoglobulina G klase u krvi neonatalne teladi različite telesne mase na rođenju, Veterinarski glasnik, 63, 321-329.

Kirovski D, Šamanc H, Vujanac I, Prodanović R, Đurić Miloje, Sladojević Ž (2012). Effect of negative energy balance on IGF system in dairy cows, 3rd International Scientific Meeting, Ohrid, Macedonia, September 2nd to 4st 2012, Book of Proceeding, 48 – 51.

Kirovski D (2015). Endocrine and metabolic adaptations of calves to extra-uterine life, Acta veterinaria-Beograd, 65, 297-318.

Koutroubakis IE, Zavos C, Damilakis J, Papadakis GZ, Neratzoulakis J, Karkavitsas N, Kouroumalis EA (2011). Low bone mineral density in Greek patients with inflammatory bowel disease: prevalence and risk factors, Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology, 24(1), 41.

Locatelli V, Bianchi VE (2014). Effect of GH/IGF-1 on bone metabolism and osteoporsosis, International journal of endocrinology, 1-25.

Lombardi G, Tauchmanova L, Di Somma C, Musella T, Rota F, Savanelli MC, Colao A (2005). Somatopause: dismetabolic and bone effects, Journal of endocrinological investigation, 28(10), 36-42.

Malekinejad H, Rezabakhsh A (2015). Hormones in dairy foods and their impact on public health-a narrative review article. Iranian journal of public health, 44(6), 742.

Marangoni F, Pellegrino L, Verduci E, Ghiselli A, Bernabei R, Calvani R, ... Poli A (2019). Cow's milk consumption and health: a health professional's guide, Journal of the American College of Nutrition, 38(3), 197-208.

Mawson A, Lai A, Carroll JS, Sergio CM, Mitchell CJ, Sarcevic B (2005). Estrogen and insulin/IGF-1 cooperatively stimulate cell cycle progression in MCF-7 breast cancer cells through differential regulation of c-Myc and cyclin D1. Molecular and cellular endocrinology, 229(1-2), 161-173.

Melnik B (2009). Milk consumption: aggravating factor of acne and promoter of chronic diseases of Western societies, JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 7(4), 364-370.

Mir MA, Sharma H, Singh J (2021). Applications and Future Prospects of Colostrum, Annals of the Romanian Society for Cell Biology, 4537-4548.

Molodecky NA, Soon S, Rabi DM, Ghali WA, Ferris M, Chernoff G, Kaplan GG (2012). Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology, 142(1), 46-54.

Nikolić JA, Ratković M, Nedić O (1996). Determination of insulin-like growth factor – I by radioimmunoassay, Journal of Serbian Chemical Society, 61, 1149-1157.

O'Neill C, Kiely AP, Coakley MF, Manning S, Long-Smith CM (2012). Insulin and IGF-1 signalling: longevity, protein homoeostasis and Alzheimer's disease, Biochemical Society Transactions, 40(4), 721-727.

Park YW, Nam MS (2015). Bioactive peptides in milk and dairy products: a review, Korean journal for food science of animal resources, 35(6), 831-840.

Philipps AF, Dvorák B, Kling PJ, Grille JG, Koldovský O (2000). Absorption of milk-borne insulin-like growth factor-I into portal blood of suckling rats, Journal of pediatric gastroenterology and nutrition, 31(2), 128-135.

Renaville R, Hammadi M, Portetelle D (2002). Role of the somatotropic axis in the mammalian metabolism, Domestic animal endocrinology, 23(1-2), 351-360.

Šamanc H, Stojić V, Kirovski Danijela, Pušić M, Jakić-Dimić Dobrila, Vujanac I (2009). Insulin-like growth factor-I (IGF-I) and thyroid hormone concentrations in colostrum of Holstein-Friesian cows before and after calving, Acta Veterinaria Beograd, 59, 17-23.

Snoj T, Majdič G (2018). MECHANISMS IN ENDOCRINOLOGY: Estrogens in consumer milk: is there a risk to human reproductive health?, European journal of endocrinology, 179(6), 275-286.

Sutariya S, Sunkesula V, Kumar R, Shah K (2018). Review: Milk and milk products, insulin-like growth factor-1 and cancer, EC Nutrition, 13, 696-705.

Tate PL, Bibb R, Larcom LL (2011). Milk stimulates growth of prostate cancer cells in culture, Nutrition and cancer, 63(8), 1361-1366.

Thissen JP, Pucilowska JB, Underwood LE (1994). Differential regulation of insulin-like growth factor I (IGF-I) and IGF binding protein-1 messenger ribonucleic acids by amino acid availability and growth hormone in rat hepatocyte primary culture, Endocrinology 134(3):1570–1576.

Turck D (2013). Cow's milk and goat's milk, Evidence-Based Research in Pediatric Nutrition, 108, 56-62.

Ungvari Z, Csiszar A (2012). The emerging role of IGF-1 deficiency in cardiovascular aging: recent advances, Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 67(6), 599-610.

Vega JR, Gibson CA, Skaar TC, Hadsell DL, Baumrucker CR (1991). Insulin-like growth factor (IGF)-I and-II and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows, Journal of Animal Science, 69(6), 2538-2547.

Ventura ER, Konigorski S, Rohrmann S, Schneider H, Stalla GK, Pischon T, Nimptsch K (2020). Association of dietary intake of milk and dairy products with blood concentrations of insulin-like growth factor 1 (IGF-1) in Bavarian adults, European journal of nutrition, 59(4), 1413-1420.

Westwood AJ, Beiser A, DeCarli C, Harris TB, Chen TC, He XM, ... Seshadri S. (2014). Insulin-like growth factor-1 and risk of Alzheimer dementia and brain atrophy, Neurology, 82(18), 1613-1619.

Yadav R, Angolkar T, Kaur GS, Buttar H (2016). Antibacterial and antiinflammatory properties of bovine colostrum, Recent patents on inflammation & allergy drug discovery, 10(1), 49-53.

Zatorski H, Marynowski M, Fichna J (2016). Is insulin-like growth factor 1 (IGF-1) system an attractive target inflammatory bowel diseases?, Benefits and limitation of potential therapy. Pharmacological Reports, 68(4), 809-815.

DETERMINATION OF EXTERNAL, INTERNAL AND EGGSHELL QUALITY PARAMETERS

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Abstract

Eggs of class "L" produced of Isa Brown genotype were used for the research needs. The research aimed to determine the external (length, width, shape index and mass of eggs), internal (proportion of main parts of eggs, albumen height, Haugh units and yolk color) and eggshell quality parameters (purity, thickness and strength). The eggshell strength was determined by applying the direct method of puncture, using a specially constructed measuring and acquisition device. The results of the research showed that the average egg length was 58.33 mm, width 45.17 mm, and shape index 77.44. Of the average egg mass (65.79 g), the albumen proportion was 59.87%, yolk 27.33% and eggshell 12.90%. Albumen height ranged from a minimum of 4.42 mm to a maximum of 6.76 mm, with a coefficient of variation of 16.90%. Accordingly, values of the Haugh units ranged from 60.2 to 80.2 and the coefficient of variation of 10.96%. The average yolk color was 13 Roche. The purity of the eggshell was rated 4.44. The eggshell quality of the tested eggs was quite uniform because the average puncture force of the eggshell was 25.88 N, with a coefficient of variation of 4.20% and an average thickness of 0.40 mm. Knowledge of the values of external, internal and properties of eggshell quality can be used in a selection of laying hens hybrids with increased eggshell strength, as well as for cage construction, design of egg collection equipment and design of egg packaging.

Keywords: *hen eggs, egg quality, eggshell puncture force.*

Introduction

The cage rearing system is the dominant way of rearing of laying hens all over the world, and this situation probably will continue for a long time. The reason for this is because the rearing of hens in the cage is the most economical way of production, and it provides that the price of eggs is low and that product be accessible to all segments of the population (Rakonjac et al., 2017). In Serbia, more than 90% of eggs for consumption are produced in this - industrial way, in closed facilities with automatic feeding and watering systems, with control of all ambient conditions and the use of artificial lighting and ventilation.

In recent years, great attention has been paid to the quality and health safety of eggs, as one of the most important foodstuffs. In addition to the chemical composition of eggs and their external and internal quality properties, a special attention is paid to the quality of the eggshell, which can be crucial for success in hen eggs production. Thus Coucke et al. (1999) state that 6-8% of produced eggs break up before it comes to use, which makes financial losses of millions of dollars. A large percentage of this damage is caused by the inadequate quality of the shell because eggs cracks during manipulation.

The research aimed to determine the external (length, width, shape index and mass of eggs), internal (proportion of main parts of eggs, albumen height, Haugh units and yolk color) and eggshell quality parameters (purity, thickness and strength). In addition, the research aims to test a device that was constructed for the experiment, for determining the stress of the eggshell by direct puncture method.

Material and Methods

A total of 30 "L" class eggs (produced of 63 weeks old Isa Brown hens on the "Grbović" farm in the vicinity of Čačak) were used in this research. These eggs (one day old) were evaluated for external (length, width, shape index and egg mass), internal (proportion of main parts of eggs, albumen height, Haugh units and yolk color) and eggshell quality properties (purity, thickness and strength).
The shape index was calculated by formula according to the research of Panda (1996):

$$\textit{Shape index (\%)} = \textit{egg width} / \textit{egg length} * 100$$

Haugh units were calculated according to the following formula (Haugh, 1937):

$$HU = 100 * \log (h - 1.7w^{0.37} + 7.6)$$

where h = albumen height, mm, w = egg weight, g.

The yolk color was determined by comparison with the color on the "Roche-Yolk Color Fan 1969" range, classified by the intensity of color shades on a scale from 1 to 15. The length and width of eggs and eggshell thickness were measured by Fowler Pro-max electronic caliper with an accuracy of 0.01 mm. The mass of whole eggs and main parts of eggs were measured using an analytical balance Kern EMS 3000-2 with an accuracy of 0.01 g. The purity of the eggshell was visually assessed from 1 (very dirty) to 5 (completely clean).
A special measuring and acquisition device was constructed to determine the eggshell strength using the direct puncture method. The device for measuring the eggshell stress by determining the puncture force of eggshell was constructed in the Laboratory for Mechatronics of the Faculty of Technical Sciences and the Faculty of Agronomy in Čačak. A detailed description of the measuring acquisition device for measuring the mechanical properties of the puncture force of cracking and breaking of rapeseed fruits was given by Božić et al. (2014) and Koprivica et al. (2021). The procedure for determining stress of the eggshell is performed in such a way that the egg is placed on a support plate in a horizontal position (along the length) perpendicular to the direction of the force. With a 2 mm diameter probe, the eggshell puncture is performed on the equatorial part of the egg. At the moment of eggshell puncture, the minimum puncture force expressed in N is registered and represents the maximum of the eggshell strength. The device registers and displays data on measured values of the eggshell puncture force in Excel table and graphic. The tabular report provides data on eggshell puncture forces, as well as basic statistical data (minimum, maximum, mean, coefficient of variation, standard deviation).

Results and Discussion

Egg quality was determined based on obtained values of 4 external, 3 internal and 3 properties of eggshell quality. The properties of the external quality of eggs are shown in Table 1.

Table 1. External quality of eggs

Egg properties	Mean	St. Dev.	CV in %	Min.	Max.
Length (mm)	58.33	1.05	1.79	56.65	59.72
Width (mm)	45.17	1.06	2.34	43.96	46.94
Shape index (%)	77.44	3.91	5.01	74.63	81.04
Mass (g)	65.79	3.01	4.57	62.89	70.79

The average length of eggs was 58.33 mm and width 45.17 mm, so the shape index was 77.44%. Based on the shape index and mass index, the eggs are graded and packaging is designed. The shape index is important from an economic point of view because, with an irregular shape, the cracking of eggs during collection, packaging and transport is much higher. The results for shape index is in agreement with the results published by Kralik et al. (2013), Ahammed et al. (2014), Dikmen et al. (2017), Rakonjac et al. (2018) and Turker and Alkan (2019), and greatest than the value it states by Nedomova et al. (2009). The mass is the main indicator of egg quality and it ranged from 62.89 g to 70.79 g, with an average of 65.78 g, so the eggs were classified in the "L" class (large eggs). These values are following the technological norm for Isa Brown laying hens in the later stages of the production cycle (Isa Brown Management Guide, 2018), and the results determined by Đukić-Stojčić et al. (2009) and Rakonjac et al. (2017).

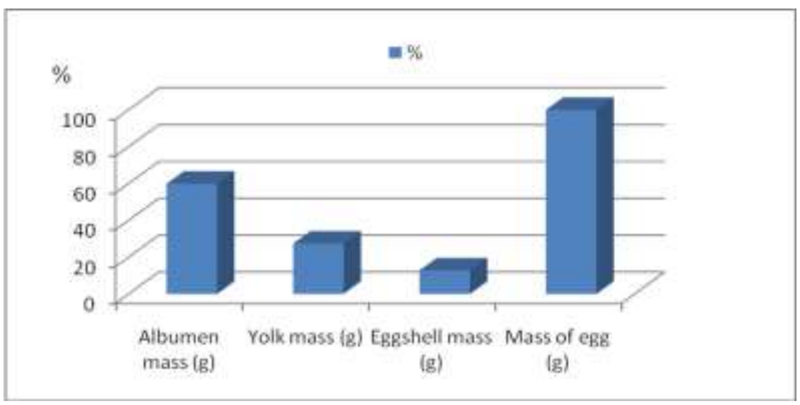

Figure 1. The average share of basic parts of egg

The proportion of the main parts of eggs is shown in Figure 1. The obtained values are in accordance with the results given by Rakita et al. (2016), Rakonjac et al. (2017) and Keta and Tumova (2018). Internal egg quality parameters are presented in Table 2.

Table 2. The internal quality of eggs

Egg properties	Mean	St. Dev.	CV in %	Min.	Max.
Albumen height (mm)	5.57	0.94	16.90	4.42	6.76
The Haugh units	70.77	7.76	10.96	60.2	80.2
Yolk color (Roche)	13.00	0.87	6.66	12	14

The values of albumen height ranged from a minimum of 4.42 mm to a maximum of 6.76 mm, with a coefficient of variation of 16.90%. Accordingly, the values of the Haugh units varied from 60.2 to 80.2 and with a coefficient of variation of 10.96%. Similar results for the same hybrid and approximately the same age of the hens were also determined by Minelli et al. (2007) and Rakonjac et al. (2018), who state that the hen's age is one of the main factors that affecting the value of these parameters.
The yolk color depends on a large number of factors, primarily on genotype (Kucukyilmaz et al., 2012), age of the laying hens (Škrbić et al., 2011) and nutrition (Mugnai et al., 2009). The average value of the yolk color in this study was 13 Roche, which is similar to the results published by Perić et al. (2016), and significantly higher than the results reported by Kralik et al. (2013) and Rakonjac et al. (2018). Eggshell quality parameters are presented in Table 3.

Table 3. The quality of eggshell

Properties of eggshell	Mean	St. Dev.	CV in %	Min.	Max.
Purity eggshell (points)	4.44	0.73	16.35	3.0	5.0
Eggshell thickness (mm)	0.40	0.02	6.16	0.37	0.45
Puncture force (N)	25.88	1.09	4.20	23.41	26.46

For eggs to be sold, the eggshell must be clean, without visible cracks. On average, the purity of the eggshell was rated 4.44. The quality of the eggshell is determined by the thickness and eggshell strength. The strength of the eggshell is the ability of the eggshell to resist the action of external forces that would break it. The eggshell strength values obtained by the direct puncture method represent the stress force to load the eggshell to the limit when it is punctured or cracked. The quality of the tested eggs was uniform because the average puncture force of the eggshell was 25.88 N, and varied from 23.41 N to 26.46 N. These values of eggshell strength measured by puncture force are in agreement or approximate with the results published by Krawczyk (2009), Ahammed et al. (2014) and Turker and Alkan (2019), and less than measured values by Hunt et al. (1977), Voisey et al. (1979), Hamilton and Thompson (1986) and Rakonjac et al. (2019). Inconsistency of the obtained results for values of eggshell puncture force presented in the research with the results of other authors can be explained by using different genotypes, different rearing and feeding conditions, different ages of laying hen, but also by using different methods to determine values of this parameter.
The average eggshell thickness with membranes on the equatorial part of the egg was 0.40 mm with a coefficient of variation of 6.16%. These values are in agreement with the values presented by Ahammed et al. (2014), Dikmen et al. (2017) and Keta and Tumova (2018).

Conclusion

The most important parameters for determining the quality of egg are external, internal and eggshell quality properties. Knowing the value of these parameters is of great importance for both egg producers and consumers, as well as for science and practice. Only by knowing the mentioned properties, it is possible to create new or improve existing hybrids of laying hens by the selection, which are characterized by a better external and internal quality of eggs and

eggshell strength increased. The obtained values of some parameters are important for cage construction, design of collection equipment and design of egg packaging.
The research shows that the device for measuring egg strength by eggshell puncture method is simple, light construction, easy to use, non-invasive, efficient, reliable in work, and the obtained results of puncture force are precise, concrete and comparable with the results of other authors. The constructed device for measuring the strength of eggs and the method of puncture eggshell have application not only in laboratories of scientific institutions but also in practice on farms.

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References

Ahammed A., Chae B.J., Lohakare, J., Keohavong, B., Lee M.H., Lee SJ., Kim DM., Lee JY., Ohh S.J. (2014). Comparison of aviary, barn and conventional cage raising of chickens on laying performance and egg quality. Asian-Australian Journal of Animal Science, 27, 1196-1203.

Božić M., Koprivica R., Bošković N., Veljković B. (2014). Data acquisition system for measurement the force craking of harvested crops, Tractors and power machines, 19(4), 98-106.

Coucke P., Dewil E., Decuypere E., De Baerdemaeker J. **(**1999). Measuring the mechanical stiffness of an eggshell using resonant frequency analysis. British Poultry Science, 40, 227-232.

Dikmen B.Y., İpek A., Şahan Ü., Sözcü A., Baycan S.C. (2017). Impact of different housing systems and age of layers on egg quality characteristics. Turkish Journal of Veterinary and Animal Science, 41, 77-84.

Đukić-Stojčić M., Perić L., Bjedov S., Milošević N. (2009). The quality of table eggs produced in different housing system. Biotechnology in Animal Husbandry, 25(5-6), 1103-1108.

Hamilton R.M.G., Thompson B.K. (1986). The Effects of the Egg Shell Strength Puncture Test on the Subsequent Hatchability of Eggs from White Leghorn and Broiler Hens. Poultry Science, 65, 1502-1509.

Haugh R.R. (1937). The Haugh Unit for measuring egg quality. U. S. Egg Poultry Magazine, 43, 522-555.

Hunt J.R., Voisey P.W., Thompson B.K. (1977). Physical properties of eggshells: A comparison of the puncture and compression tests for estimating shell strength. Canadian Journal of Animal Science, 57, 329-338.

Isa Brown Management Guide (2018).

Ketta M. Tumova, E. (2018). Relationship between egg shell thickness and other egg shell measurements in eggs from litter and cages. Italian Journal of Animal Science, 17(1), 234-239.

Krawczyk J. (2009). Quality of eggs from Polish native Greenleg Partridge chicken-hens maintained in organic vs. backyard production systems. Animal Science Papers and Reports, 27(3), 227-235.

Kralik Z., Radišić Ž., Grčević M., Kralik G. (2013). Comparison of quality of table eggs produced in various systems of keeping laying hens. XV European Symposium on the Quality of Eggs and Egg Products and XXI European Symposium on the Quality of

Poultry Meat, Proceedings World's Poultry Science Journal, Volume (69), Supplement, ID 101, Bergamo, Italy, 15-19.09.2013.

Kucukyilmaz K., Bozkurt M., Herken E.N., Cinar M., Cath A.U., Bintas E., Coven F. (2012). Effects of rearing systems on performance, egg characteristics and immune response in two layer hen genotype. Asian - Australasian Journal of Animal Sciences, 25(4), 559-568.

Minelli G., Sirri E., Folegatti A., Melluzzi A., Franchini A. (2007). Egg quality traits of laying hens reared in organic and conventional systems. Italian Journal of Animal Science, 6 (SUPPL. 1), 728-730.

Mugnai C., Dal Bosco A., Castellini C. (2009). Effect of rearing system and season on the performance and egg characteristics of Ancona laying hens. Italian Journal of Animal Science, 88, 175-188.

Nedomova Š., Severa L., Buchar J. (2009). Influence of hen egg shape on eggshell compressive strength. International Agrophysics, 23(3), 249–256.

Panda P.C. (1996). Shape and Texture. In Textbook on Egg and Poultry Technology. First Edition, New Delhi, India.

Perić L., Đukić-Stojčić M., Bjedov S. (2016). Effect of production systems on quality and chemical composition of table eggs. Contemporary Agriculture, 65(3-4), 27-31.

Rakita S., Spasevski N., Čolović D., Popović S., Ikonić P., Čolović R., Lević J. (2016). The influence of laying hens diet enriched with omega-3 fatty acids, paprika and marigold on physical properties of eggs. Journal on Processing and Energy in Agriculture, 20(2), 58-62.

Rakonjac S., Bogosavljević-Bošković S., Škrbić Z., Perić L., Dosković V., Petrović M.D., Petričević V. (2017). The effect of the rearing system, genotype and laying hens age on the egg weight and share of main parts of eggs. Acta Agriculture Serbica, Vol XXII**,** 44, 185-192.

Rakonjac S., Petrović M.D., Bogosavljević-Bošković S., Škrbić Z., Perić L., Dosković V., Petričević V. (2018). Effect of age and season on production performance and egg quality of laying hens from different rearing systems. The Journal of Animal and Plant Science, 28(6), 1602-1608.

Rakonjac S., Bogosavljević-Bošković S., Škrbić Z., Lukić M., Dosković V., Petričević V., Petrović M.D. (2019). The effect of the rearing system and the genotype of laying hens on the eggshell quality at different phases of the laying period. International Symposium on Animal Science, Herceg Novi 3-8. June, Montenegro, 58-64.

Turker I., Alkan S. (2019). Comparisons of Physical and Chemical Characteristics of Eggs Obtained using Hens Reared in Deep Litter and free-range Systems. Tarim Bilimleri Dergisi – Journal of Agricultural Sciences, 25**,** 181-188.

Škrbić Z., Pavlovski Z., Lukić M., Vitorović D., Petričević V., Stojanović LJ. (2011). Changes of egg quality properties with the age of layer hens in traditional and conventional production. Biotechnology in Animal Husbandry, 27(3), 659-667.

Voisey P.W., Hamilton R.M.G., Thompson B.K. (1979). Laboratory measurement of eggshell strength. 2. The quasi-static compression, puncture, non-destructive deformation, and specific gravity methods applied to the same egg. Poultry Science, 58 (1979), 288-294.

Koprivica R., Veljković B., Rakonjac S., Božić M., Vujičić V., Radivojević D., Marković D., Topisirović G. (2021). Direct methods for determination of the egg shell strength, Agricultural Engineering, 46(2), 1-10, doi: 10.5937/POLJTEH2102001K

SPOILAGE POTENTIAL OF PASTEURIZED MILK MICROBIOTA

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Abstract

The aim of this study was to evaluate the spoilage patterns of pasteurized milk microbiota. Additionally, the microbiological quality of raw and pasteurized milk regarding total aerobic and psychrotrophic counts was assessed. Samples were collected from two dairies at the milk reception point and different sites along pasteurized milk production line. Total aerobic and psychrotrophic counts in raw milk samples from Dairy 1 and 2 were at similar levels (approximately 6-7 log CFU/ml). The pasteurization step significantly reduced the total mesophilic bacteria counts. Samples from both dairies did not contain a detectable level of culturable psychrotrophic bacteria before the filling. However, psychrotrophic count in milk packages from Dairy 1 increased from 0.66±0.26 log CFU/ml to 3.76±0.19 at the end of the shelf-life. Forty-eight isolates with the different colony morphology selected from plate-count agar plates were screened for spoilage potential under laboratory conditions. The most prominent enzymatic trait (64.58% isolates) was proteolytic activity. Out of 17 enzymatically active isolates, 10 isolates were successfully identified as following: *Bacillus pumilus* (n=3), *Bacillus licheniformis* (n=2), *Leifsonia aquatica* (n=2), *Pseudomonas oryzihabitans* (n=2) and *Bacillus megaterium* (n=1). More stringent hygiene is required at each step of the dairy production chain to achieve a high microbiological quality of raw and pasteurized milk and to control spoilage microbiota.

Key words: *pasteurized milk, microbiota, spoilage potential.*

Introduction

The shelf life of pasteurized milk is largely dependent on the number of post process contaminants and storage temperature. Since pasteurized milk must be stored under refrigeration, psychrotrophic bacteria are favored and due to their ability to produce extracellular heat stable enzymes contribute to spoilage of heat-treated milk even when the microorganisms that produce enzymes are destroyed (Marchand et al., 2009). Proteolytic enzymes can lead to the development of bitter flavour, and physico-chemical instability (increased viscosity and milk gelation) (Datta and Deeth, 2003), while lipolytic enzymes cause rancid, butyric, or soapy flavors and also may lead to a reduction in milk foaming properties (Deeth and Fitzgerald, 2006).
Spoilage-associated microbiota may enter the pasteurized milk either as part of raw milk microbiota or as recontaminants during the dairy plant operations. In the first case, spoilage of pasteurized milk may occur as a consequence of contamination at the farm level, when psychrotolerant spore-forming bacteria, mainly *Bacillus* spp. and *Paenibacillus* spp., enter the fluid milk continuum, survive pasteurization, and subsequently germinate and outgrow to spoilage levels during refrigerated storage. Although transmission of spores into to end product

does occur, the psychrotolerant sporeformers may established themselves as in-house microbiota leading to the different recontamination sites throughout the pasteurization production line. Recontamination of milk after pasteurization, mainly with gram-negative bacteria such as *Pseudomonas* spp. may also lead to product's spoilage (Martin et al., 2021). Despite the fact that adherance to good manufacturing practices, sanitation and cleaning protocols have always been a cornerstone of dairy everyday practice, postpasteurization contamination is still very much an issue in the dairy industry. According to Martin and co-workers (2018), almost 50% of the fluid milk supply shows evidence of postpasteurization contamination with heat-labile gram-negative bacteria that originate from the dairy processing environment.
In Serbia fluid milk (pasteurized and sterilized milk) accounts for approx. 45% of the total production (Djekic et al., 2013). Pasteurized milk is one of the most preferred dairy product due to its flavor and „fresh" image with average consumption of 1075 ml a week (153.6 ml/day) (Djekic et al., 2020). The research activities that capture diversity and spoilage potential of pasteurized-associated microbiota are on the rise. Therefore, in order to contribute to this body of knowledge, the aim of the present study was to evaluate the spoilage patterns of pasteurized milk microbiota. Additionally, the microbiological quality of raw and pasteurized milk was assessed.

Materials and methods

Milk sample collection

The study was conducted in two dairies in the Republic of Serbia. Milk samples were obtained to represent the processing continuum from the raw milk reception point to packaged product. Raw and pasteurized milk samples were taken out at different sites along the production line (raw milk storage tank, just after the pasteurization step, sealed milk packages). Packaged pasteurized milk was also analyzed at the end of the shelf life.

Microbiological testing of milk samples

Milk samples were diluted with 0.1% sterile buffered peptone water (Oxoid, Basingstoke, UK) and aliquots of 1ml of the appropriate decimal dilutions were plated in duplicates on the plate count agar (PCA, Oxoid, Basingstoke, UK). Plates were incubated aerobically at 30°C for 48h and 7°C for 10 days for the enumeration of mesophilic and psychrotrophic bacteria, respectively. After viable counts, total of 48 colonies with different morphology were randomly isolated from countable plates and purified on PCA plates. The purified isolates are preliminary characterized by microscopic observation, Gram staining, catalase and oxidase reaction.

Proteolytic and lipolytic activity

Ability of selected isolates to produce extracellular enzymes was determined by agar diffusion assays. The following experimental setup was used: bacterial colonies were picked from nutrient agar (Oxoid, Basingstoke, UK) and suspended in the sterile saline solution to achieve cell density corresponding to McFarland 1. Aliquots of 10 µl of bacterial suspensions were then spotted on the surface of solid media. Skim milk agar (2x nutrient agar + 10% (w/v) skim milk powder) was used for screening of protease activity; Tributyrin Agar (Oxoid, Basingstoke, UK) was used for screening of lipolytic activity, and 2x PCA (Oxoid, Basingstoke, UK) with addition of 10% yolk emulsion was used for screening of lecithinase. Plates were incubated at 30°C for 5 days. At the end of the incubation period the Petri dishes were checked for enzymatic activity indicated by a clear halo.

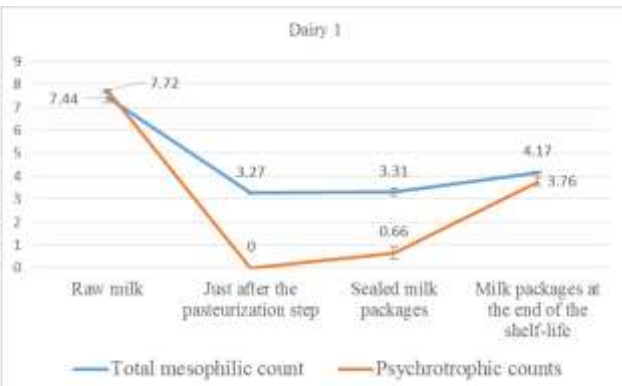
Identification of isolates

Based on their enzymatic activity, a total of 17 isolates were selected for the identification. Isolates were identified using commercial assays according to manufacturer's instruction. The Gram positive cultures (n=11) were identified using the Gram-Positive (GP) Identification (ID) kit (Beckton Dickinson, Sparks, USA), while the Gram negatives cultures were identified using the BD BBL CRYSTAL™ Enteric/Nonfermenter (E/NF) Identification (ID) kit (Beckton Dickinson, Sparks, USA). Identification was performed using BD BBL ™ Crystal ™ MIND identification software by entering the ten-digit code obtained from the results of performed tests.

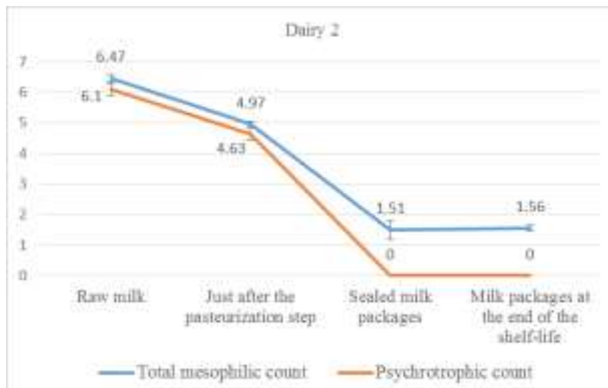
Results and discussion

The microbiological quality of pasteurized milk is traditionally estimated by the counts of both total mesophilic and psychrotrophic microbial load.

The total mesophilic and psychrotrophic counts determined in milk samples collected during the pasteurization production line in two dairies were presented in Graphs 1 and 2.



Graph 1 – Total mesophilic and psychrotrophic counts in milk samples collected from Dairy 1



Graph 2 - Total mesophilic and psychrotrophic counts in milk samples collected from Dairy

High total mesophilic count in samples taken from raw milk storage tank in both dairies indicate poor microbiological quality of raw milk. According to Serbian legislation (The Official Gazette of the Republic of Serbia no. 25/11 and 27/2014; The Official Gazette of the Republic of Serbia no. 106/17), raw milk from both dairies did not meet required quality criteria. Psychrotrophic counts were high in both dairies – 7.72±0.08 log CFU/ml in Dairy 1 and 6.1 log CFU/ml in Dairy 2. Contrary to our results, studies by other authors (Hantsis-Zacharov et al., 2007; Ercolini et al., 2009; Vithanage et al., 2014) showed the psychrotrophic microbial load of raw milk up to 10% of the total number of microorganisms. However, when milk is produced in poor hygiene conditions and/or contains increased numbers of somatic cells, the number of psychrotrophic microorganisms can make up 75% of the total raw milk microbiota (Hantnis-Zacharov and Halpren, 2007; Malacarne et al., 2013), or for more than 90% of the total microbial population in cooled raw milk (Catanio et al., 2012). Pasteurization step significantly decreased total mesophilic and psychrotrophic counts in both dairies. It is worth mentioning that both dairies have internal pasteurization procedures that differ from the HTST pasteurization process that is conventionally applied. In Dairy 1, pasteurization is performed at temperatures over 80°C for few minutes, while in Dairy 2, pasteurization at temperature higher than 80°C is performed twice before the filling step. Although psychrotrophic bacteria in milk samples from Dairy 1 were not detectable right after pasteurization, they were present at 0.66±0.26 log CFU/ml after milk packaging, and increased to 3.76±0,19 at the end of the shelf-life. According to Eneroth et al. (2000) increase in the number of microorganisms in milk packages, can occur as a result of contamination by microorganisms originating from the air in the filling machines, water used for washing, or aerosols generated during cleaning. Moreover, milk pasteurization can cause psychrotrophic bacteria to switch to the viable but non-culturable state, so the application of culture-independent methods is recommended in order to get reliable information about reducing effect of pasteurization on psychrotrophic microbiota (Oliviera et al., 2015). Culturable psychrotrophic bacteria were not detectable in samples from Dairy 2 after the packaging and during the product's shelf-life. In none of the samples microbial load reached the number of 6 log CFU/ml, which represents the limit above which pasteurized milk spoilage can occur (Doll et al., 2017). A total of 48 colonies showing different macromorphology on PCA agar were tested for their ability to produce extracellular enzymes at 30°C (Table 1). Extracellular enzymatic activity was visualized by agar diffusion assays. The most prominent enzymatic trait was proteolytic activity, which was present in 31 isolates (64.58%). Lipolytic activity showed 28 isolates (58.3%), and lecithinase activity 12 isolates (25.0%). Proteolytic activity, as the most common enzymatic trait in milk associated microbiota, was reported by other authors (Vithanage et al., 2014; von Neubeck et al., 2015). Moreover, proteolytic and lipolytic activity, and the less pronounced lecithinase activity, were characteristic of bacteria from the genus *Bacillus* (Hantnis-Zacharov and Halpern, 2007), which are the most frequently identified isolates covered by this study.Isolates obtained from different points in pasteurized milk production process were selected for the identification, based on their enzymatic activity. Out of 17 selected isolates, 10 were successfully identified at the species level (Table 1).

Table 1. Identified isolates and their enzymatic traits

Origin of isolate	**Species**	**ID accuracy (%)**	**Protease**	**Lipase**	**Lecithinase**
Raw milk	*Pseudomonas oryzihabitans*	84.70	+	+	-
Just after the pasteurization step	*Bacillus pumilus*	99.10	+	+	+
Just after the pasteurization step	*Pseudomonas oryzihabitans*	84.70	+	+	-
Just after the pasteurization step	*Bacillus megaterium*	99.90	+	+	-
Just after the pasteurization step	*Leifsonia aquatica*	99.99	+	+	-
Just after the pasteurization step	*Bacillus licheniformis*	98.70	+	+	+
Sealed milk packages	*Leifsonia aquatica*	99.99	+	+	-
Sealed milk packages	*Bacillus pumilus*	99.90	+	+	+
Sealed milk packages	*Bacillus pumilus*	99.10	+	+	+
Sealed milk packages	*Bacillus licheniformis*	96.70	+	+	-

The largest proportion of successfully identified gram-positive isolates were members of the genus *Bacillus*, ie *Bacillus pumilus*, *Bacillus licheniformis* and *Bacillus megaterium*. All *Bacillus* isolates originated from heat-treated milk, which is in accordance with the results from other studies where members of *Bacillus* spp. are identified as one of the main causes of pasteurized milk spoilage, in cases where recontamination with gram-negative bacteria is ruled out (Coorevits et al., 2008; Ivy et al., 2012; Masiello et al., 2014). *Bacillus* spp. have numerous routes of contamination of milk, such as through water, air, soil, feces, udders and milking equipment (De Jonghe, 2010). As thermoduric microorganisms, they have the ability to survive the pasteurization process, but there is also the possibility of recontamination during the pasteurized milk production process (Kumari and Sarkar, 2016). As mentioned before, in both dairies, the pasteurization process is carried out at higher temperatures than conventional pasteurization. According to the study of Ranieri et al. (2009), higher pasteurization temperatures favor spore germination and stimulate the growth of *Bacillus* spp, especially *Bacillus pumilus* and *Bacillus licheniformis*. Also, *Bacillus pumilus* and *Bacillus licheniformis*, in addition to being thermoduric, are also psychrotolerant (Ranieri et al., 2009).
Pseudomonas spp. is most common psychrotrophic microorganisms isolated from raw milk stored in the cold chain (Eneroth et al., 2000; Hantsis-Zacharov et al., 2007; Ercolini et al., 2009; von Neubeck et al., 2015. Yuan et al., 2017). However, since the pasteurization process removes all gram-negative bacteria (Ranieri et al., 2009), isolation of *Pseudomonas oryzihabitans* from pasteurized milk indicates post process recontamination.

Conclusion

The majority of enzymatically active isolates are identified as *Bacillus* spp. As sporeformers primarily enter into the milk chain on farms, control strategies must start at the farm level and continue throughout processing. Thus, the key strategy in ensuring the desired shelf life of pasteurized milk is to meet increased raw milk quality standards. This would be very hard and challenging task as high initial mesophilic and psychrotrophic plate counts of raw milk samples related to unhygienic practice during the production, storage and handling of milk is observed in this study.

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References

Catanio F.S., Inay O.M., Silva A.S., Pereira J.R., Tamanini R., Beloti V., de Rezende Costa M., de Souza C.H.B., Aragon-Alegro L., de Sanatana E.H.W. (2012). Refrigerated raw milk quality of a processing plant in the north of Parana after the implementation of changes imposed by NI 62 of 2011. Semina, 33, 3171–3180.

Coorevits A., De Jonghe V., Vandroemme J., Reekmans R., Heyrman J., Messens W., De Vos P., Heyndricx M. (2008). Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms, Systematic and Applied Microbiology, 31, 126–140.

Datta N., Deeth H.C. (2003). Diagnosing the cause of proteolysis in UHT milk, LWT - Food Science and Technology, 36, 137-182.

De Jonghe V., Coorevits A., De Block J., Van Coillie E., Grijspeerdt K., Herman L., De Vos P., Heyndrickx M. (2010). Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk, International Journal of Food Microbiology, 136, 318-325.

Deeth H.C., Fitzgerland C.H. (2006). Lipolytic enzymes and hydrolytic rancidity. In: Fox P.F., McSweeney, P.L.H. (Eds.). Advanced Dairy Chemistry Volume 2 Lipids. Springer, US, 481-556.

Djekic I., Miocinovic J., Pisinov B., Ivanovic S., Smigic N. (2013). Quality characteristics of selected dairy products in Serbia, Mljekarstvo, 63 (4), 228-236.

Djekic I., Petrovic J., Jovetic M., Redzepovic-Djordjevic A., Stulic M., Lorenzo J.M., Iammarino M., Tomasevic I. (2020). Aflatoxins in Milk and Dairy Products: Occurrence and Exposure Assessment for the Serbian Population, Applied Science, 10, 7420.

Doll E.V., Scherer S., Wenning M. (2017). Spoilage of microfiltered and pasteurized extended shelf life milk is mainly induced by psychrotolerant spore-forming bacteria that often originate from recontamination, Frontiers in Microbiology, 8, 135.

Eneroth Å., Ahrné S., Molin G. (2000). Contamination routes of Gram-negative spoilage bacteria in the production of pasteurised milk, evaluated by randomly amplified polymorphic DNA (RAPD), International Dairy Journal, l10, 325–331.

Ercolini D., Russo F., Ferrocino I., Villani F. (2009). Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk, Food Microbiology, 26(2), 228-231.

Hantsis-Zacharov E., Halpern M. (2007). Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits, Applied and Environmental Microbiology, 73 (22), 7162-7168.

Ivy R.A., Ranieri M.L., Martin N.H., Den Bakker H.C., Xavier B.M., Wiedmann M., Boor K.J. (2012). Identification and characterization of psychrotolerant sporeformers associated with fluid milk production, Applied and Environmental Microbiology, 78(6), 1853-1864.

Kumari S., Sarkar P.K. (2016). *Bacillus cereus* hazard and control in industrial dairy processing environment, Food Control, 69, 20–29.

Malacarne M., Summer A., Franceschi P., Formaggioni P., Pecorari M., Panari G., Vecchia P., Sandri S., Fossa E., Scotti C., Mariani P. (2013). Effects of storage conditions on physico-chemical characteristics, salt equilibria, processing properties and microbial development of raw milk, International Journal of Food Microbiology, 29, 36–41.

Marchand S., Vandriesche G., Coorevits A., Coudijzer K., De Jonghe V., Dewettinck K., De Vos P., Devreese B., Heyndrickx M., De Block J. (2009). Heterogeneity of heat resistant proteases from milk *Pseudomonas* species, International Journal of Food Microbiology, 133, 68-77.

Martin N.H., Boor K.J., Wiedmann M. (2018). Symposium review: Effect of post-pasteurization contamination on fluid milk quality, Journal of Dairy Science, 101, 861–870.

Martin N.H., Torres-Frenzel P., Wiedmann N. (2021). Invited review: Controlling dairy product spoilage to reduce food loss and waste, Journal of Dairy Science, 104, 1251-1261.

Masiello S.N., Martin N.H., Watters R.D., Galton D.M., Schukken Y. H., Wiedmann M., Boor K.J. (2014). Identification of dairy farm management practices associated with the presence of psychrotolerant sporeformers in bulk tank milk, Journal of Dairy Science, 97, 4083–4096.

Oliviera G.B., Favarin L., Luchese R.H., McIntosh D. (2015). Psychrotrophic bacteria in milk: How much do we really know? Brazilian Journal of Microbiology, 46 (2), 313-321.

Ranieri M.L., Huvk J.R., Sonnen M. Barbano D.M., Boor K.J. (2009). High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk, Journal of Dairy Science, 92(10), 4823-4832.

The Official Gazette of the Republic of Serbia no. 106/17. Rules on the quality of raw milk

The Official Gazette of the Republic of Serbia no. 25/11 and 27/2014. 10. Rules on veterinary and sanitary conditions, in regard to general and special conditions of hygiene of food of animal origin, as well as conditions of hygiene of food of animal origin.

Vithanage N.R., Yeager T.R., Jadhav S.R., Palombo E.A., Datta N. (2014). Comparison of identification systems for psychrotrophic bacteria isolated from raw bovine milk, International Journal of Food Microbiology, 189, 26-38.

Von Neubeck M., Baur C., Krewinkel M., Stoeckel M., Kranz B., Stressler T., Fischer L., Hinrichs J., Scherer S., Wenning M. (2015). Biodiversity of refrigerated raw milk microbiota and their enzymatic spoilage potential, International Journal of Food Microbiology, 211, 57-65.

Yuan L., Sadiq A.F., Liu T., Flint S., Chen J., Yang H., Gu J., Zhang G., He G. (2017). Psychrotrophic bacterial populations in Chinese raw dairy milk, LWT- Food Science and Technology, 84, 409-418.

USING PROBIOTICS IN POULTRY NUTRITION: OPTIMIZING PERFORMANCE AND FEEDING COSTS

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Abstract

Poultry is one of the major animal protein sources for human nutrition. In recent years, the interest of consumers and the pressure against the use of the antibiotics in animal production have been increasing for safety and healthy foods. Due to ban of the antibiotics as growth promoters, the alternative feed additives have been suggested to improve growth rate and feed efficiency, stimulates intestinal health, and inhibit the pathogenic diseases. One of these alternatives is probiotic which is defined as live microbial that produce beneficial effects in the host animal by improving microbial balance of the intestines. Probiotics consist of living bacteria's, fungi's, yeast, and enzymes which support the gastrointestinal flora and health of digestive system. Commercial probiotic products include *Lactobacillus* spp. and *Bifidobacterium* spp. and other microorganisms (*Streptococcus thermophilus*, *Escherichia coli*, *Bacillus cereus*) and yeast (*Saccharomyces boulardii*, *Saccharomyces cerevisiae*) in poultry nutrition. There is an emerging preference for Bacillus-based probiotics in the poultry industry because this genus has characteristics that overcome the challenges associated with conventional probiotics. Their endospore forming ability enables them to be stable and viable during feed manufacturing, storage, and gut transit. The expected target of the usage of the probiotics in poultry nutrition is to maximize profitability with the lowest economical losses in production chain. The supplementation of probiotic could have potential to decrease mortality due to some infectious diseases, because it stimulates the immunity, intestinal health, and digestibility. The objective of this paper is to evaluate the beneficial effects of probiotic additives on performance parameters, nutrient digestibility, intestinal histomorphology and immunity, and the feeding cost in broiler and layer hens.

Key words: *Poultry industry, Bacillus-based probiotics, performance parameters, nutrient digestibility, Intestinal health.*

The identification and characteristics of probiotics

A probiotic is defined as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. They have a special role among functional foods and form a major part of the functional foods market, especially in the last decades. Probiotics contain lactic acid bacteria (LAB) or *Bifidobacterium* spp. The functional probiotics in dairy products must be more than 108 (cfu/ml) (Homayouni, 2014). Probiotic-containing products include dairy products such as yogurt, cheese, ice cream, acidophilus bifidus milk, ayran, kefir, kumiss, probiotic-containing doogh and milk drinks (both fermented and unfermented).

Probiotics could been classified as *Bifidobacterium, Lactococcus, Lactobacillus, Bacillus, Streptococcus and Candida yeasts*. Besides bacterias, fungis or yeasts are among the probiotics that enhance the gastrointestinal flora and the maintenance of a healthy digestive system. *Lactobacilli* and *Enterococci* have been widely used of microbial species as probiotics (Elam *et al.,* 2003; Patterson and Burkholder, 2003; Russell and Grimes, 2009). These species have recently gained more interest for use as probiotics. Their endospore forming ability enables them to be stable and viable during feed manufacturing, storage, and gut transit. It have been influenced the intestinal composition and metabolism (Huyghebaert *et al.,* 2011; Das *et al.,* 2012). It has been found that the addition of live microorganisms to the ration in animal nutrition increases immunity by strengthening the immune system. (Koenen *et al.*, 2004; Placha *et al.*, 2010).

The reason of probiotics usage in poultry nutrition

Probiotic microorganisms, even once ingested with living microbial cells, can regulate the basal and intestinal balance and activities of the gastrointestinal microbiota (Chaucheyras-Durand and Durand, 2010).

The impacts of probiotics appear in different ways after consumption by animals;

1) Maintenance of normal intestinal microflora with competitive exclusion and antagonist effect (Nurmi and Rantala., 1973; Jin *et al*., 1998).

2) Increasing digestive enzymes activity, regulating metabolism by decreasing ammonia production and bacterial enzyme activity (Jin *et al.,* 2000),

3) Improving feed consumption and digestion, and stimulating the immune system (Jin *et al*., 1998).

Probiotic microorganisms may regulate the in intestine microflora benefically.

Microflora activity may provide several benefits;

• Increased weight gain and feed effiency
• Increased the intestinal health and immunity of the poultry
• Suppressing pathogens such as Campylobacter and Salmonella species (Vilà *et al.,* 2009).

The ability of probiotics to utilize feed efficiency includes positive change in intestinal flora, enhanced growth of non-pathogenic facultative anaerobic and gram-positive bacteria, lactic acid formation, suppression of growth of intestinal pathogens, and enhanced digestion and utilization of nutrients. In addition to probiotics contribute positively impact to the development of the immune system (Coconnier *et al.,* 1993; Yeo and Kim, 1997).

Table 1. Description of the reasons using probiotics in poultry nutrition (Bahadıroğlu,1997; Kumprechtova *et al.,* 2000).

Usage of a potential alternative to antibiotics.
Contribute to the improvement of digestion and effective utilization of nutrients.
Intestinal flora in poultry have been improved by the increased feed conversion efficiency.
Prevention of intestinal pathogens growth rate.
Survival rate of poultry have been increased by using probiotics.
Benefical effecs in lowering the pH of the intestine.
Producing some symbiotic enzymes such as cellulase, xylanase, lipase, protease, β-glucanase and amylase.
Prevent the accumulation of toxic substances in the intestine.
Promotes the growth of non-pathogenic facultative anaerobic and gram-positive bacteria that form lactic acid and hydrogen peroxide.

By producing enzymes such as cellulase, xylanase, lipase, protease, β-glucanase and amylase, which works symbiotically with the enzymes produced by the digestive system cells of the poultry, that enhances the digestion of nutrients especially in chicks digestive system. Besides, these various microorganisms have been used to contribute to digestion system by synthesizing B group vitamins (Niacin, Biotin, Pyridoxine, Folic Acid, Pantothenic Acid) (Hooper, 1990; Vanbelle *et al*.,1990). Probiotics reduce the pH of the intestine (pH below 4-4.5) with organic acids such as lactic acid and acetic acid that prevents the growth of pathogenic microorganisms living at neutral or basic pH (Jernigan *et al.,* 1985).
In addition to these advantages; the significant impact of adding probiotics in poultry nutrition which is enables animals to gain resistance against pathogens (Mountzouris, 2014; Mountzouris *et al.,* 2015). To protect the intestines from pathogenic microorganisms, strengthen intestinal function and contribute to the development of the immune system could be possible by using probiotics in poultry (Ng *et al.,* 2009). Probiotics strengthen the intestinal barrier function and preserve against pathogens by improving the intestinal environment, enhancing the beneficial intestinal microflora (Ganguly (2013).
In poultry nutrition, probiotics should be include tolerance to gastrointestinal conditions, ability to protect the gastrointestinal mucosa from pathogens. As a result of various studies, the impacts of probiotic use in reducing pathogenic microorganisms in poultry, probiotic supplementation in poultry rations revealed that *Salmonella enteritidis, S. typhimurium, Escherichia coli, Clostridium perfringens, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus aureus, Eimeria sp.* and Actinomyces have been reported to have antibacterial effects on many pathogens (Gadde *et al.,* 2017). After probiotic supplementation, non-pathogenic bacteria from probiotics inhibit the harmful bacteria in the intestines and secrete digestive enzymes (galactosidase, amylase, etc.) (Jadhav *et al.,* 2015).

Egg production and quality

The inclusion of probiotics in laying hens increases daily feed intake, nitrogen and calcium retention, and egg production by reducing intestinal length (Jha *et al.,* 2020). The dietary supplementation with probiotics is not only capable of increasing egg production, but also increasing feed conversion ratio and productivity, chicken performance and eggshell quality (Mikulski *et al.,* 2012). The addition of *Lactobacillus acidophilus* (0.1%) and *Bacillus subtilis* (0.05%) to the ration of laying hens resulted an improvement in performance, in antibody production, and a decrease in the amount of blood triglycerides and cholesterol (Forte *et al.*, 2016). It has been determined that probiotics (L. acidophilus) in laying hen rations reduces the plasma cholesterol level (Mohan *et al.,* 1995).
Zhang *et al.,* (2013) found that higher density lipoprotein (HDL) cholesterol in laying hens fed diets containing probiotics compared to chickens fed diets not supplemented with probiotics. As a result, 0.01% probiotic dietary supplementation to the ration improved egg production and egg quality and reduced fecal ammonia emission in this research. Besides it has been stated that *Lactobacillus salivarius* and *Bacillus subtilis* to the diets of layer hens, could be caused increases in egg production, daily egg yield, Haugh unit, damaged egg ratio (Zhang *et al.,* 2012). Mátéová *et al.,* (2009) found that Hyssex layer hybrids treated with probiotics did not cause significant changes in egg yolk cholesterol.

Feed intake, feed efficiency and growth rate

Probiotics could improve feed conversion, reduce morbidity or mortality, and improve product quality. It has been reported that probiotic consumption causes suppression of pathogenic microorganisms in the intestine, exclusionary competition and a growth in some digestive enzymes. The increase in feed efficiency is due to the use of nutrients lost by pathogenic microorganisms for growth and efficiency and the development in some digestive enzymes increases the dry matter digestibility (Tortuero and Fernandez, 1995; Yeşilbağ and Çolpan, 2006).
Usage of probiotics in improvements for growth performance and feed efficiency have been reported in broiler chickens (Kabir *et al.,* 2004; Mountzouris *et al.,* 2007; Samli *et al.*, 2007). Kurtoğlu *et al.,* (2004) found that feed conversion ratio were positively affected by supplementation of 250 and 500 mg kg (-1) probiotic compared with controls in their study. On the contrary to this research, Pruszynska- Oszmalek *et al.*, (2015) found in their research, the use of probiotics in roosters increased body weight but the feed conversion ratio was not changed. In Awad *et al.*, (2006) study indicates that probiotics supplemantation was found uneffective for feed efficieny in broilers.

Carcass yield and quality in broiler

Many studies have been conducted to investigate that the use of various probiotics to enhance performance characteristics in broilers (Patterson and Burkholder, 2003; Amerah *et al.*, 2013; Fuentes *et al.*, 2013; Zhang and Kim, 2014). *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and yeast such as *Saccharomyces cerevisiae* probiotics has been effected on the different performance parameters in chickens including feed efficiency in broilers (Endo and Nakano, 1999). Similarly, the use of *C. butyricum* and *S. boulardii* in poultry nutrition could improve growth performance in broilers. The addition of probiotics (*Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces and Candida spp*.) to broiler rations increased the carcass and meat quality of broiler chickens (Zhao *et al.*,2013; Rajput *et al.*, 2013).
In another study, the inclusion of *Lactobacillus acidophilus* and *Streptococcus faecium* probiotics in broiler ration increased protein, ash, water holding capacity, emulsion capacity and stability in meat in broiler nutrition (Mahajan *et al.,* 2000).
Supplementation of probiotics to the diet in broilers has been associated with increased body weight gain and feed intake (Mountzouris *et al.*, 2007). Al-Zenki *et al.*, (2009) examined the effect on performance in broiler chickens by using commercial probiotics *Saccharomyces cerevisiae* and *Pediococcus acidilactici*. In the results of this research probiotic treatments was found be uneffective on the production parameters of broiler chickens.
Yang *et al.*, (2010) researched that *Clostridium butyricum* supplementation reduced the ratio of n-6:n-3 fatty acids in breast muscles and enhanced eicosapentaenoic acid (EPA(and total n-3 fatty acids. For another study, it was reported that adding bacteria (Rhodobacter capsulatus) to the broiler diet could improve the fatty acid content of broiler chicken meat (Salma *et al.*, 2007).
In the contrary to this researches that the effect of probiotic dietary supplementation probiotic was found no significant on carcass yield, breast meat and lymphoid organs weight (Karaoğlu and Durdağ, 2005; Cengiz *et al.*, 2015). Due to differences of animal material and variety and

amount of probiotics could be differences in the results of the studies (Kumprechtova *et al*, 2000).
Furthermore, *Bacillus subtilis*, on musculoskeletal health and related meat quality in broilers. In relation to meat quality and musculoskeletal development in broilers, the *Bacillus subtilis* probiotic has been widely used in other animals, including broilers. (Mohammed *et al*., 2021). It is also stated that growth in poultry is accelerated by increasing probiotic doses (from 0.5 g to 1.5 g per 10 kg feed) (Zulkifli *et al.*, 2000).

Nutrient digestibility and intestinal histomorphology

Probiotic microorganisms have been found to maintain on intestinal microflora in poultry nutrition. Regulation the digestion of intestinal enzymes and nutrients and impromevent pH and flora could be possible by probiotics. In addition to that the intestinal amylase activity have been increased with the enzyme secretion of lactobacilli colonization in the intestine with the addition of probiotics (Duke, 1977; Dierck, 1989). The addition of probiotics to the ration in poultry has beneficial effects on digestion and absorption in the intestines. Probiotics have a growth-promoting effect due to their benefits on the growth of intestinal microbiota and their contribution to intestinal functions (Jin *et al.*, 1998; Fioramonti *et al.*, 2003; Guillot., 2003).
The growth of lactic acid bacteria occurs in the gastrointestinal organs after probiotic consumption of the animals including poultry. These microorganisms change the intestinal environment and produce enzymes and other beneficial substances in the intestine Also, lactic acid could been produced by some bacterias such as *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus* (Marteau *et al*., 1993).
Probiotics regulate the intestinal pH by producing organic acids such as lactic acid and formic acid, thus preventing the growth of gram (-) pathogenic microorganisms that live in a neutral and basic environment and are harmful. *Staphylococcus* species used as feed additives cause competition between gram (-) bacteria and vibrio species living in the intestines and lactic acid producing microorganisms, thereby reducing the effect of microorganisms that are pathogenic for animals such as *E. coli, Salmonella, Proteus, Pseudomonas, Klebsiella* (Ülger *et al.*, 2016).
Due to antibacterial effects, *bacteriocins* have been obtained by the probiotic with the competitive effect of bacteria in the intestine (Patterson and Burkholder, 2003). Some studies in laying hens and broilers have indicated that probiotics may be beneficial for improving intestinal health (Deng *et al.*, 2012; Bai *et al.*, 2013).
The inclusion of *C. butyricum* in the ration contributes to the performance of laying hens and the development of normal intestinal morphology. Besides in the laying hens, *Butyricum* and *S. boulardii* and *P. acidilactici* combination was found effective in enhancing of the intestine (Xiang *et al.*, 2019). In addition to other studies results, probiotic supplementation in the diet increases villus height and villus height/crypt depth ratio in ileum and cecum. As the result better epithelial growth after probiotic supplementation could been found (Kim *et al.*, 2011; Sen *et al.*, 2012).

The economical impacts and targets of the probiotics

The major component of feed cost in poultry production could be reach until %70. Recently probiotic supplementation has proven to be more profitable and economical in order to gain maximum profit from poultry production. The use of probiotics as feed additives could be caused to reduce production costs by increasing feed efficiency (Rufino *et al.,* 2015).

Probiotic to the ration as a nutritional supplement also in broilers is among the significant impacts leading to a decrease in production costs. It has been determined by Anjum *et al.*, (2005) study, the experimental group fed with the broiler diet supplemented with probiotics could be obtained at a lower cost than the control group. As the result of this study the using of probiotics was found more economical and productive for broiler production. Besides in many studies have found that probiotic supplementation in broiler production enhances growth performance (Fuentes *et al.*, 2013; Zhang and Kim, 2014).

Ray *et al.,* (2019) found in the research that probiotics to the ration increases economic profitability and the useage of probiotics containing *Bacillus subulans* and *Bacillus soagulans (PB-2)* positively affects the cost. Similarly, Patel *et al.,* (2015) found that various probiotic supplements to the ration could contribute to more economical production. Besides, adding probiotics in egg layers feeding, many economic benefits have been provided to increase eggshell breaking strength and egg quality (Sakai *et al.,* 2006).

There is an emerging preference for Bacillus-based probiotics in the poultry industry because this genus has characteristics that overcome the challenges associated with conventional probiotics (Ramlucken *et al.,* 2020).

Conclusion

In conclusion, there are many points that need to be explained regarding the full understanding of the mechanism of action in probiotics. Both consumer preferences and the limitation of using antibiotic will make the probiotics and prebiotics inevitable for today and the future. Usage various types of microorganisms that can be used as probiotics in the future will lead to profitability in yield and economic gain.

References

Abd El-Hack, M. E., Mahgoub, S. A., Alagawany, M., Ashour, E. A. (2017). Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn DDGS with or without Bacillus subtilis probiotic. Journal of Animal Physiology.

Al-Zenki, S.F., Al-Nasser, A.Y., Al-Saffar, A.E., Abdullah, F.K., Al-Bahouh, M.E., Al-Haddad, A.S., Alomirah, H., Mashaly, M. (2009). Effects of using a chicken-origin competitive exclusion culture and probiotic cultures on reducing Salmonella in broilers. Journal of Applied Poultry Research, 18: 23-29.

Amerah AM., Quilesb A., Medelc P., Sánchezc J., Lehtinend MJ., Graciac MI. (2013). Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. Animal Feed Science and Technology, 180:55-63.

Anjum MI., Khan AG., Azim A., Afzal M. (2005). Effect of dietary supplementation of multi-strain probiotics on broiler growth performance. Pakistan Veterinary Journal, 25(1): 25 - 29.

Awad WA., Ghareeb K., Abdel-Raheem S., Böhm J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science, 88:49-55.

Bahadıroğlu E. (1997). Aviguard (Doğal sindirim sistemi florası). Hayvancılık Yan Sanayi ve Veteriner Hekimliği Dergisi, 17(1):5-8.

Bai SP., Wu AM., Ding XM., Lei Y., Bai J., Zhang KY., Chio JS. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science, 92: 663-670.

Cengiz Ö., Köksal BH., Tatlı O., Sevim Ö., Ahsan U., Üner AG., Ulutaş PA., Beyaz D., Büyükyörük S., Yakan A. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poultry Science, 94:2395-2403.

Chaucheyras-Durand F, Duran H. (2010). Probiotics in animal nutrition and health. Beneficial Microbes, 1:3–9.

Coconnier MH., Bernet MF., Chauvie`re G., Servin, L. (1993). Adhering heat-killed human Lactobacillus acidophilus, strain LB, inhibits the process of pathogenicity of diarrhoeagenic bacteria in cultured human intestinal cells. Journal of Diarrhoeal Diseases Research. 11:235-242.

Das L., Bhaumik E., Raychaudhuri U., Chakraborty R. (2012). Role of nutraceuticals in human health. Journal Food Science and Technology, 49:173-183.

Deng W., Dong XF., Tong JM., Zhang Q. (2012). The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poultry Science, 91: 575-582.

Dierck, N.A. **(**1989). Biotechnology aids to improve feed and feed digestion: Enzymes and fermentation. Archives of Animal Nutrition, 39: 241-261.

Duke, G.E. (1977). Avian digestion. In physiology of domestic animals, 9th ed.; Duke, G.E., Ed.; Cornell University Press: Ithaca, NY, USA: pp. 313-320.

Elam NA, Gleghorn JF, Rivera JD, Galyean ML, Defoor PJ, Brashears MM, Younts-Dahl SM. (2003). Effects of live cultures of Lactobacillus acidophilus (strains NP45 and NP51) and Propionibacterium freudenreichii on performance, carcass, and intestinal characteristics, and Escherichia coli strain O157 shedding of finishing beef steers. Journal of Animal Science, 81:2686-2698.

Endo T., Nakano M., Shimizu S., Fukushima M., Miyoshi S. (1999). Effects of a Probiotic on the Lipid Metabolism of Cocks Fed on a Cholesterol-enriched Diet. *Bioscience, Biotechnology, and Biochemistry*, 63(9): 1569-1575.

Fioramonti J., Theodorou V., Bueno L. (2003). Probiotics and their effect on gut physiology. Best Practice and Research Clinical Gastroenterology,17:711-24.

Forte C., Moscati L., Acuti G., Mugnai C, Franciosini MP., Costarelli S., Cobellis G., Trabalza-Marinucci M. (2016). Effects of dietary Lactobacillus acidophilus and Bacillus subtilis on laying performance, egg quality, blood biochemistry and immune response of organic laying hens. Journal of Animal Physiology and Animal Nutrition. 100:977–987.

Fuentes MC, Lajo T, Carrión JM, Cuñé J. (2013). Cholesterol-lowering efficacy of Lactobacillus plantarum CECT 7527, 7528 and 7529 in hypercholesterolaemic adults. British Journal of Nutrition, 109(10):1866-72.

Gadde UD., Kim WH., OH ST., Lillehoj HS. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. Animal Health Research Reviews, 18, 26–45.

Ganguly S. (2013). Supplementation of prebiotics, probiotics and acids on immunity in poultry feed: a brief review. World's Poultry Science Association, 69:639-647.

Guillot JF. (2003). Probiotic feed additives. Journal of Veterinary Pharmacology and Therapeutics, 26: 52–55.

Homayouni RA, (2014). Therapeutical effects of functional probiotic, prebiotic and synbiotic foods. Firsted. Tabriz University of Medical Science; Tabriz, Iran, 3-35.

Hooper, R. (1990). Probiotics-İntestinal İnoculants For Production Animals. (1990). In: Probiotics in Animal Nutrition Of Animals. Sbornik Prednasek. 19-21 November, Brno.Pp.69-88.

Huyghebaert G., Ducatelle R., Immerseel FV. (2011). An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal, 187(2): 182-188.

Jadhav K., Katoch S., Sharma VK., Mane BG. (2015). Probiotics in Broiler Poultry Feeds: A Review. Journal of Animal Physiology and Animal Nutrition, 1:4–16.

Jernigan MA., Miles RD., Arafa AS. (1985). Probiotics in poultry nutrition. World's Poultry Science Journal, 41:99-107.

Jha R., Das R., Oak S., Mishra P. (2020). Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. Animals, 10:1863.

Jin LZ., Ho YW., Abdullah N., Jalaludin S. (1998). Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poultry Science, 77: 1259-1265.

Jin LZ., Ho YW., Abdullah N., Jalaludin S. (2000). Digestive and bacterial enzyme activities in broilers fed diets supplemented with Lactobacillus Cultures. Poultry Science, 79: 886-891.

Kabir SML., Rahman MM., Rahman MB., Rahman MM., Ahmed SU. (2004). The dynamics of probiotics on growth performance and immune response in broilers. International Journal of Poultry Science, 3: 361-364.

Karaoğlu M., Durdağ H. (2005). The influence of dietary probiotics (*Saccharomyces cerevisiae*) Supplementation on different slaughter age on the performance, slaughter and carcass properties of broilers. International Journal of Poultry Science, 4(5): 309-316.

Kim JS., Ingale SL., Kim YW., Kim KH.,. Sen S., Ryu MH., Lohakare JD., Kwon IK., Chae BJ. (2012). Effect of supplementation of multi-microbe probiotic product on growth performance, apparent digestibility, cecal microbiota and small intestinal morphology of broilers. Journal of Animal Physiology and Animal Nutrition, 96: 618–626.

Kim GB., Seo YM., Kim CH., Paik IK. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poultry Science, 90: 75-82.

Koenen M., Kramer J., Van der Hulst R., Heres L., Jeurissen SHM., Boersma WJA. (2004). Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. British Poultry Science, 45: 355-366.

Kumprechtova D., Zobac P., Kumprecht I. (2000). The effect of Saccharomyces Cerevisiae Sc47 on chicken broiler performance and nitrogen output. Czech Journal of Animal Science, 45: 169-177.

Kurtoğlu V., Kurtogluz F., Şeker E., Çoşkun B., Balevi T., Polat S. (2004). Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Additives and Contaminants, 21(9): 817-823.

Mahajan P., Sahoo J., Panda, P. C. (2000) Effect of probiotic (Lacto-Sacc) feeding, packaging methods and seasons on the microbial and organoleptic qualities of chicken meat balls during refrigerated storage. Journal of Food Science and Technology, 37:67-71.

Marteau P., Rambaud JC. (1993). Potential of using lactic acid bacteria for therapy and immunomodulation in man. FEMS Microbiology Reviews, 12: 207-220.

Mateova S., Gaalova M., Saly J., Fialkovicova M. (2009). Investigation of the effect of probiotics and potentiated probiotics on productivity of laying hens. Czech Journal of Animal Science, 1:24-30.

Mikulski D., Jankowski J., Naczmanski J., Mikulska M., Demey V. (2012). Effects of dietary probiotic (Pediococcus acidilactici) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poultry Science, 91:2691-2700.

Mohammed AA., Zaki RS., Negm EA., Mahmoud MA., Cheng HW. (2021). Effects of dietary supplementation of a probiotic (*Bacillus subtilis*) on bone mass and meat quality of broiler chickens. Poultry Science, 100(3).

Mohan B., Kadirvel R., Bhaskaran, M., Natarajan, A. (1995). Effect of Probiotic Supplementation on Serum/Yolk Cholesterol and on Egg Shell Thickness in Layers. British Poultry Science, 36(5): 799-803.

Mountzouris KC. (2014). Probiotics as alternatives to antimicrobial growth promoter (AGPs) in broiler nutrition: modes of action and effects on performance. Pages 129- 157 in Probiotics in Poultry Production Concepts and Applications. W.H.A.Abdelrahman, and M.Mohnl eds, 5 m Publishing Ltd, Sheffield, UK.

Mountzouris KCP., Tsirtsikos E., Kalamara S., Nitsch G., Schatzmayr, Fegeros K. (2007). Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococ-cus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Science, 86:309–317.

Mountzouris KCI., Palamidi P., Tsirtsikos M., Mohnl G., Schatz-mayr, Fegeros K. (2015). Effect of dietary inclusion level of amulti-species probiotic on broiler performance and two biomarkers of their caecal ecology. Animal Production Science, 55:484–493.

Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC (2009) Mechanisms of action of probiotics: recent advances. Inflammatory Bowel Diseases, 15(2):300–310.

Nurmi E., Rantala M. (1973). New aspects of Salmonella infection in broiler production. Nature, 241: 210-211.

Patel R., DuPont HL. (2015). New Approaches for Bacteriotherapy: Prebiotics, New-Generation Probiotics, and Synbiotics. Clinical Infectious Diseases, 60(2): 108-121.

Patterson JA., Burkholder KM. (2003). Application of Prebiotics and Probiotics in Poultry Production. Poultry Science, 82:627–631.

Placha IM., Simonova P., Cobanova K., Laukova A., Faix S. (2010). Effect of Enterococcus faecium AL41 and Thymus vulgaris essential oil on small intestine integrity and antioxidative status of laying hens. Research in Veterinary Science, 89:257–261.

Pruszynska-Oszmalek, E., Kolodziejski PA., Stadnicka K., Sassek M., Chalupka D., Kuston B., Nogowski L., Mackowiak P., Maiorano G., Jankowski J., Bednarczyk M. (2015). In ovo injection of prebiotics and synbiotics affects the digestive potency of the pancreas in growing chickens. Poultry Science, 94:1909 -1916.

Rajput IR., Li LY., Xin X., Wu BB., Juan ZL., Cui ZW., Yu DY., Li WF. (2013). Effect of Saccharomyces boulardii and Bacillus subtilis B10 on intestinal ultrastructure modulation and mucosal immunity development mechanism in broiler chickens. Poultry Science, 92: 956–965.

Ramluckena U., Lalloo R., Roets Y., Moonsamy G. Rensburg JV., Thantsha MS. (2020). Advantages of Bacillus-based probiotics in poultry production. Livestock Science 241 104215.

Ray BC, Chowdhury SD., Khatun A. (2019). Productive performance and cost effectiveness of broiler using three different probiotics in the diet. Bangladesh Journal of Animal Science, 48 (2):85-91.

Rufino JPF, Cruz FGG, Miller WPM, Melo RD, Feijó JC, Chagas EO. (2015). Análise econômica da inclusão de farinha do resíduo de tucumã (Astrocaryum vulgare, Mart) na alimentação de poedeiras comerciais. Revista Brasileira de Saúde e Produção Animal, 16:1-9.

Russell SM., Grimes JL. (2009). The effect of a directfed microbial (Primalac) on turkey live performance. Journal of Applied Science Poultry Research, 18(2):185-192.

Sakai Y., Tsukahara T., Bukawa W., Matsubara N., Ushida, K. (2006). Cell preparation of Enterococcus faecalis strain EC-12 prevents vancomycinresistant enterococci colonization in the cecum of newly hatched chicks. Poultry Sci. 85, 273–277.

Salma U., Miah UG., Maki T., Nishimura M., Tsujii H. (2007). Effect of dietary Rhodobacter capsutalus on cholesterol concentration and fatty acid composition in broiler meat. Poultry Science, 86:1920-1926.

Samli HE, Senkoylu N., Koc F., Kanter M., Agma A. (2007). Effects of Enterococcus faecium and dried whey on broiler whey on broiler performance, gut histomorphology and microbiota. Archive of Animal Nutrition, 61:42-49.

Sen S., Ingale SL., Kim YW., Kim JS., Kim KH., Lohakare JD., Kim EK., Kim HS., Ryu MH., Kwon IK. (2012). Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. Research in Veterinary Science, 93: 264–268.

Tortuero F., Fernandez E. (1995). Effect of inclusion of microbial culture in barley-based diets fed to laying hens. Animal Feed Science and Technology, 53: 255-265.

Ülger İ., Büyükkılıç Beyzi S., Kaliber M., Konca, Y. (2016). Kanatlı Sektöründe Probiyotiklerin Etkinliği ve Geleceği. Tavukçuluk Araştırma Dergisi, 12 (2): 7-12.

Vanbelle N., Teller E., Focant, M. (1990). Probiotics in Animal Nutrition. A Review. Archiv Animal Nutrition, 40:543-567.

Vilà B., Fontgibell A., Badiola I., Esteve-garcia E., Jimenez G., Castillo M., Brufau J. (2009). Reduction of *Salmonella Enterica* var. Enteritidis colonization and invasion by Bacillus cereus var. toyoi inclusion in poultry feeds. Poultry Science, 88:975-979.

Xiang Q ., Wang C., Zhang H., Lai W., Wei H., Peng J. (2019). Effects of Different Probiotics on Laying Performance, Egg Quality, Oxidative Status, and Gut Health in Laying Hens.

Yang X, Zhang B, Guo Y, Jiao P, Long F. (2010). Effects of dietary lipids and Clostridium butyricum on fat deposition and meat quality of broiler chickens. Poultry Science, 89:254-260.

Yeo J., Kim K. (1997). Effect of Feeding Diets Containing an Antibiotic, a Probiotic, or Yucca Extract on Growth and Intestinal Urease Activity in Broiler Chicks. Poultry Science, 381-385.

Yeşilbağ D., Çolpan I. (2006). Effects of Organic Acid Supplemented Diets on Growth Performance, Egg Production and Quality and on Serum Parameters in Laying Hens. Revue de Médecine Vétérinaire, 175: 280-284.

Zhang JL., Xie QM., Ji J., Yang WH., Wu YB., Li C., Ma JY., Bi YZ.(2012). Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. Poultry Science, 91: 2755 – 2760.

Zhang Z., Kim I. (2014). Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poultry Science, 93(2): 364–370.

Zhang ZF., Cho JH., Kim IH. (2013). Effects of Bacillus subtilis UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livestock Science, 155:343–347.

Zhao X., Guo Y., Guo S., Tan J. (2013). Effects of Clostridium butyricum and Enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. Applied Microbiology and Biotechnology, 97:6477–6488.

Zulkifli, J., Abdullah, N., Azrim, N.M., Ho, Y.W. (2000). Growth performance and immune response of two commercial broiler strains fed diet containing Lactobacillus culture and oxytetracycline under heat stress conditions. British Poultry Science, 41: 593- 597.

THE RELATIONSHIP BETWEEN CATTLE METABOLISM AND QUALITY OF FEED RATION

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Abstract

The purpose of given research was to study the quality of grass fodder (hay, silage, haylage), harvested in the farms of Perm Region in 2017-2020 and to determine the relationships between cattle metabolism and quality of feed ration. According to the results of the studies, corn silage from 56% of the samples did not comply with national standards for dry matter (DM) - the content was below 20%. Thirteen batches have high content of acetic and butyric acid. Herbaceous fodders with higher quality were obtained from leguminous plants. Haylage in plastic packaging is the highest quality forage among the fodders harvested in the farms of the region – 92% of total volume batches of tested feed received the high quality degree. This type of fodder is characterized by high energy concentration – up to 13.33 MJ/kg per kg DM, as well as an increased content of crude protein in DM – up to 23.29%, especially batches from goat's-rue (*Galega orientalis*), alfalfa and mixture of clover and alfalfa. Unbalanced diet and low nutrient digestibility led to metabolic processes violation in cows organism, which was expressed in the form of ketosis and alkalosis noted for 90-95 % animals of the dairy herd. The scientific novelty of the work enclose detailed study of biochemical composition of fodder prepared in Perm Region and their influence on the metabolic processes in the body of animals.

Key words: *herbaceous fodders, legume hay, corn silage, haylage, exchange energy, protein.*

Introduction

The cows breeding stock with genetic potential of milk productivity from 10 000 to 14 000 kg per lactation, with elevated milk fat and protein was created in Perm Region as a result of breeding work to improve the black-and-white breed using Holstein producers. However, in 2020, the average milk yield for all farms in region was 6633 kg from one cow (Volgin et al.,2016). Realizing the genetic potential of that breed depends largely on the biological usefulness of the herbaceous fodders used in feeding. Home grown fodder obtained in most farms in Perm Region have significant deviations from the requirements of national standards (GOSTs) in regard of basic nutrient content.

It is impossible to provide balanced feeding for highly productive livestock without having high-quality herbaceous fodders in accordance with the detailed diets, since it depends by 50% on providing the animals with metabolic energy, 25% - by protein and 25% - by minerals and vitamins (Volgin et al.,2016, 2018). So, it is necessary to have certain set of forage crops characterized by different content of animal nutrition elements for every soil and climatic zone of Russia. Technological approaches to the fodder preparation and storage also have different specifics. A lot of analyses of different fodder types from farms of Perm Region are carried out annually in the analytical laboratory of Perm Federal Research Center Russian Academy of

Sciences. From 1949 samples of grass forage tested in the last 4 years (2017-2020), 4% of silage batches, 3% of haylage and 17% of hay batches were classified as low quality ones.
The purpose of the work is to study the quality of bulky herbaceous fodders harvested in the farms of Perm Region, to reveal the reasons for obtaining low-quality herbaceous forage and give recommendations for the fodder preparation with a high content of exchange energy and digestible protein.

Materials and methods

The analyses were carried out in certified analytical laboratory of Perm Agricultural Research Institute - division of Perm Federal Research Center using national standards, (GOSTs), conventional techniques and modern laboratory equipment: the infrared feed analyzer SpectroStar 2600 XT-1, the Unico spectrophotometer, Fibretherm-12 (device for automatic fiber analysis) , etc. The laboratory fulfilled analytical studies to determine the chemical composition of raw materials and prepared fodders on the basis of the conclusion No. 07-10/18-18 on the state of measurements in the laboratory dated May 31, 2018, issued by the Perm CSM FBU.
The study of biochemical parameters of animal blood and metabolic processes was fulfilled on the dairy farms in Perm Region: «Rus» ltd; «Hohlovka» ltd; «Lipovaya Gora» ltd with control and experimental groups of dairy cows. The analyses of the biochemical composition of the cows blood were carried out in Veterinary Center «Permskiy».

Results and discussion

The detailed analysis summarized the studying a large number of samples of hay, silage and haylage, prepared in 67 farms during 2017-2020, for the main parameters of feed quality was fulfilled. Information about the forage quality is given in terms of absolutely dry matter, as it accepted by the relevant standards; the data for the content of exchange energy, crude protein, feed units and other parameters of feed quality is provided.
The main volumes of herbaceous fodders are prepared in Perm Region from perennial legume-cereal grass mixtures, legumes (red clover *Trifolium pretense*, L. alfalfa *Medicago varia* goat's-rue, *Galega orientalis*, L.), , perennial poaceous grasses and corn.
Hay is prepared from red clover and its mixtures with timothy grass (*Phleum pretense*, L.), poaceous-legumes grasstands and poaceous perennial grasses. The significance share of hay is prepared from natural meadow grasses. In general, 26 sources of raw materials were used for hay making during the analyzed period. Hay has the lowest nutritional value among all kinds of forage prepared in Perm Region: 66.47% of the tested batch of feed belonged only to third duality class and even out of quality classes (Table 1).
The tested hay samples have a low concentration of exchange energy (EEC). Only 8% of hay legume samples have EEC more than 10 MJ/kg of dry matter. There were no samples among samples from poaceous-legumes mixtures and poaceous grasstands with such EEC. Crude protein (CP) deficit was noted for all hay samples. CP content of more than 15% in dry matter of legume hay was noted only for 42.4% of samples, in poaceous-legumes batches - for 4.0%, in poaceous batches -1.40%. Crude fiber content in hay varied within 24.97% - 44.78 % for absolutely dry matter (optimal value 27-29%). The main reason of the hay low quality is the late harvesting time and the deterioration of the botanical composition of old age grass stands.

Table 1. Chemical composition of hay prepared in Perm Region, 2017-2020

Type of hay	Tested samples amount	Dry matter content, %	Content in dry matter, %				Feed class	
			crude fiber,	Crude protein,	EEC , MJ/kg	feed. units/kg	I+II, %	III+ non%
Legume	23	72,30-88,20*	25,03-36,45	7,12-21,20	8,08-10,72	0,56 – 0,66	75	25
GOST		83,00	27,00	15,00				
Poaceous-legume	127	67,60-90,15	24,97-44,78	6,48-21,31	6,94-9,46	0,54 – 0,64	55	45
GOST		83,00	28,00	14,00				
Poaceous	82	70,88-88,95	27,88-38,81	5,93-15,01	7,76-9,25	0,52 – 0,63	55	45
GOST		83,00	29,00	13,00				

Note: * - here and further –the range of values for forage batches.

In order to reach balanced diet for cows the hay should have 9.0-9.5 MJ and 14-15% digestible protein in one kg of hay DM (Morozkov et al., 2015).

Analyzing the data of the chemical composition of hay, it is necessary to pay attention to the fact that the carotene content in hay dry matter was 12.73 mg/kg in average with deviations from 6.40 to 26.65 mg/kg (optimum level 25.00 mg/kg). The most important condition for obtaining high-quality forage is mowing time. Now it is well-known fact. The optimal time means not only date or phase of plant development, but also times of day. The best time for mowing is considered to be in the morning, starting from dawn until 11 o'clock a.m. Grasses have the highest possible carotene content during this period and dry out faster. Hay making using the method of forced ventilation provides obtaining high-quality fodder. Due to the rapid drying and elimination of losses method permits to increase the hay yield by 15-20%, the yield of feed units and digestible protein - by 20-25%, and carotene – by 2-3 times compared to conventional drying in the field. Feeding the hay prepared by the method of forced ventilation allows to raise the cows productivity by 10-12% (Guryanov et al., 2007; Faritov, 2010).

The largest share of silage making is prepared from clover-timothy mixtures, perennial legume-poaceous mixtures, red clover and corn. High quality of silage (I-II classes) was noted for 85.50% of the tested batches (Table 2). Low quality silage occupy 14.50% in total amount. It should be noted that butyric acid was present in 572 samples (48.97%), including 16 samples (1.37%), that they are not allowed to be fed according sanitary rules. The most valuable silage was obtained from legumes, 68 % of the tested feed contains 10 or more MJ/kg of exchange energy and over 15 % of crude protein in dry matter.].

Table 2. Chemical composition of silage prepared in Perm Region, 2017-2020

Type of silage	Tested samples amount	Dry matter content, %	Content in dry matter, %				Feed class	
			Crude fiber,	Crude protein, %	EEC, MJ/kg	feed. units/kg	I+II, %	III+ non, %
Legume	322	15,31-37,92*	23,04-34,86	9,35-23,80	7,96-13,33	0,79 – 0,85	85	15
GOST		27,00	28,00	15,00				
Poaceous-legume	712	15,81-39,89	23,74-41,24	7,92-23,55	7,38-12,67	0,78 – 0,86	83	17
GOST		25,00	28,00	13,00				
Poaceous	46	16,50-35,41	24,13-35,39	9,61-19,06	8,30-12,60	0,73 – 0,84	82	18

GOST		20,00	28,00	12,00				
Corn	88	16,60-28,01	24,94-33,79	9,45-14,90	9,22-11,25	0,74 – 0,86	92	8
GOST		26,00	28,00	8,00				

The phenological phase during mowing has a major impact on the quality of prepared silage (Nagibin, 2018).In 47 tested corn silage samples from total amount 88 samples of corn silage dry matter content was less than 20 %, while the feed mixture should contain 50-55% dry matter for lactating cows. The average EEC for all samples was 10.22 MJ/kg in dry matter. Silage, prepared in Perm region, is traditionally characterized by an acute shortage of crude protein, only nine samples had more than 14 % in dry matter.
High milk productivity is possible with a concentration of exchange energy in a corn silage at least 10.5 MJ/kg (Zezin, 2017). That level of EEC may be achieved in corn silage when the ear content in the silage mass is at least 40-50% and provided their ripeness milk-wax and wax (Zscheischler, 1984). The technological minimum of dry matter content according in the green mass of corn before ensiling should be at least 25% (Zezin N., 2017).
The predominant amount of haylage is harvested from perennial legumes and legume-poaceous grass mixtures. 474 batches (92%) from total amount 549 were assigned to I and II quality classes (Table 3).

Table 3. Chemical composition of haylage, prepared in Perm Region, 2017-2020.

Type of haylage	Tested samples amount	Dry matter content, %	Concentration in dry matter				Feed class	
			Crude fiber, %	Crude protein, %	EEC, MJ/kg	feed. units/kg	I+II, %	III+ non %
Legume	247	25,41-69,82*	20,85-34,67	9,61-23,79	7,80-13,37	0,77 – 0,83	98	2
GOST		45,00-55,00	27,00	16,00				
Poaceous legume	240	19,79-71,18	23,26-36,36	7,58-21,06	7,81-12,38	0,72 – 0,84	96	4
GOST		45,00-55,00	29,00	15,00				
Poaceous	62	22,18-76,54	24,56-37,71	6,88-16,17	7,81-11,09	0,61 – 0,66	54	46
GOST		45,00-55,00	30,00	14,00				

Haylage making in trenches deserves special attention. The share of haylage in trenches was about 16% of the tested haylage samples. The predominant amount of haylage in trenches is prepared from perennial poaceous-legume mixtures, red clover, clover-timothy mixtures, goat's-rue, and alfalfa. Compared with silage, the tested batches contain 2.5 times less batches with the presence of butyric acid and only 2% of the batches do not comply with GOST R 55452-2013 for this parameter. Haylage from legumes is the most valuable forage in this type in terms of the of exchange energy and crude protein in dry matter.
Haylage in plastic packaging (rolls) has the highest feed quality among the grasses fodders harvested in the farms of the region – 92% of the batches of the total volume were classified as belonging to I-II quality classes. This forage type is characterized by high energy concentration – up to 13.33 MJ/kg in dry matter, as well as an increased content of crude protein – up to 23.29% dry matter, especially batches from goat's-rue, alfalfa and clover- alfalfa mixture. The safety of carotene in the haylage in plastic rolls was 12-15 times higher compared with the hay in usual

rolls, which testifies that the safety of all biologically active substances of haylage in plastic packaging was also higher. Therefore, it is necessary to give preference to that kind of fodder. However, haylage in plastic accounts only 2,60% of herbaceous forage total volume prepared in Perm Region. According to research data (Bondarev, 2008; Kosolapov et al, 2008,2009), in order to have 10 MJ of exchange energy in dry matter, grass should be harvested in time when fiber content does not exceed 26%. These parameters correspond to the phase of the beginning of earing of poaceous grasses, the beginning of budding of legumes (clover, alfalfa). Harvesting grasses in more late phases leads to a decrease in energy nutrition by 1% daily. At the same time, the average protein loss per day is 0.25%, and the fiber content increases by absolute 0.33% per dry matter. According to data of Korelina (2019), the enlargement of forage crops assortment the replacement of low-yielding varieties with more productive and nutritionally valuable ones is a significant reserve for increasing feed production and improving their quality.

