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Original article

Modification of light intensity influence essential oils content, composition and antioxidant activity of thyme, marjoram and oregano

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ABSTRACT

Thymus vulgaris L. (thyme), *Origanum majorana* L. (marjoram), and *Origanum vulgare* L. (oregano) were used to determine whether light modification (plants grown under nets with 40% shaded index or in un-shaded open field) could improve the quantity and quality of essential oils (EOs) and antioxidant activity. The yield of EOs of thyme, marjoram, and oregano obtained after 120 min of hydrodistillation was 2.32, 1.51, and 0.27 mL/100 g of plant material, respectively. At the same time under shading conditions plants synthetized more EOs (2.57, 1.68, and 0.32 mL/100 g of plant material). GC/MS and GC/FID analyses were applied for essential oils determinations. The main components of the thyme essential oil are thymol (8.05–9.35%); γ-terpinene (3.49–4.04%); p-cymene (2.80–3.60%) and caryophyllene oxide (1.54–2.15%). Marjoram main components were terpinene 4-ol (7.44–7.63%), γ-terpinene (2.82–2.86%) and linalool (2.04–2.65%) while oregano essential oil consisted of the following components: caryophyllene oxide (3.1–1.93%); germacrene D (1.17–2.0%) and (E)-caryophyllene (1.48–1.1%). The essential oil from thyme grown under shading (EC_{50} value after 20 min of incubation) have shown the highest antioxidant activity – 0.85 mg mL^{-1} in comparison to marjoram and oregano (shaded plants EC_{50} 19.97 mg mL^{-1} and 7.02 mg mL^{-1} and unshaded, control plants EC_{50} 54.01 mg mL^{-1} and 7.45 mg mL^{-1}, respectively). The medicinal plants are a good source of natural antioxidants with potential application in the food and pharmaceutical industries. For production practice, it can be recommended to grow medicinal plants in shading conditions to achieve optimal quality parameters.

1. Introduction

Aromatic plants such as Thymus vulgaris L(common thyme), Origanum majorana L. (sweet marjoram), and Origanum vulgare L. (oregano) have a long tradition of use in both folk and conventional medicine (Mimica-Dukić et al., 2004). They are native plants from the Mediterranean region, widely distributed in Balkan countries, as well as in Serbia, with great health importance (Ilić et al., 2021). These plants can also be said to belong to culinary herbs due to their characteristic odor, and they are added as a spice in the preparation of stews, soups, meat, fish, and vegetable dishes. Thyme is considered one of the fine herbs of French cuisine, oregano in Italian and marjoram in all Mediterranean regions (Wiese et al., 2018).

The essential oils and their main components such as carvacrol, thymol, and linalool isolated from medicinal plants, contained phenols and other components, are being implemented in pharmaceutical and cosmetology industries as the carriers of antibacterial (De Falco et al., 2013), antiviral and antifungal activity (Liu et al., 2019). At the same time, they are distinguished as anti-inflammatory, antidiabetic and cancer suppressor agents (Leyva-López et al., 2017). Common thyme, sweet marjoram, and oregano essential oils - natural and liquid secondary plant metabolites-are gaining importance for their use in the protection of foods, since they are accepted as safe and healthy (Gavarić et al., 2015). The use of essential oils or plant extracts of thyme as a significant source of natural additives affects stability and reduces lipid oxidation

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during storage of foods such as meat, meat products, milk, fish and their products (Nieto, 2020).

The accumulation and the final composition of essential oils in the plants are highly influenced by the cultivation conditions, climate, and growth stage at the time of harvest (Murillo-Amador et al., 2013). Light quantity and quality play an important role in the plant production and synthesis of essential oils and have been shown to affect volatile compounds in herbs (Carvalho et al., 2016). Shading nets are characterized by different mechanical, physical, and optical properties, which allow for the modulation of light (quality and quantity of sunlight radiation), but at the same time they affect temperature regulation, humidity, and wind velocity levels around the crops, allowing the greater efficiency of herbs production and quality parameters of medicinal plants in screenhouse cultivation (Milenković et al., 2019).

Studies about the cultivation of aromatic plants have found different responses concerning the content and composition of essential oil, according to the light spectrum control during cultivation (Martins et al, 2008; Costa et al., 2012; Oliveira et al, 2016; Milenković et al., 2019). The spectral changes provided by colored shade nets resulted in increments of 30% in the yield of essential oil in lemon balm plants. These plant's qualities make the use of blue net a cultivation practice suitable for commercial use (Oliveira et al., 2016). These results are in agreement with Martins et al. (2008), who showed that *Origano gratissimum* plants under the blue net were taller and had higher essential oil content. Buthelezi et al. (2016) present in their research that aromatic herbs grown under black nets achieve higher antioxidant content than herbs from open field and other shade nets.

The present paper aims to investigate the effect of light intensity modification on the yield, chemical composition of essential oils, and antioxidant activity of thyme, marjoram and oregano cultivated under different light conditions. It is important to determine what conditions the plants need for their optimum quality parameters.

2. Material and methods

2.1. Plant material and growing conditions

The experiment was conducted during 2019–2020 in an experimental garden at the village Moravac in South Serbia (21°42′E, 43°30′N, altitude 159 m). *Thymus vulgaris* L. (thyme), *Origanum majorana* L. (marjoram), and *Origanum vulgare* L. (oregano) were used to determine whether shading conditions (plants cover by color nets) could improve essential oils and antioxidant activity in plants.

The production and establishment of medicinal plants meant sowing seeds in the field at a distance between rows of 40 cm on a plot of only 3–6 mm deep on 25 May in raised beds (20 cm high), 1.2 m wide and 3 m long (3.6 m^2 plot size). in 2019.After 6–8 days, the plants began to germinate, and then thinning was performed (at 5 cm distance of plants in the row) to achieve an optimal plant density (50 plants/m^2). According to soil analyses, all quantities of phosphorus and potassium (50 kg ha^{-1}) and 50% of total nitrogen (80 kg ha^{-1}) is incorporated into the soil before sowing. The other half of nitrogen fertilizers were applied the first time 8 weeks (25 % N) after sowing and the second time after the first harvest (25 % N) using calcium ammonium nitrate (28 % N). Combinations of plant species treatments were replicated 3 times with shading (net house with shade nets from an Israel company Polysack Plastics Industries, with a shade index of 40%) and un-netted control treatment in a split-plot design. The shade nets were mounted on a structure placed about 2.0 m above the plants (net house) at middle of June until end of August.

Thyme, marjoram, and oregano plants in the second year (2020) after the establishment of the crop were harvested at the stage of commercial maturity (in full bloom stage). The plants were harvested in early August. Uniform shoots without disease with leaves without any injuries or defects were selected and dried without the presence of light and ventilation at room temperature (about 25–30 °C) as air-dry plants for analysis.

2.2. Light interception by nets

The Sun Scan probe SS1-UM-1.05 (Delta-T Devices Ltd., UK) was used for light intensity measurement (PAR-photosynthetically active radiation - μmol m^{-2} s^{-1}) under the pearl nets and open field condition (control). Solar radiation was measured at different intervals during the days with Solarimeter-SL 100 (KIMO, France).

2.3. Clevenger-hydrodistillation

Preparation of plant material involves choping (milled air-dried aerial part of thyme, marjoram, and oregano plants: *Thymi herba, Origani majoranae herba,* and *Origani herba* was used for essential oil isolation by Clevenger-type hydrodistillation, with hydromodulus (ratio of plant material:water) 1:10 m/V during 120 min. The content of essential oil is displayed in % (v/m), which conforms to mL/100 g of air-dried plant material.

2.4. Gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization detection (GC/FID) analysis

Injected in split/splitless injector set at 250 °C in 40:1 split mode. Oil constituents identification was based on the comparison of their retention indices (RI^{exp}) with those available in the literature (Adams, 2007) (RI^{lit}); their mass spectra with those of authentic standard as well as with those from Willey 6, NIST2011, and RTLPEST3 libraries and wherever possible, by co-injection with an authentic standard (Co-I). Quantification was done by external standard method using standards in the concentration ranges as follows: β-pinene (0.125–2 mg/mL), 1,8-cineole (0.25–3 mg/mL), citral (0.56–10 mg/mL), limonene (0.5–4 mg/mL), linalool (1.67–15 mg/mL), thymol (1.78–16 mg/mL) and γ-terpinene (0.75–5 mg/mL).

The response factor (RF) for each standard used was calculated as follows:

$$RF = \frac{Area_{std}}{C_{std}}$$

where $Area_{std}$ is the peak area of the analyte standard and C_{std} is the concentration of the standard used. Then the mean of the RFs was calculated (RF_{mean}) and used to quantitate each of the analytes in the samples using the equation:

$$C_X = \frac{Area_X}{RF_{mean}}$$

where C_x is the concentration of the analyte in the sample, $Area_x$ is the peak area of the analyte, and RF_{mean} is the mean response factor (Sparkman et al., 2011).

