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Research Article

Essential Oil Chemical Composition of Lavender Varieties Cultivated in an Untraditional Agro-Ecological Region

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Abstract: A field experiment has been conducted in the region of Dobrudja with the aim to investigate the essential oil yield and its chemical composition by four lavender varieties: Hemus, Sevtopolis, Yubileyna and Druzhba. The experiment has been arranged according to the Randomized Complete- Block Design in four replications and a plot size of 10 m^2. The essential oil yields varied from 90 l ha^{-1} by the variety Hemus to 131 l ha^{-1} by the variety Sevtopolis. The major constituents of the essential oil were linalyl acetate (25.5-39.7%) and linalool (27.6- 38.9%). The content of linalyl acetate was the highest by the variety Hemus, as only by this variety the ratio between linalyl acetate and linalool is 1:0.7, which defined the essential oil as high qualitative.

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Geleneksel Olmayan Bir Agro-Ekolojik Bölgede Yetiştirilen Lavanta Çeşitlerinin Uçucu Yağ Kimyasal Bileşimi

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Anahtar Kelimeler

Lavanta,
Linalool,
Linalil asetat

Öz: Dobruca bölgesinde, (Hemus, Seuthopolis, Jubilee ve Druzhba) dört çeşit lavantanın uçucu yağ verimini ve kimyasal bileşimini incelemek için bir saha deneyi kuruldu. Deneme, deneme parseli 10 m^2 büyüklüğünde ve dört tekerrürlü olarak kesirli parseller yöntemine göre düzenlenmiştir. Uçucu yağ verimleri Hemus için 90 l ha^{-1}'den Seuthopolis için 131 l ha^{-1}'e kadar değişir. Uçucu yağın ana bileşenleri (% 25.5-39.7) linalilasetat ve (% 27.6-38.9) linalool dur. Linalilasetat içeriği Hemus çeşidinde en yüksektir ve sadece bu çeşitte linalilasetat ile linalool arasındaki oran 1: 0.7'dir, bu da uçucu yağın yüksek kalitede olduğunu belirler.

1. Introduction

The *Lamiaceae* family is one of the most important herbal families with a cosmopolitan distribution, which includes a wide variety of plants of great industrial significance and multiple applications. The genus *Lavandula*, a member of the *Lamiaceae* family, with its 39 species, numerous hybrids, and about 400 officially registered varieties, are cultivated around the world and highly appreciated in many production directions because of the content and quality of the essential oil extracted mainly from the flowers. Only three Lavandula species are with commercial values *Lavandula angustifolia* Mill, *Lavandula latifolia L*, and *Lavandula x intermedia* a sterile hybrid developed by crossing *L. angustifolia x L. latifolia*. The essential oil chemical composition of the Lavandula species is the same, but with a different proportion of the compounds (Kıvrak, 2018). The quality of the essential oil is determined through the levels of linalool, linalyl acetate, and camphor in it. According to the ISO 3515:2002 standard, lavender essential oil contains linalool (25–38%), linalyl acetate (25–45%) and camphor (0.5–1.0%), lavandin essential oil contains linalool (24–35%), linalyl acetate (28–38%) and camphor (6–8%) according to the ISO 8902:2009 (Kıvrak, 2018). While lavender essential oil is used in the perfumery, cosmetics, and pharmaceuticals (Kara and Baydar, 2020), due to the high linalool and linalyl acetate content, the essential oil of lavandin is used in hygiene products, detergents, and insecticides, because of the higher amount of camphor. Lavender essential oil quality and price (85–150€/kg) is higher than lavandin essential oil (19 € kg^{-1}), although lavandin essential oil is produced in higher yields than lavender essential oil (120 kg ha^{-1}, 40 kg ha^{-1}, respectively) (Carrasco et al., 2016). Compounds such as linalyl acetate, linalool, 1,8- cineol, terpinen-4-ol, camphor have been reported to possess antimicrobial, antioxidant, and anticholinesterase activities (Gonçalves and Romano, 2013). Many studies in the last years evaluated the insecticidal activity of the essential oils against plant pests (Soares et al., 2019) and store product pests (Akami et al., 2019). Essential oil is stored in the oil glands of flowers and extracted from fresh or dried flowering tops by steam distillation or hydro-distillation. Various factors influence the content and composition of the lavender essential oil, like the genotype, climatic conditions, growing location, stage of development, storage conditions, distillation conditions. Many studies in the last years evaluated the insecticidal activity of the essential oils against plant pests (Isman and Seffrin, 2018) and store product pests (Akami et al., 2019). Many scientists researched the composition of the essential oil and the antioxidant activities of lavender (Bajalan et al., 2016; Kıvrak, 2018). Antioxidants neutralize free radicals, protect cell molecules, and preserve food quality for a longer time (Pourabdal el al., 2021).