The results of research in Perm Agricultural Research Institute (Maysak, Voloshin, 2015) showed that the optimal period for grasses mowing is determined taking into account the intensity of average daily changes in the chemical composition of forage crops green mass and balance between relatively high yield and sufficient feed quality. The optimal mowing period for winter rye was 15 days in average (for the years of research), for goat's-rue and alfalfa -16 days, for early maturing variety of red clover Trio mixed with poaceous grasses -7-17 days, for late maturing variety of red clover Perm local -20 days, perennial legume-poaceous mixtures -14 days in average, annual grasses -11 days from the date when crops reach the yield of 9-10 t / ha of green mass, or the branching of legumes, stem elongation– for poaceous grasses. Harvesting in a later phase leads to reduction of the exchange energy content by 0.1 MJ / kg daily. The losses of digestible protein are 0.36 %, and the fiber content increases by 0.46 % (absolute) per dry matter. The experiments fulfilled by scientists of Perm Agricultural Research Institute in the farms of Perm Region over the last 10 years revealed that unbalanced diet and low nutrient digestibility of applied fodders led to metabolic processes violation in cows organism, which was expressed in the form of ketosis and alkalosis, reported in some cases for 90%-95 animals of the dairy herd (Morozkov et al., 2015). In later studies the content of beta-carotene in the blood serum of cows in the first phase of lactation was lower than the physiological rate for the entire studied livestock and varied in the range of 4.39-7.40 mmol/l (optimum 7.50-18.6 mmol/l.) Carotene content in blood of cows confirmed the actual carotene deficiency in their diet (Morozkov, Sukhanova, 2020).

Conclusion

The fodders made in the farms of Perm Region does not provide the needs of highly productive cattle for basic nutrients, especially silage. Disturbance in technological requirements for harvesting grass fodders leads to metabolic processes violation in cows organism, decrease in productivity and, as a result, to essential economic damage for the farms in the region.That is why, the main objective of feed production is provision of forage with a high level of exchange energy concentration and digestible protein, since energy-saturated, high-protein fodders are the main condition for high dairy productivity. The reality of this objective is confirmed by the results of testing the forage harvested in the region – among the silage batches from legume perennial grasses and haylage from legumes, there were samples with a EEC 12-13 MJ/kg and with crude protein content of more than 15 % in dry matter. Haylage in rolls with plastic packaging obtained from legumes is the most valuable forage in this type. Urgent task in feed production is to expand the set of forage crops, to increase the share of most productive species and cultivars with valuable biochemical content, comply with the recommended time of perennial grasses use and timely replace old age grass stands with poor-quality botanical composition by highly productive species and mixtures.

References

Bondarev V. A. (2008). Improving the quality of feed from perennial grasses. *Bulletin of the Russian Academy of Agricultural Sciences.* 4: 54-55.

Volgin V. I., Romanenko., Bibikova A. S., Fedorova Stetsenko N. P. (2016). Realization of the genetic potential of productivity in dairy cattle breeding based on the optimization of the feeding system (recommendations). *Scientific review*. 5: 120-121.

Volgin. V. I.,. Romanenko L.V., Fedorova Z. L., Plemyashov K. V., Korochkina E. A. (2018). Optimization of feeding of highly productive livestock St. Petersburg: Prospekt nauki:360 p.

Faritov T. A. (2010) Feed and feed additives for animals A text.book.- St. Petersburg: Publishing house "Lan": 304 p.

GOST R 55986-2014. Silage from forage plants (General technical conditions). Moscow: Standartinform: 9 p.

GOST R 55452-2013. Hay and haylage (technical conditions). Moscow: Standartinform: 8 p

Guryanov A.M., Artemyev A. A., Lyalichkin O. A., Prytkov Yu. N.(2007). Handbook: feed technology /Under the general ed. of A.M. Guryanov. Saransk: 354 p.

Kosolapov V. M., Bondarev V. A., Klimenko V. P. (2008). Improving the quality of feed is an indispensable condition for the successful development of animal husbandry. *Agricultural science*. 1: 27-29.

Kosolapov V. M., Bondarev V. A., Klimenko V. P. (2009). Efficiency of new technologies for preparing feed from herbs. *Achievements of science and technology of the agro-industrial complex.*7: 39-42.

Korelina V. A. (2019). Creation of legume-grass stands using blue hybrid alfalfa in the conditions of the subarctic zone of the Russian Federation. *Efficient animal husbandry*. 154 (6): 76-79.

Maysak G. P., Voloshin V. A. (2015). Dynamics of the formation of the yield of green mass and its quality. *Agrotechnologies of the XXI century: materials of the All-Russian scientific and practical conference with international participation dedicated to the 85th anniversary of the founding of the Perm State Agricultural Academy and the 150th anniversary of the birth of Academician D. N. Pryanishnikov.* Perm: 54-58.

Morozkov N. A., Tretyakov S. V., Voloshin V. A.(2015). The system of balanced feeding of black-and-white cattle at milk production complexes, providing an increase in milk productivity and improving the quality of milk. Perm: 74 p.

Morozkov, N. A., Sukhanova E. V. (2020). An improved system for assessing the quality of feed and feed additives as affected the productivity raise of farm animals. Perm: 62 p.

Nagibin A. E. (2018). Tormozin M. A., Zyryantseva A. A .Grasses in the feed production system in the Urals (monograph). Yekaterinburg: 784.

Zezin N. N. (2017).Corn in the Urals (monograph).Yekaterinburg. Ural publishing house: 204 p.

Zscheischler J., Estler M.C., Gross F., Burgstaller G., Neumann H., Geissler B. (1984). Handbuch Mais Anbau Verwertung Futterung. Frankfurt DLG-Verlag: 253 p.

THE INFLUENCE OF AGE ON QUALITATIVE AND QUANTITATIVE PARAMETERS OF STALLION EJACULATE

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Abstract

Qualitative and quantitative parameters of stallion ejaculate are influenced by various factors. The age of stallion is one of them. The aim of this study was to evaluate the effect of stallion age on the ejaculate gel-free volume, concentration, motility and sperm morphology. The total number of 15 stallions of various breeds housed in the Tlumačov Regional Stud Farm were included in this research. The total number of evaluated ejaculates was 103. Stallions were divided into three age categories (3-7 y, 10-14 y, 18-20 y). The highest values of ejaculate gel-free volume were reached by the group aged 10 to 14 years (57.33 ml) and the lowest values were reached by the group of the oldest stallions (34.17 ml) ($p \leq 0.05$). Stallions from the age category 10-14 y also achieved the highest values of sperm concentration (254.21 * 10^6 / ml). The lowest sperm concentration was measured in the age group of the oldest stallions (197.07 * 10^6 / ml). The oldest age group (18-20 y) achieved the highest values of sperm motility (86.57%) while the lowest values were achieved within he youngest age group of stallions (79.27%). Differences in sperm concentration and motility were not statistically significant. The highest values of spermatozoa with normal morphology were reached by the oldest age group (89.64%), while the lowest values were reached by the youngest group from 3 to 7 years (85.22%) ($p \leq 0.05$). The effect of age on ejaculate parameters was demonstrated in both sperm volume and morphology. Therefore, the age of the stallion should be taken into consideration when evaluating stallion ejaculate.

Keywords: *Stallion ejaculate, Age, Sperm, Volume, Concentration, Motility, Morphology.*

Introduction

Artificial insemination is a widely spread biotechnical method of reproduction in animal breeding (Foote et al., 2020). Its use in the field of horse industry is constantly increasing (Janett et al., 2003a). Fresh and frozen semen have become the most used tools for breeders (Aurich and Aurich, 2006).

Artificial insemination brings many advantages such as reducing the risk of injury and transmission of infectious venereal diseases, the accelerating of genetic progress by making the best stallions available and to eliminate the stress factors caused by animal transportation (Aurich and Aurich, 2006; Aurich, 2012; Daigneault, 2012; Janett et al., 2003a; Parkinson and Morrell, 2019). The rate of fertilization of AI is the same even higher than with natural breeding (Janett et al, 2003a; Janett et al, 2003b; Langlois and Blouin, 2004). These results are significant also with problematic mares (Pickett and Shiner, 1994).

The success of artificial insemination mainly depends on the quality of the ejaculate used (Samper, 2001), which is influenced by many physiological and external factors (Kandiel and El

Khawagah, 2018). One of these factors is the procedure for the preparation of insemination doses (Rečková a Filipčík, 2020), seasonality, (Janett et al, 2003a), stallion individuality, nutrition, breed and the age of the stallion (Dowsett and Knott, 1996). Factors such as nutrition, age of the stallion when we start using him for reproduction and the process of preparing insemination doses can be easily influenced. On the other hand, the influence of the season, individuality and changes in the quality of ejaculate caused by the age can be only partially influenced. Therefore, it is important to know how age influences the quality of an ejaculate. This information is important to take into consideration when choosing the right stallion and the subsequent success rate of insemination.

The aim of this work was to evaluate the effect of age on the quantitative and qualitative parameters of ejaculate, such as gel-free volume, concentration, motility and morphological changes of sperm.

Materials and Methods

For this study we used 15 stallions of various breeds in the age from 3 to 20 years, housed in the Tlumačov Regional Stud Farm (Czech Republic). Stallions were routinely used for the production of short-term stored insemination doses and were included in the offer for an artificial insemination in the year 2019. The stallions were divided into age categories from 3 to 7 years old, from 10 to 14 years old and from 18 to 20 years old. The total number of evaluated ejaculates was 103. Ejaculates were collected during breeding season (April-August 2019).

Each ejaculate was macroscopically and microscopically evaluated in the Reproduction Center of Tlumačov Stud Farm. Immediately after the collection, gel fraction was removed from the native ejaculate by filtrating it through the sterile gaze. Ejaculate gel-free volume, sperm concentration and motility were determined, and smears for morphological evaluation of ejaculate were prepared. Morphological evaluation of sperm was executed in the laboratory of Mendel University in Brno, Department of Animal Breeding.

The gel-free volume of ejaculate was determined by weighing and it is given in millilitres. Sperm concentration was determined by an objective method CASA (Computer Assisted Sperm Analyzer), a minimum of 500 sperm were evaluated in a minimum of 5 fields of view. Total and progressive sperm motility was assessed using CASA (Computer Assisted Sperm Analyzer), minimum of 500 sperm were evaluated in a minimum of 5 fields of view. Smears of native ejaculates were fixed and dyed according to the Farelly method. At least 200 sperm were evaluated and the percentage of sperm of normal morphology to the percentage of pathological sperms were determined. Sperm without visual deformations or other pathological changes were classified as those with a normal morphology.

Pathologically formed sperm included immature sperm (containing protoplasmic drop), sperm with developmental anomalies (double tails, double head, deformity), spermatozoa with head defects (non-standard head shape, acrosome defects) and tail defects (crooked and curled tails, mid-piece defects, neck defects).

Program STATISTICA CZ 12 was used for the statistical evaluation through variational analysis. The HSD test was used to determine statistical signification within each factor.

Results and discussion

Table 1. Qualitative and quantitative parameters (means ± SEM) of stallion ejaculate for different age groups

Age categories	*n* - number of collection	Gel-free volume [ml]	Sperm concentration [10^6 / ml]	Sperm motility [%]	Sperm of normal morphology [%]
3-7 years	37	37.86 ± 0.48	214.68 ± 20.24	79.27 ± 4.63	85.22 ± 1.85 a
10-14 years	24	57.33 ± 2.54 a	254.21 ± 31.60	85.63 ± 3.45	89.04 ± 1.02
18-20 years	42	34.17 ± 0.39 b	197.07 ± 16.96	86.57 ± 1.57	89.64 ± 0.73 b

a, b Differences are significant for $p < 0.05$

In the oldest age category (18-20 y), the gel-free volume of ejaculate was 34.17 ± 0.39 ml, statistically demonstrably lower ($p \leq 0.05$) than in stallions in the age range from 10 to 14 years (57.33 ± 2.54 ml). The youngest age category of stallions (3- 7 y) had gel-free volume of 37.86 ± 0.48 ml.

The oldest age category of stallions had the lowest average sperm concentration (197.07 ± 16.96 x 10^6 / ml). In the contrast, the age category from 10 to 14 years had the highest average sperm concentration (254.21 ± 31.60 x 10^6 / ml). The youngest stallions reached the average sperm concentration 214.68 ± 20.24 x 10^6 / ml. Differences in sperm concentration among age categories were not statistically significant.

In the youngest category of horses was determinate the lowest average sperm motility 79.27 ± 4.63 %. Stallions of the age range from 10 to 14 years had the average motility of 85.63 ± 3.45 %. %. In the oldest stallions, the average value of motility reached 86.57 ± 1.57 %. Differences in sperm motility among age categories were not statistically significant.

In the youngest age category, the proportion of sperm of normal morphology (85.22 ± 1.85 %), was statistically demonstrably lower ($p \leq 0.05$), than in the oldest age category (89.64 ± 0.73 %). The age category of stallions from 10 to 14 years had a proportion of sperm of normal morphology 89.04 ± 1.02 %.

The parameters of ejaculate such as volume and sperm concentration can directly affect the total number of sperm and thus the number of insemination doses that can be produced (Samper, 2008). Sperm motility and morphology are common methods for assessing the quality of stallion ejaculate (Samper, 2008; Varner, 2008).

Ejaculate parameters are influenced by many factors (Kandiel and El Khawagah, 2018). One of them is the age of the stallion (Dowsett and Knott, 1996; Gottschalk et al., 2016; El Sisy et al., 2016; Kandiel and El Khawagah, 2018).

Based on the results, a lower ejaculate volume and sperm concentration and a higher average motility and the proportion of sperm with normal morphology were observed in the oldest age category of stallions. The highest average sperm volume and concentration were recorded in the category of stallions from 10 to 14 years, while the average values of motility and the proportion of sperm with normal morphology were slightly lower than in the oldest group of stallions. The average values of motility and the proportion of sperm with normal morphology were the lowest in the youngest group of stallions.

There were statistically significant differences in the volume of ejaculate, which was higher in the middle age category than in the oldest age category, and in the proportion of sperm with normal morphology which was higher in the oldest group than in the youngest group of stallions. Despite the differences in the monitored parameters, all stallions met the requirements for native ejaculates.

Dowsett and Knott (1996) proved that a higher proportion of immotile and abnormal sperm may be observed in younger stallions. The effect of age on ejaculate parameters is associated with less effective spermiogenesis of colts and testicular degeneration due to stallion aging (Brito, 2007). Despite the decrease in volume and concentration in oldest category of stallions, a higher proportion of sperm with normal morphology were observed than in the age category from 3 to 7 years. This agrees with statement of Gottschalk et al. (2016), who state that stallions can be used to high age while constantly monitoring ejaculate quality.

Conclusion

Age affects the parameters of ejaculate, especially the volume and the portion of sperm with normal morphology. Although all stallions met the established requirements for further ejaculate processing, the age factor must be taken into consideration when evaluating stallion reproduction characteristics.

References

Aurich, J. (2012). Artificial Insemination in Horses – More than a Century of Prectice and Research, Journal of Equine Veterinary Science, 32 (8): 458–463.

Aurich, J., Aurich, Ch. (2006). Developments in European Horse Breeding and Consequences for Veterinarians in Equine Reproduction, Reproduction in Domestic Animals, 41 (4): 275–279.

Brito, L.F.C. (2007). Evaluation of Stallion Sperm Morphology, Clinical Techniques in Equine Practice, 6 (4): 249–264.

Daigneault, B.W. (2012). Equine Artificial Insemination, Affiliation: University of Illinois – Equine Extension.

Dowsett, K.F., Knott, L.M. (1996). The influence of age and breed on stallion semen, Theriogenology, 46 (3): 397–412.

El Sisy, G.A., Abo El-Maaty, M., Rawash, Z.M. (2016). Comparative blood and seminal plasma oxidant/antioxidant status of Arab stallions with different ages and their relation to semen quality, Asian Pacific Journal of Reproduction, 5 (5): 428–433.

Foote, R.H., Layek, S.S., Parks, J.E. (2020). Reference Module in Food Science, Artificial Insemination, Elsevier.

Gottschalk, M., Sieme, H., Martinsson, G., Distl, O. (2016). Analysis of breed effects on semen traits in light horse, warmblood, and draught horse breeds, Theriogenology, 85 (8): 1375–1381.

Janett, F., Thun, R., Niederer, K., Burger, D., Hassig, M. (2003a). Seasonal changes in semen quality and freezability in the warmblood stallion, Theriogenology. 60 (3), 453–461.

Janett, F., Thun, R., Bettschen, S., Burger, D., Hassig, M. (2003b). Seasonal changes in semen quality and freezability in franches–montagnes stallions. Animal Reproduction Science, 77 (3-4): 213-221.

Kandiel, M.M.M., El Khawagah, A.R.M. (2018). Evaluation of semen characteristics, oxidative stress, and biochemical indices in Arabian horses of different ages during the hot summer season, Iranian Journal of Veterinary Research, 19 (4): 270–275.

Langlois, B., Blouin, C. (2004). Statistical analysis of some factors affecting the number of horse births in France, Reproduction Nutrition Development, 44 (6): 583–595.

Parkinson, T.J., Morrell, J.M. (2019). Artificial Insemination, Veterinary Reproduction and Obstetrics, Elsevier, 43: 746–777.

Pickett, B.W., Shiner, K.A. (1994). Recent developments in artificial insemination in horses, Livestock Production Science, 40 (1): 31–36.

Rečková, Z., Filipčík, R. (2020). An analysis of Selected Aspects of Sperm Quality in Fresh and Cooled-Storage Stallion Semen, Iranian Journal of Applied Animal Science, 10 (3): 405–408.

Samper, J.C. (2001). Management and fertility of mares bred with frozen semen, Animal Reproduction Science, 68 (3-4): 219–228.

Samper, J.C. (2008). Equine Breeding Management and Artificial Insemination, 2nd Edition, Saunders - Elsevier Health Sciences, Missouri.

Varner, D.D. (2008). Developments in stallion semen evaluation, Theriogenology, 70 (3): 448–462.

THE BASIC CHARACTERISTICS OF EGG STRUCTURE AND CORRELATION WITH EGG MASS

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Abstract

The aim of this study is to examine the influence of the age of laying hen hybrid on the basic characteristics of the egg structure (the yields and the share of shell, egg white and yolk). Additionaly, the aim of this paper is to examine what happens to certain egg structure characteristics after the established 72nd week of laying hen age, until the end of 79th week of age of the Lohmann Brown hybrid hens. The examination of the egg structure characteristics was performed on light-line hybrid Lohmann Brown, on the farm"Agrovet" Foca, at four periods of laying hen age (27th, 46th, 72nd and 79th week) with 60 individually measured eggs in each period. The basic parameters of egg structure included the egg mass (g), the share of the shell (g, %), the share of the yolk (g, %) and the share of egg white (g,%). The influence of the egg mass on the basic characteristics of the egg structure was also determined by calculating the correlation coefficients between those parameters. Based on the results of examination, it was concluded that the average absolute mass of the shell, yolk and egg white also increased along with the age of laying hen. The relative values of the shell very around 13% through all periods, while the share of the yolk gradually increased through the periods, and ranged from 24.76% to 25.77%, with minor exception at the last period. The mass of egg white was determined by calculating the difference between the total mass of egg on one side and the mass of yolk and shell on the other side. The share of egg white in total egg mass was the highest in the first period (61.83%) and the lowest at the end of the production cycle (61.05%). It can be concluded that the egg mass of analyzed commercial flock of light-line Lohmann Brown hybrid, had influenced basic characteristics of the egg structure, in all examined periods.

Key words: *Lohmann Brown, Egg structure, Egg mass, Correlation.*

Introduction

The characteristics of egg structure, i.e. the percentage of egg components in the total egg mass, is determined by genetic and paragenetic factors.

The egg is made of the shell, egg white and yolk. The purpose of the shell is to protect the sensitive parts of egg from external influences, and it enables the the gas exchange and heat transfer. The shell is built from calcium carbonate, which makes it solid and porous. The inside of the shell is covered by three-layer membrane, and by separating the inner membrane from the outer membrane on the top of the egg, the air chamber is created, which grows bigger as the egg grows older, due to evaporating of water from the egg content.

The eggs are an excellent source of nutrients. The basic structure of eggs consists of shell, egg whites and yolk. The average egg weight of 64.5 g contains 57.18% of egg white, 29.25% of yolk and 13.57% of shell (Kralik et al., 2013).
In the research performed on the Lohmann Brown hybrid in the period from 20^{th} to 72^{nd} week of age, Pandurevic (2011) obtained results that shows the average values of shell mass increased through examination periods (WA_{20} – 6.12 g, WA_{28} – 8.03 g, WA_{72} – 8.11 g), except around the middle of production cycle (WA_{48} – 7.93 g). The percentage share of the shell in egg weight through the examination periods was: WA_{20} – 13.27%, WA_{28} – 13.41%, WA_{48} – 11.85% and WA_{72} – 11.78%. Along with the age, the average absolute values of egg whites mass increased (WA_{20} – 29.16 g, WA_{28} – 36.40 g, WA_{48} – 42.79 g, WA_{72} – 44.28 g) as well as the yolk mass (WA_{20} – 10.88 g, WA_{28} – 15.47 g, WA_{48} – 16.40 g, WA_{72} – 16.72 g). The average relative values of the egg whites mass in the same periods were: 63.13%; 60.69%; 63.66% and 63.97%, while the yolk share in total egg mass by periods were: 23.60%; 25.90%; 24.49% and 24.25%. Heavier eggs from older hens had significantly higher absolute mass of egg whites and yolks ($P<0.01$ and $P<0.001$) than lighter eggs from younger hens, while the differences in shell were statistically significant ($P<0.001$), except for the difference between the second and third, ie second and forth sampling, when the significance was not confirmed ($P>0.05$).
Examining the eggs of Lohmann Brown hybrid hens at the age of 25 weeks, Pavicic (2014) found the average shell mass was 7.84 g, yolk mass 12.83 g and egg white mass 34.63 g, while at the age of 69 weeks old hens, the shell mass was 8.79 g, yolk mass 17.23 g and egg white mass 35.51 g. Based on the obtained results, it can be concluded that the age of hen has statistically significant influence ($P<0.001$) on the weight of the shell, yolk and egg white.
According to Zaheer (2015), the shell makes 9-12% of total egg mass, the egg white around 60% and the yolk from 30% to 32% of total egg mass.
Examining the egg production of Hisex Brown hybrid, Gjorgovska et al. (2016) found the average egg mass of 67.53 g, of which the average mass of egg white was 40.11 g, the average yolk mass 19.28 g and the average shell mass 8.14 g. Expressed in percentages, the average egg was consisted of 59.33% of egg white, 28.31% of yolk and 12.06% of shell.
The average egg mass of Lohmann Brown hybrid, kept in conventional cage from 20^{th} to 60^{th} week of age, obtained in research by Dikmen et al. (2017), amounted 58.35 g. The average mass of shell was 5.68 g (9.78%), while the average yolk mass was 14.07 g (23.94%) and egg white mass 38.60 g (66.29%).
The aim of this study is to examine the influence of the age of laying hen hybrid on the basic characteristics of the egg structure (the yields and the share of shell, egg white and yolk). Additionaly, the aim of this paper is to examine what happens to certain egg structure characteristics after the established 72^{nd} week of laying hen age, until the end of 79^{th} week of age of the Lohmann Brown hybrid hens.
Based on the obtained results, the phenotypic correlation between the egg mass and egg structure characteristics was determined.

Material and Methods

In order to examine the influence of the age of laying hens on the basic characteristics of the egg structure, an appropriate research was performed by organizing proper experiment and statistical processing of the obtained data. As the initial material for the research, we have used hens of

light-line hybrids Lohmann Brown, housed on the farm "Agrovet" Foca, Bosnia and Herzegovina. The production period lasted for 61 weeks, i.e. 427 days.
In the course of raising (exploitation) of the commercial flocks, a technology proposed by the selector of laying hens hybrid was used (http://www.ltz.de). All technological phases (feeding, power, temperature, lighting, ventilation, drainage system and collecting the eggs) are automatically regulated.
The eggs research, that included examination of basic characteristics of the egg structure, was performed in the appropriate, acredited, laboratory of Faculty of Agriculture in East Sarajevo. There were four control measurements during the production cycle, with 60 individually measured eggs in each period, with total of 240 eggs. The eggs were selected by random sample method (3 eggs from each row x 5 floors x 4 rows).
The examination of the egg structure characteristics was performed at the following age of laying hens: 27 weeks (WA_{27}), 46 weeks (WA_{46}), 72 weeks (WA_{72}) and 79 weeks (WA_{79}).
The following parameters of egg structure were defined:

- Egg mass (g)
- The share of shell in egg mass (g, %)
- The share of egg white in egg mass (g, %)
- The share of yolk in egg mass (g, %).

The egg mass was determined by measuring each egg on special technical weighing scale of the brand "$KERN_{PFB}$", which is accurate to 0.01 g.
The mass of the raw shell was measured on the same scale, after the egg was broken, the egg content removed, and the shell measured together with egg membranes. The obtained values are expressed both in grams (absolute values) and as percentages (relative values).
The mass of yolk was measured on the same technical scale, after the yolk was previously separated from the egg whites by a separator.
The mass of egg white was determined by calculating the difference between the total mass of egg on one side and the mass of yolk and shell on the other side.
Percentages of raw shell, yolk and egg white in total mass of egg were obtained by calculation.
Based on the obtained data, the appropriate databases were formed. The software package SPSS - Statistical Package for Social Sciences was used for statistical analysis. Statistical processing of the obtained data included the calculation of common variational-statistical data and the assessment of the significance of the obtained differences with the implementation of appropriate mathematical-statistical methods (Hadzivukovic, 1991).
Based on the experimental data and the defined frequency distributions of the examined occurrences, the following parameters were calculated for the most of the monitored egg structure characteristics:
Mean ($\bar{X}$),
Standard deviation (S)
Based on the obtained results, a phenotypic correlation was determined between the mass of egg and egg structure characteristics.
The coefficient of phenotypic correlation is calculated according to pattern (Latinovic, 1996):

$$r_{xy}=\frac{\sum xy-\frac{\left(\sum x\right)\left(\sum y\right)}{n}}{\sqrt{\left[\sum x^{2}-\frac{\left(\sum x\right)^{2}}{n}\right]\left[\sum y^{2}-\frac{\left(\sum y\right)^{2}}{n}\right]}}$$

The strength of phenotypic correlation coherence is discussed on the basis of the Roemer-Orphal classification (Latinovic, 1996). The testing of significance of differences between various ages of laying hens, in terms of monitored characteristics, was performed using an appropriate t-test model with the same number of repetitions according to the following pattern:

$$t=\frac{\bar{x}_1-\bar{x}_2}{Sp\sqrt{1/n_1+1/n_2}}$$

$$S_p=\sqrt{\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}}$$

Results and Discussion

The average values and variabilities of egg structure in different ages of laying hens are shown in table 1.

Table 1. The average values and variabilities of egg structure in certain phases of the production cycle

Indicators	Week of age (WA)	$(\bar{x})$	S
The shell mass (g)	WA_{27}	8.14	0.86
	WA_{46}	8.50	0.91
	WA_{72}	8.61	0.88
	WA_{79}	8.98	1.17
The share of the shell (%)	WA_{27}	13.41	1.27
	WA_{46}	13.42	1.15
	WA_{72}	12.92	1.27
	WA_{79}	13.29	1.59
The yolk mass (g)	WA_{27}	14.96	1.16
	WA_{46}	16.06	1.36
	WA_{72}	17.18	1.53
	WA_{79}	17.33	1.48
The share of the yolk (%)	WA_{27}	24.76	1.88
	WA_{46}	25.36	1.66
	WA_{72}	25.77	1.86
	WA_{79}	25.66	2.17
The egg white	WA_{27}	37.70	4.12

mass (g)	WA_{46}	38.78	3.53
	WA_{72}	40.87	3.89
	WA_{79}	41.24	4.75
The share of the egg white (%)	WA_{27}	61.83	2.39
	WA_{46}	61.22	1.97
	WA_{72}	61.31	2.29
	WA_{79}	61.05	3.01

As it can be seen from the table 1, the average shell mass increased over the periods of examination (WA_{27} – 8.14 g, WA_{46} – 8.50 g, WA_{72} – 8.61 g, WA_{79} – 8.98 g). The share of the shell in total egg mass waried through the control periods. During WA_{27} it averages at 13.41%, in WA_{46} it was 13.42%, WA_{72} 12.72%, while the average share of the raw shell in total egg mass in WA_{79} was 12.92%. A slightly lower values in terms of the shell mass (4.92-6.64 g) and the share of the shell in total egg mass (11.36-12.69%) were found by Zita et al. (2009). We can find lower values in other authors research: Pandurevic (2011) 6.12-18.11 g and 11.78-13.41%, Dikmen et al. (2017) 5.68 g and 9.78%, and Gjorgovska et al. (2016) 8.14 g and 12.06%. Lower values for the shell mass were found by Taha (2012) 6.27 g, and Kralik and Ljuboja (2017) 6.68 g, while the similar results were obtained in research by Rajicic et al. (2008) 8.20 g, and Pavicic (2014) 7.84-8.79 g. A slightly lower values in the share of the shell in total egg mass were found by Ren et al. (2010) 10.50%, Djogatovic and Maric (2014) 9.67-12.23% and Zaheer (2015), while the slightly higher value was found by Kralik et al. (2013) 13.57%.

Along with the age, the average absolute values of the yolk mass and egg white mass also increased. When it comes to the share of the yolk in total egg mass, it increased over the first three periods (24.76%, 25.36%, 25.77%) while it slightly decreased in the last period than the previous measurement (25.66%). The highest share of the egg white in total egg mass was in the first period (WA_{27} – 61.83%) and the lowest at the eng of the production cycle (WA_{79} – 61.05%). In the second and third period, the egg white had the share of 61.22% in WA_{46}, and 61.31% in WA_{72}. Similar values in terms of the yolk mass and share can be found in research by Zita et al. (2009) 11.94-19.64 g and 23.12-30.13%. A slightly lower values were recorded by Pandurevic (2011) 10.88-16.72 g and 23.60-25.90%, Pavicic (2014) 12.83-17.23 g, Dikmen et al. (2017) 14.07 g and 23.94%, while the higher values of the yolk were found by Taha (2012) 19.13 g, Kralik and Ljuboja (2017) 17.51 g, and Gjorgovska et al. (2016) 19.28 g and 28.61%.

When it comes to the mass and the share of the egg white, the similar results were obtained by Pandurevic (2011) 29.16-44.28 g and 60.69-63.97%, Djoganovic and Maric (2014) 59.53-61.53%, Gjorgovska et al. (2016) 40.11 g and 59.33%, Dikmen et al. (2017) 38.60 g and 66.29%. A slightly lower values are found by Zita et al. (2009) 32.78-38.49 g and 58.50-64.57%, Ren et al. (2010) 58.50%, Taha (2012) 35.60 g, Kralik et al. (2013) 57.18%, Pavicic (2014) 34.63-35.51 g, Kralik and Ljuboja (2017) 31.59 g.

The results of testing the significance of differences for the examined characteristics of the egg structure is given in table 2.

Table 2. Significance of differences of the egg structure in certain phases of the production cycle

Indicators	The age of laying hens (weeks)	Average value	Difference	Significance (p)
The shell mass (g)	27-46	8.14-8.50	-0.36	***
	27-72	8.14-8.61	-0.47	***
	27-79	8.14-8.98	-0.84	***
	46-72	8.50-8.61	-0.11	ns
	46-79	8.50-8.98	-0.48	***
	72-79	8.61-8.98	-0.37	***
The share of the shell (%)	27-46	13.41-13.42	-0.01	ns
	27-72	13.41-12.92	0.49	*
	27-79	13.41-13.29	0.12	ns
	46-72	13.42-12.92	0.50	*
	46-79	13.42-13.29	0.13	ns
	72-79	12.92-13.29	-0.37	ns
The yolk mass (g)	27-46	14.96-16.06	-1.10	**
	27-72	14.96-17.18	-2.22	***
	27-79	14.96-17.33	-2.37	***
	46-72	16.06-17.18	-1.12	***
	46-79	16.06-17.33	-1.27	***
	72-79	17.18-17.33	-0.15	ns
The share of the yolk (%)	27-46	24.76-25.36	-0.60	***
	27-72	24.76-25.77	-1.01	***
	27-79	24.76-25.66	-0.90	***
	46-72	25.36-25.77	-0.41	*
	46-79	25.36-25.66	-0.30	ns
	72-79	25.77-25.66	0.11	ns
The egg white mass (g)	27-46	37.70-38.78	-1,08	**
	27-72	37.70-40.87	-3.17	***
	27-79	37.70-41.24	-3.54	***
	46-72	38.78-40.87	-2.09	***
	46-79	38.78-41.24	-2.46	***
	72-79	40.87-41.24	-0.37	ns
The share of the egg white (%)	27-46	61.83-61.22	0.61	**
	27-72	61.83-61.31	0.52	*
	27-79	61.83-61.05	0.78	***
	46-72	61.22-61.31	-0.09	ns
	46-79	61.22-61.05	0.17	ns
	72-79	61.31-61.05	0.26	ns

***$p<0.001$; **$p<0.01$; *$p<0.05$; $^{ns}p>0.05$

The results in table 2 show that the age of laying hen has a statistically very significant influence ($p<0.001$) on the shell mass in all examined periods except between 46th and 72nd week of age, while the absolute values of the shell mass increased along with the age of laying hen. When it comes to the share of the shell, the age of laying hen has statistically significant ($p<0.05$) impact on this structural characteristic of the egg in two periods: between 27th and 72nd, and between

46th and 72nd week of age. In other comparisons, no statistically significant difference was found ($p>0.05$).

The absolute values of the yolk mass increased along with the age of laying hen. The yolk mass has changed statistically very highly significant ($p<0.001$) in examined periods between 27th week of age in relation to 72nd and 79th week, as well as between 46th week of age in relation to 72nd and 79th week. The difference was highly significant ($p<0.01$) between 27th and 46th week of age, while no statistically significant difference was found ($p>0.05$) between 72nd and 79th week of age. The age of laying hen had statistically significant influence ($p<0.05$) on the share of the yolk in examined period between 46th and 72nd week of age, and statistically very high significance ($p<0.001$) between 27th week of age and all other periods. No statistically significant difference was found ($p>0.05$) between 46th and 79th week of age, and between 72nd and 79th week of age.

When it comes to the egg white mass, the absolute values increased along with the age of laying hen. The age had no statistically significant influence ($p>0.05$) on this structural characteristic of egg only between 72nd and 79th week of age. Statistically highly significant difference ($p<0.01$) was noted between 27th and 46th week of age, while statistically very highly significant difference ($p<0.001$) was noted between all other periods. The mean values the egg white share was highly significant ($p<0.01$) between 27th and 46th week of age, significant ($p<0.05$) between 27th and 72nd week of age, while the difference was very highly significant ($p<0.001$) between 27th and 79th week of age. No statistically significant difference was found ($p>0.05$) between 46th and 72nd, between 46th and 79th, and between 72nd and 79th week of age.

The results in table 3 show coefficients of phenotypic correlation between the egg mass of four age groups of laying hens (WA_{27}, $WA_{46,}$ $WA_{72,}$ WA_{79}) and examined characteristics of egg structure.

Table 3. Phenotypic coefficients of correlation between the egg mass and the shares of the shell, yolk and egg whites

Indicators	The age of laying hens (WA)	r_p	$t_{exp.}$
The shell mass (g)	WA_{27}	0.062^{ns}	0.870
	WA_{46}	0.078^{ns}	1.001
	WA_{72}	0.315**	3.493
	WA_{79}	0.416**	4.102
The share of the shell (%)	WA_{27}	-0.187*	1.302
	WA_{46}	-0.182*	1.803
	WA_{72}	-0.482**	5.378
	WA_{79}	-0.511**	9.045
The yolk mass (g)	WA_{27}	0.331**	5.221
	WA_{46}	0.472**	6.007
	WA_{72}	0.432**	5.856
	WA_{79}	0.584***	9.880
The share of the yolk (%)	WA_{27}	0.178*	1.021
	WA_{46}	0.289*	2.218
	WA_{72}	0.433**	7.357
	WA_{79}	0.792***	12.190

The egg white mass (g)	WA_{27}	0.410**	6.285
	WA_{46}	0.309***	4.301
	WA_{72}	0.587***	10.001
	WA_{79}	0.386**	5.990
The share of the egg white (%)	WA_{27}	-0.219*	1.003
	WA_{46}	-0.352**	6.952
	WA_{72}	-0.431**	8.349
	WA_{79}	-0.690***	11.872

***p<0.001; **p<0.01; *p<0.05; ^{ns}p>0.05

Older laying hens have show better correlation than young hens. The coefficient of correlation between the egg mass and the share of shell in older hens amounted r_p= 0.416, and it was statistically highly significant (p<0.01). In young hens, the coefficient of correlation was r_p= 0.062, and was not statistically significant. Although all correlation coefficients between egg mass and shell mass (g) were positive, the coefficients between the egg mass and the share of shell (%) were all negative and statistically significant (p<0.05), while in older hens the coefficients were statistically highly significant (p<0.01).
Positive cofficients of correlation were determined between the egg mass and mass of both the yolk and egg whites, and they were all statistically highly significant (p<0.01) or very highly significant (p<0.001). Almost all coefficients showed medium strong to very strong correlations. Both old and young hens had statistically significant (p<0.05; p<0.01) coefficients of correlation calculated between the egg mass and the shares of both yolk and egg white. With the age of laying hens, the coefficients were significant at level p<0.001, and between the egg mass and egg white share (%) they were positive, while the coefficients were negative between the egg mass and yolk share (%) as shown in table 3.

Conclusion

Based on the examination of basic characteristics of egg structure of light-line hybrid Lohmann Brown, during experiment that lasted for 61 production week, the following conclusions can be drawn: Along with the age of laying hen, the average absolute mass of the shell, yolk and egg white also increased. Statistical significance was not found in the shell mass between 46th and 72nd week of age, as well as for the mass of the yolk and egg whites between 72nd and 79th week of age. When it comes to the relative values (percentage share) of the egg shell, it varied through the examined periods, but was pretty much steady around 13%. The share of the yolk gradually increased through the periods, and ranged from 24.76% to 25.77%, except in the last period when it was slightly lower than the previous.
The share of egg white in total egg mass was calculated, and it was the highest in the first period (WA_{27} - 61.83%) and the lowest at the end of the production cycle (WA_{79} - 61.05%). Statistically significant differences were found between WA_{27} and all other periods.
The coefficient of correlation between the egg mass and the share of shell was statistically significant (p<0.01) in older hens, while no statistical significance was found in younger hens. The coefficient of correlation between the egg mass and shell mass (g) were positive, while the coefficients of correlation between the egg mass and the share of shell (%) were negative and statistically significant (p<0.05; p<0.01). Positive cofficients of correlation were also determined between the egg mass and both yolk and egg whites. Almost all coefficients showed medium

strong to very strong correlations. Both old and young hens had statistically significant ($p<0.05$; $p<0.01$) coefficients of correlation calculated between the egg mass and the shares of both yolk and egg white. With the age of laying hens, the coefficients were significant at level $p<0.001$, and between the egg mass and egg white share (%) they were positive, while the coefficients were negative between the egg mass and yolk share (%).

It can be concluded that the egg mass of analyzed commercial flock of light-line Lohmann Brown hybrid, had influenced basic characteristics of the egg structure, in all examined periods, as shown by correlation between egg mass and examined characteristics of the egg structure.

References

Dikmen B. Y., Ipek A., Sahan U., Sozcu A., Can Baycan S. (2017): Impact of different housing systems and age of layers on egg quality characteristics. Turkish Journal of Veterinary and Animal Sciences (2017), 41., 77-84 p.

Djogatovic V., Maric A. (2014): Kvalitet konzumnih jaja različite mase [Quality of table eggs of different masses]. III International Symposium and XIX Scientific Conference of Agronomists of Republic of Srpska, 196-197 str., Trebinje.

Gjorgovska N., Filev K., Levkov V., Gjorgjievski S., Kostov V., Nastova R. (2016): The effect of feed rich in dha on egg production, egg components and dha content in yolk. Bulgarian Journal of Agricultural Science 22 (Supplement 1), 10-14 r.

Kralik G., Janjecic Z., Kralik Z., Skrtic Z. (2013): Stanje u peradarstvu i trendovi njegova razvoja. Poljoprivreda 19, 2, 49-58 str.

Kralik I., Kralik Z., Zelic S. (2013): Preferencije potrošača konzumnih jaja. 49. Hrvatski i 9. Međunarodni simpozij agronoma, Zbornik radova, 156-160 str., Dubrovnik.

Kralik Z., Ljuboja B. (2017): Kvaliteta jaja različitih vrsta peradi. 52. Hrvatski i 12. Međunarodni simpozij agronoma, Zbornik radova, 534-538 str., Dubrovnik.

Latinovic D. (1996): Populaciona genetika i oplemenjivanje domaćih životinja. Praktikum, Univerzitet u Beogradu Poljoprivredni fakultet Beograd.

Pandurevic T. (2011): Uticaj starosti na proizvodnost, kvalitet i hemijski sastav jaja lakog linijskog hibrida kokoši. Doktorska disertacija. Poljoprivredni fakutet Istočno Sarajevo.

Pavicic H. (2014): Kvaliteta konzumnih jaja u ovisnosti o dobi nesilica. Diplomski rad, Sveučilište Josipa Jurja Strossmayera, Poljoprivredni fakultet u Osijeku.

Rajicic V., Mitrovic S., Tolimir N., Peric L. (2008): Uticaj genotipa i uzrasta nosilja na kvalitet ljuske jajeta. „Savremena poljoprivreda" vol. 57, 1-2, 201-206 str., Novi Sad.

Ren Y, Wu J., Renema R. (2010): Nutritional and health attributes of eggs. Handbook of Poultry Science and Technology, Primary Processing, Volume 1, 535-578 p.

Taha S. Y. (2012): A comparison of egg quality from hens reared under organic and commercial systems. 2nd Organic Animal Husbandry Conference, Special Issue No 362, 164-167 r., Hamburg.

Zaheer K. (2015): An Updated Review on Chicken Eggs: Production, Consumption, Management Aspects and Nutritional Benefits to Human Health. Food and Nutrition Science, 2015, 6, 1208-1220 p.

Zita L., Tumova E., Stolc L. (2009): Effects of Genotype, Age and Their Interaction on Egg Quality in Brown-Egg Laying Hens. Acta vet. Brno 2009, 78: 85-91 p.

http://www.ltz.ba (Leyer managament program Lohmann Brown. Lohmann Tierzucht G. M. B. H., Cuxhaven Germany)

DEFINING THE ECONOMIC VALUE OF THE TRAITS INCLUDED IN THE METHODS FOR THE ASSESSMENT OF PIGS GENETIC POTENTIAL

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Abstract

The objective of this paper was to describe a methodological procedure for defining the economic value of primiparous sows production and reproductive traits of which some were used for assessment of sow`s breeeding value. This is a novel methodological procedure whose purpose is to reduce the influence that constant changes both in production cost and price of fatenners may have on selection work and to enable planning of long-term selection strategy in pig breeding. The obtained economic value is presented as a relative relationship between the changes in costs per unit of the trait and a primary trait, when the traits achieve the values defined by selection goal wherein the expenses are unknown value regarded for one feeding day. In this way the obtained economic value defines selection pressure and gives the trait the significance wished for by a selectionist without regarding it through the profit realized in the production expressed in money, which is still of a variable character in developing countries, thus allowing for its use as an indicator in calculating the genetic parameteres.

Key words: *trait economic value, selection index, pigs, methodological procedure to obtain economic value, genetic potential.*

Introduction

The proces of defining the economic value of the traits included in the models for assessment of animal breeding value is a real problem even in the countries with developed pig breeding with stable parity between the price of fatenners and the prices of inputs included into pig breeding industry, while this branch of livestock production in developing countries is characterized by continual variable relation between the production costs and the price of product *(Vukelić et al.,2004)*, what additionally complicates the determination of the economic value of the traits included in the method for assessment of animal breeding value. The value of some traits which cannot be expressed as a real market value presents an additional problem. For example, the traits such as liveborn piglet or backfat thickness have no fixed market value, because they cannot as such be found in the market, therefore the value of these traits cannot be properly expressed in monetary units. On the other hand, even if there are stable relationships between the input costs included into production and the value of the products obtained, the market value of the trait expressed in monetary units determines a selection goal *(Dube et al., 2013)*, in which some traits that have higher monetary value can be favoured, while at the same time, the traits

which are also very significant, but of lower market value difficult to determine (backfat thickness, percent of intramuscular fat and similar) may be neglected in selection.
Regarding the equation of selection index as the method for assessment the breeding value of pigs and other animals, the animal breeding value is obtained by multiplying partial regression coefficients and phenotypic difference of the traits of each individual. These partial regression coefficients express relative importance of the features included in the index, and are obtained by solving the system of matrices which includes phenotypic and genetic variances and covariances and the economic value of each of the traits *(Vidović, 2009)*. Respecting the fact that the values of genetic and phenotypic parameteres cannot be influenced in the moment of construction of selection index, but that they represent factual state displayed through mathematical solution, the only way to realize a desirable selection pressure on each of the traits in accordance with set selection goal is by way of defining the trait economic value and on that base to assess the animal breeding value. All this shows that the trait economic value represents not only a market value of some trait expressed in money but that it is mostly the sublimation of monetary value of the traits, their mutual relative relationship, desired selection goal or profit to be realized in the production *(Quinton et al., 2006)*.
Literature data indicate various possibilities of expressing traits economic value on the basis of which breeding value of farm animals is being assessed. Thus *Quinton et al. (2006)* express the economic value of sows` reproductive traits as the profit expressed in dollars which is being realized when the trait value has been improved by one genetic standard deviation, regarding the profit through kg of live weight of fatenners sold at the market. *Houška et al. (2010)* determine the economic value of swine production and reproductive traits by means of bio-economic model based on the relationship between the profit realized per unit of product and the profit which would be realized supposing the value of the trait changed for a defined size of that trait where we obtain marginal economic value which multiplied by genetic standard deviation can provide the economic value of the trait expressed in euros per trait unit. *Suzuki et al. (2005)* define the economic value of production traits by assessing the breeding value of performance tested boars and gilts of Durok breed as a relative relationship of profit of studied traits when defined value of the trait is being for desired selection goal.
On the other hand, *Radojković (2000)* and *Vukelić et al. (2004)* obtain the economic value of the traits in the conditions of a continual disparity of the cost of input and output in pig breeding production as a relative relationship of changes in the expenses per trait unit and the changes of expenses per unit of primary trait when the value of the traits is changed for one unit. The methodology described by previously mentioned two authors was used as a basis for methodological procedure displayed in this paper.
The objective of this paper was to describe a methodological procedure for defining the economic value of primiparous sows production and reproductive traits of which some were used for assessment of sow`s breeeding value.

Material and method

Data set on whose basis we determined the economic value of production and reproductive traits of the sows called fertile meaty breeds (Swedish Landrace and Great Yorkshire) and their cross-breds is formed on the basis of realized production results of 3912 sows raised on one of the pig farms in Serbia. The economic value is calculated for following production traits measured at the end of performace test: backfat thickness (SL1), sidefat thickness (SL2), depth of *m. longissimus* *dorsi* (MLD), carcass meat percentage (%MES), liveweight gain (DP), and for the reproductive traits defined after the first farrowing such as: number of total born piglets (BUP), number of liveborn piglets (BZP) and number of raised piglets (BOP). The traits mean values and duration of the period expressed in feeding days in which the traits were accomplished, and which served for calculating the economic value of traits are shown in Table 1.

Table 1. Traits mean values ($\bar{X}$) and duration of the period expressed in feeding days* in which the traits were realized

Trait	SL1 mm	SL2 mm	MLD mm	%MES %	DP g	BUP Piglet	BZP Piglet	BOP Piglet
Mean value($\bar{X}$)	12.27	10.16	48.11	59.36	541.13	9.88	9.22	8.48
No. of feed. days*	200.98	200.98	200.98	200.98	200.98	151.01	151.01	180.74

This is a research methodology paper where in the section "Results and discussion" there is a detailed methodological procedure of how to obtain the economic value of the traits of primiparous sows, on the basis of which the breeding value of animals can be defined.

Results and discussion

In this paper we tried to express the economic value of production and reproductive traits of primiparous sows in which some traits might be included into the methods for the assessment of their breeding value through relative relationship between the changes of the expenses realized (regarded through one feeding day) per product unit when the values of the traits reach the level defined by selection goal.

A primary presumption which served as a starting point of this methodological procedure is that the costs of one feeding day as an economic value are the same during a whole examined period what is not the case in practice, but smaller costs in the initial phase of breeding compensate for greater costs in later phases. These costs are unknown value T and they include the costs of care, nutrition, housing, health care, replacement and similar, for the entire life period in which the traits have been determined lasting from sow`s birth up to the weaning of her first litter of pigs (381.72 days). Out of this came the result that the cost of one feeding day is T/381.72.

Further on we determined the costs of each of the phase of the analysed life span in which we established the value of the traits, this period being divided into the period 1, from birth to the end of performance test, period 2, from the end of test until the first farrowing and period 3, from the end of test up to the first weaning, taking the view that periods 2 and 3 should also include, besides the costs of pregnancy and lactation, the costs related to the period from the end of test up to the successful conception, because the gilts practically started their reproduction in that period (the occurrence of the first puberty oestrus and so on). On the basis of the analysed records it was found that period 1 lasted on average 200.98 days, period 2 lasted 151.01 days and period 3 lasted 180.74 days. On the basis of these records the calculated costs were as follows:

for period 1: 200.98 / 381.72T = 0.5265T
for period 2: 151.01 / 381.72T = 0.3956T
for period 3: 180.74 / 381.72T = 0.4735T

The obtained values of the costs for the periods 1, 2 and 3, respecting the biological characteristics of the traits established in these periods, served to calculate the costs per unit of products, obtained by means of following pattern:

$$T_i \ / \ \overline{X} = T_j$$

Where: T_i – are the costs of the period in which the value of the trait was measured, $\overline{X}$ - mean value of the trait, T_j – costs per products unit.
On the basis of previous pattern the costs (T_{jx}) per unit of products were calculated and shown in Table 2.

Table 2. Costs per unit of products in realized production

Trait	SL1 mm	SL2 mm	MLD mm	%MES %	DP g	BUP Piglet	BZP Piglet	BOP Piglet
T_{jx}	0.04291	0.05182	0.01094	0.00887	0.00097	0.04004	0.04291	0.05584

The next step was to establish the costs T_{jy} per unit of trait when it has reached the value set as selection goal. Table 3 shows relative change of the traits according to set goal, the value of the trait defined by selection goal and the costs per unit of traits when they reach the values defined by selection goal.

Table 3. Selection goal, value of the traits defined by selection goal and the costs per unit of traits when they reach the values defined by selection goal

Trait	SL1 mm	SL2 mm	MLD mm	%MES %	DP g	BUP Piglet	BZP Piglet	BOP Piglet
Selection goal %	-6.00	-5.00	6.00	5.00	15.00	12.00	14.00	16.00
Value of trait defined by selection goal	11.53	9.65	51.00	62.36	622.30	11.07	10.51	9.84
T_{jy}	0.04565	0.05455	0.01032	0.00845	0.00085	0.03575	0.03764	0.04814

Then we determined the difference between the costs T_{jx} per unit of the traits realized in production and the costs T_{jy} per traits unit when they have reached the values defined by selection goal. Difference (T_{jx}-T_{jy}) between these costs is shown in Table 4.

Table 4. Difference (T_{jx}-T_{jy}) between the costs per unit of the traits realized in production and the costs T_{jy} per unit of the traits when they have reached the values defined by selection goal

Trait	SL1 mm	SL2 mm	MLD mm	%MES %	DP g	BUP Piglet	BZP Piglet	BOP Piglet
T_{jx}-T_{jy}	-0.00274	-0.00273	0.00062	0.00042	0.00012	0.00429	0.00527	0.00770

The obtained differences displayed in Table 4 were used in calculating the economic value of the traits, the primary trait being determined first. Knowing the biological characteristics of pig production where fatenner is the final product, the number of raised piglets was determined as the most important one among the traits displayed or as the primary trait because it is the trait that most affects the number of fatenners. It should be pointed out that any of the traits could be chosen as primary one because a relative relationship of economic value remains unchanged. By dividing the difference of costs per unit of trait displayed in previous table with the difference of costs of primary trait, when the traits reach the level defined by selection goal, the economic value of that trait has been obtained. Table 5 shows the traits economic values in which some served for the assessment of the breeding value in primiparous sows.

Table 5. Traits economic values

Trait	SL1	SL2	MLD	%MES	DP	BUP	BZP	BOP
Economic value of the trait	-0.356	-0.355	0.081	0.055	0.016	0.557	0.684	1

Having completed a whole metodological procedure of obtaining the economic value of the traits included in the model for assessment of the breeding value of primiparous sows, the economic value can be defined as a relative relationship between the changes of costs per trait unit and primary trait when the traits have reached the values defined by selection goal. In this way the economic value determines the selection impact on each of the trait, on the basis of which the animal breeding value is being assessed. The costs on whose basis we obtain economic value in this way expressed in money can be very different, but their relative relationship observed through feeding day is always the same, so that the obtained economic value and set selection goal are not dependent upon constant change of the price of production process and market price of fatenners as final product of pig breeding production. Also it should be pointed out that the traits for which we envisage greater relative change, contribute, in a greater extent, to the animal assessed breeding value, affecting such defined economic value to give such contribution of traits in animal overall breeding value which is wanted for by a selectionist, according to the importance of some traits defined by a selectionist.

Comparing the methodological procedure of obtaining economic value displayed in this paper where it is expressed as a relative relationship of reducing the costs per trait unit by means of methodological procedures *(Brkić, 2002; Suzuki et al., 2005; Quinton et al., 2006; Houška et al., 2010; Dube et al., 2013)* in which the economic value of different traits is expressed in money units (dollar, euro, dinar and likewise) of the realized profit, the advantage of such obtained economic value of the traits is that it represents a relative relationship of traits in set selection goal, which is not under the significant impact of constant fluctation of production prices and obtained final product. On the other hand, a negative side of this metodological procedure in relation to the methodology of obtaining economic value shown by some other authors *(Brkić, 2002; Suzuki et al., 2005; Quinton et al., 2006; Houška et al., 2010; Dube et al., 2013)* is that it starts from the presumption that the unknown costs of one feeding day are the same during a whole analysed period what cannot be confirmed knowing the biological and economic characteristics. This shortcoming might be solved by using the function of bio-economic model used by *Houška et al. (2010)* and thus try to define a relative value of each feeding day during production life, expressed through the value of average feeding day and then on that basis try to define the costs made in every period in which the traits were realized.

Methodological procedures suggested by *Radojković (2000)* and *Vukelić et al. (2004)* served as the basis of methodological procedure for obtaining economic value of swine traits described in this paper. However, the advantage of this methodological procedure in relation to the methodology of *Radojković (2000)* and *Vukelić et al. (2004)* is in the fact that in this way by set selection goal the selectionist himself defines selection pressure and contribution of some traits in animal estimated breeding value while the selection pressure and contribution of some traits in animal estimated breeding value in the methodology suggested by previous authors is exclusively defined by a relative value of one unit of the trait out of total value of that same trait because the economic value of the traits is obtained as a difference of costs between realized value of the trait and the value when that trait has been changed for one unit. This actually means that the traits with low relative value of one unit (gramme of liveweight daily gain) participate far less in estimated breeding value of the animal in relation to the traits with high relative value of one unit in total value of the trait (raised pig), and their importance regarding selection is most often almost equal.

Conclusion

On the basis of the facts displayed it can be concluded that this is a simple methodological procedure for obtaining economic value of the traits on the basis of which the animal breeding value is being estimated and which can be widely used in livestock production. It should also be pointed out that in this way the obtained economic value and the very models for assessing the animal breeding value are not under the constant influence of the costs fluctuations in livestock production and final products, which is charateristic of developing countries, as well as the fact that through such defined economic value we can clearly define and realize selection goal which in a considerable degree is not dependent upon constant market fluctuations while the selection can be directed into desired direction and within wanted scope.

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References

Brkić, N. (2002). Evaluation of breeding value of sows by integrated selection indexes. PhD dissertation. University of Belgrade, Faculty of Agriculture, Serbia. 1-137.

Dube, B., Mulugetal, D. S., & Dzama, K. (2013). Integrating economic parameters into genetic selection for Large White pigs. Animal. 7-8, 1231–1238.

Houška, L., Wolfová, M., Nagy, I., Csörnyei, Z., & Komlósi, I., (2010). Economic values for traits of pigs in Hungary. Czech J. Anim. Sci. 55(4), 139–148.

Radojković, D. D. (2000). Estimation of breeding value of sows and effects of direct and indirect selection. MSc thesis. University of Belgrade, Faculty of Agriculture, Serbia. 1-152.

Suzuki, K., Kadowaki, H., Shibata, T., Uchida, H. & Nishida, A. (2005). Selection for daily gain, loin-eye area, backfat, thickness and intramuscular fat based on desired gains over seven generations of Duroc pigs. Livest. Prod. Sci. 97, 193-202.

Vidović, V. (2009). Principi i metodi oplemenjivanja životinja /Principles and methods of animal breeding/. Poljoprivredni fakultet, Univerzitet u Novom Sadu. 1-348.

Vukelić, G., Radojković, D. & Petrović M. (2004). Method for determination of relative relations between economical values and reproductive traits in production of piglets. Biotechnol. Anim. Husb. 20 (3-4), 81-84.

RURAL DEVELOPMENT AND AGROECONOMY

MONITORING THE CONDITION OF AGRICALTURAL LAND AS A TOOL OF THE PRECISION FARMING SYSTEM

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Abstract

We regard agricultural land monitoring as part of the system of "precision farming", which is positioned as the future of agriculture by all the leading economies of the world. The constituent elements of the system are: technology of parallel driving and autopilot of agricultural machinery based on GPS (Global Positioning System) and GNSS (Global Navigation Satellite System) navigation systems; monitoring and assessment of the biological state of plants (construction of maps of vegetation indices) and correction of agrotechnical measures; assessment of soil condition and construction of maps of fertility, productivity and profitability of agricultural land. The most important element of monitoring territories is the use of UAVs (Unmanned Aerial Vehicle) with multispectral cameras, agricultural drones and neural network data analysis in order to form recommendations for correcting agricultural technology. Currently, OOO "Intelligent Systems of Agriculture" together with the Vitebsk State University named after P.M. Masherov, using the example of a model farm, develops a fundamental methodology for monitoring agricultural land using UAVs (DJI) (Dajiang Innovation Technology). The proposed information and analytical monitoring system includes hardware-software, natural science and legal blocks. The ultimate goal of using the system is to increase labor productivity in crop production in the north of Belarus by 10-35%. The basis of such growth is the saving of chemicals, fuel and the most accurate calculation of the place, time and volume of agrotechnical measures. In addition, the introduction of this system will increase the "environmental friendliness" of agricultural production.

Keywords: *monitoring, precision farming, agriculture, multispectral cameras, UAV*

Introduction

The development of agriculture is the most important factor in guaranteeing the food and economic independence of any country. Precision farming technologies are the leading direction in the development of this industry and monitoring of the state of crops is an integral part of them. The introduction of unmanned aerial vehicles takes the monitoring process to a completely new level, makes it possible to monitor the indicators of the state of vegetation in almost any conditions and observe the changing environmental conditions.

The ultimate goal of this project is to increase labor productivity in agricultural production, reduce costs and create new jobs for highly qualified specialists and, ultimately, increase the profits of agricultural producers. The basis for achieving this goal is the extremely accurate calculation of the time and volume of agrotechnical measures (sowing time, weed control, fertilization, etc.) and the systematic nature of assessment and control measures during monitoring observations.

The main tasks performed during the implementation of the project are: selection of options for monitoring software and hardware that meets the needs and capabilities of agricultural producers in the context of the realities of Belarusian agriculture; providing an information and cartographic base, assessing the regulatory and legal aspects of the use of unmanned aircraft and precision farming technologies in the country's agriculture; development of methodological aspects of the observation and operation of the system (criteria for assessing the state, frequency and objects of observation, development of recommendations and correction of agrotechnical measures based on monitoring data, etc.); preparation of options for commercial solutions for the implementation of the project.

Material and Methods

The project is being developed by a team of students, undergraduates and teachers of Vitebsk State University named after P.M. Masherov for LLC "Intelligent Systems of Farming", focused on the agricultural market of the Republic of Belarus. The work is carried out on the basis of the model farm of Sushchevo Agro LLC (Vitebsk region, Belarus). The object of the study is a pair of fields similar in terms of conditions of winter rapeseed, maize and winter cereals. In each pair, a control field was determined, where the farm agronomist determined the need for activities based on his data obtained in the traditional way, and an experimental one, where the correction of agricultural technology was planned to be carried out based on the data of unmanned and satellite monitoring using multispectral survey data and, ultimately, vegetation indexes such as NDVI (Normalized Difference Vegetation Index). The initial data were farm materials on the history of experimental and control fields, high-precision survey data using UAVs, information on fertilizers and plant protection products used in the farm, a database of plant pests and diseases, materials of the Land Information System of the Republic of Belarus, soil research, meteorological observations.

Results and Discussion

The precision farming system includes three main components: technologies of parallel driving and autopilot system based on GPS and GNSS navigation systems, which provide the necessary accuracy of guidance of aggregates on sowing cereals, planting potatoes, ridge formation, etc.; current assessment of the biological state of plants using UAVs, satellite data (construction of vegetation indices) and, based on the processing of the obtained data, the management of "point" application of plant protection products and correction of agrotechnical measures in general; long-term assessment of soil conditions, construction of maps of fertility, yield, and in the long term, maps of profitability of each specific plot of agricultural land on the basis of data accumulated in the process of monitoring observations.

At the current stage of research, efforts have been focused on the ongoing assessment of the biological state of plants using UAVs, satellite data and the use of these materials in the cultivation of some crops. In the process of analyzing the options for organizing the monitoring software and hardware, we selected a complex that would meet the tasks of the experimental stage of the project. An unmanned system based on a Phantom 4 multispectral quadrocopter equipped with a multispectral camera and software for data analysis was chosen as a tool for monitoring observations on a number of parameters. An important element of the analytical block of the system was the use of technologies for neural network analysis of cartographic data, which made it possible to automatically draw the boundaries of lands and areas with different conditions of crops according to maps of vegetation indices. An example of using a neural network for the automatic creation of high-precision maps is the allocation of characteristic "technological stripes" - parallel lines on the map, formed as a result of the operation of agricultural machinery, row planting of crops and other factors.

Shenzhen Dajiang Baiwang Technology (DJI) software Terra and DJI Assistant 2 For Phantom were selected for data processing. However, in order to integrate monitoring materials into the economic activities of the model farm, experiments were carried out on the use of cloud platforms for organizing agricultural activities "Geomir" and "Interra", which made it possible to synchronize databases for all elements of the farm (equipment, fields, accounting, etc.), visualize any information in a convenient form, carry out an automated calculation of indicators of the state of vegetation and corrective measures.

During the implementation of the project, it is also planned to test Agros agricultural drones for targeted agrotechnical measures, which will allow adjusting the state of crops based on automatically generated flight tasks based on the monitoring drone data. The most important characteristic of the use of unmanned aircraft is also the environmental friendliness of its use in comparison with traditional methods of monitoring and agricultural work. UAVs are used to monitor the state of the territory, accurately apply extremely dangerous chemicals in ultra-small volumes, as well as spot fertilizers and chemicals to correct the situation in certain areas of the fields, which allows: to reduce the consumption of fertilizers and chemical plant protection products to the minimum required volumes, which in turn reduces the pollution of soils and agroecosystems in general; to abandon the use of heavy agricultural machinery, this allows to reduce the risk of erosion and other dangerous geomorphological crops on agricultural land, as well as the operation of drones on batteries minimizes the emission of pollutants into the atmosphere, which occurs during the operation of internal combustion engines.

As part of the project to date: completed scientific research to prepare for the field stage of work; the structure and main components of the projected information and analytical system have been determined; the technical and technological aspects of the project have been developed - the configuration of the software and hardware complex has been selected, the system of information exchange between the blocks of the projected system has been modeled; a concept for the operation of a neural network has been developed, tests have been carried out for the algorithm for differentiating the territory by aerial photographs; high-precision survey of the territory of the model farm and the purchase of equipment are carried out.

Practical advantages of using / implementing the proposed system: saving expensive plant protection products (no overlapping of application zones, precise control of consumption during spraying, selectivity of treatment sites); saving time for processing crops (speed of deployment, mobility); extremely accurate determination of the timing of agrotechnical measures. It is especially important with a large number of small or, conversely, very large fields in area; the possibility of carrying out work in case of waterlogged soil, when the equipment cannot enter the fields; work without mechanical damage to plants and tramlines on tall crops (corn, rapeseed, etc.); reduction of environmental risks (environmental pollution, impact on health, provocation of erosion as a result of the actions of heavy equipment, etc.).

Conclusions

The territory monitoring system makes it possible to facilitate the process of tracking changes in certain areas and to make a clear analysis of the requested criteria necessary for successful farming. The projected system represents a full cycle from information support to the implementation of agricultural solutions. Due to high productivity, accuracy and mobility, it will significantly reduce the cost of work. Accordingly, the costs of the customer will decrease, and a high level of profitability of the enterprise will be ensured. In addition, optimization of the agrotechnical work map will have a positive effect on the environmental aspects of agricultural production countries (reduction of fertilizer and herbicide costs, technological load on soils, etc.).

TRANSITION TOWARDS BLUE ECONOMY IN THE EUROPEAN UNION

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Abstract

Oceans cover more than two-thirds of the earth's surface and contain 97% of the planet's water. In addition to producing half of the oxygen in the atmosphere and absorbing about 25% of CO_2 emissions, they are home of vast biodiversity and a source of food, water and jobs. In recent decades, pressure on the water ecosystems have intensified as a result of climate change, excessive pollution, increasing exploitation of water resources and the inability to tackle illegal activities. This leads to an aggravation in the quality of water resources and their productivity, to an endangerment of species, to habitat destruction and to overall deterioration in the quantity and quality of all resources extracted from oceans and seas. The interconnectedness of oceans and their specific characteristics and requirements impose that the responsibility for their conservation and restoration should be shared globally. According to The European Union (EU) Blue Economy Report 2020, the directly employed in these sectors are close to 5 million people and they have generated around €750 billion in turnover and €218 billion in Gross Value Added (GVA) in 2018. This determines their great significance for the economic development of the EU. The main objective of the paper is to represent the international efforts aimed at the protection of water resources and to evaluate the measures taken to stimulate the EU transition towards blue economy through a review of available official documents, strategies, reports, plans, communications and information provided by the EU and United Nations (UN).

Keywords: *Blue Economy, Water Resources, European Union, Sustainable Development Goals*

Introduction

Oceans cover more than two-thirds of the earth's surface, contain 97 % of the planet's water and 80 % of all life forms. They are the source of life of the planet and have an extremely important role for humanity, for its existence and development. (EU, 2018 and EU, 2021). However, at global level, measures related to protection of water and aquatic life, to the fight against climate change, water pollution, overexploitation of oceans and seas and illegal activities related to them are still lagging behind. Improper water management leads to deficit of drinking water, droughts, floods, to an aggravation in the quality of water resources and their productivity, to an endangerment of population of a number of species, to habitat destruction and to overall deterioration in the quantity and quality of all resources extracted from oceans and seas.

Ecosystems, including marine ones, provide a wide number of services like climate regulation, food, water and so they have a vital role for human survival and for the world's sustainable development aspirations (UNEP, 2016). Water conservation and sustainable use of water resources is vital to human survival and is an integral part of the overall environmental protection and the achievement of sustainable development. As stated in the first EU Blue Economy Report (BER) 2018, oceans can produce investment, jobs and economic growth – and the heathier they are, the more productive they'll be (EU, 2018).

The interconnectedness of oceans and their specific characteristics and requirements impose that the responsibility for their conservation and restoration should be shared globally. The adverse effects of climate change on the ocean (UN, 2017), including the temperature rise, ocean and coastal acidification, deoxygenation, sea level rise, the decrease in polar ice coverage, coastal erosion and extreme weather events necessitate the need to mitigate the adverse impacts or the crucial ability of the ocean to act as climate regulator, source of marine biodiversity and as key provider of food and nutrition, tourism and ecosystem services and as an engine for sustainable economic development and growth will be lost.

The origins of the blue economy concept can be traced back to the 2012 UN Rio+20 conference and the report on 'Green Economy in a Blue World' (EU, 2020). The term "Blue Economy" includes the economic sectors and related policies that together determine whether the use of oceanic resources is sustainable. The Blue Economy encompasses all sectoral and cross-sectoral economic activities based on or related to oceans, seas and coasts divided into two groups – Marine-based and Marine-related activities (EU, 2021).

The Blue Economy established sectors are Marine living resources, Marine non-living resources, Marine renewable energy, Port activities, Shipbuilding and repair, Maritime transport and Coastal tourism and the Blue Economy emerging and innovative sectors include Marine renewable energy, Blue bioeconomy and biotechnology, Marine minerals, Desalination, Maritime defence, security and surveillance, Research and Education and Infrastructure and maritime works (submarine cables, robotics). The emerging sectors offer significant potential for economic growth, sustainability transition, as well as employment creation. (EU, 2021).

The main objective of this paper is to represent the international efforts aimed at the protection of water resources and to evaluate the measures taken to stimulate the EU transition towards blue economy.

Materials and methods

This paper is based on a thorough review of available reports, analysis of official documents as action plans, declarations, communications, programs and provided by the EU and UN data related to Blue Economy, its sectors and the policies aimed at supporting the transition towards Blue Economy at EU level in the context of global efforts for achieving sustainable development.

For the period 1949 – 2021, UN and EU put considerable efforts towards nature conservation and sustainable development, including water resources management, water conservation and development of water-related sectors and activities. The current state of development of the Blue Economy is a result of many international summits, conferences, programs and significant work at international level to adopt common goals and policies to ensure the sustainability of natural resources and the world's oceans in particular and to prevent habitat destruction, species extinction, increasing emissions and the overall deterioration of natural conditions worldwide.

In some of the reviewed documents, the information related to water protection and development of ocean-related activities and sectors is represented in the context of the overall progress towards sustainable development. However, for the purpose of the analysis, only data related to Blue Economy sectors and water conservation is taken into account.

Results and discussion

During the first session of the International Law Commission in 1949, the regimes of both territorial waters and waters of high seas were put as topics for codification (UN, 1949). In 1956, the Commission adopted its final report on the territorial sea (UN, 1956) and scheduled the United Nations Conference on the Law of the Sea in 1958. In 1960 the second United Nations Conference on the Law of the Sea was held during which the topics which had not been agreed upon at the previous Conference were considered and the third conference was held from 1973 to 1982, resulting in the adoption of the United Nations Convention on the Law of the Sea, which came into force in 1994 (UN, 1982). The Convention (UN, 2012) outlines the responsibilities and the rights related to the use of world's oceans, their management and preservation of countries which ratified it. Each of the XVII Parts is focused on a different aspect of ocean and marine resources use. The first considerable steps towards sustainable development and nature conservation were taken during the United Nations Conference on the Human Environment, which was held in 1972. It led to the creation of government environment agencies and the UN Environment Program and to the adoption of the first Environment Action Program by EU later that year. The Declaration of the United Nations Conference on the Human Environment (Stockholm Declaration) was adopted in 1972 and it is known to be the first international document in which the right to a healthy environment is brought to the fore. It states 26 principles which form the framework of responsibilities and point out the main issues the world is facing and Principle 7 is directly focused on protecting the world's oceans and aquatic organisms and it points out that „States shall take all possible steps to prevent pollution of the seas by substances that are liable to create hazards to human health, to harm living resources and marine life, to damage amenities or to interfere with other legitimate uses of the sea." (UN, 1972). The UN Environment Programme (UNEP) states that prevention is preferable to follow-up actions to eliminate the consequences for nature and brings to the fore the 'polluter pays' principle and assists governments to quantify and understand the value of ecosystems and how to best use that information in their national decision-making processes (UNEP, 2016). Since 1972, UNEP has been setting the global environmental agenda, promoting the coherent implementation of the environmental dimension of sustainable development within the United Nations system (UNEP, 2019). In 1992, 2002 and 2012 were held the second, the third and the fourth UN Conferences on Sustainable Development, the main outcomes of which are the adoption of Agenda 21, the United Nations Framework Convention on Climate Change (1992), the Kyoto Protocol (1997) and the UN Action Plan for Sustainable Development (UN, 2002). A milestone for sustainable development is the Paris Agreement (UN, 2015). It is the first legally binding international treaty on climate change and it brings to the fore the need of international ambitious efforts in order to combat climate change and to help adapting to its effects by lowering the greenhouse gas emissions as soon as possible and to achieve a climate neutral world by mid-century. The Sustainable Development Goals (UN, 2015) were set up in 2015 by the United Nations General Assembly and are intended to be achieved by the year 2030. Sustainable Development Goal 14 ("Life below water") is aimed at sustainable development of oceans, seas and marine resources. Its ten targets outline the timeline for reaching the different aspects of ocean sustainability and impose the need of preventing and significantly reducing the negative impacts as well as increasing the knowledge and research capacity, conservation and sustainable use of oceans and their resources and providing access for small-scale artisanal fishers to marine resources and markets.

According to the World Bank (World Bank, 2020), "the Blue Economy concept seeks to promote economic growth, social inclusion, and the preservation or improvement of livelihoods while at the same time ensuring environmental sustainability of the oceans and coastal areas.
Oceans, seas and coastal areas form an integrated and essential component of the Earth's ecosystem and are critical to sustaining it. Conservation and sustainable use of oceans and seas and of their resources is vital for sustainable development, including through their contributions to poverty eradication, economic growth, food security and creation of sustainable livelihoods and decent work, while at the same time protecting biodiversity and the marine environment and addressing the impacts of climate change (UN, 2012). One of the targets of The European Green Deal, directly linked to ocean preservation, is to ratify by 2021 an action plan for achieving zero pollution for water, air and soil (European Comission, 2019).
Since 2009 there is a growth in levels of the EU Blue Economy main indicators (Table 1), but when divided by main sector, the results are not so encouraging (Table 2). Although the increase of 13-15% of turnover, GVA and gross profit and the high levels of investments for the period, there are some sectors which are lagging behind.

Table 1: EU Blue Economy, main indicators for the established sectors, 2009-2018

	2009	2018	Δ 2018-2009
Turnover, € billion	577,180	649,708	13%
GVA, € billion	153,643	176,067	15%
Gross Profit, € billion	59,573	68,067	14%
Employment, number	4 451 560	4 475 141	1%
Net investments in tangible goods, € billion	*	6,4	*
Net investment ratio, %	*	3.6%	*
Average annual salary, €	*	24020	*
Personnel costs per employee, € billion	93,598	107,499	15%

Source: EU Blue Economy report 2021 (Data from Table 1 and Annex 2, Tables A1-A6)
**no information*

Table 2: EU Blue Economy, change 2009-2018 for the established sectors, %

	Persons employed, Δ 2018-2009	Turnover, Δ 2018-2009	GVA, Δ 2018-2009	Gross Profit, Δ 2018-2009	Gross investments in tangible assets, Δ 2018-2009
Marine living resources	-3%	26%	29%	43%	13%
Marine non-living resources	-68%	-80%	-62%	-65%	-39%
Marine renewable energy	2246%	5621%	3582%	4114%	599%
Port activities	1%	18%	15%	8%	-6%
Shipbuilding and repair	-5%	11%	30%	93%	9%
Maritime transport	11%	31%	12%	5%	-22%
Coastal tourism	1%	20%	21%	44%	*

Source: EU Blue Economy report 2021 (Data from Annex 2, Tables A1-A6)
**no information*

As visible from Table 2, the most significant increase is observed in the Marine renewable energy sector where for the 2009-2018 period the turnover has increased by 5621 %, the gross profit – by 4114 %, the GVA by more than 3500 %, the number of employed – by more than 2000 %, all this in the context of only about 600 % growth of investments. The situation in other sectors differ as there is a huge decrease in the levels of all indicators for the Marine non-living resources sector, as well as for the number of employees in sectors of Marine living resources and Shipbuilding and repair, and in investments in sectors of Port activities and Maritime transport.

The contribution of the Blue Economy emerging and innovative sectors (EU, 2021) is significant with increasing capacity, huge investments in nature-based solutions and new opportunities like floating solar photovoltaic energy, hydrogen generation offshore, new innovations and technology for the marine minerals sector.

- The ocean energy sector has 12 GW capacity in 2020 (incl. 12 MW for wave energy and 22.4 MW for tidal energy) with expectation of increase up to at least 60 GW by 2030 and more than €78 million to R&D projects on floating offshore wind solutions via FP7 and H2020 funding programs since 2009 and €3.84 billion between 2007 and 2019, for R&D on wave and tidal energy;
- Since 2014, around €262 million have been invested through the European Regional Development Fund (ERDF) and Horizon 2020 in projects supporting Blue biotechnology;
- In January 2021, there were 2309 operational desalination plants in the European Union, producing about 9.2 million cubic meters per day and the investments for 2010-2019 period are about €630 million;
- The total defence expenditure in 2019 amounted to €186 billion, The European Maritime Security Agency (EMSA) had a budget of €81.2 million for 2020 and the whole European Defence Industry had 440 000 jobs ;
- Horizon 2020 (2013-2020) funded €79 billion to Research and Innovation and €4.9 million for skills and education were funded under the Marine Technology Skilling Strategy (MATES) project;
- In 2019, 205 out of a total of 400 submarine cable networks, responsible for 99% of international data transfer and communication, were connected to EU Member States, with a 564000 km length; and in 2019, the global underwater robotics market was valued at €2 209 million and forecasted to reach €4 390 million by 2025;

In a number of the regions, dependent of the Blue Economy sectors` development, there are not many other alternatives for livelihood and occupation. The Blue Economy activities have a great impact on the marine environment, including the effects of climate change and greenhouse gas emissions, over-exploitation and pollution which all result in biodiversity loss (EU, 2020). Many of the current activities need to reduce their carbon footprint, while new, carbon-neutral activities need to be stimulated. The Blue Economy can play a vital role in many aspects of the work to combat pollution and can benefit from new opportunities arising from that. Over the past two decades, the EU put a solid foundation for an integrated and synergetic maritime policy in order to create a sustainable Blue Economy (European Comission, 2021). Although achieving the European Green Deal objectives imposes big investments, by 2030 one third of investments in the Blue Economy could still be unsustainable (EU, 2020) and the reasons for that include focus on short-term profits, inadequate impact assessments, weak regulatory frameworks and businesses' inability to attract impact investments.

The ocean policy should be integrated into Europe's economic policy and the green and the blue policies should be better connected. The ocean, and the 'blue economy' it supports, is indispensable to achieving the transformation set out in the European Green Deal. The contribution of a healthy ocean is essential for a sustainable economy. A sustainable blue economy will create tangible opportunities for new jobs and businesses (European Comission, 2021).

Conclusion

The current state of development of the Blue Economy is a result of significant long-term work at international level. The EU is taking the necessary steps to protect water resources and ensure their sustainable use but still there is a great number of actions that should be taken on a global level so the transition towards Blue Economy could become possible. A sustainable blue economy is a global challenge that requires coordinated global action, international policies and common goals.

The Blue Economy established sectors, except Marine non-living resources, mark an increase for the last two decades. The largest increase is observed in the Marine renewable energy sector. The Blue Economy emerging and innovative sectors offer a huge number of opportunities for new activities, jobs, and nature-based solutions.

The Blue Economy activities have a great impact on the marine environment and they need to be governed in a sustainable way so the negative impacts are mitigated or eliminated. To achieve this and to reduce the unsustainable activities, EU should switch to sustainable financing in the Blue Economy sectors.

References

EU (2018), EU Blue Economy Report, PDF ISBN 978-92-79-81757-1

EU (2020), EU Blue Economy Report, PDF ISBN 978-92-76-19726-3

EU (2020), Unsustainable Finance in the Blue Economy: Where Does the Money Come From? Recommendations Report, ISBN 978-92-9460-268-8

EU (2021), EU Blue Economy Report, PDF ISBN 978-92-76-37879-2

European Commission (2019), Communication on the European Green Deal Roadmap, COM (2019) 640 final, Brussels; https://www.unep.org/resources/annual-report/letter-executive-director-2020-review

European Commission (2021), Communication on a new approach for a sustainable blue economy in the EU Transforming the EU's Blue Economy for a Sustainable Future, COM (2021) 240 final, Brussels;

UN (1949), Report of the International Law Commission, vol. I, A/CN.4/13 and Corr. 1-3, available at http://www.un.org/law/ilc/index.htm;

UN (1956), Regime of the high seas and regime of the territorial sea, Document A/CN.4/97 of the 8th session of International Law Commission, available at http://www.un.org/law/ilc/index.htm;

UN (1972), Report of the United Nations Conference on the Human Environment, A/CONF.48/14/Rev.1, available at http://www.un-documents.net/aconf48-14r1.pdf;

UN (1982), United Nations Convention on the Law of the Sea (UNCLOS), available at https://www.un.org/Depts/los/convention_agreements/texts/unclos/closindx.htm

UN (2002), Plan of Implementation of the World Summit on Sustainable Development, A/CONF.199/20, available at https://undocs.org/en/A/CONF.199/20;
UN (2012), The future we want, Outcome document, United Nations Conference on Sustainable Development, available at https://sustainabledevelopment.un.org/content/documents/733FutureWeWant.pdf;
UN (2015), Report of the Conference of the Parties, Paris, 2015 (The Paris Agreement), FCCC/CP/2015/10/Add.1, available at https://www.un.org/en/development/desa/population/migration/generalassembly/docs/globalcompact/FCCC_CP_2015_10_Add.1.pdf
UN (2015), Transforming our world: the 2030 Agenda for Sustainable Development, A/RES/70/1, available at https://www.un.org/en/development/desa/population/migration/generalassembly/docs/globalcompact/A_RES_70_1_E.pdf;
UN (2016), UNEP Annual Report 2016, ISBN: 978-92-807-3638-0), available at https://wedocs.unep.org/bitstream/handle/20.500.11822/19529/UN%20Environment%202016%20Annual%20Report.pdf?sequence=1&amp%3BisAllowed=;
UN (2019), UNEP Annual Report 2019, available at https://wedocs.unep.org/bitstream/handle/20.500.11822/32374/AR2019.pdf?sequence=1&isAllowed=y;
UN (2017), Our ocean, our future: call for action, Resolution adopted by the General Assembly, A/RES/71/312, available at https://undocs.org/en/A/RES/71/312;
World Bank (2020), Bulgaria: Toward Blue Economy Development, Washington DC, USA

CURRENT SITUATION AND TRAINING NEEDS ON HERB SECTOR: EVIDENCE FROM GREECE, MOLDOVA, ARMENIA AND GEORGIA

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Abstract

Herbs are an integral component of the Mediterranean culture. Many farmers changed their old crops into new cultivations like herbs. This development requires investments, training programs, studies, projects in order to enrich the knowledge and skills of all the participants involved in the value chain. HEGO is a Black Sea Project, funded by the European Union and one of its main goals is the modernization of enterprises associated with cultivation, production and promotion of diversified, sustainable, value-added herb products and the enhancement of cross-border trade opportunities for local herb enterprises in participating Black Sea Basin countries (Greece, Moldova, Georgia and Armenia). During the first year of the project, Market Research survey has been performed, one in each Project country, with stakeholders from several target groups (farmers, advisors, collectors, SMEs, public authorities, organizations etc) in order to identify: (a) the current situation with reference to the collection/cultivation, processing and promotion practices used for herb products as well as legislative issues and the niche market segments for sustainable and ethical herbs products, (b) the current skills and expertise towards herbs of the participants in each country and (c) the specific future training needs of end-users of Project Outputs in relation to the above mentioned topics. The findings of these surveys provide important insights in the research field of herbs and led to conclusions, suggestions, recommendations and specific guidelines for target groups' members that will be used as feedback for the formulation of policy implementations including the development of training curricula and courses.

Keywords: *Black Sea, Extension, Herbs, Survey, Training.*

Introduction

In recent years there is a strong interest in herbs. The trend towards new alternative crops, the search for products with environmentally friendly production methods as well as the valuable properties of herbs are the main reasons for the increased interest of farmers and consumers (Papamixalopoulos, 2020). Herbs have been used extensively for their healing properties across history and cultures (Barata et al., 2016). During the Hippocratic Corpus (5th – 2nd century B.C., Greece), almost 380 medicinal herbs useful for various illnesses were recorded (Petrakou et al., 2020). Hundreds of millions of people collect plant material to fulfill their needs for substances for personal uses or for trade (Cheminal et al, 2020). The Mediterranean Basin is considered as a "Global biodiversity hotspot" and the largest of the world's five Mediterranean type climatic regions (Skoufogianni et al, 2019). According to WHO (2015), 60.000 species of aromatic and medicinal plants are used globally and more than 500.000 tons of materials from those species are traded every year. The collection and use of nature products and especially herbs is a common practice. One of the main concerns globally is the uncontrollable harvest of wild herbs which results in a severe decrease in the population of these species (Lange, 2004). Due to overharvesting and habitat loss, approximately 15.000 species are now endangered (Schippmann et al., 2006).