2.5. DPPH assay

The ability of the essential oil to scavenge free DPPH radicals was determined using the DPPH assay. Absorption was measured at 517 nm immediately after adding the DPPH radical and after 20 min of incubation with the radical. Free radical scavenging activity was calculated according to the formula (Stanojević et al., 2015):

$$\text{DPPH radical scavenging capacity}\ (\%) = 100 - \left[(A_S - A_B) \times \frac{100}{A_C}\right]$$

A_S – Absorption of the "sample" at 517 nm. "Sample" – ethanolic solution of the essential oil treated with DPPH radical solution
A_B – Absorption of the "blank" at 517 nm. "Blank" – ethanolic solution of the essential oil which is not treated with DPPH radical solution
A_C – Absorption of the "control" at 517 nm. "Control" – ethanolic solution of the DPPH radical

All absorptions were measured on Perkin Elmer Lambda 25, Spectrophotometer.

The essential oil concentration needed for the neutralization of 50% of the initial DPPH radical concentration is called EC_{50} value. This value was determined by using linear regression analysis of the different concentrations range of essential oil added to the reaction mixture.

2.6. Statistical analysis

Statistical analysis of obtained data was performed using software Statistica (TIBCO Software Inc. 2018, version 13). ANOVA was used to analyse the significance of influence of shading conditions on medicinal plants with Duncan's multiple range test used for analysis of significance of differences between means.

3. Results

3.1. Climatic conditions

The climatic conditions of southern Serbia are very favorable for the production of thyme, marjoram, and oregano throughout the growing season (Table 1a).

Net houses have the potential to create an appropriate microclimate that positively affects plants productivity and quality. Photosynthetically active radiation (PAR) was significantly lower under pearl nets with 40% shading (1100 μmol $s^{-1}m^{-2}$) compared to the control - open field condition (2242 μmol $s^{-1}m^{-2}$). Shading with pearl nets affects the reduction of photosynthetically active radiation, slightly lower in the morning (31.2%), grows during the day, and provides the highest reduction (53.9%) in the afternoon. Results from Table 1b show that the maximum solar radiation in the open field, during a sunny day in July reached 874 Wm^{-2}. Compared to control, the solar radiation at noon was significantly reduced under pearl nets (459 W m^{-2}).

3.1.1. Thyme essential oil yield

Thyme has a characteristic odor of thymol and is used as a culinary herb with typical spicy aroma.

The yield of essential oils (EOs) from thyme was 2.32 mL/100 g of plant material from shaded condition and 2.57 mL/100 g of plant material from unshaded open field Table 2.

3.1.2. Sweet marjoram essential oil

The yield of essential oil (EOs) from the aerial part (herb) of marjoram obtained after 120 min of hydrodistillation was 1.51 mL/100 g of plant material. Shaded plants showed higher EOs content (1.68 mL/100 g of plant material) than unshaded-control plants (Table 2).

The dependence of the yield of marjoram essential oil from the hydrodistillation time was presented in Fig. 1.

3.1.3. Oregano essential oil yield

The yield of essential oil (EOs) from the aerial part (herb) of oregano obtained after 120 min of hydrodistillation was 0.27 mL/100 g of plant material. Shaded plants showed higher EOs content (0.32 mL/100 g of plant material) than un shaded-control plants. The yield of essential oils in oregano is much lower than that of thyme and marjoram (Table 2).

The dependence of the yield of oregano essential oil from the hydrodistillation time was presented in Fig. 1.

3.2. Essential oils composition

3.2.1. Thyme essential oils composition

The composition of thyme essential oils depends on the geographical origin, variety, growth phase, watering, harvest time and method of drying the plant mass. The chemical profile of thyme essential oils constituents determines their interactions and activity. Thyme EOs vary, which influences the physicochemical properties of the oil.

In our research the main components of the thyme essential oil are thymol (8.05–9.35 mg/mL); γ-terpinene (3.49–4.04%); p-cymene (2.80–3.60%) and caryophyllene oxide (1.54–2.15%) - Table 3 and Fig. 2.

The majority of the compounds in the thyme essential oil in unshading and shading conditions were aromatic compounds (63.4–67.2%), followed by oxygen-containing monoterpenes (20.8–22.0%), oxygen-containing sesquiterpenes (10.5–5.6%), and sesquiterpene hydrocarbons (1.7–1.9%) respectively.

It has been noticed that only one component (linalool formate) has been registered in unshaded plants.

3.2.2. Marjoram essential oils composition

The majority of EO compounds in the marjoram cultivated in unshaded and shaded conditions were terpinene 4-ol (7.44–7.63%), γ-terpinene (2.82–2.86%) and linalool (2.04–2.65%), Table 4 and Fig. 3.

Thymol (0.06 % mg/mL) and (Z)-β-Ocimene (0.01%) were detected only in shaded plants (Table 4).

3.2.3. Oregano essential oil composition

In our research, the presence of as many as 111 components of oregano essential oils was detected. Oregano EOs consist of the following components: caryophyllene oxide (3.1–1.93%); germacrene D (1.17–2.0%) and (E)-caryophyllene (1.48–1.1%) Table 5 and Fig. 4.

Table 1a
Parameters of climatic conditions during the growing season in south Serbia (Aleksinac).

Month	Temperature					MSR	RH	PR
	TX	TM	TS	EMd	EMnd	MJ/m^2	%	mm
May	26.6	12.6	19.6	31.0	9.0	279.0	62	66.8
June	26.9	14.4	20.6	34.0	7.0	223.0	68	83.1
July	28.4	16.7	22.5	32.0	13.0	218.3	72	99.3
August	32.7	16.7	24.7	34.1	13.2	283.1	65	51.6
September	26.7	10.5	18.6	34.3	−3.0	230.8	64	9.8

TX- temperature maximum; TM-temperature minimum; TS-mean monthly air temperature (°C); EMd-extreme maximum daily temperture; EMnd- extreme minimun daily temperyture; MSR, mean daily solar radiation (number of hours); MSR, mean daily solar radiation (MJ/m^2); RH -Relative humidity (%); PR precipitation amount (mm).

Table 1b
Influence of shading on growing environment (average day in July).

Time (h)	PAR* (μmol m^{-2} s^{-1})		Solar radiation (W m^{-2})		Temperature °C		Relative Humidity %	
	Non-shading	Shading Reduction %	Non-shading	Shading	Non-shading	Shading Reduction %	Non-shading	Shading Reduction %
6:00	182.5	31.2	162.5	40.5	16.7	0.0	74.7	−4.1
9:00	1325.6	46.0	513.8	281.0	24.7	−0.4	71.8	0.0
12:00	2242.2	49.1	874.5	459.5	31.4	−2.2	47.3	−2.1
15:00	1684.1	51.9	790.5	351.0	31.5	−3.4	48.2	−1.2
18:00	672.0	53.9	375.5	90.9	28.3	−1.0	50.4	−0.2

* PAR.-Photosynthetically active radiation.

Table 2
Yield of essential oil yield obtained after 120 min of hydrodistillation (hydromodule 1:10 m/v) and EC_{50} values of essential oil from the aerial part (herb) of thyme, marjoram and oregano.

Plant species	Shading	Essential oil yield, mL/100 g p.m.	EC_{50}, mg mL^{-1}/20 min incubation
Thyme	Unshaded	2.32^{b} ± 0.03	0.944^{a} ± 0.001
	Shaded	2.57^{a} ± 0,09	0.852^{a} ± 0.005
Marjoram	Unshaded	1.51^{d} ± 0.03	54.012^{e} ± 1.051
	Shaded	1.68^{c} ± 0.03	19.972^{d} ± 0.199
Oregano	Unshaded	0.27^{e} ± 0.01	7.449^{c} ± 0.018
	Shaded	0.32^{e} ± 0.01	7.023^{b} ± 0.054
ANOVA (p values)			
Plant species		0.00000	0.00000
Shading		0.00000	0.00000
Plan species · shading		0.00518	0.00000

Values followed by the same letter do not significantly differ between the treatments, at P = 0.05 according to Duncan's multiple range test.
[c] EC_{50}, mg·mL^{-1} = concentration of extract necessary to neutralize 50% of initial concentration of DPPH radicals.

Among monoterpenes, hydrocarbons ranged between 3.7 and 11.8% whereas oxygenated monoterpenes (i.e., monoterpenoids) showed a range of 12.2–21.1%. Total sesquiterpenes were always represented in the highest level (24.3–31.4%). Aromatic compound are present in quate small levels (4.0–4.2%). The other compounds always reached amounts lower than 1%.

Even though no literature data could be found to compare with our EO profile, the characterizing and new compounds of the species were also detected in our study. It is very interesting to point out that some components of essential oils are present only in plants that are shaded, while some others are present only in unshaded plants that grow in full light.

It has been noticed that a number of components have only been registered in unshaded plants. Among these arebenzaldehyde (0.01%); rose furan (0.03%); rosefuran epoxide (0.02%); coahuilensol, methyl ether (0.01%); *cis*-sabinene hydrate acetate (0.02%); citronellol (0.01%); β-cubebene (0.02%); (Z)-jasmone (0.02%); aromadendrene (0.03%); *cis*-muurola-4(14),5-diene (0.02%); *endo*-1-bourbonanol (0.03%) and β-colacorene (0.02%).