Traditionally, lavender cultivation in Bulgaria is concentrated in the region of the Thracian valley. The total lavender areas in the last years have expanded, which is related to increasing cultivation outside the traditional areas, for example, the region of Dobruja - known as the granary of the country. The experience of the farmers indicates that the lavender grown in this area can realise its productive potential, but there is no comparative study on the essential oil chemical composition of the lavender varieties cultivated in this untraditional region. In this connection, the study aims to define which variety demonstrates the highest content of essential oil with the better proportion of the compounds.

2. Material and Methods

During the period from 2014 to 2017 in the region of Dobruja, village Kapinovo (43.759° N 27.977° E)– Northeastern Bulgaria, a field experiment was conducted to achieve the aims of the study. There were used four varieties of lavender - "Hemus,Yubileyna", "Druzhba", and "Sevtopolis. The experiment was arranged according to the randomized complete block design in four replications and a plot size of 10 m^2. Fresh plant material was collected from five years old plants at a flowering stage when the concentration of essential oil reached its maximum. The plants were planted in rows at the spacing of 1.4 m x 0.35 m (Yankov et al., 2013) and grown under conventional practice. Flowers were cut and directly transported to the laboratory of the Department of Crop Science at the Agricultural university-Plovdiv, where distillation took place. The essential oil content was determined with the help of a Clevenger type distillation apparatus. Fifteen grams of lavender flowers were subjected to 240 mL of water for 105 min (hydrodistillation). The procedure was repeated three times for each sample. The obtained essential oil was stored in the refrigerator (4 ^{0}C) until the gas chromatograph analysis. The essential oil of lavender was analyzed on a Hewlett Packard 6890 gas chromatograph with an autosampler (carrier gas helium, 40 cm s^{-1}; 11.7 psi [60 ^{0}C]; 2.5 mL min $^{-1}$ constant flow rate; injection: split [60:1], 0.5 µL, inlet 220 ^{0}C; oven temperature program: 60 ^{0}C for 1 min, 10 ^{0}C min $^{-1}$ to 250 ^{0}C; column: HP-INNOWAX [cross-linked polyethylene glycol], 30 m x 0.32 mm x 0.5 µm; flame ionization detector temperature 275 ^{0}C). The gas chromatograph analysis detected and identified 12 different compounds in the lavender oil: Limonene (L), cis-β-Ocimene (cis), β-phellandrene (β), 1,8-cineole (cin), Linalool (L), Camphor (Cam), Linalyl acetate (LA), Terpinen-4-ol (T), Lavandulol acetate (LVA), Lavandulol (LV), α-Terpineol (α-T), trans-β-Ocimene (O).

The statistical processing of the data was carried out through ANOVA, as the differences between the variants were established by the multi-rank LSD test. The correlation analysis is performed with XLSTAT Version 2016.02.

2.1. Soil and climatic characteristics of the region

Gyurov and Artinova (2015) defined the soils in the region as slightly leached chernozem. The humus horizon is relatively strong, extending on average in the range of 60-80 cm. In the surface layers, the total nitrogen reserve varies from 156 to 166 mg 100 g^{-1} soil by Kjeldahl, which characterizes the soil as moderately stocked with nitrogen. These soils belong to the group of poorly stocked with phosphorous and medium stocked with potassium ().

In terms of the climate conditions, the average monthly temperatures in the survey years did not differ significantly from those of the long-term period (Table 1). For the triennial period, the average daily temperature values exceed the perennial ones. According to the amount of precipitation, the values are close to or slightly higher than those of the long-term period.