This paper is part of one of the main Deliverables of the project's first work package: Market Research for current situation and training needs on herb sector. The main aim of this deliverable is to describe the current situation of herb sector, in the Black Sea HEGO countries, and to identify the training needs of end-users from several target groups (farmers, advisors, collectors, SMEs, public authorities, organizations etc). During this deliverable Market Research survey has been performed, one in each Project country, using both quantitative and quantitative instruments (Michailidis et al, 2011). This research has been performed under the Deliverable "Final Cross-country Report in Market Research Surveys results" and aims at providing the final common findings, conclusions and suggestions/recommendations on training needs of herb products among Project countries.

Materials and Methods

The research in all four project countries was conducted during March and April 2021. The questionnaire was developed by the Greek team and completed: (a) in Greece by 30 responders, (b) in Moldova by 49 responders, (c) in Georgia by 33 responders and (d) in Armenia by 40 responders (Fig. 1). The sample was representative consisting of all target groups, of different age groups and of different educational background (more details are presented below). The primary collection method was face-to-face/personal interviews. But in some cases, due to weaknesses (pandemic, national reasons), interviews were done by phone, Skype, e-mail or via Google Forms. The collected national data were gathered in data excel files and were statistically analyzed using the SPSS for Windows (ver. 27). The survey instruments were based on a previous work of Valero et al. (2021).

Figure 1. Distribution of the sample by country

The following Tables 1 and 2 present a short description of the research sample. According to Table 1, Moldova had the biggest sample, followed by Armenia, Georgia and Greece. Greece's main target groups were Farmers of herbs (53,33%), whereas in Moldova, Georgia and Armenia, were SMEs (42,68%, 33,33% and 35,00% respectively), The main group of the total sample of the survey were SMEs (31,58%), followed by farmers of herbs with 23,03%.

Table 1. Description of the sample

Distribution of the sample by country	
Greece (30 questionnaires)	19,74 %
Moldova (49 questionnaires)	32,24 %
Georgia (33 questionnaires)	21,71 %
Armenia (40 questionnaires)	26,32 %

Table 2. Description of the target groups

Target groups	Greece	Moldova	Georgia	Armenia (%)
Farmer of herbs	53,33	20,41	12,12	12,50
Collector of herbs	6,67	0,00	27,27	15,00
Local public authorities	6,67	4,08	0,00	0,00
Regional public authorities	0,00	2,04	0,00	22,50
National public authorities	3,33	4,08	3,03	0,00
Sectoral agencies	0,00	2,04	0,00	2,50
Interest groups including NGOs	3,33	6,12	12,12	5,00
Education/ training centers and schools	13,33	8,16	9,09	5,00
SMEs	6,67	42,86	33,33	35,00
Business support Organisations	6,67	10,20	3,03	2,50

Results and discussion

According to all participants, current skills and expertise towards herbs are higher in processing techniques and lower in wildcrafting practices and technological expertise. Table 3 presents the mean values per country and per total survey sample.

Table 3. Grade the level of your skills and expertise today towards herbs (1: none, 5: very high)

Skills and expertise	Greece	Moldova	Georgia	Armenia	Mean	St. Dev.
Cultivation practices (planting, irrigation, fertilization, weed and pest control, harvesting, propagation)	3.57	3.15	3.00	2.80	3.13	0.33
Knowledge on cultivation needs (from planting till harvesting)	3.87	3.38	3.15	3.00	3.35	0.38
Wildcrafting practices (do you implement these methods of harvesting?)	2.83	2.46	3.58	3.30	3.04	0.50
Knowledge on ethical wildcrafting (improve the process, follow regulations and make it more sustainable and environmental-friendly)	3.37	2.38	3.61	3.20	3.14	0.53
Knowledge on the biodiversity conservation of endemic herb plant species (do you know that many endemic herb plants are forbidden to harvest as they are protected, red-listed?)	3.47	2.76	3.48	3.30	3.25	0.34
Knowledge about the ecology and sustainable management methods of herb species	3.37	3.15	3.39	3.40	3.33	0.12

Processing techniques (drying herbs, herbal mixtures, distillation, extracts, food products etc)	3.50	3.46	3.48	3.30	3.44	0.09
Technological expertise (like value chain, precision agriculture, innovations, application of technology in the cycle of production etc)	3.13	3.00	3.18	2.90	3.05	0.13
Trading skills (marketing skills, certification etc)	3.03	3.30	3.42	3.10	3.21	0.18
Business management skills (value chain development, legislative expertise, finance etc)	3.17	3.53	3.21	3.30	3.30	0.16

Future needs in training towards herbs should target the gain of plant health conditions-weed control and managerial-commercial skills (Fig.2). The next figure summarizes the averages of those mean values in an ascending order.

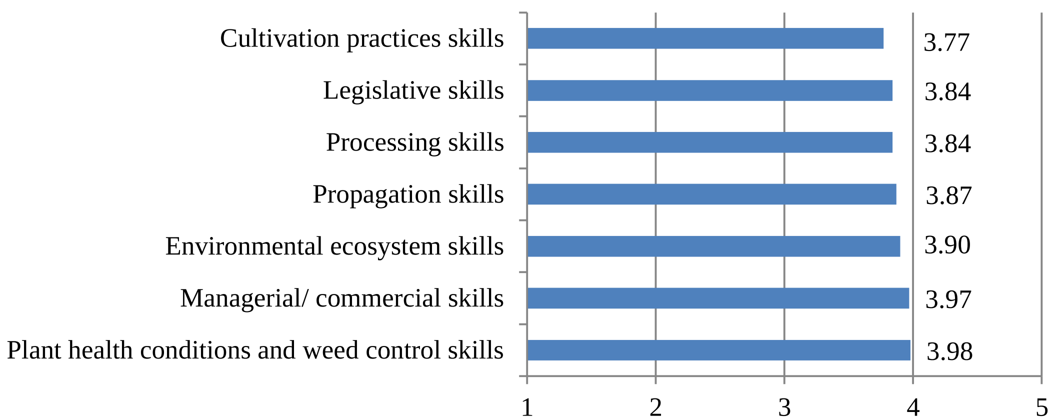

Figure 2. Future needs in herbs training

Table 4 presents the mean values per country and per total survey sample in every subcategory of skills. In Greece, training for managerial/commercial skills was seen as most needed, whereas cultivation practices skills were graded as a lesser necessity. Moreover, the most important skills that need to be developed are marketing, trading and cooperating internationally. In Moldova, training for propagation skills was more important, whereas managerial/commercial skills were less important. In Georgia, most needed skills were training for plant health conditions and weed control, while training for legislative skills seems to be a lesser necessity. Finally, in Armenia, training for plant health conditions and weed control skills seems to be very important whereas managerial/commercial skills were less important.

Table 4. Rate your need for training to the following (1:not important, 5:extremely important)

Training needs	Greece	Moldova	Georgia	Armenia	Mean	St. Dev.
a. Training for cultivation practices skills						
On planting process	3.20	4.07	3.97	3.67	3.37	0.39
On site selection like soil composition, pH level, drainage	3.40	4.15	4.27	3.82	3.91	0.39
On fertilizing	3.00	4.07	4.03	3.72	3.71	0.50
On irrigation	3.00	4.07	4.15	3.8	3.76	0.53
On relevant equipment and tools for cultivation and wildcrafting practices	3.37	3.76	4.12	3.72	3.74	0.31

Average Mean values	**3.19**	**4.02**	**4.11**	**3.75**	**3.77**	
b. Training for plant health conditions and weed control skills						
On identification of plant health problems	3.83	4.15	4.21	4.10	4.07	0.17
On weed control	3.47	4.00	4.06	3.82	3.84	0.27
On pest control	3.50	4.00	4.27	3.97	3.94	0.32
On disease control	3.83	4.07	4.39	3.97	4.07	0.24
Average Mean values	**3.66**	**4.06**	**4.23**	**3.97**	**3.98**	
c. Training for propagation skills						
On establishing and operating an herb nursery	3.23	4.23	4.06	4.00	3.88	0.44
On selection of propagation methods and materials (growing structures, cuttings, seed, separation, division)	3.30	4.00	4.27	3.77	3.84	0.41
On knowledge of each method characteristics	3.53	4.23	4.12	3.72	3.90	0.33
Average Mean values	**3.35**	**4.15**	**4.15**	**3.83**	**3.87**	
d. Training for processing skills						
On harvesting	3.03	4.00	4.12	3.87	3.76	0.49
On storage/ post-harvest practices	3.13	4.00	4.09	4.15	3.84	0.48
On distillation techniques	3.57	4.07	3.97	3.47	3.77	0.29
On drying techniques	3.53	4.15	4.24	4.05	3.99	0.32
Average Mean values	**3.32**	**4.06**	**4.11**	**3.89**	**3.84**	
e. Training for legislative skills						
On understanding legislation for products, cultivation, propagation, taxes etc	3.97	4.38	4.00	3.55	3.98	0.34
On dealing with bureaucracy	3.83	4.00	4.03	2.97	3.71	0.50
On regulations and fines about wildcrafting	3.40	3.76	3.85	3.47	3.62	0.22
On regulations about certifications	3.97	4.38	4.15	3.45	3.99	0.40
On organic certification	3.87	4.15	4.15	3.50	3.92	0.31
Average Mean values	**3.81**	**4.13**	**4.04**	**3.93**	**3.84**	
f. Training for environmental ecosystems skills						
Knowledge about the biodiversity conservation of endemic herb plant species	3.57	4.00	4.09	3.75	3.85	0.24
Knowledge about the ecology and sustainable management methods of herb species	3.60	4.23	4.18	3.67	3.92	0.33
New sustainable cultivation practices	3.97	4.08	4.09	3.75	3.97	0.16
New sustainable wildcrafting practices	3.50	4.08	4.09	3.72	3.85	0.29
Average Mean values	**3.66**	**4.09**	**4.11**	**3.72**	**3.90**	
g. Training for managerial/ commercial skills						
On business management	3.90	4.00	4.09	3.52	3.88	0.25
On innovation management	4.10	4.15	4.18	3.70	4.00	0.21
On technological management	4.13	4.15	4.15	3.65	4.02	0.25
On marketing	4.33	4.00	4.12	3.67	4.03	0.28
On trading	4.30	4.00	4.12	3.57	4.00	0.31
On evaluating market demand	4.13	4.31	4.21	3.75	4.10	0.24
On cooperating internationally (language, trade terminology)	4.17	3.15	4.03	3.62	3.74	0.46
Average Mean values	**4.15**	**3.95**	**4.13**	**3.64**	**3.97**	

However, the specification and ranking of training needs per country is of greater value. Figure 3 shows the mean values in each country.

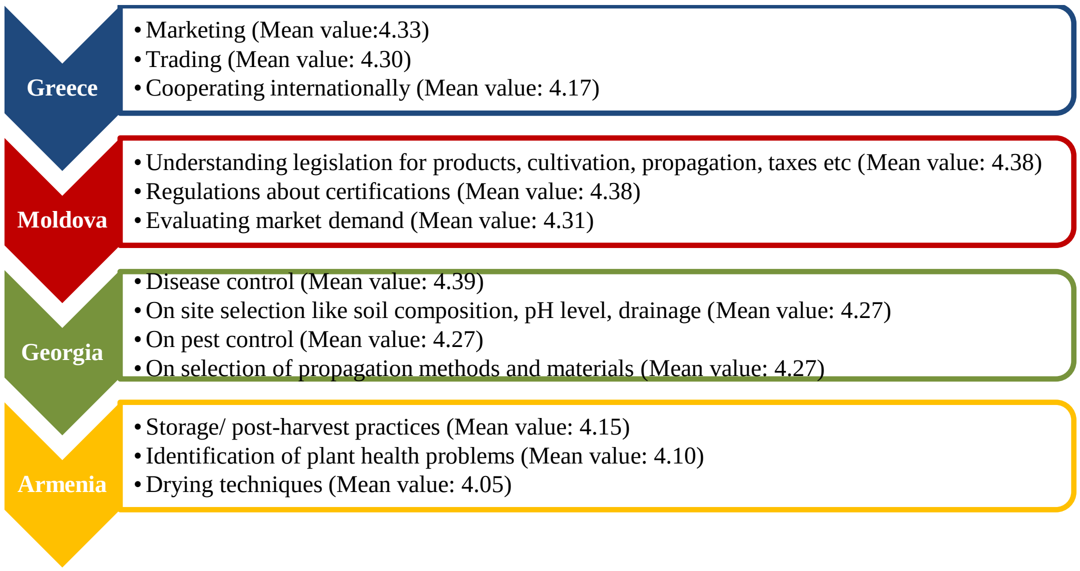

Figure 3. Most important training needs by country

From the above mentioned figure the following interesting results are obtained:

a) all three major training needs of Greek responders are included in the broader category "Training for managerial/ commercial skills",

b) major training needs of Moldavian responders are included in the broader categories "Training for managerial/ commercial skills" and "Training for legislative skills",

c) major training needs of Georgian responders are included in the broader categories "Training for plant health conditions and weed control skills", "Training for cultivation practices skills" and "Training for propagation skills",

d) major training needs of Armenian responders are included in the broader categories "Training for plant health conditions and weed control skills" and "Training for processing skills".

Figures 4-10 show the future training needs of all target groups of each country with a different color, for cultivation practices, plant health conditions-weed control, propagation, processing, legislative, environmental ecosystems and managerial/commercial skills.

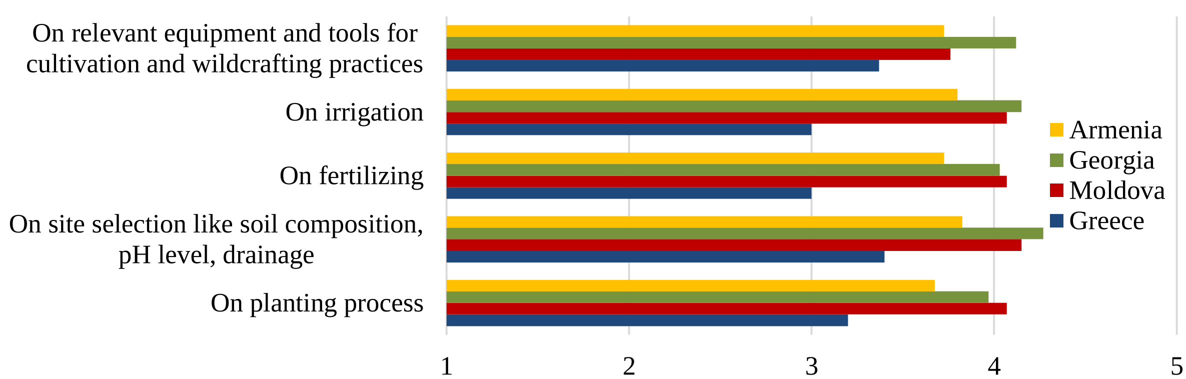

Figure 4. Future training needs for cultivation practices skills

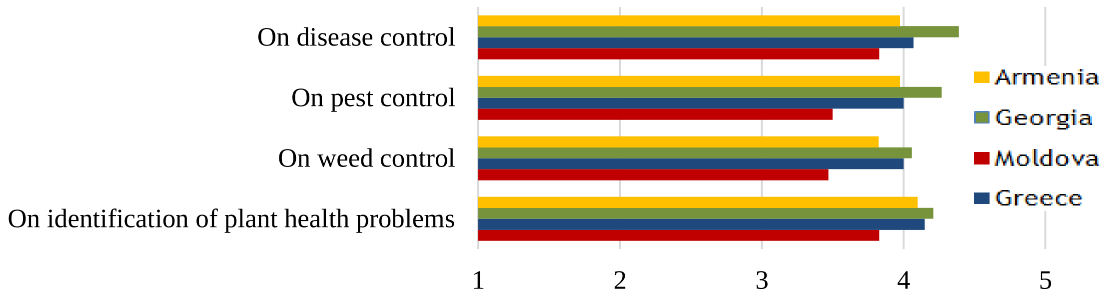

Figure 5. Future training needs for plant health conditions and weed control skills

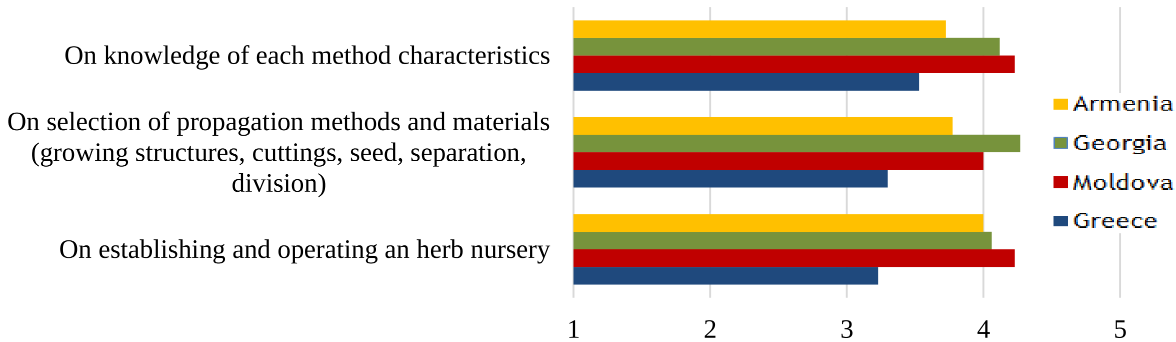

Figure 6. Future training needs for propagation skills

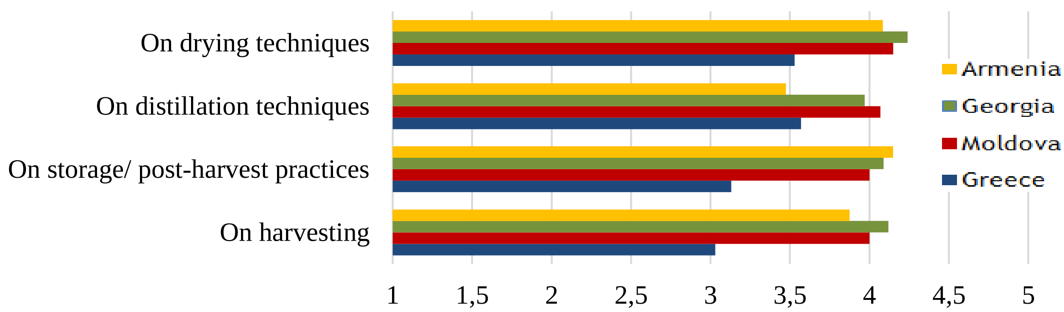

Figure 7. Future training needs for processing skills

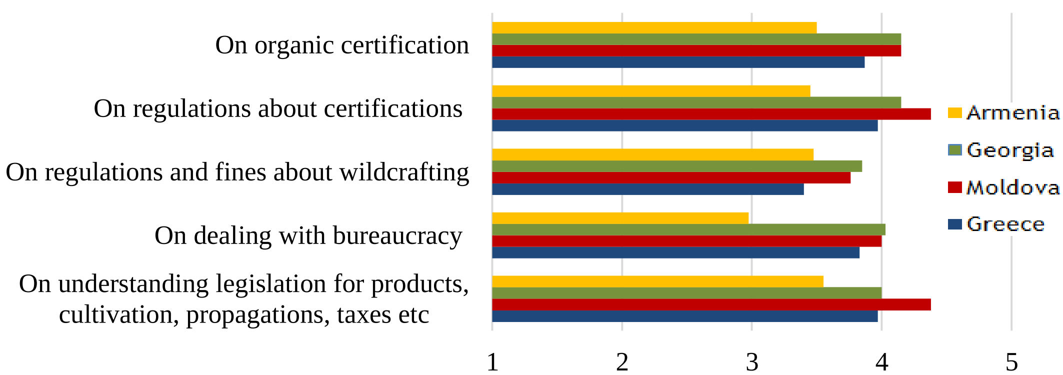

Figure 8. Future training needs for legislative skills

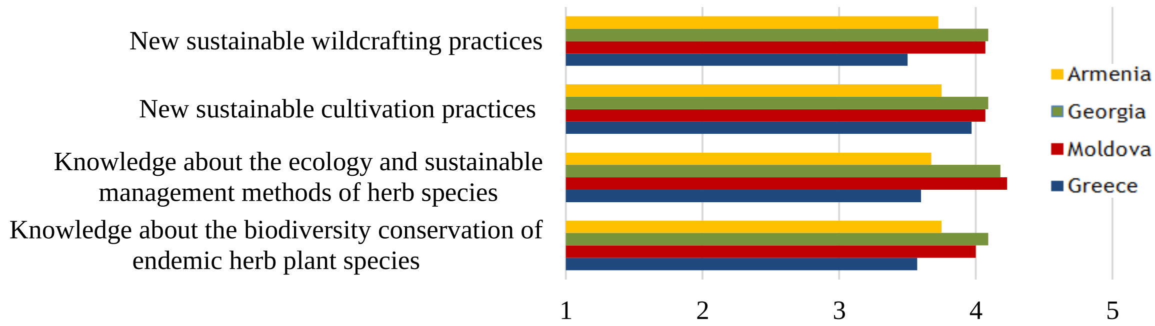

Figure 9. Future training needs for environmental ecosystems skills

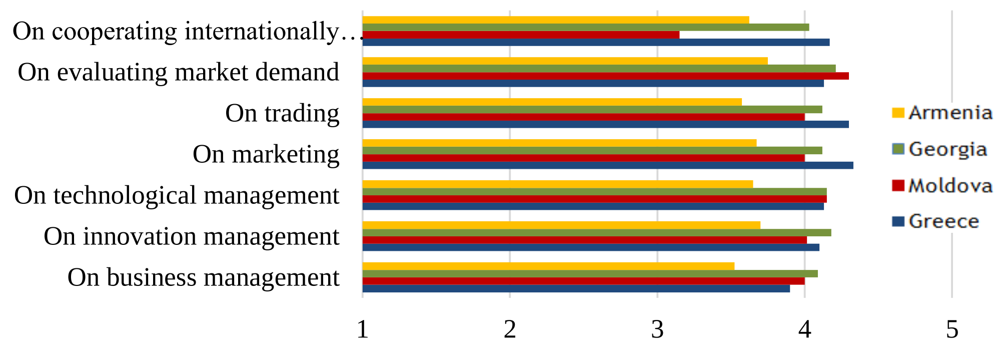

Figure 10. Future training needs for managerial/commercial skills

Conclusion

This paper provides important insights in the research field of herbs. From content point of view, this paper helps bring the sector of herbs a step closer to all different target groups participated in the survey and reduce their training gap. More specifically, results of this paper can be used to design a common training framework for all Project countries, in order to cope with the potential threats of the herb sector. The most important gap detected was knowledge/skills, especially in wildcrafting practices and technological expertise, among participants of the survey. In addition, almost all of the responders recognize the importance of education/training for herbs' cultivation. The most important skills regarding training were managerial/ commercial and plant health conditions-weed control. More specifically, the major future training needs are "evaluating market demand", which belong to managerial/commercial skills and "identification of plant health problems and disease control", which belong to plant health conditions-weed control skills. Based on the implemented questionnaire and performed analysis of the collected data from all countries, we conclude into a series of recommendations on training needs which can be the basis to design the training programme:

- Training on wildcrafting practices.
- Training on plant health conditions and weed control skills.
- Training for processing skills.
- Training on sustainable development of the business.
- Training on recognition and adoption of innovation and modernization practices current and future practices in growing, harvesting, production and trade of herbs (i.e. during the entire cycle of business).
- Training in developing skills for market review demand and supply.

- Training on product quality improvement and traceability.
- Training in accessing financing and investments.
- Training on labeling and certification.
- Training on increasing capacities to cooperate locally and internationally.
- Training in trading and commercial skills.
- Training for the development of the value chains and clusters of the herbaceous sector.
- Training for internationalization of companies and development of export activities.
- Training of new qualified specialists in herbal sector.

Acknowledgements

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References

Barata, A. M., Rocha, F., Lopes, V., & Carvalho, A. M. (2016). Conservation and sustainable uses of medicinal and aromatic plants genetic resources on the worldwide for human welfare, Industrial Crops and Products, 88, 8–11.

Cheminal, A., Kokkoris, I. P., Strid, A., & Dimopoulos, P. (2020). Medicinal and aromatic lamiaceae plants in greece: Linking diversity and distribution patterns with ecosystem services, Forests, 11(6), 1–14.

Lange, D. (2004). Medicinal and aromatic plants: Trade, production, and management of botanical resources. In Acta Horticulturae (Vol. 629, pp. 177–197). International Society for Horticultural Science.

Michailidis, A., Partalidou, M., Nastis, S. A., Papadaki-Klavdianou, A., & Charatsari, C. (2011). Who goes online? Evidence of internet use patterns from rural Greece, Telecommunications Policy, 35(4), 333-343.

Papamixalopoulos. (2020). Papamixalopoulos. Accessed on 15/09/2021 http://repository.library.teimes.gr/xmlui/handle/123456789/8644

Petrakou, K., Iatrou, G., & Lamari, F. N. (2020). Ethnopharmacological survey of medicinal plants traded in herbal markets in the Peloponnisos, Greece, Journal of Herbal Medicine, 19, 100305.

Schippmann, U., Leaman, D., Cunningham, A.B., (2006). Cultivation and wildcollection of medicinal andaromatic plants under sustainability aspects. In: Bogers, R.J., Crakerand, L.E., Lange, D. (Eds.), Medicinal and Aromatic Plants:Agricultural, Commercial, Ecological, Legal, Pharmacological and SocialAspects. Springer Dordrecht, Wageningen, The Netherlands, pp. 75–95.

Skoufogianni, E., Solomou, A. D., & Danalatos, N. G. (2019). Ecology, Cultivation and Utilization of the Aromatic Greek Oregano (Origanum vulgare L.): A Review, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(3), 545–552. https://doi.org/10.15835/nbha47311296

Valero, C., Krus, A., Barreiro, P., Diezma, B., Garrido-Izard, M., Hernández, N., ... & Pulignani, G. (2021). Teaching precision farming and entrepreneurship for European students: Sparkle online course. In Precision agriculture'21 (pp. 177-190). Wageningen Academic Publishers.

World Health Organization (WHO), (2015). Connecting global priorities: biodiversity and human health: a state of knowledge review. ISBN 978 92 4 150853 7.

THE POLICY REFORMS THAT SHAPED CONTEMPORARY RURAL SOCIETY IN GREECE

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Abstract

Rural communities remain an integral part of contemporary Greek society which is active, takes actions and resists, trying to maintain its distinctive character. These are communities in a continuous endeavor to negotiate terms and manage their integration in society, very much in the way it was envisaged in the policy reforms firstly introduced in the early decades of the 20th century. The objective of this paper is to explore and highlight the importance of policies introduced by policy makers and decisions taken by Governments of Greece at that time, in order to stimulate the agricultural economy and introduce rural education in the country. The options that were available, the radical reforms implemented during the period 1910-1920, reforms that led to the social transformation of the rural sector in Greece will be analyzed and presented. Legislative decrees and laws of 1911, the educational reform that had already begun in 1913, the establishment of new ministries and the rural reform of 1917, were the result of a dynamic relationship between the rural population and the policy initiated by the Greek Government. The decisions taken and the Bills passed in the period 1910-1920 concerning agricultural economy and rural life, particularly promoted agricultural education. The primary sector in the country, although it employed a large percentage of the active Greek population and brought sizable revenues to the state treasury, had been neglected in previous decades. It is important to point out that a result of the radical reforms that occurred in the agricultural sector was the active participation of rural population in the social and economic life of the country.

Key words: *rural economy, agriculture,20th century, agricultural education.*

Introduction

Ruralism is defined on the basis of the interaction between internal social links and external policies. In this context, a dominant role in shaping ruralism is played by the social integration processes of the rural population in modern society, which are detected at three levels: the economic, political and cultural. (Zakopoulou, 2008, p.29) At the political level, which also concerns the present study, what is being investigated is the conversion of farmers into citizens of an equal standing to others, the functionality of rural order in the current system, the importance of rural confrontations, integration policy, the importance of family agriculture, the maintenance of property by farmers and the importance of the agricultural sector in modernizing the Greek state in the first decades of the 20th century is investigated.

The rural community is an important part of the newer Greek society that acts, affects and resists. The local rural society and surrounding society developed an unprecedented two-way relationship for Greek standards in this period. The radical decisions of the Greek Governments during the first decades of the 20th century and their modernity led to the social transformation of the rural sector in Greece. The aim of the research is to investigate the contribution of the Greek Governments'' decisions to the promotion and development of the rural economy in Greece.

Materials &Methods

The agricultural sector has been the main employer of Greek working force during the period of this research (1910-1920) and its support has significantly helped both in the country's finances and in avoiding social tensions in times of social crisis.

The more general assumptions of this research are as follows: a) through the study of historical archives of the time period (Benaki Museum, Archive of Eleftherios Venizelos) a substantial promotion of the rural economy will emerge b) the decisions of the governments on the agricultural sector will be more and more substantial during the periods 1914-1917 and 1922-1924 given the historical circumstances (end of the Balkan wars and the Asia Minor disaster)

The methodology chosen for this research is qualitative. Through qualitative research, data are collected that describe problems and concepts of people's lives. The data can emanate from interviews, observations, participatory observations, stories, interactions, case studies, personal experiences, life stories, file analyzes, visual material and endoscopies.

This research uses the Grounded Theory as a methodological tool. Grounded Theory appeared in 1967 when Barney Glaser and Anselm Strauss introduced it in their book *The Discovery of Grounded Theory*. It is a systematic research method according to which the generated theory derives inductively from the study of empirical data and is not based on previous ideas or ideas of the researcher. Its basic principle is that the researcher is not encouraged to do some initial bibliographic research but to suspend his personal theoretical stereotypes in order for theory to emerge exclusively from empirical data. It is a purely inductive research approach. As its name suggests, this research method has the effect of forming a theory that is based on the empirical data it interprets. Grounded theory belongs to the qualitative methods of social research and consists in the analysis of data, the methods of production of quality material, the use of theory and ultimately the presentation of the results (Iosefides, 2006: 212) and is mainly used in research issues that have not yet been investigated or are under investigation.

Results and Discussion

The Constitution of 1911 aimed, on the one hand, to regulate relations between the large landowners and farmers and on the other to support agricultural production. These intentions can be seen through many governmental decisions such as:

- "the establishment in Athens of a Veterinary Microbiological Laboratory",
- "the establishment of a State stallion Station",
- "the establishment of veterinary regions and the appointment of veterinarians as civil servants",
- "the supply by the State of agricultural supplies to villagers",
- "the reconstruction of the agricultural service of the State ",
- "the implementation of land reclamation projects draining of swamps and rearrangement of river beds and torrents etc." (Peliouras, 2017, p.185).

The revision of the Constitution imposed arrangements (such as Article 17 which provided for the possibility of expropriation of properties not only for reasons of "public necessity" but also for "public benefit"), which opened up the way for the expropriation of estates (Patronis, 2015, p. 110). Moreover, the addition to Article 11 of the Constitution where it states that a cooperative cannot be dissolved due to a breach of the provisions of the laws, or by judicial decision,' removed from the administration the right to approve the establishment or revoke the approval of a Union.

The Interim Government of Thessaloniki 1917 proceeded to issue a series of legislative decrees that have been the first real and radical effort to resolve the agricultural issue in the country. The Agricultural reform with five legislative decrees gained force, gave impetus to the whole of the Greek state and concerned the following issues:

-2466: The transference of government properties to farmers so as to create small land ownerships

-2467: Concession of Macedonian estates to those who participated in the National great effort

-2468: Adjustment of legal relationships regarding public land, as defined by Ottoman laws

-2469: Compulsory expropriation of rural real estate

-2470: Issuing of bonds to compensate for the expropriation of farm estates which were expropriated (Tsiros, 2013, p.131).

In 1917, the Venizelos government takes a decision of crucial importance and establishes the Ministry of Agriculture. The main endeavor of the newly established ministry was to apply the agricultural reform that the prime minister had promised, but at the same time it dealts with expropriations, public estate management, land distribution, land registration and agricultural insurance (Petmezas, 2012, p.145)

An important decision and a basic principle underlying legislation were to assemble farmers in rural cooperatives to eliminate the disadvantages of small ownership. The expropriations made in the 1724 estates restored 130,000 landless families or poor farmers (Martinos, 2004, p. 245).

In addition, the agricultural reform that started in 1917 allowed the rural rehabilitation of about 600,000 refugees thus preventing social tensions, opened up the road to the development of the system of agricultural insurance, almost eliminating the usurious system of major landowners and has significantly contributed to the establishment of cooperatives (Vergopoulos, 1975, p .177).

This modernizing attitude that characterized Greek governments of this period is also detected in the field of education. Main purpose of the Prime Minister was to provide to educational programs a technical and practical content. Indicatively, we refer to the bills of the Minister of Education in 1911, that established among other things, monastery farms, a technical-vocational education network was envisaged. In the same year, the institution of the county agronomist is being introduced. The Averofio Agricultural School also contributed significantly to the improvement of agricultural production in the country. It should be noted that while the original purpose for the establishment and operation of the School was to create agronomists to fill the positions of directors in the agricultural industry, in 1911 the institutional framework changed. More specifically, two departments were established: a senior one to train agronomists to fill managerial positions in large estates and agricultural industries, and a lower one to train young farmers from rural families (Kandila, 2004, p.426).

In 1913, the Minister of Public Education submits to Parliament eight new bills. In the introductory report, it is characteristically mentioned that ' The urban school as we have said should provide the necessary general education to young people who either immediately or after their graduation and having completed their studies in vocational, commercial, agricultural, naval or other specialized school should start their profession in life.As foreseen by the 1913 bills there were six years of mandatory education and, among other things, curriculum had a direct

relationship with agricultural production with subjects such as "horticulture, arboriculture, livestock, beekeeping" (Eliades, 2010, p. 65).
In 1914, lower level agricultural schools are founded with a purely practical orientation. These schools would be founded in each county and be attended for 2 years by graduates of the elementary school aged 14-19 years as boarding school students. (Kandila, 2004, p. 426-427).
The first "senior Agricultural School of Athens" (AGSA) was established in 1920. The founding law stated amongst others things that the graduates should be able to serve as senior executives of state agricultural service departments and manage agricultural and associated businesses, to promote Greek scientific research to the various sectors of agricultural production. Moreover: "the graduates of the school will have a great qualification, such that immediately after the end of their studies they will know the conditions of Greek agriculture in contrast to graduates from foreign schools, who are ignorant of the land, its climatic, economic and social conditions, thus needing a lot of time to orient themselves and be able to beneficially apply their knowledge in Greece. "
Considering the texts of the decisions of the Greek Governments during the period 1910-1920, the projects implemented in the period 1910-1920 by the competent Ministries and Ministers, but also the Archive of Eleutherios Venizelos it is clear that the Prime Minister sought to modernize the agricultural sector and strengthen farmers and livestock farmers thus emphasizing the importance of the primary sector in the economic development of the country.
The so-called agricultural issue had preoccupied the previous Greek governments without them finding, however, ways to resolve or even address it until 1910 when the Prime Minister relieved farmers from the fear and the threat of eviction. Until then, landowners reserved the right to evict their farmers in cases where the latter did not meet the -often strict and unbearable- obligations that had been set. This prevented the impoverishment and humiliation of the rural population. In addition, the Constitution (1911) provided for the possibility of expropriating property not only for reasons of "public necessity" but also for reasons of "public benefit". This paved the way for land expropriations and rural rehabilitation of landless farmers. The result of this decision was the improvement of living conditions of the country's farmers, the increase in agricultural production and at the same time it offered motivation for future growers.
The Prime Minister's concern for the strengthening and improvement of the agricultural sector in Greece is evident from a series of decisions. In particular, the decision to send scholarship holders to study Forestry reveals that the Ministers of Agriculture and Finance realized the importance of specialization and education in order to strengthen the country's agricultural sector. Hiring trained scientists in the Forestry Service would contribute substantially and practically to the guidance and dissemination of information to farmers. Informed farmers would in turn improve both the quality of their agricultural production and their living standards.
The institution of the veterinarians is another example that demonstrates the interest of the Government to strengthen all sectors of the agricultural economy of Greece. Livestock breeding has traditionally been a main occupation of Greek farmers and the existence of scientists able to inform, help and support them with specialized knowledge has strengthened this sector of the agricultural economy. With the Veterinarians, not only would animal diseases be effectively treated but the quality and quantity of the livestock products would be improved. The establishment of a veterinary microbiological center additionally contributed to the improvement of animal husbandry in Greece. At the same time, the institution of the agriculturist introduced by the Constitution of 1911 provided the necessary advice to Greek farmers to improve their

production. The agriculturalists would act as advisors, supporters and would provide their scientific knowledge in order to improve the quality and quantity of products produced.

Conclusions

The decisions taken and the Bills passed in the period 1910-1920 concerning the agricultural economy and life particularly promoted agricultural education. The primary sector of the country, although it employed a large percentage of the active Greek population and brought huge revenues to the state treasury, had been neglected in previous decades. It is important to point out that a result of the radical changes that occurred in the agricultural sector during this period was the active participation of the rural population in the social and economic life of the country. As the rural population increased, crops important to the national economy were strengthened such as tobacco and cotton. The Law 301 of 30 September 1914 established lower level agricultural schools with a purely practical orientation. It was therefore realized that Greece as an agricultural country should care for the agricultural education of its citizens. The establishment of agricultural schools in each prefecture of the country was envisaged for children aged 14-19 to qualify to acquire knowledge and appropriate skills regarding the cultivation of the land. Knowledge was the vehicle for the development of the agricultural sector. In addition, the Constitution of 1911 provided for the establishment of model monastic farms, the establishment of a network of technical and vocational education. However, the establishment of the first School of Agriculture is the greatest proof that the intention of the Greek Government was to establish agricultural education in the country. In the founding statute of the School it becomes clear that the purpose of its establishment is to promote systematic agricultural holdings and related industries, to establish agricultural enterprises and to approach agriculture in Greece a scientific way, which was missing until then. Research, studies and scientific training of personnel in state agriculture service departments would give a new impetus to the agricultural economy, improving the living conditions of farmers and strengthening the state budget.

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References

Vergopoulos, K. (1975). The Agricultural Issue in Greece. The social integration of agriculture. Athens: Exantas

Zakopoulou, E. (2008). The position of Stathis Damianakos on the rurality of Modern Greek society. In Agriculture, Society and Space. Athens: Plethron

Eliades, D. (2010). The basic education in the Prefecture of Attica in the period 1897-1922, (Unpublished Doctoral Thesis). University of Ioannina, Department of Kindergarten Teachers, Ioannina.

Iosifides Th. And Spyridakis M. (2006), Qualitative Social Research Methodological Approaches and Data Analysis, Kritici.

Kandila, I. (2004). The organization of the lower and middle mental nature (professional and not) in the years of the Venizelist governments: expectations and results. The Greek agricultural society and economics during the Venizelian years (424-437) Proceedings. Athens: Greek Letters.

Martinos N. (2004). Agricultural policy in the interwar period: its limits and possibilities. In The Greek agricultural society and economy during the Venizelist period (pp. 240-251): Minutes. Athens: Greek Letters.

Patronis, V. (2015). Greek economic history. Economy, Society and State in Greece (18th-20th). Hellenic Academic Libraries Link (HEAL)

Peliouras, G. (2017). Eleutherios Venizelos in the Athenian press. His first government terms (1910-1912). Athens: Papazisis publications

Petmezas, S. (2012). Proverbs in the history of the Greek agricultural economy of the interwar period. Athens: Alexandria

Tsiros, N. (2013). The legislation of Eleutherios Venizelos during the period 1911-1920 in the context of his reform policy and the socio-political data of the time. Athens: Papazisis publications

TENDENCIES AND PREDICTION OF GRAPE PRODUCTION CHARACTERISTICS IN SERBIA

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Abstract

The research in this paper deals with the main production characteristics of grape production in Serbia. Based on the quantitative analysis, the aim of the research was to determine the trends in the production characteristics of grapes, and to predict these movements for the following period. The production characteristicsof grapes(vineyard area, annual production and yield) were analysed for the period 2005-2019. The quantitative analysis was performed by using the methods of descriptive statistical analysis and the average annual rate of changes to determine the trends in the analyzed period and to predict the movements in the following period2020-2024. The average vineyard area in the analyzed period in Serbia was 22,173 ha;the annual change rate of the vineyard area was -1.59%. The average annual production of grapes in Serbia was 174,976t;the annual change rate was 0.64%. The average yield of grapes was 7.88t/ha;the annual change rate of the grape yield was 2.32%. According to the forecast results,it is expectedthat the vineyard area in 2024 in Serbia will be 18,917 ha, the annual production will be 168,971 t, and the grape yield will amount to 8.97 t/ha.

Keywords:*grape, production, prediction,vineyard area, grapeannual production, grape yield, Serbia.*

Introduction

Grapes have great nutritional, dietary and medicinal value in the human diet. A kilogram of grapes contains from 700 to 1200 calories, depending on the sugar content, which can provide the body with around 30% of the required daily intake of calories.
Grapes contain a large number of very important ingredients: sugars, organic acids, vitamins and minerals, aromatic substances, tannins, etc. The most important ingredient in grapes is sugar – glucose and fructose, which are easily absorbed by the human body. In the right proportion with sugar, acids in grapes providea pleasant and refreshing taste. In the form of salts, they have an important physiological role in the body.
Minerals found in grapesare also very important for the human body. In addition, because of a small amount of nitrogenous substances, extremely small content of chloride and absence of fatin grape juice, grapes have a special role in the treatment of some diseases. Grapes contain a higher number of vitamins, which also contribute to their value in the human diet.
Owing to the beneficial effects of grapes on the human body, consumption of fresh grapes in the world is constantly increasing. Only a small amount of grapes is consumed fresh, while they are

most commonly used as raw material for the production of wine, grape juice, raisins, grape honey and other food products. The largest amount of grapes is processed into wine (Golovic,2021).

Grape pomace, which remains after processing of grapes and extraction of must or wine, is used for the production of komovica brandy or pure alcohol, which is used in the chemical industry, for medicinal purposes, etc. After the production of brandy, pomace can be used as animal feed or for production of organic fertilizers, by composting.

In terms of labor needed for establishing and maintaining vineyards, grapevine is one of the most intensive crops.

Theresearch in this paper analyzes the trends forthe production characteristics of grapes –the vineyard area, annual production and yield of grapes in the Republic of Serbia and the Autonomous Province of Vojvodina (AP Vojvodina).

The aim of the research is to perform analysis for the previous period and determine the trends for the observed characteristics using the quantitative methods, as well as to predict the analyzed production characteristicsfor the following period. Based on the results of the implemented forecasts, it will be possible toplan appropriate measures which would lead to improved grape production. The aim of the paper is also to perform a comparative analysis of the obtained results for Serbia and Vojvodina.

The authors of this paper have recently studiedsimilar problems. Novković et al. (2011) analyzed changes in the sown areas, yield and total production of several important vegetable crops in Vojvodinafor the period 2000-2009. The study results indicated that owing to increased yields per hectare, the total production increased, despite reductions in the total sown area.

A great number of authors have published findings concerning analyses and forecasts for production of different crops (Novković et al. 2013, 2014, 2014a, 2015, 2018; Miljanović et al. 2014; Mutavdžić et al. 2013).

Material and Methods

The methods applied in this study are quantitative methods. The data used for analyzing the grape production from the previous period include data on the vineyardarea, total production and yield of grapes in the Republic of Serbia and AP Vojvodina for the period 2005-2019. The research data were acquiredfrom the official publications of the Statistical Office of the Republic of Serbia.

Analysis of the grape production was performed by using the methods of descriptive statistical analysis, which includedthe basic statistical indicators: arithmetic mean, i.e. average value of occurrence, extreme values of occurrence (min and max), coefficient of variation (Cv) and annual rate of change (r).

Given the values of a time series Y with length n, the average index of change is:

$$G = \left(\frac{Y_n}{Y_1}\right)^{\frac{1}{n-1}}$$

and the average rate of change:

$$r = (G - 1)$$

where:

- r is the average annual rate of change
- G is the average annual index of change

- Y_1 is the absolute value of the first member of the time series
- Y_n is the value of the last number of the time series
- n is the length of the series (number of years)

By applying the calculated rates of change to the production characteristics in the last year of the analyzed period (2019), prediction (extrapolation) was conductedfor the period of the following five years (2020-2024).

Results and Discussion

Tendencies and prediction of grape production characteristics in the Republic of Serbia

The results of the descriptive statistics for the grape production characteristicsare presented in **Table 1**. The parameterwith the greatest variability in the period 2005-2019 in Serbia wasthe annual production. High variability was determined also for the yield, while it was significantly lower for the vineyard area. The area showed a decreasing trend, while the yield hada more pronounced growth rate, so the annual production of grapes showed a tendency of increase.

Table 1. Basic indicators of grape production characteristicsin Serbia (2005-2019)

Production parameters	Average value	Variation interval		Coefficient of Variation (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	22,174	20,333	25,676	6.79	-1.595
Yield (t/ha)	7.88	5.8	10.6	15.78	2.324
Production (t)	174,976	122,489	240,369	30.94	0.637

Source: Authors' calculations

The results of the forecast for the area, annual production and yield of grapes in Serbia for the period 2020-2024 are presentedin **Table 2**. The vineyard area will decrease and in the last year of the observed period (2024)it will occupy less than 19 thousand hectares, which is by1,584 ha or 7.7% less than the vineyard area in 2019.

Table 2.Predictionsfor the vineyard area, annual production and yield of grapes in Serbia (2020-2024)

Year	Area (ha)	Production (t)	Yield (t/ha)
2020	20,174	164,558	8.18
2021	19,852	165,606	8.38
2022	19,536	166,661	8.57
2023	19,224	167,722	8.77
2024	18,917	168,791	8.97

Source: Authors' calculations

In the forecast period 2020-2024, the grape production will increase. In 2020, the production will reach 164.6 thousand tons, which is only 1 ton more than in the previous year (2019). In the last year of forecasting, the annual production of grapes in Serbia will be close to 169 thousand tons, which is by 5,200 tor 3.2% more than the production in 2019. The forecast for the grape yield on the territory of Serbia indicates that the yield will increase, so in 2024 it will be around 9 t/ha, which is significantly higher than the average value of 7.88 t/ha. The difference is around 1.1 t/ha or 14%.

Tendencies and prediction of grape production characteristics in AP Vojvodina

The results of the descriptive statistics forthe grape production characteristicsin AP Vojvodina (**Table 3**) showed that the average vineyard area was slightly more than 4.8 thousand hectares. The annual production of grapes in the analyzed period was around 42 thousand tons, while the yield was more than 8.5 tons per hectare. There were great variations in the annual production, caused primarily by variations in the yield, and significantly less by variations in the area. The highest yield was achieved in 2009, while the lowest yield was in 2005.

Table 3. Basic indicators of grape production characteristics in the region of Vojvodina (2005-2019)

Production parameters	Average value	Variation interval		Coefficient of Variation (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	4,838	3,267	5,693	10.79	-3.889
Yield (t/ha)	8.65	5.4	11.9	18.85	1.521
Production (t)	41,920	25,546	59,705	22.5	-2.423

Source: Authors’ calculations

The vineyard area and annual production hada decreasing tendency, while the yields hada tendency of increase.

Table 4 presents the results of predictions for the vineyard area, annual production and yield of grapes on the territory of AP Vojvodina for the period 2020-2024.

The vineyard area will decrease, so it is expectedthat in the last observed year (2024) the vineyard area in AP Vojvodina will be around 2.7 thousand hectares, which is by 588 haor 18% less than in 2019.

Table 4.Predictions for the vineyard area, annual production and yield of grapes in AP Vojvodina (2020-2024)

Year	Area (ha)	Production (t)	Yield (t/ha)
2020	3,140	26,710	8.53
2021	3,018	26,063	8.66
2022	2,900	25,431	8.79
2023	2,788	24,815	8.92
2024	2,679	24,214	9.06

Source: Authors’ calculations

In the forecast period, the annual production of grapes will also decline. According to these forecasts, the grape production in 2024 will decline to around 24.2 thousand tons, which is by 3,159 tons or 11.5% less than in 2019.

Unlike the areas and annual production, the yield of grapes will grow. By the end of 2024,the yield of grapes will be over 9 tons per hectare, which is higher by 410 kilograms per hectare or 4.7% than the average value achieved in the analyzed period 2005-2019.

Comparative analysis Serbia-Vojvodina

The vineyard area in the period 2005-2012 was constantly decreasing, both in AP Vojvodina and in Serbia.The area stagnated in the period 2012-2017, butthen continued to decline in Vojvodinain 2018 and 2019. In the Republic of Serbia, a decline in 2018 was followed by an increase in the vineyard area in 2019 (compared to the previous year) for the first time in 15 years.

During the period 2005-2017, the percentage share of the vineyard area on the territory of AP Vojvodina in the total area at the national level was constant, amounting to around 22%.

In 2018, there was a slower decline in the area in AP Vojvodina in relation to Serbia, so itsshare in the total area in Serbia slightly increased. In 2019, this percentage dropped to less than 16%.

Table 5 shows the predictions for the vineyard area in AP Vojvodina and Serbia, as well as the percentage share of the areain Vojvodina in relation to Serbia for the period 2020-2024.

The vineyard area in AP Vojvodina will decrease at 2.4 times higher annual rate than in the Republic of Serbia. The share of the vineyard areain Vojvodina in the total vineyard area in Serbia will constantly decrease and in 2024 it is expected to be around 14%, which is as much as 36% less than it was in 2005.

Table 5. Predictionsfor the percentage share of the vineyard area in AP Vojvodina inthe total vineyard area in Serbia for the period (2020-2024)

Year	**AP Vojvodina (ha)**	**Percent (%)**	**Republic of Serbia (ha)**
2020	3,140	15.56	20,174
2021	3,018	15.20	19,852
2022	2,900	14.84	19,536
2023	2,788	14.50	19,224
2024	2,679	14.16	18,917

Source: Authors' calculations

The average share of the grape production in Vojvodina in relation to Serbia was 23.96%. The highest production, both in Vojvodina and in Serbia, was achieved in 2009. The lowest production in Vojvodina and Serbia was achieved in 2014.

The largest share of the grape production in Vojvodina in the total grape production in Serbia was in 2011, while the lowest percentage share was recorded in 2019.

The production in Vojvodina will decline, while at the national level it will increase. The percentage share of the grape production in Vojvodina in the total grape production in Serbia will constantly decrease and in 2024 it is expected to amount to 14.35%.

The highest yields recorded in both Vojvodina and at the national level were achieved in 2009 (11.9 t/ha in Vojvodina, 10.6 t/ha in Serbia). In the period 2005-2019, the yield in Vojvodina was two times below the national average (in 2014 and 2015). In addition, 2014 was also the year in which the lowest yields were recorded (Vojvodina 5.4 t/ha, Serbia 5.8 t/ha).

By analyzing the predictionsforthe grape yield for the following five years in Vojvodina and Serbia, it can be concluded that the yield will increase over the years, both in Vojvodina and in Serbia. If this trend continues, it can be expected that in 2025 the yield in Vojvodina will be almost identical to the yield in Serbia, amounting to approximately 9.2 t/ha.

Conclusions

Based on the presented research results, the following conclusions can be reached:
- In the Republic of Serbia, there is a trend of increasing the grape yield and grape production, while the vineyard area shows a decreasing trend. The average area was 22,173 ha, the average yield was 7.88 t/ha, and the average production was 174,976 t in the period 2005-2019. In 2024, the expected vineyard area in the Republic of Serbia will be around 18,900 ha, the production will be approximately 168,800 t, and the yield will be 8.97 t/ha.
- On the territory of AP Vojvodina,there is a trend of decreasing the vineyard area and grape production, but also atrend of increasing the grape yield. The average vineyardarea was 4,838 ha, the average yield was 8.65 t/ha, and the average production was 41,920 t. In 2024, the area is expected to be around 2,700 ha, the yield will be around 9.06 t/ha, and the production will be around 24,200 t.
- The vineyards area in Vojvodina will decrease at a faster rate than in Serbia. The yield shows an increasing trend both in Vojvodina and in Serbia, but a largerincrease is expected at the national level. Unlike the yield and area, which have the same trends in Vojvodina and Serbia, the grapeproduction in AP Vojvodinais expected to fall, while at the national level the grape production shows an increasing trend.

References

Golovic, M. (2021): Analysis and Prediction Grape Production in Serbia, Faculty og Agriculture, University of Novi Sad, Serbia

Miljanovic G., Mutavdzic B., Novkovic, N., Janosevic, M. (2014): Prediction of Tomato Production Characteristics in Serbia, Book of proceedingsFifth International Scientific Agricultural Symposium "Agrosym 2014", Jahorina, October 23 – 26.

Mutavdzic B., Drinic, Lj., Novkovic, N., Ostojic, A., Rokvic, G. (2013): Prediction of Vegetable Production in Republic of Srpska, FourthInternational Scientific Symposium "Agrosym 2013" – Book of Proceedings, University of East Sarajevo, Jahorina, pp. 1276-1282.

Novković N., Mutavdžić B., Drinić L., Sedlak O., Mihajlović Š. (2018): Potato Production Characteristics – Comparative Analysis: Serbia, Macedonia and Entity of Republic of Srpska (Bosnia and Herzegovina), Book of proceedings, IX International Scientific Agricultural Symposium, University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, Bosnia,University of Belgrade, Faculty of Agriculture, Serbia, Jahorina, 4-7 October, pp. 1962-1967.

Novkovic, N., Mutavdžić,B., Ilin, Ž., Lazić, D. (2014): Red Pepper Production Characteristics Analysis in European Union Countries, Book of Abstracts, III International Symposium and XIX Scientific Conference of Agronomists of Republic of Srpska, pp. 87-88.

Novković, N., Mutavdžić B., Ilin, Ž.,Ivanišević, D. (2013): Potato Production Forecasting, Agroznanje, 14(3): 345-355

Novkovic, N., Mutavdzic, B., Janosevic, M., Miljanovic, G. (2014a): Potato ProductionCharacteristics – Comparative Analysis in European Countries and Serbia, Book of proceedings Fifth InternationalScientific Agricultural Symposium "Agrosym 2014", Jahorina, October 23 – 26;pp. 1062-1067.

Novković, N., Mutavdžić, B., Krasnić, T. (2015): Red Pepper Production Characteristics – Comparative Analysisin EU Countries and Serbia, Book of Proceedings, 6th International Scientific Agricultural Symposium, AGROSYM 2015, 15-18 October 2015,pp. 1955-1960.
Novković, N., Mutavdžić, B., Vukelić, N. (2011): Vegetable Production Tendencies in Vojvodina, Proceedings of 22nd International Symposium Food Safety Production, Faculty of Agriculture, Novi Sad, Trebinje 19-25 June, pp.163-165.

ANALYSIS OF THE PARTICIPATION OF INCENTIVES IN LIVESTOCK IN RELATION TO THE AGRICULTURAL BUDGET OF THE REPUBLIC OF SERBIA

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Abstract

One of the most difficult chapters in the EU accession negotiations, based on the experience of other members, is the chapter on agriculture and rural development. The agrarian budget is a joint and consolidated form of state support for agriculture, which is implemented through subsidies for agricultural production. The subject of research in this paper is the analysis of agricultural policy and formed incentive measures in various areas of agricultural production in Serbia. Also, the budget of the Republic of Serbia, the agrarian budget of the Republic of Serbia and the planned and implemented incentive measures in agriculture for the period 2016-2020 will be compared. Based on the budget of the Republic of Serbia for 2017, the funds distributed to the Ministry of Agriculture amounted to 43.8 billion rsd, which is 3.3 billion rsd more than in 2016. On the other hand, the share of the budget of the Ministry of Agriculture in the total budget of the Republic of Serbia shows a decreasing trend and in 2017 it amounted to about 4.8%. Budget funds for agriculture in 2018 amounted to 44.1 billion rsd, which is approximately the level of the previous year (0.7% more), but with the trend of decreasing the share of the budget of the Ministry of Agriculture in the total budget of the Republic of Serbia, which reaches a value of 4.46% in 2018. The financial resources determined by the agrarian budget in 2019 were lower for 0.3%, while the difference in 2020 was 3.13%, with the announced possibility of rebalance and increase of funds as in 2019. Solving the problem of financing has a direct impact on the development of agriculture, observed from the aspects of primary agricultural production, agriculture in a broader sense, multifunctional agriculture, as well as rural development.

Key words: *agrarian budget, incentives, agriculture, animal husbandry, development*

Introduction

A very important economic activity that has economic, social and political significance is agriculture. In market developed economies, agriculture is "privileged", ie it has financial support, whether viewed in a narrower sense, as primary agricultural production or more broadly as multifunctional agriculture. Examples of this are the measures of the Common Agricultural Policy (CAP) of the European Union, which are mostly based on incentive (non-refundable) funds, as well as the way of financing agriculture in the United States based on favorable farm loans.

The Common Agricultural Policy (CAP) is one of the oldest policies of the European Union. Agrarian policy developed in parallel with the development of the Union during long-term processes, continuous changes, integration of a large number of entities that took place within it. The common agricultural policy has influenced: increasing agricultural production and productivity, changing the production structure, stabilizing the internal market, increasing exports, independence from imports, protecting and increasing producers' incomes, security of supply.

All of the above has led to the fact that the European Union is today at the very top of the list of the largest importers and exporters of agricultural products in the world. The Common Agricultural Policy has always played an important role in the EU budget. Large funds were allocated for financing agriculture and rural development of the EU (about 43% of the EU budget). The CAP is also the largest expenditure in the EU budget.

The agricultural sector in the Republic of Serbia in the past decades, after all the negative challenges it has faced in the past, has experienced a serious economic downturn and economic crisis. Serbian agriculture has significant comparative advantages due to its strategic position, favorable geographical terrain, favorable climatic conditions, good education of the population, etc. The Republic has large areas of arable land that is of high quality (80% belongs to the good and 20% to a high supply of humus). By implementing the EU agricultural policy and applying its laws in Serbian agriculture, a safe business environment would be created, and the highest level of efficiency and competitiveness would be achieved. There would be an increase in the process of food production and processing, and thus, there would be a recovery and development of the entire economy.

One of the most difficult chapters in the EU accession negotiations, based on the experience of other members so far, is the chapter on agriculture and rural development. The EU's agricultural policy is constantly evolving, quite complicated and subject to constant change. It takes a lot of time and effort, good coordination around policy harmonization. Therefore, the chapter on agriculture should be focused on priorities, ie work on the implementation of all regulations related to the accession of Serbian agricultural policy to the common agricultural policy of the EU.

The Ministry of Agriculture, Forestry and Water Management (MAFWM) has the largest role in the integration and association of agriculture in Serbia and the EU. However, due to the seriousness and scope of work, it is necessary to involve other bodies and institutions in the cooperation.

The agrarian budget as a source of financing for agriculture was established by the Decision on the formation of the agrarian budget, which was adopted at the end of 1995, and practically became an integral part of the state budget in 1996. "The agrarian budget is a joint and consolidated form of state support for agriculture, which is implemented through subsidies for agricultural production" (Miličić et al., 2018).

The Republic Government, at the proposal of the Ministry of Agriculture, Forestry and Water Management and in accordance with the Law on the Budget of the Republic of Serbia, determines the amount of the agrarian budget every year. By analyzing the quantitative data on investments in the agricultural budget in the period from 1996 to 2015, it can be concluded that in the observed period, the average share of agriculture in the total state budget was only 4.8%. The Law on Incentives in Agriculture and Rural Development stipulates that the agrarian budget cannot be less than 5% of the budget of the Republic of Serbia starting from 2014 (MAFWM, 2017).

The term "incentives" in the Law on Incentives in Agriculture and Rural Development is defined as funds provided in the budget of the Republic of Serbia, as well as funds provided from other sources allocated to farms and other persons in accordance with this law in order to achieve agricultural policy goals and rural development policy Živadinović, Milovanović, 2011).

The intention of this law was to directly affect all agricultural producers, who will be motivated to invest in agricultural production by establishing legally regulated standards for transparent use of budget funds, and thus improve production in terms of quantity and quality. In order to plan the production cycle, it is necessary for agricultural producers to be informed in advance about the possibility of subsidizing their production by the state. In this way, the planned volume of production in the next production cycle is significantly influenced, and thus the final offer of final products on the market after the completed production cycle (Pejanović et al., 2011).

This law has achieved a higher level of harmonization with the regulations of the European Union, given that the Republic of Serbia is in the process of joining the European Union and that in accordance with the obligations arising from that process, is working to harmonize its legislation with EU legislation. A higher degree of harmonization of the agrarian policy of the Republic of Serbia with the Common Agricultural Policy of the European Union is being achieved. This law defines the obligation of users to comply with regulations governing environmental quality standards, public health protection, animal and plant health protection, animal welfare protection and protection of agricultural land, which are also requirements that must be met by a farmer in the European Union. would exercise the right to use incentives (MAFWM, 2018).

The agrarian budget of the Republic of Serbia is conceived in accordance with the overall real financial possibilities of the country, and with the assumed macroeconomic parameters for the same year. In difficult economic conditions, where the funds of the agrarian budget are not enough to meet the real needs of domestic agriculture, the Ministry of Agriculture is forced to redefine the priorities of its activities, and at the same time provide basic conditions for achieving strategic goals in the livestock sector. The funds in the limit are distributed by first planning funds for the IPARD program, then for incentives for rural development measures, special incentives and, finally, the remaining funds are directed to direct payments. Of the total funds planned for direct payments, funds for incentives for plant production are planned first, because they can be precisely planned (Pejanović et al., 2013). Then, funds are planned for outstanding obligations from the previous period, and the remaining funds are directed to incentives in animal husbandry. The Law on Agriculture and Rural Development established the Directorate for Agrarian Payments and Article 8 defines its activities. The Law on Ministries defines the competence of the Ministry of Agriculture in creating agricultural policy, and as a direct user of the budget of the Republic of Serbia, it is responsible for the process of planning, providing, implementing and monitoring the effects of realized funds for livestock incentives (Popović, 2014). The budget planning process starts from the Proposal of priority areas of financing, information on the realization of funds for incentives in livestock in the current year and projections of funds for requests submitted in the current year, which will be resolved in the next year. The input parameters in the process of planning the necessary funds for incentives in animal husbandry are: realization from the previous period; the situation in the processing of received requests, the projection of the number of requests that will be submitted by the end of the current year and the necessary funds for them, and the projection of obligations under the requests from the current year that will be transferred to the next year; the total potential of requests that can be processed in one year (Stojanović et al., 2017).

The aim of this paper is to apply modern knowledge and economic measures from the new agricultural and rural economy in order to create a basis for long-term planning of agricultural production by direct beneficiaries of incentives and define new types of incentives, incentive beneficiaries, minimum levels of incentives and conditions of their use.

Material and methods

The subject of research in this paper is the analysis of agricultural policy and created incentive measures in various areas of agricultural production in Serbia. Also, the budget of the Republic of Serbia, the agrarian budget of the Republic of Serbia and the planned and implemented incentive measures in agriculture for the period 2016-2020 will be considered comparatively.

Results and discussion

Funds intended for incentives in agriculture and rural development are determined by the Law on the Budget of the Republic of Serbia for the current year, and are distributed through the Decree on the distribution of incentives in agriculture and rural development for the current year. The financial basis for the implementation of incentives in agriculture and rural development is the Decree on the distribution of incentives in agriculture and rural development for the current year, which defines the amount of funds, types of incentives and maximum amounts per type of incentives for the current year. Detailed conditions and criteria for exercising the right to incentives in agriculture and rural development for certain types of measures are prescribed by the regulations and the Law on Incentives in Agriculture and Rural Development.

Table 1. Share of the agrarian budget in the budget of the Republic of Serbia in the period 2016-2020

Year	Total RS budget (in 1.000.000 dinars)	RS agrarian budget (in 1.000.000 of dinars)	The share of the agricultural budget in the total RS budget (%)
2016	1,659.678	40,466	4,89
2017	1,951.754	43,788	4,80
2018	1,854.185	50.955	4,46
2019	1.246.200	51.700	4,14
2020	1.314.500	40.500	3,08

Source: Work newsletter, Documentation of the Ministry of Agriculture of the Republic of Serbia. Author's calculation

For each budget year, the Government prescribes the amount of funds, types and maximum amounts by type of incentive, in accordance with this law and the law governing the budget of the Republic of Serbia, within 30 days of the law governing the budget of the Republic of Serbia. The amount of funds is determined within the budget of the Ministry of Agriculture, and the Law prescribes that the budget of the Ministry of Agriculture cannot be less than 5% of the budget of the Republic of Serbia for a given year in terms of the law governing the budget system.
The maximum amount by type of incentive depends on: available funds determined by the law governing the budget of the Republic of Serbia, the volume of these funds by type of incentive, the number of beneficiaries and the unit of measure depending on the type of incentive (Karajović et al., 2020). Based on the Law on Budget for 2016, the funds distributed to the Ministry of Agriculture amounted to 40.5 billion dinars, which represented 4.9% of the budget of the Republic of Serbia for 2016. Based on the Law on the Budget of the Republic of Serbia for 2017, the funds distributed to the Ministry of Agriculture amounted to 43.8 billion dinars, which is 3.3 billion dinars more than in 2016. On the other hand, the share of the budget of the Ministry

of Agriculture in the total budget of the Republic of Serbia shows a decreasing trend and in 2017 it amounted to about 4.8%.

Budget funds for agriculture in 2018 amounted to 44.1 billion dinars, which is approximately the level of the previous year (0.7% more), but the trend of decreasing the share of the budget of the Ministry of Agriculture in the national budget, which in 2018 reaches the value of 4.46%.

Budget funds for agriculture in 2019 amounted to 51.7 billion dinars, which is approximately the level of the previous year (0.3% less), but the trend of decreasing the share of the budget of the Ministry of Agriculture in the national budget, which in 2019 reaches the value of 4.14%.

Budget funds for agriculture in 2020 amounted to 40.5 billion dinars, which is approximately the level of the previous year (1.06% less), but the trend of decreasing the share of the budget of the Ministry of Agriculture in the national budget, which in 2020 reaches the value of 3.08%.

Of the total funds allocated by the budget of the Republic of Serbia for the Ministry of Agriculture in 2017, 28.6 billion dinars are planned for incentives in agriculture and rural development, while in 2018, 30.4 billion dinars are planned. The right to incentives have: agricultural holdings and family agricultural holdings that are registered in the Register of Agricultural Holdings, local self-government units, and other persons and organizations (Pešić, 2020). In order to initiate the procedure for exercising the right to incentives, it is necessary for the beneficiary of the incentive to submit a request to the Directorate for Agrarian Payments. Also, the procedure can be initiated through a competition, a request to the bank for approval of credit support, etc. The decision on exercising the right to incentives is made by the director of the Directorate for Agrarian Payments by a decision. The beneficiary of the incentive is obliged to: adhere to the regulations governing environmental quality standards, protection of public health, protection of animal and plant health, protection of animal welfare and protection of agricultural land; acts in the manner and under the conditions provided by a special regulation which regulates certain types of incentives in more detail; provides accurate data and documentation for exercising the right to incentives; purposefully uses incentive funds as well as to purposefully use, not alienate and not enable another person to use the object of incentives, within the period determined in accordance with a special regulation; keeps documentation related to the exercise of the right to incentives for at least five years from the day of their collection; return the paid funds if the obligation from the law is not complied with; return unjustifiably paid funds due to an administrative error; return excess funds in accordance with the law. The National Strategy for Sustainable Development defines development as a long-term and goal-oriented, comprehensive and synergistic process that affects all aspects of life - economic, social, environmental and institutional. Sustainable development is oriented towards the formation of a model that does not have a quality way to meet the socio - economic needs and interests of citizens, while reducing the impact that poses a threat to the environment and natural resources. For these reasons, the Budget of the Republic of Serbia for 2020 will undergo certain changes. These changes will be reflected through an appropriate rebalance of funds that will be directed to agricultural producers, sustainable development and improvement of agricultural production.

The expected amount allocated for agricultural production, after the budget rebalance, will be increased by approximately 1.13%, which is a steady amount that is determined for agricultural producers in the territory of the Republic of Serbia.

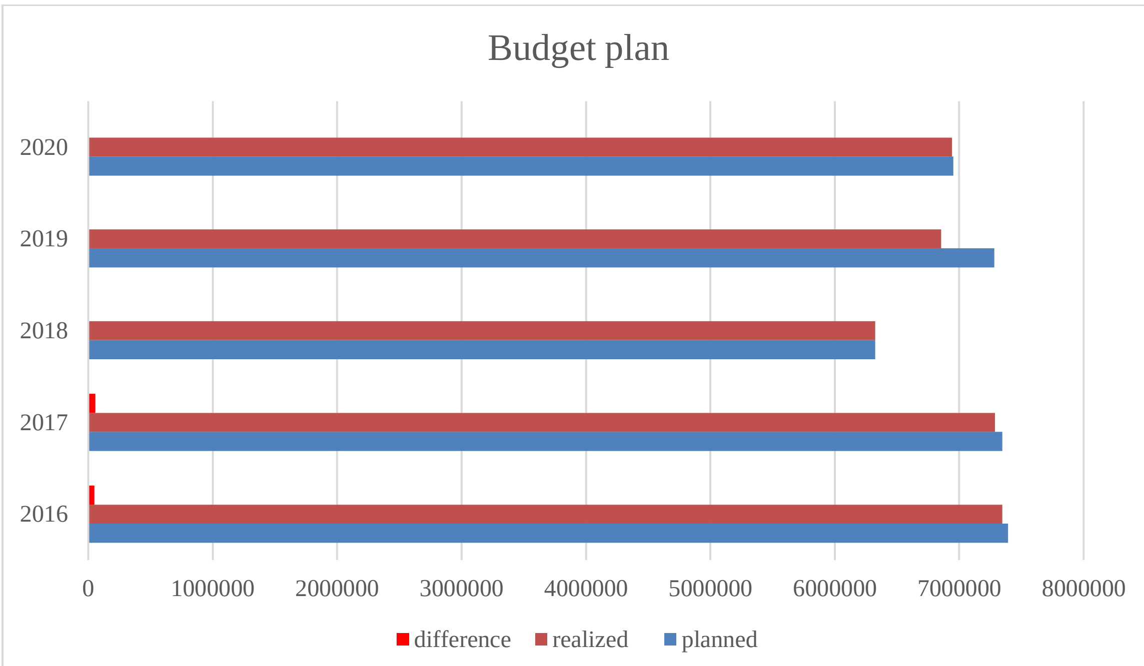
Table 2. Overview of planned and realized funds for incentives in animal husbandry for the period 2016-2020. years (in thousands of dinars)

	2016.		2017.		2018.		2019		2020	
	Planned	**Imple mented**	**Planned**	**Imple mented**	**Planned**	**Imple mented**	**Planned**	**Imple mended**	**Planned**	**Imple mended**
Breeding dairy cows	4.471.000	4.465.069	4.456.000	4.430.751	3.460.332	3.460.196	3.501.870	3.501.870	2.566.742	2.566.542
Fat pig	835.024	835.024	726.566	726.461	600.353	600.353	663.105	663.105	676.188	676.187
Fat cattle	591.799	591.788	843.219	843.077	559.019	558.959	908.299	908.299	1.234.382	1.234.382
Breeding sheep and goats	672.582	663.364	338.000	333.740	635.814	635.814	499.377	371.309	523.947	523.206
Beehives	420.000	399.211	550.000	548.415	500.000	499.985	640.000	520.938	600.000	599.988
Breeding sows and boars	215.000	214.410	176.500	172.960	236.850	236.440	286.752	280.752	112.047	112.035
Fattening lambs	134.915	134.915	154.619	154.619	135.295	135.295	293.190	181.280	189.129	189.129
Breeding cows and calves for fattening	0	0	69.744	52.365	150.000	149.860	447.708	389.640	530.000	530.000
Parent chickens heavy type	21.975	19.601	11.000	8.938	18.339	18.338	14.000	11.657	492.800	485.400
Suckler cows	20.000	12.380	100	0	12.600	12.420	20.000	18.426	20.000	19.978
Breeding fattening cows	6.025	6.000	10.000	9.850	13.300	13.300	7.000	7.000	5.040	5.040
Parent chickens easy type	3.200	2.945	7.000	5.759	3.570	3.569	1.319	711	0	0
Parent turkey	800	0	500	0	1	0	1	0	25	25
Fat goat	262	262	596	596	332	332	406	382	800	800
Consumable fish	1.000	0	100	0	1	0	1	0	1	0
Trout farming fish	100	0	500	0	1	0	1	0	0	0
Breeding queens of carp fish	100	0	500	0	1	0	1	0	0	0
In total	***7.393.782***	***7.344.969***	***7.344.944***	***7.287.531***	***6.325.808***	***6.324.860***	***7.283.030***	***6.855.369***	***6.953.101***	***6.942.712***

Source: Work newsletter, 2016-2020 year, Ministry of Agriculture (http://uap.gov.rs/wp-content/uploads/2021/03/informator-februar-2021.-god.pdf

In the period 2016-2020, the level of planned funds for incentives in animal husbandry is declining. Based on the data of the Ministry of Agriculture, in the observed three years in 2017, there was a slight decrease in the level of funds compared to 2016, but in 2020 that amount is one billion dinars less than in 2017. In the following years, in 2019 and 2020, the funds for financing livestock production were increased, regardless of the structure of planned and realized funds.

A graphical representation of these assets is shown in Graph 1. Livestock production in the Republic of Serbia is facing numerous problems that result in a decrease in the number of head and a decrease in livestock production.



Graph 1. Incentives in livestock 2016-2020
(Izvor: http://www.minpolj.gov.rs/dokumenti/2021)

Conclusion

In Serbia even today, agriculture provides about 12 percent of the gross value added of agriculture. Natural potentials are insufficiently used and there is still accelerated deagrarization, a high level of senilization of the active agricultural population and a low level of motivation of employees due to low average wages.

In the past period, Serbia has started structural reforms of agricultural policy by implementing various strategies and programs and has made significant steps. Harmonization of the standards of the common agricultural policy and the agrarian policy of Serbia is not only a condition for membership in the EC. Its application would increase production, improve product quality, market competitiveness, increase imports and exports, modernize equipment and introduce new technologies, etc.

Solving the problem of financing has a direct impact on the development of agriculture, observed from the aspects of: primary agricultural production, agriculture in a broader sense, multifunctional agriculture, as well as rural development.
In the current period in the Republic of Serbia, financing the development of all aspects of agriculture, as well as financing rural development, is necessary to be realized with the dominant support of the state. The need for a dominant role of the state is also caused by the underdevelopment of financial markets and financial institutions that would enable greater activation of market mechanisms in the system of financing agriculture. The financial support of the state should be manifested in a larger allocation from the state budget for the agrarian budget, and it is necessary to redefine its structure in order to increase the participation of incentives aimed at rural development.
The agricultural sector is large and very demanding, it brings with it a lot of laws and regulations, the implementation of which takes a lot of time and a large organization. Participation in the reform and harmonization of the standards of the common agricultural policy is not only in the power of the muscles of the Government and the Ministry, but of all those involved in agricultural and rural development and production.

Referencess

Karajović, M., Kaličanin, M., Kaličanin, Z., Budgeting and Sustainable Development, Ecologica, 27 (100), pp. 707-714, Belgrade. 2020

Ministry of Agriculture and Environmental Protection-MAFWM (2017). IPARD program for the Republic of Serbia, for the period from 2014-2020. year, 2020

Milinčić, M., Tucović, M., Mandić, B., Some aspects of the impact of agriculture on the environment. In: Proceedings - Faculty of Geography, University of Belgrade. 2018.

National program for agriculture for the period from 2018-2020, http://www.minpolj.gov.rs/download/Uredbe/Zakljucak-i-Nacionalniprogram-za-pol poljoprivredu-za-period-2018-2020.pdf

Pejanović, R., Njegovan, Z. (2011): Principles of Economy and Agrarian Policy, Faculty of Agriculture, Novi Sad. 2011.

Pejanović, R., Njegovan, Z., Maksimović, G., Ekonomika poljoprivrede, agrarna politika i ruralni razvoj, Monografija, Drustvo agrarnih ekonomista Balkana, Beograd. 2013

Pešić, B., Analysis of organic livestock and crop production in Serbia, Ecologica, 27 (98), pp. 195-203, Belgrade. 2020

Popović, R., Stockbreeding in the Republic of Serbia. Census of Agriculture 2012. Republic Statistical Office. White City. 2014

Stojanović, I., Manić, A., Nerić, N., Agrarian policy and rural development of the European Union and Serbia. In: Megabusiness. 2017

Živadinović, B., Milovanović, M., Guide through EU policies - Agriculture, Belgrade, European Movement in Serbia. 2011

Work newsletter, Ministry of Agriculture, Belgrade, 2021.
http://uap.gov.rs/wp-content/uploads/2021/03/informator-februar-2021.-god.pdf

ASSESSING THE IMPACTS OF COVID-19 ON AGRICULTURE FROM THE PERSPECTIVE OF AGRICULTURAL ORGANIZATIONS: A CASE STUDY FROM GÜMÜŞHANE, TURKEY

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Abstract

The main purpose of this study is to determine the impact of the COVID-19 outbreak on agricultural production from the perspective of agricultural organizations in Gümüşhane province in Turkey. Agricultural organizations in the province include provincial and district agriculture directorates, agricultural credit cooperatives and the chamber of agriculture. The survey was conducted in February and March of 2021. Simple descriptive statistics were used in the analysis of the data. Agricultural change in the research area compared to the period before the COVID-19 outbreak was shown with proportional distributions. Findings show that the COVID-19 outbreak has not had a negative impact on agriculture in many ways in the study area. However, the increase in agricultural input prices during the pandemic period is the most unfavourable situation encountered. As expected during the pandemic period, the demand for crop and animal products increased. However, the supply of agricultural products did not increase at the same level as expected. Another finding from the study is that the COVID-19 outbreak has not reduced the sown area in the region. The reason why producers do not reduce their production areas despite the increase in production costs can be attributed to the increase in agricultural subsidies provided by the government during the pandemic. However, it is important for governments to work on structural reforms in order to overcome the shocks that occur in COVID-19 and similar epidemics.

Keywords: *Agricultural organizations, COVID-19, impact, production, Turkey.*

Introduction

There is no beneficial truth that besides its impact on human health, COVID-19 and the lockdown that was done beginning in March 2020 in an effort to hold its spread have had a major economic impact that has affected whole sectors including the agricultural sector and agricultural market in the world. The Anatolian geography meets the important needs of Turkey with its arable land. The agricultural population in Turkey is quite high and it plays an important role in feeding, rural development and providing raw materials to the industrial sector. The agricultural sector covers 1/4 of the national income. Like many other developing countries, this sector in Turkey is the single largest source of livelihoods. Varshney et al. (2020) stated that the agricultural sector in India accounts for 60% of all rural employment.
According to the Turkish Statistical Institute (TurkStat) data; growth in the agricultural sector was 4.8% in Turkey in 2020. But, as agriculture grew, per capita income fell to 8 thousand 599 dollars, the lowest level in the last 11 years (TurkStat, 2020). During the pandemic; farmers, agricultural workers, food producers, just like healthcare workers have been at work 24 hours a

day. Even if there is a curfew, agricultural production activities continued to be carried in the fields, vineyards, gardens and barns. Yavuz (2020) emphasized that with the COVID-19 outbreak, the strategic importance of the agricultural sector increased and created social awareness in the country. On the other hand, Kayabaşı (2020) pointed out that although there were gradual transitions, those working in both agriculture and industry would be under the risk due to the COVID-19. The impact of the pandemic can manifest itself in the form of agricultural production and yield decrease as well as supply shocks. During the pandemic, stabilizing agricultural production and ensuring farmers' incomes became important tasks for governments (Hai-ying and Chang-wei, 2020). To minimize the impact of the outbreak on agricultural production, Turkey has introduced incentives to encourage farmers to produce.

Some issues need to be addressed regarding the impacts of the outbreak on agriculture: one is to ensure the production and supply and the other is to stabilize farmers' incomes. At this point, it is very vital to analyse its sustainable impacts before and after the pandemic in Turkey, because the Turkish Republic Ministry of Health is still reporting a continuous rise in the number of cases, with the outbreak now spreading to virtually in the country. Although there is an increase in agricultural production according to the data of 2020, the full impact of the outbreak on agriculture is not known in future production periods. The impacts of the outbreak on agriculture is a big risk especially in rural areas in Turkey. For this reason, it is clear that much bigger improvements with the right agricultural policies will cause new developments during the pandemic. No doubt, while COVID-19 is influencing agriculture such as crop harvest, plantation, transportation, marketing of agricultural products, labour mobility, exporting and the food system in the world (Hai-ying and Chand-wei, 2020; Varshney and Meenakshi, 2020; Jambor et al., 2020; Pan et al., 2020; Kayabaşı,2020; Doğan and Doğan, 2020), in Turkey, reaching internal and external input and output markets, lack of temporary labour, changing marketing channels have been the main problems (Ceylan and Özkan, 2020). The main agricultural product in crop production is grain and wheat is the main stable grain. The lockdown and social distancing did not create a significant problem for wheat production because, it was already planted before the outbreak and it can also be harvested by machinery (Ceylan and Özkan, 2020). Nevertheless, the case of the production in fruit and vegetables is different due to the seasonal/migrant labourers. So, urgent measures have affected production of fruit and vegetable. In some periods, there has been permanent lockdown in Turkey and this affected seriously district bazaars. Thus, family farmers have been affected since they sell their products regularly in these bazaars.

As mentioned in the literature above the impacts of this virus in the world and on Turkey's agricultural economy must be taken seriously now and in the near future. The studies relating to the impact of the pandemic on Turkey's agricultural economy are not in detail. These studies give macro data about agricultural products in Turkey and the world. Qualitative analysis or surveys are not used to understand the real problem in the field. Then, the results are similar in the current literature. Moreover, the results do not reflect the situation at a certain point, which can trace the vital impacts of the virus on Turkey's agricultural economy. This study goes beyond these constraints in the literature with the oriented research questions relating to the perspective of agricultural engineers on the impact of COVID-19. We explore COVID-19's impacts on Gümüşhane province which is in the north of Turkey. By providing impacts of COVID-19 on breeding and crop production, makes an appreciable contribution to the public authorities, NGOs, etc. Gümüşhane is a province where most of the sectors cannot exist due to its geographical conditions so livestock has been a source of livelihood for most households for many years. As can be understood from the preliminary study conducted on the province, although the time-honoured tradition of animal production offers potential, it does not currently reach the desired level due to recent problems experienced in the sector. Thus we search the impacts of COVID-19 in light of the ongoing and widespread of the virus in the province. In our paper on the COVID-19 impacts, we have a specific focus on the agricultural production in Gümüşhane through the eyes of the agricultural organizations.

Materials and Methods

Primary data in this study were obtained from Gümüşhane, face-to-face interviews with the agricultural engineers were carried out to collect qualitative and quantitative primary data. Agricultural organizations in the province of Gümüşhane include provincial and district organizations affiliated with the Ministry of Agriculture and Forestry, Agricultural Credit Cooperatives and the Chamber of Agriculture. During the survey, fifteen agricultural engineers were interviewed. The agricultural engineers interviewed consist of the managers of the organizations they work with or the experienced people in these organizations who have information about the agricultural structure of the region. Surveys were conducted in February and March 2021. To address the impacts on the Gümüşhane agriculture, we developed a survey that consists of two parts. In the first part of the survey, we have used the questions which reflect the impact of the pandemic using three criteria ("Increased", "Decreased", and "Unchanged") on the production in the province.

Input and product prices, production cost, demand and supply, amount of production, sown area, number of animals, access to agricultural information and credit opportunities, migration movements, agricultural supports and farmer income are some of these indicators. In the second part, the perceptions of the fifteen agricultural engineers working in the agricultural organisations in Gümüşhane regarding the impact of the COVID-19 outbreak on agriculture were presented. We developed a survey using a 5-point scale, from 1= "strongly disagree" to 5= "strongly agree" and its design followed standard implementation in the field.

Simple descriptive statistics were used in the analysis of the data. Agricultural change in Gümüşhane compared to the period before the COVID-19 outbreak was shown with proportional distributions. In the introductory instructions to the survey, the experts were informed that participation was voluntary, that the information they shared would not be linked to them and that the data would be reported in aggregate form only. These instructions were followed by the first part, which asked participants to what extent they agree regarding the impact of the pandemic. Overall, the content of the survey was designed to capture perceptions regarding their agronomic, breeding, horticulture practices. As it is, we used the spread of the virus (using data on caseloads in each district), as distinct from the lockdown itself, to provide the impacts. So, the agricultural experts' perceptions, concerns and expectations from COVID-19 were based on a merger of access to data, media and other information in Turkish society, as well as their own experiences because of the outbreak.

Results and Discussion

Change in agricultural indicators compared to the period before COVID-19

In this section, the aspect of agricultural change in Gümüşhane compared to the period before the COVID-19 outbreak was analysed with some indicators. In this context, the aspects of the change in main agricultural indicators were presented such as input and product prices, production cost, demand and supply, amount of production, sown area, number of animals, access to agricultural information and credit opportunities, migration movements, agricultural supports and farmer income. The opinions of the agricultural engineers working in the agricultural organisations in the region about the aspect of the change in the agricultural indicators are shown in Table 1. As the table reflects, a significant number of agricultural engineers participating in the survey stated that agricultural input prices had increased compared to the period before the COVID-19 outbreak. All of the participants (100%) stated that fertilizer prices had increased. While the rate of those who stated that the prices of seeds and pesticides increased was 93.3%, this rate was 86.3% for fuel and 93.3% of the participants stated that the prices for feed, which is an important input for animal production, had also increased. At the same time, 86.7% of the participants stated that the cost of energy (electricity) used in irrigation increased. From this standpoint, we can say that in general, the increase in agricultural input prices compared to the period before the COVID-19 outbreak can be considered as a negative change. This has led to an increase in production costs. As a matter of fact, all of the participants said that the cost of both crop and livestock production had increased. As it was found by Pan et al. (2020), COVID-19 had the most significant impact on the livestock after the crop production and they also indicated in their study that the shortage of crop production materials had become one of the main obstacles in agricultural production during the outbreak of this pandemic. It was mentioned in a similar study by Middendorf et al. (2021) that in three farming systems (cropping, livestock, and horticulture) significant majorities expressed concerns, especially related to input prices.