Conversely, α-thujene (0.02%); α-terpinene (0.04%); viridene (0.03%); cryptone (0.01%); *cis*-verbenyl acetate (0.03%) and *cis*-cadina-1(6),4-diene (0.02%) were present only in shaded plants.

3.3. Antioxidant activity of thyme, marjoram, and oregano EOs

Efficient concentration – EC_{50} values of essential oil during 20 min incubation from plants covered by shade nets were: 0.852 mg mL^{-1}, 19.97 mg mL^{-1}, and 7.023 mg mL^{-1} of thyme, marjoram, and oregano essential oil, respectively. The efficient concentration (EC_{50}) values from the unshaded plants were: 0.944, 54.01, and 7.450 mg mL^{-1}, respectively (Table 2).

Medicinal plant (thyme, marjoram and oregano) essential oils samples from shaded plants showed higher antioxidant activity compared to the unshaded control plants. The results in Table 2 revealed that the highest antioxidant activity was seen in thyme EOs from shaded plants.

Comparing it with the activity after 20 min incubation with a radical (when compared all the samples), the activity decreases in the following order (the smaller the EC_{50} value, the better the antioxidant): shaded thyme (0.852) > unshaded thyme (0.944) > shaded oregano 7.023) > unshaded oregano (7.450) > shaded marjoram (19.97) > unshaded marjoram (54.01). Based on the results given in Table 6 the highest antioxidant activity was observed in a thyme EOs from plants covered by nets (Fig. 5). Marjoram and oregano essential oils samples from shaded plants showed higher antioxidant activity than the unshaded control plants (Figs. 6 and 7).

4. Discussion

Thyme, marjoram, and oregano were cultivated by direct sowing to the open field, but different biotic and abiotic challenges during the summer (high temperatures, hail, wind, pathogens, pests, birds) forced producers to protect plants by covering and shading with nets. Unless additional protective measures are taken, high temperatures and solar radiation may affect crop growth and performance including the appearance of abiotic disorders, limited metabolism and productivity (Ilić and Fallik, 2017).

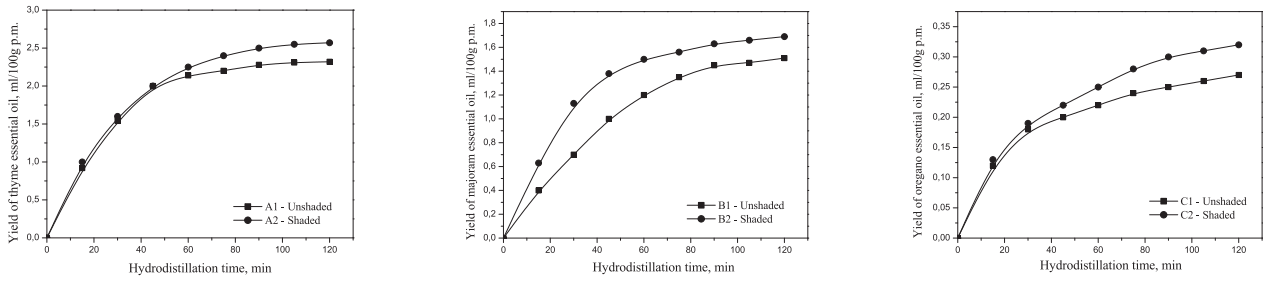

Fig. 1. The dependence of the yield of thyme, marjoram and oregano essential oil from the hydrodistillation time.

Table 3
Chemical composition of thyme essential oil.

No.	t_{ret}, min	Compound	Molecular formula	RI^{exp}	RI^{lit}	Method of identification	Area%		**Content %**	
							Unshade	Shade	Unshade	Shade
1	7.10	α-Thujene	$C_{10}H_{16}$	927	924	RI, MS	0.8	1.1	0.18	0.28
2	7.32	α-Pinene	$C_{10}H_{16}$	934	932	RI, MS, Co-I	0.5	0.7	0.12	0.17
3	7.78	Camphene	$C_{10}H_{16}$	949	946	RI, MS, Co-I	0.3	0.3	0.07	0.07
4	8.52	Sabinene	$C_{10}H_{16}$	974	969	RI, MS	0.1	tr	0.03	0.01
5	8.59	1-Octen-3-ol*	$C_8H_{16}O$	979	974	RI, MS	1.2	1.7	0.27	0.44
6	8.64	β-Pinene*	$C_{10}H_{16}$	978	974	RI, MS, Co-I				
7	8.85	3-Octanone	$C_8H_{16}O$	985	979	RI, MS	0.1	0.1	0.01	0.02
8	9.01	Myrcene	$C_{10}H_{16}$	991	988	RI, MS	1.5	1.7	0.34	0.44
9	9.12	3-Octanol	$C_8H_{18}O$	994	988	RI, MS	0.1	0.1	0.02	0.03
10	9.53	α-Phellandrene	$C_{10}H_{16}$	1006	1002	RI, MS	0.2	0.2	0.04	0.05
11	9.74	δ-3-Carene	$C_{10}H_{16}$	1012	1008	RI, MS	0.1	0.1	0.02	0.02
12	9.96	α-Terpinene	$C_{10}H_{16}$	1018	1014	RI, MS	2.0	2.2	0.45	0.55
13	**10.28**	**p-Cymene**	**$C_{10}H_{14}$**	**1026**	**1020**	**RI, MS**	**12.4**	**14.2**	**2.80**	**3.60**
14	10.41	Limonene*	$C_{10}H_{16}$	1029	1024	RI, MS, Co-I	0.6	0.6	0.05	0.06
15	10.43	β-Phellandrene*	$C_{10}H_{16}$	1030	1025	RI, MS				
16	10.51	1,8-Cineole	$C_{10}H_{18}O$	1032	1026	RI, MS, Co-I	0.5	0.4	0.05	0.03
17	11.07	(E)-β-Ocimene	$C_{10}H_{16}$	1047	1044	RI, MS	0.1	0.1	0.02	0.02
18	**11.58**	**γ-Terpinene**	**$C_{10}H_{16}$**	**1060**	**1054**	**RI, MS, Co-I**	**14.3**	**14.8**	**3.49**	**4.04**
19	11.85	*cis*-Sabinene hydrate	$C_{10}H_{18}O$	1068	1065	RI, MS	1.1	0.9	0.24	0.24
20	12.67	Terpinolene	$C_{10}H_{16}$	1089	1086	RI, MS	0.3	0.2	0.07	0.04
21	13.09	Linalool	$C_{10}H_{18}O$	1100	1095	RI, MS, Co-I	3.1	2.3	0.32	0.21
22	14.01	dehydro-Sabina ketone	$C_9H_{12}O$	1122	1117	RI, MS	0.2	0.1	0.05	0.02
23	14.74	*trans*-p-Menth-2-en-1-ol	$C_{10}H_{18}O$	1140	1136	RI, MS	0.2	tr	0.04	0.01
24	15.00	Camphor	$C_{10}H_{16}O$	1146	1141	RI, MS,Co-I	0.1	0.1	0.01	0.02
25	15.86	Borneol	$C_{10}H_{18}O$	1167	1165	RI, MS, Co-I	1.0	0.8	0.23	0.19
26	16.38	Terpinen-4-ol	$C_{10}H_{18}O$	1179	1174	RI, MS	3.7	0.8	0.85	0.20
27	16.98	α-Terpineol	$C_{10}H_{18}O$	1194	1186	RI, MS, Co-I	0.3	0.1	0.06	0.03
28	*17.72*	*Linalool formate*	*$C_{11}H_{18}O_2$*	*1211*	*1214*	*RI, MS*	*0.1*	–	*0.01*	–
29	18.73	Thymol, methyl ether	$C_{11}H_{16}O$	1235	1232	RI, MS	0.3	0.4	0.07	0.10
30	19.13	Carvacrol, methyl ether	$C_{11}H_{16}O$	1244	1241	RI, MS	0.3	0.3	0.07	0.09
31	19.76	Linalool acetate	$C_{12}H_{20}O_2$	1259	1254	RI, MS, Co-I	0.2	0.1	0.04	0.03
32	**21.38**	**Thymol**	**$C_{10}H_{14}O$**	**1298**	**1289**	**RI, MS, Co-I**	**47.4**	**49.2**	**8.05**	**9.35**
33	21.64	Carvacrol	$C_{11}H_{16}O$	1304	1298	RI, MS	2.9	2.9	0.65	0.73
34	23.77	Thymol acetate	$C_{12}H_{16}O_2$	1355	1349	RI, MS	0.1	0.1	0.01	0.02
35	23.91	Eugenol	$C_{10}H_{12}O_2$	1358	1356	RI, MS, Co-I	–	0.1	–	0.01
36	26.54	(E)-Caryophyllene	$C_{15}H_{24}$	1423	1417	RI, MS	1.2	1.3	0.27	0.33
37	27.90	α-Humulene	$C_{15}H_{24}$	1457	1452	RI, MS	0.1	0.1	0.02	0.02
38	28.64	Geranyl propanoate	$C_{11}H_{22}O_2$	1475	1476	RI, MS	0.2	0.1	0.04	0.03
39	28.79	γ-Muurolene	$C_{15}H_{24}$	1479	1478	RI, MS	–	0.1	–	0.02
40	29.60	Bicyclogermacrene	$C_{15}H_{24}$	1499	1500	RI, MS	0.2	0.1	0.04	0.01
41	30.25	γ-Cadinene	$C_{15}H_{24}$	1516	1513	RI, MS	0.1	0.1	0.02	0.03
42	30.62	δ-Cadinene	$C_{15}H_{24}$	1526	1522	RI, MS	0.1	0.2	0.02	0.04
43	32.92	Caryophyllene oxide	$C_{15}H_{24}O$	1586	1582	RI, MS	0.3	0.2	0.06	0.06
44	35.02	epi-α-Cadinol	$C_{15}H_{26}O$	1644	1638	RI, MS	0.1	0.1	0.03	0.03
				Total identified (%)			98.4	99.0		
Grouped components (%)										
Monoterpene hydrocarbons (1–4, 6, 8, 10–12, 14, 15, 17, 18, 20)							20.8	22.0		
Oxygen-containing monoterpenes (16, 19, 21, 23–28, 31, 38)							10.5	5.6		
Sesquiterpene hydrocarbons (36, 37, 39–42)							1.7	1.9		
Oxygen-containing sesquiterpenes (43, 44)							0.4	0.3		
Aromatic compounds (13, 29, 30, 32–35)							63.4	67.2		
Others (5, 7, 9, 22)							1.6	2.0		