Table 1. Meteorological data of the experimental field*

Year	Temperature (^{0}C)											
	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII
2014/2015	23.2	17.5	11.2	5.0	2.0	1.4	2.0	5.0	10.0	16.4	19.4	23.0
2015/2016	22.8	19.5	10.9	5.6	3.1	-0.8	7.3	6.8	13.2	14.7	20.9	23.7
2016/2017	22.7	18.1	10.6	6.5	-0.6	-4.1	2.0	7.3	8.7	15.0	20.0	22.5
long-term average	20.6	16.2	11.5	6.1	1.8	-0.5	0.6	3.8	9.4	14.8	18.8	21.0
	Precipitation (mm)											
2014/2015	34	31.4	58	33.2	101.4	33.2	79.5	67.7	46.3	12.9	31.3	30.2
2015/2016	42.0	21.0	78.3	33.2	2.0	86.3	40.7	52.7	20.8	117	55.7	2.8
2016/2017	12.4	35.8	72.0	43.0	12.5	48.4	27.4	49.0	38.4	29.0	87.7	66.5
long-term average	44.0	31.0	37.4	50.0	38.7	30.5	40.7	32.7	44.6	50.0	67.3	50.0

*Meteorology Station Kapinovo.

3. Results and discussion

The increased requirements in recent years to the quality, safety, and authenticity of essential oils can be met with an objective characteristic of their chemical composition. Table 2 presents the results of the gas chromatographic analysis of lavender oils from the tested varieties during the three experimental years. The main components in lavender essential oil are linalool and linalyl acetate. Linalool, linalyl acetate, camphor, and lavandulol were determined as the main constituents in the fresh stem flower essential oil of lavender reported from Blažeković et al. (2018). The same authors registered higher linalool content in all tested varieties of lavender. The most important ingredient when determining the quality of lavender oil is linalyl acetate. Its high content is an indicator of good quality.

This content is highest by the Hemus variety (from 35.98 to 42.00%) and meets the requirements of the standard for lavender oil (from 25 to 45%). By the variety Sevtopolis the content of linalyl acetate over the years is slightly below the values of the standard, while by the varieties Yubileyna and Druzhba in 2015 and 2017 experimental years, the values are like by the standard, and in 2016 - are about 17% lower. The results determined in the present study are in line with the observations of Zagorcheva et al. (2013) and Stanev et al. (2016). Of great importance for the quality of the essential oil is the ratio between linalyl acetate and linalool, which for the typical Bulgarian lavender oil should be in a ratio of

1: 0.7 (Stanev and Dzhurmanski, 2011). The content of linalool by the studied varieties between 27.66-38.92% and is within limits and slightly above the standard (25 - 38% according to ISO 3515: 2002). The ratio between the two components is observed only by the variety Hemus, while by Yubileyna, Druzhba, and Sevtopolis, the amount of linalyl acetate is less than that of linalool. Many authors also stated that the main components of lavender essential oil were linalool and linalyl acetate and the contents varied between 37.0-54.0% and 21.0-36.0% (Carrasco et al., 2016), 28.10-36.80% and 29.09-46.88% (Kıvrak, 2018), 23.51-27.39% and 26.60- 40.66% (Bogdan et al., 2020) respectively. Camen et al. (2016) stated that linalool (30.39%) and linalyl acetate (23.60%) exceeded half of the total content of essential oil components. According to Özel (2019) the dominating constituents of the essential oil of *Lavandula angustifolia* leaves were 1.8 cineol 35.24% and camphor 21.79%. Yildirim et al. (2019) reported that the percentage of the essential oil components was influenced by the time of harvest, as the highest essential oil (10.27%) was obtained from flowers harvested at 15:00 with linalool as the main compound. Moreover, the essential oil contents changed between 6.73-10.27% for flowers, 0.29-0.76% for peduncles, and 0.08-0.42% for leaves.

Other chemical ingredients that determine the quality of the essential oil are lavandulyl acetate, lavandulol, α-terpineol, 1,8-cineole, and limonene. In terms of the content of lavandulol, α-terpineol, 1,8-cineole, and limonene, all varieties meet the standards.