The rate of participants who stated that the demand for crop and animal products increased compared to the period before the COVID-19 outbreak was 60%. The remaining participants reported that the demand had not changed much. It was reported in the study published by Lioutas and Charatsari (2021) that the pandemic experience taught us that the restrictions posed by governments during major crises, in combination with the changing consumers' behaviour, can cause market fluctuations with considerable effects on agri-food production. The situation is slightly different in terms of supply. Varshney et al. (2020) searched the impact of the pandemic by type of commodity and their results point to considerable resilience in agricultural markets in dealing with COVID-19 shock. One case study (Bereir, 2020) found that the restrictions of COVID-19 were hindering the Sudanese farmers especially small-scale farmers in their supply of agricultural products. In our study, the rate of the participants stating that the supply of crop and animal products had increased was 46.7% and 40%, respectively. On the other hand, more than one-third of the participants stated that the supply had fallen. The rise in demand more than supply is a reason for the increase in prices of agricultural products compared to the period before the COVID-19 pandemic. As a matter of fact, 80% of the participants stated that the price of the crop products had increased, and this rate was 85.8% for the animal products.

In considering the access to credit it was seen that there was no negative development. In fact, nearly 50% of the participants stated that access to credit opportunities had increased. More than 50% of the participants stated that there was not much change in terms of access to agricultural information for the farmers compared to the pre-COVID-19 outbreak period.

It can be said that there is a positive development in terms of the sown area. Because 53.3% of the participants stated that the sown area had increased. This can be attributed to the increased agricultural subsidies provided by the government due to COVID-19 restrictions. So that, 73.3% of the participants stated that agricultural supports had increased compared to the period before the COVID-19 outbreak. However, in the study by Kumar et al. (2020), it was emphasized that farmers of high-value commodities had suffered higher losses due to the pandemic in India, so they suggested that supporting SMEs like credit opportunities would help the farmers to survive.

Regarding the amount of the crop production in the study area, 60% of the participants reported that the amount of crop production had increased compared to the period before the COVID-19 pandemic. It can be said that the increase in crop products is mainly due to field crops. As a matter of fact, more than 50% of the participants claimed that there was not much change in the amount of fruit and vegetable production. A different conclusion was found in a study by Hai-ying and Chang-wei (2020) that stated the pandemic had an impact on all stages of the vegetable supply chain in Shanghai. In our study, according to the opinions of the participants, we can say that the rate of increase in the amount of animal production is less than crop production. While 42.9% of the interviewed participants found that the amount of animal production had increased compared to the period before the COVID-19 epidemic, 35.7% of them said that it did not change. The opinion of the participants for milk, red and poultry meat, eggs and honey production is similar to the general average. On the other hand, according to Pu and Zhong (2020) in China, the marketing quantity of agricultural products dropped dramatically during the pandemic.

In terms of productivity, there is no negative development for the crop and animal production in the study area. While 60% of the participants indicated that the productivity in the crop production was increasing, this rate was 50% in the animal production. A similar result was found in Andrieu et al. (2021) in Burkina Faso, France and Colombia. They reported that surveyed crop farmers in rural areas were not affected in their productive activities as in our study. When an evaluation was made in terms of the number of animals in the research area, there was not much change. Only 35.7% of the participants expressed that the number of cattle and small ruminants (sheep and goats) had decreased compared to the period before the COVID-19 outbreak. However, the remaining participants argued that the number of cattle and small ruminants had either increased or had not changed. In terms of the number of poultry and beehives, there was no negative development compared to the period before the COVID-19 outbreak. Conversely in the study of Seleiman et al. (2020) was stated that livestock farmers already suffer from a shortage of labour concerning both farms and packing plants, since animals need to be fed daily and the production cycle is short for some animals.

One of the most curious issues in the period before the COVID-19 pandemic is migration movements. A more positive development had been experienced in terms of the migration movements compared to the pre-pandemic period. 60% of the participants remarked that the reverse migration from the city to the rural areas had increased, and 40% pointed out that there had been no change. On the other hand, 40% of the participants said that the migration from the rural areas to the city decreased compared to the period before the COVID-19 outbreak, and 53.3% reported that it did not change. The findings of this research also partially confirm this. A similar finding was stated in the study by Kayabaşı (2020) that less prevalence of the pandemic in rural areas would further increase migration to rural areas. What kind of change occurred in terms of the farmer income compared to the period before the COVID-19 pandemic was another curious issue. In print and social media, statements about the decrease in the income of the farmers are frequently encountered in this period. 40% of the participants stated that the income of the farmers decreased, 26.7% of them reported that it did not change. The rate of participants who stated that the income of the farmers increased compared to the period before the COVID-19 outbreak was 33.3%.

Table 1. The opinions of the agricultural engineers on the change in agricultural indicators compared to the period before COVID-19

Agricultural indicators	Increased (%)	Decreased (%)	Not changed (%)
Seed prices	93.3	-	6.7
Fertilizer prices	100.0	-	-
Agricultural pesticide prices	93.3	-	6.7
Petrol prices	86.7	-	13.3
Water prices	40.0	6.7	53.3
Cost of energy (electricity) used in irrigation	86.7	6.7	6.7
Feed prices	93.3	6.7	-
Crop production cost	100.0	-	-
Animal production cost	100.0	-	-
Demand for crop products on the market	60.0	-	40.0
Demand for animal products on the market	60.0	13.3	26.7
Amount of crop products supplied	46.7	33.3	20.0
Amount of animal products supplied to the market	40.0	33.3	26.7
Crop product prices on the market	80.0	13.3	6.7
Animal product prices on the market	85.8	7.1	7.1
Access to credit	46.7	6.6	46.7
Access to agricultural information	20.0	26.7	53.3
Sown area	53.3	20.0	26.7
Number of crop products produced	33.3	13.3	53.4
Total amount of crop production	60.0	20.0	20.0
Field crops production amount	60.0	20.0	20.0
Fruit production amount	20.0	13.3	66.7
Vegetable production amount	33.3	13.3	53.4
Overall productivity in crop production	60.0	13.3	26.7
Total amount of animal production	42.9	21.4	35.7
Milk production	35.7	21.4	42.9
Red meat production	35.7	14.3	50.0
White meat production	45.5	9.0	45.5
Egg production	35.7	7.1	57.2
Honey production	30.8	15.4	53.8
Overall efficiency in animal production	50.0	21.4	28.6
Number of cattle	35.7	35.7	28.6
Number of small ruminants	50.0	35.7	14.3
Number of poultry	38.5	23.0	38.5
Number of beehives	23.1	23.1	53.8
Reverse migration from urban to rural areas	60.0	-	40.0
Urban migration from rural areas	6.7	40.0	53.3
Agricultural supports	73.3	6.7	20.0
Farmer Income	33.3	40.0	26.7

Perceptions on the agricultural impact of the COVID-19

In this section, the perceptions of the agricultural engineers in the region regarding the impact of the COVID-19 outbreak on agriculture were presented. In this context, various proposals were directed to the participants. Likert scale averages of the responses to the proposals are shown in Table 2. The perceptual responses of the participants to the proposals confirm their views in the previous section. As it was indicated in the previous section, the participants were asked about the absolute direction of the agricultural change in Gümüşhane. In this section, perceptual expressions are included. According to the findings in Table 2, the proposals that most of the participants agree with the increase in input prices. It was understood that the participants mostly agreed with the proposals that "prices of agricultural pesticides and animal feed have increased", followed by the propositions about the increase in fertilizer and seed prices, respectively. Participants underlined that they strongly agreed with the proposals regarding the increase in the cost of agricultural production due to the COVID-19 outbreak.

It can be said that the participants agree with the proposal that "there has been immigration from the city to rural areas" significantly. In the previous section, it was stated by the participants that the reverse migration from urban to rural areas had increased in the region. It is possible to say that the participants agree, but do not strongly agree, with the proposal regarding the increase in agricultural subsidies compared to the period before the COVID-19 outbreak. As it was indicated in the previous section, more than 70% of the participants stated that agricultural subsidies were increased. Participants were undecided about the decline in income of farmers compared to the period before the COVID-19 pandemic. This result is consistent with the findings in Table 1. Compared to the pre-COVID-19 outbreak, it was seen that the participants had an undecided attitude in their proposal regarding the increase in the cost of energy (electricity) used in irrigation and fuel prices. However, according to the findings in Table 1, a significant majority of the participants stated that both the cost of energy used in irrigation and fuel prices increased. Participants have been in an undecided attitude regarding the COVID-19 outbreak causing problems in finding workforce, access to agricultural information, and transportation of products to market places after harvest, product storage, and the number of small ruminants. As it was found in the research of Cariappa et al. (2021), the pandemic-induced lockdown exacerbated food loss at production, marketing, distribution and wastage on the Indian agricultural system. On the other hand, according to the perceptions of the participants; the COVID-19 outbreak did not pose a significant problem in terms of planting and harvesting times, access to credit opportunities, the number of beehives, animal and plant product prices, migration from rural areas to cities, demand for animal and plant products and the amount of plant products supplied to the market.

Table 2. Perceptions of agricultural engineers in agricultural organizations about the impact of the COVID-19 outbreak on agriculture

Statement	Mean	SD
Agricultural medicine prices increased	4.13	1.3558
Animal feed prices increased	4.07	1.3870
Production cost increased	4.00	1.3628
Fertilizer prices increased	3.93	1.4376
Seed prices increased	3.87	1.5523
Urban migration to rural areas happened	3.60	1.3522
Agricultural supports increased	3.40	1.2421
Farmers' income decreased	3.27	1.2799
Cost of energy (electricity) used in irrigation has increased	3.20	1.4243
Fuel prices increased	3.13	1.4075
There were problems in finding workforce	2.93	1.3848
Producers had problems in accessing agricultural information	2.93	1.5796
Problems occurred in transporting products to market places after harvest	2.87	1.5055
There were problems with product storage	2.67	1.4960
Number of cattle decreased	2.64	1.6919
Number of small ruminants decreased	2.57	1.6968

Quality seed has become harder to find	2.43	1.2839
Problems occurred in fertilizer supply	2.43	1.4525
Agricultural areas decreased	2.40	1.4541
Problems occurred in procurement of agricultural pesticides	2.36	1.3927
Number of poultry decreased	2.36	1.7805
Productivity fell in animal production	2.21	1.6257
Productivity fell in crop production	2.14	1.4601
Amount of animal products supplied to the market decreased	2.13	1.3020
Farmers changed their production pattern during the pandemic period	2.13	0.8338
Animal production decreased compared to the pre-pandemic period	2.13	1.5976
Water prices increased	2.07	1.4376
Crop production decreased compared to the pre-pandemic period	2.07	1.5796
Problems in accessing water resources for irrigation	2.00	1.4142
Sowing or planting delayed	1.93	1.2688
There was a delay in the harvest	1.93	1.1411
Access to credit facilities decreased	1.93	1.2799
Number of beehives decreased	1.93	1.5424
Animal products prices fell in the market	1.87	1.3558
Crop product prices fell in the market	1.73	1.3345
Rural to urban migration ??	1.73	1.4376
Demand for animal products fell in the market	1.60	0.9856
Demand for herbal products fell in the market	1.53	0.9155
Amount of crop products supplied to the market decreased	1.53	0.9155

Mean: mean score of likert scale (1: Strongly Disagree - 5: Strongly Agree); SD: standard deviation

Conclusions

This study was carried out to determine the impacts of COVID-19 on agricultural production in Gümüşhane province in Turkey and also compared to the pre-pandemic period. Findings show that the COVID-19 outbreak has not had a negative impact on agriculture in many ways. However, the increase in agricultural input prices during the pandemic period is the most unfavourable situation encountered. Restrictions during the epidemic period have a significant effect on the increase in agricultural input prices. Thus, this rise increased the cost of farmers. As expected during the pandemic period, the demand for the crop and animal products has increased. However, the supply of agricultural products did not increase at the same level as expected. This has contributed to the increase in prices of agricultural products.
COVID-19 outbreak did not reduce agricultural production area in the region. The reason why producers do not reduce their production areas despite the increase in production costs can be explained by the increase in agricultural subsidies provided by the government. This shows that the shocks that occur in COVID-19 and similar epidemics can be prevented with government support. However, it is controversial that government support can be a sustainable solution. Not every country in the world has a strong budget to afford such epidemics. The World Health Organization (WHO) points out that there may be a hunger problem in many countries in the coming period. For this reason, it is important for governments to work on structural reforms in order to overcome the shocks that occur in COVID-19 and similar epidemics.

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References

Andrieu, N., Hossard, L., Graveline, N., Dugue, P., & Chirinda, N. (2021). COVID-19 management by farmers and policymakers in Burkina Faso, Colombia and France: lessons for climate action. Agricultural Systems, 190, pages?

Bereir, A.M. (2020). Impact of the COVID-19 on Sudan Agriculture: the role of agricultural extension during the pandemic era. International Journal of Agricultural Science, Research and Technology in Extension Systems, 10(1), 43-49.

Ceylan, R.F., & Özkan, B. (2020). Assessing impacts of COVID-19 on Agricultural production and Food systems in the World and in Turkey. Gaziantep University Journal of Social Sciences, Special Issue, 472-485.

Doğan, Y., & Doğan, S. (2020). Coronavirus pandemic and its effect on crops production in Turkey. Artuklu Kaime International Journal of Economics and Administrative Researches, 3(1), 41-45.

Hai-ying, G., & Chang-wei, W. (2020). Impact of the COVID-19 pandemic on vegetable production and countermeasures from an agricultural insurance perspective. Journal of Integrative Agriculture, 19(12), 2866-2876.

Jambor, A., Czine, P., & Balogh, P. (2020). Impact of the Coronavirus on agriculture: first evidence based on global newspaper. Sustainability, 12, pages/DOI?

Kayabaşı, E. (2020). The effect of COVID-19 on agricultural production. Eurasian Journal of Researches in Social and Economics, 5(5), 38-45.

Kumar, A., Padhee, A.K., & Kumar, S. (2020). How Indian agriculture should change after COVID-19. 12, 837-840.

Lioutas, E. D., & Charatsari, C. (2021). Enhancing the ability of agriculture to cope with major crises or disasters: what the experience of COVID-19 teaches us. Agricultural Systems, 187, pages?

Middendorf, B., Faye, A., Middendorf, G., Steward, Z., Jha, P. K., & Vara Prasad, P.V. (2021). Smallholder farmer perceptions about the impact of COVID-19 on agriculture and livelihoods in Senegal. Agricultural Systems, 190, pages?

Pan, D., Yang, J., Zhou, G., & Kong, F. (2020). The influence of COVID-19 on agricultural economy and emergency mitigation measures in China: A text mining analysis. Plos One, 15(10), pages?

Pu, M., & Zhong, Y. (2020). Rising concerns over agricultural production as COVID-19 spreads: lessons from China. Global Food Security, 26, pages?

Seleiman, M.F., Selim, S., Alhammad, B.A., Alharbi, B.M., & Juliatti, F.C. (2020). Will novel coronavirus (COVID-19) pandemic impact agriculture, food security and animal sectors? Bioscience Journal, 36(4), 1315-1326.

TurkStat. (2020). Turkish Statistical Institute News. Retrieved 01.03.2021 http://www.tuik.gov.tr/Bulten

Varshney, D., Roy, D., & Meenakshi (2020). Impact of COVID-19 on agricultural markets: assessing the roles of commodity characteristics, disease caseload and market reforms. Indian Economic Review, 55, 83-103.

Yavuz, F. (2020). Impact of coronavirus on agriculture. Kriter Journal, 5(46), 86-89.

INVOLVEMENT OF FARMERS IN LOCAL PARTNERSHIPS: STUDY OF LOCAL ACTION GROUPS STRUCTURE IN THE CZECH REPUBLIC

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Abstract

The contribution aims to evaluate the importance and role of farmers in 78 local action groups (LAGs) in the Czech Republic. The LAGs have gradually established themselves as important players in the EU's rural development. Therefore, it is a question of how active the farmers are in these local partnerships. Farmers represent one group of actors (non-profit organizations, municipalities, schools, non-agricultural entrepreneurs), which create the LAG. The research is based on the analysis of the created database of LAG partners (3,960 entities) (1) and the database of supported projects in LAGs in the period 2007-2013 (2). The database of LAG partners contains information on: sector of the partners, role in the LAG or localization. The database of projects supported by the LAG contains information about the applicant, the aim of the project, the focus and priority of the support. The results show that 61-70% of the partners of all LAGs are members of a private sector (including agriculture). Behind representatives of municipalities, farmers are the second most frequently represented group, as they accounted for between 12 to 23% of LAG partners. However, decision-making powers are most often held by representatives of the public sector. Supported projects (2007-2013) of LAG partners were most often implemented by the private non-profit sector (45%). Farmers, together with other entrepreneurs who were partners of the LAG, were also significantly supported (40% of projects).

Keywords: *Farmers, Local Action Group, Rural Development, Community-Led Local Development, Partnership Structure.*

Introduction

Local Action Groups (LAGs) are well-established institutions in local development, composed of public and private sector partners. This private sector can be further subdivided into the non-profit, business, and private sectors. And it is often farmers who are part of this sector. The initial involvement of farmers in LAGs in the Czech Republic was examined by Delín (2012). Research focusing on the partnership structure of LAGs is then primarily oriented towards the involvement of the partners in the board and the relationships between them (Thuesen, 2010; Zajda, 2014; Patkós, 2018).

Local partnership is one of the principles of the LEADER method (*Liaison Entre Actions de Développement de l'économie Rurale*). Therefore, the development of the territory should be addressed by the LAG based on the principle of endogenous development and so-called bottom-up approach, whereby it is an effort to activate the local potential of the territory through local actors. As many studies reveal, the implementation of bottom-up principles is often very limited (Hudečková and Balzerová, 2010; Chevalier and Maurel, 2013; Konečný *et al.*, 2021). The motivation of local actors to be part of partnerships is sometimes criticised, as Pátkos (2018, p.

97) shows in the example of Hungary: *"Action groups were motivated to involve as many actors as possible since the amount of subsidy mostly depended on the size"*.
Getting support from the LAG is very important for many actors in the LAG and the distribution of support is a key role for many LAGs (Binek *et al.*, 2020). Therefore, the topic of LAG support for local projects is widely represented in the literature (Lošťák and Hudečková, 2010; Volk and Bojnec, 2014). As farmers are an important target group of LAG support for rural development, their involvement in partnerships is welcome (Furmankiewicz, Thompson and Zielin, 2010; Volk and Bojnec, 2014).
We want to ask how different types of partners are involved in LAGs and what role they play here. For this paper, we want to consider the role of farmers. The main objective of the paper is therefore to assess the current structure of LAG partners and to uncover the representation of different types of partners in LAGs and decision-making bodies, with an emphasis on the role of farmers. A sub-objective is to reveal the influence of the partnership structure of LAGs in supporting projects in the 2007-2013 programming period. We are therefore interested in the extent to which active and successful farmers are among the supported applicants among the LAG partners.

Materials and Methods

The data needed for the key analyses of the partnership structure of 78 Local Action Groups (as of 2020) from five selected regions of the Czech Republic (South Moravian, Moravian-Silesian, Olomouc, Pardubice and Zlín regions) were collected individually from the websites of the Local Action Groups and their mandatory published information. It was drawn both from the traceable information on the websites and from the annual reports or minutes of the meetings of the LAGs' bodies. In total, a database of 3,690 LAG partners was created containing the following information:

- Name of partner.
- Legislative form.
- Sector (public sector and private sector (further subdivided into non-profit, business and individuals)).
- Role in the LAG (participation in the LAG bodies (an audit committee, a selection committee, or a board)
- Interest group/topic of focus (primarily identified agriculture).

We were also interested in who is the chairperson of the decision-making body (committee/council) of the LAG. Identification was made for 75 out of 78 LAGs. It was possible to classify partners by interest group/theme of focus for 2,312 partners (63% of partners).
We further assessed the importance of farmers in the partnership structure according to their representation among successful applicants of projects funded through the LAG. Therefore, we had to link the partner database of selected LAGs (11 LAGs) with the database of projects supported by Local Action Groups in the 2007-2013 programming period (it contained 854 projects). The databases were linked through the organisation identification numbers (IČO) of the partners. In terms of interpretation of the results, it should be noted that we are fully aware that the actual number of identified partner projects varied to some extent. This is because the project support is related to the partnership structure of the LAG in 2020 (we do not have the partnership structure from 2013 and it is not possible to find out additionally). However, we know from partial research that the partnership structure changes only gradually over time and

rather there is a slight increase in the number of partners (Binek *et al.*, 2020). Sadloňová (2021) published comprehensive research on the issue.

Results and Discussion

LAG partnership structure

A higher proportion of private sector partners in the LAG is expected, as it is already specified in the LAG standards that the number of public sector partners in the LAG must not exceed 49%. However, the rules over time in different EU countries vary (Thuesen, 2011; Chevalier, 2012). In all analysed regions, the dominance of private sector LAG partners was evident, accounting for between 60-70% of LAG partners in the study areas. This means that the average representation of the public sector is comparable to the situation in Hungary (Pátkos, 2018).
However, the composition of the private sector in LAGs varies across the regions studied. In three regions (Olomouc, Pardubice and South Moravian Region) it is possible to see a significant share of the private non-profit sector. However, the business sector, also represented by farmers, accounted for at least one-third of the private sector (35-49%). Individuals who are not representatives of non-profit organizations or in business have the smallest share of representation in all regions. Although these are lower proportions of representation of individuals in the LAG membership base, it means that the individuals are interested in influencing the development of the area in which they live. Based on the additional information, which we know for the 2,312 partners (63%), farmers are very common representatives of the private business sector. They accounted for 12-23% of LAG partners in the regions analysed.
The average number of LAG partners in each sector in the regions shows slight differences between the formed LAG partnerships. Representatives of the public sector had the largest average number of partners (13-24 partners), while the non-profit sector had on average between 11-21 partners in LAGs in each region. The number of entrepreneurs and among them, farmers in LAG partnerships was lower (11-15). Czech LAGs are much smaller in terms of the average number of partners than e.g. in Hungary (Pátkos, 2018).
However, the key information is to find out how active the partners are in the LAG bodies, as by serving in them they have the greatest opportunity to influence the functioning and direction of the LAG (Thuesen, 2010). Zajda (2014) show that there can be considerable mistrust between private and public sector representatives, which affects the functioning of LAGs. Of the 3,690 LAG partners in the analysed regions, 1,395 (38%) are part of the LAG bodies: the council/committee, the control committee, and the selection committee. These are mandatory bodies whose organisational structure must be published on the LAG website according to the standards.
The LAG partners are expectedly represented in the bodies mostly from the private sector, except for the Zlín Region, the largest share of partners is from the private non-profit sector. For the only Zlín Region, the private business sector dominates the private sector in the LAG bodies. Partners from the public sector were represented in the bodies between 32-42%. Their representation is thus slightly higher than could be inferred from their representation within the overall partnership. From this perspective, we can say that the risk of public sector dominance highlighted by Pollermann et al. (2013) has been minimised.
Public sector representatives play an even greater role in the role of LAG leaders. In all regions, at least half of the LAG chairpersons were representatives of the public sector, predominantly municipal representatives (mayors). They were chairpersons in 54-75% of LAGs in each region.

Thus, we confirmed the findings of other studies (Furmankiewicz et al., 2010; Marquardt et al., 2012; Chevalier and Maurel, 2013) that show the key role of this sector in the decision-making functions of LAGs. The reason for this lies in:

- Efforts to control financial flows and support local projects (1) (Furmankiewicz et al., 2010).
- historical inertia, as municipal representatives were the most frequent initiators of the establishment of LAGs (2) (Pátkos, 2018), and,
- the natural roles of sectors/actors in the region, as for mayors, concern for the development of municipalities is an integral and key role in their performance (3).

On the other hand, Thuesen (2010), who examined the composition of 55 Danish LAGs, showed that there was no predominance of the public sector on the boards (14% of the partners in this body were from the public sector).

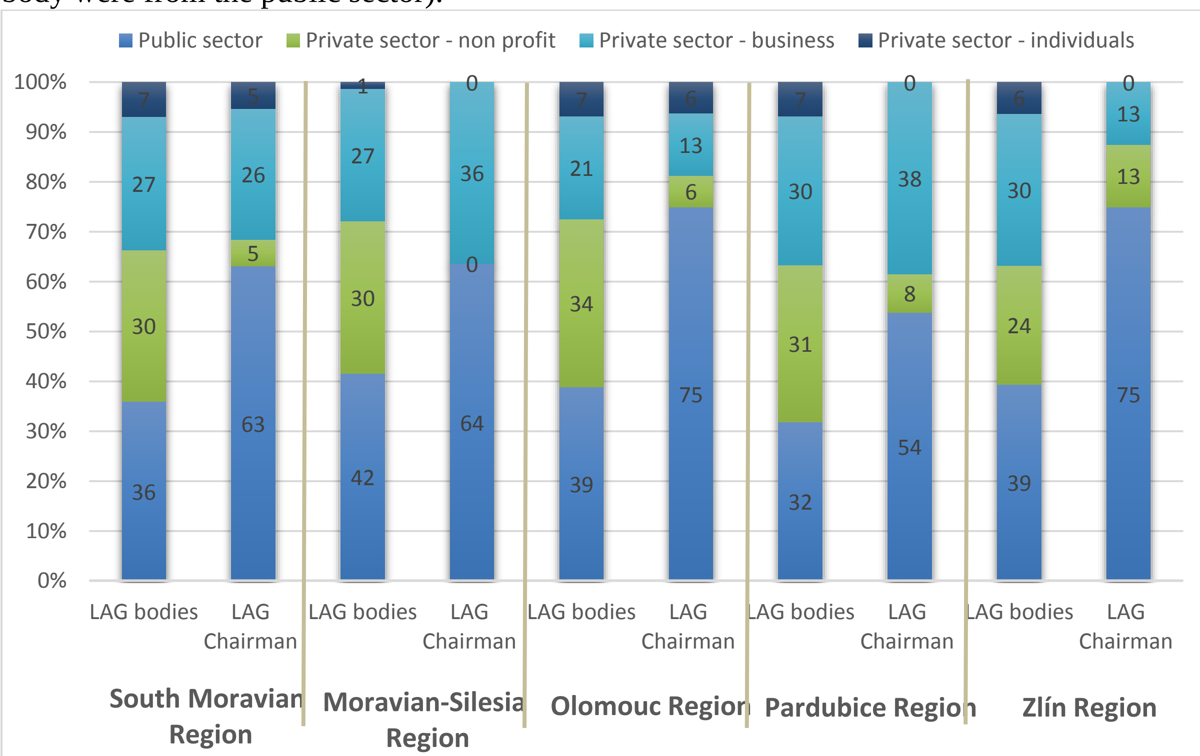

Figure 1. Representation of selected types of partners in bodies of LAGs and among the chairmen of LAGs in selected Czech regions

Source: Author's elaboration based on the created database.

The results showed that the second most frequent representatives as chairpersons were entrepreneurs. Compared to their representation within partnerships or bodies, their role as chairs is much greater. In 13-39% of the LAGs surveyed, entrepreneurs were the chairperson of the LAG executive body. However, in only five LAGs out of 75, we identified that they were farmers. Thus, we confirmed the persistence of the trend of weaker representation of farmers in key roles and support of LAGs in the Czech Republic, as previously pointed out by Lošťák and Hudečková (2010) or Delín (2012), given the influence they have in other countries, such as Italy or Spain (Chevalier, 2012).

LAG supported projects

We want to evaluate the role of farmers in the LAG not only based on their representation in the LAG, its bodies and decision-making functions. We will focus on the projects supported through the LAG in the period 2007-2013 and look at how successful farmers were among the applicants from among the LAG partners. It should be noted that the LAG supports not only projects of LAG partners, but also individual applicants outside the partnership structure who are within the territorial scope of the LAG. There were significant differences between the 11 LAGs surveyed in the total amount they could use to support local applicants (a total amount of EUR 0.8-3.6 million). In total, the LAGs supported 854 projects with a total of EUR 25.5 million in total project expenditure (of which EUR 14.3 million in subsidies).

Among the successful applicants for projects supported through LAGs, actors outside the LAG partners were significantly predominant, accounting for 77% of applicants and their projects accounted for 4/5 of the total expenditure of supported projects. In terms of differences across LAGs, the share of LAG partners in supported projects ranged between 7-37% and between 4-58% of total project expenditure.

If we focus on the 172 supported projects of the 11 LAG, the largest representation is of applicants-partners from the non-profit private sector (47%). The private business sector was on average 41% represented. Although public sector representatives are very important in the structure of LAG partners, representation in the decision-making bodies and leaders of the LAG, they accounted for only 9% of the projects. It should be added that the actual dimension of municipalities as applicants is much larger, as municipalities are often part of the LAG partnership structure as part of a micro-region (institution) but apply for projects independently. Therefore, they are not found in the results. This is a common practice, which is often required by LAGs themselves to meet the prescribed quota of a maximum of 49% public sector representation within the partnership (Binek *et al.*, 2021). For example, even thirty municipalities can be represented in a LAG through one micro-region that has one representative in the partnership. Micro-regions are an important part of LAGs, as studies from different countries show (Hudečková and Balzerová, 2010; Marquardt et al., 2012).

A review of the overall spectrum of applicants clearly shows that municipalities were very dominant among applicants outside the LAG partners. Supported municipal projects accounted for 42% of all projects outside the LAG partners. In all LAGs, it was at least 30% and in three LAGs more than half of the LAG projects were supported by municipalities. Using Slovenia as an example, Volk and Bojnec (2014, p. 368) showed that *"in the years 2008 and 2009, the final beneficiaries in 58% of all projects approved for co-financing with the LEADER funds were from the public sector, 27% of the final beneficiaries were civil societies and only 15% of the final beneficiaries came from the private sector"*.

The importance of farmers among the successful applicants among the LAG partners can be inferred by knowing the distribution of partners within the interest groups/theme of focus that the LAG partners represent and the project name. We assume that the projects of these partners will themselves be linked to the interest group to which the partner belongs. The largest number of projects of the partners of the analysed LAGs can be seen in the interest group Agriculture and Environment, with 58 out of 172 projects (one-third of the projects). It is the relative success of farmers and the good absorption capacity of the territory in supporting agriculture that was reflected in the 2014-2020 period, as almost every LAG included support for agricultural entrepreneurship in its strategies (Konečný et al., 2021).

The results show the surprising finding that in most cases projects of partners without any official role in LAGs (not represented in the LAG bodies - control committee, selection committee or committee/council) were supported. The proportions of these partners with supported projects ranged between 38-90% in the LAGs addressed. Only eight projects were implemented by partners belonging to a committee/council (5% of partners' projects).

Conclusion

The results of a study of 78 LAGs in five Czech regions show that farmers are an important part of local partnerships. The business sector, also represented by farmers, accounted for at least one-third of the private sector in all regions (35-49%). On average, one to two LAG partners out of ten were directly farmers. The private sector accounted for at least one-fifth of the representatives in the LAG bodies in the regions. However, key positions in the LAG are held by the public sector and this is most evident in the LAG committee/council chair. Indeed, the public sector partner was chairperson in 54-75% of LAGs in each region. The second most common representatives among the chairpersons are entrepreneurs, as the non-profit sector holds few of these positions. However, farmers at the head of LAGs are very rare (only in 5 LAGs).

Even so, farmers can be very active in terms of subsidy support for projects for their activities. The claim can be supported by the fact that about one-third of the partner projects supported by the LAG were implemented by farmers. Farmers were an important group of beneficiaries targeted by LEADER support under the 2007-2013 rural development program (RDP). In conclusion, however, it should be said that although municipalities were insignificant applicants among the LAG partners (only 9%), projects of municipalities as non-partners of the LAG prevailed. They accounted for 42% of all non-LAG partner projects.

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References

Binek, J., Konečný, O., Šilhan, Z., Svobodová, H., Chaloupková, M. (2020). Local Action Groups: rural leaders? Mendel University in Brno: Brno. 166 pp.

Delin, M. (2012). The role of farmers in Local Action Groups: The case of the national network of the Local Action Groups. Agricultural Economics - Czech, 58(9), 433–442.

Furmankiewicz, M., Thompson, N., & Zielin, M. (2010). Area-based partnerships in rural Poland: The post-accession experience. Journal of Rural Studies, 26(1), 52–62.

Chevalier, P., & Maurel, M. (2013). The Leader Programme in Central Europe. A New Local Development Policies. Acta Universitatis Lodziensis, 44, 39–53.

Hudečková, H., & Balzerová, H. (2010). The adaptability of stakeholders to new approaches in rural development in the Czech Republic. Agris On-Line Papers in Economics and Informatics, 2(2), 3–13.

Chevalier, P. (2012). What effect does national regulation have on LEADER programming in the EU-27? Wieś i Rolnictwo, 156(3), 9–29.

Lošťák, M., & Hudečková, H. (2010). Preliminary impacts of the LEADER+ approach in the Czech Republic. Agricultural Economics - Czech, 56(6), 249–265.
Konečný, O., Šilhan, Z., Chaloupková, M., & Svobodová, H. (2021). Area-based approaches are losing the essence of local targeting: LEADER/CLLD in the Czech Republic. European Planning Studies, 29(4), 619–636.
Marquardt, D., Möllers, J., & Buchenrieder, G. (2012). Social Networks and Rural Development: LEADER in Romania. Sociologia Ruralis, 52(4), 398–431.
Pollermann, K., Raue, P., & Schnaut, G. (2013). Rural Development experiences in Germany: opportunities and obstacles in fostering smart places through LEADER. Studies in Agricultural Economics, 115, 111–117.
Sadloňová, P. (2021). Involvement of local actors in local action groups in selected regions of the Czech Republic, Mendel University in Brno: Brno. Diploma thesis.
Thuesen, A. A. (2011). Partnerships as associations: Input and output legitimacy of LEADER partnerships in Denmark, Finland and Sweden. European Planning Studies, 19(4), 575–594.
Thuesen, A. A. (2010). Is LEADER Elitist or Inclusive? Composition of Danish LAG Boards in the 2007 – 2013 Rural Development and Fisheries Programmes. Sociologia Ruralis, 50(1), 32–45.
Volk, A., & Bojnec, Š. (2014). Local action groups and the LEADER co-financing of rural development projects in Slovenia. Agricultural Economics - Czech, 60(8), 364–375.
Zajda, K. (2014). Problems of functioning of Polish local action groups from the perspective of the social capital concept. Eastern European Countryside, 20, 73–97.

BIOECONOMY IN LIVESTOCK SECTOR THROUGH BIBLIOMETRIC NETWORK ANALYSIS: A CASE FROM GREECE

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Abstract

Bioeconomy is becoming increasingly one of the most popular topics on the agenda of policy and research worldwide. Its importance has been demonstrated by the immediate strategic goals and resources that have been provided to finance its implementation at international, regional, and local levels. Our planet faces great environmental, social, and economic challenges. The livestock sector is a fundamental element of the global economy as it maintains and supports the economy of urban and marginal areas. Meanwhile, it supports human demands for food, clothing, numerous other essential products for human survival, and contributes to the recycling of nutrients, and enhances biodiversity and resource sustainability. This paper provides a thorough review of the scientific literature on the bioeconomy of livestock production. By means of bibliometric network analysis (i.e. software VOSviewer), 550 relevant documents were identified in publications between 2004 and 2021 and used in the analysis. This research visualizes the strongest links with bioeconomy with emphasis on livestock production, main research domains, most influential countries, as well as research collaborations among countries and organizations. Among the findings of this research, 5 keywords with the highest frequency during 2020 were identified. These outcomes would be useful for understanding the latest development of global research about the bioeconomy in the livestock sector and guiding future research in this subject.

Keywords: *Bioeconomy, livestock production, bibliometric analysis, VOSviewer.*

Introduction

The world's population is expected to exceed 9.7 billion by 2050 and 11.2 billion by 2100 (United Nations, 2015). This increase in population combined with the emergence of the COVID-19 disease, has led to a worsening of global food insecurity (Kusmayadi et al., 2021). There are three big challenges that the humanity is facing today in relation to the future of our planet: (a) reducing dependence on fossil fuels, (b) mitigating the effects of climate change, and (c) achieving a sustainable, adequate and safe food chain (Papadopoulou et al., 2018). To overcome these challenges, bioeconomy has to play a key role for the reconciliation of the environmental and social difficulties and the enhancement of the economic development within the primary and secondary production sectors (D'Amato et al., 2020). Bioeconomy is based on the use of research and innovation in the biological sciences to create economic activity and benefits for the society (Staffas et al., 2013). Focusing on the agri-food sector, by 2050 food production will have to increase by 50% to meet demand from the growing global population (Rodias et al., 2021). Intensified global food production contributes to the greenhouse gas emissions and utilizes large amounts of natural resources and contributes to the loss of biodiversity, given the fact that it is based on monocultures and aims for high yields (Benton et al., 2021). While livestock production also contributes to these aspects, there is undoubtedly many sustainable ways to mitigate its negative effects, including the use of agro-ecological approaches, adoption of principles of organic farming, modern technology and increased circularity (Mbow et al., 2019). The aim of bioeconomy practices for agriculture includes: a) minimizing the use of primary natural resources (such as water and energy) throughout cultivation and animal production, b) minimizing polluting activities and unsustainable practices (such as the use of synthetic fertilizers and unsustainable use of chemicals), and c) recycling, transformation and reuse of agricultural waste for the production of bioenergy, nutrients and bio-fertilizers (Rodias et al., 2021). In this context, a transition from a fossil-based economy to a circular bioeconomy is the way to secure a sustainable future (Tsimitri et al., 2018).

Although the importance of the bioeconomy has been recognized worldwide, in Greece it has not yet received the appropriate attention. This is evidenced by the complete lack of an implemented national strategy for bioeconomy, despite the fact that the Greek government seems to recognize the need for resource efficiency and low carbon emissions (Papadopoulou et al., 2018). The agri-food sector in Greece is one of the most important sectors in exports, with a strong presence in Europe and a growing presence in the American food markets (BRIDGES, 2017). The livestock production sector in Greece is one of the two main sectors of primary production and represents about 25% of the total gross agricultural production of Greece (Zervas et al., 2015).

Given its location, the formation of the landscape, the rich water resources and the vast coastline, Greece has considerable prospects to develop an economy based on agro-ecological approaches for sustainable food production and agri-tourism. Indeed, there is an enormous potential of the agricultural sector for development and Greece can claim a significant role in the development of the bioeconomy both in local and European level. Bioeconomy is the central theme of the 21st century which will ensure the prosperity of our planet.

One of the most critical methodologies which are widely used to show the research development in a field concerned is the literature review (Zhang & Yuan, 2019). The objective of this paper is to visualize the definition of bioeconomy towards livestock production, through the bibliometric networks of international scientific literature.

Materials and Methods

The bibliometric analysis is defined as the process of identifying, analyzing and measuring the scientific productivity of individuals, groups, institutions and countries on a specific topic (Konstantinis et al., 2018). For the purpose of this study, bibliometric analysis was performed using VOSviewer version 1.6.16 (Van Eck & Waltman, 2014). The VOSviewer software was developed by Van Eck and Waltman (2014) for the creation, visualization, and exploration of maps based on the bibliometric network data (Biancolillo et al., 2020).

The literature review was made with the aim of analyzing peer-reviewed publications (books, book chapters, articles, and papers published in conference proceedings) concerning the livestock production bioeconomy issue. The peer-reviewed publications were retrieved from the Scopus database (https://www.scopus.com) on May 29th 2021 using *("animal production" OR "livestock production") AND bioeconomy* as the search keyword. This search keyword was selected, firstly because there wasn't any other literature on this subject and secondly, both terms "animal production" and "livestock production" are used in literature, so they were both included as keywords. This keyword was searched in all fields of the publications. The time frame was set from 2004 to 2021 (May 29th). All the data were exported as "comma-separated values" (csv) files and processed through a bibliometric network analysis using the VOSviewer software.

Results and discussion

Comprehensive search in the Scopus database on the bioeconomy of the livestock production resulted in 550 publications. There is a notable, almost linear increase, in scientific publications on bioeconomy from year 2014 onwards, achieving the highest peak in year 2019 for about 150 publications (Figure 1).

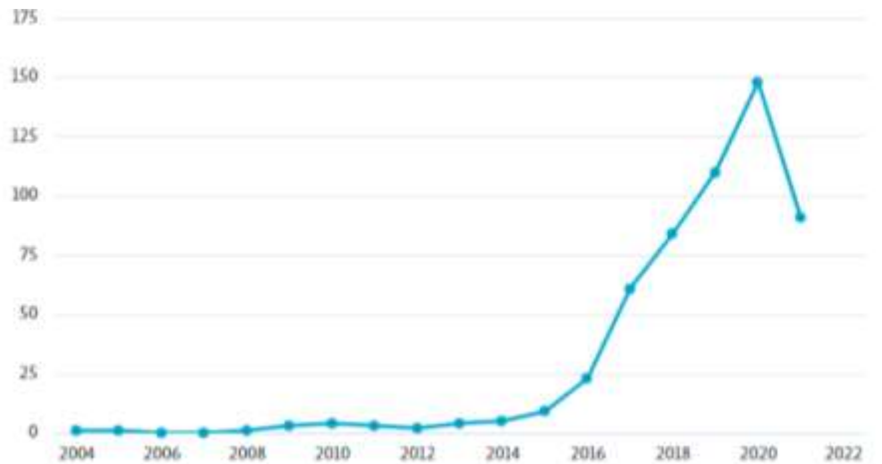

Figure 1. Scopus publications on the livestock production bioeconomy at the international level (2004-2021)

These publications, based on their research area that deal with can be categorized as follows: a) agriculture and biological sciences (288 papers, 26.8%), b) environmental science (193 papers, 18.0%), and c) veterinary (113 papers, 10.5%). Although the bioeconomy of the livestock production has attracted an international interest, the intensity of the research seems to vary among countries. It is therefore important to demonstrate its distribution to research globally through an analysis of papers published in different countries. This can also serve as an indicator to imply the demand of more studies in the regions concerned. It is easily observed that Poland, Germany, Norway and Italy are the most active countries with 151, 91, 84 and 62 papers, respectively (Figure 2). Similar research on the bioeconomy of the livestock production in Greece seems to be less frequent, as currently there are only 6 scientific publications available. Based on the inter-connections among different countries, this proves that cooperation is prevailing in Europe when research is conducted.

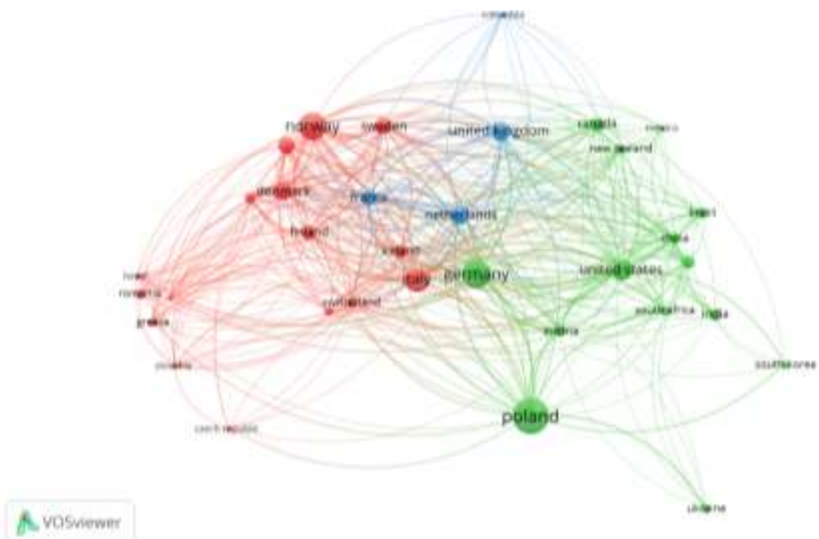

Figure 2. Network demonstration of main countries in livestock production bioeconomy research (Minimum number of documents of a country: 5)

The number of publications produced within the period 2004-2021 on the bioeconomy of the livestock production is derived from 160 research organizations (universities and research institutes). Specifically, the 5 most productive organisations are the University of Life Sciences in Lublin, Poland (112 documents), The Norwegian Institute of Bioeconomy Research (67 documents), Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam, Germany (46 documents), Wageningen University & Research in the Netherlands (33 documents) and Norwegian University of Life Sciences (28 documents).

According to Zhang & Yuan (2019), keywords are used to identify the aspect/topic of each paper and are useful for readers to comprehend the researching contexts. Author keywords in our research sample were also explored and analyzed by using the VOSviewer software to demonstrate the keywords with a high frequency as well as keyword relationships. The analysis of the keywords generated 2,003 results. However, only 54 keywords have at least five co-occurrences. The most important keywords are bioeconomy (1.20%), agriculture (1.10%) and sustainability (1.10%) (Table 1).

Table 1. Top ten keywords related to the livestock production bioeconomy

Keyword	Occurrences	Frequencies (%)	Total link strength	Links
Bioeconomy	24	1.20	28	15
Agriculture	22	1.10	26	16
Sustainability	22	1.10	38	25
Anaerobic digestion	11	0.55	8	7
Circular economy	11	0.55	16	9
Climate change	11	0.55	16	10
Biomass	10	0.50	11	8
Dairy cow	10	0.50	4	4
Microalgae	10	0.50	11	10
Bioenergy	9	0.45	11	8

It is also very interesting to find out the development of high-frequency keywords in the past decades, which will help researchers to understand the main trends in this research topic. The co-occurrence network map of keywords related to the livestock production bioeconomy (Figure 3) shows the 51 most used keywords. The size of the circle is relative to the co-occurrence of that item, the shorter the distance is among the items, the stronger their relation is. The color of the circle shows the happened time slot. Keywords with lighter colors are highlighted more recent. It is easily observed that researches started to pay more attention to the terms "sustainability" and "bioeconomy" from 2019. The three red arrows show the lighter areas with 5 keywords, "air pollution", "waste management", "biorefinery", "manure", "circular economy". Those have the highest frequency among all the keywords analyzed in our research during 2020, indicating the new focusing directions in livestock production bioeconomy research.

Figure 3. The overlay visualization map of high-frequency keywords in livestock production bioeconomy research (Minimum number of occurrences of a keyword: 5)

Conclusion

This study presents publications that deal with the bioeconomy of the livestock production sector, through a bibliometric network analysis. The main advantage of this method is that it is capable to analyze the multidimensional nature of bioeconomy through a large literature database. On the other hand, the main disadvantage lies with the fact that some publications may be missing in our study because they do not precisely use our search keywords. Furthermore, the publications analyzed with this method do not include literature such as working papers, Masters' and Bachelors' thesis, project reports and deliverables.

During the last decades, publications on the research area of bioeconomy have increased rapidly confirming that both research community and international policies have recognized bioeconomy as an important activity for the production of goods, services, and energy and as one of the main solutions towards sustainable economic growth of the livestock sector. This paper summarized the main research topics in livestock production bioeconomy and revealed the most important keywords that can be the new directions in livestock production bioeconomy research. However, it revealed limited studies and projects that will be able to support and diffuse the bioeconomy development with particular focus on Greece. It is necessary to further intensify research towards this direction and attempt a deeper content analysis in the future on the bioeconomy of the livestock production. From the political side, new legislation and national policies are needed to address challenges that the livestock production sector is going to encounter, protect national resources and introduce efficient waste management. Finally, we suggest that policy directions in Greece should encourage multi-disciplinary programs, innovation and knowledge developments, to provide skilled experts in the field of the livestock production and its associations with bioeconomy. This may contribute to addressing the acceptability and legitimacy of issues that are central to sustainability science and practice.

Acknowledgements

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References

Benton, T. G., Bieg, C., Harwatt, H., Pudasaini, R., & Wellesley, L. (2021). Food system impacts on biodiversity loss: Three levers for food system transformation in support of nature, The Royal Institute of International Affairs Chatham House. London.

Biancolillo, I., Paletto, A., Bersier, J., Keller, M., & Romagnoli, M. (2020). A literature review on forest bioeconomy with a bibliometric network analysis, Journal of Forest Science, 66(7), pp. 265–279.

BRIDGES (2017). Mapping and understanding the innovation potential of bio-economy businesses in the partner regions. The case of W. Macedonia, Greece, focused in Agri-Food.

D'Amato, D., Bartkowski, B., Droste, N. (2020). Reviewing the interface of bioeconomy and ecosystem service research, Ambio, 49(12), pp. 1878–1896.

Konstantinis, A., Rozakis, S., Maria, E.-A., & Shu, K. (2018). A Definition of Bioeconomy through the Bibliometric Networks of the Scientific Literature, AgBioForum, 21(2), pp. 64–85.

Kusmayadi, A., Leong, Y. K., Yen, H. W., Huang, C. Y., & Chang, J. S. (2021). Microalgae as sustainable food and feed sources for animals and humans – Biotechnological and environmental aspects, Chemosphere, 271, 129800.

Mbow, C., Rosenzweig, C., Tubiello, F., Benton, T., Herrero, M., Pradhan, P., & Xu, Y. (2019). Food security. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security and greenhouse gas fluxes in terrestrial ecosystems, PLoS ONE, 8.

Papadopoulou, E., Vaitsas, K., Fallas, I., Tsipas, G., Chrissafis, K., Bikiaris, D., Kottaridi, C., Vorgias, K. E. (2018). Bio-economy in Greece: Current trends and the road ahead, The EuroBiotech Journal, 2(3), pp. 137–145.

Rodias, E., Aivazidou, E., Achillas, C., Aidonis, D., & Bochtis, D. (2021). Water-energy-nutrients synergies in the agrifood sector: A circular economy framework, Energies, 14(1), pp. 159.

Staffas, L., Mccormick, K., Gustavsson, M. (2013). A Global Overview of Bio-economy Strategies and Visions, Swedish Knowledge Centre for Renewable Transportation Fuels (f3 Centre).

Tsimitri, P., Michailidis, A., Loizou, E., Mantzouridou, F. T., Gkatzionis, K., & Mugampoza, E. (2018). Bioeconomy and the production of novel food products from agro- industrial wastes and residues under the context of food neophobia, AgBioForum, 21(2), pp. 97–106.

United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Prospects: 2015 Revision, Key Findings and Advance Tables, Working Paper No. ESA/P/WP.241.

Van Eck, N. J., Waltman, L. (2014). Visualizing Bibliometric Networks. In: Ding Y., Rousseau R., Wolfram D. (eds) Measuring Scholarly Impact, Springer International Publishing, Cham, pp. 285–320.

Zervas, G., Bizelis, I., Papadomichelakis, G., Tsiplakou, E., Symeon, G., Mitsiopoulou, C., Katsoura, P., Papakonstantinou, D. (2015). Recharging Greek Youth to Revitalize the Agriculture and Food Sector of the Greek Economy, Final Report Sectoral Study 4. Livestock farming. Athens.

Zhang, W., Yuan, H. (2019). A bibliometric analysis of energy performance contracting research from 2008 to 2018, Sustainability (Switzerland), 11(13), pp. 3548.

POTENTIALS OF IRRIGATION ALONG TELWA VALLEY IN TCHIROZERINE DEPARTMENT, AGADEZ AREA (NIGER)

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Abstract

The study focuses on the constraints and potentials of irrigation in three municipalities crossed by the Telwa valley in the department of Tchirozérine, Region of Agadez. The valley passes through the towns of Agadez, Dabaga and Tchirozérine. It is one of the Aïr valleys that carry significant amounts of rainwater collected from the Aïr Mountains. The article highlights the constraints potential of irrigation in this Telwa valley. The study involved a sample of 48 producers chosen from 12 villages in the three communes. It appears that the total areas of irrigable land are variable with an average of 1.66 ha. Likewise, the average cultivated area is 0.92 ha. The availability of irrigated land takes 47% of producers operate an area of less than 1 ha, around 31% of producers operate more than 1 ha per year. The municipalities have 46% of the irrigated sites in the region. There is an experienced human capital in irrigation management formed by 12.908 producers in 2018, including 4.427 for the three municipalities studied, which represents more than 34% of the region's workforce. Among the producers, we find both sexes but largely dominated by men 97% against 3% of women. Access to groundwater for irrigation is through two main systems, shallow PVC wells and boreholes (varying at varying depths). The dewatering is provided by motor pumps and the crops are irrigated according to the gravity and Californian type.

Keywords: *Irrigation Constraints, Potentials, Telwa valley, Borehole, PVC.*

Introduction

Niger, due to its location, is subjected to Sahelian - arid type of climate, the main characteristics of which are low rainfall, variable in time and space, high temperatures and strong winds, tending to accentuate its aridity (Aliou *et al.*, 2014). Mainly rain-fed, Nigerien agriculture is practiced in very hostile conditions, due to the country's arid climate regime characterized by low rainfall, a short rainy season and high temperatures. Despite these unfavorable constraints, agriculture remains the most important sector of Niger's economy. More than 80% of the population is active in agriculture (INS, 2012), while the primary sector represents around 45% of the Gross Domestic Product (GDP).

This primary sector dominated by agricultural activities has in fact grown faster than other sectors of the economy. It is also the second sector contributing to export earnings after mining (Ministry of Agriculture, 2014). However, cereal yields in Niger have declined in recent decades due to climate change causing flooding, erratic rains and poor distribution in time and space. Irrigated crops such as vegetable production are therefore considered as an alternative to food

security in the face of climate change (Yacoubou, 2009). As water is the first limiting factor for agricultural production in most of the country, the long-term development of irrigation in Niger has always been considered as an essential aspect which makes it possible to stabilize yields, especially in the event of irregular rainfall (Alizee *et al*., 2011; Patrick and Issa, 2009).
In the region of Agadez located in an arid zone where the climate of the arid type (with very low rainfall averages hardly exceeding an annual cumulative of 200 mm (PRODEX, 2013), only irrigated crops are practiced, provided from the surface water table, which is fed by runoff from wadis or *koris* during the short rainy season, water collection being done through wells and manual drilling, the depth of which generally varies from 0 to 18 meters depending on the area (Moumouni, 2014).
Irrigated agriculture is developing more and more in the Aïr mountain areas, thanks to the intensification and the orientation of production towards national and regional markets. However, this vegetable production faces enormous constraints associated with biophysical factors which unfortunately slow down its development. Despite these constraints, the Agadez region, particularly the communes of Agadez, Dabaga and Tchirozerine crossed by the Telwa valley, have great potential in terms of irrigation. The paper aimed to assess the main constraints and potentialities of irrigation along Telwa *kory* stream in three municipalities of Tchirozerine Department, Agadez Region.

Materials and Methods

The main materials used in this study consists of list of all irrigation sites in the region, including their respective area coverage provided by the regional directorate of agriculture; a GPS for the geo-referencing of sites and surface measurements; individual discussion and focus group questionnaires for the field survey and a smart phone for taking pictures. The study relies on the use of primary and secondary data. Primary data was collected through field observations and interviews with public technical services and staff of Non-Governmental Organizations (NGOs) working in the field of irrigation in the study area. Questionnaires were administered to farmers and market gardeners to assess the constraints and potential of irrigation in the area. Secondary data comes from documentary that deals with themes and subjects related to the issue of irrigation. Also, the use of GPS made it possible to collect the geographical coordinates of all the villages and gardens sampled in order to measure the total surface area of all the gardens representing the samples to highlight the irrigated land potential of the area of 'study.

Sample size

The study carried out in the department of Tchirozérine concerned three (3) communes of the region of Agadez which are the commune of Agadez; of Tchirozerine and of Dabaga. In each commune, four (4) villages were selected on the basis of the importance of market gardening activity and the size of the cultivated areas. Then, four (4) producers were surveyed in each village, which resulted in a sample of twelve (12) villages and forty-eight (48) farmers/producers questioned (table 1).

Table 1. Sample frame and distribution of respondents for interview and Focus Group Discussion (FGD)

Municipalities	Villages	Sample for individual interviews	Sample for FGD
Agadez	Tchiguefen	4	8 farmers
	Eladeb	4	
	Toudou Bila	4	
	Azamalan	4	
Tchirozerine	Azzelkelawey	4	8 farmers
	Azzel Ecole	4	
	Tchimbiram	4	
	Emassaknan	4	
Dabaga	Baital	4	8 farmers
	Intatat	4	
	Tassalamsalam	4	
	Babaré	4	
Total	**12**	**48**	**24**

For the purposes of the study, individual interviews and collective discussions with the operators (48) were carried out in order to obtain detailed and precise information. The selection of respondents was made at random, a list of all the producers in the village was drawn up, then the respondents were drawn at random. However, criteria attributable to age; the experience and level of responsibility of the irrigator were considered. That is, each respondent must be an active gardener at least twenty (20) years old and have at least five (5) years of experience in market gardening. both qualitative and quantitative analysis. The qualitative data were discussed and interpreted by the research team. To determine the extent and area of Kory-Telwa, a supervised classification map was produced using ARCGIS software and the watershed was digitized from Google Earth. To assess the potentials of irrigation in this area, quantitative data from the questionnaire results were analyzed and interpreted using descriptive statistical techniques including percentage, ratio, average and analysis presented in tables and graphs. This analysis is performed using Microsoft Excel software.

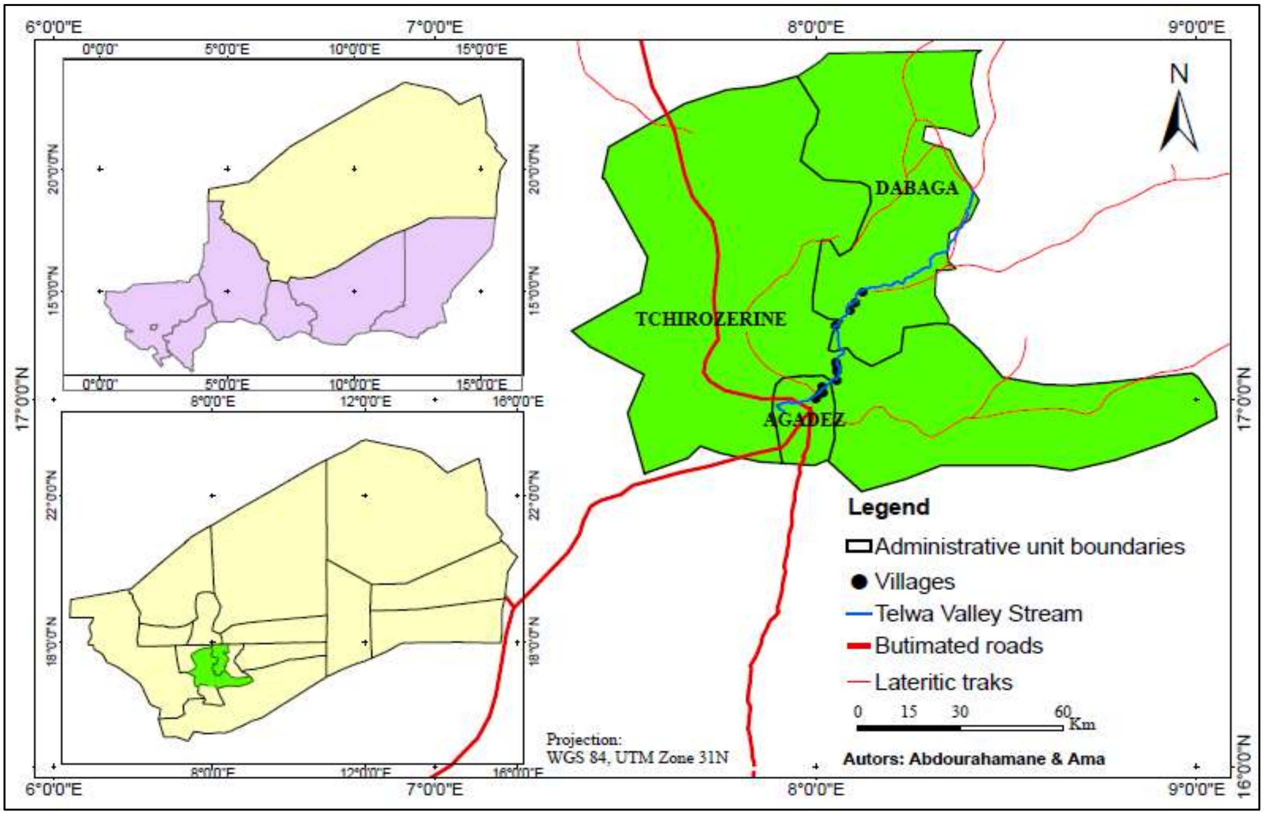

Figure 1. The Study location, the three municipalities and the selected villages

Results and discussion

Potentials of irrigation

Despite the various constraints encountered by irrigation production in the area, which are common biophysical constraints hindering the development of irrigation farming in the area. They can be divided into two: the biotic such as parasitic and pest attacks, weed infestations and diseases associated with insects, birds and rodents; for abiotic constraints, these relate mainly to water erosion, siltation, leaching, soil gullying and destruction of crops during floods. Others include early drying up of the wells, the presence of bedrock. The latter contains (abiotic) retard enormous potential which, if developed, could boost the development of vegetable production and the income of producers.

Land potential

The irrigable land potential of Niger is estimated at 270,000 ha on the basis of exploitable water and soil resources (FAO-AQUASTAT, 2015 and Moussa, 2016). Agriculture of Agadez in 2018, the irrigable areas are estimated at 21,510 ha for the whole region with about 7,000 hectares cultivated areas which represents 32% of the regional irrigable potential. Most of the region's land resources (51%) are concentrated in the department of Tchirozérine where irrigated production seems to be developing well with more than 50% of cultivable areas being developed.

Table 2. Sample characteristics

	Mean	**Median**	**Minimum**	**Maximum**
Total surface (ha), N=48	1.66	1.3	0.4	6
Exploited surface (ha), N=48	0.92	0.8	0.3	2

One can therefore note from table 3, as an indication, that the size of the areas used for irrigation in this zone is very random and varies considerably from one farmer to another. Table 3 also presents the distribution of exploitable and exploited areas of the zone.

Table 3. Distribution of Exploitable and Cultivated Farmland in the Study Area

Size class (ha)	Frequency	Relative frequency (%)	Cultivable land (ha)	(%)	Cultivated land) (ha)	(%)
< 1	13	27.1	9.1	11	20.6	47
1-1.99	19	39.6	24.8	31	19.5	44
2-2.99	10	20.8	21.6	27	4	9
3-6	6	12.5	24.4	31	0	0
Total	48	100	79.9	100	44.1	100

The table (3) shows that gardens with less than one (1) ha represent 27% of all gardens, then 11% of the total exploitable area of the sample studied. Gardens with areas between 1 to 1.99 occupy approximately 40% in number and 30% in area. Farms with an area greater than two (2) hectares represent 33% in workforce and approximately 58% in area. It is noticed that the largest areas occupy more of the cultivated areas. This proves the presence of significant land potential that could be developed. However, for the areas actually exploited, more than 64% of farmers interacted, cultivate an area of less than one (1) ha per year, corresponding to 47% of the total area exploited, approximately 31% of producers have an average holding of more of one (1) ha and only 4% manage to exploit two (2) ha per year. These figures show a predominance of small-scale irrigation despite the importance of land availability in the area. This depends on the one hand on the financial resources of the operator (financial capacity for investment in development) and on the other hand on the availability of water, which remains a limiting factor along the valley.

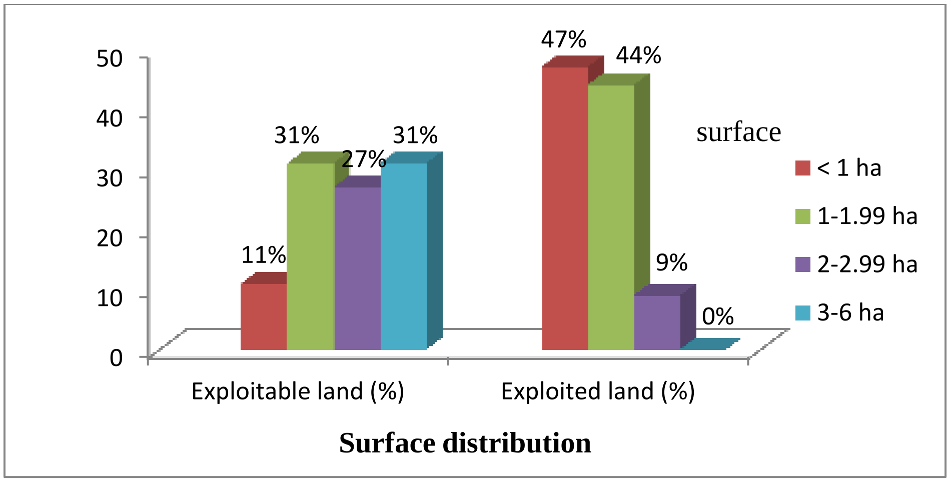

Figure 2. Exploitable and Exploited land (%) in the three Communes as farmland distribution

Water potential

The water potential of the study area consists of the water resources used in the Aïr, especially in the Telwa valley, mainly come from alluvial acquires which are in turn supplied by the floods received from the watersheds during the rainy season. These shallow aquifers, whose recharges already depend on the quantity and duration of the flows, have recently suffered from early exhaustion which could be linked to the intense drawdowns of water for irrigation, which remains the main activity of the populations of the area. . The development of irrigation encouraged by the advent of the use of motor pumps has influenced the balance of this alluvial water table. In the vast majority of cases, the latter no longer ensures the water supply necessary for irrigation for the entire agricultural season in the area.

In addition, the presence of deep water tables in this area was noticed, though not very much known. There is lack of reliable scientific data that could help in estimating the water potential or in understanding how the aquifer works. The presence of the base in places and the lack of technical and financial means limit access to deep water tables for farmers, whose average depth of wells does not exceed twelve (12) meters. However, deep boreholes have recently been tested in the Aïr with depths varying from twenty-five (25) to one hundred and fifty-two (152) meters, but these holes show a weak relationship between the depth and the magnitude of flow (Illias, 2018). As for the alluvial aquifers of the Telwa valley, although superficial, they have the potential to store large quantities of water, the recharge of which is sometimes spontaneous, following the first flows depending on the proximity of the water and this well relative to the minor *koris* bed (Illias, 2018).

With each irrigation time, thousands of cubic meters (m3) of water flow and infiltrate through this valley. The latest estimates of the water potential of this valley come from studies carried out by scientific experts from the Office for Overseas Scientific and Technical Research (ORSTOM) who spent several years in the Aïr. Indeed, for the hydrological studies of the Telwa valley, hydrometric stations had been installed along this *kory* and the data were collected and analyzed for each rainy season (Gallaire, 1995). Table 3 shows the volumes sold in Dabaga and the volumes lost between Dabaga and Azzel (village in the municipality of Tchirozerine) from 1980 to 1988 were considered.

The number of producers constitutes a human potential, one of the real factors of production at the level of a farm. Indeed, the region of Agadez has 278 irrigated cultivation sites spread over fifteen (15) municipalities, of which 128 sites are located in the municipalities of Tchirozerine, Dabagaet and Agadez, i.e. 46% of the irrigated sites in the region (table 3). There were 12,908 producers in 2018, including 4,427 for the three municipalities mentioned, which represents more than 34% of the region's workforce. These farmers are represented by both sexes but largely dominated by 97% men against 3% of women.

Table 3. Farmers and Irrigation Sites Proportions in Agadez Region

Communes	Number of sites		Number of producers	
Tchirozerine	80	(28.8%)	2613	(20.2%)
Dabaga	36	(12.9%)	1566	(12.1%)
Agadez	12	(4.3%)	248	(1.9%)
Autres communes	150	(54%)	8481	(65.7%)
Total	278	(100%)	12908	(100%)

Socio-demographic characteristics of farmers

The study area is characterized by a large demographic representation as shown in table5. The household size of the sample studied varies from 3 to 15 people with an average of 8 people per household. The age of the producers surveyed varies from 24 to 74 years, with an average age of 44 years. Market gardeners are generally married (93.75%). Market gardening constitutes the main activity for all the producers surveyed (100%), 27% of whom have a secondary activity. The average experience of practicing irrigation is 28. In addition, 58% of these respondents have at least once received agricultural training, which testifies to the good mastery of agricultural practice in the area. This signifies a good workforce in the region.

Market Gardening as labor activity

Representing the main activity in the Aïr zone, market gardening is a major employer of labor, mainly coming from the southern part of the country. The laborious tasks, especially manual weeding, require the use of hard labor which often works for the entire crop season. Most producers do not work alone, family labor is the first to be called upon, but in the absence of this, farmers employ easily accessible external manpower, although most often without agricultural experience. In some cases, depending on the importance of the work and farmers' uses, both family and hired manpower are involved (figure 3).

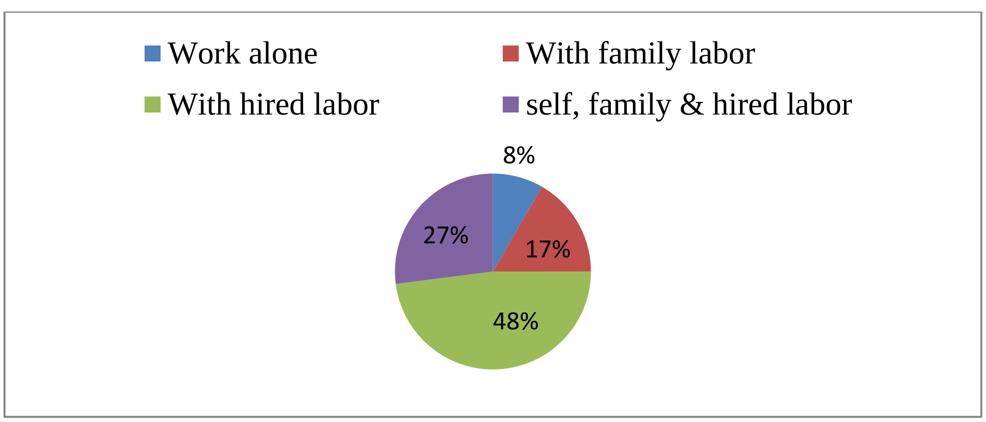

Figure 3. Relative Labor (manpower) Proportion for Irrigation Farming in the Three Communes

Irrigation system
Due to the absence of surface water in the study area, only groundwater is used for irrigation. Access to groundwater for irrigation is through two main systems, shallow PVC wells and boreholes (varying at varying depths). The watering is provided by motor pumps and the crops are irrigated according to the gravity and Californian type.

The diversity and Importance of Cultivated Crop Species

Irrigated crops are characterized by a large number of cultivated species in the Aïr zone. This study identified 24 species of market gardening and 2 species of cereal crops divided into 11 families. The most represented families are *Solanaceae* (5 species), *Cucurbitaceae* (4 species), *Apiaceae* (4 species), *Malvaceae, Liliaceae, Fabaceae* and *Poaceae* (with 2 species each).
For the most cultivated crops, it was considered of those with a relative frequency greater than 30%. Fruit vegetables, with the exception of eggplant, are among the most widely cultivated crops. They have the advantage of giving rise to several harvests per crop cycle. Indeed, these crops are produced during the period of high water availability in the area. Salad, cabbage and moringa are the most widely cultivated leafy vegetables. The salad has a very short cycle (less than 40 days) and shares with moringa the advantage of being well produced in all seasons and as for cabbage, it is generally appreciated for its good yields. For root vegetables, carrots and beets are better represented. Onions, on the other hand, dominate bulb vegetables and generate significant economic value.

Conclusion and Recommendations

This study allowed us to highlight the irrigation potential along the Telwa Valley in the northern part of the Agadez region. Thus, it emerged that there is an important land potential which is not significantly exploited, as farmers are unable to grow large areas of land (more than 3 ha). Despite the importance of water flows, farmers are not effectively exploiting this water potential due to the lack of water retention structures. A part this, there is also significant irrigated crop biodiversity and human potential in the area.
As a recommendation, technical support in terms of training (organic fertilization, biological control, etc.) and infrastructure (water retention and mobilization structures) is necessary for these irrigators.

References

National Institute of Statistics of Niger, 2012. General Census of Population and Housing. Niamey: National Institute of Statistics of Niger.

Ministry of Agriculture of Niger, 2014. Final results of the 2014 Winter Agricultural Season and 2014-2015 Food Outlook. Niamey, Directorate of Statistics, Ministry of Agriculture of Niger.

Yacoubou, M. 2009. Climate Change and Food Security in Niger: Acting through Irrigation.

Alizée, E., Lawali, D. and Ronald, J. 2011. Irrigation Development Projects and Programs in Niger (1960-2010): Elements for an Assessment. Center for Studies and Information on Small-scale irrigation.

Projects for the Development of Exports of Agro-sylvo-pastoral Products (PRODEX), 2013. Environmental and Social Impact Study Report of Three Thresholds for Recharging the Abseck, Bogol, Misène aquifer in the Agadez Region, Niger.
Moumouni, D. M. 2014. A Contribution to the Study of Small-Scale Irrigation in the Aïr: Case of the Alexess Zone, in the Urban Commune of Agadez, Niger, Master's degree in Sustainable Agriculture and Water Resources Management. Higher Institute of Agronomy and Veterinary Medicine (ISAV) of Faranah, Republic of Guinea, 79p
FAO-AQUASTAT, 2015. Country Profile – Niger. Food and Agriculture Organization of the United Nations. Rome, Italie.
Moussa, M., Philippe, L., Boubacar, Y. (2016). Land Issue around the Lands Recovered in the Region of Tahoua in Niger. University of Agadez, Agadez, Niger (1) 2Ulg–Gembloux Agro Bio Tech, Economic and Rural Development unit, Gembloux, Belgium (2) 3Abdou Moumouni University of Niamey, Niamey, Niger (3). *International Journal AgroFor, Vol. 1 (No. 1), 2016.*
Illias, A. 2018. Assessment of groundwater resources in the Timia basin (Aïr massif, North of Niger), Impacts of climate variability and anthropogenic activities. Universite Abdou Moumouni De Niamey: Doctoral School of Life and Earth Sciences, Thesis, 238p.
Gallaire, R. 1995. Hydrology in a high altitude sub-desert environment: The case of Aïr (Niger), thesis, University of Paris, Paris XI, institute of science and research for Development and Cooperation, Paris, France, 1995.

INCREASING THE AGRICULTURAL PRODUCT COMPETITIVENESS AS A FACTOR IN THE AGRICULTURAL ENTERPRISE EXPORT DEVELOPMENT

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Abstract

In order to achieve the set tasks to increase the level of GDP growth, a significant increase in investments is required, while the agribusiness faces the challenge of increasing exports to USD 45.0 billion by 2024, which is also impossible without serious investments in the development of foreign trade activities of enterprises. The existing measures of state support for the development of foreign trade activities do not provide the required level, as well as the existing investment mechanisms have not lead to a significant increase in the volume of agricultural exports. At the same time, the potential of private investment in the agribusiness development directly or indirectly through funds and stock exchanges is estimated at the level of 10% to 12% of the population of Russia. Currently, Russia has only 1.7% of private investors or about 2.5 million people, while this figure is 11% of private investors in China, it is an average of 39% in Japan, and it is more than 52% of private investors from the total population in the USA. Therefore, the recent years' reforms in the sphere of the Russian agribusiness have led to an increase in agricultural production, profitability of enterprises and the development of export activities. The necessity of analyzing and substantiating the essence of the economic category of competitiveness, which is largely based on the external manifestations of the competitiveness of agricultural products, in particular the cost, has been justified. The measures to increase competitiveness at the economic level are substantiated based on the use of high-tech, nature and soil-protective agriculture and new technologies in animal husbandry and crop production in terms of the development of export potential. Some reasons have been identified that impede the development of the agricultural sector, in particular, the insufficient level of investment activity in the agricultural sector and the weakness of the economic positions of agricultural enterprises. It has been proposed to apply budgetary support to normalize their financial situation. The risks that must be taken into account when bringing agricultural products to foreign markets have been identified.

Keywords: *Agricultural enterprises, Products, Competitiveness, Export activities, Development.*

Introduction

A number of studies have identified the export of agricultural products as a driving force of economic growth. Stimulating the development of exports is the primary task of national economies (Ayob Freixanet, 2014; AboagyeGunjal, 2000; Khanal et al., 2020; Altukhov et al.,

2019). Mania and Rieber (2019) found that export baskets have different potential for growth and economic development and that the quality of export diversification should be assessed according to a country's ability to develop its production structure.
In recent years, significant institutional and functional reforms have taken place in the Russian agribusiness that are focused on changing the structure of agricultural production, its re-equipment, diversification and development of agricultural exports. At the same time, these years were a period of development of agriculture and obtaining competitive products of the industry. The most important result of this work should be considered an increase in agricultural production and the profitability of agricultural enterprises including those through the implementation of export activities (KhokhlovaYushina,2019; Deloitte Survey, 2019).
A chronically unprofitable industry has become profitable. The aggregate level of profitability of agricultural organizations increased almost 3 times, although incomes are not high enough to ensure the maintenance of expanded reproduction in most farms. It is known that profit stimulates business to make investments while the industry becomes more attractive for Russian and foreign investors. Nevertheless, the overall level of investment activity in the agribusiness remains low. However, their share in the total volume of investments in the Russian economy is slightly less than 3%, while the share of agriculture in the gross domestic product is much higher and is equal to about 5.5%. Investments in the country's agribusiness has resulted in the growth of the labor productivity being the most important economic indicator. If an agricultural employee produced products worth 72,000 rubles in 2010, then currently, it is 1.5 times more then that in 2010 (On the progress and results of the implementation…, 2020, ShagaidaUzun, 2020). However, the dynamics of development of the agribusiness industries suggests that most of them have exhausted their reserves of internal growth and cannot develop further at the existing rates without a radical change in the investment and government policy of regulation. Over the past three years, state support for the agribusiness has increased 1.4 times. However, the positive effect of state support for the agricultural economy is significantly brought to naught by its insufficient volume and lack of solutions to all existing problems. The state of affairs in the agricultural sector of the economy can be corrected only by increasing its investment and innovation attractiveness. An example of this is the development of food and processing sectors of the agribusiness economy, where production fixed assets have been actively updated in recent years, i.e. good conditions have been created there for domestic and foreign capital. It is necessary to achieve the same situation in agriculture. Further development and growth of the Russian agribusiness (at least 4% to 5% annually) is possible only within the framework of the implementation of strategic target programs for effective and accelerated reform of agriculture and increasing the competitiveness of products of the industry's.
The purpose of the study is to analyze the factors hindering the development of the agricultural sector and export activities of agricultural enterprises, as well as to develop measures to increase competitiveness at the economic level in terms of developing export potential.

Materials and Methods

The materials for the study were those of the State Program for the Development of Agriculture and Regulation of the Markets of Agricultural Products, Raw Materials and Food, the priority project titled 'Export of Agricultural Products', regulations governing the export of agricultural products, data from the Ministry of Agriculture of Russia and other federal and regional authorities. In the course of the research, the methods of system and structural-dynamic analysis, expert assessments, extrapolation and others methods were used.

Results and discussion

In recent years, the competitiveness of agricultural products has become more significant. The transition to the model of export-oriented development of agricultural enterprises presented them with a fait accompli: a significant difference in prices for agricultural products when selling them in Russia and abroad and insufficient demand due to the low purchasing power of the Russian population. It should be borne in mind that even before the recent transformations, the level of labor productivity in agriculture, the material and technical base of product processing and its implementation lagged behind those level of the developed countries, which determined the higher cost of production of the agricultural industry of this country. Of course, at the same time, it should be noted that the bioclimatic potential of domestic agriculture is relatively worse. However, there is a certain part of the farms that successfully compete in the domestic and foreign markets, and their products are in constant demand (Kovaleva, 2019 Kovalenko Chekuno, 2019).

Such a contradictory situation requires analysis and substantiation of the essence of the economic category of competitiveness and search for ways to improve it for domestic producers. In the current market, one should not absolutize in many respects the external manifestations of competitiveness or non-competitiveness of agricultural products, which are mainly determined by the price factor. It is necessary to clearly find out which market one is talking about and where the competitiveness of the products should and can be shown. It goes without saying that the domestic market of Russia features extremely low paying capacity of the bulk of the population. In addition, the food market is practically not regulated by the government, which allows the trade sector to dictate its terms to agricultural producers and contributes to the growth of the disparity in prices for agricultural products. Agricultural exports are not adequately supported and imports are not sufficiently limited.

Measures to improve competitiveness at the economic level follow from the experience of profitable agricultural enterprises. The basis of their success is the use of high-tech, nature and soil-protective agriculture and new technologies in animal husbandry and crop production. The increase in the number of such agricultural enterprises is the key to the competitiveness of the industry. After all, intensive technologies are designed for deeper knowledge, require active involvement in the production of agricultural products of mineral fertilizers (150 kg of active agent per 1 ha or more), low-volume use of plant protection products against diseases, pests and weeds, differentiated application of agents in different phases of plant development and equipment operation along the tramline. At the same time, a programmed product quality can be obtained that meets the requirements of both processing and the domestic and foreign markets.

The next step is high (high-intensity) technologies. This is the strategic future of competitive agriculture in Russia. These technologies not only require high-quality varietal crops and seed varieties, but also need to take into account various aspects of their natural and climatic growing conditions, soil cultivation and seeding systems, fertilization and protection against diseases, and requirements for determining the parameters of cultivated areas and product quality. High-techs have been created and in many respects provide accurate (precise) control of the production process of growing and storing competitive products. As a rule, these technologies take into account and control the quality of performed process steps based on changing landscape conditions and optimize the use of intensification resources. Many elements of these technologies require revision or adaptation of their European counterparts to local conditions.

Obviously, the task of intensifying production in agriculture with the use of new technologies requires a sharp, 3-4 times, increase in labor productivity by way of transferring the production of the agricultural sector to the use of agricultural machinery of new generations and then precision technology that accurately fulfills the production processes specified. In general, one can conclude that each agricultural enterprise needs to turn to the technical design of production of specific types and with given parameters of competitive products for sale in the foreign market.

It should also be added that agricultural enterprises should pay more attention to marketing and look for the most profitable channels for selling products in the foreign market. These channels should have a stable financial position and the ability to pay for purchased goods on time. The implementation of cluster strategies will also contribute to increasing the competitiveness of agricultural enterprises (Kovshov et al., 2019; Cherkesova et al., 2019; Voytyuk et al., 2021).

In the regions, the increase in the competitiveness of agricultural products is due to the fact that the regional market develops on specific types of products and their sale profitability is associated with the costs of transporting products to foreign markets. In Russia, transporting agricultural and food products are required, because the conditions for growing some types of agricultural products are very different. However, due to the lack of an extensive infrastructure for the distribution of products and high tariffs for the transportation of products, agricultural enterprises cannot take full advantage of the opportunity for their products to enter the foreign market at the lowest cost. As a result, there was a huge difference in prices for agricultural products in different regions of Russia.

Considering the issue of the competitiveness of the products of agricultural enterprises in the industry in the domestic market, it is necessary to predict possible changes in export-import activities associated with the dynamics of the world market. Thus, ensuring the competitiveness of agribusiness products and foodstuffs cannot be considered only as a function of an economic entity, agricultural or other producer in the agricultural sector.

Federal and regional state authorities are responsible for the current processes and regulation of domestic and foreign markets. The measures used for these purposes include the organization of information support and the formulation of forecasts, subsidies and compensations to support profitability and reduce costs in the production of competitive products of agricultural enterprises, as well as interventions to keep prices in a certain range; the formation of reserves, including strategic ones, when the world market conditions are undergoing major changes. At the same time, the most important source of risks for the implementation of the agricultural development policy is the low competitiveness of the industry in its economic relations with other sectors of the Russian economy, i.e. unsatisfactory competitive position of agricultural producers in their relations both with processors of raw materials, trade, infrastructure and transport enterprises, and with manufacturers of industrial products necessary for the reproduction of fixed assets and ensuring current production activities.

The price proportions of the Russian economy were formed mainly during the period of price liberalization and foreign trade at the initial stage of reforms. At the same time, a number of sectors of the country's economy used market advantages and achieved an outstripping rise in prices for their products in comparison with prices for agricultural products. The price proportions that changed as a result of the abovementioned came into conflict with the existing technological structure of production and the structure of its distribution throughout the country, which determine the level of current material costs and capital intensity in the agricultural sector.

The weakness of the economic positions of agricultural enterprises in their relations with enterprises of related industries allows them neither to resist the rise in prices for the resources they consume, nor to adapt to it by increasing the selling prices of agricultural products. The growing lag of the selling prices of agricultural products from the prices of consumed production resources leads to a shortage of financial resources in agriculture and a reproduction crisis. Consequently, while maintaining the prevailing prices for the industry's products, the problem of normalizing the financial situation of an agricultural enterprise cannot be solved and the industry cannot switch to a mode of sustainable export-oriented development without any budgetary support.

In addition, there is a lack of effective tools and scientific methods to ensure the competitiveness of enterprises. Inattention to the issues of restructuring and modernization of most production subsectors lead to low efficiency of agricultural production (Ushachev, 2009).

To reduce the influence of negative factors, the Strategy for the development of the agricultural and fishery sectors of the Russian Federation for the period up to 2030 has been approved, one of the purpose of which is creating a high-performance export-oriented sector that develops based on up-to-date technologies and is provided with highly qualified personnel. The State Program for the Development of Agriculture and Regulation of Markets for Agricultural Products, Raw Materials and Food for the Period of 2013-2020, the Federal Scientific and Technical Program for the Development of Agriculture for 2017-2025, and the Doctrine of Food Security are also being implemented.

However, the agricultural production management mechanisms used in the framework of development programs are focused on solving individual local problems and turn out to be insufficiently effective (Bakhtin et al., 2017; Kotov et al., 2017).

The "International cooperation and export" national project is implementing the "Export of agricultural products" priority project, within the framework of which the regional authorities develop programs with appropriate funding and set targets. So, the "Export of agricultural products" project of the Saratov region sets the task of increasing the supply of regional agricultural products to USD 773 million by 2024, the project for the development of exports in Udmurtia assumes an increase to USD 49.1 million, Kurgan region also plans an increase up to USD 49 million, Moscow region plans an increase up to USD 1.7 billion, Stavropol Territory plans an increase up to USD 1.093 billion, and Bashkiria plans to increase up to USD 230 million.