$t_{ret.}$: Retention time; RI^{lit}-Retention indices from literature (Adams, 2007); RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C_8-C_{20}) on the HP-5MS column. MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching; Co-I: constituent identity confirmed by GC co-injection of an authentic sample; tr = trace amount (<0.05%); *co-eluting compounds. Compounds marked in *italic* are present only in sample from unshade plants Compounds marked in under line are present only in sample from shade plants

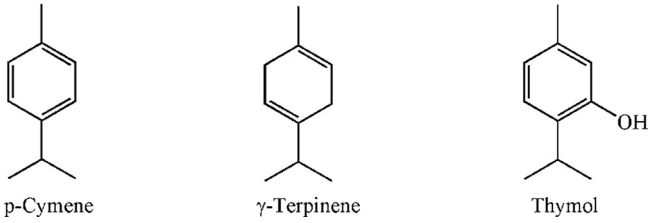

Fig. 2. Structures of the most abundant components identified in thyme essential oil.

Net houses have the potential to create a appropriate microclimate that positively affects plants productivity and quality. Growth, development, and accumulation of the secondary metabolites of medicinal plants may be significantly influenced by genetic (internal) and environmental (external) factors. Light intensity play an important role in oil biosynthesis and accumulation. The effect of the light spectrum transmitted by shade nets confirms the effect of the accumulation of secondary metabolites of medicinal plants (Stagnari et al., 2018). Therefore, it is possible to alter the composition of the bioactive compound in basil and other plants, through the exposure of plants to a specific light spectrum (Hosseini et al., 2018; Milenković et al., 2019).

Table 4
Chemical composition of marjoram essential oil.

No.	t_{ret}, min	Compound	Molecular formula	RI^{exp}	RI^{lit}	Method of identification	Area%		**Content %**	
							Unshade	Shade	Unshade	Shade
1	7.11	α-Thujene	$C_{10}H_{16}$	927	924	RI, MS	0.4	0.3	0.07	0.08
2	7.33	α-Pinene	$C_{10}H_{16}$	934	932	RI, MS, Co-I	0.3	0.4	0.06	0.08
3	8.53	Sabinene	$C_{10}H_{16}$	974	969	RI, MS	4.4	4.8	0.89	1.06
4	8.66	β-Pinene	$C_{10}H_{16}$	979	974	RI, MS, Co-I	0.3	0.3	0.18	0.21
5	9.02	Myrcene	$C_{10}H_{16}$	991	988	RI, MS	1.4	1.5	0.28	0.33
6	9.54	α-Phellandrene	$C_{10}H_{16}$	1006	1002	RI, MS	0.2	0.2	0.04	0.04
7	9.99	α-Terpinene	$C_{10}H_{16}$	1018	1014	RI, MS	6.9	6.6	1.41	1.47
8	10.25	p-Cymene	$C_{10}H_{14}$	1025	1020	RI, MS	0.6	0.6	0.12	0.12
9	10.43	Limonene*	$C_{10}H_{16}$	1030	1024	RI, MS, Co-I	2.4	2.5	0.36	0.42
10	10.44	β-Phellandrene*	$C_{10}H_{16}$	1030	1025	RI, MS				
11	10.51	1,8-Cineole	$C_{10}H_{18}O$	1032	1026	RI, MS, Co-I	0.1	0.1	0.05	0.03
12	10.68	(Z)-β-Ocimene	$C_{10}H_{16}$	1037	1032	RI, MS	–	tr	–	0.01
13	11.08	(E)-β-Ocimene	$C_{10}H_{16}$	1047	1044	RI, MS	0.1	0.1	0.01	0.01
14	**11.58**	**γ-Terpinene**	**$C_{10}H_{16}$**	**1060**	**1054**	**RI, MS, Co-I**	**13.0**	**12.0**	**2.82**	**2.86**
15	11.87	*cis*-Sabinene hydrate	$C_{10}H_{18}O$	1068	1065	RI, MS	3.0	3.3	0.60	0.74
16	12.70	Terpinolene	$C_{10}H_{16}$	1090	1086	RI, MS	2.9	2.6	0.58	0.57
17	**13.14**	**Linalool**	**$C_{10}H_{18}O$**	**1102**	**1095**	**RI, MS, Co-I**	**12.9**	**14.8**	**2.04**	**2.65**
18	14.04	*cis*-p-Menth-2-en-1-ol	$C_{10}H_{18}O$	1123	1118	RI, MS	1.9	2.2	0.38	0.48
19	14.77	*trans*-p-Menth-2-en-1-ol	$C_{10}H_{18}O$	1141	1136	RI, MS	1.3	1.5	0.25	0.33
20	**16.55**	**Terpinen-4-ol**	**$C_{10}H_{18}O$**	**1183**	**1174**	**RI, MS**	**36.8**	**34.4**	**7.44**	**7.63**
21	16.98	α-Terpineol	$C_{10}H_{18}O$	1194	1186	RI, MS, Co-I	4.6	4.4	0.94	0.98
22	17.14	γ-Terpineol	$C_{10}H_{18}O$	1198	1199	RI, MS	0.4	0.5	0.08	0.11
23	17.21	*cis*-Dihydro carvone	$C_{10}H_{16}O$	1199	1191	RI, MS	0.3	0.2	0.06	0.04
24	17.51	*trans*-Dihydro carvone	$C_{10}H_{16}O$	1206	1200	RI, MS	0.2	0.2	0.03	0.03
25	17.63	Linalool formate	$C_{11}H_{18}O_2$	1209	1214	RI, MS	0.6	0.7	0.13	0.16
26	18.47	Nerol	$C_{10}H_{18}O$	1229	1227	RI, MS	0.1	0.1	0.02	0.02
27	19.64	Linalool acetate	$C_{12}H_{20}O_2$	1257	1254	RI, MS, Co-I	0.9	1.0	0.19	0.21
28	*21.14*	*Thymol*	*$C_{10}H_{14}O$*	*1292*	*1289*	*RI, MS, Co-I*	–	*0.4*	–	*0.06*
29	21.54	Terpinen-4-ol acetate	$C_{12}H_{20}O_2$	1301	1299	RI, MS	0.4	0.4	0.08	0.08
30	24.18	Neryl acetate	$C_{12}H_{20}O_2$	1365	1359	RI, MS	0.1	0.1	0.01	0.02
31	24.99	Geranyl acetate	$C_{12}H_{20}O_2$	1384	1379	RI, MS	0.1	0.2	0.02	0.04
32	26.54	(E)-Caryophyllene	$C_{15}H_{24}$	1423	1417	RI, MS	1.3	1.5	0.25	0.34
33	27.89	α-Humulene	$C_{15}H_{24}$	1457	1452	RI, MS	0.1	0.1	0.01	0.02
34	29.60	Bicyclogermacrene	$C_{15}H_{24}$	1500	1500	RI, MS	0.8	0.9	0.16	0.20
35	32.69	Spathulenol	$C_{15}H_{24}O$	1581	1577	RI, MS	0.2	0.1	0.05	0.01
36	32.92	Caryophyllene oxide	$C_{15}H_{24}O$	1586	1582	RI, MS	0.3	0.1	0.07	0.02
				Total identified (%)			99.3	99.1		
Grouped components (%)										
Monoterpene hydrocarbons (1–7, 9, 10, 12–14, 16)							32.3	31.3		
Oxygen-containing monoterpenes (11, 15, 17–27, 29–31)							63.7	64.1		
Sesquiterpene hydrocarbons (32–34)							2.2	2.5		
Oxygen-containing sesquiterpenes (35, 36)							0.5	0.2		
Aromatic compounds (8, 28)							0.6	1.0		

$t_{ret.}$: Retention time; RI^{lit}-Retention indices from literature (Adams, 2007); RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C_8-C_{20}) on the HP-5MS column. MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching; Co-I: constituent identity confirmed by GC co-injection of an authentic sample; tr = trace amount (<0.05%); *co-eluting compounds. Compounds marked in *italic* are present only in sample from unshade plants Compounds marked in under line are present only in sample from shade plants

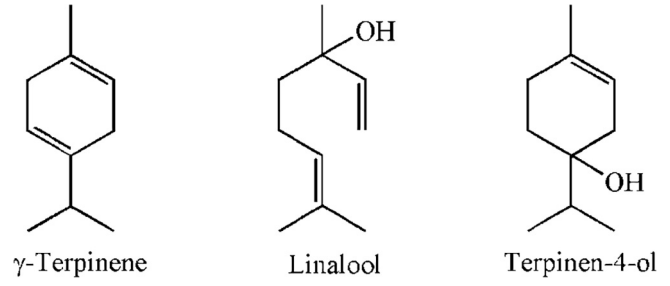

Fig. 3. Structures of the most abundant components identified in marjoram essential oil.