Table 2. Essential oil composition (%) in fresh stem flowers of lavander

Essential oil components	RT	Cultivar											
		Hemus			Druzhba			Sevtopolis			Yubileyna		
		2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Limonene	19.7	0.25	0.34	0.43	0.27	0.30	0.39	0.25	0.49	0.66	0.36	0.31	0.43
cis-β-Ocimene	20.3	2.82	3.11	3.82	2.09	4.47	3.96	2.55	1.95	3.09	2.45	2.92	9.01
β-phellandrene	48.9	0.08	0.36	0.30	0.43	0.52	0.40	0.27	0.42	0.95	0.56	0.77	0.38
1,8-cineole	19.9	0.06	0.40	0.94	0.70	0.43	0.86	0.55	0.75	0.15	0.64	0.40	0.91
Linalool	24.7	27.6	37.1	36.7	34.2	28.0	31.1	36.2	35.1	38.9	31.5	29.6	36.9
Camphor	27.7	0.11	0.13	0.16	0.14	0.20	0.18	0.18	0.28	0.15	0.20	0.15	0.21
Linalyl acetate	35.5	39.7	42.0	35.9	32,1	24.9	33.0	29.3	25.5	28.0	30.0	26.5	33.0
Terpinen-4-ol	30.0	0.14	0.86	1.61	3.15	3.43	3.20	3.21	4.36	0.62	3.39	4.78	3.83
Lavandulol acetate	37.7	3.78	2.10	2.24	3.64	3.20	3.34	3.54	4.36	2.32	3.06	3.48	2.93
Lavandulol	49.2	0.55	0.39	0.48	1.54	1.42	1.39	1.42	1.36	0.35	1.14	1.14	1.16
α-Terpineol	30.9	1.53	1.70	1.60	1.14	1.16	1.22	1.30	1.77	1.88	1.29	1.40	1.27
trans-β-Ocimene	21.0	3.33	1.32	3.92	2.55	2.12	3.41	2.57	2.84	3.33	3.20	4.00	3.94

A serious problem in the last few years is the low content of lavandulyl acetate in Bulgarian lavender oil intended for export. In the region of Northeastern Bulgaria, all tested varieties of lavender meet the standard (2-5%). Undesirable ingredients in the essential oil are terpinene-4-ol and camphor. According Baydar (2009), the camphor content in a quality lavender oil must be between 0.5 and 1%. The modern breeding in Bulgaria was aimed at creating varieties with a very low content of terpin- 4-ol. That is why its content is very low by all Bulgarian varieties. The ISO 3515: 2002 requirement for camphor content is up to 1%. Depending on the year, by the tested varieties, the amount of camphor varies from 0.11 to 0.28%, which is significantly lower than the maximum permissible quantity according to ISO 3515: 2002.

The yield of essential oil (Y) varies depending on the climatic conditions of the year and the genotype (Table 3).

In the first year of the study, the values are higher by all tested varieties, which could be explained with the favourable wetter conditions - optimal temperature values and evenly distributed rainfall, as the values vary between 179 l ha^{-1} by Sevtopolis to 120 l ha^{-1} by the variety Hemus. More abundant rainfall in May and June in 2016 and 2017 led to a decrease in the average yields of essential oil by the tested varieties with respectively 31% and 47.5%.

Table 3. Yield of essential oil l ha^{-1})

Variety	2015	2016	2017	Average
Hemus	120c	80c	70c	90c
Yubileyna	150b	100b	80b	110b
Druzhba	170a	120a	85a	125a
Sevtopolis	179a	127a	90a	131a
LSD 5%	*25.3*	*19.8*	*9.4*	*18.2*

*Values with the same letters do not differ significantly.

Table 4. Two-way ANOVA analysis of variance

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Years	42867	2	21433	1148.24	0.00*	3.26
Varieties	12093	3	4031	215.95	0.00*	2.87
Interaction	2473	6	412	22.09	0.00*	2.36
Within	672	6	18			
Total	58106	47				

*Significance at 0.05 level.

In the second year the maximal yield of essential oil was observed by Sevtopolis (127 l ha^{-1}), and the minimal by the variety Hemus (80 l ha^{-1}). During the third year are registered the lowest values of the examined parameter by all varieties due to the highest precipitation of 66.5 mm and the lowest temperature of 22.5^{0} C during the harvest period. On average for the three years, the highest yield of essential oil was reported by the variety Sevtopolis - 131 l ha^{-1}, followed by Druzhba with 125 l ha^{-1}, Yubileyna with 110 l ha^{-1}, and at least the variety Hemus with a value of 90 l ha^{-1}. The mathematical processing of the data shows that the differences between the varieties Sevtopolis and Druzhba are statistically non-significant, not only by years but also for the three years of the experiment. Giannoulis et al. (2020) reported smaller essential oil yields ranging between 14 kg ha^{-1} and 85 kg ha^{-1}, as the highest values were observed after treatment with bio-stimulator. As per the essential oil content, the values varied depending on the variety and the climate conditions of the year, and on average, for the period, the varieties could be arranged in the following descending order: Sevtopolis (2.1%), Druzhba (1.9%), Yubileyna (1.8%) and Hemus (1.6%) (Georgieva et al., 2021). The lowest values were reported in the third year of the investigation when the content of essential oil in the tested varieties had similar values of 1.3% by the varieties Druzhba and Hemus, 1.4% - by the variety Yubileyna, and 1.5% by the variety Sevtopolis. Many researchers reported that the essential oil content may vary due to soil and climatic factors or genetic characteristics of the variety (Hassiotis et al., 2014; Dudaa et al., 2015). Chrysargyris et al. (2016) stated that because of different fertilization treatments, the lavender essential oil ranged from 0.71 to 1.14%. In an experiment conducted in Ankara, Turkey the reported lavender essential oil content ranged between 0.7 and 2.6 % (Kirimer et al., 2017), while Rasheda et al. (2017) reported smaller contents (0.61- 0.81%).