The goal of the regional project in the Kaluga region is to triple the volume of exports of agricultural products of the region until 2025. The "Volume of exports of agricultural products" target indicator for 2024 is planned in the amount of USD 72 million (Ministry of Agriculture of the Kaluga Region…, 2020).

Two hundred and fifty six agricultural enterprises represent the agrarian sector of the Kaluga region, more than 150 of which are involved in export activities (Voytyuk Marinchenko, 2021). They sold products worth over 107 billion rubles in 2019. The industrial production index was 110.0% (Export of agricultural products…, 2021).

The total amount of financing for the activities of the regional export development project amounted to 9.33 million rubles in 2019, of which 8.96 million was from the federal budget and 0.37 million was from the regional one.

The indicator of the program implementation is the volume of export products sold (Table 1). The indicator exceeded USD 52.1 million (130.3% of the target) in 2019.

Table 1. Indicators of the implementation of the Export of Agricultural Products project of the Kaluga region

Item	Export volume, USD million	Period / year							
		2017	2018	2019	2020	2021	2022	2023	2024
1	Agricultural products, including:	23.9	38.4	40.0	42.0	45.0	50.0	59.0	72.0
1.1	Fat and oil products	0.5	0.1	0.2	0.1	0.1	0.2	0.2	0.2
1.2	Cereals	0.0	0.3	0.4	0.3	0.3	0.5	0.8	1.5
1.3	Fish and seafood	1.1	1.3	1.0	1.4	1.5	1.6	1.8	2.0
1.4	Meat and milk products	0.8	0.9	0.4	0.9	1.0	1.7	5.6	12.7
1.5	Food and processing industry products	11.9	22.7	32.5	25.0	26.8	29.2	32.1	35.3
1.6	Other agricultural products	9.6	13.1	17.6	14.3	15.3	16.8	18.5	20.3

Source: the official portal of the authorities of the Kaluga region

When planning the entry of agricultural products into foreign markets, one should take into account risks, including macroeconomic ones. These include, for example, uncertainty regarding:

- Prospective conjuncture of the foreign energy market (return of oil prices to the corridor of normal values can sharply reduce the financial capabilities of the budget);
- Prospective trends in the real ruble exchange rate (strengthening the ruble will help weaken the effect of measures to protect the domestic market);
- Ways and rates of implementation of reforms in the social sphere and housing and communal services (under some scenarios, these reforms may lead to a sharp reduction in the growth rate of real incomes of the population).

With the industrialization of the livestock sector, the risks of epizootics objectively increase. It is necessary to develop and implement a system of preventive measures to damp social risks that may be generated by the uneven development of agriculture in the constituent entities of the Russian Federation, therefore, the different severity of the socio-economic crisis caused by the ousting of inefficient economic entities from the market, or the transformation of modern hidden unemployment into open.

It is also necessary to consider the following risks:

- Institutional risks (uncertainty of the actual timing of the formation and establishment of viable unions and associations of producers, which will provide an opportunity for intra-industry and inter-sectoral coordination of development programs);
- Legislative risks (uncertainty of the timing of the formation of the legal and regulatory framework for agricultural activities and state regulation of agriculture);

Man-made, ecological and natural-climatic risks (the possibility of large-scale disasters, the elimination of which requires the diversion of funds from all sectors of the national economy, including that from the agricultural sector of the economy).

This is the main range of issues and tasks to be solved in the country in order to increase the competitiveness of the agribusiness and bring the industry to the dynamics of sustainable development.

Conclusion

There are a number of unresolved problems in the industry that impede the development of the agricultural sector, such as the inability to reduce the shortage of financial resources resulting from the constant rise in prices for consumed resources and the insufficient level of investment activity through higher prices for products. At the same time, the relatively low competitiveness in comparison with developed foreign countries is due to lower labor productivity in agriculture because of the less developed material and technical base of enterprises in the agricultural sector, as well as processing and product selling enterprises. In recent years, thanks to government support, many enterprises have been upgraded, which has increased the competitiveness of agricultural products and has a positive effect on the export potential of enterprises.

To increase the competitiveness of the agribusiness and individual economic entities, as well as bring the industry to the dynamics of sustainable development, it is necessary to take into account the macroeconomic risks associated with the international situation and the risks associated with socio-economic development within the country.

To reduce the negative impact of factors hindering the development of the agricultural sector, as well as to ensure further enterprise modernization that increases the competitiveness of enterprise products and export potential, it is necessary to expand government support measures. The main area of increasing the competitiveness of enterprises is their modernization using high-tech production in animal husbandry and crop production, which are based on up-to-date agricultural machinery, varieties, breeds, types and hybrids of an intensive type and modern technologies based on the engineering of programmed products for the domestic and foreign markets. That will allow reaching the specified levels of production and export.

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References

Aboagye A., Gunjal K. (2000). An analysis of short-run response of export and domestic agriculture in sub-Saharan, Africa Agricultural Economics, 23, issue 1, 41-53

Altukhov A., Kundius V., Kovaleva I. (2019). Prospects of development of export of agricultural products and foodstuffs in the countries of the Shanghai cooperation organization, Proc. AgroCon-2019: Russian Conference on Innovations in Agricultural and Rural development, Kurgan State Agricultural Academy, Kurgan, Russia

Ayob A., Freixanet J. (2014). Insights into public export promotion programs in an emerging economy: The case of Malaysian SMEs, Evaluation and Program Planning, 46, 38-46

Bakhtin P., Saritas O., Chulok A., Kuzminov I., Timofeev A. (2017). Trend monitoring for linking science and strategy, Scientometrics, 111(3), 2059-2075

Cherkesova E., Mironova D., Slatvitskaya I., Simonyan L. (2019). Development of agricultural regions in the conditions of rational import substitution policy, Proc. INTERAGROMASH

2019: 12th International Scientific Conference on Agricultural Machinery Industry, Don State Technical University, Rostov-On-Don, Russia

Deloitte Survey(2019). Russian Agriculture Market Survey, Moscow, Russia

Export of agricultural products. Kaluga region https://admoblkaluga.ru/upload/minselhoz/!!!!!_19/Export_doclad.pdf Accessed on 21/07/2021

Khanal N., Nepal P., Zhang Y., Nepal G., Paudel B., Liu L., Rai R. (2020). Policy provisions for agricultural development in Nepal: A review, Journal of Cleaner Production, 261, 12124110

Khokhlova G., Yushina Y. (2019). Improvement of the mechanism of management of investment projects, Proc. Int. Scientific Conf.: Information Society: Health, Economics and Law, Irkutsk, Russia

Kotov D., Gamilova D., Alekseeva E., Sharipov R. (2017). Conceptual provisions of regional strategies Modern Economy: Problems and Solutions, 2(86), 204-211

Kovalenko N., Chekunov A. (2019). State support for the export of agricultural products to the Russian Federation Agribusiness: Economics, Management, 11, 4-15

Kovaleva I. (2019). Prospects for the development of foreign economic activity of agriculture in the context of the implementation of the export policy of the agribusiness, Int. Journal of Humanities and Natural Sciences, 2-2, 77-81

Kovshov V., Lukyanova M., Galin Z., Faizov N., Frolova O. (2019). Methodology of strategic planning of socio-economic development of the agricultural sector of the region, Montenegrin Journal of Economics, 15(3), 179-188

Mania E., Rieber A. (2019). Product export diversification and sustainable economic growth in developing countries, Structural Change and Economic Dynamics, 51, 138-151

Ministry of Agriculture of the Kaluga Region begins the digitalization of agribusiness with applied IT solutions, https://admoblkaluga.ru/sub/selhoz/news/detail.php?ID=265332 Accessed on 12/12/2020

Government of the Russian Federation (2020). On the progress and results of the implementation in 2019 of the State program for the development of agriculture and regulation of markets for agricultural products, raw materials and food.National report, Moscow, Russia, http://council.gov.ru/media/documents/pdf/79sHkFtVtAr3PFbC3EaL3WZr7AjAAfPc.pdf Accessed on 12/06/20201

Shagaida N., Uzun V. (2020). Development Trends and Main Challenges of the Agrarian Sector of Russia (Analytical Report, Moscow, Russia)

Ushachev I. (2009). Economic growth and competitiveness of Russian agriculture, Economy of agricultural and processing enterprises, 3, 1-9

Voytyuk M., Voytyuk V., Marinchenko T. (2021). Increasing the competitiveness of agricultural enterprises based on a cluster strategy, Earth and Environmental Science, 677(2), 022083

Voytyuk V., Marinchenko T. (2021). Modelling of export development of agricultural enterprises of the Kaluga region, Earth and Environmental Science, 659(1), 012126

LIVELIHOODS OF SMALL FARM HOUSEHOLDS IN COVID-19 PANDEMIC: A CASE STUDY IN THE RED RIVER DELTA OF VIETNAM

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Abstract

The COVID-19 pandemic has been affecting the entire food system, especially small farm households. Border closures, trade restrictions and confinement measures have been preventing farmers from accessing markets, including for buying inputs and selling their produce, and harvesting crops, thus disrupting domestic and international food supply chains and placed millions of livelihoods at risk. With more than two-thirds of the population living in rural areas, rural Vietnam is strongly affected by the COVID-19 pandemic in terms of losing jobs of many agricultural labourers, reducing income sources of small farm households due to the interruption of agricultural exported products market, and the decline of domestic market spending on food. This paper aims to provide empirical evidence about the impact of the COVID-19 pandemic on the livelihoods of small farm households in Northern Vietnam. Using data collected from sample farm households in the Red River Delta in Northern Vietnam, this paper investigates the effects of the COVID-19 pandemic on livelihoods of small farm households including livelihood resources, livelihood activities, and livelihood adaptation strategies. Results from 120 farm households show that a wide decrease in their income since the COVID-19 pandemic occurred in Vietnam, in which non-farming income dropped the most in households relying on crop production and wage-earning. Farm households relying on aquacultural productions and tourist services are the most affected by the COVID-19 pandemic when total income is reduced by more than 32 percent. For more pandemic resilient livelihoods, most of the farm households expected to receive government support in forms of financial provisions, preferential loans, market opening.

Keywords: *livelihood, small farm household, COVID-19 pandemic, Vietnam.*

Introduction

The COVID-19 pandemic has led to a dramatic loss of human lives worldwide and presents an unprecedented challenge to public health, food systems and the world of work. The economic and social disruption caused by the pandemic is devastating: tens of millions of people are at risk of falling into extreme poverty, while the number of undernourished people, currently estimated at nearly 690 million, could increase by up to 132 million by the end of 2020 (ILO, 2020). The pandemic has been affecting the entire food system, especially small farm households. Border closures, trade restrictions and confinement measures have been preventing farmers from accessing markets, including for buying inputs and selling their produce, and harvesting crops, thus disrupting domestic and international food supply chains and reducing households' income. The pandemic has decimated jobs and placed millions of livelihoods at risk (FAO, 2020).

Vietnam's agricultural sector plays an important role in the economy. With its strength in agriculture production, Vietnam has the advantage of ensuring national food security and

contributing to food security in other countries. In addition, Vietnam's agricultural sector has also created many high-value export items. With more than two-thirds of Vietnam's population living in rural areas, rural Vietnam is strongly affected by the COVID-19 pandemic in terms of losing jobs of many agricultural labourers, reducing income sources of small farm households due to the interruption of agricultural exported products market, and the decline of domestic market spending on food. In this context, small farm households are vulnerable to the impacts of the COVID-19 pandemic on livelihoods, income and welfare (Tran et al., 2021).
This paper aims to provide empirical pieces of evidence about the impact of the COVID-19 pandemic on the livelihoods of small farm households in Northern Vietnam. In this paper, the COVID-19 pandemic is assumed to affect the livelihood of small farm households in terms of livelihood resources, livelihood activities, income and livelihood strategies. The following sections of this paper include material and methods, results and discussion and conclusion.

Material and Methods

Hai Phong is a coastal province in the Red River Delta, Northern Vietnam. The livelihood of small farm households in Hai Phong is mainly based on agricultural farming, aquacultural farming and fisheries catching, and tourism services. However, the COVID-19 pandemic has strongly affected the livelihoods of smallholder farmers due to the disruption of the supply chain of agricultural and aquatic products and especially the absence of tourists. Therefore, this study was conducted in 2 districts of Hai Phong province to clearly identify the impact of the COVID-19 pandemic on the livelihoods of smallholder farmers.
In order to get an overview of the livelihood transformation of small farm households under the impact of the COVID-19 pandemic, this study collected data from 120 small farm households based on 3 main livelihood groups: 30 farm households depend on agricultural production and wage earning (hereinafter referred to as agriculture - wage earning group); (2) 30 farm households depend on agricultural and aquacultural farming (hereinafter referred to as agriculture - aquaculture group); (3) 60 farm households depend on aquaculture and tourism services (hereinafter referred to as agriculture – tourism service group). These households have their own farm land of less than 0.36 ha per household, so they are identified as small farm households in Vietnam (GSO, 2019). Primary data from 120 small farm households were collected through direct interviews with household heads using semi-structured questionnaires. The content of the interview included: information on the head of the household as age, gender, education level; the number of family members, number of labour, livelihood resources, livelihood activities, income and livelihood strategies in response to the COVID-19 pandemic.
Primary data from small farm households are synthesized and analyzed using descriptive statistical methods combined with T-test and Mann Whitney U-test to examine the difference in the mean of household's income pre and during the pandemic (Dinh, 2011).

Results and Discussion

Description of the surveyed households

This section provides demographic information about the surveyed households including the age, sex and education of the household head and the number of family members as well as gender structure of the family. In small farm households in the Red River Delta of Vietnam, the age of the household head has a significant influence on the livelihood activities of the household because of the strong influence of household head on household's members (Hoang, 2009). The long production experience of the elderly householders helps them to quickly adapt to market risks and diseases in agricultural and aquacultural farming; thereby making reasonable changes in the livelihood activities of their households. Basically, the age of the household head in the agriculture - aquaculture group is the highest with an average age of 50.9 years old; the age of the household head in the aquaculture-tourism service group is lowest at 47.5 years old (Table 1). The author's observations during household interview show that young householders are less experienced but more dynamic, creative and have many bold ways of thinking for business. They have many business ideas suitable for tourism activities. This is very necessary for the group of aquaculture-tourism service households to develop livelihood activities and increase income.

Table 1 Statistical description of small farm households

Description	Unit	Agriculture – Wage earning (n=30)		Agriculture – Aquaculture (n=30)		Aquaculture- Tourism services (n=60)	
		No of HH**	Percentage (%)	No of HH	Percentage (%)	No of HH	Percentage (%)
Household member	Persons	4.3	-	4.8	-	4.6	-
- Male	Persons	2.3	56.7	2.5	50.0	2.4	58.3
- Female	Persons	2	43.3	2.3	50.0	2.2	41.7
Family labourers	Persons	2.3	-	3.0	-	2.8	-
Age of household head	Years	50.7	-	50.9	-	47.5	-
Education of household head	Years	8.7	-	9.6	-	10.3	-

*Source: Authors' own calculations from survey data in 2021; ** HH: Household.

Besides age, a high level of education is also an advantage for the household head to easily access agricultural and fishery market information as well as tourism service market information. Based on these market information, small farm households can diversify their livelihood activities. Statistical data show that the heads of households in the group of aquaculture - tourism services have the highest level of education (more than 10 years of schooling), in the remaining two groups, the heads of households have lower education levels.

Livelihood resources of small farm households during the COVID-19 pandemic

Farm land

The Red River Delta of Vietnam has a history of agricultural cultivation for thousands of years. However, with a high population density, the average farm land area per person is the lowest (500m2) compared to other regions in the country (Bergstedt, 2012). Figure 1 illustrates the area of farm land used for crop farming and aquacultural farming of the three small farm household groups. Compared to 2019, the area of land used for crop and aquacultural farming in 2020 is lower in all three groups of households. Householders believe that the decline in domestic consumption of agricultural and aquatic products is the main reason why they narrow their farming area. With the declining demand, farm householders cannot sell their products. Amongst these three groups of households, agriculture – wage earning households have the lowest decrease in land use, the householders think that they try to make full use of their farm land area to serve livelihood activities and to avoid wasting natural resources.

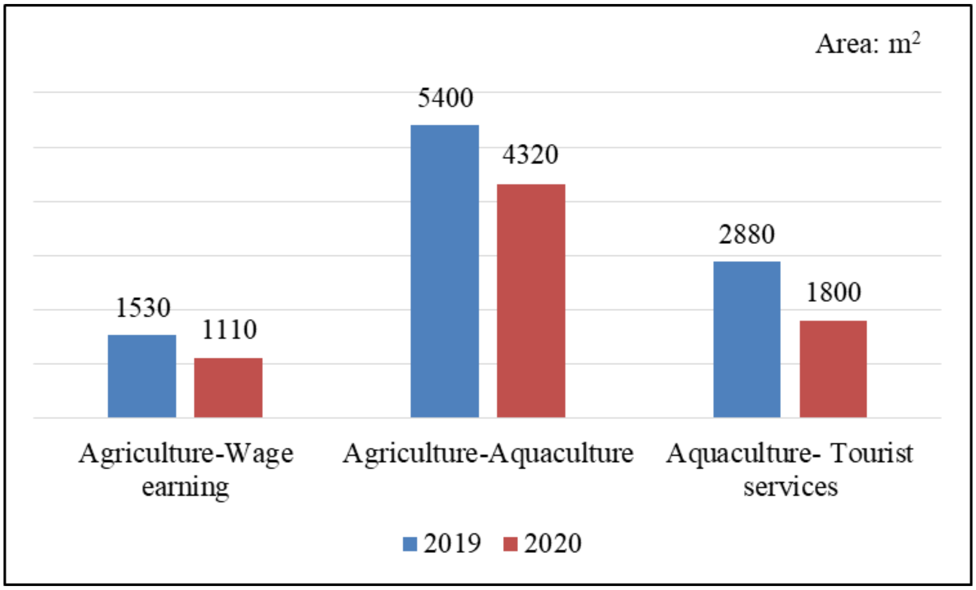

Figure 1. Land use for agriculture and aquaculture of small farm households
*Source: Authors' own calculations from survey data in 2021

Financial resource

Not only farm land, financial resource for production is also an important livelihood resource of smallholder farmers. In rural areas of Vietnam, farmers can access official loans from banks and credit institutions through farmers' union and women's union. In this study, the sources of loans for all three groups of households are mainly from banks and relatives. The group of agriculture-wage earning households has the lowest number of households with loans (10% of the households), and the group of aquaculture - tourism service households need more capital for business, so 60% of households have loans (Table 2). Some householders believe that accessing financial resources at banks is still difficult due to the requirements of mortgage of property rights such as land use rights or housing property rights, so they tend to borrow from relatives if the loan amount is small. In contrast, householders borrow money from banks mainly with large amounts. Some households borrowed to invest in production before the pandemic, so they cannot afford to pay now.

Table 2. Loan of small farm households in 2020

Description	Agriculture – Wage earning (n=30)		Agriculture – Aquaculture (n=30)		Aquaculture- Tourism services (n=60)	
	No of HH	Percentage (%)	No of HH	Percentage (%)	No of HH	Percentage (%)
Number of HH loan	3	10.0	15	50.0	36	60.0
Amount of money loan	10 (mils VND**/HH)		44 (mils VND/HH)		40 (mils VND/HH)	
Comparison to the loan of 2019						
Increase	1	33.3	3	20.0	5	13.9
Keep stable	0	0.0	2	13.3	4	11.1
Decrease	2	66.7	10	66.7	27	75.0

*Source: Authors' own calculations from survey data in 2021; **VND: Vietnam Dong, 23 thousands VND equals to 1 US dollar

In order to better understand the impact of the COVID-19 pandemic on the livelihood resources of smallholder farmers, householders were required to compare loan amounts in 2020 with the pre-pandemic period. As a result, the majority of householders answered that they reduced their loans compared to 2019. Especially the group of aquaculture - tourism services with 75% of households reduced loans in 2020. Farm households working as tourism services face the most difficulties during the COVID-19 pandemic when their livelihoods were frozen. Householders in this group reported that they must completely change their livelihoods. Some households have not fully paid off their bank loans, then they have a crisis.

> *"I just hope the COVID-19 pandemic is quickly defeated to stabilize the country's socio-economics, through which the lives of people like me can stabilize. I still owe the bank a loan before, I have to pay a lot of interest every month, the epidemic has severely reduced income from tourism services, so it is even more difficult to pay bank interest." (Interview with a head of small farm household, female, 49 years old)*

Labour resource

For smallholder farmers in the Red River Delta of Vietnam, in addition to farm land, family labour is also a resource that plays an important role in livelihood activities (Le Thai, 2011). To analyze the impact of the COVID-19 pandemic on the change in labour use, this study compared the change in family working days in 2020 compared to the time before the pandemic occurred. There are 3 levels of assessment of working days in 2020 for household heads to answer including an increase compared to 2019; remain the same as in 2019; and decreased compared to 2019. Statistical results show that aquaculture - tourism service households have the largest number of working days decrease by 33.3%; the decrease was mainly due to the frozen tourism service activities. In the group of agriculture – wage earning households, labour only decreased by 10% and mainly because tourism service households did not need to hire labourers anymore. In any group of households, this decrease leads to a decline in the income of households in general and of labourers in particular. This decrease, therefore, makes farmers' lives extremely difficult during the pandemic.

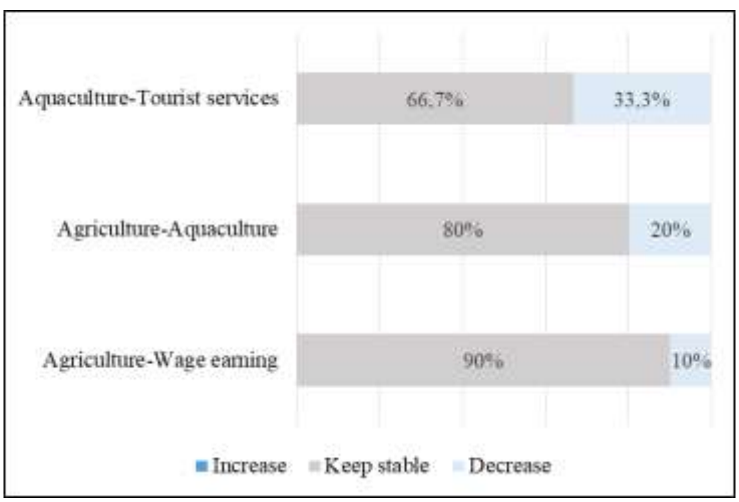

Figure 2. Labour use in small farm households in 2020 compared to 2019

*Source: Authors' own calculations from survey data in 2021

Livelihood activities

Many studies on the livelihoods of smallholder farmers in Vietnam in general and the Red River Delta in particular have come to the conclusion that diversifying livelihood activities is a way for farmers to increase their income and risk reduction (Nguyen 2015, Hoang 2009). In this study, the results show that a small farm household can simultaneously carry out different livelihood activities such as crop cultivation, animal husbandry, aquacultural farming, trade, tourism services and wage earning, etc. Nevertheless, in order to clearly identify the impact of the COVID-19 pandemic on the livelihoods of three groups of small farm households, this study only focuses on analyzing the changes of the main livelihood activities which account for the majority of households working time and main contribution to household income.

In the group of agriculture – wage earning households, before the pandemic (2019), the majority of them (over 83%) carried out all 3 main livelihood activities, namely crop farming, breeding and wage earning. However, when the pandemic occurred, the number of breeding and wage earning households decreased, especially the number of households engaged in wage employment decreased from 100% (in 2019) to 60% (in 2020) (Table 3). According to the surveyed households, livestock in this area is mainly large one such as buffaloes, cows, goats and pigs to supply restaurants to serve tourists. Therefore, when the restaurants were almost closed, the consumption market for livestock meat was a limitation, some households with insufficient capital gave up raising livestock and only focused on crop cultivation (vegetables and rice) to self-serve household consumption. In addition, the number of households with non-farm workers, such as serving at restaurants, decreased due to the social distancing. So that, only seasonal jobs such as construction workers remain, causing a significant decrease in the job supply.

Table 3. Livelihood activities of small farm households

Livelihood activities	2019		2020		Mann-Whitney U Test (2020/2019)
	No of HH	Percentage (%)	No of HH	Percentage (%)	
Agriculture – Wage earning (n=30)					
Crop farming	27	90.0	30	100	0.045**
Animal husbandry	25	83.3	22	73.3	0.087*
Wage-earning	30	100	18	60.0	0.981
Agriculture – Aquaculture (n=30)					
Aquacultural farming	10	33.3	10	33.3	0.771
Fisheries catching	26	86.7	22	73.3	0.278
Crop farming	20	66.7	25	83.3	1.239**
Wage-earning	4	13.3	15	50.0	1.428**
Aquaculture - Tourist services (n=60)					
Fisheries catching	40	66.7	35	58.3	1.121*
Tourist services	60	100	38	63.3	2.718***
Fisheries trading	40	66.7	30	50.0	0.039
Wage-earning	0	0.0	18	30.0	1.28**

*Source: Authors' own calculations from survey data in 2021

*** Confidence 99%; ** Confidence 95%; * Confidence 90%

In the group of agriculture - aquaculture households, aquacultural farming and fisheries catching are the main livelihood activities, especially fisheries catching is the livelihood of more than 86% of the surveyed households before the pandemic. Besides, many households still maintain crop farming activities (rice, vegetables) to provide food for their families (over 66%), and very few households still have wage labourers in their leisure time. Since the COVID-19 pandemic occurred, the structure of livelihood activities of this group has also changed significantly. The number of aquacultural farming households remains the same but the area of aquacultural ponds is reduced because aquacultural farming requires large financial investment, households have to borrow capital from banks or relatives. So that in the difficult situation of epidemics, no household intends to invest more. Similarly, the number of fisheries households also decreased due to the decline of the domestic market and the social distancing orders of the Government that made it difficult to sell seafood. On the contrary, the number of households cultivating rice and vegetables, and wage earning has increased significantly because households consider crop farming as a lifeline during the pandemic, which helps households to be self-sufficient in food and cut down living costs. Moreover, the number of households looking for non-farm jobs increased by nearly 40%. Despite the increase in wage employment, the income for these kinds of jobs is not high because the work is only seasonal. The wage employment only helps the household to slightly improve their income during the epidemic period.
It can be said that the group of aquaculture-tourism service households is the group that suffers the most losses due to the COVID-19 pandemic. Before the epidemic, 100% of the surveyed households did services and tours along with trading seafood products for tourists. Tourists to the locality number in the millions. This is a relatively large source of income for households in each holiday and summer vacation. Nonetheless, the epidemic caused a serious decrease in the number of visitors while the tourism service business required a large amount of money to invest, so nearly 40% of surveyed households had to close their businesses, even some. Householders think they are on the verge of bankruptcy. There were householders who reported that they rented a shop for a period of 5 years, but had encountered nearly 2 years of epidemics. The tourism business faced many difficulties, so they were in danger of bankruptcy. In parallel with the difficulties in the tourism service business, the fisheries catching activities of farmers are also difficult because of the lack of consumption markets, so the number of fisheries catching households also decreased by nearly 10%. Under such difficult conditions, 30% of households with surplus labour had to switch to wage employment to earn a living and pay debts. However, the amount of money earned is only enough for daily living, this group of households also has to spend a large amount of money to pay bank interest, so life becomes more and more difficult.

Household income and livelihood strategies

Compared with 10 other provinces in the Red River Delta of Vietnam, the average income of smallholder farmers in this research site is relatively high because of diverse sources of livelihood, especially the development of agricultural activities and tourism services before the time COVID-19 occurred. The source of household income comes from the main livelihood activities mentioned above. These are incomes from tourism services, wage employment, agricultural farming, aquacultural farming, fisheries catching and seafood trading. Compared with the other two groups of households, the average income of agriculture – wage earning household is the lowest both pre and during the COVID-19 pandemic. In this group, household's income from cattle breeding and wage earning accounted for a higher proportion than income from crop farming before the pandemic. However, during the pandemic, income from wage

employment decreased by more than 16%, instead, households grew more crops, causing the share of income from crop farming to increase to nearly 30% (Table 4). This shows a reasonable shift in the households' livelihood strategies of this group to cope with the impact of the pandemic.
The group of agriculture - aquaculture households has diversified sources of livelihood, especially since the pandemic occurred. The COVID-19 pandemic changed the livelihood strategy of this household group with the increase of crop farming and wage earning activities, and the reduction of aquaculture farming and fisheries catching activities due to the inability to find a consumer market as well as a large financial investment. With this movement, income from crop farming and wage earning accounted for more than 20% of the household income during the pandemic (compared to more than 6% before the pandemic). The timely diversion helps this group of households maintain a higher total income than the other two groups of households.

Table 4. Average household's income from different livelihood strategies (millions VND per household per year)

Income sources	2019		2020		T-test (Income)
	Household income	Income share (%)	Household income	Income share (%)	
Agriculture – Wage earning (n=30)					
Crop farming	39	23.2	34	29.3	0.343*
Animal husbandry	67	39.9	59	50.9	0.189
Wage-earning	62	36.9	23	19.8	1.809**
Total income	168	100	116	100	0.923*
Agriculture – Aquaculture (n=30)					
Aquacultural farming	105	57.7	84	54.9	0.823
Fisheries catching	65	35.7	36	23.5	1.419**
Crop farming	12	6.6	18	11.8	0.539
Wage-earning	0	0	15	9.8	0.825*
Total income	182	100	153	100	1.889**
Aquaculture - Tourist services (n=60)					
Fisheries catching	70	35.5	52	39.1	0.012
Tourist services	127	64.5	28	21.0	0.971*
Fisheries trading	0	0.0	35	26.3	1.081*
Wage earning	0	0.0	18	13.6	1.287**
Total income	197	100	133	100	1.080*

*Source: Authors' own calculations from survey data in 2021
*** Confidence 99%; ** Confidence 95%; * Confidence 90%

If the group of agriculture – wage earning and agriculture - aquaculture households are affected by difficulties in lack of consuming agricultural and aquatic products during the epidemic period, the group of aquaculture - tourism service households has to face the heavy impact from the orders of social distancing that led to no visitors. Income from tourism services decreased by up to 90%, forcing households to increase other livelihood activities such as seafood trading (especially online trading) and wage work. With the agility of young household heads, this group of households has taken advantage of information technology to serve their livelihoods. Selling seafood online is a new and effective idea during the epidemic. Consumers also prefer this form because it is both convenient to buy and ensures the health of themselves and their families.

Conclusion

The COVID-19 pandemic has caused changes in the livelihoods of smallholder farmers in the Red River Delta, Northern Vietnam, in many different directions. In order to adapt to the difficulties caused by the disruption of the consumption chain of agricultural and aquatic products, most households tend to further diversify their livelihood activities to create multiple sources of income. In addition, the COVID-19 pandemic has also changed the structure of crops for farming households and changed the form of fisheries catching and aquacultural farming.

Changes in livelihood activities and livelihood strategies depend not only on the group of the household as analyzed above, but also on the age, sex and education level of the household head. In addition, livelihood resources such as farm land, financial resource, and labour resource are also factors affecting the household's livelihood strategies. The analysis results show that in general, the group of agriculture – wage earning, agriculture - aquaculture households are less affected by the COVID-19 pandemic than the group of aquaculture - tourism service households. In order to adapt to the change of livelihood during the pandemic, each group of households has different difficulties such as lack of production capital, lack of market support and production techniques support from local authorities.

In view of the difficulties and challenges faced by smallholder farmers during the COVID-19 pandemic, this study makes a number of policy recommendations to help households stabilize their livelihoods as follows:

- Firstly, the bank simplifies procedures so that households can easily borrow capital to invest in redirecting or expanding livelihood activities in line with the ongoing epidemic situation in Vietnam.
- Secondly, local authorities need to have solutions to support people in selling agricultural and aquatic products through distribution channels such as cooperatives, local businesses, online agricultural product sales floors, etc.
- Thirdly, it is necessary for small farm households to take advantage of idle time to improve the quality of labour by learning more production techniques and market information to be able to adapt to changes in the market and consumption trends during the pandemic.

References

Bergstedt C. (2012). The Life of the Land: gender, farmwork, and land in a rural Vietnamese village. Unpublished PhD thesis. University of Gothenburg.

Dinh, P. H. (2011). Statistical methods in social-economic research. Hong Duc Publishing House, Hanoi.

FAO (2020). The impact of COVID-19 on fisheries and aquaculture food systems, possible responses. Information paper, November 2020, Rome.

General Statistic Office Vietnam (2019). Vietnam Statistical Year Book. Statistic Publishing House, Hanoi.

Hoang T.B (2009). Rural employment and life: Challenges to gender role in Vietnam's agriculture at present. Working Paper presented at FAO and ILO. Link?

ILO (2020). Ngành dệt may Châu Á – Thái Bình Dương chịu tác động nặng nề do Covid ảnh hưởng toàn bộ chuỗi cung ứng, thông cáo báo chí, ngày 21 tháng 10, Hà Nội (The Asia-Pacific textile industry is heavily impacted by COVID 19 pandemic, a press release, October 21, Hanoi)

.Lê Thái Thị Băng Tâm (2011). Một vài đặc điểm của hộ gia đình sau khi bị thu hồi quyền sử dụng đất canh tác. Tạp chí Xã hội học (Some characteristics of farm households after having their land use rights revoked. Vietnam Sociology Journal). 3(115): 47-57.

Nguyen A.T (2015). Migration and agricultural production in a Vietnamese village. Working Paper No 164 of Max Planck Institute for Social Anthropology. 42: 24.

CONSUMERS' BEHAVIOR TOWARDS FOOD WASTE: A CASE STUDY OF FAYOUM, EGYPT

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Abstract

This paper aims at exploring consumers' behavior towards food waste in Fayoum city, Egypt, through identifying the causes and degree of awareness of Food waste. A survey was collected in January 2021 where 200 respondents from Fayoum Governorate, Egypt. Who each one of them represents a family or household; they were randomly selected and interviewed online. A structured questionnaire was used to investigate whether respondents know about the concept of food waste or not, moreover, who is responsible for food purchasing and the amount of food wasted by the family. The reasons for food waste and the degree of awareness of food waste were investigated, food waste reduction practices. Finally, the impact of some explanatory variables such as Age, Sex, Education, Marital status, Job, Residence. The results showed that among respondents, there are significant differences between age groups, sex, job categories and frequency of purchase regarding knowledge of food waste. The results clarified that respondents considered offers and discounts in supermarket a first cause of high Food waste, they think it's important that I don't waste food and food waste creates economic damage to the society, where, preparing a list of purchases is considered a first practice of food waste reduction. The results of multinomial logistic model revealed that there is a significant effect of education level, residence, shopping frequency, and sex on the knowledge of food waste. The study recommends organizing campaigns to promote food waste reduction among families and students at universities, encouraging food waste recycling for its environmental, social and economic importance and impacts.

Key words: *Consumers' behavior, Food waste, Multinomial logistic model.*

Introduction

Food is considered a necessity for the resumption of people's life, and the issue of food came to be one of the biggest concerns for all countries and governments. Commonly, food consumption is influenced by economic, social, and cultural factors, economic factors comprise individual income, population, prices, availability, etc., where social factors include family size, education level, location, etc., and culture includes beliefs, ideologies, unwritten norms, motivations, attitudes and knowledge about food (Mohamed et al., 2012). Resulting from decisions and actions by retailers, food service providers and consumers, a decrease in the quantity or quality of food is called food waste. Food is wasted in many ways, for example, fresh produce that deviates from what is considered optimal, for example in terms of shape, size and color, is often removed from the supply chain during sorting operations, foods that are close to, at or beyond the "best-before" date are often discarded by retailers and consumers, and large quantities of wholesome edible food are often unused or left over and discarded from household kitchens and eating establishments. According to FAO, around one third of the world's food was lost or wasted every year. Food loss and waste has become an issue of great public concern. The 2030 Agenda for Sustainable Development reflects the increased global awareness of the problem (FAO, 2011). Pervious researches have shown interest to the issue of food loss and waste, in many of them knowledge about food waste and its causes was investigated, the study of (Barr, 2007) stated that individuals with a good knowledge of the problems caused by food waste are more likely to be aware of food waste issue and avoid it. As for demographic and socio-economic variables, many previous studies stated that the higher the income of households, the higher the food wasted and vice versa with poorer households (Buzby and Guthrie, 2002, Brook Lyndhurst, 2007; Koivupuro et al., 2012). The study of Barr (2007) showed that females more careful to reduce waste than males, while other studies found that women waste more than men (Gallo, 1980; Buzby and Guthrie, 2002). This paper focuses on household food waste, which includes food purchased from shops or takeaways and consumed within the home, and it aims at exploring consumers' behavior towards food waste in Fayoum city, Egypt, through identifying the causes and degree of awareness of Food waste, Food Waste Reduction Practices, and the relationship of independent variables and the knowledge of food waste.

Materials and methods

<u>Sampling and Sample Size</u>

The information produced in this research is based on extensive fieldwork, A survey was collected in January 2021 where 200 respondents from Fayoum Governorate, Egypt. Who each one of them represents a family or household; they were randomly selected and interviewed online. A structured questionnaire was used to investigate whether respondents know about the concept of food waste or not, moreover, who is responsible for food purchasing and the amount of food wasted by the family. The reasons for food waste and the degree of awareness of food waste were investigated, food waste reduction practices. Finally, the impact of some explanatory variables such as Age, Sex, Education, Marital status, Job, Residence, having children under 14 years old, Monthly income, shopping responsibility, and shopping frequency on the knowledge or awareness of food waste.

<u>Data Analysis</u>

To describe socioeconomic characteristics of respondents, descriptive statistics was applied. A multinomial logistic model was applied to analyze factors determining food waste knowledge and awareness, in this model, the independent variables can be either dichotomous (i.e., binary) or continuous (i.e., interval or ratio in scale). Multinomial logistic regression is a simple extension of binary logistic regression that allows for more than two categories of the dependent or outcome variable (Starkweather and Moske, 2011, Mohamed and Mahmoud, 2019).

Multinomial logistic regression is a simple extension of binary logistic regression that allows for more than two categories of the dependent or outcome variable (Starkweather and Moske, 2011).

The empirical multinomial logistic model for this study is specified as (Sofoluwe. et al, 2011):

$Y_i = f(X1, X2, ..., X10)$

Where yi, the dependent variable is polychotomous and it is the knowledge or awareness of food waste; (yi) is defined as 1 for (yes I know or I am aware), 2 for (I know or I am aware to some extent), 3 for (I don't know). Xs are the explanatory variables, where: X1 = Age, X2 = Sex, X3= Education, X4= Marital status, X5= Job, X6= Residence, X7= having children under 14 years old, X8= Monthly income, X9= shopping responsibility, X10= shopping frequency.

Results and Discussion

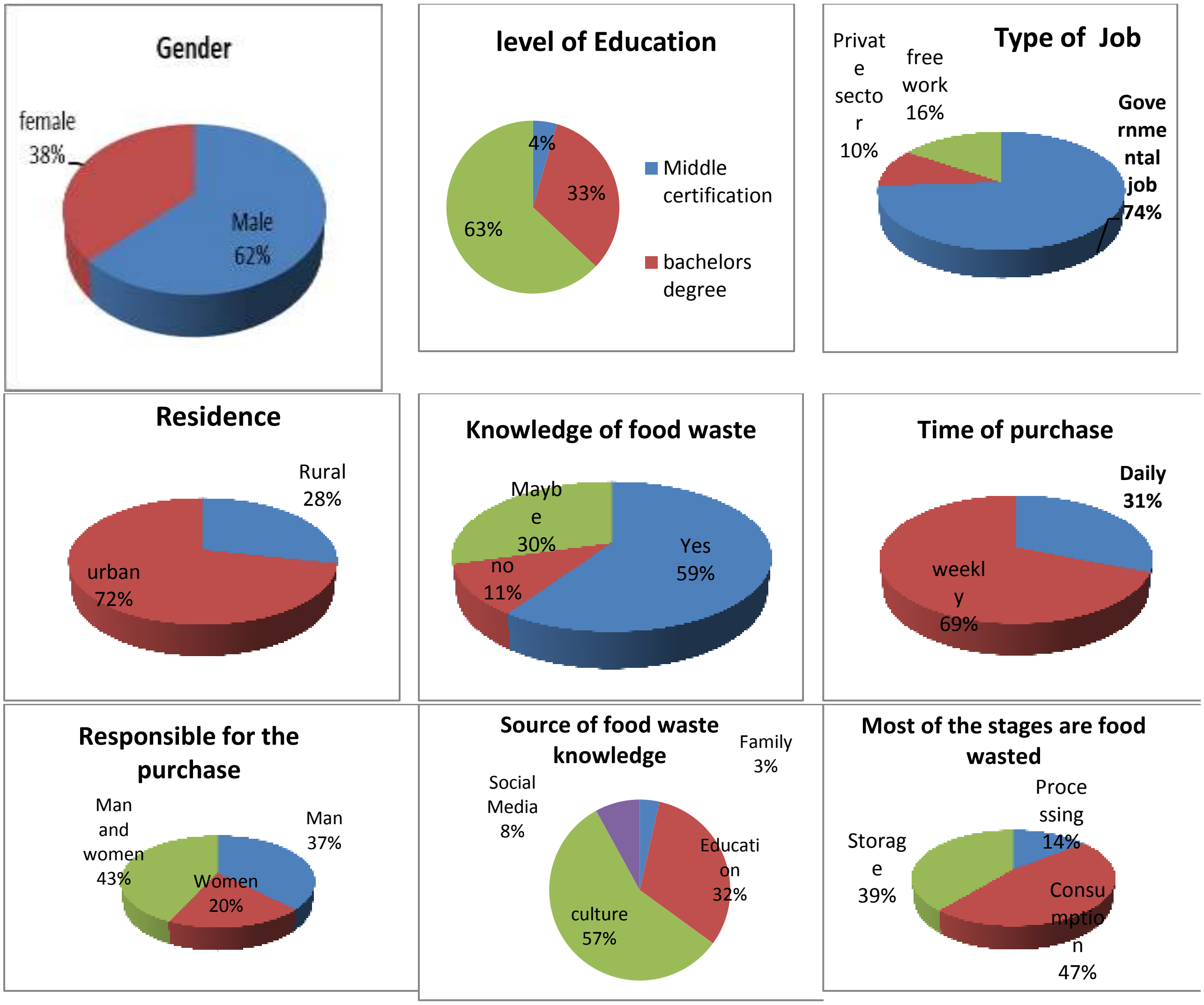
Socioeconomic characteristics

Results showed that 41% of the respondents' age range of 30-39 year, 35% of 40-49 year, and 15% were between 20-29 year, while only 9% of the respondents were in the age of more than 50 years. 5% of respondents' Monthly household income were less than 2000 L.E, 29% 2000 L.E to less than 4000 L.E, 33.5% 4000 L.E to less than 7000 L.E, 32.5% more than 7000 L.E. Results showed that 49% of the respondents interviewed get rid of food debris throw put in basket, 34.5% Transfer the surplus as food for birds, and 16.5% recycle food. Results showed that 62% of the respondents interviewed were Male, 38% were Female. As for the level of education 63% of the respondents interviewed had obtained Postgraduate qualification, 33% had obtained bachelor's degree and 4% had obtained middle certification. 74% of the respondents interviewed are working in Governmental job, 16% free work, while 10% in Private sector. Referring to Residence 28% of respondents live in Rural, 72% live in urban. 59% of the respondents have knowledge of food waste, 30% maybe have knowledge of food waste while 11% don't have knowledge of food waste. 69% of the respondents buy their needs weekly and 31% daily. For source of food waste knowledge 57% was from culture, 32% from Education, 8% from Social Media, and 3% was from Family. As for Most of the food has a percentage of waste Vegetables it the first with 50%, second was baked goods with 36%.

Table 1. Respondents' Socioeconomic Characteristics

Variable		Frequency	Percent
Age	20-29	30	15
	30-39	82	41
	40-49	70	35
	More than 50	18	9
	Total	**200**	**100**
N of family member	Less than 4	30	15
	4-6	153	76.5
	More than 6	17	8.5
	Total	**200**	**100**
Monthly household Income	Less than 2000 L.E	10	5
	2000 L.E to less than 4000 L.E	58	29
	4000 L.E to less than 7000 L.E	67	33.5
	More than 7000 L.E	65	32.5
	Total	**200**	**100**
How to get rid of food debris	Recycle	33	16.5
	put in basket	98	49
	Transfer the surplus as food for birds	69	34.5
	Total	**200**	**100**
percentage of food purchased that is wasted by households	10%	100	50
	20%	62	31
	30%	26	13
	40%	7	3.5
	More than 40 %	5	2.5
	Total	**200**	**100**

*Source: Author s' elaboration based on the questionnaire survey results



*Source: Author s' elaboration based on the questionnaire survey results

Graph (1) Information about Knowledge of Food Waste

Causes of high Food waste

Respondents interviewed were asked to rank the Causes of high Food waste according to the degree of approval. Offers and discounts in the supermarket is considered a first Causes of high Food waste which ranked by respondents with a percentage of 65.7%, I drop off items that are nearing the expiration date comes in second place with 64.8%, while Package sizes for some commodities are large compared to the requirement come in third place with 64.7%, and Lack of awareness of the economic importance of Food waste comes in fourth place with 63.5%. Not planning ahead for the shopping process comes in fifth place with 60%, while as the family income increases, I buy large amounts of food comes in sixth place with 59.8%.

Table 2. Causes of high Food waste

Statements Or variable	Degree of approval										Total marks	Weighted percentage% = (Total marks/1000 (200*5))*100	Rank
	Strongly disagree		Moderately disagree		Neither agree nor disagree		Moderately agree		Strongly agree				
	F	%	F	%	F	%	F	%	F	%			
Offers and discounts in the supermarket	7	3.5	41	20.5	65	32.5	62	31	25	12.5	657	65.7	1
Not planning ahead for the shopping process	15	7.5	65	32.5	46	23	53	26.5	21	10.5	600	60	5
I drop off items that are nearing the expiration date	9	4.5	65	32.5	31	15.5	59	29.5	36	18	648	64.8	2
As the family income increases, I buy large amounts of food	16	8	61	30.5	46	23	63	31.5	14	7	598	59.8	6
Lack of awareness of the economic importance of Food waste	16	8	57	28.5	34	17	62	31	31	15.5	635	63.5	4
Package sizes for some commodities are large compared to the requirement	8	4	51	25.5	48	24	72	36	21	10.5	647	64.7	3

Note: total mark (frequency*mark), mark= from 5 to 1, 5=ranked first, 1=ranked last.
*N= number of respondents answered each statement,
(Strongly agree =5, moderately agree =4, neither agree nor disagree =3, moderately disagree =2, strongly disagree =1)
*Source: Author s' elaboration based on the questionnaire survey results

Degree of awareness of Food waste

Respondents interviewed were asked to rank the Degree of awareness Food waste according to the degree of approval. I think it's important that I don't waste food is considered a first Statements It indicates the degree of awareness of Food waste which ranked by Respondents with a percentage of 96.7%, Food waste creates economic damage to the society comes in second place with 92.5%, while Food waste is harmful to the environment come in third place with 86.2%, and I would probably throw away less food if I had more information about Damage of Food waste comes in fourth place with 71%. I think it is better to throw away leftovers rather than to risk of use comes in fifth place with 45%, while I am not worry about the amount of food thrown away comes in sixth place with 42.5%, while I am not worried about the cost of food that I throw away comes in seventh place with 39.7%, finally Food waste is not a problem for the environment as it is natural comes in sixth place with 37.5%.

Table 3. Degree of awareness of Food waste

Statements Or variable	Degree of approval										**Total marks**	**Weighted percentage% = (Total marks/1000 (200*5))*100**	**Rank**
	Strongly disagree		Moderately disagree		Neither agree nor disagree		Moderately agree		Strongly agree				
	F	%	F	%	F	%	F	%	F	%			
I think it's important	0	0	0	0	1	0.5	31	15.5	168	84	967	96.7	1
Food waste is harmful to	2	1	13	6.5	16	8	59	29.5	110	55	862	86.2	3
Food waste creates	0	0	2	1	3	1.5	63	31.5	132	66	925	92.5	2
Food waste is not a	76	38	94	47	13	6.5	14	7	3	1.5	374	37.5	8
I think it is better to throw away leftovers	48	24	90	45	33	16.5	22	11	7	3.5	450	45	5
I am not worry about	53	26.5	92	46	34	17	19	9.5	2	1	425	42.5	6
I am not worried	59	29.5	106	53	18	9	13	6.5	4	2	397	39.7	7
I would probably throw away	6	3	24	12	50	25	94	47	26	13	710	71	4

Note: total mark (frequency*mark), mark= from 5 to 1, 5=ranked first, 1=ranked last.
*N= number of respondents answered each statement,
(Strongly agree =5, moderately agree =4, neither agree nor disagree =3, moderately disagree =2, strongly disagree =1)
*Source: Author s' elaboration based on the questionnaire survey results

Food Waste Reduction Practices

Respondents interviewed were asked to rank Food Waste Reduction Practices according to the degree of approval. Preparing a list of purchases is considered a first Practices of Food Waste Reduction which ranked by Respondents with a percentage of 91.4%, Implement good storage practices comes in second place with 89.8%, while Advance meal planning comes in third place with 88.7%, and Planning before the process of shopping comes in fourth place with 88%. To differentiate between the phrases "good before", "consumed before" comes in fifth place with 82.3%, while limited family budget comes in sixth place with 76.6%.

Table 4. Food Waste Reduction Practices

Statements Or variable	Degree of approval										Total marks	Weighted percentage% = (Total marks/1000 (200*5))*100	**Rank**
	Strongly disagree		Moderately disagree		Neither agree nor disagree		Moderately agree		Strongly agree				
	F	%	F	%	F	%	F	%	F	%			
Planning before the process of shopping	1	0.5	3	1.5	15	7.5	77	38.5	104	52	880	88	4
Advance meal planning	0	0	4	2	7	3.5	87	43.5	102	51	887	88.7	3
Preparing a list of purchases	0	0	0	0	3	1.5	80	40	117	58.5	914	91.4	1
Implement good storage practices	1	0.5	1	0.5	6	3	83	41.5	109	54.5	898	89.8	2
To differentiate between the phrases "good before", "consumed before"	0	0	3	1.5	31	15.5	106	53	60	30	823	82.3	5
limited family budget	4	2	19	9.5	36	18	89	44.5	52	26	766	76.6	6

Note: total mark (frequency*mark), mark= from 5 to 1, 5=ranked first, 1=ranked last.
*N= number of respondents answered each statement,
(Strongly agree =5, moderately agree =4, neither agree nor disagree =3, moderately disagree =2, strongly disagree =1)
*Source: Author s' elaboration based on the questionnaire survey results

The relationship of independent variables and the knowledge of food waste

Chi-Square was used to test if there are significant differences between the independent variables and knowledge of food waste. Regarding age of respondents, the calculated output value of Chi-square ($\chi2$) is 11.86, significant at the 5% level of normality, this means, among respondents, there are significant differences between age groups regarding knowledge of food waste. The calculated output value of Chi-square ($\chi2$) for the variable Sex is 6.1, significant at the 5% level of normality which means, among respondents, there are significant differences between men and women regarding knowledge of food waste. Regarding job of respondents, the calculated output value of Chi-square ($\chi2$) is 11.97, significant at the 1% level of normality, which means, among respondents, there are significant differences between job categories and knowledge of food waste. Finally, value of Chi-square ($\chi2$) for the frequency of purchase is 10.79, significant at the 1% level of normality which means, among respondents, there are significant differences between those who buy products daily or weekly regarding knowledge of food waste.

Table 5 . The relationship of independent variables and the knowledge of food waste

Variable	Categories	Yes	To what extend	No	Total	χ2 Value	P % value
Age	20-29	13	15	2	30	11.86*	0.05
	30-39	39 21	21	10	70		
	40-49	15	2	1	18		
	More than 50	52	21	9	82		
Sex	Female	43	28	4	75	6.1**	0.04
	Male	76	31	18	125		
Job	Government	96	38	14	148	11.97**	0.016
	Private sector	13	5	3	21		
	free works	10	16	5	31		
Frequency of purchase	Daily	29	20	13	62	10.79**	0.005
	Weekly	90	39	9	138		

** Values are significant at P = 0.01, * Values are significant at P = 0.05

*Source: Author s’ elaboration based on the questionnaire survey results

Results of the multinomial logistic model

Holding all explanatory variables mentioned above except education level, the odds for a respondent with university degree of being knowing about the term food waste rather than those who don’t know about it are 0.166 times (83.4% higher than) the odds for a respondent with lower education. In other words, the relative log odds of being knowing what food waste versus no knowledge of it is will decrease by 1.8 if moving from the highest level of education (university degree) to the lowest level (high school). Holding all explanatory variables mentioned above except residence, the odds for a respondent who lives in urban areas of being know about the term food waste rather than those who don’t know about it are 0.335 times (66.5% higher than) the odds for a respondent lives in rural areas. In other words, the relative log odds of being knowing what food waste versus no knowledge of it is will decrease by 1.1 if living in rural areas rather than living in urban areas. Holding all explanatory variables mentioned above except marketing frequency, the odds for a respondent who buy products weekly of being know about the term food waste rather than those who don’t know about it are 0.315 times (68.5% higher than) the odds for a respondent who buy products every day. In other words, the relative log odds of being knowing what food waste versus no knowledge of it is will decrease by 1.15 if moving from buying weekly to buying every day. Holding all explanatory variables mentioned above except Sex, the odds for a female respondent being knowing about the term food waste to some extent rather than those who don’t know about it are 5.132 times (4132% higher than) the odds for a male respondent. In other words, the relative log odds of being knowing what food waste to some extent versus no knowledge of it is will increase by 1.6 if a respondent is a female rather than a male. Holding all explanatory variables mentioned above except residence, the odds for a respondent who lives in urban areas of being know about the term food waste to some extend rather than those who don't know about it are 0.250 times (75% higher than) the odds for a respondent lives in rural areas. In other words, the relative log odds of being knowing what food waste to some extend versus no knowledge of it is will decrease by 1.39 if living in rural areas rather than living in urban areas. Holding all explanatory variables mentioned above except marketing frequency, the odds for a respondent who buy products weekly of being know about the term food waste to some extend rather than those who don't know about it are 0.043 times (95.7% higher than) the odds for a respondent who buy products every day. In other words, the relative log odds of being knowing what food waste to some extend versus no knowledge of it is will decrease by 1.23 if moving from buying weekly to buying every day.

Table 6 . Results of the multinomial logistic model

Parameter Estimates									
Food waste knowledge (dependent)		**B**	**Std. Error**	**Wald**	**df**	**Sig.**	**Exp(B)**	**95% Confidence Interval for Exp(B)**	
								Lower Bound	**Upper Bound**
yes	Intercept	3.182	.850	14.012	1	.000			
	[Education=high school]	-.129	1.500	.007	1	.932	.879	.046	16.623
	[Education=university degree]	-1.797	.699	6.610	1	**.010**	.166	.042	.652
	[Education=Master or higher]	0[b]	.	.	0	.	.	.	.
	[Residence=Rural]	-1.095	.653	2.808	1	**.094**	.335	.093	1.204
	[Residence=Urban]	0[b]	.	.	0	.	.	.	.
	[shopping frequency = everyday]	-1.154	.574	4.038	1	**.044**	.315	.102	.972
	[shopping frequency = weekly]	0[b]	.	.	0	.	.	.	.
To some extent	Intercept	1.251	.938	1.778	1	.182			
	[Sex= female]	1.635	.719	5.180	1	**.023**	5.132	1.255	20.987
	[Sex= male]	0[b]	.	.	0	.	.	.	.
	[Residence= Rural]	-1.387	.704	3.879	1	**.049**	.250	.063	.993
	[Residence= Urban]	0[b]	.	.	0	.	.	.	.
	[shopping frequency = Everyday]	-1.231	.608	4.101	1	**.043**	.292	.089	.961
	[shopping frequency = weekly]	0[b]	.	.	0	.	.	.	.

a. The reference category is: no knowledge of food waste.
b. This parameter is set to zero because it is redundant.
** and * are significant levels at 1% and 5% probabilities, respectively.

Prob > chi2 = 0.008   Log Likelihood= 281
Pseudo R2= (Cox and Snell= 0.267, Nagelkerke= 0.319, McFadden= 0.171)
*Source: Author s' elaboration based on the questionnaire survey results

Conclusion

The study examines consumers' behavior towards food waste in Fayoum city, Egypt, through identifying the causes and degree of awareness of Food waste. The results revealed that offers and discounts in the supermarket and dropping off items that are nearing the expiration date comes, and package sizes for some commodities are large compared to the requirement are considered a main cause for food waste, according to respondents, Food waste creates economic damage to the society and it is harmful to the environment, and they probably will throw away less food if they had more information about damage of Food waste. Preparing a list of purchases and implement good storage practices are considered the most important practices of Food Waste reduction. The results revealed that there is a significant effect of education level, residence, shopping frequency, and sex on the knowledge of food waste. Accordingly, the study recommends organizing campaigns to promote food waste reduction among families and students at universities, encouraging food waste recycling for its environmental, social and economic importance and impacts.

References

Barr, S. (2007), "Factors influencing environmental attitudes and behaviors: a UK case study of household waste management", Environment and Behavior, Vol. 39 No. 4, pp. 435-473.

Brook Lyndhurst (2007), "Food behavior consumer research – findings from the Quantitative Survey", briefing paper, WRAP, Banbury.

Buzby, J.C. and Guthrie, J.F. (2002), "Plate Waste in School Nutrition Programs", final report to Congress, ERS E-FAN-02-009, USDA, Washington, DC.

Gallo, A.E. (1980), "Consumer food waste in the US", National Food Review, Fall, pp. 13-16.

Greene, W. (2012), Econometric Analysis, Prentice Hall, Upper Saddle River, NJ.

Koivupuro, H.K., Hartikainen, H., Silvennoinen, K., Katajajuuri, J.M., Heikintalo, N., Reinikainen, A. and Jalkanen, L. (2012), "Influence of socio-demographical, behavioural and attitudinal factors on the amount of avoidable food waste generated in Finnish households", International Journal of Consumer Studies, Vol. 36 No. 2, pp. 183-191.

Mohamed, A., Mahmoud, A.A. (2019). "Farmers' Perception and Adaptation to Climate Change: Multinomial Logistic Model Evidence", SSRG International Journal of Economics and Management Studies (SSRG-IJEMS) – Vol. 6 No. 10, pp. 143-151.

Mohamed, M.A., Chymis, A. and Shelaby, A.A. (2012), "Determinants of organic food consumption in Egypt", International Journal of Economics and Business Modeling, Vol. 3 No. 3, pp. 183-191.

Food Loss and Food Waste, Food and Agriculture. Organization (FAO), available at: http://www.fao.org/food-loss-and-food-waste/flw-data

Sofoluwe, N. A., Tijani, A. A. and Baruwa, O. I. (2011), Farmers' perception and adaptation to climate change in Osun State, Nigeria. African Journal of Agricultural Research Vol. 6(20), pp. 4789-4794.

Starkweather, J. & Moske, A. K. (2011). Multinomial Logistic Regression. Available at: https://it.unt.edu/sites/default/files/mlr_jds_aug2011.pdf, June, 2019.

IMPACTS OF FIRST WAVE OF THE COVID 19 ON THE AGRICULTURAL PRODUCTION IN TURKEY: ASSESSING THE ROLES OF FARMERS DURING THE PANDEMIC

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Abstract

The current study provides potential expectations for the adverse effects of first wave of the COVID-19 on farmers in Turkey and also describes farmers' responses to mitigate the adverse effects pandemic on the agricultural sector. The study is based on telephone surveys with 2125 farmers in 22 provinces during April and May in 2020 in Turkey. This communication illustrates the possible implications and expected outcomes of the outbreak of coronavirus. Multistage cluster sampling was used as the sampling method in the study. Simple descriptive statistics and Likert scale averages were used to better understand smallholder farmer perceptions regarding the anticipated impacts of the pandemic. The results showed that 61.98% of smallholder farmers would not make any changes in their current agricultural production activities due to Covid-19 restrictions, while 32.14% stated that they were undecided, and another 5.88% reported they were willing to make changes. We identified contrasting responses by the agricultural production mostly driven in animal and crop production. In addition, it was determined that production costs increased in agricultural enterprises due to the excessive increase in input prices. It has been also concluded that the transition of farmers from animal and crop production to other production systems will accelerate in cases. Therefore, the decisions taken by policymakers to handle pandemic's direct and indirect effects can help smallholder farmers draw lessons on how to cope with input prices.

Keywords: *Agricultural production, First wave of COVID pandemic, Impact, Smallholder farmers, Turkey.*

Introduction

The dangerous COVID-19 pandemic, which emerged in Wuhan, China on 1 December 2019 and spread rapidly, adversely affected human life and economic areas all over the world (World Health Organization, 2020). Therefore, as a result of the high number of cases and deaths that emerged after the spread rate and strength of the COVID-19 virus, partial restrictions on economic and social life, and then unprecedented limitations in human history were introduced (Huss et al., 2021; Kumar et al., 2021). As a result of the COVID-19 pandemic and quarantine measures, the whole economic activities, including agriculture, have been significantly affected (Hossain, 2020; Inegbedion, 2020). Pu and Zhong (2020) reported that agricultural production inputs were blocked as a result of COVID-19 restrictions and thus production cycles were disrupted. This has caused many risks in agricultural production due to the COVID-19 pandemic. It has been demonstrated by various studies that it is necessary to be prepared against these risks in the coming years (FAO, 2020; Yoshida and Yagi, 2021). It was stated that if these measures were not taken, there would be lower agricultural production and further increases in food prices in the future (Cevher et al., 2021; Obayelu et al., 2021). World agricultural policy experts reported that the COVID-19 pandemic was causing major pressures on agricultural production (OECD, 2020). They also stated that most of these pressures were due to the policies implemented by the governments to contain the spread of the pandemic (Fei et al., 2020; FAO, 2020). It has been reported in a similiar study that COVID-19 caused a decrease in farmer income and an increase in rural poverty due to its effects on agricultural production branches (Xiao and Torok, 2020; Zhang et al. 2020). On the other hand, many countries have taken quick decisions to prevent COVID-19 restrictions, and thanks to these decisions, they have reported that there is no significant food shortage in the world yet (Gray 2020; Laborde et al., 2020).

The first confirmed case of COVID-19 in Turkey emerged on 28 February 2020, after which the first case of death was reported on March 28, 2020. Therefore, restrictive decisions were taken on 23 March 2020 in order to prevent the COVID-19 pandemic by the Turkish Ministry of Internal Affairs. In order to reduce the negative impact of the decisions taken on agriculture, Turkish Republic (TR) Ministry of Internal Affairs issued a circular on 25 March 25 2020. Thanks to this circular, it was possible to carry out agricultural production without interruption. Turkey has 23.1 million hectares of usable agricultural land. In this respect, agriculture has a multifaceted effect on Turkey's economic and social development. This multifaceted effect is quite remarkable in terms of providing the country's people with food, contributing to employment, continuing agricultural exports, providing raw materials to industry and contributing to national income (Cevher et al., 2021). Therefore, it is important to investigate the effects of the COVID-19 pandemic on the Turkish agricultural economy. Although there are many studies on the impact of the COVID-19 pandemic on agriculture in Turkey until the date of the article was written (Akın et al., 2020; Ceylan and Özkan, 2020; Akbudak and Özgür 2021), there are almost no field studies examining and showing the effect of the restrictions applied for the COVID-19 pandemic on agricultural production branches. For this reason, it is thought that our findings obtained in the study can be a role model in determining the policies to be implemented in the face of similar risks that may be encountered in the future of the COVID-19 pandemic experience and struggle. With this study, it was aimed to determine the effect of the first restriction measures taken to prevent the COVID-19 pandemic in Turkey on agricultural production branches.

Material and Method

The primary data of this study was obtained by telephone survey. Data has been collected from the seven regions namely Eastern Anatolia, Central Anatolia, Black Sea, Mediterranean, Aegean, Marmara, and Southeastern Anatolia in Turkey. The survey was carried out in selected 22 cities in seven geographical regions of Turkey. Survey studies in the research area were carried out between May-June 2020. Cities with a high share in terms of the amount of plant and animal production in each region were selected. Convenience sampling method was used in data collection. Pandemic conditions were influential in the selection decision of this sampling method. According to Acharya et al. (2013), convenience sampling is the most commonly used sampling method. The sample is chosen on the basis of the convenience of the researcher. Farmers (2125) were interviewed by telephone survey. Before participating in the telephone survey, the farmers were asked whether they had consented to participate or not. The design of the questionnaire used in the survey is shown in Table 1.

Table 1. Summary of the survey design and questions

Section Name	Question	Question Type(s)	Possible Responses
Consent	Q1.	Willingness to participate	Will participate/won't
Demographics	Q2	Relationship and activity with farmer organisations; age; gender; district; household size	Yes/no; amount of time; male, female, prefer not to say; age range; open-ended; household size
Farming Systems	Q3	Which best describes your farming system	Crops, Livestock, Mixed farming
The tendency of farmers to change in agricultural production during the pandemic period	Q4	Do you have a tendency to make a change in your agricultural production decision due to pandemic conditions?	Yes, No, Undecided
The level of participation of farmers in possible risks and uncertainties that may affect agricultural production during the pandemic period	Q5	Increase in input costs, Insufficient government supports, Problems in product sales, Problems in labor supply, Problem of access to agricultural land	1: Strongly disagree - 5: Strongly agree

Opinions of the farmers on possible risks and uncertainties that may affect agricultural production during the pandemic were presented with 5-point Likert scale averages. In this study, non-parametric tests were used to determine whether the analyzed data differed among farmers engaged in crop, animal and mixed production. For this purpose, Chi-square independence test was applied for the data obtained by counting and Kruskal-Wallis test was applied for the means. IBM SPSS 22 package program was used in the analysis of the data.

Results and Discussion

One of the most important questions to be answered is to what extent the COVID-19 pandemic can affect the farmers' agricultural production activities. For this purpose, the farmers were asked whether they would make any changes in their agricultural production during the pandemic period or not. Majority of the farmers (61.98%) stated that they would not make a change in their basic agricultural production activities, 32.14% of the farmers were undecided about making changes and 5.88% of the farmers stated that they would make changes (Table 2). The farmers who were undecided about making changes in the basic agricultural production branch stated that they would decide according to input access and changes in input prices. Middendorf et al. (2021) reported that due to the COVID-19 pandemic, the farmers reduced their production and they would turn to different production branches according to the change in the input prices. In another study, they mentioned that 30% of farmers would turn to other activities on their farms as a risk management strategy due to the COVID-19 pandemic (Yoshida and Yagi, 2021). According to Pan et al. (2020) identified that COVID-19 did not have a significant impact on grain production and the change in grain branch would be difficult. In a similar study by Tougeron and Hance (2021) that the fruit and vegetable sector in Europe was the most affected agricultural branch during the pandemic period and that the change from these production branches would be higher.

Table 2. Tendency of farmers to make changes in agricultural production during the COVID-19 Pandemic period

Tendency	Herbal production		Livestock		Mixed production		General		χ^2 Value	p Value
	n	%	n	%	n	%	n	%		
Doesn't think change	1040	68.69	65	45.14	212	45.40	1317	61.98	127.940	0.000*
Undecided on change	377	24.90	75	52.08	231	49.46	683	32.14		
Thinking of change	97	6.41	4	2.78	24	5.14	125	5.88		
Total	1514	100.0	144	100.0	467	100.00	2125	100.0		

*Statistically significant at 1% significance level

At the first beginning of the COVID-19 pandemic, the tendency of farmers to make changes in agricultural production branches was examined separately in terms of agricultural enterprises engaged in crop, animal and mixed production. It has been determined that there is a statistically significant difference of opinion on whether or not to make changes among the agricultural production branches examined. A significant portion (68.69%) of the farmers engaged in crop production stated that they would not make any changes in their agricultural production activities. However; The ratio of those who are undecided about changes in agricultural enterprises with animal and mixed production was found to be around 50%. It has been determined that the intensive use of labor in animal production activities is effective on this result. Vidaurreta et al. (2020) reported that although the COVID-19 pandemic had negative effects on livestock, its impact on employment was not significant. Zhang (2020) stated that animal husbandry was under serious pressure due to the shortage of agricultural labor and raw materials and insufficient animal feed during the epidemic period. In order to reduce these negative effects, it is important to create a network that connects buyers and sellers through a digital platform in order to organize them. Thus, farmers can have faster access to inputs and market information.

According to the results of this study, it has been concluded that it is unlikely that farmers will make a change in their basic production activities in a short time. It has been determined that farmers' habits from the past, soil and climatic conditions are effective in this situation. Gerasimov et al. (2020) reported in their study that organic tea producers did not make any changes in their production decisions due to the COVID-19 outbreak. Pu and Zhong (2020) stated that agricultural production cycles were disrupted and production capacity was weakened due to COVID-19 restrictions, which accelerated the process of making changes in agricultural production branches. During the COVID-19 epidemic in Turkey, grain producers showed less behavioral changes than producers in other branches. This situation is thought to have been influenced by the government's announcement of grain prices right after the COVID-19 outbreak. Therefore, it is thought that it would be beneficial for the government to announce the production and price policies of agricultural products before the production period. Thus, it will be possible to prevent negative behavior changes in the decisions of the farmers regarding the branches of production, albeit partially.

In this study, farmers' views on possible risks and uncertainties that may affect agricultural production during the COVID-19 pandemic period were also examined. In this context, some suggestions were directed to the farmers during the survey study. The level of farmers' participation in these propositions is shown in Table 3. According to likert scale averages, the most worrying issue for the farmers during the COVID-19 pandemic period is the possibility of insufficient agricultural support. Therefore, it can be said that it would be beneficial to reconsider agricultural support policies during the COVID-19 pandemic. The possibility of rising input costs is the second most important issue for the farmers during the pandemic period. Therefore, it may be beneficial to facilitate border procedures on key inputs such as fertilizers, veterinary drugs and pesticides to prevent rapid rise in agricultural input prices. The third issue that farmers were most concerned about was the possibility of the problems in the sales of the products produced. In this context, access to the buyer and logistics are seen as the most important problems. According to the farmers in our country, finding labor force during the pandemic period was not seen as a very important problem. In this situation, 80.7% of agricultural enterprises in Turkey has an impact on the small business structure (TurkStat, 2019). It can be said that it is easier to obtain labor from neighbors and relatives in such enterprises. However, it is known that the level of social solidarity, neighborly relations and cooperation is high in Turkey (Cevher et al., 2021). In conclusion, it can be said that all of these factors contribute to the reduction of behavioral change on agricultural production. Bochtis et al. (2020) In a post-COVID-19 study, they reported that the annual income of 54% of agricultural workers is at medium and high risk. In our study findings, this risk factor was found to be 17.5%. It can be said that the COVID-19 measures taken by the government were effective in keeping this result at a low level. COVID-19 has impacted the agricultural workforce, especially the pool of seasonal agricultural workers. These are often migrant workers typically employed in the crop harvesting, who use highly dexterous and physical skills (Benos et al., 2020; Mitaritonna and Ragot 2020). These consequences have particularly affected vegetable and fruit producers as well as garden nurseries and horticulture (OECD, 2020). Labor supply constraints could stimulate innovations resulting in further mechanisation within the fruit and vegetables and meatpacking sectors (Orden, 2020).

Farmers' views on possible risks and uncertainties that may affect agricultural production during the pandemic were also examined within the scope of agricultural enterprises engaged in crop, animal and mixed production. According to the Kruskal-Wallis test, a statistically significant difference was found between the responses of the three groups to the statements ($p<0.01$). The most important problem of the farmers engaged in the crop production was the support. It was determined that the sudden increase in fertilizer and crop medicine prices had an effect on this situation. In the enterprises engaged in animal production and mixed production, the concern of increasing input costs came to the fore. The most important cost element of animal production enterprises was feed costs. Owners of livestock farms thought that there would be a decrease in the production of forage crops and this would be reflected in feed prices. The fact that the number of farmers benefiting from subsidies in crop production is quite high, causes these farmers to be more sensitive to subsidies. In the initial period of the pandemic, the livestock industry was severely damaged and farmers suffered significant economic losses due to the COVID-19 measures in transportation to feed mills, animal shipments and slaughterhouses. In order to prevent this negative situation, the government soon published regulations stating that animal producers are exempt from the restriction. This enabled the producers to return to their normal production activities, thus eliminating further economic losses. As Thilmany et al. (2021) reported that animal production activities were adversely affected due to COVID-19.

In order to reduce the negative effects of the Covid-19 pandemic and similar epidemics, especially agricultural cooperatives and local municipalities should be more involved in the production, distribution and consumption system. This will shorten the supply chains between producers and consumers. This will contribute to the better evaluation of the produced product and the consumer to obtain products at an affordable price. Our findings show that especially vegetable, fruit and livestock farmers are generally struggling against short-term shocks, accepting lower prices to maintain their sales, interruptions in the flow of products to consumers, and thus increasing food prices in the country. Therefore, it has made it mandatory for governments to develop new policies against COVID-19 and future similar pandemics. In this study, important data are presented for the formation of agricultural policies to prevent the negative effects in agricultural production activities in case of COVID-19 pandemic.

Table 3. The level of participation of farmers in possible risks and uncertainties that may affect agricultural production during the pandemic period

Statements	Herbal production		Livestock		Mixed production		General		K-W test p value
	$\bar{x}$	Std Dev	$\bar{x}$	Std Dev	$\bar{x}$	Std Dev	$\bar{x}$	Std Dev	
Rising input costs	3.08	0.8208	3.97	0.4791	3.68	0.6136	3.27	0.8215	.000*
Insufficient agricultural support	3.28	0.6126	3.16	0.9287	3.54	0.6779	3.33	0.6626	.000*
Market problem	2.91	1.0889	3.83	0.4153	3.63	0.6157	3.13	1.0299	.000*
Agricultural labor supply problem	2.78	1.0857	2.63	0.7911	2.20	0.6312	2.65	1.0127	.000*
The problem of transportation to the agricultural area	2.09	1.0654	1.87	0.9377	1.65	0.7301	1.98	1.0092	.000*

$\bar{x}$: likert scale mean; 1: Strongly disagree.5: Strongly agree; Std Dev: standard deviation;
*Statistically significant at 1% significance level

Conclusions

Study results reflect perceptions and concerns of the COVID-19 pandemic in agricultural systems. It shows the necessity of developing new agricultural policies by addressing these concerns. COVID-19 restrictions have been effective in the increase in input prices, the disruption of livestock trade in animal husbandry and the increase in feed prices. Even if the impact of the pandemic decreases in the upcoming period, it is important to make the agricultural sector more resistant to pandemics and similar shocks. The fact that the epidemic caused sudden and rapid increases in agricultural input prices seriously threatens agricultural production. It does not seem possible for the farmer to make changes between agricultural production systems in a short time. It has been concluded that if the effect of the pandemic lasts for a long time and the necessary agricultural policies are not taken, there will be more transitions to other agricultural systems, especially in animal and vegetable production. Our results show that restrictions will not adversely affect agricultural production, disrupt production cycles and ultimately undermine productive capacity. However, it has been concluded that if the sudden increase in agricultural production input costs cannot be prevented, there will be transitions between agricultural production branches and production amounts will decrease. It is expected that these study experiences in Turkey will make suggestions to other countries, especially developing countries, which have serious problems in agricultural production during the pandemic period.

References

Acharya A.S., Prakash A., Saxena P., Nigam A. (2013). Indian Journal of Medical Specialities. 4(2), 330-333.

Akbudak N., Şen Ö. (2021). GLOBALGAP in the COVID-19 Epidemic Process. Turkish Journal of Agricultural Research, 8(2), 248-255.

Akın Y., Celen B., Çelen M.F., Karagöz A. (2020). Agriculture and pandemic: how should Turkish agriculture change after covıd-19? EJONS International Journal on Mathematic. Engineering and Natural Sciences, 36 (4), 904-914.

Benos L., Tsaopoulos D., Bochtis D. (2020). A Review on Ergonomics in Agriculture. Part I: Manual Operations. Applied Sciences, 10, 1905.

Bochtis D., Benos L., Lampridi M., Marinoudi V., Pearson S., Sorensen C.G. (2020). Agricultural Workforce Crisis in Light of the COVID-19 Pandemic. Sustainability, 12(19), 8212.

Cevher C., Altunkaynak B., Gürü M. (2021). Impacts of COVID-19 on Agricultural Production Branches: An Investigation of Anxiety Disorders among Farmers. Sustainability, 13(9), 5186.

Ceylan R.H., Özkan B. (2020). Assessing impacts of COVID-19 on agricultural production and food systems in the World and in Turkey. Gaziantep University Journal of Social Sciences, Special Issue, 472-485.

FAO. (2020). COVID-19 Pandemic-impact on food and agriculture food and agriculture organization of the United Nations. Available online: http://www.fao.org/2019-ncov/q-and-a/impact-on-food-and-agriculture/en/ (accessed on 4 August 2020).

Fei S., Ni J., Santini G. (2020). Local food systems and COVID-19: An insight from China. Resources. Conservation And Recycling, 105022.

Gray R.S. (2020). Agriculture, transportation and the COVID-19 crisis. The Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie, 68, 239-243.

Hossain S.T. (2020). Impacts of COVID-19 on the agri-food sector: food security policies of Asian productivity organization members. The Journal of Agricultural Sciences-Sri Lanka, 15(2), 116-132.

Huss M., Brander M., Kassie M., Ehlert U., Bernauer T. (2021). Improved storage mitigates vulnerability to food-supply shocks in smallholder agriculture during the COVID-19 pandemic. Global Food Security, 28, 100468.

Inegbedion, H.E. (2020). "COVID-19 lockdown: implication for food security". Journal of Agribusiness in Developing and Emerging Economies. https://doi.org/10.1108/JADEE-06-2020-0130.

Kumar A., Padhee A.K., Kumar S. (2021). How Indian agriculture should change after COVID-19. Food Securty, 12, 837–840.

Laborde D., Martin W., Swinnen J., Vos R. (2020). COVID-19 risks to global food security. Science. 369: 500-502.

Middendorf A., Faye A., Middendorf G., Stewart Z.S., Jha P.K., Prasad P.V.V. (2021). Smallholder farmer perceptions about the impact of COVID-19 on agriculture and livelihoods in Senegal. Agricultural Systems 190:103108.

Mitaritonna C., Ragot L. (2020). After Covid-19, will seasonal migrant agricultural workers in Europe be replaced by robots? Research and Expertise on the Wold Economy, Policy Brief No, 33.

Obayelu A.E., Obayelu O.A., Bolarinwa K.K., Oyeyinka R.A. (2021). Assessment of the ımmediate and potential long-term effects of COVID-19 outbreak on socioeconomics, agriculture, security of food and dietary ıntake in Nigeria. Food Ethics, 6, 5.
OECD. (2020). COVID-19 and the food and agriculture sector: Issues and Policy Responses; OECD, Paris. France.
Orden D. (2020). Resilience and vulnerabilities of the North American food system during the Covid- 19 pandemic. British Association for the Advancement of Science, https://doi.org/10.1111/1746-692X.12273
Pan D., Yang J., Zhou G., Kong F. (2020). The influence of COVID-19 on agricultural economy and emergency mitigation measures in China: A text mining analysis. PLoS ONE 15(10), e0241167.
Pu M., Zhong Y. (2020). Rising concerns over agricultural production as COVID-19 spreads: lessons from China. Global Food Security, 26, 100409.
Thilmany S., Canales E., Low S.A., Boys K. (2021). Local food supply chain dynamics and resilience during COVID- 19. Agricultural and Applied Economics Association, 43(1), 86-104.
Tougeron K., Hance T. (2021). Impact of the COVID-19 pandemic on apple orchards in Europe. Agricultural Systems, 190, 103097.
TurkStat. (2019). Gross domestic product. https://data.tuik.gov.tr/Bulten/Index?p=Yillik-Gayrisafi-Yurt-Ici-Hasila-2019-33671.
Vidaurreta I., Orengo J., Fe C.D.I., González J.M., Gómez-Martín Á., Benito B. (2020). Price fluctuation, protected geographical ındications and employment in the Spanish small ruminant sector during the COVID-19 Crisis. Animals, 10, 2221.
World Health Organization. (2020). Coronavirus disease (COVID-19) pandemic. World Health Organization. Regional Office for Europe. https://www.euro.who.int/ en/health-topics/health-emergencies/coronavirus-covid19/novel-coronavirus-2019-ncov
Xiao T., Torok M.E. (2020). Taking the right measures to control COVID-19. Lancet Infectious Diseases, 20 (5), 523-524
Yoshida S., Yagi H. (2021). Long-term development of urban agriculture: resilience and sustainability of farmers facing the Covid-19 pandemic in Japan. Sustainability, 13. 4316.
Zhang H., Hu L.X., Hu Z. (2020). Attach great importance to the impact of the new crown disease on the agricultural rural economy-Proper response, precise measures, insurance, supply and increase income. Farmers daily. 02, 17.

ECONOMIC AND FINANCIAL ASPECTS OF CABBAGE PRODUCTION IN FAMILY FARMS IN THE REPUBLIC OF SERBIA

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Abstract

Vegetable production in the Republic of Serbia is carried out on about 130,000 hectares, which is approximately 3.5% of total plant production. Succeeding the production of potatoes, tomatoes and peppers, cabbage is one of the most common vegetable crops grown in the Republic of Serbia. According to the Statistical Office of the Republic of Serbia, cabbage production is in the same group with kale and in 2019 both crops were grown on about 8,000 hectares in the Republic of Serbia, while in the analyzed period these crops were mostly grown on 11,000 hectares in 2015. The aim of this paper is to present the economic and financial aspects of cabbage production in farms in the Republic of Serbia in the period 2015 and 2019 based on data from the Statistical Office of the Republic of Serbia and the income and expenses survey on the family farms, which represent calculations based on variable costs, conducted by the Institute for the Science Application in Agriculture. The data for analysis have been collected from 90 farms, which are also a representative sample, due cabbage production is the dominant production on these farms. The paper also presents the influence of prices and yields on gross margin amounts in cabbage production through sensitivity analysis. The obtained results indicate a constant decrease in the amount of gross margins in cabbage production in the analyzed period, due to a significant variation in the selling price of cabbage. A significant influence of the price and yield on changes in gross margins in cabbage production was confirmed by the data obtained by sensitivity analysis.

Keywords: *gross margin, sensitivity analysis, cabbage, family farms, Serbia.*

Introduction

Vegetables are produced in almost all regions in the Republic of Serbia, comprising different crops grown. Vegetable crops are grown both in an open field and in enclosed space, depending on climate conditions, with open-field production being dominant (Ljiljanić and Rajić, 2018). Vegetable production is a very intensive and profitable branch of agriculture (Novković et al., 2012). As such, it can significantly affect the development of the agricultural sector, but this type of production also depends very much on the level of total agricultural development (Paunović, 2016). Global cabbage production amounts to approximately 71 million tones and it is the seventh largest vegetable production. Cabbage production has been increasing over the last couple of years, not only due to the growth in the global population but also due to the change in eating habits. The total areas under cabbage in the world amount to about 2.5 million hectares. The largest cabbage producers are China, India, Russia, Japan and the Republic of Korea (Petrović, 2020). The cabbage is a very important vegetable crop in the Republic of Serbia, since it is substantially used in human consumption and therefore grown on large areas. The achieved level of cabbage production has been significantly affected by high consumption (around 20 kg per capita per year). High consumption per capita is due to relatively low market price for cabbage, when compared to the price for other vegetables (Vlahović, 2015). The average area under cabbage in the analyzed period in the Republic of Serbia was about 9,650 ha with the average yield of about 25 t/ha. The main cabbage producers in Serbia are family farms. The cabbage is mostly grown in the region of Šumadija and Western Serbia, where 42% of total cabbage production is carried out, followed by the region of Southern and Eastern Serbia with 25%, Vojvodina autonomous province 24% and the region of the city of Belgrade, where 9% of total cabbage production is carried out (Červenski and Medić-Pap, 2018). The aim of this paper is to present the economic and financial aspects of cabbage production in Serbian farms in the period 2015-2019. The authors used the data retrieved from the Statistical Office of the Republic of Serbia and the data on income and expenses obtained from the survey on family farms analyzed by the Institute for the Science Application in Agriculture.

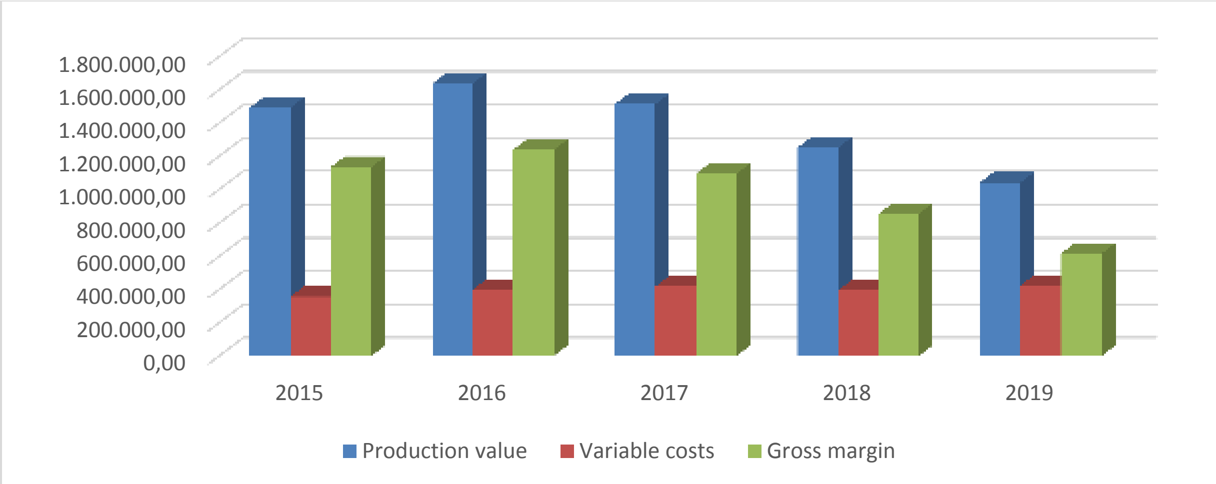
Material and Methods

This research paper contains the data from a survey on gross margins conducted by the Institute of Science Application in Agriculture for the period 2015-2019. For the purpose of this paper, 90 survey questionnaires were analyzed, all collected from family farms on which cabbage production is dominant. The family farms had been selected by the Institute for Science Application in Agriculture to be surveyed by the advisors from the agricultural advisory services of Serbia. The survey was carried out face-to-face on those farms. The main criteria for farm selection was cabbage production as the dominant type production. Due to the fruitful collaboration between the advisors and farmers in the previous years, the survey was carried out without any problems.