Light intensity can affect the EOs content, the EOs compounds, and the antioxidant activity of medicinal plants. The growing condition and cultivation methods during production of many herbs can improve the yield and quality of EOs more than gathering them from wild nature.

According to some literature data, the content of essential oil in dry herb of thyme ranges from 0.3% (Ozguven and Tansi, 1998) to 4.0% (Carlen et al., 2010). Results from our study are in agreement with Gavarić et al. (2015) who showed that the presence of essential oil in thyme leaves from Serbia also noticeably with a relatively low amount (0.8–2.6%).

Similarly to these results, also in our previous studies, we have observed significantly different yields of the EOs between shaded and unshaded plants. Thus, the lowest accumulation of essential oils in sweet basil was observed in the unshaded, control plants (1.02 mL/100 g of plant material) while the highest oil accumulation was achieved in plants from red shade nets (3.23 mL/100 g) (Milenković et al., 2019).

Method of production (open field or protected area) and environmental conditions greatly affects the quality and composition of the medicinal plants. Similarly to our research and in the results of other colleagues we can recognize the positive impact of shading on the content of EOs.

The fresh or dried highly aromatic leaves and flowering tops of marjoram are widely used to flavor many foods. Usually, marjoram

Table 5
Chemical composition of oregano essential oil.

No.	t_{ret}, min	Compound	Molecular formula	RI^{exp}	RI^{lit}	Method of identification	Area%		**Content %**	
							Unshade	Shade	Unshade	Shade
1	7.10	α-Thujene	$C_{10}H_{16}$	927	924	RI, MS	–	0.1	–	0.02
2	*8.10*	*Benzaldehyde*	*C_7H_6O*	*960*	*952*	*RI, MS*	*0.1*	–	*0.01*	–
3	8.52	Sabinene	$C_{10}H_{16}$	974	969	RI, MS	1.3	4.5	0.24	0.71
4	8.60	1-Octen-3-ol*	$C_8H_{16}O$	977	974	RI, MS	2.2	1.8	0.41	0.29
5	8.65	β-Pinene*	$C_{10}H_{16}$	979	974	RI, MS, Co-I	–	0.2	–	0.03
6	8.86	3-Octanone	$C_8H_{16}O$	986	979	RI, MS	0.2	0.2	0.04	0.03
7	9.02	Myrcene	$C_{10}H_{16}$	991	988	RI, MS	0.2	0.6	0.04	0.09
8	9.14	3-Octanol	$C_8H_{18}O$	995	988	RI, MS	0.3	0.2	0.06	0.03
9	9.75	δ-3-Carene	$C_{10}H_{16}$	1012	1008	RI, MS	0.1	0.1	0.02	0.02
10	9.96	α-Terpinene	$C_{10}H_{16}$	1018	1014	RI, MS	–	0.2	–	0.04
11	10.25	p-Cymene	$C_{10}H_{14}$	1025	1020	RI, MS	1.4	2.3	0.27	0.36
12	10.40	Limonene	$C_{10}H_{16}$	1029	1024	RI, MS, Co-I	0.4	0.6	0.01	0.01
13	10.52	1,8-Cineole	$C_{10}H_{18}O$	1032	1026	RI, MS, Co-I	0.5	0.7	0.04	0.05
14	10.68	(Z)-β-Ocimene	$C_{10}H_{16}$	1037	1032	RI, MS	1.1	2.8	0.21	0.44
15	10.93	Benzene acetaldehyde	C_8H_8O	1043	1036	RI, MS	0.2	0.3	0.04	0.04
16	11.07	(E)-β-Ocimene	$C_{10}H_{16}$	1047	1044	RI, MS	0.4	1.1	0.07	0.17
17	11.52	γ-Terpinene	$C_{10}H_{16}$	1059	1054	RI, MS, Co-I	0.1	1.4	0.01	0.21
18	11.85	*cis*-Sabinene hydrate	$C_{10}H_{18}O$	1068	1065	RI, MS	0.4	0.4	0.08	0.07
19	12.06	*cis*-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$	1073	1067	RI, MS	0.1	0.1	0.02	0.02
20	12.26	1-Nonen-3-ol	$C_9H_{18}O$	1079	1083[c]	RI, MS	0.2	0.1	0.03	0.02
21	12.68	Terpinolene	$C_{10}H_{16}$	1090	1086	RI, MS	0.1	0.2	0.02	0.04
22	*12.99*	*Rose furan*	*$C_{10}H_{14}O$*	*1098*	*1093*	*RI, MS*	*0.2*	–	*0.03*	–
23	13.10	Linalool	$C_{10}H_{18}O$	1102	1095	RI, MS, Co-I	2.8	2.9	0.16	0.11
24	13.58	1-Octen-3-yl acetate	$C_{10}H_{18}O_2$	1112	1110	RI, MS	0.1	0.1	0.02	0.01
25	14.02	*cis*-p-Menth-2-en-1-ol	$C_{10}H_{18}O$	1123	1118	RI, MS	0.2	0.2	0.04	0.04
26	14.27	*trans*-p-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	1129	1119	RI, MS	0.1	0.1	0.03	0.01
27	14.75	(Z)-Myroxide	$C_{10}H_{16}O$	1132	1131	RI, MS	0.4	0.4	0.08	0.07
28	14.99	*trans*-Verbenol	$C_{10}H_{16}O$	1146	1137	RI, MS	0.1	0.1	0.02	0.01
29	15.52	Sabina ketone	$C_9H_{14}O$	1159	1154	RI, MS	0.4	0.4	0.07	0.07
30	15.87	Borneol	$C_{10}H_{18}O$	1167	1165	RI, MS	1.0	0.2	0.13	0.03
31	15.95	Viridene	$C_{11}H_{16}$	1169	1163	RI, MS	–	0.2	–	0.03
32	*16.23*	*Rosefuran epoxide*	*$C_{10}H_{14}O_2$*	*1176*	*1173*	*RI, MS*	*0.1*	–	*0.02*	–
33	16.37	Terpinen-4-ol	$C_{10}H_{18}O$	1179	1174	RI, MS	2.2	2.9	0.42	0.45
34	16.63	Thuj-3-en-10-al	$C_{10}H_{14}O$	1185	1181	RI, MS	0.4	0.3	0.06	0.05
35	16.74	Cryptone	$C_9H_{14}O$	1188	1183	RI, MS	–	0.1	–	0.01
36	16.91	α-Terpineol	$C_{10}H_{18}O$	1192	1186	RI, MS, Co-I	1.0	1.2	0.19	0.18
37	17.02	Myrtenol	$C_{10}H_{16}O$	1195	1194	RI, MS	0.2	0.3	0.04	0.04
38	17.16	Myrtenal	$C_{10}H_{14}O$	1198	1195	RI, MS	0.2	0.2	0.03	0.03
39	17.61	γ-Terpineol	$C_{10}H_{18}O$	1209	1199	RI, MS	0.2	0.2	0.04	0.03
40	*17.96*	*Coahuilensol, methyl ether*	*$C_{10}H_{12}O$*	*1217*	*1219*	*RI, MS*	*0.1*	–	*0.01*	**–**
41	18.07	*trans*-Carveol	$C_{10}H_{18}O$	1220	1215	RI, MS	0.1	0.1	0.01	0.01
42	18.25	Dihydro myrcenol acetate	$C_{12}H_{22}O_2$	1224	1214	RI, MS	0.1	–	0.02	–
43	*18.46*	*cis-Sabinene hydrate acetate*	*$C_{12}H_{20}O_2$*	*1229*	*1219*	*RI, MS*	*0.2*	–	*0.02*	–
44	*18.68*	*Citronellol*	*$C_{10}H_{20}O$*	*1234*	*1223*	*RI, MS*	*0.1*	–	*0.01*	–
45	18.74	Thymol, methyl ether	$C_{11}H_{16}O$	1235	1232	RI, MS	0.1	–	0.01	–
46	18.99	Cumin aldehyde*	$C_{10}H_{12}O$	1241	1238	RI, MS	–	0.4	–	0.07
47	19.04	Neral*	$C_{10}H_{16}O$	1241	1235	RI, MS	3.4	–	0.53	–
48	19.17	Carvone	$C_{10}H_{14}O$	1245	1239	RI, MS	0.1	0.2	0.03	0.03
49	19.57	Geraniol	$C_{10}H_{18}O$	1255	1249	RI, MS	0.4	0.1	0.06	0.02
50	20.29	Geranial	$C_{10}H_{16}O$	1272	1264	RI, MS	4.2	0.3	0.80	0.05
51	20.48	dihydro-Linalool acetate	$C_{12}H_{22}O_2$	1276	1272	RI, MS	0.1	0.1	0.01	0.01
52	20.71	*cis*-Verbenyl acetate	$C_{12}H_{18}O_2$	1282	1280	RI, MS	–	0.2	–	0.03
53	20.87	α-Terpinen-7-al	$C_{10}H_{14}O$	1286	1283	RI, MS	0.1	0.2	0.01	0.02
54	20.96	Bornyl acetate	$C_{12}H_{20}O_2$	1288	1287	RI, MS	0.1	–	0.01	–
55	21.09	Dihydroedulan II*	$C_{13}H_{22}O$	1291	1288[b]	RI, MS	1.3	1.7	0.25	0.27
56	21.13	Thymol*	$C_{10}H_{14}O$	1292	1289	RI, MS, Co-I	–	–	–	–
57	21.55	Carvacrol	$C_{10}H_{14}O$	1302	1298	RI, MS	0.2	1.0	0.03	0.16
58	22.09	(3E)-Hexenyl tiglate	$C_{11}H_{18}O_2$	1315	1315	RI, MS	0.1	–	0.02	–
59	22.72	p-Mentha-1,4-dien-7-ol	$C_{10}H_{16}O$	1330	1325	RI, MS	0.1	0.6	0.02	0.09
60	24.32	Piperitenone oxide	$C_{10}H_{14}O_2$	1368	1366	RI, MS	1.9	0.1	0.35	0.01
61	24.51	*cis*-Carvyl acetate	$C_{12}H_{18}O_2$	1373	1365	RI, MS	0.1	–	0.02	–
62	24.74	α-Copaene	$C_{15}H_{24}$	1379	1374	RI, MS	0.4	0.3	0.07	0.04
63	24.98	Geranyl acetate	$C_{12}H_{20}O_2$	1384	1379	RI, MS	0.3	0.1	0.04	0.01
64	25.14	β-Bourbonene	$C_{15}H_{24}$	1388	1387	RI, MS	2.2	0.7	0.42	0.11
65	*25.33*	*β-Cubebene*	*$C_{15}H_{24}$*	*1393*	*1387*	*RI, MS*	*0.1*	–	*0.02*	–
66	25.40	β-Elemene	$C_{15}H_{24}$	1394	1389	RI, MS	0.2	0.2	0.04	0.03
67	*25.65*	*(Z)-Jasmone*	*$C_{11}H_{16}O$*	*1400*	*1392*	*RI, MS*	*0.1*	–	*0.02*	–
68	25.88	Italicene	$C_{15}H_{24}$	1406	1405	RI, MS	0.2	0.1	0.02	0.02
69	**26.58**	**(E)-Caryophyllene**	**$C_{15}H_{24}$**	**1424**	**1417**	**RI, MS**	**7.8**	**7.1**	**1.48**	**1.1**
70	26.91	β-Copaene	$C_{15}H_{24}$	1432	1430	RI, MS	0.4	0.2	0.08	0.03
71	27.15	α-*trans*-Bergamotene	$C_{15}H_{24}$	1438	1432	RI, MS	0.1	0.1	0.02	0.01
72	*27.52*	*Aromadendrene*	*$C_{15}H_{24}$*	*1447*	*1439*	*RI, MS*	*0.2*	–	*0.03*	–