The results from the performed two-way ANOVA analysis of variance (Table 4) confirm that both factors – "year" and "variety" proven to influence the yield of essential oil, as their interaction is also significant.

From the performed correlation analysis, it can be concluded that there exists a positive correlation between the yield of essential oil and the content of Lavandulol acetate (0.539); Limonene content and the content of β-phellandrene (0.597) and the content of α-Terpineol (0.688) (Table 5). The strongest relation is observed between the variables Terpinen-4-ol and Lavandulol (0.775). A strong negative correlation is determined between the content of Linalyl acetate and the content of Terpinen-4-ol (-0.848), as well as with the content of Lavandulol (-0.794). Analogical to the content of Linalyl acetate, the values of the variable yield of essential oil tend to decrease when the values of the Limonene, cis-β-Ocimene, and trans-β-Ocimene increase because of the determined negative correlation. A negative correlation is also determined between the content of camphor and the content of Linalyl acetate (-0.614).

Table 5. Correlation matrix (Pearson)

Variables	Y	L	cis	β	cin	L	Cam	LA	T	LVA	LV	α-T	O
Y													
L	0.466												
cis	0.501	0.043											
β	0.152	**0.597**	0.076										
cin	0.096	0.017	0.363	0.222									
L	0.196	0.565	0.158	0.160	0.277								
Cam	0.121	0.282	0.167	0.073	0.531	0.114							
LA	0.330	0.249	0.094	**0.602**	0.068	0.129	**0.614**						
T	0.223	0.117	0.128	0.509	0.398	0.037	**0.666**	**0.848**					
LVA	**0.593**	0.355	0.290	0.251	0.025	0.493	0.405	0.411	0.416				
LV	0.531	0.047	0.027	0.375	0.209	0.084	0.494	**0.794**	**0.775**	0.520			
α-T	0.414	**0.688**	0.264	0.213	0.352	0.492	0.027	0.165	0.333	0.292	0.478		
O	**0.619**	0.266	0.397	0.125	0.213	0.122	0.143	0.208	0.029	0.257	0.383	0.202	

*Values in **bold** are different from 0 with a significance level alpha =0.05.
*Abbreviations are given in the material and methods.

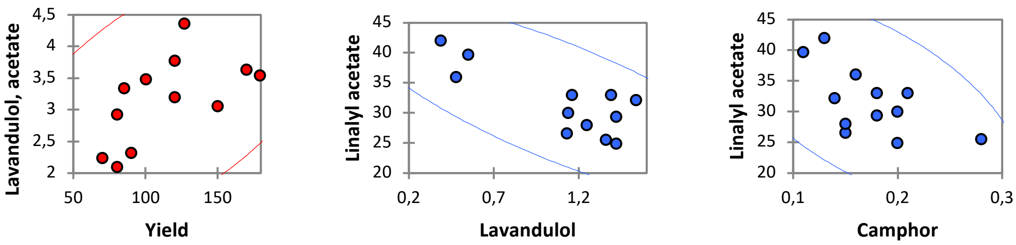

Figure 1. Correlation scatterplots between the variables.

With larger values of the yield of essential oil, the values of the variable Lavandulol acetate also had a tendency to increase. The scatterplots illustrate the determined relations (Figure 1) and define the negative relations between the Linalyl acetate and the variables Lavandulol and Camphor.

Conclusion

For the untypical lavandula growing region of Northeastern Bulgarian, the variety Sevtopolis distinguished with the highest yield of essential oil during the studied period, but the chemical composition of the essential oil was better by the variety Hemus, which determines the last one as more appropriate for oil production. The content of the linalyl acetate by the variety Hemus is the highest and meets the requirements of the international standard, as only by this variety the ratio between linalyl acetate and linalool is 1: 0.7, and the essential oil could be defined as high qualitative. The performed analysis confirmed that the yield of essential oil is dependent, not only on the weather conditions during the harvest by also on the genotype.

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