Financial indicators are the universal indicators of profitability of every production. For family farms, which are the subject-matter of this analysis, calculations based on variable costs are mostly used when determine the optimal scope and structure of farm production, and the indicators obtained from such calculation can be a good financial instrument for making business decisions on a farm (Gogić, 2014). Using a calculation based on variable costs, the following basic financial business indicators are determined: the value of production, variable costs and gross margin. The business result is expressed as a gross margin, which is a difference between the value of production and variable costs (Savić et al, 2020). A gross margin is a quick and efficient indicator for comparing different lines of production on a family farm and choosing the most profitable one (Andrić, 1998; Tomić et al., 2013). The aim of this paper is to show financial indicators of cabbage production by calculating them based on variable costs.

Results and Discussion

Given the calculations based on variable costs of cabbage production on family farms in the Republic of Serbia in the period 2015-2019, the average annual value of production, variable costs and gross margin were calculated.



Source: Authors' analysis based on the data from the selected family farms surveyed by the Institute of Science Application in Agriculture

Chart 1: Movement of financial indicators of cabbage production, based on a variable cost calculation in the period 2015-2019 (RSD/[41]ha)

Chart 1 shows the movement of financial indicators of cabbage production based on variable cost calculations in the period 2015-2019, calculated for 1 ha. Given the Chart 1, it can be concluded that the highest value of production was achieved in 2016, together with the highest gross margin, whereas the highest variable costs were recorded in 2019. The lowest value of production and gross margin were recorded in 2019, whereas the lowest variable costs were achieved in 2015.

Table 1. Financial indicators of cabbage production in the period 2015-2019 (calculated for 1 ha)

Year	2015	2016	2017	2018	2019
Value of production (RSD)	1,491,503.00	1,637,333.33	1,512,666.67	1,251,571.43	1,040,320.00
BI* (%)	-	109.78	101.42	83.91	69.75
CI**(%)	-	109.78	92.39	82.74	83.12
Variable costs (RSD)	356,326.25	398,640.83	417,882.50	394,497.07	423,706.90
BI* (%)	-	111.88	117.28	110.71	118.91
CI**(%)	-	111.88	104.83	94.40	107.40
Gross margin (RSD)	1,135,176.75	1,238,692.50	1,094,784.17	857,074.36	616,613.10
BI* (%)	-	109.12	96.44	75.50	54.32
CI**(%)	-	109.12	88.38	78.29	71.94
Share of gross margin in the value of production (%)	76.11	75.65	72.37	68.48	59.27

*BI – base indices
**CI – chained indices
Source: Authors' analysis based on the data from the selected family farms surveyed by the Institute of Science Application in Agriculture

[41] RSD(Serbian dinar) is the official currency code for the currency of the Republic of Serbia

Table 1 shows the most significant financial indicators from a variable cost calculation of cabbage production in the period 2015-2019 (calculated for 1 ha). The highest value of production was recorded in 2016, amounting to 1,637,333.33 RSD/ha, whereas the lowest value was recorded in 2019, and it amounted to 1,040,320.00 RSD/ha. Given the indicators calculated based on base and chained indices, it can be seen that in 2016 the value of production increased by 9.78% compared to 2015, which is the highest increase in the value of production in the period in question. In 2017, the value of production was 1.42% higher than in the base year, but 7.61% lower than in the previous year. The value of production kept decreasing in the following two years. In 2018, the value of production decreased by about 17% compared to the previous year (2017) and base year (2015). The lowest value of production was recorded in 2019, being 30.25% lower than in the base year and 16.88% lower than in 2018.
Unlike the value of production that declined over the analyzed years, the variable costs of cabbage production mostly increased. The lowest variable costs (356,326.25 RSD/ha) were achieved in 2015, and the highest (423,706.90 RSD/ha) in 2019. The base and chained indices have shown that the variable costs in 2016 were 11.88% higher than in 2015, whereas in 2017 there was an increase of 17.28% in the variable costs compared to the base year, and of 4.83% compared to the previous year. In 2018 the variable costs were 10.71% higher than in 2015 and 5.60% lower than in the previous year. In 2019 the variable costs were highest, 18.91% higher than in the base year and 7.40% higher than in the previous (2018) year.
The highest gross margin was 1,238,692.50 RSD/ha and it was recorded in 2016, whereas in 2015 and 2017 it was slightly lower. In 2018 the gross margin was lower and in 2019 it was lowest, amounting to 616,613.10 RSD/ha. The indicators obtained from a calculation of base indices have shown that the gross margin mostly decreased over the analyzed years. In 2016 the gross margin was 9.12% higher than in the base year. The amount of the gross margin in 2017 was 5.56% lower than in the base year, and 24.50% lower than in 2018. The lowest gross margin was recorded in 2019, almost half as much as the gross margin achieved in the base year. The indicators obtained from a calculation of chained indices have shown a continuous decrease in gross margin over the analyzed years, except in 2016, when there was an increase of 9.12% compared to the previous year. In 2017 the gross margin was about 12% lower than in 2016, whereas in 2018 it was about 22% lower than in 2017. The largest decline in gross margin over the analyzed years was recorded in 2019, when it was about 28.06% lower than in 2018.
The indicator of farm profitability expressed as the share of gross margin in the value of production was highest in 2015 (76.11%), and lowest in 2019 (59.27%). In 2016 the share of gross margin was slightly smaller than in 2015 (75.65%). In 2017, it amounted to 72.37%, and in 2018 it was 68.48%.
The sale price for the cabbage greatly affected the value of production and, consequently, the gross margin, given that this price was highest in the year when the value of production and gross margin were highest, and then declined in the following years. Cabbage prices ranged from 16.80 to 23.75 RSD/kg. The lowest price was recorded in 2019 (16.80 RSD/kg), and the highest price in 2015 (23.75 RSD/kg). In 2016 and 2017 the cabbage price amounted to about 22.5 RSD/kg, and in 2018 it was 19.43 RSD/kg. One of the elements that affect the value of production and, consequently, the gross margin of cabbage production is the sale price for cabbage. Given that cabbage yield and price significantly affect gross margin, a sensitivity analysis was conducted. The sensitivity analysis of gross margin in cabbage production in Serbia in the period 2015-2019 has shown how the gross margin is affected by changes in the factors that form the value of production. It was analyzed how changes in prices and yields affect the

change in gross margin. The sensitivity analysis took into consideration absolute changes in prices and yields if the parameters in question change for 10% and 20%.
Table 2 shows the sensitivity analysis of gross margin in cabbage production on changes in cabbage price and yield in the period 2015-2019, based on a five-year average of the price, yield and gross margin achieved on the surveyed farms.

Table 2. Sensitivity analysis of gross margin in cabbage production on changes in cabbage price and yield

Yield (kg/ha)		Price (RSD/kg)				
		-20%	-10%	Average	+10%	+20%
		16.90	19.02	21.13	23.24	25.36
-20%	50,883.05	504,646.72	612,162.60	719,678.48	827,194.36	934,710.24
-10%	57,243.43	612,162.60	733,117.96	854,073.33	975,028.69	1,095,984.06
Average	63,603.81	719,678.48	854,073.33	988,468.18	1,122,863.03	1,257,257.88
+10%	69,964.19	827,194.36	975,028.69	1,122,863.03	1,270,697.37	1,418,531.70
+20%	76,324.57	934,710.24	1,095,984.06	1,257,257.88	1,418,531.70	1,579,805.52

Source: Authors' analysis based on the data from the selected family farms surveyed by the Institute of Science Application in Agriculture

The average cabbage price in the analyzed period was 21.13 RSD/kg, the average yield was 63,603.81 kg/ha, whereas the average gross margin in cabbage production was 988,468.18 RSD/ha. If cabbage prices and yields decreased by 20%, it would lead to a significant decrease in gross margin (for about 49%), while an increase of 20% in prices and yields would result in an increase of about 60% in gross margin. It can be concluded that changes in cabbage prices and yields significantly affect gross margin in cabbage production.

Conclusion

The areas under cabbage have been shrinking, consequently leading to a decline in total cabbage production. A large decline in cabbage production happened in 2019, when along with shrinking of areas under cabbage (about 2,000 ha less than in 2016) there was also a decrease in the average yield for about 3 tones/ha. The average cabbage yield in the analyzed period was about 26 tones/ha, and in 2019 it was 22.40 tones/ha. The gross margin of the surveyed farms, where cabbage production is dominant, also decreased significantly over the analyzed period. It averaged about 1,000,000 RSD/ha, and in 2019 it was slightly more than 600,000 RSD/ha. This decrease in the gross margin was mostly due to a decrease in the average yield and price and an increase in the variable costs. The sensitivity analysis of gross margin in cabbage production on changes in cabbage price and yield has determined that an increase of 20% in prices and yields would result in about 60% higher gross margin, while a decrease of 20% in prices and yields would result in about 50% lower gross margin.

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References

Andrić J. (1998). Teorija troškova sa kalkulacijama u poljoprivrednoj proizvodnji/ The Theory of Costs with Calculations in Agricultural Production, "Savremena administracija", Belgrade. (In Serbian)

Červenski J., Medić-Pap S. (2018). Proizvodnja kupusa / Cabbage Production Monograph, Institute of Field and Vegetable Crops, Novi Sad. (In Serbian)

Gogić P. (2014). Teorija troškova sa kalkulacijama u proizvodnji i preradi poljoprivrednih proizvoda / The Theory of Costs with Calculations in Production and Processing of Agricultural Products, Faculty of Agriculture, University of Belgrade, Belgrade. (In Serbian)

Ilin Ž., Gvozdenović Đ., Boćanski J., Novković N., & Adamović B. (2014). Proizvodnja povrća u funkciji razvoja sela u Republici Srbiji / Vegetable Production for Development of Villages in the Republic of Serbia, Proceedings from the Scientific Conference "Village Development Prospects", Serbian Academy of Sciences and Arts, Belgrade. (In Serbian)

Ljiljanić N., Rajić Z. (2018). Determinants of Family Farms in the Regions of Serbia. International Conference on Competitiveness of Agro-food and Environmental Economy, Proceedings, vol. 7, Bucharest University of Economic Studies, Bucharest.

Novković N., Mutavdžić B., Drinić Lj., Ostojić A., Rokvić G. (2012). Tendency of Vegetables Development in Republic of Srpska. Third International Scientific Symposium "Agrosym Jahorina 2012", Book of Proceedings, University of East Sarajevo, Faculty of Agriculture, BIH, University of Belgrade, Faculty of Agriculture, Serbia.

Paunović T. (2016). Modeli za optimizaciju strukture proizvodnje povrća na porodičnim gazdinstvima / Models for the Optimization of the Structure of Vegetable Production on Family Farms, PhD dissertation, Faculty of Agriculture, University of Belgrade, Belgrade. (In Serbian)

Petrović, M. (2020). Ekonomsko-finansijski aspekti proizvodnje povrća na gazdinstvima u Republici Srbiji / Economic and Financial Aspects of Vegetable Production on Farms in the Republic of Serbia, Master's thesis, Faculty of Agriculture, University of Belgrade, Belgrade. (In Serbian)

Savić B., Petrović M., & Vasiljević Z. (2020). The Impact of Transportation Costs on Economic Performances in Crop Production, Economics of Agriculture, Year 67, No. 3/2020, Belgrade.

Statistical Office of the Republic Of Serbia, https://data.stat.gov.rs/Home/Result/130102?languageCode=sr-Cyrl, retrieved on 15 Sep. 2020.

Tomić V, Janković S., Kuzevski J., Ljiljanić N., Radišić R. (2013). Maize Gross Margins in Different Environmental Conditions in 2011 and 2012, 50th Anniversary of the Department of Agricultural Economics/The Seminar Agriculture and Rural Development - Challenges of Transition and Integration Processes, Faculty of Agriculture, Belgrade.

Vlahović B. (2015). Tržište agroindustrijskih proizvoda – specijalni deo / The Market of Agro-Industrial Products – Special Edition, Faculty of Agriculture, University of Novi Sad, Novi Sad. (In Serbian)

FORESTRY AND AGRO-FORESTRY

NORTHERN RED OAK (*QUERCUS RUBRA L.*) IN BELGRADE

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Abstract

In order to properly select plant species, especially dendroflora as the basis of different categories of open green spaces, of great importance is to know the characteristics of development, vitality and decorativeness of already grown specimens in similar environmental conditions. One of the very interesting species for Belgrade region is northern red oak (*Q. rubra* L.). According to the experience from Serbia, *Q.rubra* is easily adapted to different climatic and other conditions. It is suitable for habitats of sessile and common hornbeam forests, mountain beech forests and forests of Hungarian and Turkey oaks. It is relatively fast growing, tolerant on soil conditions. It does not tolerate stagnant water and floods, as also dry land. It tolerates shade better than native oaks. The species is resistant to low temperatures and polluted urban environments. In the area of Belgrade it is often planted in some urban forests, but also on different categories of open green spaces. The paper presents the researching results of development, vitality and decorativity of this species at five sites, in different environmental conditions.

Keywords: *Quercus rubra L., Belgrade, urban conditions, development, landscaping.*

Introduction

Northern red oak (*Quercus rubra* L., syn. = *Quercus rubra* var. *borealis* (Michx.f.) Farw. = *Quercus borealis* F. Mich x. = *Quercus borealis* (var.) *maxima* Sarg. = *Quercus maxima* Ashe. = *Quercus rubra* Du Roi non L.) is a medium to high deciduous tree. Belongs to the family Fagaceae, genus Quercus, secc. (Subgenus) Erythrobalanus Spach. The genus includes a large number of deciduous and evergreen trees and several shrub species, spreaded out from the cold zone to the tropical regions.

Q. rubra from nature occurs in a wide range in eastern North America, across the eastern United States and southeastern Canada, and up to 1000 m in height (Little, Elbert L., Jr. 1979) Extent of occurrence (EOO) is estimated at over 4.150.000 km^2. According to IUCN (2017), the total population size of *Quercus rubra* has not been quantified, but considering its occurrence in wide geographical range, it is estimated that the number of adult specimens of this species is probably very large. In the southern part of its natural occurrence, the number has slightly decreased. However, this is of insignificant extent and is unlikely to have the effect of significantly reducing the population of *Q. rubra* in the near future. It can be concluded that *Q. rubra* is a stable species and, according to NS (Nature Serve), it is listed as a globally safe (G5) species, and that the current population trend is stable (Haavik, L.J., Stahle, D.W., Stephen, F.M., 2011).

This species is one of the tallest and fastest growing from the group of red oaks, as well as oaks in general. It usually reaches a height of about 30 m. It is characterized by a straight, cylindrical trunk, up to 2 meters in diameter and a large wide rounded crown. The bark of the trunk is smooth and gray at a young age, later becoming thickened, with shallow fissures. Young twigs are reddish-brown; the buds are large, ovoid, reddish-brown, hairy scales. The leaves are alternate, simple, oblong or inversely ovoid, 12-20 cm long, 10-12 cm wide, cut into 7-11 lobes with sharper edges. On the face are shiny, light green, with tufts of hairs in the leaf nerves axils. The change in the color of the leaves in the fall is characteristical for this species. The color goes from green to different shades of brown and red, which makes this species very attractive in the autumn aspect. The leaves decompose better and faster than our oak species. Male flowers are yellowish green, clustered in up to 10 cm long, hanging tassels. They bloom at the same time as leafing, in late May. The fruit is a roundish acorn, 1.5-2.5 cm long, 1-2 cm wide, shallowly placed in a scaly cap. It matures 18 months after pollination. *Quercus rubra* has a wide ecological range, but it is most commonly found in a variety of moderately moist habitats, including rich forests, arid slopes, rocky habitats, sandy plains and at the edges of floodplains. It is a dominant species in a wide range of communities such as mixed mesophilic forests, pine-oak communities and south-exposed forests in the lower parts of the mountains. Species that occur with red oak are: *Quercus alba* L., *Quercus velutina* Lam., *Pinus strobus* L., *Fagus grandifolia* Ehrh. and *Acer* spp.

This North American exotic species was introduced to Europe in 1691. and currently covers over 350,000 ha, being found all over the continent, except the coldest part of Scandinavia (Nicolescu, VN et all., 2018). It is also often grown as an ornamental tree in the parks. Of the varieties, the most common is *Q. rubra* var. *maxima*, more rarely *Quercus rubra* 'Aurea'.

According to the experience from Serbia, *Q.rubra* is easily adapted to different climatic and habitat conditions (Drazic et al., 2004., Vuckovic et al., 2008). It is suitable for habitats of sessile and common hornbeam forests, mountain beech forests and forests of Hungarian and Turkey oaks. It tolerates low temperatures and thrives on poor soils that can be acidic, but avoids limestone. It is best suited to sandy loam, especially fresh. It does not tolerate stagnant water and floods, as also dry land. The species is resistant to low temperatures and polluted conditions of urban environments. It is relatively fast growing, tolerates shade, tolerant of soil conditions, and thrives on poor soils as well as acid reaction of soils, though sandy and fresh soils are most suitable. It does not tolerate flooded but also dry habitats (Hirons, A.D. and Sjöman, H., 2019. Due to the quality hardwood, it is used for interior, furniture and flooring (The values of North Carolina Trees, 1995).

Material and Methods

Selecting species for design and establishment new and reconstruction of already existing different types of urban green spaces, requires a good knowledge of not only the general ecological, but also the micro-habitat conditions of the environment. In the urban environment, all important parameters of climate, soil and habitat type have been significantly changed by anthropogenic activity, especially by the increased content of various harmful substances in air, soil and water. The climate of the city significantly differs from the climate of the surrounding area, which is a consequence of the different radiation and the water balances of the city and its surroundings. The consequence of these differences is a stronger heating of the city area and changes of some other elements such as wind, fog and smog. Furthermore, differences also occur in different parts of the city depending on its topography and structure. Air pollution has also a significant influence on climate modification in the city.

The paper presents the following climatic factors: air temperatures and their amplitudes, precipitation, relative air humidity, fog and smog; The characteristics of the localities where the red oak trees were analyzed are shown: altitude, distance from roads, soil and habitat type; The mean diameter values (cm), total height, trunk height and crown height (m) were analyzed and a general assessment of vitality and decorativeness (1-5) was given; The current annual diameter increment, the average diameter increment and the development of the diameter over a period of 15 years has been analyzed; The content of toxic metals in the wood was also investigated; Based on all of the above, recommendations for the further use of this species in Belgrade and similar environmental conditions has given.

Results and Discussion

Climatic characteristics of Belgrade region. Belgrade and its surroundings are characterized by a temperate continental climate with an average annual temperature of 11.6ºC. The coldest month is February (mean temperature 0.0ºC) and the warmest month is July (mean temperature 22.1ºC). An important climatic characteristic is the difference between winter and summer temperatures. January average temperature is 0.0ºC, while the average July temperature is 22.1º. The annual amplitude of the temperature, the difference between the warmest and coldest month was 22.1ºC. There are significant differences between urban and rural conditions due to the heavy morning frosts that occur around the city, while there are none in the city. The spatial distribution of temperatures is determined by the combined influence of the topography and heat island effect of the city. The absolute daily minimum is -26.2ºC and the absolute daily maximum is 41.8ºC. According to the classification (The degree of continentality, Gorcinski), the area of Belgrade is with a value for K = 33.6, at the lower boundary of the continental type. The average air temperature of the vegetation period is 18.3°C, the maximum mean temperature is 22.3°C, while the minimum mean temperature is 14.2°C. The average annual rainfall is 668 liters per square meter. The annual flow of rainfall has predominantly continental-type characteristics, with maximum in summer (June) and minimum in winter half-year. Although Belgrade is quite deep in the continent, it also has some characteristics of a maritime type. Thus, Belgrade has two highs and two lows in the annual rainfall. The average amount of precipitation in the vegetation period is 436 mm, i.e. 65% of the average annual amount. The average annual relative humidity is 70%. July is the month with the lowest humidity (mean relative humidity 62.7%) and the month with the highest humidity is December (81%). The average number of bright days a year is 67, and cloudy 111. The basic information about localities. The following table shows localities in the wider Belgrade area with altitude data (m), distances from roads (m), land type and habitats where existing northern red oaks were analyzed.

Table 1. Overview of localities with basic information

No.	Location	Altitude (m)	Distance from roads (m)	Soil type	Habitat type
1.	Cerak-Vigradi	158	5	Eutric cambisol	*Quercetum frainetto cerris* Rud.
2.	Zeleznik	135	200	Eutric cambisol	*Quercetum frainetto cerris* Rud.
3.	Sremčica	170	3	Eutric cambisol	*Quercetum frainetto cerris* Rud.

No.	Location	Altitude (m)	Distance from roads (m)	Soil type	Habitat type
4.	Baba Velka	265	1500	Eutric cambisol	*Quercetum frainetto cerris* Rud.
5.	Ibar motorway	150	30	Eutric cambisol	*Quercetum frainetto cerris* Rud.
6.	Highway Bg- Zg	80	15	Chernozem	*Carpino quercetum roboris* Anić
7.	Open pit coal mine "Kolubara"	185	-	Deposols	Antrophogenically degraded

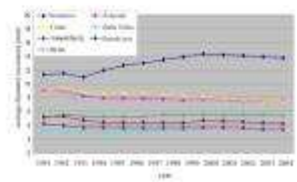
The site selection was based on the coverage of plantations located in the urban area, suburban area, relatively unpolluted environment and the specific location of the open pit coal mine "Kolubara". The first point (1) is located within the Cerak-Vineyard residential area, on the site of *Quercetum frainetto cerris* Rud., on the eutric cambisol, at an altitude of 158m, near the road. The next (2) is located in the suburban settlement of Zeleznik, on the same site and soil type and approximate altitude, but about 200m distance from the nearest road. The third point (3) is in the suburban settlement of Sremcica, on the site and soil with similar characteristics and approximate altitude, close to the road. Beside the very high-frequency Ibar road, is the point 5, on the same site, soil type and altitude. In the vicinity of Belgrade, at a slightly higher altitude (265m), on the same site and type of soil, but about 1.5km away from the road is located urban forest Baba Velka (4). Near the Belgrade-Zagreb highway is a sixth point, on the Chernozem soil and habitat of *Carpino quercetum roboris*, at a lower altitude (80m). In the very specific environmental conditions of the open pit coal mines "Kolubara" is the last analyzed plantation (7), located at 185m altitude, on Deposol (mining waste dump sites). The following table shows results of analysis of mean diameter at 1.30m height in centimeters (cm), the total height of the trees, the height of the trunk and the crown in meters (m), as well as the width of the crown. Based on health analysis (presence of harmful insects, phytopathological diseases and mechanical damages), an evaluation of the general vitality (gradation from 1 to 5), as well as the decorativeness was made in the same scale.

Table 2. Mean values of diameter (cm), total height, trunk height and crown height (m), crown width (m) with average vitality and decorativenes (grade 1-5)

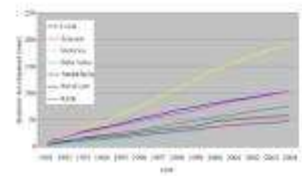
Locality	Mean value of diameter (cm)	Height (m)			Width of crown	Vitality	Dekorativenes
		total	trunk	crown	(m)	(1-5)	(1-5)
Cerak-Vineyard	32.5	**13.75**	2.95	10.63	10.65	3.92	3.88
Železnik	**58.3**	**19.50**	2.10	**17.40**	**18.33**	**4.50**	**4.50**
Sremčica	32.6	14.56	3.19	11.37	11.56	4.20	4.20
Baba Velka	35.0	16.63	3.13	13.50	9.00	3.50	3.00
Ibar motorway	29.8	15.54	**2.00**	13.54	8.30	**3.48**	3.38
Highway Bg-Zg	31.5	18.35	6.40	11.95	7.00	4.20	4.50
Open pit coal mine "Kolubara"	**20.7**	18.54	**8.57**	**9.38**	**4.50**	3.60	**2.80**

The values of trunk diameter ranges from 20.7cm in the Kolubara coal basin on deposols to 58.3cm in the suburban settlement of Zeleznik. The following graphs presents the current annual diameter increment (1), average diameter increment (2), and diameter development (3) for the period 1990 to 2004:

Graph.1 Current annual diameter increment



Graph.2 Average diameter increment



Graph.3 Diameter development

The total height ranges from 13.75 to 19.50 m, and the crown width from 4.50 to 18.33 m. The vitality of all trees is in the range of very good values, while the decorativenes varies in a slightly larger range. Studies (Vučković et al., 2008) in the area of the Majdanpek domain has shown that red oak, by its general condition and growth dynamics, has characteristics of a fast-growing species with high dendromase production. The foundation of 40 $m^2 \cdot ha^{-1}$ and the volume of the large wood 460 m^3 ha^{-1} in the year 41 indicate exceptional production possibilities. Growth in height is characterized by a high positive phototropism. Current growth culminates in year 10 with 0.9 m and average in year 18 with 0.73 m. The following table shows the content of toxic metals in the wood:

Table 3. The content of toxic metals in the wood (mg/kg)

No.	Sample	Zn	Pb	Mn	Cd
1.	**Reference values**	**300**	**100**	**-**	**3**
2.	Cerak-Vineyard	3.256649	1.177697	5.404562	**0.0**
3.	Železnik	2.493287	0.767165	4.411200	0.546605
4.	Sremčica	**6.457013**	2.391486	8.848499	**0.0**
5.	Baba Velka	2.262923	0.990028	45.331513	0.198005
6.	Ibar motorway	5.28238	**2.697386**	13.501484	0.112391
7.	Highway Bg-Zg	**0.938471**	**0.586544**	**3.519267**	**0.692122**
8.	Kolubara coal basin	3.869281	1.045751	**71.256995**	**0.0**

The results showed that the concentration of Zn in the tree ranged from 0.94 mg/kg at the locality Highway Beograd-Zagreb to 6.46 mg/kg at the locality Sremčica. The Zn deficiency in plants is indicated as 20 mg/kg (Kabata-Pendias, 2011). Based on the presented data, it is not possible to draw a conclusion about the actual Zn deficiency in the examined individuals. The concentration of Pb ranged within the normal Pb content in plants (0.1 to 5.0 mg/kg, De Vries and Bakker, 1996). However, it is important to note that the translocation of Pb from the root to the aboveground parts is limited (Kabata-Pendias, 2011). Maximum Pb concentrations of 2.70 mg/kg were detected at the locality Ibar motorway, and the lowest 0.59 mg/kg at the locality Highway Beograd-Zagreb. The results of the study also showed that the Mn concentration ranged within normal natural intervals of 30-300 mg/kg (Kabata-Pendias, 2011). As in the case of the content of other tested elements, the min. Mn concentrations (3.52 mg/kg) were determined at the locality Highway Beograd-Zagreb and the max. concentration of 71.26 mg/kg contained the wood of red oaks at the locality REIK. The concentration of Cd was in the range of average values of Cd content in plant at most localities (Kadović and Knežević, 2002). Based on the presented results, it can be concluded that all the analysed elements in the wood were in concentrations that did not interfere with various physiological and biochemical processes in red oak.

Conclusion

In the wider area of Belgrade, red oak has shown very good development results: diameter, height, crown width; the average, age and the overall diameter increment; Very good vitality and health conditions; No harmful insects or phytopathological diseases have been registered; It is characterized by exceptional decorativeness throughout the year (habitus, bark, leaf, fruit), and especially in the autumn aspect; Due to modest requirements and good success on different habitats and rapid growth in young age, red oak can be a very important species for increasing the stability of our forests and solving the problem of degraded habitats and low quality and poorly produced sessile stands. Also, especially in better quality habitats, it can contribute to the improvement of financial management effects, because of higher timber production compared to native oaks. On tourism and recreation facilities, the population can serve as an attractive species to break the monotonous landscape. All of the above recommends this species for much wider use in Belgrade (as well as other cities with similar environmental conditions), on different categories of urban open green spaces - from the tree-lined areas to forest complexes, as well as for the biological reclamation of coal mine landfills.

References

De Vries, W., Bakker, D.J. (1996): Manual for calculating critical loads of heavy metals for soils and surface waters; preliminary guidelines for critical limits, calculation methods and input data. Report 114, Wageningen, SC-DLO; pp. 173;

Dražić, D., Bojović, S., Jovanović, Lj., Čokeša V. (2004): Crveni hrast (*Quercus borealis* F. Michx) na području Beograda. Prvi Simpozijum ekologa Crne Gore. Tivat, 14-18. oktobra. Knjiga abstrakta, p. 87-88.

Haavik, L.J., Stahle, D.W., Stephen, F.M. (2011): Temporal aspects of *Quercus rubra* decline and relationship to climate in the Ozark and Ouachita Mountains, Arkansas. *Canadian Journal of Forest Research*, 2011, 41(4): 773-781, https://doi.org/10.1139/x11-018

Henin, J.M., Hernea, C. (2018): Ecology and management of northern red oak (*Quercus rubra* L. syn. *Q. borealis* F. Michx.) in Europe: a review.

Hirons, A.D. and Sjöman, H. (2019) *Tree Species Selection for Green Infrastructure: A Guide for Specifiers*, Issue 1.3. Trees & Design Action Group.

IUCN 2017. *The IUCN Red List of Threatened Species. Version 2017-3*. http://www.iucnredlist.org. Downloaded on 05 December 2017.

J.C., Brus, R., K., Rédei, K., Štefančik, Kabata-Pendias, Alina, & Mukherjee, Arun B. (2007). Trace elements from soil to human: Springer Science & Business Media.

Kabata-Pendias, Alina. (2011). *Trace elements in soils and plants* (4th edition ed.): Taylor and Francis Group, CRC press.

Kadović, R., Knežević, M. (2002): Teški metali u šumskim ekosistemima Srbije. Šumarski fakultet Beograd i Ministarstvo za za zaštitu prirodnih bogatstava i životne sredine Republike Srbije.279.

Little, Elbert L., Jr. 1979. Checklist of United States trees (native and naturalized). Agric. Handb. 541. Washington, DC: U.S. Department of Agriculture, Forest Service. 375 p.

Nicolescu, V.N., Vor, T., Mason, W., Bastien, J.C., Brus, R., Henin, J.M., Kupka, I., Lavnyy, V., La Porta, N., Mohren, F., Petkova, K., Rédei, K., Štefančik, I., Wąsik, R., Perić, S., Hernea, C. (2018): Ecology and management of northern red oak (*Quercus rubra* L. syn. *Q. borealis* F. Michx.) in Europe: a review.

Forestry: An International Journal of Forest Research, cpy032, https://doi.org/10.1093/forestry/cpy032

The values of North Carolina Trees, University of Minnesota, Forest Library, May, 1995. U.S. Forest Service, Forest Report R8-FR-48. National Forests in North Carolina, Digitized by Google.)

Vučković, M., Stajić , B., Nedeljković, J. (2008): Karakteristike rasta crvenog hrasta na području Majdanpečke domene. Šumarstvo 4, p. 79-86, Beograd.

Vučković M, Stamenković V. (1995): Karakteristike prirasta crvenog hrasta, Vajmutovog bora i ariša na staništu kitnjaka. Šumarstvo 3, s. 3-10

Vukićević, E. (1987.), Dekorativna dendrologija, Beograd: Naučna knjiga, ISBN: 86-23-48000-7

Wenzell, K. & Kenny, L. 2015. *Quercus rubra*. The IUCN Red List of Threatened Species 2015: e.T194226A2305058. http://dx.doi.org/10.2305/IUCN.UK.2015-.RLTS.T194226A2305058.en. Downloaded on 07 June 2018.

FLORISTIC COMPOSITION COMPARISON BETWEEN AUSTRIAN PINE FORESTS OF ZLATIBOR AND KOPAONIK (SERBIA)

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Abstract

Austrian pine forests represent a significant complex of azonal foerests on serpentitines of Western and central Serbia. In this research, comparison of floristic composition was conducted between Austrian pine forests (*Erico-Pinetum nigrae* Krause 1957. in Krause & Ludwig 1957) of Zlatibor (western Serbia) and Kopaonik (central Serbia). Austrian pine stands on Kopaonik were registered on altitudes between 900-963 m, NW and SW expositions and 20-35° slopes. Austrian pine stands on Tornik (Zlatibor) occupy a narrow zone of altitudes from 1120-1145 m, varying expositions (SW, S, SE, N) and 20-35° slopes. Phytocoenological table shows great degree of similarity between studied stands, which is expected, considering that they belong to the same community. Given that studied Austrian pine forests belong to different localities, each exhibiting their own specificities, CA analysis of study stands indicates that there are significant differences between them in terms of floristic composition. Records from Kopaonik also show presence of xerophilous species that exist in difficult living conditions: *Carex humilis, Juniperus oxycedrus, Minuartia verna, Euphorbia glabriflora, Polygala supina* and others. Within records from Zlatibor, presence of species that are typical indicators of community degradation is noted: *Crataegus monogyna, Brachypodium pinnatum, Danthonia calycina*. Life forms spectrum shows that phanerophytes are somewhat more numerous on Zlatibor, which is a consequence of intense grazing, while hamephytes are more prevalent on Kopaonik, which indicates more extreme living conditions. Spectrum of floral elements exhibits most differences in terms of Mediterranean-sub-Mediterranean floral element presence. This element is significantly more present on Kopaonik than on Zlatibor, which indicates that Kopaonik is subject to sub-Mediterranean influence.

Key words: *Pinus nigra, serpentinite, Serbia, floristic similarity*.

Introduction

Austrian pine (*Pinus nigra* Arnold) belongs to the group of Mediterranean pines that have disjunct areal – they are present in north Africa, in Europe spanning from Spain on the west, across north Mediterranean, all the way to the Black Sea (Afzal-Rafii and Dodd, 2007, Rubio-Moraga *et al*., 2012). Two main subspecies of Austrian pine are recognized: *Pinus nigra* subsp. *salzmannii* (Corsican pine), occurring from Morocco and Spain to South France and Corsica, and *Pinus nigra* subsp. *nigra* (Austrian pine), occurring from Austria and North-Eastern and Central Italy through Balkans, to Turkey and Crimea Peninsula (Enescu *et al*., 2016). It grows at altitudes ranging from sea level to 2000 m, most commonly between 800 and 1500 m (Govannelli *et al.*, 2019). Austrian pine forests constitute a significant complex of azonal, relict and orographic-edaphically conditioned forests on ophiolitic massifs of central and eastern Bosnia, as well as western and some of the central Serbia (Tomić, 2004). Many authors considers these forests to be relict mainly due to edificatory role of Austrian pine (*Pinus nigra* subsp. *nigra* Vid.), which represents a typical tertiary relict with some subspecies' disjunct areals (Tatić and Tomić, 2006). Considering it's a relict species, primary Austrian pine communities can still be found sporadically, mostly on shaded expositions. Since Austrian pine is a pioneer species that conquers other species habitats, secondary Austrian pine communities on habitats of Balkans sessile oak, beech-fir, beech-fir-spruce, etc. are more commonly found. Austrian pine forests are dynamic systems with pronounced progressive succession. Importance of studying natural stands of Austrian pine does not stem from the surface area of Serbian forest fund which they occupy, but from the facts that Austrian pine is among most important and valuable tree species in this part of the world and it is most commonly used for afforestation of deforested terrains in unfavorable xerothermic habitat conditions (Stojanović *et al.* 2010). The aim of the paper was to broaden the knowledge on the Austrian pine forests on serpentine bedrock and to compare the floristic data in order to determine the differences between the studied forests on two localities in Serbia.

Material and method

For analysis of Austrian pine forest (*Erico-Pinetum nigrae* Krause 1957. in Krause & Ludwig 1957) floristic composition on serpentines of Kopaonik and Zlatibor areas (table 1), data from literature (Novaković-Vuković, 2015) were used. The size of the sample plots was 200 m². Syntaxonomic names were given according to Tomić and Rakonjac (2013). CA vegetation data analysis was performed using the statistical software CANOCO 4.5 (Lepš and Šmilauer, 2003). The cover-abundance score obtained for each species within a relevé was transformed using the method of Van Der Maarel (Van Der Maarel, 1979). Phytocoenological table was made based on phytocoenological relevés. Nomenclature of life forms, floristic elements and their classification into higher phytochories corresponds to the phytogeographical division of the Balkan Peninsula into phytochoria of subregion and province rank given by Horvat, Glavač & Ellenberg (1974), adapted and modified by Stevanović (1992).

Table 1. GPS coordinates of phytocoenological relevés. Abbreviations for locations: K-Kopaonik, Z-Zlatibor; (1-5)- relevés on Kopaonik; (6-10)- relevés on Zlatibor (Tornik)

Relevé number	Gauss-Kruger	Gauss-Kruger
	X	Y
K_1	7490309	4781855
K_2	7490091	4782456
K_3	7490069	4782599
K_4	7489989	4782850
K_5	7489926	4782856
Z_6	7393309	4833937
Z_7	7393360	4833937
Z_8	7393284	4833980
Z_9	7393316	4833978
Z_10	7393354	4833967

Results and discussion

Austrian pine stands on Kopaonik were registered on altitudes between 900-963 m, NW and SWexpositions and 20-35° slopes. Austrian pine stands on Tornik (Zlatibor) occupy a narrow range of altitudes between 1120-1145 m, various expositions (SW, S, SE, N) and same slopes as stands on Kopaonik (20-35°). Austrian pine forests on Kopaonik and Zlatibor feature many mutual species, which is expected, because they belong to the same community. However, each locality has their own specific features, hence the figure (1) shows clear distinctions among phytocoenological relevés from research localities in terms of floristic composition. Relevés from Kopaonik are concentrated on the left side of the graph, with xerophilous species: *Carex humilis, Juniperus oxycedrus, Minuartia verna, Euphorbia glabriflora, Polygala supina,* etc. The right side of the graphs exhibits dominant presence of relevés from Zlatibor, which are slightly dispersed around the axis. These stands are degraded due to intense grazing, which reflects on their floristic composition. There is prominence of species which are typical indictors of community degradation: *Crataegus monogyna, Brachypodium pinnatum, Danthonia calycina*.

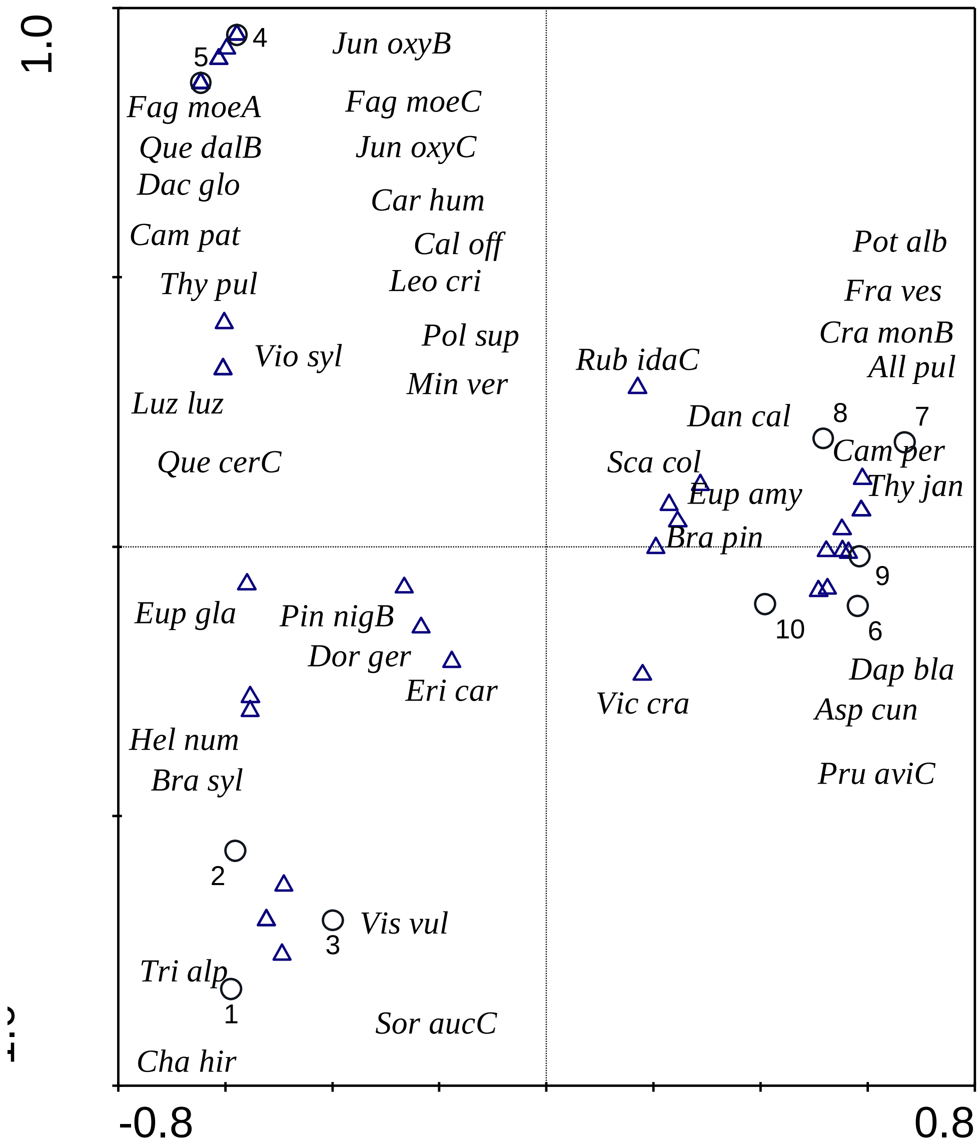

Figure 1: CA ordination biplot, fit range for the species 35-100%, 41 species (○-relevé representation, Δ- species representation; (1-5)- relevés on Kopaonik; (6-10)- relevés on Zlatibor (Tornik)
Species abbreviations: *Min ver-Minuartia verna; Jun oxy-Juniperus oxycedrus; Luz luz- Luzula luzuloides; Fam moe- Fagus moesiaca; Car hum- Carex humilis; Que dal- Quercus dalechampii; Cal off- Calamintha officinalis; Leo cri- Leontodon crispus; Cam pat- Campanula patula; Dac glo- Dactylus glomerata; Pol sup- Polygala supina; Thy pul- Thymus pulegioides; Fra ves- Fragaria vesca; Que cer- Quercus cerris; Vio syl- Viola sylvestris; Rub ida- Rubus idaeus; Cram on- Crataegus monogyna; All pul- Allium pulchellum; Dan cal- Danthonia calycina; Cam per- Campanula persicifolia; Sca col- Scabiosa columbaria; Eup amy- Euphorbia amygdaloides; Thy jan- Thymus jankae; Bra syl- Brachypodium sylvaticum; Pru avi- Prunus avium; Pot alb- Potentilla alba; Asp cun- Aslenium cuneifolium; Dap bla- Daphne blagayana; Vic cra- Vicia cracca; Eup gla- Euphorbia glabriflora; Pin nig- Pinus nigra; Bra pin- Brachypodium pinnatum; Dor ger- Dorycnium germanicum; Eri car- Erica carnea; Hel num- Helianthemum nummularium; Vis vul- Viscaria vulgaris; Tri alp- Trifolium alpestre; Cha hir- Chamaecytisus hirsutus; Sor auc- Sorbus aucuparia* (the abbreviations following the species denotes A-tree layer,B –shrub layer, C-ground flora layer)

Cade juniper (*Juniperus oxycedrus*) was registered only on Kopaonik. This species is often present on dolomites and is an indicator of xerophility of study stands on Kopaonik. Austrian pine forests of Kopaonik feature 20% of xerophytes (Novaković-Vuković, 2015), which is almost as twice as the number of xerophytes in Austrian pine forests on Zlatibor, Tara, Šargan and Pešter. *Euphorbia glabriflora*, Illyric endemic and relict, exhibits notable abundance and coverage, which is a clear indicator that Austrian pine forests on this locality have Illyric imprint. "Going south from the center of development in western Serbia, pine forests change their appearance and structure, their vitality gets weaker and their floristic composition gets poorer in most important types of pine forests" (Pavlović, 1964). Austrian pine forests on Kopaonik are lower in height and of weaker vitality compared to these forests on Zlatibor. They don't have typical Illyric species such as *Epimedium alpinum* and *Daphne blagayana,* common inhabitants of black and Scots pine forests in western Serbia, in their floristic composition – which is expected, given that pine forests of Kopaonik are on the easternmost border of Illyric floristic area.

Even though *Erico-Pinetum nigrae* Krause 1957 community on Kopaonik is xerophilous to a significant extent, it still features mesophilic species, because it is situated within climate-regional belt of *Fagetum montanum* and *Abieti-Fagetum* (Jovanović, 1972). Beech (*Fagus moesiaca*) was registered in all the layers, young Balkanic durmast oak (*Quercus dalechampii*) was also noted, which indicates syndynamic connection between pine and forests on serpentinite and more mesophilic deciduous forests. Horvat (1959) classified not only pine but also oak forests into suballiance *Orneto-Ericion serpentinicum* Ht apud. Krause et Ludw. 1957, which represent the sucession according to vegattion climate in serpentinites of Bosia. Syndynamic connection of pine and oak forest was also noted in later reasearch, one of them has been conducted on mountain Ozren in B&H (Blagojević and Govedar, 2009). Authors described *Erico-Pinetum nigrae* Stefanović 1962, localized on inner Dinarides within climate-regional community of *Querco-Carpinetum illyricum* Horvat 1963. Within the association, three subassociations have been noted: *typicum, daphnetosum blagayanae* and *callunetosum.* Durmast oak (*Quercus petraea*) was registered in all the relevés, which may indicate that Austrian pine has "inhabited" former habitats of durmast oak (Blagojević and Govedar, 2009). Subassociation *typicum* and *daphnetosum blagayanae* are characterized by significant presence of species typical for Austrian pine forests of thermophilus habitats, while subassociation *callunetosum* is abundant with mesophilous plant species. Subassociation *callunetosum* is characterized by interference of basophilic winter heath (*Erica carnea*) with acidophilic common heather (*Calluna vulgaris*), which indicates that *Erica carnea* has acido-tollerant properties (Lukić *et al*., 2019). Authors also noted that winter heath is a characteristic species for relict forest communities. Considering the area investigated in this paper, *Daphne blagayana* was noted only on Zlatibor, while *Calluna vulgaris* was not registered at all, which brings us to the conclusion that Austrian pine forests of B&H and Serbia differ significantly in their floristic composition.

Regressive succesion is commonly seen in Austrian pine forests. On Kopaonik, Austrian pine forest (*Erico-Pinetum nigrae*) on shaded expositions is being replaced with pasture community-*Erico-Seslerietum rigidae* (Jovanović-Dunjić and Jovanović, 1987).

Hemicryptophytes are most numerous in spectrum of life forms (table 2) on both research localities. Somewhat greater presence of phanerophytes is noted on Zlatibor than on Kopaonik (24%:18%), which can be a consequence of intense grazing on Zlatibor. Chamaephytes are more represented in Austrian pine forest in Zlatibor (18%:13%), which is an indicator of more extreme life conditions on Kopaonik. Geophytes have significant presence, especially on Zlatibor (10%), because geophytes accompany mesophilic beech forest, and listed communities have been registered on their habitats. Geophytes are also the indicators of low winter temperatures.

Table 2. Spectrum of life forms in Austrian pine forests on Zlatibor and Kopaonik

	Zlatibor	Kopaonik
P scap (trees)	13%	11%
P caesp (shrubs)	11%	7%
Ch (chamaephytes)	13%	18%
H (hemicryptophytes)	51%	58%
G (geophytes)	10%	4%
T (therophytes)	0%	2%
S (lianas)	2%	0%

Considering that it is the same community (both on Zlatibor and Kopaonik), spectrum of floral elements (table 3) shows many similarities, but certain differences are also noted. Group of central European floral elements is most dominant on both localities. Species of colder regions exhibit similar presence. The biggest difference is in the presence of xerophilous, Mediterranean-submediterranean floral element. It is significantly more numerous on Kopaonik than on Zlatibor (11%:3%). Kopaonik and Zlatiobr are both indisputably under Mediterranean influence. Generally speaking, xerophilous floral elements (Mediterranean-submediterranean, Mediterranean-submediterranean-pontic and pontic-Mediterranean-submediterranean) are more prominent on Kopaonik than on Zlatibor (20%:16%), which indicates that Kopaonik is under greater submediterranean influence. Comparison of floral elements in Austrian pine forests on serpentinites in Serbia and B&H (Novaković-Vuković *et al*. 2019) showed that xerophilous floral elements are more dominant in Serbia, while mesophilic floral elements are more represented in B&H, which is another indicator of submediterranean influence in Serbia.

Table 3. Spectrum of floral elements in Austrian pine forests on Zlatibor and Kopaonik

	Zlatibor	Kopaonik
CIRCHOL (circumholarctic)	5%	4%
BOR+CIRCBOR (boreal –circumboreal)	6%	5%
EAS (Eurasian(	18%	22%
CE+CE-CAUC (central European-centra European Caucasian)	41%	35%
MED-SMED-PONT (Mediterranean-submediterranean-pontic)	13%	7%
MED-SMED (Mediterranean-submediterranean)	3%	11%
PONT-MED-SMED (pontic-Mediterranean-submediterranean)	0%	2%
SEP (south European mountain)	8%	5%
CEP (central European mountain)	6%	9%

Conclusions

The paper compared floristic composition of Austrian pine forests (*Erico-Pinetum nigrae* Krause 1957. in Krause & Ludwig 1957) on Zlatibor (western Serbia) and Kopaonik (central Serbia). CA analysis of study stands shows significant differences in floristic compositions. Relevés from Kopaonik indicated notable presence of xerophilous species: *Carex humilis, Juniperus oxycedrus, Minuartia verna, Euphorbia glabriflora*, *Polygala supina,* etc. Within relevés from

Zlatibor, there is marked presence of species that are typical indicators of community degradation: *Crataegus monogyna, Brachypodium pinnatum, Danthonia calycina*.
Spectrum of life forms shows that phanerophyte are somewhat more numerous on Zlatibor (24%:18%), while chamaephyte are more represented on Kopaonik (18%:13%). Spectrum of floral elements on both localities shows most dominant presence of central European floral elements. The biggest difference is in the presence of Mediterranean-submediterranean floral element, which is significantly more represented on Kopaonik than on Zlatibor (11%:3%), indicating stronger submediterranean influence on Kopaonik. Aforementioned findings point out that study stands of Austrian pine on Kopaonik and Zlatibor are very similar, but also exabit notable differences.

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References

Afzal-Rafii, Z., Dodd., R. S. (2007): Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (*Pinus nigra*) in Western Europe. Molecular Ecology, 4(16): 723-736

Blagojević, V., Govedar, Z. (2009): Forest soils and phytocoenological characteristics of black pine in the area of Ozren. Bulletin Faculty of Forestry, University of Banja Luka, (11): 97-109

Enescu, C. M., de Rigo, D., Caudullo, G., Mauri, A., Houston Durrant, T. (2016): *Pinus nigra* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, pp. e015138+

Giovannelli, G., Scotti-Saintagne, C., Scotti, I., Roig Anne., Spanu, I., Giuseppe Vendramin, G., Guibal, F., Fady, B. (2019): The genetic structure of the European black pine (Pinus nigra Arnold) is shaped by its recent Holocene demographic history. *Biorxiv*, 535591

Horvat. I. (1959): Sistematic relations of termophylous oak and pine forests. Biološki glasnik, (12): 1-40

Horvat, I., Glavač, V., Ellenberg, H, (1974): Vegetation Südosteuropas. Geobotanica selecta 4 Stuttgart: Gustav Fischer Verlag.

Jovanović, B. (1972): Phytocoenoses of Austrian pine (*Pinus nigra* Arn.) on Kopaonik. Bulletin, Natural Sciences Museum (Belgrade), B/27: 11-29

Jovanović-Dunjić, R., Jovanović, S. (1987): The succesion of vegetation on serpentine rocky grounds on eastern spurs of the Kopaonik mountain. Arhiv bioloških nauka, 39(1-4): 93-104

Lepš, J., Šmilauer, P. (2003): Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge.

Lukić, O., Milanović, Đ., Brujić, J., Stupar, V. (2019): Contribution to the knowledge of distribution and ecology of Winter heath (*Erica carnea* L.) in Bosnia and Herzegovina. The Bulletin of the Faculty of Forestry, University of Banja Luka, (29): 75-83.

Novaković-Vuković, M. (2015): Floristic characteristics of Austrian pine and Scots pine forests on serpentinite and serpentinite in western and central Serbia. PhD thesis, University of Belgrade, Faculty of Forestry, Belgrade, Serbia, pp. 245.

Novaković-Vuković, M., Eremija, S., Lučić, A., Kadrović, S., Kapović Solomun, M., Blagojević, V., Košanin, O. (2019): Floristic composition of black pine forests on serpentinite in the territory of Serbia and Bosnia and Herzegovina (B&H). Applied Ecology and Environmental Research, 17(2): 4999-5010.

Pavlović, Z. (1964): Pine forests on serpentinites in Serbia. Bulletin, Natural Sciences Museum (Belgrade) B19: 25-65

Rubio-Moraga, A., Candel-Perez, D., Lucas-Borja, M.E., Tiscar, P.A., Vinegla, B., Linares, J.C., Gómez- Gómez, L., Ahrazem, O. (2012): Genetic diversity of *Pinus nigra* Arn. populations in southern Spain and northern Morocco revealed by inter-simple sequence repeat profiles. International Journal of molecular sciences, 13(5): 5645-5658

Stevanović, V. (1992): Classification of plant life forms in the flora of Serbia. – In: Sarić, M. (ed.), The Flora of Serbia. Serbian Acad. Sci. & Arts, Belgrade, Ed. 2. Vol. 1, pp. 37-49.

Stojanović, Lj., Krstić, M., Bjelanović, I. (2010): Stand state and thinning in natural stands of Austrian pine on Mt. Maljen. Forestry, (3-4): 23-34

Tatić, B., Tomić, Z. (2006): Forests of Austrian pine and Scots pine. In Vegetation of Serbia II, Forest Vegetation 2. Eds. Škorić D. & O. Vasić. SANU, Dept. Chemical and Biological Sciences. Beograd, 127-154.

Tomić, Z. (2004): Forestry phytocoenology, University textbook. Faculty of Forestry, University of Belgrade, Belgrade

Tomić Z, Rakonjac Lj. (2013). Forest phytocenoses of Serbia. A manual for foresters, ecologists and biologists: University Singidunum, Faculty of Applied Ecology Futura, Institute of Forestry, Belgrade. 177p.Van Der Maarel, E. (1979): Transformation of cover-abundance values in phytosociology and its effects on community similarity. Vegetatio, 39(2): 97–114.

TAXONOMICAL PROPERTIES OF GREEK MAPLE (*ACERHELDREICHII* ORPH.) ON JAHORINA MOUNTAIN IN BOSNIA AND HERZEGOVINA

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Abstract

Greek maple (*Acer heldreichii* Orph.) is subendemic tree species of Balkan Peninsula and tertiary relic. On Jahorina Mt (Entity of Republic of Srpska, Bosnia and Herzegovina), this species occurs on northwestern border of its global distribution. The aim of this research was to investigate taxonomical division of Greek maple on Jahorina, based on morphological markers. For taxonomical research, 20 representative, physiologicaly mature trees were chosen, and from every tree, 20 normally developed leaves were collected, which formed the basis for taxonomical analysis. Measured leaf properties were: leaf length, widths of upper, main and lower leaf lobes, peduncle length and angle between upper and main leaf lobes. Varieties and forms of Greek maple were separated according to leaf shape and dimensions. Two varieties were set apart, var. *heldreichii* and var. *macropterum*; within var. *macropterum* two forms were distinguished, f. *typicum* and f. *dissectum*. Results of taxonomical analysis were statistically processed. Considering that Greek maple is protected species in Republic of Srpska, this research possess special interest in increasing knowledge of variability and genetic diversity of this species, which will help in its preservation and protection.

Key words: *Greek maple, Acer heldreichii, endemic, taxonomy, Jahorina*

Introduction

Greek maple (*Acer heldreichii* Orph.) is one of the most interesting native woody species of Balkan Peninsula, being Balkan sub endemic and tertiary relic (Cvjetićanin et al., 2016). It grows as a high tree, produces good quality timber and possesses ornamental properties. It is also ecologically interesting, considering it grows only on mountain sites, and among native Balkan maples it is most suited for cold climatic conditions of higher elevations (Lakušić, 1989). Capability of Greek maple growth in ecological conditions, where only small number of tree species are able to survive, stresses its ecological importance on native sites, where it protects soil from erosion, improves ecological conditions for survival of other plant species and represents food source for significant number of animal species (Perović, 2014). Greek maple occurs at elevations between 1000-2000 m a.s.l., on mountains of Bosnia and Herzegovina, Serbia, Montenegro, Northern Macedonia, Bulgaria, Greece and Albania. In Bosnia and Herzegovina, it occurs only in Republic of Srpska on the following mountain massifs (Lakušić 1964; Fukarek 1969; Perović 2014): Jahorina, Trebević, Klek, Radomišlje, Treskavica, Zelengora, Volujak, Maglić and Bjelasnica near Gacko, which represents the westernmost world site of this species. Apart from typical subspecies *Acer heldreichii* subsp. *heldreichii* from Balkans, the second subspecies *Acer heldreichii* subsp. *trautvetteri* (Medw.) Murray) occurs on Caucasus Mountain and northeastern Turkey (van Gelderen et al. 2010)

The aim of this paper was to research taxonomical characteristics of *Acer heldreichii* on Jahorina mountain. Perović (2014), concluded that in Serbia and some localities of Bosnia and Herzegovina and Montenegro occur two Greek maple varieties: var. *macropterum* and var. *heldreichii*. Within variety *macropterum* four forms were set apart: f. *typicum*, f. *rotundiloba,* f. *dissectum* and f. *equiloba*. Considering that Greek maple is protected species in Republic of Srpska (*2014), this research is especially important because it will provide better knowledge about variability and genetic diversity of this species, which will help in protecting of Greek maple in researched region.

Material and method

Research was done in mountain Jahorina (Republic of Srpska, Bosnia and Herzegovina), within forest area „Jahorina" in compartments 117 and 120, which belong to forest management unit ,,Gornja Prača". Compartment 117 occurs at elevation 1590-1640 m a.s.l., on northern and northwestern aspect, with inclination 20-25° Compartment 120 is positioned at elevation 1400-1630 m a.s.l., on eastern and southeastern aspect, with inclination 20° (*2015).
In the aim to research taxonomic characteristics of Greek maple on Jahorina, 20 representative, physiologically mature trees were chosen for analysis. From every tree 20 normally developed leafs were collected, which were the basis for taxonomical analysis. Varieties and forms were distinguished by the leaf shape and dimensions. Following leaf properties were measured (Figure 1): leaf blade length (A), side leaf lobe width (B), basal leaf lobe width (C), upper leaf lobe width (D), length of leaf stalk (E), and angle between side and upper leaf lobes (F). Results of morphological analysis were statistically processed using statistical methods: method of correlation analysis and T-test of independent samples of measured leaf properties. Statistical analysis was done using software SPSS 23.0 (Pallant 2011; *2015).

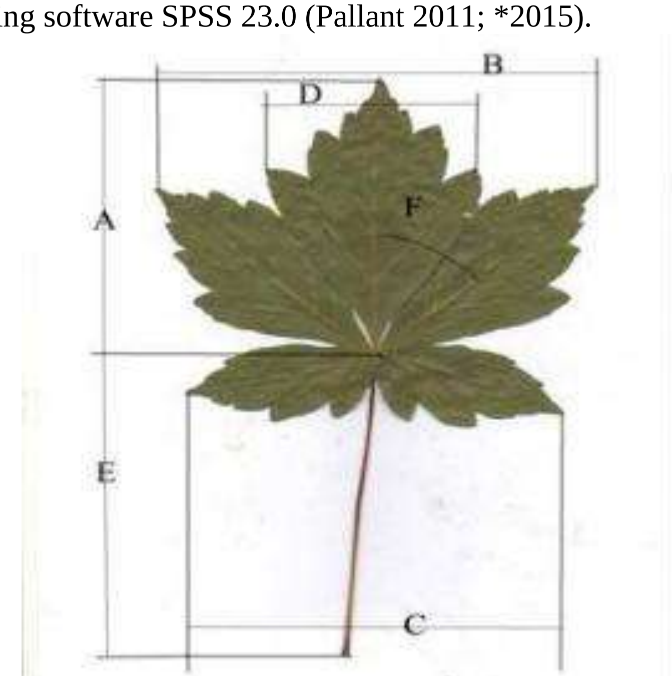

Figure 1 : Analysed morphological leaf charateristics (Perović 2014)

Figure 2. *Acer heldreichii* on researched locality on Jahorina Mt.

Results and discussion

Two Greek maple varieties vere set apart on reserached area (var. *heldreichii* and var. *macropterum*), and within var. *macropterum* two forms were determinated (f. *dissectum* and f. *typicum*).

Acer heldreichii var. *macropterum* is characterized by leaf lobes which are separated and never ovelap. 15 of 20 researched trees on Jahorina belong to this variety. Average values of measured leaf properties of *Acer heldreichii* var. *macropterum* on Jahorina are: length of leaf blade is 10.49 cm, width of side leaf lobe is 14.40 cm, width of basal leaf lobe is 11.98 cm, width of upper leaf lobe is 4.85 cm, length of leaf stalk is 10.44 cm. Within this variety, 10 individual trees on Jahorina belong to f. *typicum* (Figure 3), which is characterized by shallower lobed leaves, while 5 trees belong to the f. *dissectum* (Figure 4), with very deeply lobed leaves, almost to the leaf base. Average values of measured leaf properties of *Acer heldreichii* var. *macropterum* f. t*ypicum* are: length of leaf blade is 10.53 cm, width of side leaf lobe is 14.70 cm, width of basal leaf lobe is 12.48 cm, width of upper leaf lobe is 4.91 cm, length of leaf stalk is 10.58 cm. Average values of measured leaf properties of *Acer heldreichii* var. *macropterum* f. *dissectum* are: length of leaf blade is 10.42 cm, width of side leaf lobe is 13.78 cm, width of basal leaf lobe is 10.98 cm, width of upper leaf lobe is 4.71 cm, length of leaf stalk is 10.13 cm.

Acer heldreichii var . *heldreichii* (Figure 5) is characterized by overlapping leaf lobes. 5 researched trees on Jahorina belong to this variety. Average values of measured leaf properties of *Acer heldreichii* var. *heldreichii* are: length of leaf blade is 10.75 cm, width of side leaf lobe is 14.96 cm, width of basal leaf lobe is 12.07 cm, width of upper leaf lobe is 4.32 cm, and length of leaf stalk is 11.48 cm.

Perović (2007, 2014) researched taxonomical properties of Greek maple in Serbia (on six most representative localities), and he included in this research one site from Bosnia and Herzegovina and Montenegro respectively (localities „Perućica" primary forest on Maglić Mt. in Bosnia and

Herzegovina and "Biogradska gora" primary forest on Bjalasica Mt. in Montenegro). In this research the author determined occurrence of two *Acer heldreichii* varieties (var. *macropterum* and var. *heldreichii*) on researched localities. Interesting is to note that both varieties were represented on all eight researched localities in his work. This paper proves that both varieties occur on Jahorina also.

Figure 3. *Acer heldreichii* var. *macropterum* f. *typicum*, Jahorina Mt

Figure 4. *Acer heldreichii* var. *macropterum* f. *dissectum*, Jahorina Mt.

Figure 5 *Acer heldreichii* var. *heldreichii,* Jahorina Mt.

Corelation analysis of measured leaf properties were applied separately on trees which belong to *Acer heldreichii* var. *heldreichii*, *Acer heldreichii* var. *macropterum* f. *dissectum* and *Acer heldreichii* var. *macropterum* f. *typicum*. It is determined that measured properties occur in very different corelative relations, from strong positive corelation, to insignificant corelative relations to moderately negative corelation.

At variety *heldreichii*, all measured leaf properties show strong positive correlation, except the angle between upper and basal leaf lobes, which shows no statistically significant corelations to other measured properties except the width of basal leaf lobe. Speaking about var. *macropterum* f. *dissectum* correlation has somewhat lower intensity. Neither property shows strong correlation to other, while it is moderate to weak. The strongest correlation is between length of leaf blade and width of basal leaf lobe, as well as between width of side leaf lobe and width of upper leaf lobe. For var. *macropterum* f. *typicum* it is established that measured leaf properties are within moderately strong correlation, except the angle between upper and side leaf lobes, which show weak corelation to other measured properties.

T-test for individual samples, compared mean values of measured leaf properties between var. *heldreichii* and var. *macropterum*. Analysis was done for every leaf property separately. Difference between these two varieties on Jahorina is statistically insignificant respecting to the leaf blade length, side leaf lobe width, basal leaf lobe width, and angle between side and upper leaf lobes. Statistically significant difference between *Acer heldreichii* var. *heldreichii* and *Acer heldreichii* var. *macropterum* on Jahorina Mt. occurs regarding the width of upper leaf lobe ($t(18)=2.67$; $p=0.016$, i.e. $p<0,05$) and length of leaf stalk ($t(18)=2.11$; $p= 0.048$ i.e. $p<0,05$).

Conclusion

Analysis of morphological markers (leaves) in Greek maple (*Acer heldreichii* Orph.) population on Jahorini Mt. in Republic of Srpska, Bosnia and Herzegovina, showed occurrence of two Greek maple varieties on researched locality- var. *macropterum* and var. *heldreichii*. Within var. *macropterum*, two forms were set apart- f. *typicum* and f. *dissectum*.

Correlation analysis ascertained that measured leaf properties of Greek maple occur in positive corelative relations of various level, except the property angle between side leaf lobe and upper lef lobe, which mosty shows no clear correlation to other measured leaf properties.
Analysis made by t-test for small independent samples between individuals belonging to var. *heldreichii* and var. *macropterum* showed statistical significance by properties width of side leaf lobe and length of leaf stalk, while it is not statistically significant for other properties.
Considering that Greek maple is protected species in Republic of Srpska, this research is especially important because it will provide better knowledge about variability and genetic diversity of this species, which will help in protecting of Greek maple in researched region.

References

Cvjetićanin, R., Brujić, J., Perović, M., Stupar, V. (2016): Dendrologija (Dendrology). Univerzitet u Beogradu-Šumarski fakultet

Fukarek, P. (1969): Dendroflora nacionalnog parka „Sutjeska" (Dendroflora of the „Sutjeska" National Park). In: „Osnovne prirodne karakteristike, flora i vegetacija nacionalnog parka Sutjeska". Akademija nauka i umjetnosti BiH, Posebna izdanja, Knj. XI, Odjeljenje prirodnih i matematičkih nauka, knj. 3. Sarajevo. Str. 107-170.

Lakušić, R. (1964): Planinski javor (*Acer heldreichii* Orph) (Greek Maple (*Acer heldreichii* Orph) . Godišnjak Biološkog fakulteta Univerziteta u Sarajevu, Vol. XVII. Str. 117.-143.

Lakušić, R. (1989): Ekologija biljaka-I dio-idioekologija (Plant Ecology I part-Idiecology). Svjetlost, Sarajevo

Pallant, J. (2011). SPSS priručnik za preživljavanje (SPSS surviving Guidebook). 4 izdanje. Mikro knjiga, Beograd.

Perović, M. (2007): Taksonomske, ekološke i anatomske karakteristike planinskog javora (*Acer heldreichii* Orph.) u centralnoj Srbiji (Taxonomy, Ecology and Anatomy of Greek Maple (*Acer heldreichii* Orph.) in Central Serbia). Magistarska teza u rukopisu. Univerzitet u Beogradu-Šumarski fakultet

Perović, M. (2014): Taksonomija i uticaji staništa na karakteristike planinskog javora (*Acer heldreichii* Orph.) u Srbiji (Taxonomy and Site Influence on Greek Maple (*Acer heldreichii* Orph.) Characteristics in Serbia). Doktorska disertacija u rukopisu. Univerzitet u Beogradu-Šumarski fakultet

van Gelderen, D., de Jong, P., Oterdoom, H. (2010): Maples of the world. Timber press. Portland-London

*(2014): Zakon o zaštiti prirode Republike Srpske (Law on Nature Protection of Republic of Srpska). Službeni glasnik Republike Srpske br. 20

*(2015): Šumskoprivredna osnova za Jahorinsko šumskoprivredno područje (01.01.2015. do 31.12.2024) (Forest basis for Jahorina Forestry area (01.01.2015. do 31.12.2024). a.d. Sokolac, Istraživačko razvojni i projektni centar, Banja Luka. JPŠ „Šume RS",

*(2015): IBM SPSS Statistics for Windows. Version 23. Armonk NY: IBM corp.

EUGLEY SOIL OF FLOODPLAIN AND PROTECTED PART OF ALUVIAL PLANE AND POSSIBILITIES OF THEIR FORESTATION

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Abstract

The paper presents the properties of eugley soil in the floodplain and embankment protected part of the alluvial plain. The floodplain is under the influence of flood and groundwater, while the protected part is exclusively under the influence of groundwater. The properties of the soil in the examined parts differ depending on the method of wetting. Eugley floodplain soil is characterized by lighter granulometric composition and the texture classes of these soils are sand and loamy sand, while the protected part soil is heavier granulometric composition with texture classes: sandy loam, clay loam, sand, loamy sand and sandy loam. In the case of soil in the flood zone, the reduction sub-horizon of gley is located at a smaller depth, i.e. 30 to 35 cm, and in the protected zone it is deeper, and we find it at a depth of 45 to 90 cm. The reaction of the soil of the floodplain is neutral to slightly alkaline, while in the soil in the protected zone is moderately alkaline. The capacity of the humus horizon of the flood zone is lower and is 10 cm, with humus content up to 3.01% in the surface horizon, while the protected part had the more powerful humus horizon with depth, from 45 to 50 cm and higher humus content ranging up to 5.59 %. The examined eugley of the soil according to the depth of the gley horizon in the area of the flood zone is classified as α-gley, and in the area of the protected zone into β-gley and γ-gley. Due to the high level of groundwater and the small depth of the gley horizon, eugley soils in the floodplain are not suitable for raising forest plantations, while eugley soils in the protected zone are suitable habitats for raising white willow plantations due to the greater depth of the gley horizon.

Keywords: *Eugley, Alluvial plain, Flooding, Groundwater, Afforestation.*

Introduction

The area of the alluvial plain is characterized by hydromorphic soils that show regularity in distribution in relation to the riverbed, and can also be musically and irregularly distributed. Eugley soils as a representative of the class of gley soils (Škorić et al., 1985) are mostly found at a certain distance from the riverbed, in the area where the finest soils are deposited after flood periods. Their distribution is related to the lowest terrains and they are located in depressions. The main characteristic of these soils in the morphology of the profile is the heavier granulometric composition, and groundwater that varies to a depth of about 100 cm (Pekeč et al.. 2011a). Certain parts of the alluvial plain that are not protected by the embankment are still under the direct influence of flooding, which, along with the action of groundwater, affects the genesis and characteristics of eugley soil in this zone. The part of the alluvial plain at a greater distance from the riverbed, which is protected from flood waters by the embankment, is not exposed to the influence of surface waters, but only the groundwater affects the genesis and characteristics of this area. Eugley soils (Gleysols according to the WRB classification, 2006) develop a gley horizon of secondary oxidation and a gley sub horizon of secondary reduction with respect to variation and groundwater level, and the depth of the gley horizon is related to groundwater hydro morphism. According to the depth of the gley horizon, eugley soils are classified according to Wilde into α-gley, β-gley and γ-gley (Živanov and Ivanišević, 1986). The depth of the gley horizon is of the most important importance for the productivity of eugley soils, and soils with a greater depth of this horizon are of better credit rating class Antić et al (1982). Based on the examination of the properties of eugley soil in the floodplain of the alluvial plain and the embankment protected from flooding, the paper presents the characteristics of these soils in these two different areas, where in earlier periods afforestation of these areas depending on the depth of the gley horizon.

Material and method

The paper investigates wetland gley soil in the area of the alluvial plain of the Danube in the vicinity of Novi Sad in Serbia. Three pedological profiles were opened in the part of the alluvial plain under the influence of flood waters (N 45°14'8.17"; E 19°40'31.21") and three profiles in the part of the alluvial plane protected by the embankment from flood waters (N 45°17' 44.58; E 19°53'28.87"). External and internal profile morphology is described. Soil samples in disturbed condition were taken from open pedological profiles and the following physical and chemical analysis of soil were performed: Mechanical composition, B-pipette method with sample preparation for analysis in sodium pyrophosphate according to Bošnjak et al (1997), and the texture class of the soil was determined according to the Tommerup classification; CaCO3 content was determined volumetrically using a Scheibler calcimeter; The pH value was determined in a suspension of soil with water, potentiometrically; Humus content according to Tyurin, modified by Simakov; Based on the performed analysis, the characteristics of the examined land are presented.

Results and discussion

Analysing the data from Table 1 for the first three pedological profiles (P1-P3) which represent wetland gley soil wetted by flood and groundwater, it can be concluded that the largest share of fine sand fraction, which ranged from 65.03 to 77.93%. The content of coarse sand is lower and ranges from 7.83 to 20.43%. This soil contains the smallest fractions of clay whose values ranged from 2.63 to 7.03%, while the powder content ranged from 7.53 to 11.63%. The average value of total sand for this land is from 85.42 to 86.03%, while the value of total clay ranged from 13.96 to 14.56%. The texture classes of these soils were: sand in the surface horizon and loamy sand in the lower horizons of the profile. In the case of profiles representing wetland gley soil of the part of the alluvial plain which is protected from flooding (P4-P6) and is moistened exclusively by groundwater, the fraction of fine sand whose values are from 29.16 to 69.73% also predominates. The coarse sand fraction is smaller and ranges from 0.59 to 13.62%, while the increased fraction of powder fraction ranging from 13.91 to 39.00% and the content of the clay fraction whose values are from 12.21 to 31.26%. The average value of total sand for this land is from 29.74 to 73.88%, while the value of total clay ranged from 26.12 to 70.26%. The texture classes of these soils were: sandy clay loam to clay loam in the surface part of the profile and in the lower parts of the profile: sand, loamy sand, sandy loam and clay loam. Pekeč et al. (2011b) exploring the eugley of the soil of the protected part of the alluvial plain lists textural classes such as loam and silty loam.

Table 1. Granulometric composition of soil

No Profile	Horizon	Depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay(%)	Total sand (%)	Total clay (%)	Texture class
P1	Aa	0-10	31.3	58.7	3.8	6.2	90.0	10.0	Sand
	Gso	10-30	11.3	70.0	10.7	8.0	81.3	18.7	Loam sand
	Gr	30>	18.7	66.4	8.1	6.9	85.0	15.0	Loam sand
	average		***20.43***	***65.03***	***7.53***	***7.03***	***85.43***	***14.56***	
P2	Aa	0-10	6.3	81.3	9.3	3.1	87.6	12.4	Sand
	Gso	10-30	8.1	86.2	5.6	0.2	94.2	5.8	Sand
	Gr	30>	9.1	66.3	20.0	4.6	75.4	24.6	Loam sand
	average		***7.83***	***77.93***	***11.63***	***2.63***	***85.73***	***14.26***	
P3	Aa	0-10	1.5	89.8	5.6	3.0	91.4	8.6	Sand
	Gso	10-35	22.6	57.6	12.4	7.5	80.1	19.9	Loam sand
	Gr	35>	12.9	73.7	6.1	7.3	86.6	13.4	Loam sand
	average		***12.33***	***73.7***	***8.03***	***5.93***	***86.03***	***13.96***	
P4	Aa	0-50	6.71	39.49	26.6	27.2	46.2	53.8	Sand clay loam
	Gso	50-90	18.02	66.78	9.64	5.56	84.8	15.2	Loam sand
	Gr	90>	16.12	68.92	9.8	5.16	85.04	14.96	Loam sand
	average		***13.62***	***58.40***	***15.35***	***12.64***	***72.01***	***27.99***	
P5	Aa	0-50	7.31	43.10	25.84	23.76	50.4	49.6	Sand clay loam
	Gso	50-90	2.85	71.95	14.64	10.56	74.8	25.2	Sand clay loam
	Gr	90>	2.31	94.13	1.24	2.32	96.44	3.56	Sand
	average		***4.16***	***69.73***	***13.91***	***12.21***	***73.88***	***26.12***	
P6	Aa	0-45	0.73	21.59	45.48	32.2	22.32	77.68	Clay loam
	Gr	45>	0.44	36.72	32.52	30.32	37.16	62.84	Clay loam
	average		***0.59***	***29.16***	***39.00***	***31.26***	***29.74***	***70.26***	

On the basis of chemical properties of the soils in the floodplain, it can be stated that according to the carbonate content, these soils (P1-P3) have a range from 14.11 to 14.24%, and we classify them as strongly carbonate soils. The pH value ranged from 7.32 to 7.49, ie these are soils of neutral to slightly alkaline reaction (according to the American classification for chemical reaction of soils), and the humus content of the studied soil is on average from 1.27 to 1.43% and these soils are poorly humus (according to Scheffer-Schachtschabel classification). The chemical properties of the soil in the part protected from flooding (P4-P6) indicate a carbonate content of 7.59 to 16.55%, ie this soil is carbonate to strongly carbonate. The pH value ranges from 8.23 to 8.86%, which classifies it as moderately alkaline soils (according to the American classification for soil chemical reaction), and the humus content averages 1.05 to 5.31%, so this soil is in classes from weak to very humus. (according to the Scheffer-Schachtschabel classification).

Table 2. Chemical properties of soil

No Profile	Horizon	Depth (cm)	$CaCO_3$ (%)	pH (H_2O)	Humus (%)
P1	Aa	0-10	14.11	7.28	3.01
	Gso	10-30	14.37	7.68	0.57
	Gr	30>	14.25	7.52	0.71
	average		***14.24***	***7.49***	***1.43***
P2	Aa	0-10	14.17	7.16	3.01
	Gso	10-30	14.01	7.61	0.08
	Gr	30>	14.60	7.47	1.17
	average		***14.11***	***7.41***	***1.42***

No Profile	Horizon	Depth (cm)	$CaCO_3$ (%)	pH (H_2O)	Humus (%)
P3	Aa	0-10	14.08	7.24	3.00
	Gso	10-35	14.42	7.38	0.46
	Gr	35>	14.20	7.33	0.36
	average		***14.23***	***7.32***	***1.27***
P4	Aa	0-50	2.91	7.69	3.56
	Gso	50-90	10.40	8.87	0.12
	Gr	90>	13.95	8.86	0.10
	average		***9.09***	***8.47***	***1.26***
P5	Aa	0-50	9.44	7.87	2.02
	Gso	50-90	20.92	9.4	0.82
	Gr	90>	19.28	9.32	0.30
	average		***16.55***	***8.86***	***1.05***
P6	Aa	0-45	13.54	7.98	5.59
	Gr	45>	1.64	8.47	5.02
	average		***7.59***	***8.23***	***5.31***

According to the granulometric composition of the examined soils (Table 1), it can be seen that the eugley soils are in the flood zone of lighter mechanical composition, especially in the surface horizon where the texture class is sand, which is the result of flooding of that area during high Danube water levels. The lower parts of these soils have a slightly higher share of total clay in relation to the surface horizon. Eugley soils in the part of the alluvial plain protected from floods have a heavier mechanical composition, which is especially visible near the humus horizon, while the depth of the profile reduces the share of total clay. The reduction sub-horizon of gley (Gr) in the soil in the flood zone is located at a depth of 30 to 35 cm, which indicates a high level of groundwater, and due to the large amount of water, according to Živkovic et al. (1972) this land is overgrown with hydrophilic vegetation. In the zone protected from floods, the reduction sub-horizon of gley is at a depth of 45 cm, i.e. at a depth of 90 cm, and in this zone the groundwater is at a greater depth. Analyzing the chemical characteristics of the examined soils, the reaction of the soils (Table 2) in the floodplain is neutral to slightly alkaline due to the leaching of the soils with floodplain water, with a slight increase in alkalinity along the depth of the profile. In the flood-protected zone, the soil reaction is moderately alkaline with a significant increase in alkalinity according to the depth of the profile, which is a possible result of wetting with alkaline groundwater. Based on the poorer quality of groundwater, it is possible to increase the alkalinity of the lower parts of the eugley soil profile according to Pekeč et al. (2020). Also, the capacity of the humus horizon of the soil of the flood zone is up to 10 cm deep, and the formation of humus is often interrupted by flood waters and the application of new soil material, and the humus content in the surface humus horizon is up to 3.01%. In eugley soil protected from flood waters, the humus horizon is more powerful, with a depth of 45 to 50 cm, and a humus content of up to 5.59%, which is the result of the accumulation of organic matter that is not interrupted by fluvial sedimentation and flooding. Based on the depth of the gley horizon according to Wilde's classification, according to Živanov and Ivanišević (1986), soils in the flood zone are classified as α-gley (P1-P3), and soil in the protected zone into β-gley (P6) and γ- see (P4 and P5). Considering the depth of the gley horizon and the depth of groundwater retention of the examined areas, the dominant forest species to which such habitat conditions correspond are willows, ie white willow (*Salix alba*). As stated by Antić et al. (1982) White willow stands can have different productivity in relation to the depth of the gley horizon in the profile. The dependence of forest quality and depth of the gley horizon was determined by Antić et al (1972), Milojković et al. (1972) and Jović et al (1972) who stated that the most productive are white willow stands on γ-gley, while the middle productive stands are on β-gley, and the lowest productivity was shown by white willow stands on α/β-gley. According to the data on the productivity of white willow on eugley, we can conclude that the examined lands in the floodplain classified as α-gley are not suitable for raising white willow plantations due to high groundwater levels and small depth of gley horizon, mostly this land covered by marsh vegetation. Eugley soils examined in the part of the alluvial plain which is protected from floods by the embankment and where we find β and γ-gley, with a greater depth of the gley horizon are suitable habitats for raising white willow plantations. Depending on the groundwater level and the depth of the gley horizon, we can expect different productivity of plantations.

Conclusion

The paper presents the properties of eugley soil in the floodplain and the embankment protected part of the alluvial plain. The floodplain is under the influence of flood and groundwater, while the protected part is under the influence of only groundwater. The properties of the soil in the examined parts different depending on the method of wetting. Eugley soil of the flood part is characterized by a lighter granulometric composition, and the proportion of clay increases with the depth of the profile. The reduction sub-horizon of gley near the soil in the flood zone is closer to the surface and is located at a depth of 30 to 35 cm, and the reaction of the soil is neutral to slightly alkaline. The capacity of the humus horizon of the flood zone is smaller, i.e. up to 10 cm deep. The soil in the protected part has a heavier granulometric composition, and the proportion of clay decreases along the depth of the profile. The reduction sub horizon of gley is at a greater depth, ie from 45 to 90 cm, while the reaction of this soil is moderately alkaline. The thickness of the humus horizon is more pronounced and ranges from 45 to 50 cm. Based on the depth of the gley horizon, the soil in the flood zone is classified as α-gley, and in the protected zone into β-gley and γ-gley. The examined eugley soils in the flood zone are not suitable for raising forest plantations, while eugley soils in the protected zone are suitable for raising white willow plantations, whose productivity depends on the depth of the gley horizon.

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References

Bošnjak, Đ., Dragović, S., Hadžić, V., Babović, V., Kostić, N., Burlica, Č., Đorović, M., Pejković, M., Mihajlović, TD, Stojanović, S., Vasić , G., Stričević, R., Gajić, B., Popović, V., Šekularac, G., Nešić, Lj., Belić, M., Đorđević, A., Pejić, B., Maksimović, l., Karagić , Đ., Lalić, B., Arsenić, I. (1997). Methods of research and determination of physical properties of soil. JDPZ, Belgrade.

Antić, M., Jovanović, B., Jović, N., Munkačević, V., Nikolandić, S. (1972). Types of forests of the Belje hunting and forest area, Current problems of forestry, Faculty of Forestry, Belgrade, Serbia

Antić, M., Munkačević, V., Jović, N. (1982). Pedology, Scientific book, Belgrade, Serbia

Jović, N. Falica, M., Vasung V. (1972). Production characteristics of forest types within the Belje hunting and forest area. Deer - LSPG Bulletin, special issue No. 3, Belgrade, Serbia

Milojkovic, D., Mirkovic, D., Jovic, D. (1972). Forest economic basis of the Belje hunting and forest area, Current problems of forestry, Belgrade, Serbia

Pekeč S., Vrbek B., Orlović S., Kovačević B. (2011a). Production potential of black poplar (Aigeiros Duby Section) on eugley. Šumarski list, No 1-2: 29-37, 2011.

Pekeč S., Ivanišević P., Orlović, S., Kovačević B., Pilipović A. (2011b). Production of seedlings of white willow (*Salix alba* L.) on eugley soil, Forestry Ideas, 2011, Vol. 17, No. 1: 88-96, Sofia, Bulgaria.

Pekeč, S., Milović, M., Karaklić, V., Vasin, J., Živanov, M. (2020). Level and quality of groundwater in eugley soil of the central Danube basin, XI International Scientific Agricultural Symposium „Agrosym2020" p.1018-1024, 8-9. October, 2020. Bosnia and Herzegovina, Republic of Srpska.

Škorić, A., Filipovski, G., Ćirić M. (1985). Soil Classification of Yugoslavia, Academy of Sciences and Arts of Bosnia and Herzegovina, Special Editions, Book LXXVIII Sarajevo.