Table 5 (*continued*)

No.	t_{ret}, min	Compound	Molecular formula	RI^{exp}	RI^{lit}	Method of identification	Area%		**Content %**	
							Unshade	Shade	Unshade	Shade
73	27.90	α-Humulene	$C_{15}H_{24}$	1457	1452	RI, MS	1.1	1.0	0.21	0.16
74	28.19	allo-Aromadendrene	$C_{15}H_{24}$	1464	1458	RI, MS	1.0	0.7	0.18	0.11
75	28.27	*cis*-Cadina-1(6),4-diene	$C_{15}H_{24}$	1466	1461	RI, MS	–	0.1	–	0.02
76	*28.41*	*cis-Muurola-4(14),5-diene*	$C_{15}H_{24}$	*1470*	*1465*	*RI, MS*	*0.1*	–	*0.02*	–
77	28.82	γ-Muurolene	$C_{15}H_{24}$	1480	1478	RI, MS	0.3	0.3	0.05	0.04
78	**29.03**	**Germacrene D**	$\mathbf{C_{15}H_{24}}$	**1485**	**1487**	**RI, MS**	**6.3**	**12.6**	**1.17**	**2.0**
79	*29.13*	*(E)-β-Ionone*	$C_{13}H_{20}O$	*1488*	*1487*	*RI, MS*	*0.2*	–	*0.04*	–
80	29.29	β-Selinene	$C_{15}H_{24}$	1492	1489	RI, MS	0.1	0.1	0.02	0.02
81	29.60	Bicyclogermacrene	$C_{15}H_{24}$	1499	1500	RI, MS	0.6	3.2	0.12	0.50
82	29.72	α-Muurolene	$C_{15}H_{24}$	1501	1500	RI, MS	0.3	0.3	0.05	0.05
83	29.99	(E,E)-α-Farnesene	$C_{15}H_{24}$	1510	1505	RI, MS	1.1	2.1	0.21	0.33
84	30.27	γ-Cadinene	$C_{15}H_{24}$	1509	1513	RI, MS	0.4	0.5	0.07	0.08
85	*30.39*	*endo-1-Bourbonanol*	$C_{15}H_{26}O$	*1510*	*1518*	*RI, MS*	*0.2*	–	*0.03*	–
86	30.51	Myristicin	$C_{11}H_{12}O_3$	1512	1517	RI, MS	0.2	0.1	0.03	0.01
87	30.60	*trans*-Calamenene*	$C_{15}H_{22}$	1526	1521	RI, MS	–	–	–	–
88	30.62	δ-Cadinene*	$C_{15}H_{24}$	1513	1522	RI, MS	1.3	1.7	0.24	0.26
89	31.17	α-Cadinene	$C_{15}H_{24}$	1513	1522	RI, MS	0.1	0.1	0.02	0.02
90	31.37	α-Colacorene	$C_{15}H_{20}$	1545	1544	RI, MS	0.1	0.1	0.02	0.02
91	31.80	Salviadienol	$C_{15}H_{24}O$	1557	1560[d]	RI, MS	1.6	1.2	0.27	0.17
92	*32.16*	*β-Colacorene*	$C_{15}H_{20}$	*1567*	*1564*	*RI, MS*	*0.1*	–	*0.02*	–
93	32.29	1,5-Epoxysalvial-4(14)-ene	$C_{15}H_{24}O$	1570	1562[b]	RI, MS	0.3	0.2	0.04	0.02
94	32.82	Spathulenol	$C_{15}H_{24}O$	1584	1577	RI, MS	6.8	7.6	1.28	1.19
95	**33.04**	**Caryophyllene oxide**	$\mathbf{C_{15}H_{24}O}$	**1586**	**1582**	**RI, MS**	**16.8**	**12.3**	**3.1**	**1.93**
96	33.24	Guaiol	$C_{15}H_{26}O$	1595	1600	RI, MS	0.8	0.4	0.02	0.07
97	33.34	Salvial-4(14)-en-1-one	$C_{15}H_{24}O$	1598	1594	RI, MS	–	0.2	–	0.03
98	33.67	Ledol	$C_{15}H_{26}O$	1607	1602	RI, MS	0.4	0.3	0.06	0.05
99	33.92	Humulene epoxide II	$C_{15}H_{24}O$	1613	1608	RI, MS	2.2	2.0	0.42	0.31
100	34.09	1,10-di-epi-Cubenol	$C_{15}H_{26}O$	1618	1618	RI, MS	0.2	0.2	0.04	0.03
101	34.57	1-epi-Cubenol	$C_{15}H_{26}O$	1631	1627	RI, MS	0.3	0.3	0.02	0.03
102	35.07	epi-α-Cadinol	$C_{15}H_{26}O$	1645	1638	RI, MS	1.7	1.4	0.25	0.21
103	35.22	α-Muurolol	$C_{15}H_{26}O$	1644	1644	RI, MS	0.5	0.4	0.08	0.07
104	35.54	α-Cadinol	$C_{15}H_{26}O$	1658	1652	RI, MS	1.7	2.2	0.31	0.34
105	35.70	*cis*-Calamenen-10-ol	$C_{15}H_{22}O$	1663	1660	RI, MS	0.3	–	0.05	–
106	35.99	*trans*-Calamenen-10-ol	$C_{15}H_{22}O$	1670	1668	RI, MS	1.2	–	0.21	–
107	36.13	14-hydroxy-9-epi-(E)-Caryophyllene	$C_{15}H_{24}O$	1674	1668	RI, MS	1.1	1.1	0.21	0.17
108	36.66	Eudesma-4(15),7-dien-1β-ol (impure)	$C_{15}H_{24}O$	1694	1687	RI, MS	0.7	0.5	0.13	0.08
109	36.83	5-neo-Cedranol	$C_{15}H_{26}O$	1694	1684	RI, MS	0.6	0.8	0.10	0.13
110	39.44	Methyl ester of 2,2,5,6-tetramethylbenzotetrahydrofuran-3-carboxylic acid	$C_{14}H_{18}O_3$	1770	–	MS	0.4	0.4	0.06	0.06
111	42.04	Hexahydrofarnesyl acetone (phytone)	$C_{18}H_{36}O$	1847	1846[e]	RI, MS	0.4	0.3	0.07	0.05
				Total identified (%)			95.2	96.2		
		Grouped components (%)								
		Monoterpene hydrocarbons (1, 3, 5, 7, 9, 10, 12, 14, 16, 17, 21)					3.7	11.8		
		Oxygen-containing monoterpenes (13, 18, 19, 23, 25–28, 30, 33, 34, 36–39, 41–44, 47–54, 59–61, 63)					21.1	12.2		
		Sesquiterpene hydrocarbons (62, 64–66, 68–78, 80–84, 88, 89)					24.3	31.4		
		Oxygen-containing sesquiterpenes (85, 91, 93–104, 107–109)					35.9	31.1		
		Aromatic compounds (2, 11, 15, 40, 45, 46, 56, 57, 86, 87, 90, 92, 105, 106)					4.0	4.2		
		Others (4, 6, 8, 20, 22, 24, 29, 31, 32, 35, 55, 58, 67, 79, 110, 111)					6.2	5.5		