World reference base for soil resources (2006): IUSS Working Group WRB. 2006. World Soil Resources Reports No. 103. FAO, Rome.

Živanov, N., Ivanišević, P. (1986): Soil for growing poplars and willows. In: Poplars and Willows in Yugoslavia. Poplar Institute, Novi Sad: 103-119

Živković, B., Nejgebauer, V., Tanasijević, Đ., Miljković, N., Stojković, L., Drezgić, P., (1972). "Soils of Vojvodina", Institute for Agricultural Research, Novi Sad

COMPARISON OF THE GROUND VEGETATION IN DOUGLAS-FIR ARTIFFICIALY ESTABLISHED STANDS AND NATURAL MIXED BEECH AND SESSILE OAK FOREST IN THE AREA OF KOSMAJ (SERBIA)

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Abstract

The paper presents a study of changes in ground vegetation associated with the conversion of natural, mixed beech-sessile oak forest (*Querco petraeae – Fagetum moesiacae* Glišić 1971.) into artificially established stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the area of Kosmaj (Serbia). The study has revealed that there are differences in the floristic composition because the process of artificial stand establishment entails disappearance of some species and the appearance of new ones. The introduction of Douglas-fir increases the plant species diversity. The values determined for beech-sessile oak forest have been as follows: average number of species 22; average Shannon-Wiener index 2.32; average equality index 0.74. The values of the same indicators for artificially established stands of Douglas-fir have been: average number of species 38; average Shannon-Wiener index 2.80; average equality index 0.75. In the spectrum of floral elements, plants of the Central European range type are most represented, amounting to 49% (beech-sessile oak forest) or 59% (Douglas-fir plantations). The study of the plant life forms shows that phanerophytes are the dominant class with 41% (beech-sessile oak forest) or 46% (Douglas-fir plantations). Comparative analysis of ecological indices for plant species shows a higher percentage of plants of mesotrophic and eutrophic character in artificially established stands of Douglas-fir, which indicates a higher nutrient availability

Keywords: *Douglas-fir, beech-sessile oak forest, plant species diversity, Kosmaj.*

Introduction

During of afforestation and reclamation of degraded forests conducted in previous decades, insufficient attention was paid to complex properties of vegetation-forest ecosystems, which resulted in plantation of coniferous monocultures. The most commonly used species in Serbia in the reclamation of oak and beech forests were Austrian pine, Scots pine, spruce, Weymouth pine, Douglas-fir, larch etc.

Long-term processes of reducing the area under forests, changes in the structure of forest ecosystems and the quality of wood mass, as an inevitable consequence of economic and demographic development and the struggle of people for survival, did not pass the researched area of Kosmaj. Since the middle of the last century, in order to solve the problem of coppice and degraded forests in this area, as well as everywhere in Serbia, various types of trees, primarily conifers, have been introduced. It is known that these types of trees can quickly establish a complete set of stands, and thus fully realize their protective and hydrological function, and at the same time can cause negative consequences for the soil and vegetation. The establishment of a new forest ecosystem directly or indirectly affects plant species - in most cases there is a change in the composition and number of species of ground vegetation, ie a reduction in species

diversity. Tree species composition is a key driver of forest biodiversity, influencing structural components of the environment from soil and litter to vegetation layers and the canopy, and ecosystem processes, such as nutrient cycling (Oxbrough *et al*., 2012).
Douglas-fir is a species which was introduced to very different sites in Serbia. Its monoplantations were used in the substitution of natural forests from submontane to subalpine belts. Numerous different experiences have been reported on the effects of Douglas-fir on the soil changes after the substitution of autochthonous forests on different sites in Serbia (Miletić *et al*., 2003; 2013). Proper selection of tree species during afforestation or reclamation of degraded forests, with an appropriate management method, ensures the improvement of biological diversity, while preserving endangered and rare plant species. Therefore, the aim of this study was to determine the extent to which the establishment of artificial Douglas-fir stands (*Pseudotsuga menziesii* (Mirb.) Franco) at the site of beech and sessile oak forest (*Querco petraeae-Fagetum moesiacae*) had an impact on the change of floristic composition and diversity of these forests.

Material and Methods

The research was conducted in the area of Kosmaj Mountain, which is located 40 km southeast of the city of Belgrade (Serbia). The forest cover of the Mt. Kosmaj study area is composed of deciduous oak and beech forest communities (Stajić *et al*., 2021). The research of site conditions and floristic composition was performed by means of series of trials, conducted in mixed beech-sessile oak forest and artificially established stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Field surveys were conducted in 2016 and 2017. Floristic sampling was done on a floristically homogeneous surface area with a plot size of 900 m^2 (30 x 30 m). In each plot, a complete floristic list of all vascular plants (tree, shrub, and herb layers separately) was recorded using the Braun-Blanquet scale (Braun-Blanquet, 1964). The transformation of abundance and cover estimation of each species within phytocoenological relevés was performed according to the Van Der Maarel method (1979). Species diversity was measured using *the Shannon-Wiener* diversity index (Shannon and Weaver, 1963); the Pielou Index (E) was used for the estimation of species evenness (1975). JUICE 7.0 software was used to calculate these indices (Tichý, 2002). The spectrum of floral elements was determined according to the systematization of geo-floristic elements by Gajić (1980), the spectrum of life forms according to the Method of Kojić *et al.* (1997), and the indicator values of plants and ecological optimums were determined using the Method of Kojić *et al.* (1997).

Results and Discussion

Mixed beech and sessile oak forests – *Querco petraeae-Fagetum moesiacae* Glišić 1971. on Kosmaj occur in a wide range of altitudes (relevés range from 373 to 585 m a.s.l.), cold northern aspects (northern, north-western, and north-eastern) and slopes of 16° – 26° (Stajić *et al*., 2020). The cover of the ground flora layer ranges from 0.2 to 1.0. Mesophilic species of beech forests occur with the highest degree of presence: *Lamium galeobdolon* (L.) Crantz, *Carex sylvatica* Huds., *Dryopteris filix-mas* (L.) Schott and *Rubus hirtus* Waldst. & Kit. (Stajić *et al.*, 2020). Based on their floristic composition, it is concluded that the Douglas-fir stands were established at the site of beech and sessile oak forest *(Querco petraeae-Fagetum moesiacae* Glišić 1971). The cover of the ground flora layer ranges from 0.7 to 0.8. The study has revealed that there are differences in the floristic composition because the process of artificial stand establishment entails disappearance of some species and the appearance of new ones (Table 1).

Table 1. Floristic composition of the study forests

Species	*BS*	*DF*	*Species*	*BS*	*DF*
Acer campestre L.	+		*Lamium galeobdolon* (L.) Crantz	+	+
Acer negundo L.		+	*Lamium maculatum* L.	+	
Acer platanoides L.	+		*Lathyrus vernus* (L.) Bernh.	+	
Acer pseudoplatanus L.	+	+	*Lilium martagon* L.	+	
Ajuga reptans L.	+		*Lonicera caprifolium* L.	+	
Alliaria petiolata (Bieb.) Cavara & Grande	+		*Lysimachia punctata* L.	+	
Allium ursinum L.	+		*Malus sylvestris* (L.) Mill.	+	
Arum maculatum L.	+	+	*Melica uniflora* Retz.	+	+
Asarum europaeum L.	+		*Moehringia trinervia* (L.) Clairv.	+	+
Asperula odorata L.	+	+	*Mycelis muralis* (L.) Dum.	+	+
Athyrium filix-femina (L.) Roth	+		*Polygonatum multiflorum* (L.) All.	+	
Clinopodium vulgare L.	+		*Polygonatum odoratum* (Mill.) Druce	+	
Campanula patula L.	+		*Polystichum setiferum*(Forsk.) Woynar	+	
Campanula persicifolia L.	+		*Populus tremula* L.	+	
Cardamine bulbifera (L.) Crantz	+	+	*Prunus avium* L.	+	+
Carex pendula Huds.	+		*Prunus spinosa* L.	+	
Carex pilosa Scop.	+	+	*Pseudotsuga menziesii* (Mirb.) Franco		+
Carex sylvatica Huds.		+	*Pteridium aquilinum* (L.) Kuhn	+	
Carpinus betulus L.	+	+	*Quercus cerris* L.	+	+
Cephalanthera longifolia (L.) Fritsch	+		*Quercus farnetto* Ten.	+	
Circaea lutetiana L.	+	+	*Quercus petraea* (Matt.) Liebl.	+	+
Clematis vitalba L.	+	+	*Ranunculus cassubicus* L.	+	+
Cornus mas L.	+		*Rosa canina* L.	+	
Cornus sanguinea L.		+	*Rubus canescens* DC.	+	
Corylus avellana L.	+	+	*Rubus hirtus* Wald. & Kif.	+	+
Crataegus monogyna Jacq.	+		*Ruscus aculeatus* L.	+	
Crataegys nigra Waldst. & Kit.	+		*Ruscus hypoglossum* L.	+	+
Cystopteris fragilis (L.) Bernh		+	*Sambucus nigra* L.	+	+
Dryopteris filix-mas (L.) Schott.	+	+	*Scrophularia nodosa* L.	+	+
Euonymus europaeus L.	+		*Stachys alpina* L.	+	+
Euphorbia amygdaloides L.	+		*Stachys sylvatica* L.	+	+
Fagus sylvatica L.	+	+	*Tamus communis* L.	+	+
Fragaria vesca L.	+		*Tilia platyphyllos* Scop.		+
Fraxinus excelsior L.	+	+	*Tilia tomentosa* Moench.	+	
Fraxinus ornus L.	+		*Tilia cordata* Mill.		+
Galium aparine L.		+	*Ulmus minor* Mill.	+	+
Galium sylvaticum L.	+		*Urtica dioica* L.		+
Geranium robertianum L.	+	+	*Veronica montana* L.	+	
Glechoma hirsuta Waldst. & Kit.	+		*Viola alba* Bess.		+
Hedera helix L.	+	+	*Viola hirta* L.	+	
Helleborus odorus Waldst. & Kit.	+		*Viola odorata* L.	+	
Heracleum sphondylium L.	+		*Viola sylvestris* Lam.	+	+
Juglans regia L.	+				

Legend: BS- Beech-sessile oak forest; DF- Douglas-fir artificially established stands

The mean number of species in the artificially established stand of Douglas-fir is higher than in the natural beech-sessile oak forest, which is mainly due to the more open canopy of the tree layer in the artificially established Douglas-fir stands (Table 2). Light availability may affect the species richness of the herb layer both directly through species pool effects and indirectly by its positive effect on productivity (Axmanová *et al*., 2012).

Table 2. Average species richness, Shannon diversity index and species evenness (mean ± SE)

Forest community type	*Species rihness*	*Shannon-Wiener diversity index (H')*	*Evenness*
	Mean ± SE	Mean ± SE	Mean ± SE
Beech-sessile oak	21.60±1.740	2.32±0.123	0.74±0.022
Douglas - fir	38.33±2.081	2.80±0.045	0.75±0.006

Mixed beech–sessile oak community have the lower average value of the Shannon-Wiener diversity index (*H′*) and it amounts to 2.32. Evenness index values are slightly lower in natural beech-sessile oak forest (0.74) compared to artificially raised Douglas-fir stand (0.75).
Regarding the spectrum of plant life forms (Table 3), both the natural forest and the artificially-established stand are dominated by phanerophytes and hemicryptophytes. Phanerophytes are the dominant class with 41% (beech-sessile oak forest) or 46% (Douglas-fir plantations). The high share of geophytes indicates favourable soil conditions (moisture, structure, and depth of soil). The intermediate group of plants between therophytes and chamaephytes accounts for 5%, but there is a higher percentage of therophytes in the artificially established stand (3%).

Table 3. Spectrum of life forms

Life forms	Beech-sessile oak		Douglas - fir	
	Share (%)		Share (%)	
Phanerophytes	27	41	33	46
Nanophanerophytes	13		10	
Phanerophytic lianas	1		3	
Herbaceous chamaephytes		3		3
Hemicryptophytes		28		23
Geophytes		23		21
Therophytes		-		3
Therophytes /Chamaephytes		5		5

The spectrum of floral elements (Table 4) is dominated by the central European group, accompanied by other species typical of cold and humid regions, which leads us to the conclusion that both the forest of beech-sessile oak and the artificially established stand of Douglas-fir are dominated by mesophilous species.

Table 4. Spectrum of floral elements

Cumulative range types	Beech-sessile oak		Douglas - fir	
	Share (%)			
Pontic	6	19	-	12
Sub-Mediterranean	9		10	
Balkan	4		2	
Central European	40	49	51	59
Subatlantic	9		8	

<table>
<tr><td>Desert</td><td>1</td><td>1</td><td>-</td><td></td></tr>
<tr><td>Eurasian</td><td>20</td><td rowspan="2">25</td><td>13</td><td rowspan="2">18</td></tr>
<tr><td>Cosmopolitan</td><td>5</td><td>5</td></tr>
<tr><td>Circumpolar</td><td>5</td><td>5</td><td>8</td><td>8</td></tr>
<tr><td>Adventive</td><td>-</td><td>-</td><td>2</td><td>2</td></tr>
</table>

Ground vegetation under Douglas-fir had higher indicator values for moisture and nitrogen than under beech-sessile oak (Table 5).

Table 5. Average ecological indicator values

	Moisture	Soil reaction	Nitrogen	Light	Temperature
Beech-sessile oak	3.03	3.30	2.86	2.71	3.42
Douglas-fir	3.10	3.28	3.05	2.49	3.38

Comparative analysis shows a higher percentage of plants of mesotrophic and eutrophic character in artificially established stand of Douglas-fir, which indicates a higher nutrient availability (*Galium aparine* L., *Urtica dioica* L.).

Conclusions

Forest plant richness, diversity and evenness were higher in Douglas-fir established stands than in beech-sessile oak forests. In the spectrum of floral elements, plants of the Central European range type are most commonly represented, amounting to 49% (beech-sessile oak forest) or 59% (Douglas-fir plantations). The study of the plant life forms shows that phanerophytes are the dominant class with 41% (beech-sessile oak forest) or 46% (Douglas-fir plantations). Comparative analysis of ecological indices for plant species shows a higher percentage of plants of mesotrophic and eutrophic character in artificially established stands of Douglas-fir, which indicates a higher nutrient availability.

The choice of tree species in forest management has economical, biogeochemical and ecological consequences over the long term. With the plantations of forest cultures, the native vegetation changes, as well as the species diversity of ground flora. In the further actions on the reconstruction of coppice forest of beech-sessile oak in this region, autochthonous species should be forced, which are, in any case, bio-ecologically more adapted to these site conditions. In the case when coniferous species are used, whether native or introduced coniferous species, it is recommended to cover the smaller areas, taking into account the bioecological characteristics of the conifers.

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References

Axmanová I.M., Chytrý D., Zelený C.F., Li M., Vymazalová J., Danihelka M., Horsák M., Kočí S.. Kubešová Z., Lososová Z., Otýpková L.,Tichý V.B., Martynenko E.Z., Baisheva B., Schuster B., Diekmann M. (2012). The species richness productivity relationship in the herb layer of European deciduous forests. Global Ecology and Biogeography 21: 657-667.

Braun-Blanquet J. (1964). Phytosociology – Broad Vegetation Science. 3rd ed. Springer, Wien (in German).

Kojić M., Popović R., Karadžić B. (1997). Vascular plants of Serbia. "Siniša Stanković" Institute for Biological Research, Belgrade.

Gajić M. (1980). An overview of flora of SR Serbia with herbal geographical indications. Bulletin of the Faculty of Forestry, 54: 111-141.

Miletić Z., Veselinović M., Stajić S. (2003). The Changes of Soil Properties after Beech Stand Substitution with Douglas-Fir Plantation. Proceedings of Scientific Papers Vol. 2, Bulgarian Academy of Sciences, Sofia, pp 301-305.

Miletić Z., Stajić S., Čokeša V. (2013). Changes in soil characterstics resulting from the substitution of the forest of Hungarian oak and Turkey oak with hornbeam (*Carpino betuli-Quercetum farnetto-cerris* (Rud. 1949) Jov. 1979) with Douglas-fir plantations. International scientific conference: „Forestry science and practice for the purpose of sustainable development of forestry"1-4. November 2012, Banja Luka, Bosnia and Hercegovina. Proceedings pp. 153-164.

Oxbrough A., French V., Irwin I., Kelly T., Smiddy P., O'Halloran J. (2012). Can mixed species stands enhance arthropod diversity in plantation forests? For. Ecol. Manage. 270, 11-18.

Pielou E.C. (1975). EcologIcal diversity. John Wiley& Sons, New York.

Shannon C.E., Weaver W. (1963). The mathematical theory of communications. University of Illinois Press, Urbana.

Stajić S., Čokeša V., Rakonjac Lj., Miletić Z., Babić V., Kanjevac B., Marković M. (2020). Mixed forests of beech and sessile oak (*Querco petraeae-Fagetum moesiacae* Glišić 1971) in Kosmaj protected area (Serbia) – floristic and edaphic characteristics. XI International Scientific Agriculture Symposium"AGROSYM 2020", Virtual Conference, October 8-9, 2020, Jahorina, Bosnia and Herzegovina. Book of proceedings, pp.1042-1048.

Stajić S., Cvjetićanin R., Čokeša V., Miletić Z., Novaković-Vuković M., Eremija S., Rakonjac Lj. (2021). Plant species richness and diversity in natural beech and oak dominated forests of Kosmaj protected area (Serbia). Applied Ecology and Environmental Research 19(4): 2617-2628.

Tichý L. (2002). JUICE - software for vegetation classification. Journal of Vegetation Science 13: 451-453.

Van Der Maarel E. (1979). Transformation of cover-abundance values in phytosociology and its effects on community similarity. Vegetatio 39 (2): 97–114.

MORPHOLOGICAL CHARACTERISTICS AND VARIABILITY OF THE SEEDLINGS OF WILD CHERRY (*PRUNUS AVIUM* L.) IN SERBIA

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Abstract

Wild cherry (*Prunus avium* L.) is one of the most important forest fruit species. It is very valuable forest tree species which grows in mixed forests of South, Central and West Europe. In forests of Serbia it belongs to the group of endangered species which enough attention is not paid to. The main characteristics of seedlings, their individual variability as well as the variability between and within populations were shown using Comparative Morphometric Analysis of half-sib lines of wild cherry originating from seven natural populations. Seed for seedling production was collected in nine natural populations of wild cherry in its natural distribution area in Serbia. The trial was established in the seedling nursery of Institute of Forestry in Belgrade (Serbia). On the sample of 30 seedlings as per half-sib line at their age of 30 days the following characteristics were measured: length of a root, length of a epicotyl, a hypocotyl and a cotyledon, a cotyledon width, mass of a seedling, root collar diameter and number of cotyledons. Descriptive and multivariate statistical methods were used in the study. The obtained results contribute to the knowledge of the analyzed characteristics, the preliminary assessment of the genetic variability at the level of the studied half-sib lines and populations. The presented results are the basis for the continuation of the research which is necessary to be carried out in order to provide guidelines and recommendations for the preservation and targeted use of wild cherry genetic resources on the territory of Serbia.

Keywords: *Wild cherry, seedling, half-sib line, variability, population.*

Introduction

Wild cherry (*Prunus avium* L.) is the most important tree species from the Rosaceae family in Europe and occupies a position within temperate forest belt (Praciak et al., 2013). It is a fast-growing species which mostly grows in isolated positions and forest edges and rarely occurs in dense mixed stands (Santi et al., 1998). In the southern part of the range, the limiting factor is the amount of precipitation during the summer period, while in the northern part it is limited by the cold conditions of Eastern and Northern Europe (Russell, K., 2003). It has a wide elevation amplitude, from lowland areas up to the submontane belts. On larger mountain massifs it can be found up to 2000 m of elevation (Russell, K., 2003). In Serbia it is an autochthonous, indigenous, single-tree mixed secondary species in mesophilic forests *Aceri-Fraxinetum, Fagetum submontanum, Fagetum montanum calcicolum*, as well as in various forests of oak belt and lower beech belt (Tomić, Z., 2004). Due to its widespread use, many European countries have wild cherry gene pool conservation and/or cultivation programs (Russell, K., 2003). In Serbia it is categorized as *species at risk* (Banković et al., 2008) due to a number of threatening factors (Russell, K., 2003; Stjepanović, S., 2012; Jagodić, A., 2014). The wild cherry endangerment at the European level is primarily based on its disjunct range and difficult natural regeneration in selection forests in which sciophyte species are favored (Jarni et al., 2012). Regardless of the distribution in the form of scattered single-tree or group distribution, contrary to previous knowledge, gene flow within and between populations takes place freely and quickly resulting in a low rate of genetic differentiation of populations (Ducci et al., 2013). In addition to a variety of factors (pollinators, anthropogenic impact, seed dispersion by birds, etc.), self-incompatibility during pollination plays a dominant role in maintaining a high level of genetic diversity. However, given its shooting vigor, the genetic constitution of small groups of individuals can be of very low diversity, i.e., it may consist of one or several genotypes (Frascaria et al., 1993; Ducci, F., Santi, F., 1997). The basis for learning about the adaptive potential of wild cherry in an area is a research into genetic diversity and structure (Popović, V., Kerkez, I., 2016). A comprehensive study in the territory of the entire range of the species has not been conducted yet and previous research conducted mainly at the regional level has determined significant diversity of morphological and phenological characteristics of wild cherry (Meier-Dinkel et al., 1997; Kleinschmit et al., 1999; Ballian, D., 2000; Kleinschmit et al., 2003; Russel, K., 2003; Mikić et al., 2004; Tančeva Crmarić et al., 2006; Mikić, T., 2007; Ballian, D., Čabaravdić, A., 2007; Nonić et al., 2013; Popović, V., Kerkez, I., 2016; Popović et al., 2020). Vitality and survival of natural populations of forest woody species in altered environmental conditions depends on the degree of their genetic variability (Šijačić-Nikolić, M., Milovanović, J., 2012). The occurrence of a high degree of variability within populations is typical of most forest tree species (Bogdan, S., 2009). Selection and conservation of key populations and individuals in marginal parts of the range are the basis for the preservation and targeted use of the wild cherry gene pool in Europe, especially in the context of global climate change (Russell, K., 2013). Serbia is located near the southern border of the wild cherry range, and it is predicted that most of its territory will be in conditions ranging from negligible to medium low survival success of this species (Welk et al., 2016), so the assessment of gene-environmental potential of the selected individuals and half-sib lines is of great importance in this area (Popović et al., 2020). The objective of this research was a preliminary assessment of the genetic variability at the level of researched half-sib lines and populations on the basis of the pattern of phenotypic variability of the analyzed characteristics. The obtained results are the basis for the continuation of the research which is necessary to be carried out in order to provide guidelines and recommendations for the preservation and targeted use of wild cherry genetic resources on the territory of Serbia.

Materials and methods

The fruits were collected in nine natural populations (provenances) of wild cherry in the Republic of Serbia (Figure 1, Table 1). Each population was represented by ten trees, while about 1 kg of mature and fully developed fruits was collected from each tree. Only physiologically mature trees that bore fruit in July 2017 were sampled. The fruits were collected from trees in order to raise half-sib lines, in which the mother is known but not the other parent, according to the method of genetic analysis of trees (Isajev, V., Mančić, A., 2001). After collection, the fruits were macerated to prevent fermentation of the mesocarp. Freshly cleaned seeds were washed and dried at room temperature for two days. After that, it was packed in marked plastic bags and stored in the refrigerator at a temperature of 0 to 5 ° C until the moment of sowing. In order to avoid the occurrence of dormancy, the seeds were sown directly in the prepared bed at the beginning of September 2017. The sowing space was divided into fields of equal dimensions so that each field represents one half-sib line. The seeds were sown in furrows about 1 to 2 cm deep. The distance between the furrows was 20-30 cm, and the density was about 60 pieces per meter along the furrow. Measurements of the morphometric characteristics, and the analysis of seedling variability within and between population were performed at the age of 30 days. The following parameters were measured on a sample of 30 seedlings in each of the half-sib lines: root length, epicotyl length, hypocotyl length, cotyledon length, cotyledon width, seedling mass and root collar diameter. The normality of the data collected by the measurements was analyzed by the Kolmogorov-Smirnov test, and the homogeneity of variances by the Levene test in R. Descriptive analysis was performed using the MEANS procedure in SAS to calculate: arithmetic means (x), standard deviations (SD) and coefficients of variation (CV%). Analysis of variance (ANOVA) was performed using the MIXED procedure in SAS, with the aim of determining statistically significant differences between populations and within populations. The analyzed sources (factors) of variability were population and tree, with the tree factor being nested within the population factor. The share of representation of individual studied sources of variability in the total variance (between populations, between trees within a population, within trees) was determined by REML method (Restricted Maximum Likelihood Method). All the above statistical analyses were performed using the statistical program STATISTICA 7.0 (StatSoft Inc. 2004).

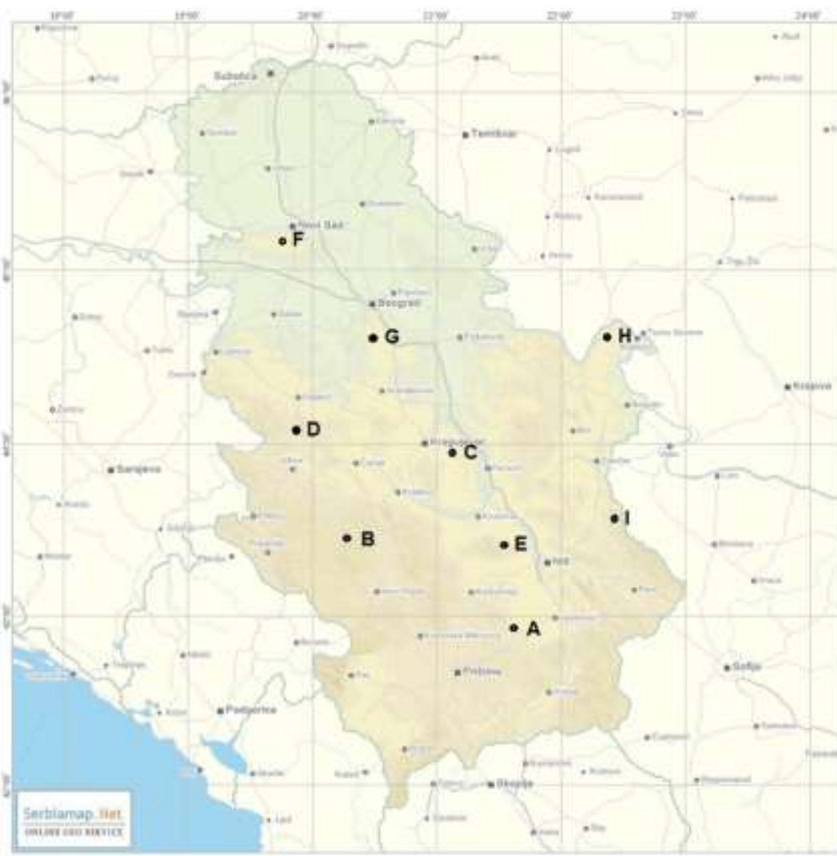

Figure 1. Map of spatial distribution of the studied provenances (A – Radan, B – Javor, C – Bešnjaja, D – Divčibare, E – Jastrebac, F – Fruška Gora, G – Lipovica, H – Đerdap, I – Stara Planina).

Table 1. Basic data on the wild cherry provenances studied.

Provenance Mark	Provenance	Elevation (m)	Latitude WGS84	Longitude WGS84
A	Radan	1159	43° 00' 09"	21° 31' 26"
B	Javor	1178	43° 29' 05"	20° 01' 50"
C	Bešnjaja	512	43° 59' 12"	21° 03' 38"
D	Divčibare	815	44° 06' 31"	19° 54' 43"
E	Jastrebac	693	43° 26' 47"	21° 26' 28"
F	Fruška Gora	473	45° 09' 18"	19° 53' 09"
G	Lipovica	240	44° 38' 10"	20° 25' 06"
H	Đerdap	473	44° 31' 44"	22° 18' 36"
I	Stara Planina	737	43° 34' 44"	22° 26' 45"

Results and Discussion

The results of descriptive statistics of morphological parameters of wild cherry seedlings at the level of the studied populations are shown in Table 2. The cotyledon width (36.2%) stands out as the most variable trait, while the root collar diameter is the characteristic with the lowest variability (10.5%). The average value of root length at the level of all populations is 95.2 mm. The average value of hypocotyl length is 33.2 mm. The average value of epicotyl length at the level of the studied populations amounts to 29.7 mm. The average value of the cotyledon length is 17.5 mm, while the average value of the cotyledon width is 11.6 mm. The mass of seedlings is 1.1 g on average at the population level, and the average value of the root collar diameter amounts to 1.9 mm.

Table 2. Basic indicators of descriptive statistics of morphological parameters of seedlings

Characteristic	No. of specimens	Mean value	Minimum	Maximum	Standard deviation	Coefficient of variation
Root length (mm)	270	95.2	42	118.6	28.7	30.1
Hypocotyl length (mm)	270	33.2	19	49	8.5	25.6
Epicotyl length (mm)	270	29.7	12	31	7.3	24.6
Cotyledon length (mm)	270	17.5	8.3	22.4	5.9	33.7
Cotyledon width (mm)	270	11.6	5.4	17.9	4.2	36.2
Weight (g)	270	1.1	0.4	1.9	0.3	27.3
Root collar diameter (mm)	270	1.9	1.2	2.3	0.2	10.5

Table 3. Statistical significance (p-value) of trees nested within populations effect (i.e., variability within populations) for analyzed characteristics per provenance and percentage of variance component of the same effect calculated by combined analysis of all provenances (the last column).

Characteristic	Within populations									Variance component (%)
	A	B	C	D	E	F	G	H	I	
Root length	p<0.01	p<0.01	p<0.01	p<0.01	0.53	p<0.01	p<0.01	p<0.01	p<0.01	43.2
Hypocotyl length	p<0.01	p<0.01	p<0.01	0.56	p<0.05	p<0.01	p<0.05	p<0.01	p<0.01	41.4
Epicotyl length	p<0.01	0.62	p<0.05	p<0.01	0.11	p<0.01	p<0.05	p<0.01	p<0.01	45.6
Cotyledon length	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01	p<0.05	p<0.01	p<0.01	p<0.05	39.7
Weight	p<0.01	p<0.01	0.66	p<0.05	p<0.01	p<0.05	p<0.05	p<0.05	p<0.01	48.4
Root collar diameter	p<0.05	p<0.01	p<0.05	p<0.01	p<0.01	0.52	p<0.05	p<0.01	p<0.01	50.2

The obtained results show that the level of variability within populations was high, occupying from 39% to 50% of the total variance (Table 3). The trees within populations differed significantly with respect to most of the researched morphological characteristics (Table 3).

Table 4. Provenance effect variance component percentage (i.e., variability between populations) and its statistical significance for investigated morphological characteristics.

Characteristic	Effect (%)	
	Population	p-value
Root length	6.43	<0.01
Hypocotyl length	7.4	<0.01
Epicotyl length	11.2	<0.01
Cotyledon length	9.4	<0.01
Weight	7.1	<0.01
Root collar diameter	7.9	<0.01

Based on the obtained results of the analysis of variance (ANOVA), it can be concluded that statistically significant differences were found between the studied populations, for all analyzed morphological characteristics of seedlings (Table 4). The level of variability between populations was not high, occupying from 6% to 11% of the total variance.
The of phenotypic diversity of wild cherry leaves, fruits and seeds in this part of Europe has also been confirmed by other studies (Ballian, D., 2000; Ballian, D., Čabaravdić, A., 2007; Ballian et al., 2012; Mratinić et al., 2012; Ballian, D., Mujagić-Pašić, A., 2013, Popović, V., Kerkez, I., 2016, Popović et al., 2020). It has long been considered that the variability parameters of tree seedlings are not of special importance, therefore seedlings characteristics have been presented only on a typological basis (Anić, M., 1983.; Vasilchenko, T.I., 1960). This view is not accepted today. The variability of seedlings is an adaptive trait and is controlled by natural selection, because genetic factors determine the extent and direction of the allowed phenotypic plasticity (Tucović, A., 1990). Seedling variability must be interpreted as the product of the interaction between genetic inheritance and selection. Age changes during the life cycle are the result of two opposing tendencies. One tendency is towards greater differentiation from germination to maturity. The other tendency is towards adaptation at each stage of the life cycle. Some studies have shown that morphological variability is apparently the result of an adaptive response to the environment; e.g., variation of some characteristics related to latitude and elevation

(Kleinschmit, J., 1993). Determining the contribution of heritage or environment to a particular character is of great importance in the field of production and cultivation of species (Tucović, A., Isajev, V., 2000.). The first, often the strongest selection takes place when the plant is in the germination phase. Thereby, larger dimensions of cotyledons, characteristics of hypocotyls or epicotyls, supernumerary cotyledons, characteristics of the root system are later manifested by better characteristics of mature trees (Vasilchenko, T.I., 1960). Some morphological differences may be caused by each genotype adapting to its own environment (Abrams, M.D., 1990). Peripheral or isolated populations found in habitats that differ in soil, climate, and competitors may be a source of new adaptations, and conservation efforts should be made toward such populations (Lessica, P., Allendorf, F.W., 1995). As a result, research into these seemingly insignificant characteristics of seedlings is gaining increasing importance (Tucović, A., Isajev, V., 2000.).

The natural regeneration of wild cherries is weak, and game grazing and poor competitiveness in relation to other species, primarily common beech, contribute to the difficult regeneration. It cannot withstand competition from sciophytic species in selection forests (Jarni et al., 2012). Auxiliary measures for natural regeneration are, for the above reasons, necessary, because wild cherry is an economically and ecologically very important woody species (Stjepanović, S., 2012). In this regard, the importance of the correct choice of reproductive material, along with the correct breeding interventions, is considered the main prerequisite for achieving maximum profit from forestry production (Kingswell, G., 1998; Coello et al., 2013).

There is also an increased risk of extinction in small populations, where reduced genetic diversity may be the result of genetic drift. In subsequent generations, the loss of heterozygosity resulting from genetic drift and inbreeding may lead to reduced fitness in these otherwise stable populations (Bruschi et al., 2003). Peripheral or isolated populations found in habitats that differ in soil, climate, and competitors may be beneficial to the protection of the evolutionary process , and conservation efforts should be made toward such populations (Lessica, P., Allendorf, F.W., 1995). Appropriate *ex situ* conservation measures should be taken to preserve such populations, using generative and vegetative methods of reproduction. Seed propagation is more justified because it is the least harmful to existing populations and would involve the widest range of genetic variability (Bruschi et al., 2003).

The results of this study showed a high level of variability within populations, but also differentiation between populations. It can be stated that the available wild cherry gene pool in Serbia is characterized by a satisfactory degree of genetic variability, and represents a good starting point for the process of further breeding. In order to preserve and improve the ecological adaptability and evolutionary potential of wild cherry populations in Serbia in the long term, it is necessary to start implementing adequate *in situ* and *ex situ* conservation measures.

Conclusion

The results obtained in this research have shown there is a significant level of variability of morphological characteristics of seedlings within populations and differentiation between populations.

The available gene pool of wild cherry in Serbia is characterized by genetic variability, therefore it can be utilized and improved in future breeding efforts.

Planning and implementation of appropriate measures of gene pool *in situ* and *ex situ* conservation will contribute to the preservation and improvement of the condition of wild cherry in part of the range.

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References

Abrams, M.D. (1990): Adaptation and responses to drought in Quercus species of North America. Tree Physiology 7: 227-238.

Anić, M. (1983): Klijanci, Šumarska enciklopedija, Zagreb, 2, 246-259. [Anić, M. (1983): Seedlings, Forestry Encyclopedia, Zagreb, 2, 246-259.]

Ballian, D. (2000): Početna istraživanja varijabilnosti morfoloških svojstava sjemena divlje trešnje (*Prunus avium* L.), Sumar. list, 124 (5-6): 271-278. [Ballian, D. (2000): Initial research of variability of morphological characteristics of wild cherry (*Prunus avium* L.) seeds, Šumarski List, 124 (5-6): 271-278.]

Ballian, D. (2004): Varijabilnost mikrosatelitne DNK u populacijama divlje trešnje (*Prunus avium* L.) iz središnje Bosne, Sumar. list, 128 (11-12): 649-653. [Ballian, D. (2004): Variability of microsatellite DNA in wild cherry (*Prunus avium* L.) populations from Central Bosnia, Šumarski List, 128 (11-12): 649-653.]

Ballian, D., Čabaravdić, A. (2007): Neki korelacijski odnosi između svojstava pupova, cvijeta i sjemena divlje trešnje (*Prunus avium* L.) iz populacije Mrkovići, Works of the Faculty of Forestry University of Sarajevo, 1: 29 - 38. [Ballian, D., Čabaravdić, A. (2007): Certain correlations between characteristics of buds, flowers and seeds of wild cherry (*Prunus avium* L.) from Mrkovići population, Works of the Faculty of Forestry University of Sarajevo, 1: 29 - 38.]

Ballian, D., Bogunić, F., Čabaravdić, A., Pekeč, S., Franjić, J. (2012): Population differentiation in the wild cherry (*Prunus avium* L.) in Bosnia and Herzegovina, Period. Boil., 114 (1): 43-54.

Ballian, D., Mujagić-Pašić, A. (2013): Morphological variability of the fruit and seed of wild cherry (*Prunus avium* L.) in a part of its natural distribution in Bosnia and Herzegovina. Biologica Nyssana, 4 (1-2).

Banković, S., Medarević, M., Pantić, D., Petrović, N. (2008): National Forest Inventory of the Republic of Serbia: the forest fund of the Republic of Serbia, Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the Forest Administration, Belgrade: p. 57.

Bogdan, S. (2009): Genetika s oplemenjivanjem drveća i grmlja (interna skripta). Šumarski fakultet Sveučilišta u Zagrebu. 1-207. [Bogdan, S. (2009): Genetics with breeding of trees and shrubs (internal script). Faculty of Forestry of the University of Zagreb. 1-207]

Bruschi, P., Vendramin, G., Bussotti, F., Grossoni, P. (2003): Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae). Annals of Botany 91: 707-716.

Ducci, F., Santi, F. (1997): The distribution of clones in managed and unmanaged populations of wild cherry (Prunus avium), Canadian Journal of Forest Research 27: 1998-2004.

Ducci, F., De Cuyper, B., De Rogatis, A., Dufour, J., Santi, F. (2013): Wild cherry breeding (*Prunus avium* L.). In forest tree breeding in Europe. Springer, Dordrecht. 463-511.

Frascaria, N., Santi, F., Gouyon, P. H. (1993): Genetic differentiation within and among populations of chestnut (*Castanea sativa* Mill.) and wild cherry (*Prunus avium* L.)., Genetical Society of Great Britain: 634-644.

Isajev, V., Mančić, A. (2001): Šumsko semenarstvo, Šumarski fakultet, Univerziteta u Banja Luci, Univerzitet u Beogradu - Šumarski fakultet, Banja Luka, Beograd (1-283). [Isajev, V., Mančić, A. (2001): Forest Seed Production, Faculty of Forestry, University of Banja Luka, University of Belgrade - Faculty of Forestry, Banja Luka, Belgrade (1-283).]

Jagodić, A., 2014: Program genetičke konzervacije šumskih voćkarica na području ŠG „Visočnik". Master rad. Šumarski fakultet, Univerzitet u Beogradu, Beograd (53-66). [Jagodić, A., 2014: Forest fruit trees genetic conservation program in the area of Visočnik Forest Enterprise. Master's thesis, Faculty of Forestry, University of Belgrade, Belgrade (53-66).]

Jarni, K., Cuyper, B., Brus, R. (2012): Genetic variability of wild cherry (*Prunus avium* L.) seed stands in slovenia as revealed by nuclear microsatellite loci. Plos one 7: 1-5.

Kleinschmit, J., Stephan, R., Wagner, I. (2003): European Forest Genetic resources Programme: Wild Fruit Trees Genetic Resources Conservation Strategy.

Kleinschmit, J., Stephan, B.R., Ducci, F., Rotach, P., Matyas, C. (1999): Inventoriesof Noble Hardwoods genetic resources: basic requirements in Noble Hardwoods Network. Report of the Third Meeting, 13–16 June 1998, Sagadi, Estonia. IPGRI (92–97).

Lessica, P., Allendorf, F.W. (1995): When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Meier-Dinkel, A., Svolba, J., Kleinschmit, J. (1997): Selektierte, mikrovermehrte Vogelkirschen – Klone. AFZ – Der Wald, Allgemeine Forst Zeitschrift fur Waldwirtschaft und Umweltvorsorge, 52: 963–964. [Meier-Dinkel, A., Svolba, J., Kleinschmit, J. (1997): Selected, micro-propagated wild cherry - Clone. AFZ – Der Wald, Allgemeine Forst Zeitschrift fur Waldwirtschaft und Umweltvorsorge, 52: 963–964.]

Mikić, T. (2007): Analiza morfoloških parametara lista divlje trešnje (*Prunus avium* L.) u Bosni i Hercegovini, Doktorska disertacija, Šumarski fakultet, Univerzitet u Banja Luci, Banja Luka, 1-118. [Mikić, T. (2007): Analysis of morphological parameters of wild cherry (*Prunus avium* L.) leaves in Bosnia and Herzegovina, Doctoral thesis, Faculty of Forestry, University of Banja Luka, Banja Luka, 1-118.]

Mratinić E., Fotirić Akšić, M., Jovković, R. (2012): Analysis of wild sweet cherry (*Prunus avium* L.) germplasm diversity in south-east Serbia. Genetika, 44(2), 259-268.

Nonić, M., Popović, V., Kerkez, I., Šjiačić-Nikolić, M. (2013): Varijabilnost morfometrijskih karakteristika semena različitih test stabala divlje trešnje (*Prunus avium* L.) sa područja Beograda, Šumarstvo, 65 (1-2), 113-123. [Nonić, M., Popović, V., Kerkez, I., Šjiačić-Nikolić, M. (2013): Variability of morphometric characteristics of seeds of various test trees of wild cherry (*Prunus avium* L.) from the territory of Belgrade, Šumarstvo, 65 (1-2), 113-123.]

Praciak, A. (2013): The CABI encyclopedia of forest trees (CABI, Oxfordshire, UK).

Popović, V., Kerkez, I. (2016): Varijabilnost populacija divlje trešnje (*Prunus avium* L.) u Srbiji prema morfološkim svojstvima listova. Sumar list, 7-8, 347-355. [Popović, V., Kerkez, I.

(2016): Variability of wild cherry (*Prunus avium* L.) populations in Serbia according to morphological characteristics of leaves. Sumar List, 7-8, 347-355.]

Popović, V., Lučić, A., Kerkez Janković, I., Rakonjac, L., Bogdan, S. (2020): Variations in fruit traits of wild cherry (*Prunus avium* L.) provenances in Serbia. Šumarski list, 144(11-12), 585-596.

Russell, K. (2003): EUFORGEN Technical guidelines for genetic conservation and use for wild cherry (*Prunus avium*). International Plant Genetic Resources Institute, Rome, Italy. p. 6.

Santi, F., Muranty, H., Dufour, J., Paques, L.E. (1998): Genetic parametersand selection in a multisite Wild cherry clonal test, Silvae Genet., 47: 61–67.

Stjepanović, S. (2012): Pokazatelji kvaliteta jednogodišnjih sadnica divlje trešnje (*Prunus avium* L.), Master rad. Šumarski fakulet, Univerzitet u Beogradu, Beograd (8-45). [Stjepanović, S. (2012): Indicators of quality of one-year-old wild cherry (*Prunus avium* L.) seedlings, Master's thesis. Faculty of Forestry, University of Belgrade, Belgrade (8-45).]

StatSoft Inc., 2004. STATISTICA, version 7.

Šijačić–Nikolić, M., Milovanović, J. (2012): Conservation and sustainable use of forest genetic resources throught an example of watland ecosystems, Agriculture and Forestry, 57 (1): 23-31.

Tančeva Crmarić, O., Štambuk, S., Šatović, Z., Kajba, D. (2011): Genotipska raznolikost divlje trešnje (*Prunus avium* L.) u dijelu prirodne rasprostranjenosti u Hrvatskoj, Sumars list, 130 (11–12): 543–555. [Tančeva Crmarić, O., Štambuk, S., Šatović, Z., Kajba, D. (2011): Genotypic diversity of wild cherry (Prunus avium L.) in the part of natural range of distribution in Croatia, Šumarski List, 130 (11–12): 543–555.]

Tomić, Z. (2004): Šumarska fitocenologija, Šumarski fakultet Univerziteta u Beogradu, Beograd, (133-174). [Tomić, Z. (2004): Forest Phytocoenology, Faculty of Forestry, University of Belgrade, Belgrade, (133-174).]

Tucović, A. (1990): Oblici promenjivosti i njihova klasifikacija, Genetika sa oplemenjivanjem biljaka, Naučna knjiga, Beograd, 124-154. [Tucović, A. (1990): Forms of variability and their classification, Genetics with plant breeding, Naučna knjiga, Belgrade, 124-154.]

Tucović, A., Isajev, V. (2000): Karakteristlke i varijabilnost klijavaca bagrenca (Amorpha fruticosa L.) – korovske vrste plavnih staništa, Acta herbologica, Vol. 9, No1, 101-111. [Tucović, A., Isajev, V. (2000): Characteristics and variability of false indigo-bush (Amorpha fruticosa L.) – weed species of floodplain habitats, Acta herbologica, Vol. 9, No.1, 101-111.]

Welk, E., de Rigo, D., Caudullo, G. (2016): Prunus avium in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, pp. e01491d.

VARIABILITY OF MORPHOMETRIC CHARACTERISTICS OF SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL) ACORN

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Abstract

The research of intrapopulation variability on the basis of morphometric characteristics of acorn was conducted for the purpose of conservation of the available gene pool of sessile oak in the population in the area of Avala Landscape of Outstanding Features and controlled use of genetic resources. Fifty trees of the best quality based on phenotype characteristics, the healthy bearers of sessile oak (*Quercus petraea* (Matt.) Liebl) reproductive material production were selected at the population level. In October 2019, ca. 5 kilograms of healthy, normally developed acorns were collected from each of the test trees. On the sample of 50 acorns per tree, length, width and mass of the acorn were measured. The volume and the shape index of acorns were calculated on the basis of measured values of length and width. The mean values of morphometric characteristics obtained in the research indicate that there is a high variability among the studied genotypes. The latter is confirmed, also, by the analysis of variance which determined statistically significant differences among the sampled mother trees for all the observed morphometric characteristics. The obtained results represent a good starting point for future research in breeding, long-term conservation and improvement of ecological adaptability and evolutionary potential of sessile oak population by means of implementing adequate measures of in situ and ex situ conservation.

Keywords: *Sessile oak, acorn, population, genetic variability.*

Introduction

In the forest growing stock of the Republic of Serbia, sessile oak (*Quercus petraea* /Matt./ Leibl.) is the second most valuable oak species after pedunculate oak. It participates in the total volume with 5.9%, i.e., it occupies an area of 173.200 ha. Pure and mixed stands represent 57.5% and 42.5% of the total volume of sessile oak, respectively. Sprout forest stands are dominant at approximately 75% of the total area (Banković et al., 2009). It mainly occurs on warmer, southern exposures within the alliance *Quercion petraeae-cerris* Lakš. et Jov. 1980. Sessile oak forests in Serbia occur within the special oroclimatogenic altitudinal range, above the climatogenic forests of Hungarian oak *Quercus frainetto* syn. and Turkey oak (*Quercus cerris*). The complex of sessile oak forests encompasses upper part of submontane belt and lower montane belt at the altitudes from 300 m up to 1300 m (Stojanović et al., 2005). In the last several decades forest stands, groups and individual trees of sessile oak on almost whole range have been dying. The causes of forest decline are still insufficiently studied and systematized. The phenomenon is most probably caused by the influence of a complex of factors whose effect is cumulative (Marinković et al., 1990). On the basis of the results achieved so far in the studies relating to the phenomenon of decline of sessile oak forests it could be considered the

consequence of the global climate change, population structure change in sessile oak forests, air pollution, plant diseases, insect gradations, etc. (Isajev et al., 2005).
In sessile oak as in most oak species a haploid number of chromosomes is found, 12, while polyploidy occurs rarely and ranges about 0.5%. It is characterised by great individual and group variability which was confirmed by means of provenance and progeny tests (Isajev et al., 2005). Variability was found in seedling growth, tree formation, resistance to powdery mildew and insect attacks. Significant differences in agglutinins content have been determined in biochemical studies of fruits from different provenances, which can serve to facilitate identification of oak provenances (Jovanović, M., Tucović, A., 1975). In addition to the significant differences between provenances, they have also been determined within the provenances, and between individual trees. The morphological characteristics of trees and acorns are under considerable genetic control. In the scientific literature there are data on the existence of spontaneous hybrids described under various names among several species of oaks: *Quercus petraea* /Matt./ Leibl. and *Quercus robur* L.; *Quercus petraea* / Matt./ Leibl and *Quercus dalechampii* Ten; *Quercus petraea* /Matt./ Leibl and *Quercus polycarpa* Schur. The progeny of the mentioned stock species is characterised by a pronounced variability of quantitative and qualitative characteristics. The high variability of morphological and physiological characteristics of spontaneous hybrids creates great difficulties in production of forest reproductive material and the use of the available gene pool of sessile oak (Isajev et al., 2005).
The high level of diversity in terms of genetic resources, abundance of species and habitat diversity classifies forests under the most complex ecosystems on the Earth (Geburek, T., Konrad, H., 2008). Due to the constant pressure on forests in the last few centuries and the disappearance of natural forests, biodiversity is endangered (Carabeo et al., 2016). The long-term survival of species is closely related to their genetic diversity (Gapare, W.J., 2014). Under the influence of the altered environmental conditions, biotic pathogens and damage the survival and evolution of species depend on the level of genetic diversity (Reed, D.H., Frankham, R., 2003). The research dealing with genetic diversity and identifying populations characterized by high genetic variability can help in reducing the risk of biological diversity loss (Souto et al., 2015). The vitality and survival of natural populations of forest woody species in altered environmental conditions depend on the degree of their genetic variability as a basis for adaptation and undisturbed evolution (Šijačić-Nikolić, M., Milovanović, J., 2012). The occurrence of a high degree of intrapopulation variability is typical of most forest tree species (Bogdan, S., 2009).
The object of research in this study was to determine intrapopulation variability of sessile oak in the population in the area of Avala Landscape of Outstanding Features according to the morphological characteristics of acorn. The results obtained in the research can serve for preliminary knowledge of the genetic variability of the studied population, proposal of measures for conservation of the available gene pool and the improvement of quality seed and planting material production.

Materials and methods

For the research in this study acorns from the 2019 harvest were used. In order to determine genetic variability and evaluate the condition of genetic resources in the area of Avala Landscape of Outstanding Features, 50 trees of sessile oak (*Quercus petraea* (Matt.) Liebl). were selected and sampled. Representative trees of phenotypically highest quality which have been the bearers of healthy reproductive material production were sampled. The trees were evenly distributed over the entire area of the protected area, at least 50 m apart in order to avoid relatedness. About 3 kg of acorns ocularly estimated as healthy and undamaged were collected per each tree, regardless of their dimensions. After the collection acorns were dried at 35% of humidity and stored at temperature of 3 to 5 ºC.

The analyses of measured and derived morphometric characteristics were carried out on a random sample consisting of 50 acorns per each mother tree. Length, width at the widest part and mass of acorns were measured. The length and width of acorns were measured by a vernier calliper with an accuracy of 0.01 mm and mass by an electronic scale with an accuracy of 0.01 g. The shape index, volume of acorns and average number of acorns per 1 kg were calculated based on the measured values. The shape index was calculated as a ratio of the length and the width of the acorn. The volume of the acorn was calculated according to the formula for volume of a cylinder. (The shape of the acorn approximates to the shape of cylinder). The average number of acorns in 1 kg was calculated based on the average mass of one acorn, separately for each sampled mother tree.
Morphological characteristics of acorns were described by means of descriptive statistical indicators such as arithmetic mean (x), standard deviation (SD), coefficient of variation (CV %). The one-way analysis of variance (ANOVA) was used for the purpose of determining intrapopulation variability. The analysed factor of variability was a mother tree. All the aforementioned statistical analyses were carried out using the statistical program STATISTICA 7.0 (StatSoft Inc. 2004).

Results and Discussion

The mean value of acorn length at the population level amounts to 24.7 mm. The acorn length ranges from 20.9 mm (Tree No. 34) to 31.2 mm (Tree No. 49), with the standard deviation ranging from 1.4 to 3.6 mm (Table 1).
The mean value of acorn width at the widest part amounts to 14.5 mm. The acorn width ranges from 12.1 mm (Tree No. 5) to 19.0 mm (Tree No. 48) and the standard deviation ranges from 0.6 mm to 3.3 mm (Table 1).
The highest mean value of acorn mass amounts to 6.6 g (Tree No. 49) and the lowest is 2 g (Tree No. 5), while the mean value at the forest stand level amounts to 3.3 g with the standard deviation ranging from 0.3 g to 3.2 g (Table 1).
The acorn shape index ranges from 1.5 (Tree No. 25) to 2.3 (Tree No. 39), with the mean value of 1.7 at the forest stand level and the standard deviation ranging from 0.1 to 0.3 (Table 1).
The mean value of the acorn volume at the forest stand level amounts to 4.3 cm^3 and ranges from 2.6 cm^3 (Tree No. 5) to 9 cm^3 (Tree No. 48) with the standard deviation ranging from 0.3 cm^3 to 3.2 cm^3 (Table 1).

Table 1. Descriptive statistics of the studied acorn characteristics.

Tree	Length (mm)			Width (mm)			Mass (g)			Shape Index			Volume (cm^3)		
No.	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
1	21.7	2.3	10.6	14.0	1.0	6.9	2.8	0.7	23.7	1.5	0.1	8.3	3.4	0.7	21.4
2	23.9	2.3	9.8	15.4	1.2	7.5	3.4	0.8	24.5	1.6	0.1	8.0	4.5	1.0	22.1
3	24.1	1.8	7.6	12.9	0.9	7.3	2.5	0.6	22.2	1.9	0.1	5.4	3.2	0.7	21.5
4	25.1	1.4	5.6	14.2	3.3	23.6	3.0	0.5	15.9	1.8	0.2	12.2	4.2	3.0	70.6
5	22.5	1.5	6.5	12.1	0.6	4.6	2.0	0.3	15.0	1.9	0.1	5.9	2.6	0.3	13.3
6	22.9	1.6	7.0	14.7	1.4	9.3	3.0	0.7	22.4	1.6	0.1	7.0	4.0	1.0	24.6

7	21.2	1.7	8.2	13.8	1.3	9.2	2.5	0.6	22.1	1.5	0.2	11.0	3.2	0.7	21.6
8	21.8	2.1	9.4	13.6	1.8	13.4	2.6	1.0	40.1	1.6	0.1	8.6	3.3	1.1	35.1
9	24.5	2.5	10.4	14.9	0.8	5.1	3.4	0.7	20.2	1.6	0.2	10.1	4.3	0.7	16.5
10	24.7	2.5	10.0	16.4	1.2	7.3	4.4	1.3	30.3	1.5	0.1	7.2	5.3	1.2	23.0
11	26.8	2.4	8.9	13.9	1.3	9.0	3.3	0.6	19.4	1.9	0.2	9.2	4.1	1.0	25.2
12	23.6	2.0	8.4	15.1	1.0	6.7	3.3	0.7	22.8	1.6	0.1	7.6	4.3	0.8	18.8
13	21.1	1.8	8.5	12.9	1.6	12.0	2.2	0.6	28.0	1.6	0.2	12.5	2.8	0.8	29.5
14	22.1	1.4	6.1	13.5	1.0	7.5	2.3	0.5	20.7	1.6	0.1	6.2	3.2	0.6	19.3
15	24.2	3.0	12.3	13.9	1.4	10.1	2.4	1.2	49.9	1.7	0.1	6.8	3.8	1.2	31.3
16	22.5	1.7	7.4	12.8	1.4	10.6	2.3	0.6	26.2	1.8	0.2	11.4	2.9	0.7	25.1
17	24.5	2.1	8.5	14.3	1.2	8.6	3.3	0.9	27.2	1.7	0.1	7.6	4.0	1.0	24.8
18	21.7	1.6	7.5	14.3	1.0	6.8	2.7	0.8	28.2	1.5	0.1	7.7	3.5	0.6	17.9
19	24.4	3.5	14.2	13.6	1.3	9.8	2.6	0.7	26.9	1.8	0.3	17.7	3.6	0.9	25.2
20	21.6	1.4	6.7	12.7	1.2	9.8	2.2	0.6	26.7	1.7	0.1	6.9	2.8	0.7	26.5
21	22.6	2.3	10.4	13.5	1.2	8.6	2.6	0.7	28.7	1.7	0.1	7.7	3.3	0.9	27.1
22	25.7	2.3	8.8	14.9	1.2	8.0	4.3	3.2	74.2	1.7	0.1	6.6	4.5	1.0	22.4
23	24.3	1.8	7.5	14.9	1.6	10.9	2.8	0.9	33.3	1.6	0.1	8.8	4.3	1.1	26.6
24	22.4	2.1	9.5	13.1	1.6	12.1	2.4	0.6	26.4	1.7	0.2	9.8	3.1	1.0	33.1
25	21.8	2.0	9.2	14.9	1.0	7.0	3.2	0.5	16.7	1.5	0.2	12.3	3.8	0.6	16.6
26	24.4	2.8	11.3	14.5	1.6	11.1	3.2	1.1	34.5	1.7	0.2	11.2	4.1	1.3	31.0
27	24.1	1.8	7.4	13.8	0.7	5.2	3.1	0.5	15.2	1.7	0.1	6.3	3.6	0.6	15.6
28	26.6	2.1	8.0	15.3	1.0	6.8	3.9	0.8	21.0	1.7	0.1	8.2	4.9	0.8	17.1
29	27.3	2.4	8.7	14.4	0.9	6.0	3.6	0.7	19.7	1.9	0.2	8.1	4.5	0.8	17.7
30	22.1	2.0	9.1	13.8	1.1	8.0	2.8	0.8	28.8	1.6	0.1	6.8	3.4	0.8	23.3
31	22.5	2.7	12.2	14.4	1.9	12.9	2.8	1.0	34.6	1.6	0.2	15.5	3.8	1.2	32.6
32	23.6	2.3	9.9	14.2	1.1	8.0	2.9	0.7	25.4	1.7	0.1	8.1	3.8	0.9	23.3
33	26.0	2.0	7.5	15.9	1.2	7.7	4.0	1.0	23.6	1.6	0.1	6.2	5.2	1.1	20.9
34	20.9	1.6	7.4	13.3	0.9	6.9	2.3	0.5	20.7	1.6	0.1	5.2	2.9	0.6	19.7
35	26.2	2.1	8.1	14.7	1.2	8.3	3.4	0.9	24.9	1.8	0.1	7.1	4.5	1.0	23.0
36	23.1	1.6	6.9	14.7	1.2	8.3	3.1	0.8	25.1	1.6	0.1	5.4	4.0	0.9	21.5
37	24.7	2.4	9.6	15.1	0.9	5.7	3.6	0.7	18.4	1.6	0.1	7.4	4.5	0.8	18.4
38	24.3	1.6	6.6	15.1	1.1	7.4	3.4	0.7	19.0	1.6	0.1	6.8	4.4	0.9	19.7
39	30.4	2.0	6.5	13.3	1.0	7.3	3.3	0.7	19.8	2.3	0.2	9.5	4.2	0.7	16.2
40	23.4	2.0	8.6	14.7	1.1	7.3	3.4	0.7	21.1	1.6	0.1	8.8	4.0	0.8	19.8
41	29.1	2.4	8.4	15.9	1.4	8.5	4.4	0.9	20.2	1.8	0.2	11.8	5.8	1.1	18.3
42	27.6	2.0	7.2	16.4	1.2	7.3	4.2	1.2	27.7	1.7	0.1	7.6	5.9	1.1	18.4
43	23.3	3.5	15.0	14.5	1.3	8.9	3.0	1.1	38.2	1.6	0.2	12.4	3.9	1.2	30.1
44	29.6	2.1	7.0	17.2	1.3	7.6	5.6	1.1	18.9	1.7	0.1	5.9	6.9	1.4	19.8
45	30.3	3.6	12.0	15.7	3.1	19.9	5.0	2.4	48.9	2.0	0.3	14.4	6.3	3.2	50.8
46	25.8	2.5	9.6	15.1	1.9	12.6	3.8	1.2	32.7	1.7	0.2	12.3	4.7	1.5	31.6
47	26.8	2.9	10.9	13.5	1.1	7.8	3.2	0.7	22.8	2.0	0.2	10.4	3.9	0.8	21.7
48	30.9	2.8	9.1	19.0	2.2	11.7	6.4	2.0	30.5	1.6	0.2	9.6	9.0	2.5	28.0
49	31.2	2.1	6.6	18.0	1.5	8.4	6.6	1.4	21.5	1.7	0.1	6.8	8.1	1.7	21.6
50	28.7	2.3	8.0	14.5	1.0	7.0	3.8	0.8	21.2	2.0	0.1	7.0	4.8	0.9	18.8
Mean	24.7	3.5	14.2	14.5	1.9	13.0	3.3	1.4	41.9	1.7	0.2	12.8	4.3	1.7	39.9

The most variable characteristic at the level of the forest stand is the acorn mass (41.9 %), while the shape index is the characteristic showing the lowest variability (12.8 %) (Table 1).
The average number of acorns in 1 kg at the level of the forest stand amounts to 301 pcs and ranges from 151 pcs/kg (Tree No. 49) to 489 pcs/kg (Tree No. 5).

Table 2. The analysis of variance for measured morphometric characteristics of acorn.

Parameter	df	SS	MS	F	P
Acorn length	49	11203.2	228.6	45.8	0.0000
Acorn width	49	2550.2	52.0	26.6	0.0000
Acorn mass	49	1417.07	28.92	27.93	0.0000
Shape index	49	34.400	0.702	27.6	0.0000
Acorn volume	49	2336.25	47.68	34.76	0.0000

The results of the conducted analysis of variance are presented in the Table 2. The studied source of variability was a tree (family) within a population. The results obtained showed there are statistically significant differences caused by the observed source of variability for all studied morphometric characteristics of acorn, thus confirming the assumption of significant intrapopulation differentiation.

High statistically significant differences determined between individual stock trees (genotypes) within the population indicate a high level of intrapopulation genetic variability. The occurrence of high level intrapopulation variability is typical of the majority of forest tree species and it can be explained by the process of gene flow (exchange of genes between different populations by means of natural processes, but also by human activity) (Bogdan, S., 2009).

The research of genetic variability of sessile oak populations which cover the major part of its natural range showed wedge-shaped variation trends, decrease of variability with an increase of the researched area. (Zanetto, A., Kremer, A., 1995). Certain studies have shown that the morphological variability is apparently the result of an adaptive response to the environment; e.g., variation of some characteristics related to latitude and altitude (Kleinschmit, J., 1993). Some of the researched morphological traits have been variable among populations without showing any geographical trends. The trend of morphological variability indicates there has been adaptation to local climatic conditions which is confirmed by a significant correlation with climatic parameters and there is no association with population distance (Jurkšiene, G., Baliuckas, V., 2014). Some morphological differences may be caused by adaptation of each genotype to its own environment (Abrams, M.D., 1990). In addition to direct exposure to sunlight, morphological variability can also be affected by water stress and the nutrient content in the soil (Carter et al., 1987). From a conservation perspective large genetic variability of tested populations is encouraging, but adequate ecological management is necessary for *in situ* conservation of populations (Bruschi et al., 2003). In case of excessive felling of trees, which would result in reduction in population size, the risk of extinction would increase (Gilpin, M.E., Soule, M.E., 1986). The increased risks of extinction exist also in small populations, in which reduced genetic diversity may be the result of genetic drift. In subsequent generations the loss of heterozygosity which is the result of genetic drift and inbreeding may lead to reduced condition in this otherwise stable populations. (Bruschi et al., 2003). Peripheral or isolated populations found in habitats that differ in soil, climate and competitors can be the source of new adaptations so an effort should be made to conserve such populations (Lessica, P., Allendorf, F.W., 1995). Appropriate measures of *ex situ* conservation should also be taken to preserve such populations using generative and vegetative means of reproduction. Seed propagation is more justified because it is the least harmful for existing populations and it would involve the widest range of genetic variability (Bruschi et al., 2003).

Conclusion

The results obtained in this research have showed the existence of significant variability of morphological characteristics of acorns and indicated high phenotypic variability of the researched traits in the sessile oak population in Avala Landscape of Outstanding Features. Based on the obtained results it can be concluded that the available gene pool of sessile oak in Avala Landscape of Outstanding Features is characterised by a satisfactory degree of genetic variability so it represents good starting point for the process of further breeding and conservation of natural population. For the purpose of long-term conservation and improvement of ecological adaptability and evolutionary potential of sessile oak population it is necessary to initiate implementing measures of *ex situ* conservation of genetic resources.

The isolated and analysed test trees can serve as a source of planting material for establishment of *ex situ* conservation facilities. The established facility will be of great importance for conservation of genetic resources of sessile oak from the area of Avala Landscape of Outstanding Features and it can also serve as a source of reproductive material for establishing cultivated forests or as a support in natural regeneration of pure or mixed stands of sessile oak in the future.

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References

Abrams, M.D. (1990): Adaptation and responses to drought in Quercus species of North America. Tree Physiology 7: 227-238.

Bogdan, S. (2009): Genetika s oplemenjivanjem drveća i grmlja (interna skripta). Šumarski fakultet Sveučilišta u Zagrebu. 1-207. [Bogdan, S. (2009): Genetics with breeding of trees and shrubs (internal script). Faculty of Forestry of the University of Zagreb. 1-207]

Bruschi, P., Vendramin, G., Bussotti, F., Grossoni, P. (2003): Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae). Annals of Botany 91: 707-716.

Carabeo, M., Simeone, M.C., Cherubini, M., Mattia, C., Chiocchini, F., Bertini, L,, Caruso, C., La Mantia, T., Villani, F., Mattionia, C. (2016): Estimating the genetic diversity and structure of Quercus trojana Webb populations in Italy by SSRs: implications for management and conservation. Can J Forest Res 47: 331-339.

Carter, S.P., Proctor, J., Slingsby, D.R. (1987): Soil and vegetation of the Keen of Hamar serpentine. Shetland. Journal of Ecology 75: 21-42

Gapare, W.J. (2014): Merging applied gene conservation activities with advanced generation breeding initiatives: a case of study of Pinus radiate D. Don. New For. 45: 311–331.

Geburek, T., Konrad, H. (2008): Why the conservation of forest genetic resources has not worked. Conserv. Biol. 22(2): 267–274.

Gilpin, M.E., Soule, M.E. (1986): Minimum viable populations processes of species extinctions. In: Soule M. E. ed. Conservation biology: the science of scarcity and diversity. Sunderland: Sinauer. 19-34.

Isajev, V., Ivetić, V., Vukin, M. (2005): Veštačko obnavljanje šuma hrasta kitnjaka. Šumarstvo 3. Udruženje šumarskih inženjera i tehničara Srbije. 37-51. [Isajev, V., Ivetić. V., Vukin, M. (2005): Artificial regeneration of sessile oak forests. Forestry 3. Association of Forestry Engineers and Technicians of Serbia. 37-51]

Jovanović, M., Tucović, A. (1975): Genetics of common and sessile oak /*Quercus robur* L. and *Q. petraea* Liebl./. Anales Forestales 7/2. Academia scientiarum et artum slavorum meridionalum. Zagreb. 1-83.

Jurkšienė, G., Baliuckas, V. (2014): Leaf morphological variation of sessile oak (*Quercus petraea* (Matt.) liebl.) and pedunculate oak (*Quercus robur* L.) in Lithuania. Research for rural development 2014. volume 2. 63-69.

Kleinschmit, J. (1993): Intraspecific variation of growth and adaptive traits in European oak species. Annales des Sciences Forestieres 50 (Suppl. 1): 166-185.

Lessica, P., Allendorf, F.W. (1995): When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Marinković, P., Popović, J., Karadžić, D. (1990): Uzroci epidemijskog sušenja hrasta. značaj i mogućnosti saniranja žarišta zaraze. Šumarstvo 2-3. Tematski broj „Sušenje šuma". 7-17. [Marinković, P., Popović, J., Karadžić, D. (1990): Causes of epidemic dying of oak. importance and possibilities of remediation of the infection hotspots. Forestry 2-3. Thematic issue *Forest Decline*. 7-17.]

Reed, D.H., Frankham, R. (2003): Correlation between fitness and genetic diversity. Conserv. Biol. 17: 230–237

Souto, C., Mathiasen, P., Acosta, M. (2015): Identifying genetic hotspots by mapping molecular diversity of widespread trees: when commonness matters. J. Hered. 106: 537–545.

Stojanović, Lj., Krstić, M., Bjelanović, I. (2005): Proredne seče u šumama hrasta kitnjaka na području severoistočne Srbije. Šumarstvo 3. Udruženje šumarskih inženjera i tehničara Srbije. 1-24. [Stojanović, Lj., Krstić, M., Bjelanović, I. (2005): Thinning in sessile oak forests in northeast Serbia. Forestry 3. Association of Forestry Engineers and Technicians of Serbia. 1-24.]

StatSoft Inc., 2004. STATISTICA, version 7.

Zanetto, A., Kremer, A. (1995): Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. I. Monolocus patterns of variation. Heredity 75: 506-517.

Šijačić-Nikolić, M., Milovanović, J. (2012): Conservation and sustainable use of forest genetic resources through an example of wetland ecosystems. Agriculture and Forestry. 57 (1): 23-31.

SUITABILITY OF WOOD OF NATIVE OAK SPECIES (*QUERCUS* spp.) FROM THE IBERIAN PENINSULA NORTHWEST FOR COOPERAGE

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Abstract

In the northwest of Iberian Peninsula, the oak wood is scarce used in the forestry industry. Small plots and many owners do not facilitate its exploitation, as well as the later industrial development. Forest management of oak forests is virtually non-existent and the use of oak wood is limited for firewood. This is one of the main reasons why it would be essential to develop a specific industry that contributes to the valorisation of these species. The aim of our research was to study the characteristics and/or properties of wood of native oak species for the manufacture of wine aging barrels. For more than 15 years, from 1993 to 2010 approximately, several inventories were carried out in oak forests of the most abundant species of the *Quercus* genus in the northwest of the Iberian Peninsula, in Galicia, particularly. Subsequently, we calculated the essential parameters of the wood using samples of different oak species felled in the inventoried forests to assess the suitability of their wood for cooperage. At first, this would allow us to even propose in advance whether to carry out certain silvicultural treatments to achieve our goal. The overall objective was to make a description of the most important properties of the wood of these species on the barrel manufacture for aging wines. The current study about wood properties of *Quercus robur* L. and *Q. pyrenaica* Willd. is based on the determination of bark, sapwood, and heartwood to assess their variation in the Galician oaks. *Quercus robur* has a higher proportion of bark (22.9%) than *Quercus pyrenaica* (17.6%), but the high coefficient of variation associated with this value (62.7%) moderate this statement.

Keywords: *Atlantic oaks, Bark, Heartwood, Sapwood, Wine aging.*

Introduction

Hardwood trees differentiate with softwood trees, and they are not necessarily harder than these (Hoadley, 1990; Alden, 1995). This terminology does not necessarily reflect the wood properties; "balsa" (a hardwood) is much softer than average softwood (Plomion *et al*., 2001). There are many anatomical differences between hardwood and softwood species; however, wood parts of a tree system are common to both (Alden, 1997; Zhang *et al*., 2014). The development of the tree trunk is reached by two growth types. The first is produced by the apical meristem and is responsible for the common and ascending primary growth of all vascular plants. Unlike non-tree plants, there is also secondary growth that results in increased trunk thickness (Ramagea *et al*., 2017). This increase is controlled by the multiplying activity of the vascular cambium, i.e., the cambium is only one cell thick that produces bark on one side and wood on the other (Dinwoodie, 2000; Schweingruber, 2007).
Accumulating extracts provides the heartwood of many hardwood species a darker colour than the sapwood (Plomion *et al*., 2001). On the oak trees, the heartwood can be seen easily visible thanks to its dark coloration. (Bary-Lenger and Nebout, 1993; Diaz-Maroto and Sylvain, 2016).

During the fall and winter months, when deciduous trees have no foliage, it is the bark that becomes the focal point. The bark is an essential part of the tree because protects it and provides with the necessary nutrients and moisture to grow (Schweingruber, 2007).
This is why it is important to protect a tree bark by using tree guards, especially over the winter months when rodents are hidden under the snow and eat the bark of the tree. If a tree's bark is deeply eaten in a continuous band (girdled), chances are the tree will not survive. Bark covers the outside of the tree and its branches and is formed by several layers. The outer bark (epidermis) protects the interior of the tree from insects, fungi, humidity, drought, wind, and sun, and during the winter months it insulates from the cold, and in summer from heat (Fernandez-Parajes *et al*., 2005). The inner bark or phloem is how the tree gets its nutrition. It is like a pipeline transporting nutrients made by photosynthesis from the leaves to rest of the tree, carries them to the branches, trunk and down to the roots of the tree. It is short lived and when it dies it becomes part of the outer bark (Myburg *et al*., 2013; Diaz-Maroto and Sylvain, 2016) (Figure 1).

Figure 1. Image of the bark, sapwood, and heartwood of a slice of *Quercus robur* trunk. As we mentioned, the heartwood can be seen easily thanks to its dark coloration.

Sapwood is the tree's pipeline for water moving up to the leaves. Sapwood is new wood. As newer rings of sapwood are laid down, inner cells lose their vitality and turn to heartwood (Hacke and Sperry, 2001). Heartwood is the central, supporting pillar of the tree. Although dead, it will not decay or lose strength while the outer layers are intact.
Trees produce annual rings that reflect the changing environment. Rapid growth during the spring produces "earlywood" which is less dense and composed of large cells with thinner walls allowing for efficient water transport to support photosynthesis. This period is followed by slower growth, yielding "latewood", characterised by more densely packed, smaller cells, production of which stops for winter (Ramagea *et al*., 2017). In softwoods, the transition can be

gradual, distinct, or a combination of each. In hardwoods, vessels may have a different size in early- and latewood (Fernandez-Parajes *et al*., 2005; Myburg *et al*., 2013).

The objectives of this study were: i) to make a description of the most important properties of wood of *Quercus* spp. –*Quercus robur* L., *Q. pyrenaica* Willd., and possible hybrids of both species– for their use in the barrel manufacture industry; ii) to analyse and assess those characteristics and/or properties of the native oaks wood with a greater influence on the manufacture of barrels for aging quality wines.

Material and Methods

Study area, sampling, data collection, and software for image analysis

Study area includes the Autonomous Community of Galicia, located in the northwest of Iberian Peninsula, with an area of ~ 3 million hectares (Figure 2). The average altitude is 508 m, and slopes of more than 20% are present in half of the territory. The lithological conformation is heterogeneous, although siliceous substrates predominate. The climate is miscellaneous and is generally classified as humid oceanic but with different Mediterranean influence in certain areas. The annual precipitation varies between 600 and more than 3000 mm (Diaz-Maroto *et al*., 2005). For more than 15 years, from 1993 to 2010 approximately, several inventories were carried out in oak forests of the most abundant species of the *Quercus* genus in the northwest of the Iberian Peninsula, in Galicia, particularly. The zone under study was considered as a single unit, where regions for sampling were selected, taking care to incorporate a representative number of oak stands with a minimum area ranged between 0.5 and 1 ha, which avoids edge effect problems. In total, 31 trees were selected in 11 different stands located of Galician provinces of Lugo and Ourense, from which we obtained 30 wood slices at 60 cm height on the tree trunk. Around 70% of the Galician territory is 600 m above sea level and many of the stands have steep slopes. Therefore, there could be an important problem in selection of the oak wood for cooperage industry because a steep slope favours the formation of tension wood with a heart outside centre, which is not suitable for the manufacture of barrels (Bary-Lenger and Nebout, 1993; Lehringer *et al*., 2008). Table 1 shows the main characteristics of the trees felled in the oak stands (from normal tree to heart strongly off centre).

Figure 2. Study area: Autonomous Community of Galicia and its four provinces (A Coruña, Lugo, Ourense and Pontevedra), northwest of Iberian Peninsula, in the world context.

Using a ruler with millimetre appreciation we can calculate the length of the different parts of the trunk (Bakour, 2003). However, it is advisable to work with a particular type of software, e.g., ImageJ, given its advantages because it is a public domain image processing program. It is possible calculate the area of bark, sapwood, and heartwood, as well as their relative percentage. The precision is greater, being possible to use a bigger number of samples considering the singularity of each one. ImageJ was created with an open architecture and is multithreaded, so time-consuming functions such as image file reading can be performed in parallel. Age measurement also was done by ImageJ with a scanning resolution of 600 dpi. Spatial calibration is available to provide real measurements in mm.

Table 1. Main characteristics of the trees felled and the wood slices.

Species	Normal tree	Heart a little off centre	Heart off centre	Heart strongly off centre	Total
Quercus robur	7	4	4	2	17
Quercus pyrenaica	5	2	3	3	13
Total	12	6	7	5	30

Results and Discussion

Measurement of the age of wood slices

Through the sample selection program, the following criteria were followed:

- Two oak species: *Quercus robur* and *Q. pyrenaica*.
- Two wood types: sapwood and heartwood; additionally, the bark.
- Four tree types and wood slices: heart a little off centre, heart off centre, heart strongly off centre, and tree with a normal heart (Table 1).

Since Image J software offers a special pointer to assign a number to each ring of growth, the age was calculate done with it, using a scanning of a resolution of 600 dpi. On the whole, the determination of the age was moderately easy. However, some samples showed periods of very slow growth, a characteristic found in many hardwood trees in the study area. In these cases, the latewood is almost absent, and it is very difficult to identify the separation of each growth ring (Bakour, 2003; Myburg *et al*., 2013).

Variability of bark, sapwood and heartwood depending on age

Quercus robur has a higher percentage of bark (22.9%) than *Quercus pyrenaica* (17.6%), but the high-level coefficient of variation (CV) linked with this value (62.7%) moderate this statement. Also, as expected, the accuracy of bark measurements is relatively low because of the irregularities. However, bark has value, be it from selling as landscaping mulch, burning it to heat dry kilns, making charcoal briquettes or carbon filters, or converting it to biomass energy (Hoadley, 1990). The method used when a log is scaled in a log yard excludes the bark content from the purchase price, resulting in it being a free commodity. Every log processed incurs a cost

in separating the bark from the wood. Hopefully, the value of the bark exceeds this cost plus the cost of transportation to its end user. Proximity to a market to minimize transportation costs plays an important role in deciding a profitable end use for the bark. Most sawmills have an idea how much bark is produced by how many trucks they fill over a period of time, thus it is volume based or weight based if the trucks are weighed as part of the sales agreement (Zhang *et al*., 2014). Age does not seem, in this case, to have a significant influence in the bark proportion.
On the contrary, there is a significant relationship between age and the proportion of the heartwood. The relationship of the heartwood age, i.e., the number of rings in the heartwood area, and the proportion of heartwood makes it possible to obtain the best correlation for each of the species. A lower width ring in oak species results in a lower texture (Bakour, 2003; Diaz-Maroto and Sylvain, 2016); the proportion of earlywood is more important than the latewood (Pasztory *et al*., 2014; Zhang *et al*., 2014).

Conclusions

The estimation of the different percentages of bark, sapwood and heartwood of the Galician oaks and its relationship with the age has allowed knowing the following characteristics:
- The proportion of heartwood is positively proportional to the age of the tree.
- When a particular tree grows quickly, the proportion of heartwood is higher at the same age than in other individuals.
- The fast growth of *Quercus pyrenaica* produces the formation of large proportion of heartwood in a few years.
- *Quercus robur* has an intermediate growth between *Quercus pyrenaica* and other oaks.
- In the Galician oaks, the heartwood proportion is between 21.8 and 62.8% (maximum and minimum values ± standard deviation ± accuracy). However, the knowledge of the tree age and the heartwood age makes it possible to improve this range by using different models.
- On the contrary, data from this study do not make it possible to respond to the existence of a relationship between age and proportion of bark and sapwood.

References

Alden H.A. (1995). Hardwoods of North America, FPL-GTR-83, USDA Forest Service, Madison, WI, USA.

Alden H.A. (1997). Softwoods of North America, FPL-GTR-102, USDA Forest Service, Madison, WI, USA.

Bakour R. (2003). Influence de l'espèce de chêne et de la provenance des deux principaux chênes français (*Quercus robur* L. and *Q. petraea* Liebl.) sur la structure anatomique et les propriétés physiques du bois de merrain [Influence of the species of oak and the origin of two major French oak (*Quercus robur* L. and *Q. petraea* Liebl.) on the anatomical structure and physical properties from wooden staves], Thèse présentée pour l'obtention du titre de Docteur de l'ENGREF, France.

Bary-Lenger A., Nebout J.P. (1993). Le chêne pédonculé et sessile en France et en Belgique [Pedunculate and sessile oak in France and Belgium], Gerfaut Club Editions du Perron, Alleur-Liège, Belgique.

Diaz-Maroto I.J., Vila-Lameiro P., Silva-Pando F.J. (2005). Autecology of oaks (*Quercus robur* L.) in Galicia (Spain). Annals of Forest Sciences, vol. 62, pp. 737-749.

Diaz-Maroto I.J., Sylvain T. (2016). Analysis of physical properties of wood in three species of Galician oaks for the manufacture of wine barrels. Part I: Wood infradensity. Wood Research, vol. 61, pp. 683-696.

Dinwoodie J.M. (2000). Timber: its nature and behaviour, Taylor and Francis, Abingdon, Unite Kingdom.

Fernandez-Parajes J., Diaz-Maroto I.J., Vila-Lameiro P. (2005). Physical and mechanical properties of rebollo oak (*Quercus pyrenaica* Willd.) wood in Galicia (Spain). Wood Research, vol. 50, pp. 1-15.

Hacke U.G., Sperry J.S. (2001). Functional and ecological wood anatomy. Perspectives in Plant Ecology, Evolution and Systematics, vol. 4, pp. 97-115.

Hoadley R.B. (1990). Identifying Wood: Accurate results with simple tools, Taunton Press, Newtown, CT, USA.

Lehringer Ch., Gierlinger N., Koch G. (2008). Topochemical investigation on tension wood fibres of *Acer* spp., *Fagus sylvatica* L. and *Quercus robur* L. Holzforschung, vol. 62, pp. 255-263.

Myburg A., Lev-Yadun S., Sederoff R. (2013). Xylem structure and function, John Wiley and Sons Ltd., Chichester, United Kingdom.

Pasztory Z., Borcsok Z., Ronyecz I., Mohacsi K., Molnar S., Kis S. (2014). Oven dry density of sessile oak, turkey oak and hornbeam in different region of Mecsek Mountain. Wood Research, vol. 59, pp. 683-693.

Plomion Ch., Leprovost G., Stokes A. (2001). Wood formation in trees. Plant Physiology, vol. 127, pp. 1513-1523.

Ramagea M.H., Burridgeb H., Busse-Wicherc M. et al. (2017). The wood from the trees: the use of timber in construction. Renewable and Sustainable Energy Reviews, vol. 68, pp. 333-359.

Schweingruber F.H. (2007). Wood structure and environment, Springer, Berlin, Germany.

Zhang J., Nieminen K., Alonso J.A., Helariutta Y. (2014). The formation of wood and its control. Current Opinion in Plant Biology, vol. 17, pp. 56-63.

DETERMINATION OF *SALVIA OFFICINALIS* L. VOLATILE CONSTITUENTS BY SPME METHOD

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Abstract

Sage (*Salvia* L.) is a valuable medicinal and aromatic plant from the *Lamiaceae* family. Although there are about 900 taxa of the genus *Salvia*, which is affiliated to the *Lamiaceae* (*Labiatae*) family, the most commercially valuable ones are *Salvia officinalis* L., which is called Medicinal sage or Dalmatian sage. In this study, volatile components of *Salvia officinalis* sage were determined by solid-phase micro extraction (SPME) technique. The aim of this study is to examine the effect of different brewing times on the change of volatile compounds in sage leaves.The extraction of volatile compounds (VCs) was carried out in a water bath at 50 degrees Celsius. After the samples were kept in the water bath for 10, 20 and 30 minutes respectively, the fiber was placed in the headspace bottle and held for 30 minutes. In these techniques, major volatile components were determined as thujone, eucalyptol and camphor. Major components were determined as thujone 28.65%, eucalyptol 15.03% and camphor 11.94% in vials kept in water bath for 10 minutes. In vials kept in water bath for 20 minutes, major components were determined as thujone 37.29%, eucalyptol 15.88% and camphor 14.12%. In vials kept for 30 minutes, major components were determined as thujone 44.21%, eucalyptol 15.66% and camphor 10.88%, respectively. It was observed that thujone ratios increased according to the waiting times of the vials in the water bath.

Keywords: *Salvia officinalis L., SPME, Sage. Lamiaceae*

Introduction

Sage is an herb that has been used for medicinal purposes since ancient times. The most commonly used species is *Salvia officinalis* L, known as medicinal sage. The genus Salvia, which belongs to Lamiaceae, is represented by more than 900 species worldwide (Tundis et al., 2020). Anatolia is an important center for Salvia, with 47 of the 90 species endemic to Turkey (Kan et al., 2007).