$t_{ret.}$: Retention time; RI^{lit}-Retention indices from literature (Adams, 2007; [b]Milosevic et al., 2010, [d]Đorđević et al., 2011; [e]Balogun et al., 2017); RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C_8-C_{20}) on the HP-5MS column. MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching; Co-I: constituent identity confirmed by GC co-injection of an authentic sample; tr = trace amount (<0.05%); *co-eluting compounds. Compounds marked in *italic* are present only in sample from unshade plants Compounds marked in under line are present only in sample from shade plants.

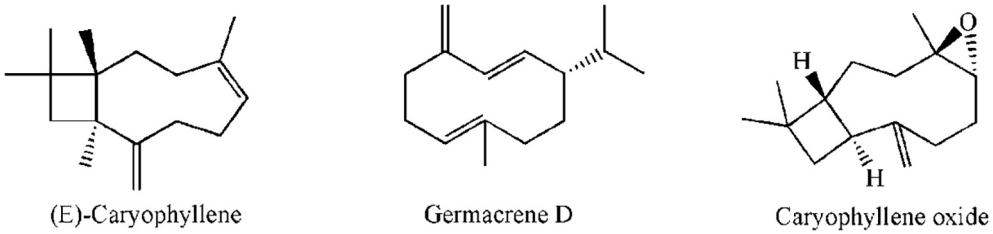

Fig. 4. Structures of the most abundant components identified in oregano essential oil.

contains 0.5–3.5% of essential oil in the dry herb (Tabanca et al., 2014). EO content in oregano of approximately 3.7% on dry weight obtained by Azizi et al. (2009), corresponding to approximately 1.52% on a fresh weight basis obtained by Tibaldi et al. (2011). The much higher EO content than our result (0.27–0.32%) could partially be due to the different cultural practices and environmen-

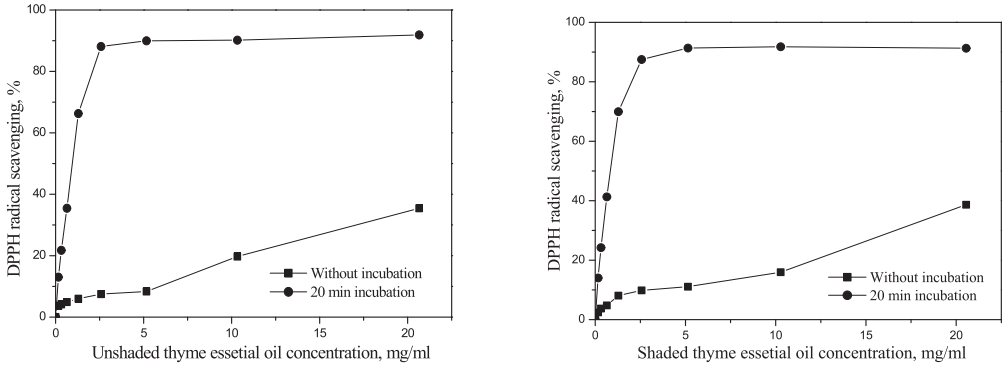

Fig. 5. Antioxidant activity of unshaded (A1) and shaded (Sample A2) thyme essential oil.

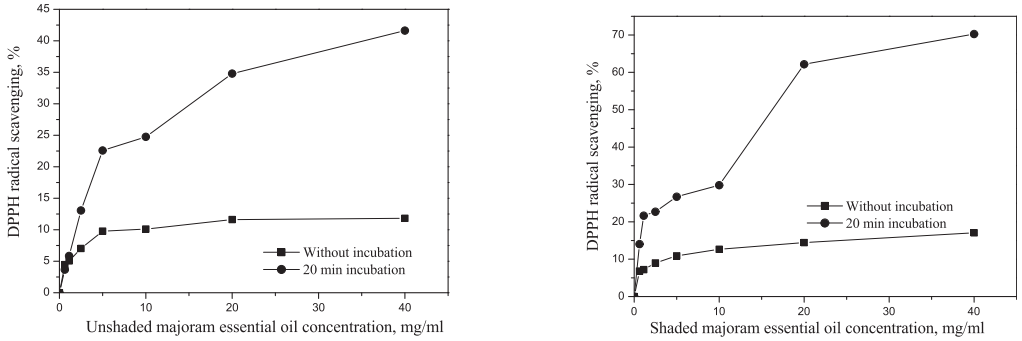

Fig. 6. Antioxidant activity of unshaded (B1) and shaded (B2) marjoram essential oil.

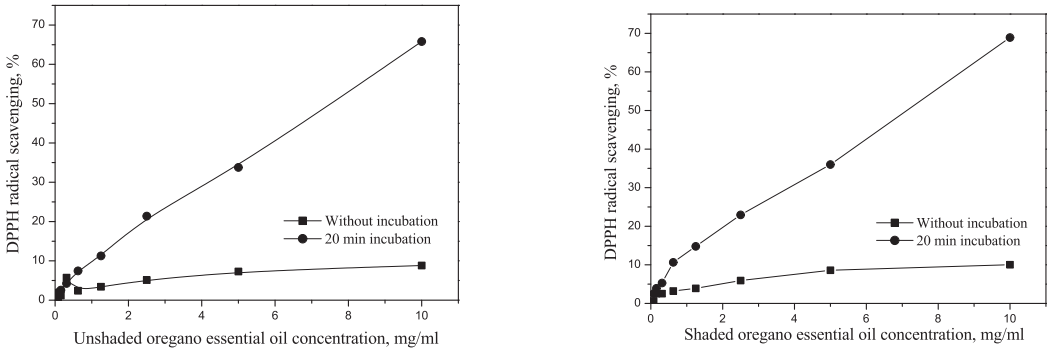

Fig. 7. Antioxidant activity of unshaded (C1) and shaded (C2) oregano essential oil.

tal conditions, and also to the different distillation method and material used by Azizi et al. (2009).

At the same time, we can find in the literature a lower yield of oregano EOs than in our research. Thus, EO content (0.16%) of oregano plants grown in the pot exposed to full light was around 50% higher than the EO from the plants grown in the soil exposed with full light or the pot plants cover with 50% shade nets (Tibaldi et al., 2011).

Light conditions and solar radiation can affect the differently to the accumulation of essential oils EO and EO profile in plants. Thus, Li et al. (1996) found that *Salvia officinalis* L., grown in shading condition (shade index-55%), reached the highest total EO content (0.38%) compared to the plants grown in the open field with full light (0.34%) conditions. EO content decreased with shading intensity by 55% to 85% shade. Opposite, some herbs like *Thymus vulgaris* L. the maximum total EO content obtained at full light (0.49%).

Thyme EO contains at least 60 bioactive compounds with powerful antioxidant properties. The antioxidant properties of thyme extract from Spain has been analyzed by Rota et al. (2008), and they concluded that main phenolic compounds are thymol (68.1%), p-cymene (11.2%), γ-terpinene (4.8%), and carvacrol (3.5%). In Hungary, the dominant compounds also was thymol (32.2%). In the agro-climacteric condition of Serbia the main compound of essential oil in thyme were thymol (25–50%) and carvacrol (3–10%) (Gavarić et al., 2015).