There are many types of sage used in treatment and grown in Turkey. Some of these are: *Salvia triloba* L. (anatolian sage), *S. aethiopis* L. (woolly sage), *S. dichroantha* Stapf., *S. multicaulis* Vahl., *S. sclarea* L., *S. tomentosa* Miller, *S. verbenaca* L. , *S. virgata* Jacq. and *S. viridis* L. species (Baytop, 1984).The leaves of this plant, which has versatile uses, are used fresh or dry. The essential oil obtained from the plant is also registered in the codex and has an important place in pharmacy; In addition to the effect of facilitating digestion in nutrition, tea prepared with sage has a cough suppressant effect. Sage is also used as an additive in various cheeses and breads made in Europe. It is known that it restores the lost energy of the body, strengthens the memory and liver, and the tea obtained from the plant is used to strengthen the nervous system. Even in recent years, this plant has started to be used in hair-strengthening shampoos, deodorants, hair dye and skin care creams (Gürbüz, 1993). It has medicinal properties such as

strengthening, carminative, stimulant, antiseptic, against oral wounds and infections (Demirci et al. 2002). In addition to the use of the plant for food, tea and medicinal purposes, it is also used as an ornamental plant in horticulture and landscape architecture. Sage, which produces very showy and abundant flowers, is also important in honey bee breeding. In a study conducted by Sıralı and Deveci (2002), some *Salvia* species it was stated that *S. glutinosa, S. officinalis, S. sclarea, S. verbanaca, S. verticillata* and *S. viridis* species are important for bees. This plant is used in different fields due to its essential oil.
The essential oil content of medicinal sage leaves is between 1.0-2.5%. The essential oil components are 30-50% thujon, 15% cineole and 10% borneol (Baytop, 1984). There are many studies on the essential oil ratio and components of species belonging to the genus Salvia in the world and in Turkey. Along with these studies, the existence of some antimicrobial and antifungal effects of the essential oils and components of these plants has been demonstrated by many studies (Joshi et al. 2021). As a matter of fact, there are studies conducted with some of the species planned to be collected within the scope of this project.

Material and methods

Plant material was collected from the trial fields of Hatay Mustafa Kemal University, Department of Field Crops. The leaves were dried under +35 ^{o}C in oven. VCs analyze of leaves was performed according to the procedures described by Guler with minor modification. Dried samples were cut into in small pieces in cold mortar and taken into headspace vials. Three mL NaCl solution (3 % concentration) was added in samples. The adsorption of VCs was carried out using Solid Phase Microextraction Technique (SPME) by a divinylbenzene/carboxen/polydimetylsilioxan fiber. The extraction of VCs was done in water bath at 50 ^{o}C. The samples were hold in water bath for 10, 20 and 30 min than fiber was inserted to headspace vial and kept at 30 min. For desorption of VCs, fiber hold in injection port at 250 ^{o}C for 5 min. The VCs were analyzed by gas chromatography-mass spectrometry (GC-MS) using TR-FAME model, 5 % Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter x 60 m length, 0.25 μm film thickness column was used. Helium (99.9 %) was used as the carrier gas at a flow rate of 1 mL/min. Mass spectra were recorded at 70 eV, the mass range was from 1.2-1200 m/z. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50°C and the temperature was increased to 220°C with a rate of heat increase of 3°C/min. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9) 35. The individual compositions were determined by comparing their retention index and with Wiley Library (Wiley Interscience, New York). The relative quantities of individual compounds were calculated with the Xcalibur Report program.

Results and discussion

The main components determined according to the three different absorption times (10, 20 and 30 minutes) of SPME fiber are respectively: thujone, eucalyptol and camphor. When the volatile components of the fiber, which was kept in the vial for 10 minutes, were examined, the main component was determined as 28.65% thujone, followed by eucalyptol with 15.63% and camphor with 11.94%, respectively. The main components determined as a result of 20 min absorption of fiber were determined as 37.29% thujone, 15.88% eucalyptol and 14.12%

camphor, respectively. In vials that were absorbed for 30 minutes, the main components were determined as 44.21% thujone, 15.66% eucalyptol and 10.88% camphor (Table 1.). The other studies reported that *Salvia officinalis* compositions were determined as camphor, α-thujone, 1,8-cineole, viridiflorol, borneol, camphene, limonene, salvene, α-pinene, β-pinene, β-thujone and γ-terpinene (Celik et al., 2018). In another study, Raal et al. (2007) determined the major components of *S. officinalis* as 1,8-cineole, camphor, thujone, borneol, and viridiflorol.

Table 1. Main components determined according to three different absorption times of SPME fiber.

RT	**Compound Name**	**SI**	**RSI**	**Cas #**	**10 m**	**20 m**	**30 m**
4.55	cis-Salvene	977	991	NA	0.6	0.45	0.35
5.85	α-Pinene	990	992	80-56-8	2.66	1.87	1.52
7.04	Camphene	988	990	79-92-5	2.63	1.62	1.44
7.98	β-Pinene	975	983	127-91-3	2.45	1.58	1.43
8.61	Myrcene	986	989	123-35-3	5.56	4.63	4.35
9.09	α-Phellandrene	979	980	99-83-2	0.1	0.05	0.06
9.55	α-Terpinene	980	986	99-86-5	0.22	nd	0.06
9.77	Limonene	987	989	138-86-3	5.18	3.89	3.35
10.46	cis-Ocimene	983	991	6874-10-8	0.1	nd	0.06
10.8	Sabinene	987	989	3387-41-5	0.16	0.24	0.18
10.99	γ-Terpinene	994	995	99-85-4	0.67	0.66	0.54
12.05	**Eucalyptol**	**996**	**996**	**470-82-6**	**15.03**	**15.88**	**15.66**
12.66	Cymene	986	991	25155-15-1	2.54	2.45	1.92
17.35	1-Octen-3-ol	988	992	3391-86-4	0.13	0.07	0.07
17.93	α-Cubebene	932	957	17699-14-8	0.08	0.06	0.05
18.04	α-Methoxytoluene	802	871	538-86-3	0.09	nd	nd
18.48	o-Allyltoluene	961	969	1587-04-8	0.44	0.2	0.22
19.38	α-Copaene	958	966	3856-25-5	0.71	0.13	0.13
20.04	Aristolene	858	869	6831-16-9	0.78	nd	nd
20.74	β-Bourbonene	953	965	5208-59-3	0.47	0.25	0.3
21.04	Linalool	986	988	78-70-6	0.21	0.11	0.13
22.32	**Thujone**	**986**	**992**	**471-15-8**	**28.65**	**37.29**	**44.21**
23.54	Alloaromadendrene	971	974	25246-27-9	0.85	0.81	1.03
24.53	Caryophyllene	997	997	87-44-5	6.02	6.41	5.87
25.81	Bornyl acetate	994	995	76-49-3	2.51	1.59	nd
26.14	Germacrene D	932	951	23986-74-5	0.1	nd	nd
26.53	**Camphor**	**990**	**994**	**76-22-2**	**11.94**	**14.12**	**10.88**
26.91	Humulene	924	935	6753-98-6	2.53	1.63	1.38
27.48	Aromadendrene	890	896	489-39-4	0.46	0.25	0.18
27.66	Junipene	921	921	475-20-7	0.13	nd	0.18
28.05	Borneol	995	995	10385-78-1	1.85	1.03	0.73
31.12	cis-Calamenene	819	941	483-77-2	0.29	nd	0.1

32.16	p-Cymen-8-ol	815	889	1197-01-9	0.14	nd	0.05
39.37	Veridiflorol	947	971	552-02-3	1.18	nd	nd
40.45	Carvacrol	951	968	499-75-2	0.07	nd	nd
40.76	Caryophyllene oxide	973	982	1139-30-6	0.56	0.25	0.28
45.8	2,5-Bornanedione	900	902	4230-32-4	0.62	nd	0.36

Conclusion

Changes in volatile components and composition may have resulted from various different factors such as climatological, seasonal, geographical and geological conditions. Also, different harvest periods, different harvesting stages and different harvest times of plants can affect essential oil content and composition, even if they are grown in the same ecological conditions. In this study, different waiting times and volatile components of sage leaves obtained by harvesting under the same conditions were determined by SPME. Thus, in addition to the standardization in harvesting and growing conditions, volatile components can be determined by different methods and standardization in components will be achieved.

References

Baytop T. (1984). Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün) (Herbal Treatment in Turkey (Past and Present).). İ.Ü.Yayınları,No: 3255, s: 520, Sanal Matbaacılık, İstanbul.

Celik, S.A., Ayran, I., Kan, A., Kan, Y. (2018). Elemental characterization of buckwheat (Fagopyrum esculentum Moench) cultivated in Turkey. Int. J. Agric. Environ. Food Sci., 2 (Special Isseue 1), 193-195. DOI: 10.31015/jaefs.18034.

Demirci B., Tabanca N., Başer K.H.C. (2002). Enantiomeric distribution of some monoterpenes in the essential oils of some Salvia species. Flavou and Fragrance Journal 17: 54–58.

Gürbüz B. (1993). Türkiye'de tıbbi adaçayı (*Salvia officinalis* L.) yetiştirme çalışmaları, Tarım ve Köy İşleri Bakanlığı Dergisi (Studies on growing medicinal sage (*Salvia officinalis* L.) in Turkey, Journal of the Ministry of Agriculture and Rural Affairs) sayı:93, 51–52.

Joshi, S., Pandey, R. D., Bhattarai, R., Gharti, B. B. (2021). Antimicrobial Activity of Essential Oil and Crude Organic Extracts of *Salvia officinalis* L. Leaves from Nepal. International Journal of Innovative Science and Research Technology. Volume 6, Issue 2, February.

Kan, Y., Gökbulut, A., Kartal, M., Konuklugil, B., Yılmaz, G. (2007). Development and Validation of a LC Method for the Analysis of Phenolic Acids in Turkish Salvia Species, Chromatographia, 66, 147-152

Raal, A., Anne Orav, A. and Arak, E. (2007) Composition of the essential oil of *Salvia officinalis* L. from various European countries, Natural Product Research, 21:5, 406-411, DOI: 10.1080/14786410500528478

Sırali R. ve M. Deveci (2002). Bal arısı (Apis melifera L.) için önemli olan bitkilerin Trakya bölgesinde incelenmesi, Uludağ Arıcılık Dergisi (Investigation of plants important for honey bee (*Apis mellifera* L.) in Thrace region, Uludağ Journal of Beekeeping). 2(1):17–26.

Tundis, R.; Leporini, M.; Bonesi, M.; Rovito, S.; Passalacqua, N.G. (2020). *Salvia officinalis* L. from Italy: A comparative chemical and biological study of its essential oil in the mediterranean context. Molecules, 25, 5826.

CHANGE OF ESSENTIAL OIL RATIO AND COMPONENTS OBTAINED BY DIFFERENT METHODS IN LAUREL AND MYRTLE PLANTS

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Abstract

Medical and aromatic plants are divided into two groups, those collected from nature and those that are cultivated. Medical and aromatic plants collected from nature are plants that grow spontaneously in natural places such as forests. Two of these plants commonly grown are *Laurus nobilis* L. and *Myrtus communis* L. This study was carried out in order to determine the ratio and components of essential oils obtained by different methods (hydrodistillation, steam distillation and boling distillation) of laurel trees and myrtus growing densely in Hatay flora of Turkey. Hydrodistillation method is a traditional method widely used in obtaining essential oils made with a Clevenger type apparatus in small-scale productions. Whereas, steam and boiling distillation method is carried out in large distillation boilers (retort) in industrial applications. Previously it was determined that the essential oil ratios in Clevenger were between 2.5-3% in laurel, 1.5-2% in myrtle plant. In the essential oils obtained by retort methods (steam and boiling distillation), these ratios were observed between 0.8-1.2% in laurel and 0.3-0.7% in myrtle plant. When the essential oil components were examined, the main components of laurel were determined as eucalyptol, α-terpinyl acetate and sabinene in all methods. Main components of laurel essential oil in the Clevenger were determined as eucalyptol 54.85% α-terpinyl acetate 18.52% and sabinene 3.25%, respectively. Whereas, components obtained by retort methods (steam and boiling distillation) were determined as 57.53-44.48% eucalyptol, 12.06-8.94% sabinene and 7.59-17.98% α-terpinyl acetate. The main components for the myrtle plant were determined as α-pinene, eucalyptol, linalool. Linalool was replaced by limonene in the retort methods.

Key words: *Laurus nobilis L., Myrtus communis L., Essential oil, Clevenger, Retort.*

Introduction

Laurel is a plant belonging to the *Lauraceae* family intensely found in Turkey, especially in the Mediterranean, Aegean and Black Seas. As a species, there are mostly Mediterranean Laurel (*Laurus nobilis* L.). This species is a 3-10 meters high, evergreen tree with yellow flowers in winter. The leaves of the plant are 5-10 cm long, 2-5 cm wide, hard and short-stalked, green-colored; its fruits are in the form of small olives (Baytop, 2000). All parts of the laurel plant are used in terms of the use of leaves, wood and fruits. Spices and essential oil are produced from its leaves, fuel is produced from its wood and fixed fruit oil is extracted from its fruits (Safak and Okan 2004).
Myrtle is a shrub-shaped plant from the *Myrtaceae* family. It is a plant that can rise up to 2-3 meters in height and is seen in places where the Mediterranean climate prevails, especially in coastal areas, sometimes in the form of a small tree. The color of the leaves is light bright green and old leaves are dark green, leathery, egg-shaped or lanceolate with a pointed tip and whole

edges. Its flowers consist of five white oval or egg-shaped segments and have small white knobs at the tip. Its fruits are round or egg-shaped, pea-sized, bluish-black or rarely white. Immature fruits are bitter while mature ones are sweet. Since it is a drought-resistant plant, it blooms throughout the summer (June-September) (Sümbül et al., 2011). The essential oils of the fresh and/or dry leaves of the myrtle plant are used in the cosmetics, confectionery and beverage industries (Akgül, 1993; Akgül and Bayrak, 1989; Boelens and Jimenez, 1992). The fruit of the myrtle plant has an aromatic flavor and can be eaten fresh when ripe or dried for later use as a flavoring agent. The leaves are also suitable for use. Its dried fruits and flower buds are used to flavor sauces and syrups. The essential oils of the leaves and branches are used as a flavoring agent, especially when mixed with other spices. In addition, the flowers have a pleasant taste and are used in salads in Italy (Genders, 1994; Farah et al., 2006).

Material and methods

Essential oil isolation and hydrodistillation

The essential oil was obtained from dried leaves. A total of 25 g of each of the ground plant samples was used for the separate hydrodistillation experiment. Weighed samples were placed into a 1L flask, individually and carefully. Then distilled water was added until it covered completely the sample. Essential oils were obtained in an all-glass Clevenger-type distillation according to hydrodistillation method. The trial was carried out at three times. The essential oils were evaporated with anhydrous sodium sulfate and stored at 4°C in dark bottles until analysis time.

Steam Distillation

Twenty liters of water was put into a 40-liter stainless steel steam generator with 3000 Watt dimmer belt heater. The 10 kg sample was placed in a 40-liter stainless steel distillation vessel with a dome-designed cover, distillation column and cooler made entirely of copper. The water inlet and outlet of the cooler part are provided and the cooling process is started. When the steam generator was started and the temperature rose to 100°C, the distillation process started and the process continued for 4 hours. After the distillation, the oil obtained with a separating funnel was separated from the water and the process was completed.

Boiling Distillation

20 liters of water and 10 kg of sample were put into the 40-liter stainless steel steam generator used as a herbal boiler with a 3000-watt dimmer belt heater with a dome-designed cover, distillation column and cooler made entirely of copper. The water inlet and outlet of the cooler part were provided and the cooling process started. When the steam generator was started and the temperature rose to 100°C, the distillation process started and the process continued for 4 hours. After distillation, the oil obtained with a separating funnel was separated from the water and the process was completed.

Figure 1. Retort distillation methods (steam and boling)

GC/MS analysis

The essential oil components obtained from plants were determined by the Gas-chromatographic method. Determination of the components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatographic device under the following conditions. TR-FAME model column consisting of 5 % Phenyl Polysilphenylene-siloxane (0.25 mm inner diameter x 60 m length, 0.25 µm film thickness) was used. Helium (99.9 %) was used as a carrier gas at a flow rate of 1 mL/min. Mass spectra were recorded at 70 eV, the mass range was from 1.2-1200 m/z. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C. The column temperature was initially 50°C and the temperature was increased by a ramp of 3°C/min up to 220°C. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9) 35. The individual compositions were determined by comparing their retention index and with Wiley Library (Wiley Interscience, New York). The relative quantities of individual compounds were calculated with the Xcalibur Report program.

Results and discussion

Essential oil ratios were determined between 2.5-3% in the Clevenger according to hydrodistillation method. On the other hand, this ratio varied between 0.8-1.2% in retort boiling and 1.5-2% in steam distillation method. When the laurel essential oil components obtained by different methods were examined, the main components were determined as eucalyptol, α-terpinyl acetate and α-pinene. While the highest eucalyptol ratio was determined with 57.53% in retort boiling, the lowest ratio was determined with 44.48% in the steam distillation method. The highest value for α-terpinyl acetate was determined in hydrodistillation method with 18.52%, and the lowest ratio was detected in retort boiling with 7.59%. While α-pinene, another main component, was determined at the highest ratio with 7.18% in retort boiling, at the lowest ratio with 3.9% in the hydrodistillation method (Table 1). Özcan and Chalchat, (2005), in their study on laurel essential oil, reported that the essential oil ratios of laurel leaves collected from seven different regions varied between 1.4% and 2.6%. In the component analyzes performed in GC-MS, researchers reported that the main components were eucalyptol (51.73-68.48%) and α-terpinyl acetate (4.04-9.87%), respectively.

Table 1. Laurel essential oil components obtained by different methods

RT	Compound Name	Cas #	Hydrodistillation	Retort distillation	Steam distillation
6.62	α-Pinene	80-56-8	3.9	7.18	6.46
7.66	Camphene	79-92-5	0.11	0.36	0.53
8.48	β-Pinene	127-91-3	1.68	2.66	2.5
8.77	Sabinene	3387-41-5	3.25	12.06	8.94
8.92	Myrcene	123-35-3	nd	0.24	0.55
9.46	α-Phellandrene	99-83-2	0.13	0.28	nd
10.08	Limonene	138-86-3	1.07	1.04	1.38
11.03	β-Phellandrene	555-10-2	0.36	nd	nd
11.18	γ-Terpinene	99-85-4	0.73	0.25	nd
12.12	Eucalyptol	470-82-6	54.85	57.53	44.48
12.72	p-Cymene	99-87-6	1.68	1.09	1.26
19.74	trans Sabinene hydrate	17699-16-0	0.38	nd	nd
20.72	Linalool	78-70-6	1.54	0.27	1.06
22.59	β-Elemene	515-13-9	0.07	nd	0.83
22.79	1-Terpinenol	586-82-3	0.15	nd	1.29
24.26	Terpinen-4-ol	562-74-3	3.4	2.74	3.06
26.52	Ocimenyl acetate	NA	0.6	0.19	0.86
27.05	α-Terpineol	98-55-5	3.41	2.56	1.29
27.58	α-Terpinyl acetate	80-26-2	18.52	7.59	17.98
38.01	Methyleugenol	93-15-2	2.12	0.47	1.09
38.85	Junipene	475-20-7	0.08	nd	nd
40.28	Caryophyllene oxide	1139-30-6	0.23	0.09	0.19
42.59	Cinnamyl acetate	103-54-8	0.12	nd	0.24
43.1	Guaiol	489-86-1	0.07	0.02	0.11
43.74	Eudesmol	473-15-4	0.22	0.02	0.46

When the essential oil ratios of myrtle were examined, the highest essential oil ratio was obtained in the hydrodistillation method with 1.5-2%. On the other hand, the lowest ratio was observed in the retort boiling with 0.3-0.7%, this ratio varied between 0.9-1.2% in steam boiling. When the changes of essential oil components were examined, the highest α-pinene ratio with 52.56% was determined in retort boiling method while the lowest ratio with 28.32% in hydrodistillation method (Table 2). Eucalyptol, another main component, was detected with the highest ratio as 34.25% in hydrodistillation method, while the lowest ratio with 19.78% in steam distillation method.

Table 2. Myrtus essential oil components obtained by different methods

RT	Compound Name	Cas #	Hydrodistillation	Retort distillation	Steam distillation
6.64	α-Pinene	80-56-8	28.32	52.56	45.75
8.47	β-Pinene	127-91-3	0.27	0.23	0.17
8.77	4-Caren	NA	0.11	0.12	0.16
10.07	Limonene	138-86-3	6.01	10.17	7.23
11.18	Sabinene	3387-41-5	0.07	nd	nd
12.12	Eucalyptol	470-82-6	34.25	24.2	19.78
12.73	p-Cymene	99-87-6	0.79	0.43	0.45
14.31	1-Dodecene	112-41-4	0.04	nd	nd
15.37	Octanal	124-13-0	0.04	nd	nd
18.24	Cyclooctanone	502-49-8	0.04	0.04	0.03
18.85	Linalool oxide	5989-33-3	0.03	nd	nd
20.7	Linalool	78-70-6	7.56	1.41	10.71
22.36	Linalyl acetate	115-95-7	1.91	nd	2.06
22.6	β-Elemene	515-13-9	0.05	nd	nd
24.16	Caryophyllene	87-44-5	0.68	nd	0.1
24.28	Terpinen-4-ol	562-74-3	0.33	nd	nd
24.72	trans-Caryophyllene	87-44-5	0.03	nd	0.75
25.3	trans-Pinocarveol	547-61-5	0.14	nd	0.06
26.28	Estragole	140-67-0	0.39	nd	nd
26.54	Humulene	6753-98-6	0.28	nd	0.51
27.04	α-Terpineol	98-55-5	6.65	2.53	3.07
27.28	trans-Caryophyllene	87-44-5	0.04	0.51	0.15
27.58	α-Terpinyl acetate	80-26-2	4.57	2.26	2.37
27.7	Myrtenyl acetate	1079-01-2	0.97	nd	0.1
28.65	Geranyl acetate	105-87-3	1.85	0.43	2.23
29.61	Myrtenol	515-00-4	0.59	0.37	0.1
30.4	Geraniol	106-24-1	0.83	0.15	0.25
40.28	Caryophyllene oxide	1139-30-6	0.26	0.12	0.13

Conclusion

Laurel and Myrtle play an important role in the essential oil trade. Therefore, it is very important to standardize the essential oils of these plants. As a conclusion, it was determined that the highest essential oil ratio was obtained by hydrodistillation method. When an evaluation is made in terms of the components, it is seen that there are differences according to the methods. Since it is clear that the cultivation of these plants collected from nature and the standardization of essential oil ratios and contents are very important for the essential oil trade, these studies should increasingly continue.

References

Akgül, A., Bayrak, A. (1989). Essential oil content and composition of myrtle (Myrtus communis L.) leaves. Doga TU Tar ve Or. D., 13-: 143–147.

Akgül, A. (1993). Spice Science and Technology (Publ. No. 15). Turkish Association of Food Technologists (in Turkish).

Baytop T. (2000). Türkiye'de Bitkiler ile Tedavi. İstanbul Üniversitesi Eczacılık Fakültesi Yayınları (Treatment with Herbs in Turkey. Istanbul University Faculty of Pharmacy Publications)(3.), İstanbul, No.3255, Nobel Tıp Kitapevleri.

Boelens, M. H., Jimenez, R. (1992). The chemical composition of Spanish myrtle oils. Journal of Essential Oil Research, 4: 349–353

Farah, A., Afifi, A., Fechtal, M., Chhen, A., Satrani, B., Talbi, M. ve Chaouch, A. (2006) Fractional distillation effect on the chemical composition of Moroccan myrtle (*Myrtus communis* L.) essential oils Flavour and fragrance Journal 21: 351–354.

Genders, R. (1994). Scented Flora of the World. Robert Hale, London

Özcan M., Chalchat JC. (2005). Effect of different locations on the Chemical composition of essential oils of laurel (*Laurus nobilis* L.) leaves gowing wild in Turkey. Journal of Medicinal Food, Vol. 8, No 3; pp. 408-411.

Safak, I., Okan, T., (2004). Kekik, Defne ve Çam Fıstığının Üretimi ve Pazarlaması, Doğu Akdeniz Ormancılık Araştırma Müdürlüğü (Production and Marketing of Thyme, Laurel and Pine Nuts, Eastern Mediterranean Forestry Research Directorate). Doa Dergisi(Journal of Doa) Sayı: 10 Sayfa: 101-129.

Sümbül S., Ahmad AM., Asif M. & Akhtar M. (2011). *Myrtus communis* Linn. – A review. Indian Journal of Natural Products and Resources, Vol. 2, No 4; pp. 395-402.

CHEMICAL COMPOSITION OF YELLOW GENTIAN ROOT (*Gentianae radix*) FROM BOSNIA AND HERZEGOVINA

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Abstract

It has long been known that certain parts of yellow gentian have medicinal and edible usage value and that as such they find their application in pharmaceutical and food industries as well as in traditional medicine. Yellow gentian (*Gentiana lutea* subsp. *symphyandra* (Murb.) Hayek) is a protected (sub) species in Bosnia and Herzegovina because it is considered endangered. The main reasons for this are over-exploitation and relatively slow natural regeneration. Working on the project of its mass reproduction, we collected and analyzed samples of yellow gentian`s rhizome and root from the mountain Šator in B&H. We found that *Gentianae radix* contains about 66.9% water and about 33.1% dry matter. Total sugars are represented by 5.63%, fats with 3.14% and pectins with 1.04%. Among analyzed elements, the most prevalent was calcium with an average concentration of 3208.7 mg/kg and the least represented was chromium with an average concentration of 0.44 mg/kg. The main part of the mineral spectrum consists of three elements: Ca, K and Mg, with a share of 93.4%. In *Gentianae radix*, 25 fatty acids were detected, of which 12 were sturated and 13 unsaturated. The ratio of saturated to unsaturated fatty acids is 43.16 % : 56.84 %. The most common among saturated fatty acids is palmitic with a share of 26.52 %, while the most common unsaturated fatty acid is a mixture of oleic and elaidic fatty acids with a share of 31.99%. Regarding phytochemicals, *Gentianae radix* contains on average: total phenols – 3820.54 μgGAE/g, total flavonols – 568.22 μgQE/g, non-flavonoids – 4280.49 μgGAE/g and anthocyanins 710.71 μg/g.

Key words: *yellow gentian, rhizome and root, chemical composition, phytochemicals.*

Introduction

Taxon *Gentiana lutea* L. is distributed from the Iberian Peninsula to the mountains of Asia Minor. It consists of four subspecies: the type spp. *lutea*, spp. *symphiandra*, spp. *monserratii* and spp. *vardjanii* (Rossi, 2011). The type spp. *lutea* has a wide range stretching from the Cantabrian Mountains and the Pyrenees in the west to the Central Alps in the east (Stevanović et Jakovljević, 2014), going south to Gennargentu Massif on Sardinia (Chiappini et Angiolino, 1983; Gentili et al., 2013). The exclaves of this area are located on the Mt. Šar in Northern Macedonia, on the mountain massifs on the border of Albania and Greece and on the high mountains within Sterea Hellas Region of Central Greece (Hartvig, 1991; Matevski et Wraber, 2010). The subspecies *symphiandra* also has a wide range covering the Eastern Alps, the Balkan Peninsula, and the Carpathian Mountains, as well as the mountains of the North West Asia Minor. The other two subspecies have significantly narrower ranges. The subspecies *monserratii* inhabits the Central Pyrenees, while spp. *vardjanii* occupies the South East Alps. Yellow gentian grows on upper montane and subalpine belts, above 800-1000 m., where, as a distinct heliophyte, it occurs in the grasslands and mountain barren lands.

The root, *i.e*. rhizome, is used from the yellow gentian. *Gentianae radix* is a part of our and European traditional medicine and its use has been described in most of the world's pharmacopoeias. In folk medicine, it is used to improve appetite and stimulate digestion (Nastasijević, 2016). It is also used against coughs and diarrhea, and very often to strengthen the decrepit organism (Tucakov, 1984). It was previously used in veterinary medicine for stomach diseases and problems with digestion of domestic animals (Jakovljević, 1951). *Gentianae radix* is used in official medicine as well as in the pharmaceutical and food industries. It is used in lack of appetite, in the treatment of anorexia, atonic dyspepsia, gastrointestinal atony, especially in dyspepsia with anorexia (Tasić et al., 2009; Anon., 2014).

Due to its medicinal properties, gentian root has been collected in nature for thousands of years. Since it is a slow-growing species, by non-selective root extraction, the plant can be completely destroyed, so it takes a long time for the damaged populations to recover. Yellow gentian is considered an endangered species in our country and its collection from natural sites is out of the question (Anon., 2013). However, the affirmative results with its artificial cultivation (Bjelić, 2012; Cuena-Lombraña, 2015; Galambosi, 1996; Holobiuc et al. 2006, 2008; Kušar et Baričevič, 2006; Lamproye et al., 1987; Petrova et al., 2006, 2019; Radanović et al. 2005, 2007, 2008, 2013; Skrzypczak et al., 1993; Wesolowska, 1985; and other sources) have restored interest in this medicinal plant and created realistic preconditions for its wider exploitation in our region, especially in the area of its natural habitats along the Dinaric Alps. Bearing in mind that very modest sources dealing with the chemical composition of gentian root from B&H, from which its useful value derives, we analyzed the chemical elements, fatty acids and phytochemicals of *Gentianae radix* from the locality Šatorsko Lake on the Mt. Šator.

Material and method

Material

Yellow gentian is an herbaceous perennial with rhizomatous and branched roots. The stem is erect, unbranched, up to 1.5 m high, round, and hollow. The upper leaves are oblong, wide lanceolate to elliptic, oppositely arranged, with 5-7 parallel nerves. The ground leaves are gathered in a rosette. The flowers are large, golden yellow in color, grouped in the apex of the stem in compact pseudo whorls inflorescences (Fig. 1).

Figure 1: Yellow gentian in full bloom on the slopes of Mt. Jahorina (photo S. Ljubojevć)

Figure 2: Root and rhizomes of yellow gentian from Mt. Šator (photo S. Ljubojevć)

Gentian roots were extracted in October 2020 on Mt. Šator, after the flowering phenophase was completed (Fig. 2). The older root of the adult plant was selected, cleaned of soil and rotten parts. After digging, the heads of the rhizomes with buds were returned to the ground together with the excavated soil and covered with sod. Thus, the plant is preserved from destruction and a new harvest is provided in a few years. Otherwise, the root of the yellow gentian is more or less cylindrical, often times over 10 cm long, up to 5 cm thick. It is reddish-brown on the outside and dark yellow on the inside. It swells very much in water. Its taste is first discreetly sweet and then very bitter, smelling like figs.

According to the Red list of vascular flora of B&H (Anon., 2013), yellow gentian is distributed in the following localities: Mt. Šator, Mt. Cincar, Mt. Dinara, Mt. Treskavica, Mt. Prenj, Mt. Plasa, Mt. Čvrsnica, Mt. Velež, place Petrovac, place Vakuf, Mt. Veliki Cincar, village Prolog near Livno, Rosni Dolac – the ridge of Mt. Kamešnica, Mt. Vlašić, Mt. Bjelašnica, Mt. Vlahinja, Mt. Jahorina, Mt. Maglić, Mt. Volujak, Mt. Zelengora, Mt. Ivan, and has the status: endangered species (EN). Material for analysis was taken in the immediate vicinity of Šatorsko Lake (1448 m a.s.l.). This area is inhabited by dwarf mountain pine (*Pinus mugo* Turra) as the main edificator (Fig. 3). The lake is of glacial origin and one of the main natural beauties of this region. According to the ecological-vegetation regionalization of B&H (Stefanović et al, 1983.), Mt. Šator belongs to two areas. The north-exposed parts belong to the Area of Inland Dinarides (including research site), while the South-exposed parts of this mountain belong to the Area of Mediterranean Dinarides.

Figure 3: Research site next to the Šatorsko Lake – in the foreground is a community of dwarf mountain pine inhabits with yellow gentian (Photo: S. Ljubojević)

Method

Taxon determination was performed using two sources: *Flora Europae*, Vol. 3: *Diapensiaceae* to *Myoporaceae*, CXL *Gentiantaceae*, page 60 (Tutin et al., 1972) and Mountain plants, page 108 (Lakušić, 1982). Gentian root was excavated in mid-October 2020. Immediately after extraction, the root was cleaned and transferred to the laboratory. There it is washed in distilled water, peeled and dried. The collected material of fragmented underground organs of *Gentiana lutea* ssp. s*ymphyandra* complies with the European Pharmacopoeia (Anon., 2019).

The water content was determined by drying fresh tissue to constant mass at 105^{o}C (935.29, AOAC, 2016). Dry weight, total ash and total sugar were determined by the standard AOAC methods (Horwitz & AOAC, 2000). Fats content was determined by the Soxhlet method (920.39, AOAC, 2016). Total pectin substances were precipitated with ethanol. After the addition of carbazole, a red color appears which was measured spectrophotometrically at 535 nm. Total phenol content was determined by the modified Folin-Ciocalteu method (Wolfe et Liu, 2003). Gallic acid was used as a standard compound and the results were expressed as a phenol equivalent to the gallic acid (GAE), i.e. µg GAE/mg. The total flavonoids are determined by the method of Kumaran & Karunakaran (2007), and the total flavonols according to the method of Ordoñez *et al*. (2006). Quercetin was used as the standard compound, and the results were expressed as µgQcE/mg. Anthocyanins were determined by the spectrophotometrically modified "single" pH method (Sun, 2002). Fatty acid methyl esters of the tested samples were prepared by direct esterification with a saturated solution of methanol with KOH, according to Majors (2013). Obtained fatty acid methyl esters were separated in a gas chromatography from Perkin Elmer, model Clarus 680, equiped with flame ionization detector and a capillary column Elite-

WAX, 60 m long, with inner diameter 0.32 mm and film thickness 0.50μm. Initially, the column temperature was set at 60 ºC for 2 min, than raised to 200 ºC at a rate of 10 ºC/min; again, it was raised at 240 ºC at a rate of 5 ^{o}C/min and maintained at 240 ^{o}C for 30 min. Thus, total chromatographic run time was 54 min. The flow rate for the carrier (H_2), auxilary (N_2) and detector flame (H_2 and synthetic air) gases was 1.5 mL/min. The composition of fatty acid methyl esters and the resulting acids is shown as a percentage of individual fatty acids in the total amount of identified fatty acids (g/100 g of total fatty acids). All samples were studied three times and averaged. When determining the chemical elements, 2.5 ± 0.0001 grams of crushed sample was burned in a mixture of nitric and perchloric acid by volume (10 mL of concentrated nitric acid, 10 mL of diluted nitric acid [1: 1], 10 mL of concentrated perchloric acid) and evaporated to dryness. The dry matter was dissolved with 10 mL of diluted hydrochloric acid [1:3] (Trajković et al., 1983). The solution was then filtered into a 50.00 mL volumetric flask and the filtrate was made up with deionized water. ICP OES measurements were performed three times for each duplicate using Optima 8000 Optical Emission Spectrophotometer (Perkin Elmer, USA). The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin Elmer auto-sampler model 510. For the calibration of instrument, a CRM solution proposed by the Instrument Calibration standard 2 was used, with a minerals concentration of 100 mg/L (Pb, Cd, ... Perkin Elmer, USA). The obtained results are presented as the mean value from three repetitions.

Results and discussion

The results of the chemical analysis are shown in tables 1 & 2. Root of yellow gentian (*Gentiana lutea* subsp. *symphyandra* (Murb.) Hayek) from B&H contains about 66.93% water and about 33.07% dry matter. The most common group of compounds are sugars (5.63%), followed by fat (3.14%) and pectins (1.04%), while ash has only 0.9%. In this regard, Tucakov (1964) is one of the few authors to mention that gentian root also contains some fat, stating that their content can go up to 5%.

Among the 16 elements analyzed in this study, the most prevalent was calcium with an average concentration of 3208.79 mg/kg_{DW} and the least represented was chromium with an average concentration of 0.44 mg/kg_{DW} (Tab. 1). Cd, Fe, Mo and Pb were below the limit of detection in all samples, while As and Se were not detected at all. The main part of the mineral spectrum consists of three elements: Ca, K, and Mg, with a share of 93.5%, while all other detected elements have 6.5% of total composition. For comparison, elemental concentrations obtained from several locations along the Dinaric Arc are also summarized in Table 1. In the material originating from the Mt. Velebit in neighboring Croatia (Zeiner et al., 2015), there is a slightly different constellation of elements. Calcium is the most abundant element here as well, but it is four times more abundant than in our material from Mt. Šator. At the same time, the concentration of potassium is 10 times lower in the samples from Mt. Velebit compared to our material. There are significant differences in the concentrations of Al and Na. There is twice as much sodium and five times as much aluminum in the material from Mt. Velebit in relation to the material from the Mt. Šator. There are also significant differences in the content of Zn which is 7.5 times more in our material compared to the samples from Mt. Velebit. Comparing the elemental composition of gentian root from the Mt. Klekovača, Mt. Ocječenica and Mt. Vranica in B&H (Predić et al, 2012) and materials from the seven mountains of northern Montenegro (Balijagić et al, 2012), minor differences in the concentrations of individual elements are observed (Tab. 1). In relation to Mt. Šator and Mt. Velebit, the material from these localities is characterized by a significantly higher concentration of Fe and Mn. Concentrations of As, Cd and Pb in our and other observed samples do not exceed the permissible level established by WHO for medical raw plant materials (1.0, 0.3 and 10 mg / kg, respectively), (WHO, 1991, 2007).

Table 1: Elements in yellow gentian root from different localities along the Dinaric Arc

Element	Mt. Šator, B&H		Northern part of Mt. Velebit, Croatia(1)		Western and Central Dinarides in B&H(2)		Mountains in the north of Montenegro (3)	
	Unit	Content	Unit	Content	Unit	Content	Unit	Content
N	mg/kg$_{DW}$	N.A.	mg/kg$_{DW}$	N.A.	%$_{DW}$	0.94	%$_{DW}$	0.52
P		94.96		N.A.		0.07		0.18
K		1169.85		116		0.27		0.39
Ca		3208.79		12,350		1.14		N.A.
Mg		1047.98		1070		0.16		N.A.
Na		178.86		302	mg/kg$_{DW}$	N.A.	mg/kg$_{DW}$	N.A.
Al		34.68		155		N.A.		N.A.
As		N.D.		0.030		N.A.		N.A.
Cu		2.79		1.10		21.5		6.91
Co		N.A.		0,0083		N.A.		0.50
Fe		N.D.		25,7		517.5		352.86
Mn		3.47		2,02		34.33		25.66
Sr		N.A.		4.09		N.A.		N.A.
Zn		62.46		8.35		30.33		27.51
Cd		N.D.		0.11		N.A.		N.A.
Cr		0.44		N.D.		N.A.		N.A.

(1) Zeiner et al., 2015; (2) Predić et al., 2012; (3) Balijagić et al.,2012. N.A. – not analyzed, N.D. – analyzed but not detected.

In the root of the yellow gentian, excavated near Šatorsko Lake, 25 fatty acids were detected, of which 12 were saturated and 13 unsaturated (Tab. 2). The ratio of saturated to unsaturated fatty acids is 43.16% : 56.84%. The most common among saturated fatty acids is palmitic acid with a share of 26.52%, while the most common unsaturated fatty acid is a mixture of oleic and elaidic fatty acids with a share of 31.99%. Two more fatty acids are significantly present, stearic with 8,08 % and linelic with 14.81 %.

Root of the yellow gentian also contains chemical compounds from the category of phytochemicals, such as: phenols - 3820.54 μgGAE/g, flavonols - 568.22 μgQcE/g, non-flavonoids - 4280.49 μgGAE/g, anthocyanins - 710.71 μg/mg.

Table 2: Assortment of fatty acids in the root of the yellow gentian, excavated near Šatorsko Lake

Saturated fatty acids				Unsaturated fatty acids			
No	Common name	C:D	%	No	Common name	C:D	%
1	Butyric acid	4:0	0.82	1	Myristoleic acid	14:1	0.33
2	Caproic acid	6:0	0.58	2	Pentadecenoic acid	15:1	0.17
3	Capriylic acid	8:0	0.26	3	Palmitoleic acid	16:1	1.85
4	Capric acid	10:0	0.69	4	Heptadecenoic acid	17:1	0.96
5	Lauric acid	12:0	0.41	5,6	Oleic + Elaidic acid	18:1	31.99
6	Myristic acid	14:0	1.78	7	Linoleic acid	18:2	14.81
7	Pentadecylic acid	15:0	0.32	8	α-Linolenic acid	18:3	0.91
8	Palmitic acid	16:0	26.52	9	Eicosatrienoic acid	20:3	0.46

9	Margaric acid	17:0	0.50	10	Timnodoic acid	20:5	0.80
10	Stearic acid	18:0	8.08	11	Erucic acid	22:1	1.46
11	Arachidic acid	20:0	1.69	12, 13	Nervonic+ Docosahexaenoic acid	24:1/ 22.6	3.11
12	Lignoceric acid	24:0	1.51				
	Σ		43.16				56.84

Conclusions

The results of the research confirmed that yellow gentian is a valuable medicinal plant species. Gentian root, among other things, contains an interesting set of fatty acids, whose potential has yet to be expressed. At the same time, no harmful substances were found in the root of the gentian. By further improving plantation cultivation, the pharmaceutical and food industries could obtain significant quantities of high-value raw materials of wide use value.

References

Anonymus (2013): Red List of Flora of Bosnia and Herzegovina, Book 2 [Crvena lista flore Bosne i Hercegovine, Knjiga 2]. Federalno ministarstvo okoliša i turizma. 1-348

Anonymus (2014): *Gentianae radix*, Gentian Root. European Scientific Cooperative on Phytotherapy (ESCOP). 1-6

Anonymus (2019): European Pharmacopoeia, Tenth Edition. Council of Europe, Strasbourg. Volume I, II & III. 1-4370

AOAC (2016) : Official methods of analysis of AOAC International - 20th ed., Editor: Latimer W.G. Research BLVD, Rockville, Maryland. 1-3172

Balijagić J., Radanović D., Antić-Mladenović S., Crnobarac J., Bošković J., Zečević V., Dozet G., Jovančević M. (2012): Mineral content of autochthonous gentian (*Gentiana lutea* L.) root from natural habitats in northern parts of Montenegro. Proceedings of the 7th CMAPSEEC, 27-31 May, 2012, Subotica, Republic of Serbia. 255-262

Bjelić V. (2012): Guide to the cultivation of medicinal and aromatic plants in Bosnia and Herzegovina [Vodič za uzgoj ljekovitog i aromatičnog bilja u Bosni i Hercegovini]. Udruženje "GEA" – Centar za istraživanja i studije. 1-41

Chiappini M., Angiolino C. (1983): La *Gentiana lutea* L. in Sardegna. Morisia 5. 89-94

Cuena-Lombraña A. (2015): Integrated *in situ* and *ex situ* approach for conservation of *Gentiana lutea* L. subsp. *lutea*. Università degli Studi di Cagliari. 1-112

Galambosi B. (1996). Experiences of cultivation *Gentiana lutea* L. in Finland. In: Attidel Convegno Genziana e specie amaroaromatiche. Ricerche ed Applicazioni. Camerino, 8-10 giugno 1995. L'uomo e l'ambiente. 139-142

Gentili R., Ardenghi N.M.G., Armiraglio S., Bacchetta G., Bartolucci F., Cogoni D., Conti F., Fenu G., Fisogni A., Galloni M., Gigante D., Maneli F., Parolo G., Rossi M., Santangelo A., Selvaggi A., Wagensommer R.P. (2013): *Gentiana lutea* L. e relative sottospecie in Italia. Informatore Botanico Italiano 45. 115-193

Hartvig P. (1991): *Gentianaceae*. In: Strid A., Tan K. (eds) Mountain flora of Greece, Vol 2. Edinburgh University Press. 1-9

Holobiuc I., Blindu R. (2006). In vitro culture of the protected rare species *Gentiana lutea* L. for conservative purpose. Contributii Botanice Vol. XLII. 125-134

Holobiuc I., Blindu, R., Brezeanu A. (2008): Ex situ conservation in *Gentiana lutea* L. through somatic embryogenesis. Proceeding of the International Symposium "New Research in Biotechnology", USAMV Bucharest, Romania, 2008, 325-334

Horwitz W. (2000): Official methods of Analisys AOAC International, Vol. 1. 17th edition, AOAC International. 1-2200

Jakovljević J. S. (1951): Systematics of medicinal plants [Sistematika lekovitih biljaka]. Narodna knjiga, Beograd 1-339

Kumaran A., Karunakaran R. J. (2007): In vitro antioxidant activities of methanol extracts of *Phyllanthus* species from India. Food Science and Technology, Vol. 40 (2). 344-352

Kušar A., Baričevič D. (2006): Cultivation trials of yellow gentian (*Gentiana lutea* L. subsp. S*ymphyandra* Murb.) in west part of Slovenia. Acta Agriculturae Slovenica, 87, 213-22

Lakušić R. (1982): Mountain plants [Planinske biljke]. "Svjetlost", OOUR Zavod za udžbenike i nastavna sredstva, Sarajevo. 1-204

Lamproye A., Crevecoeur M., Kevers C., Gaspar Th. (1987): Multiplication vegetative in vitro de *Gentiana lutea* et de *Gentiana pneumonante*. Ned. Fac. Landbuoww Rijkuniv Gent. 52, 1255-1257.

Majors ER 2013: Sample preparation fundamentals for chromatography. Agilent Technologies, Inc.1-356

Matevski V., Wraber T. (2010): *Gentiana* L. In: Matevski V (ed) The flora of the Republic of Macedonia, Vol 2(1). Macedonian Academy of Sciences and Arts, Skopje. 31–40

Nastasijević J.B. (2016): Biological effects of Gentian root extracts (*Gentiana lutea*) – enzyme inhibition, antioxidative and antimicrobial activity. Doctoral Dissertation. University of Belgrade, Faculty of technology and metallurgy. 1-176

Ordoñez A.A.L., Gomez J.D., Vattuone M., Isla M.I. (2006): Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry, Vol. 97 (3). 452-458

Petrova M., Zagorska N., Tasheva K., Evstatieva L. (2006): In vitro propagation of *Gentiana lutea* L. Genetics and Breeding. Vol. 35. No. 1-2. 63-68

Petrova M.I., Zayova E.G., Dimitrova L.I., Geneva M.P., Miladinova-Georgieva, K.D. (2019): Micropropagation studies and antioxidant analysis of the endangered plants of Bulgarian yellow gentian (*Gentiana lutea* L.). Acta Scientiarum Polonorum, Hortorum Cultus 18(3). 71–78

Predić T., Pećanac D., Radanović B., Docić-Kojadinović T., Radanović D. (2012): Root and leaf mineral content of wild growing yellow gentian (*Gentiana lutea* L.) from natural habitats in western part of Bosnia and Herzegovina. Proceedings of the 7th CMAPSEEC, 27-31 May, 2012, Subotica, Republic of Serbia. 68-73

Radanović D., Stepanović B., Nastovski T. (2005): Some experiences in nursery plants production of Yellow gentian (*Gentiana lutea* L.) with an accent on its seed dormancy. Contemporary Agriculture, 54. 474-481

Radanovic D., Markovic T., Jankovic T. (2007): Morphological and chemical parameters of importance for cultivation of *Gentiana lutea* L. in mountain region of Serbia. 1st Int. Sci. Conf. on Medicinal, Aromatic and Spice Plants. Nitra, Slovakia. Proceedings. 28-32

Radanovic D., Markovic T., Jankovic T., Pljevljakusic D. (2008): Yield and quality of Yellow gentian (*Gentiana lutea* L.) root grown in mountaneous region of Serbia. V Conference on Medicinal and Aromatic Plants of Southeast European Countries (V CMAPSEEC), Brno, 2-5.9.2008. Czech Republic. Book of abstracts, 119.

Radanović D., Marković T., Antić Mladenović S. (2013): Production of Yellow Gentian (*Gentiana lutea* L.) Nursery Plants Suitable for Transplanting and Cultivation under Dry Farming Conditions in Mountain Region of Serbia. Ratarstvo i Povrtarstvo, Institute of Field and Vegetable Crops, Novi Sad, 50:3. 13-21

Rossi M. (2011): Taxonomy, phylogeny and reproductive ecology of *Gentiana lutea* L. Ph.D. in biodiversity and evolution, Faculty of mathematical, physical and natural sciences, Department of Experimental Evolutionary Biology, University of Bologna. 1-125

Skrzypczak L., Wesołowska M., Skrzypczak E. (1993): *Gentiana* species: in vitro culture, regeneration and production of secoiridoid glucosides. Biotechnology in agriculture and forestry. Bajaj YPS [ed.], vol. 21. Medicinal and aromatic plants IV, Springer. 172-186

Stefanović V., Beus V., Burlica Č., Dizdarević H., Vukorep, I. (1983): Ecological-vegetation regionalization of Bosnia and Herzegovina [Ekološko-vegetacijska rejonizacija Bosne i Hercegovine] Šumarski fakultet u Sarajevu, Posebna izdanja 17. 1–49

Stevanović V., Jakovljević K. (2014): Distribution, ecology, and some taxonomical notes of the genera *Gentiana* L. and *Gentianella* Moench (*Gentianaceae*) in the Balkans. In: The *Gentianaceae* - Volume 1: Characterization and Ecology. Springer. 1-329

Sun J., Chu Y.F., Wu X., Liu R.H. (2002): Antioxidant and antiproliferative activities of common fruits, Journal of Agricultural and Food Chemistry, 50. 7449–7454

Tasić S., Šavkin K., Menković N. (2009): A guide through the world of medicinal herbs [Vodić kroz svet lekovitog bilja]. Alexandria, Beograd. 1-202

Tucakov J. (1964): Pharmacognosy [Farmakognozija]. Zavod za izdavanje udžbenika Socijalističke Republike Srbije. 1-523

Tucakov J. (1984): Herbal treatment, Phytotherapy [Lečenje biljem, Fitoterapija]. Izdavačka radna organizacija "Rad". Beograd. 1-717

Trajković J., Baras J., Mirić M., Šiler S. (1983): Food analyzes [Analize životnih namirnica]. Faculty of Technology and Metallurgy, University of Belgrade. 1-786

Tutin T.G., Heywood V., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (1972): *Flora Europae,* Vol 3 *Diapensiacae* to *Myoporaceae*. Cambridge University Press. 1-370

Wesolowska M., Skrypczak L., Dudzinska R. (1985): Genus *Gentiana lutea* in *in-vitro* culture. [Rodzaj Gentiana lutea kulturze in vitro]. Acta Poloniae Pharmaceutica, 42. 79-83

WHO (1991): Guidelines for Assessment to Herbal Medicines. Geneva. 1-4

WHO (2007): WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva. 1-105

Wolfe K., Wu X., Liu R.H. (2003): Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry, Vol. 51 (3). 609-614

Zeiner M., Juranović Cindrić I., Požgaj M., Pirkl R., Šilić T., Stingeder G. (2015): Influence of soil composition on the major, minor and trace metal content of Velebit biomedic plants. Journal of Pharmaceutical and Biomedical Analysis. 153-161

CHANGES IN FRUIT SIZE AND BIOCHEMICAL COMPOSITION OF *VIBURNUM OPULUS* FRUITS DURING RIPENING

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Abstract

Fruits of *Viburnum opulus* are very popular not only in folk medicine but they are also used for production of food and medicinal products. Biologically active compounds of these fruits could help in heart diseases and colds, digestive disorders and internal bleeding. In this study, the dynamic of fruit biochemical compounds during ripening and fruit size changes were determined. It was found that the average weight of one fruit increases no longer after the stage of technical maturity. The average ascorbic acid content increased during fruit ripening. The largest amounts of ascorbic acid were accumulated at the biological maturation in fruits of *V. opulus* var. *sargentii* and the clone P1, respectively, 58.4 mg/100g and 56.0 mg/100 g. Quantity of ascorbic acid ranged from 27.0 mg/100 g to 44.3 mg/100 g on average. At the stage of biological maturation, sugars and soluble solids were detected in large quantities also. The soluble solids content must be assessed with a refractometer to determine the exact harvest time. However, the highest titratable acidity was determined in unripe fruits (ripening stage I). Summarizing the performed biochemical studies of *V. opulus* fruits, it can be stated that amounts of biochemical compounds varied greatly during ripening. In order to determine precisely the most appropriate harvest time for *V. opulus* fruits, these characteristics need to be assessed. It is important to define when fruits accumulate large amounts of biologically active substances and acquires good taste also.

Keywords: *cultivar, biochemical compound, fruit, maturation.*

Introduction

Fruits containing high amounts of bioactive compounds are the focus of different studies. The growing interest in less known plants and their fruits has been observed in recent years (Kraujalytė et al., 2013). The species *Viburnum opulus* L. (European cranberry bush) is widespread in eastern, northeastern, western, and central Europe and in western and eastern Siberia. Different phytochemicals were identified as important components in fruits and seeds, for example, ascorbic acid (Rop et al., 2010), α-tocopherol, linoleic and oleic fatty acids (Yang et al., 2011), various polyphenolics, including phenolic acids (Velioglu et al., 2006), flavonoids (Erdogan-Orhan et al., 2011) and anthocyanins (Velioglu et al., 2006; Česonienė et al., 2009). As reported by Velioglu et al. (2006) and Kraujalytė et al. (2013), *V. opulus* fruits are a good source of chlorogenic acid (0.54-6.93 mg/mL of juice) which is characteristic by anti-inflammatory, antiviral, antimicrobial, antimutagenic and anticarcinogenic activities. Traditionally bark, leaves, flowers and fruits of *V opulus* has been used for food and folk medicine in European and Asian countries (Česonienė et al., 2009). Fruits of *V. opulus* are also used for food in Scandinavia, while in Canada they are used as surrogates of cranberries (*Vaccinium macrocarpon* Aiton)

(Velioglu et al., 2006). Studies of frozen fruits have shown different amounts of phenolic acids, anthocyanins (Altun and Yilmaz, 2007; Deineka et al., 2005), organic acids (Çam and et al., 2007), and carotenoids (Česonienė et al., 2008). The antimicrobial properties of these fruits have also been studied and it has been found that their ethanolic extracts inhibit the growth of some pathogenic bacteria (Sagdic et al., 2006). As Kraujalytė et. al (2013) has reported, the phytochemical composition of *V. opulus* fruits is complex and depends on genotype. So, the breeding of new cultivars is very promising. Cultivars of *V. opulus* were usually developed by selecting plants according to the fruit size, yield, colour of leaves and fruits. Russian scientists have achieved good results and have nurture cultivars distinguish by a better taste of fruits and big yield (Česonienė et al., 2009). To date, more detailed research has been conducted in Ukraine also (Gavrilin et al., 2007). The aim of the study was to determine the dynamic of fruit weight and chemical composition of *V. opulus* during the ripening process.

Materials and methods

Fruit samples were collected at the *Viburnum* L. genus collection in Botanical Garden of Vytautas Magnus University in Lithuania. Fruit samples were collected during four stages of fruit ripening: stage I - fruits begin to colour; stage II – fruits are coloured but not yet softened (technical maturity); stage III – fruits are coloured and begin to soften (consumption maturity); ripening stage IV – overripe fruits (biological maturity). Samples were collected from different parts of the plant (from well-lit and middle of the crown places), each sample weighing 0.6-0.7 kg. The average weight of a fruit was measured by using an analytical balance with a sensitivity of 0.01 g (ISHIDA company, Japan, model DJ-150E). The maximum capacity of the balance was 150 g. For each genotype, 50 fruits in three replicates were estimated. Total sugar content was determined by the Bertran method, reducing sugars - by the inversion method, and sucrose content - by the pre- and post-inversion methods (AOAC[a], 1990). Ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol sodium salt solution and using chloroform (for intense coloured extracts), titratable acidity by titration with 0.1 N NaOH solution, converted to citric acid content (AOAC[b], 1990). Dry matter content was determined gravimetrically, the berries were dried at 105 °C to constant weight (Food ..., 1986). Total soluble solids were determined by digital refractometer (ATAGO PR-32, Atago, Japan).
The data were analysed using SPSS Statistics version 24.0. Duncan's multiple range test was used to assess the data for significant differences. A p-value of 0.05 was set as the limit for significant differences.

Results and Discussion

V. opulus fruit is a juicy stone fruit with a round and flat stone. During the processing of these fruits, the pulp should be separated so it is important to assess not only the weight of fruits but also what a part of fruit weight forms the pulp. It was found that during the first ripening stage, the average weight of one fruit was 46.1 g (Table 1). During the other two ripening stages, fruit weight increased but no statistically significant differences were found between ripening stages II, III, and IV. During the first three ripening stages, the fruit pulp did not change however the largest proportion of a pulp was detected at the stage IV - 73.5%.

Table 1. Changes of *Viburnum opulus* fruit weight and pulp proportion during ripening

Maturity stage	Average fruit weight, g	Fruit pulp, %
I	46,1 a	64,8 a
II	58,5 bc	65,5 a
III	60,3 bc	65,0 a
IV	61,2 c	73,5 b

Means followed by the same letter in the column are not significantly different at $\alpha \leq 0.05$

Unripe fruits are characteristic by an unpleasant bitter taste and are not suitable for either fresh consumption or processing. It is therefore important to determine the optimal harvesting time to ensure an adequate amount of biologically active compounds.

Fruits of *V. opulus* accumulated on average 6.22% of total sugars at the first stage of ripening. The total sugar content increased significantly in fruits at the ripening stage II (technical maturity) and the average amount was 7.98%. Later, overall increase in sugar content was not substantially higher compared to technical maturity stage (Figure 1).

The average content of reducing sugars in fruits was 5.56% at the ripening stage I and the highest content was found at technical maturity (ripening stage II) - 7.46%. The amount of reducing sugars in fruits did not changed significantly during ripening stages III and IV. Sucrose was found in small amounts that varied slightly during the ripening stages (Figure 1).

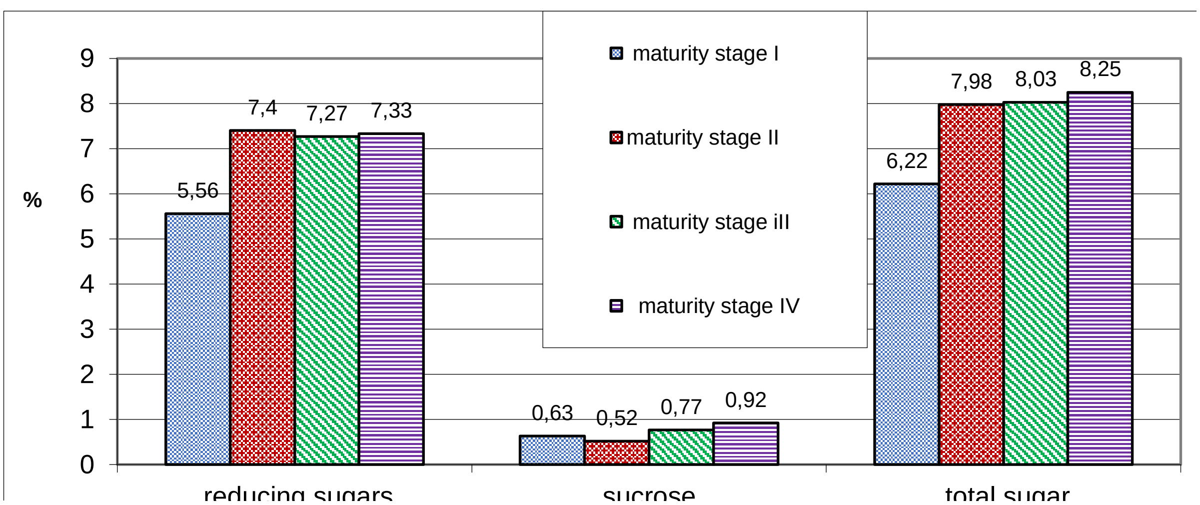

Figure 1. Variation in average amounts of total sugars, reducing sugars, and sucrose of *V. opulus* fruits during ripening, $p \leq 0.05$.

The dynamic of the average dry matter content was not significant during fruit ripening and varied from 13.3% (ripening stage I) to 13.5% (ripening stages III and IV) (Figure 2).

The highest titratable acidity was found in the fruits of ripening stage I and significant differences were not detected during ripening stages II-IV. At the first ripening stage, the total soluble solids content in fruits of *V. opulus* was 8.0%. During ripening, the average soluble solids content of the fruit increased to 12.2% (ripening stage IV).

An important property of fruits is their taste which depends on the ratio of sugars and acids, i.e., sweetness index. The average sweetness index of the tested samples was 3.76 at the ripening stage I. It increased to 6.84 during ripening stage II but did not change significantly with further ripening. The sweetness index was 6.36 and 6.56 at the maturity stages III and IV, respectively.

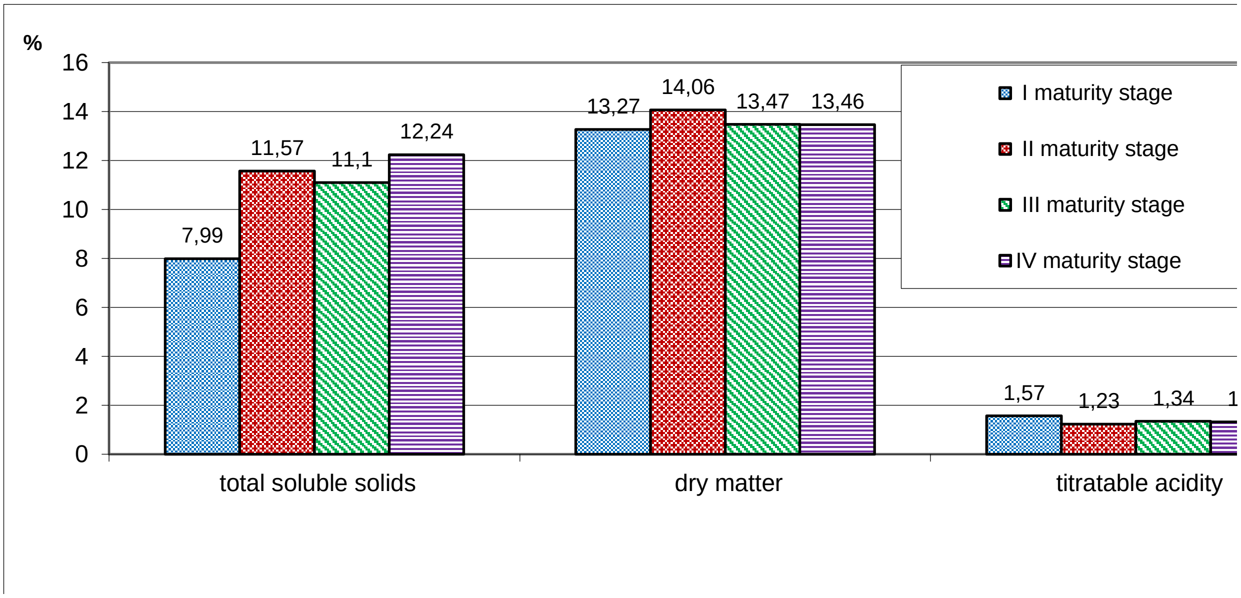

Figure 2. Variation in average amounts of total soluble solids, dry matter and titratable acidity of *V. opulus* fruits during ripening, p≤0.05.

During the I and II ripening stages, fruits of *V. opulus* accumulated the average ascorbic acid content from 18.3 to 19.6 mg /100g (Figure 3).

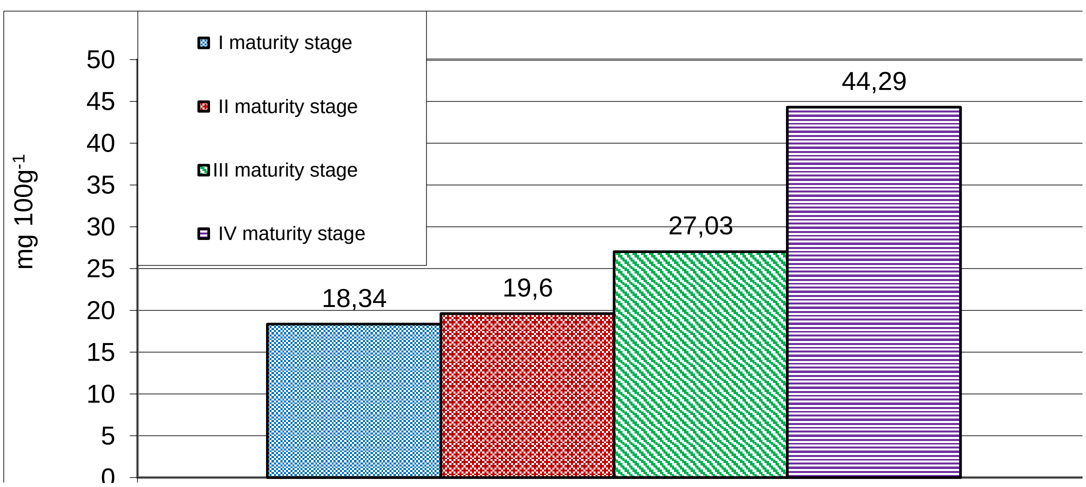

Figure 3. Variation in average ascorbic acid amounts of *V. opulus* fruits during ripening, p0≤.05.

The average ascorbic acid content increased significantly at the ripening stage III. Ascorbic acid was found on average of 27.0 mg/100 g in ripe fruits (ripening stage III) and 44.3 mg/100 g at the stage IV (Fig. 4).

Summarizing the investigations on *V. opulus*, it can be stated that sugars, dry matter, total soluble solids and ascorbic acid amounts varied during different maturity stages. Interest in the chemical composition of *V. opulus* fruit is stimulated by the fact that other biologically active compounds found in them are strong antioxidants (Kraujalytė et al., 2013; Rop et al., 2010). In the food industry, the pulp of fruits is commonly used. Our study has shown that pulp forms more than 60% of the total weight of ripe fruit. However, according to some authors (Çam et al., 2007), *V. opulus* seeds are also a source of valuable biologically active compounds, so the seeds could be used in the pharmaceutical industry for fruit processing.

Conclusions

V. opulus fruits accumulated various amounts of sugars, acids, dry matter, total soluble solids, and ascorbic acid. The content of different biochemical compounds varied during fruit ripening. The largest amount of total reducing sugars and sucrose were detected starting at the technical maturity stage. Ascorbic acid levels increased gradually and the highest levels were found in fruits of biological maturity. Fruit weight changed more slowly with the beginning of technical maturity, but the largest proportion of fruit pulp was detected at the stage of biological maturity.

References

Altun M. L., Yilmaz B. S. (2007). HPLC method for the analysis of salicin and chlorogenic acid from *Viburnum opulus* and *V. lantana*. Chemistry of Natural Compounds, Vol. 43, pp. 205–207.

AOAC[a]. (1990). Sucrose in fruits and fruit products. In: K. Helrich (ed.), Official Methods of Analysis, 15th ed., AOC Inc., Arlington, VA.

AOAC[b]. (1990). Vitamin C (ascorbic acid) in vitamin preparations and juices. In: K. Helrich (ed.), Official Methods of Analysis, 15th ed., AOC Inc., Arlington, VA.

Çam M., Hişl Y., Kuscu A. (2007). Organic acid, phenolic content, and antioxidant capacity of fruit flesh and seed of *Viburnum opulus*. Chemistry of Natural Compounds, Vol. 43, pp. 460–461.

Česonienė L., Daubaras R., Viškelis P. (2009). Biochemical and agro-biological diversity of *Viburnum opulus* genotypes. Central European Journal of Biology, Vol. 5(6), pp. 864–871.

Česonienė L., Daubaras R., Viškelis P. (2008). Evaluation of productivity and biochemical components in fruit of different *Viburnum* accessions. Biologija, Vol. 54(4), pp. 93–96.

Deineka V. I., Sorokopudov V. N., Deineka I. A., Shaposhnik E. I., Kol'tsov S. V. (2005). Anthocyanins from fruit of some plants of the *Caprifoliaceae* family. Chemistry of Natural Compounds, Vol. 41 (2), pp. 162–164.

Erdogan-Orhan I., Altun M. L., Sever-Yilmaz B.,& Saltan, G. (2011). Anti-acetylcholinesterase and antioxidant assets of the major components (salicin, amentoflavone and chlorogenic acid) and the extracts of *Viburnum opulus* and *Viburnum lantana* and their total phenol and flavonoid contents. Journal of Medicinal Food, Vol. 14, pp. 434-440.

Food analysis: general techniques, additives, contaminants, and composition. (1986). Rome, FAO.

Kraujalytė V, Venskutonis P. R, Pukalska A, Česonienė L., Daubaras R. (2013). Antioxidant properties and polyphenolic compositions of fruits from different European cranberrybush (*Viburnum opulus* L.) genotypes. Food Chemistry, Vol. 141, pp. 3695-3702.

Rop O., Reznicek V., Valsikova M., Jurikova T., Mlcek J., Kramarova D. (2010). Antioxidant properties of European cranberrybush fruit (*Viburnum opulus* var. *edule*). Molecules, Vol. 15, pp. 4467–4477.

Sagdic O., Aksoy A., Ozkan G. (2006). Evaluation of the antibacterial and antioxidant potentials of cranberry (gilaburu, *Viburnum opulus* L.) fruit extract. Acta Alimentaria, Vol. 35(4), pp. 487–492.

Velioglu I. S., Ekici L., Poyrazoglu E. S. (2006). Phenolic composition of European cranberry bush (*Viburnum opulus* L.) and astringency removal of its commercial juice. International Journal of Food Science, Vol. 41, pp. 1011–1015.

Yang B., Ahotup M., Määttä P., Kallio, H. (2011). Composition and antioxidative activities of supercritical CO2-extracted oils from seeds and soft parts of northern berries. Food Research International, Vol. 44, pp. 2009-2017.

RESPONSE OF LIFE FORMS OF UNDERSTORY PLANTS TO CLEAR-CUT LOGGING

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Abstract

Anthropogenic disturbances of the boreal forest ecosystems are defined as important ecological processes with significant long-term influence on biogeochemical cycles and vegetation. The development of understory plants immediately after clear-cut logging was studied in Lithuanian boreal Scots pine forests. Experiments were carried out in *Vacciniosa* forest types. The mature forest stands and the clear-cutted areas were compared. We have determined the different response of Ericaceae shrubs *Vaccinium vitis-idaea, Vaccinium myrtillus* and *Calluna vulgaris* to changed environmental conditions after clear-cuttings. *Lycopodium annotinum* and *Goodyera repens* were defined as the most sensitive because small populations of these species disappeared in the cutted areas. Juvenile populations of *L. annotinum* most likely were destroyed after soil preparation for new forest planting. Mosses were damaged exceptionally strong, some species disappeared in the clear-cutted areas as well. Considerable decline of dwarf shrub layer confirmed the negative reaction of this dominated understory life form however, the dynamic of different vascular plants demonstrated species-specific response to changing environmental conditions. Because of rapid changes of light as well as damage of rhizomes by soil disturbances, the values of mean cover of the studied Ericaceae species decreased. Spread of *C. vulgaris* renewed through seedlings and the number of new annual and perennial light-demanding and nitrophilic herbs species was significant in both forest types in two years after cuttings. This study corroborated the importance of evaluation of understory plants before cuttings and will promote the implementation of protection of sensitive species and non-wood forest resources.

Keywords: *clear-cutting, life form, understory plants, soil*

Introduction

Deforestation and logging have a very strong impact on forest soil and cause significant changes in the understory vegetation. After clear-cutting, intensive soil preparation and subsequent planting of saplings affect forest biodiversity as these activities significantly disrupt the natural dynamic of forest ecosystems (Bergstedt et al., 2008; Paillet et al., 2010). Clear-cuttings destroy the natural forest structure, plant species composition, distribution and abundance of shrubs and herbaceous plants. Understory plants are particularly drastically affected by clear-cutting, where various felling waste was collected. Vanha-Majamaa et al. (2017) conducted investigations in spruce forests and determined that vascular plant species suffer less and recover better compared to moss species. The results of other studies confirmed that the response of understory plants to the removal of trees from the forest ecosystem depends on the characteristics of a particular species, and some authors also note the different response of plant life forms to clear-cutting (Tonteri et al., 2016). The results presented in the scientific literature confirm that invasive plant species can also enter forest ecosystems after major changes (Gilliam, 2007). Meanwhile, studies

of mature forests demonstrated the resistance of forest communities to invasive species even when there are deforested areas nearby where invasive plants are already recorded (Luken, 2003; McCarthy, 2003). Studies of the primary response of forest plants to clear-cutting can explain the characteristics of species resistance to drastic changes in the environment and justify the restoration of forest ecosystems. Vanha-Majamaa et al. (2017) reported that the state of understory plant species should be determined immediately after cutting. On the other hand, long-lasting assessments are needed to determine the ability of these species to rapidly restoration of population size. Research on a development of herbaceous plants and shrubs in temperate forest ecosystems is essential because the soil cover species make up about 90% of all plant species and are very significant in forest soil nutrient metabolism (Gilliam, 2007).
The aim of these investigations was to determine the changes of understory vascular plant species in Scots pine *Pinus sylvestris* L. forests due to changes in environmental conditions after clear-cutting.

Materials and methods

Ten Scots pine forest plots were selected in Southern Lithuania. According to the nearest meteorological station, the average annual temperature in this region is 6.8 °C, with an average annual rainfall of 700 mm. The average temperature in July is 17.9 °C and the average temperature in January is -3.7 °C. The average age of mature Scots pine stands ranged from 120 to 130 years in the research plots. The stand volume was 330 m^3/ha, the average diameter of trees was 31 cm. The size of selected plots ranged from 2.0 to 2.6 ha. Each plot was divided into two parts. One part of the study plot was cleared, with tree stems and big branches removing in 2015. Our study was implemented on cleared subplots and compared with control subplots in intact (mature) forest sites. The research was conducted in 2016 and 2017. Based on the classification of Lithuanian forest types, plots of mature stands were selected, taking into account the predominant plant species (Navasaitis et al., 2003). The plots of *Pinetum vaccinio-myrtillosum (PVM) and Pinetum vacciniosum (PV)* forest types are located in very infertile soils where the humus fraction is up to 2%, the thickness of the forest floor is on average 2-4 cm. Lingonberry *Vaccinium vitis-idaea* L., bilberry *V. myrtillus* L., common heather *Calluna vulgaris* L. (Hull) are the predominant understory vascular plant species. The total coverage of this species typically ranges from 10% to 16%. Mosses are dominant in the understory vegetation layer covering, on average, from 70% to 85%. The most abundant moss species are *Pleurozium schreberi* (Willd. Ex Brid.) Mitt. and *Dicranum polysetum* Sw. The average cover of understory plant species was determined in the cutting site and mature forest. The cover was assessed in transects using a 1 m^2 frame (with a net with a 1 dm^2 square mesh) with 30 replications. The cover of each plant species was determined by a visual estimation as percent cover. In analysing the response of plants, species abundance was discussed in terms of ecological indicator values (Ellenberg, et al., 1991). During the study, changes in plant life forms (deciduous and evergreen shrubs, annual and perennial herbaceous plants) were also identified.
The data were analysed using SPSS Statistics version 24.0. Duncan's multiple range test was used to assess the data for significant differences. A p-value of 0.05 was set as the limit for significant differences.

Results and Discussion

One year after clear-cutting, the cover of predominant *Ericaceae* Juss. shrubs decreased from 11.2% to 3.0%, i.e., almost 4 times in PVM forest type and from 41% to 13.7% in PVM forest type (Figure 1). Already in the second year after clear-cutting, the total cover of these species increased to 5.7% in PV forests.

The response of *Ericaceae* plants to changes in environmental conditions after clear-cutting depended on properties of each species. A stronger negative effect of clear-cuttings was observed on deciduous *V. myrtillus* compared to evergreen *V. vitis-idaea* and *C. vulgaris* shrubs (Table 1). In the second year after felling, the cover of *V. myrtillus* did not reach 1% at the felling sites. The cover of *V. vitis-idaea* decreased from 16.3% (mature forest) to 1.2% in 2016 but already a significant increase of lingonberry cover to 8.3% was observed in 2017. An increase in the cover of *C. vulgaris* was also recorded in the second year after felling i.e., in 2017. Such changes can be

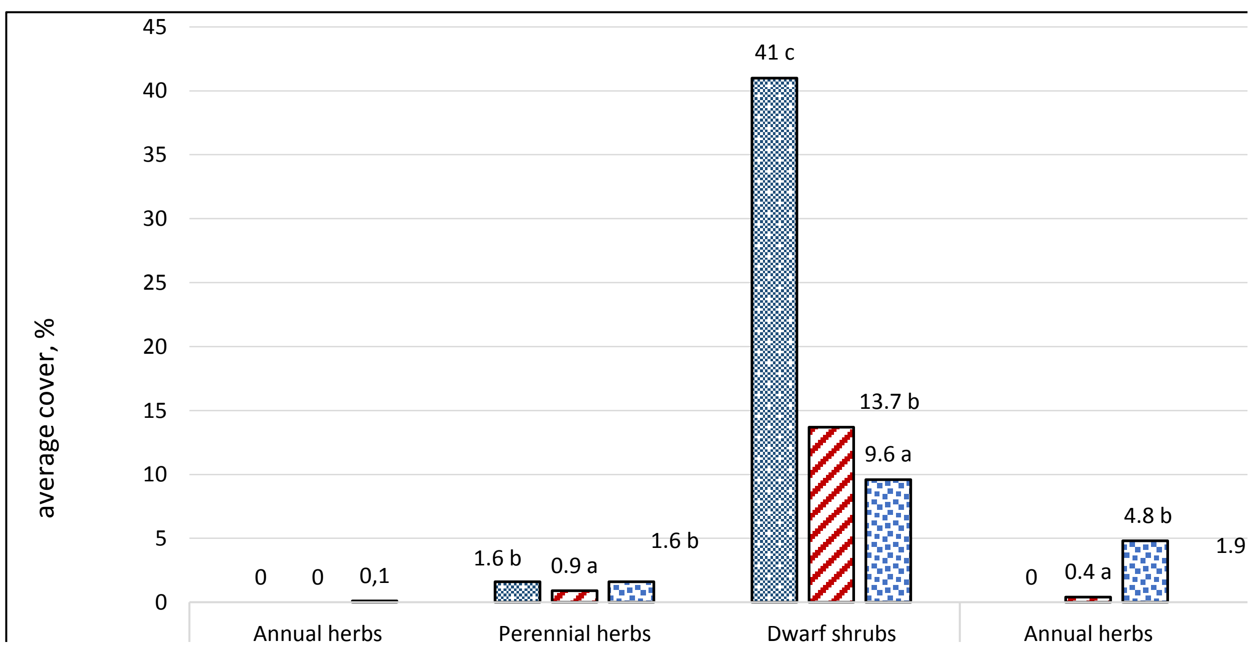

Figure 1. Dynamic of cover of herbaceous plants and shrubs in Scots pine forests after clear-cuttings, p≤0.05. PVM -*Pinetum vaccinio-myrtillosum*, PV-*Pinetum vacciniosum*

explained by the fact that common heather seedlings appeared in the felling site because the indicator value of this species for light reaches as high as 8 (Table 1).

The species diversity of perennial herbaceous plants in mature Scots pine forests was low: a total of six species were recorded in forest plots, with an average cover of 1.9%. In mature forest stands, the cover of perennial herbaceous plants was not significant. This can be explained by the need for light of these species, as their indicator values for light were quite high: from 5 (*Solidago virgaaurea* L.) to 8 (*Lycopodium clavatum* L.). As *Luzula pillosa* (L.) Willd. the indicator value for light is 2, this species was not recorded at the felling site. After felling, new species of perennial herbaceous plants were found, which were not detected in the mature forest: *Epilobium angustifolium* L., *Veronica officinalis* L., and *Rumex acetosella* L. Thus, the prevalence of *R. acetosella* in the first year after felling was low (projection cover was less than 1%), and already in the second year the projection cover was as high as 9.9% (Table 1).

Table 1. Cover and indicator values of understory vascular plant species in mature Scots pine forests and clear-cutting sites

Species	Cover			Ecological indicator values*	
	Mature forest	Clear-cutting, 2016	Clear-cutting, 2017	L	N
Vaccinium myrtillus	6,5	3,3	+	5	3
V. vitis-idaea	16,3	1,2	8,3	5	1
Calluna vulgaris	2,4	+	2,6	8	1
Festuca ovina	1,8	+	1,7	7	1
Luzula pillosa	+			2	4
Arctostaphylos uva-ursi	+			6	2
Lycopodium clavatum	+	+	+	8	2
Corynephorus canescens		+	+	8	2
Pilosella officinarum	+		+	7	2
Spergula arvensis		+	+	7	5
Senecio vulgaris		+	+	7	8
Solidago virgaurea	+	+		5	4
Epilobium. angustifolium			+	8	6
Veronica officinalis		+	+	6	4
Rumex acetosella		+	9,9	8	2
Carex caryophyllea			+	8	2

+ - cover ≤ 1
L – *ecological indicator value for light,* N – *ecological indicator value for soil fertility, according Ellenberg et al., 1991*

In the second year after clear-cutting, annual herbaceous plants also appear, with a cover of 4.8% in PV (Figure 1, Table 1). Even the light-demanding nitrophilous species *Senecio vulgaris* L. has been recorded, with an indicator value of 7 for light and an indicator value of 8 for soil fertility. Other authors have indicated also that the adaptability plants is particularly influenced by their need for lighting and soil moisture (Heinrichs and Schmidt, 2009; Tonteri et al., 2016). The response to fast changes in the environment depends not only on the characteristics of each species, but also on the type of forest. It was found that lingonberry and heather populations were more resistant in Scots pine forests compared to bilberry (Česonienė et al., 2018). Herbaceous

plants recorded in the felling sites are characterized by a high demand for light and a high amount of nutrients in the soil (Table 1).
Similar results were presented in studies by other authors (Atlegrim and Sjöberg 1996; Tonteri et al., 2016). Summarizing the results of the research, it can be stated that new anemochory species *R. acetosella*, *E. angustifolium* and *S. vulgaris*, were particularly widespread. As Pykälä (2004) have presented, the rapid spread of such species in degraded forest ecosystems is driven by the presence of long-term seed banks and air flow. Intensive regeneration of common heather population could also be influenced by species-specific characteristics i.e., ambophilous pollination and anemochory seed distribution (Česonienė et al., 2018). *L. clavatum* was found to have survived in the first and second years after clear-cutting but this species was recorded only in mechanically undamaged areas of the felling site. According to Rimgailė-Voicik and Naujalis (2016), this species is particularly sensitive to changes in the forest ecosystem. Thus, if adult individuals are destroyed, juvenile populations can only occur on forest plots that are free of significant damage. *L. clavatum* is estimated as a light-demanding species because according to Ellenberg et al. (1991), its indicator value for light is 8.

Conclusions

The cover of *Ericaceae* shrubs predominating in Scots pine forests begins to increase already in the second year after clear-cutting due to the significant spread in the common heather populations. Rapid regeneration of lingonberry shrubs has also been observed however, bilberry populations were decreasing in the second year. Comparing mature forest (control) and one- or two-year-old felling sites, annual herbaceous plants were recorded only after clear-cuttings. It was found that light-preferring and nitrophilous species appeared in the first year after felling. Clear-cuttings promoted the rapid spread of perennial and annual herbaceous plant species that are not typical of Scots pine forest ecosystems and are characterized by anemochorous seed dispersal.

References

Atlegrim O., Sjöberg K. (1996). Response of bilberry (*Vaccinium myrtillus*) to clear-cutting and single-tree selection harvest in uneven-aged boreal Picea abies forests. Forest Ecology and Management, Vol.86, pp. 39-50.

Bergstedt J., Hagner M., Milberg P. (2008). Effects on vegetation composition of a modified forest harvesting and propagation method compared with clear-cutting, scarification and planting. Applied Vegetation Science, Vol. 1, pp. 159-168.

Česonienė L., Daubaras R., Kaškonas P., Kaškonienė V., Maruška A.S., Tiso N., Zych M. (2018). Effects of clear-cut logging on dynamic of understory vascular plants and pollinators in Scots pine-dominated forests in Lithuania. Turkish Journal of Agriculture and Forestry, Vol. 42, pp. 433-443.

Ellenberg H., Weber H., Düll R., Wirth V., Werner W., Paulissen D. (1991). Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica, Vol 18, pp. 1-248.

Gilliam F.S. (2007). The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. BioScience, Vol.57, pp. 845-856.

Heinrichs S., Schmidt W. (2009). Short-term effects on selection and clear cutting on the shrub and herb layer vegetation during the conversion of even-aged Norway spruce stands into mixed stands. Forest Ecology and Management, Vol. 258 (5), pp. 667-678.

Luken J.O. (2003). Invasions of forests of the eastern United States. In: Gilliam FS, Roberts MR, editors. The Herbaceous Layer in Forests of Eastern North America. New York:Oxford University Press, 2003, pp. 283-301.

McCarthy B.C. (2003). The herbaceous layer of eastern old-growth deciduous forests. In: Gilliam FS, Roberts MR, editors. The Herbaceous Layer in Forests of Eastern North America. New York: Oxford University Press, pp. 163-176.

Navasaitis M., Ozolinčius R., Smaliukas D., Balevičienė J. (2003). Dendroflora of Lithuania, Kaunas, Lututė (In Lithuanian).

Paillet Y., Berges L., Hjältén J., Ódor P., Avon C., Bernhardt-Römermann M., Bijlsma R.-J., De Bruyn L., Fuhr M., Grandin U., et al. (2010). Biodiversity differences between managed and unmanaged forests: meta-analysis of species richness in Europe. Conservation Biology, Vol. 24, pp. 101-112.

Pykälä J. (2004). Immediate increase in plant richness after clear-cutting of boreal herb-rich forests. Applied Vegetation Science, Vol. 7(1), pp. 29-34.

Rimgailė-Voicik R., Naujalis J. R. (2016). Presence of juvenile club moss (Lycopodiaceae) sporophytes and gametophytes in relation to vegetation cover in dry pine forests. American Fern Journal, Vol. 106, pp. 242-257.

Tonteri T., Salemaa M., Rautio P., Hallikainen V., Korpela L., Merilä P. (2016). Forest management regulates temporal change in the cover of boreal plant species. Forest Ecology and Management, Vol. 381, pp. 115-124.

Vanha-Majamaa I., Shorohova E., Kushnevskaya H., Jalonen J. (2017). Resilience of understory vegetation after variable retention felling in boreal Norway spruce forests – A ten year perspective. Forest Ecology and Management, Vol. 393, pp. 12-28.

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