Marjoram essential oil (MEO) is obtained by steam distillation of dry marjoram leaves which contain 0.7–3.5% essential oil (Kumar et al., 2011). Considerable variations in the content and compositional pattern of MEO are observed depending on the species, growth stages, the origin of herb, climatic and drying conditions (Baatour et al., 2012).

The characteristic compounds of the commercially exploited marjoram essential oils are terpinen-4-ol, α-terpinene, γ-

terpinene, α-terpineol, and cis-sabinene hydrate, occurring in variable quantities. Marjoram also produces essential oils with different compositions, which are either rich in linalool or p-cymene and its biosynthetically related compounds thymol and carvacrol (Kokini et al., 2003).

It was postulated that the marjoram essential oils exist in two forms. In the first chemotype, terpinene-4-ol either alone or together with sabinene hydrate, α- terpineol, α- and γ-terpinene were found to be main constituents of the essential oils (Vera et al., 1999; Banchio et al., 2008), and the other chemotype with thymol and/or carvacrol as predominant compounds (Daferera et al., 2003). Marjoram volatile oil is rich in terpinen-4-ol, sabinene hydrate, γ-terpinene, p-cymene, α-terpinene, and α-terpineol (Lis et al., 2007). However, terpinene-4-ol alone or along with sabinene hydrate is responsible for the characteristic flavor and fragrance of marjoram oil (Vági et al., 2005). Spicy "*marjoramy*" aroma derived from compound like *cis*-sabinene hydrate (Raina and Negi, 2012).

The essential oil composition of marjoram showed terpinen-4-ol (31.15%), *cis*-sabinene hydrate (15.76%), p-cymene (6.83%), sabinene (6.91%), *trans*-sabinene hydrate (3.86%), and α-terpineol (3.71%) as the main constituents. Precursors of phenolic components like p-cymene and γ-terpinene were much higher in *O. vulgare* compared to *O. majorana*, whereas sabinene, cis- and *trans*-sabinene hydrate, and α-terpineole were much higher in *O. majorana*. The amounts of oxygenated monoterpenes were higher in *O. majorana* (Raina and Negi, 2012).

Egyptian marjoram oil belonged to terpinen-4-ol /sabinene-hydrate chemotype. Studies also indicated that the oil was dominated by monoterpene-hydrocarbons (75.79%), followed by oxygenated-monoterpenes (21.50%) and sesquiterpene-hydrocarbons (2.34%), (Badee et al., 2013). Terpinen-4-ol (29.13–32.57%), *cis*-sabinene hydrate (19.9–29.27%) and *trans*-sabinene hydrate (3.5–11.61%) were the main components of this fraction in *O. majorana* essential oils harvested in Tunisia, (Sellami et al., 2009) whereas 1,8-cineole (58.59 ± 0.85%), linalool (13.05 ± 0.04 %) and α-terpineol (3.33 ± 0.10%) were the main compounds in commercial natural marjoram analyzed in Spain (Ibáñez and Blázquez, 2017). The main components of the essential oil from marjoram collected from Greece were terpinen-4-ol (37.1%), p-cymene (12.05%), α-terpineol (7.15%), carvacrol (3.60%), *trans*-sabinene hydrate (2.41%), *cis*-sabinene hydrate (1.43%) and thymol (0.7%), (Komaitis, 1992).

Our marjoram essential oil composition was found to be close to that reported by Badee et al. (2013) except for some minor variations. The oil quality was close to the one produced in Europe and southern India.

Diverse concentrations of the main constituents are reported in oregano essential oils (OEOs) from different *Origanum* variety, geographic region, and origin, environmental conditions, harvest time, etc. The carvacrol content of different chemotypes of O. vulgare is variable and it can be up to 95% (Gounaris et al., 2002). Thymol (36.91–60.14%), γ-terpinene (11.59–24.14%), and p-cymene (2.56–9.38%) were the major components in all OEOs (Napoli et al., 2020).

The effect of light intensity through alteration in photosynthesis, physiological, and morphological processes of plants and methods of production in oregano plants affect essential oil constituents. The soil full-light treatment plant gave the essential oil mainly composed of 4-terpineol, γ-terpinene, carvacrol, and p-cymene. The pot 50%-shade treatment of the plant gave the essential oil mainly composed of γ-terpinene, 4-terpineol, carvacrol and p-cymene (Tibaldi et al., 2011).

Origanum species shared a similar aroma profile, described as spicy, phenolic and minty, as many of them contain thymol and carvacrol in varying amounts (Meyers, 2005).

Phenolic compounds (thymol and carvacrol) and their biogenetic precursors γ-terpinene and p-cymene are the main compounds in oregano essential oils, but with great variability in the percentage depending on the geographical origin. The *Origanum* species from Saudi and Jordanian indicate that the cymyl chemotype should predominate from these regions. Saudi *Origanum* contain carvacrol as the major component (79.5%-71.9%) while Jordanian *Origanum* contain thymol (68.7%) as the main constituents (Khan et al., 2018). High content of carvacrol and thymol has been determined also for oregano EO from Italy (De Martino et al., 2009) while, Armenian oregano consisted mainly of sesqui- and monoterpenes (β-caryophyllene epoxide – 13.3%; β-caryophyllene – 8.2%; o-cymene – 5.2%), (Moghrovyanet al., 2019).

In certain regions of India, *O. vulgare* produces an essential oil rich in p-cymene (6.7–9.8%), γ-terpinene (12.4–14.0%), thymol (29.7–35.1%), and carvacrol (12.4–20.9%) (Pande et al., 2012), whereas Turkish oregano essential oil together with the phenolic compounds thymol (15.66%) and carvacrol (24.52%) contain high amounts of linalool (50.53%) (Ozkan & Erdoğan, 2011). Carvacrol (72.06%) and thymol (4.98%) were the major oil constituents in the EO of *O. vulgare* from Serbia too, followed by *trans*-caryophyllene (3.61%) and *p*-cymene (2.08%) (Karaman et al., 2017).

Many herbs like thyme, sweet marjoram, oregano, and their extracts have been added to a variety of foods to improve their sensory characteristics and extend shelf-life (Burt, 2004). In recent years, the use of natural plant preservatives to increase the shelf-life of food products is promising technology since they derived substances having antioxidant and antimicrobial properties.

In our research thyme EO was reported to be the best antioxidant in a comparison of the antioxidant activity with other plants from Fam. Lamiaceae in the following order: thyme > oregano > marjoram. Our results are consistent with research of Roby et al. (2013).

Thyme, compared to other medicinal plant species, has better antioxidant properties of volatile oils with an inhibitory effect similar to the effect of α-tocopherol or BHT (butylhydroxytoluene), (Lee and Shibamoto, 2002).

Thymol and carvacrol, are main constituents of the thyme and oregano essential oil with antioxidant activity (Rodriguez-Garcia et al., 2016). Several *Thymus* species were found rich in both carvacrol and thymol and observed to have a high antioxidant activity including *Thymus vulgaris* L. (Alsaraf et al., 2020). The different position of the phenolic group in thymol relative to that in carvacrol makes it most likely that thymol is a better antioxidant and more efficient in oxidizing lipids at room temperature (Yanishlieva et al.,1999).

Shade nets provide greater presence and biosynthesis of polyphenolic compounds known to exhibit antioxidant properties (Milenković et al., 2019).

Thyme essential oil, thymol and carvacrol, are generally recognized as safe (GRAS status) and have been registered by the European Commission for use as flavoring agents in foods (FAD, 2010). These compounds are also potent antioxidants, EOs could be directly used in food products with some novel applications such as encapsulation, edible films, and edible coatings (Mutlu-Ingok et al., 2020).

Essential oils, especially from thyme and oregano are a potential source of natural antioxidants, as a possible alternative to synthetic antioxidants in food products and can prevent their oxidative deterioration. Most of these phytochemicals from the mentioned medicinal plants should soon be included in the regular dietary procedure and side effects on human health that can be caused by classic drugs and antibiotics should be avoided (Roychoudhury and Bhowmik, 2020). More abundant use of natu-

ral antiviral bioactive supplements in the form of hot drinks like tea, can help strengthen the immune system in protection against the current COVID-19 pandemic, as well as against possible newer viruses (Roychoudhury and Bhowmik, 2020).

5. Conclusion

Our data show that shade nets can be incorporated into the protected cultivation practices currently used for producing of medicinal plants. All three plant species under shade nets increase the yield of essential oils, but thyme and marjoram resulting in statisticaly higher levels of essential oil yield. In the light of this investigation, it is evident that the modification of ligh intensity can act as a physiological tool via the shade nets to improve the phytochemical quality and antioxidant activity of these plants. Our observation confirms that the antioxidant scavenging activity of different herbs in this study depends on the light modification to a greater and lesser level depending on the plant species. Among the three plant species investigated in this work, thyme plants are characterized by the highest level of antioxidant activity. Marjoram and oregano tolerate shading well, so it is recommended to grow them under shading nets.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Z.S.I., and Lj. S.Head of the research group planned the research, analyzed, and wrote the manuscript; L.M. N.T. and L.S conducted the experiment in the field; and J.C. and D.C. performed analyses of physical properties and chemical composition in the laboratory. All authors have read and agree to the published version of the manuscript.

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