Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 24 (3):268-278, 2019
(*Mustafa Kemal University Journal of Agricultural Sciences* 24 (3):268-278, 2019)
e-ISSN: 2667-7733
http://dergipark.org.tr/mkutbd

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Diurnal and nocturnal variability of essential oil content and components of *Lavandula angustifolia* Mill. (Lavender)

Diurnal ve nocturnal varyabilitenin *Lavandula angustifolia* Mill. (Lavender) uçucu yağ içeriğine ve bileşenlerine etkisi

Mehmet Uğur YILDIRIM[1], Ercüment Osman SARIHAN[1], Havva KUL[1], Khalid Mahmood KHAWAR[2]
[1]*Usak University, Faculty of Agriculture and Natural Sciences, Department of Field Crops, Uşak, Turkey.*
[2]*Ankara University, Faculty of Agriculture, Department of Field Crops, Dışkapı-Ankara, Turkey.*

MAKALE BİLGİSİ / ARTICLE INFO

Makale tarihçesi / Article history:
Geliş tarihi /Received:11.07.2019
Kabul tarihi/Accepted:23.10.2019

Keywords:
Harvest time, essential oil, compositions, Mediterranean (Csa type) climate.

Corresponding author: M. Uğur YILDIRIM
✉: ugur.yildirim@usak.edu.tr

Ö Z E T / A B S T R A C T

Aims: The study aimed to make a comprehensive comparison in the effects of both diurnal and nocturnal variations at full bloom on dried flowers, peduncles and leaves were checked on the essential oil contents and components of lavender under hot-summer Mediterranean (Csa type) climatic conditions of Uşak province (Turkey).

Methods and Results: The samples of flowers, peduncles and leaves were harvested at full blooming (14 July) using eight different times (06:00, 09:00, 12:00, 15:00, 18:00, 21:00, 00:00; 03:00) in 24 hours. The air-dried (shade) 100 g each of flowers, peduncles and leaves were taken from the plants and water distilled for 3 h with usage of a Clevenger-type apparatus. Than, the essential oil samples were subjected to GC-MS analysis for their components.

Conclusions: The percentage of essential oil components varied and was influenced by the type of sample and the time of harvest. The results demonstrated that 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. According to GC-MS analysis; *Linalool, terpinene-4-ol, camphor, borneol, 1,8 cineole, lavandulol, β-pinene* and *β-farnesene* were determined as the main components during full flowering period. The highest essential oil (10.27%) was obtained from flowers harvested at 15:00 with *linalool* as main compound of Lavender essential oil.

Significance and Impact of the Study: It was concluded that the harvest at 09:00 should be preferred for production of high quantity of camphor and nocturnal harvest at 03:00 should be preferred for production of high quality linalool from Lavender essential oils. The results depicted that the amount of linalool was higher in flowers and peduncles and camphor was higher in leaves.

Atıf / Citation: Yıldırım MU, Sarıhan EO, Kul H, Khawar KM (2019) Diurnal and nocturnal variability of essential oil content and components of *Lavandula angustifolia* Mill. (Lavender). *MKU. Tar. Bil. Derg.* 24(3) : 268-278

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill.) in Lamiaceae family is an aromatic perennial shrub with narrow and linear leaves and violet-blue flowers (Upon, 2002) are highly aromatic in nature (Harborne and Williams, 2002) and are grown and cultivated in several parts of the World, especially Mediterranean countries (Beetham and Entwistle, 1982; Ilieva-Stoilova et al., 2002; Kara and Baydar, 2013.) and South-East India. There are about 30 species of lavender, with dozens of subspecies, varieties, and hybrids that are selected for culture (Koulivand et

al., 2013; Prusinowska and Smigielski, 2014; Camen et al., 2016). Lavandula species are being cultivated over two hundred thousand hectares in Europe since a long time (Hassiotis et al., 2010). Only 3 taxa are used in trade to produce essential oils for usage in cosmetics and perfume industries. These taxa (Two species *L. angustifolia* Mill., *L. latifolia* Medik and one hybrid lavandin *L. intermedia* Emeric ex Loisel) are widely cultivated in Turkey (Tucker, 1985; Kara and Baydar, 2013). Turkish province of Isparta is the most prominent place where lavender is widely cultivated. (Kara and Baydar, 2011). They produce complex mixtures of essential oils from glands on the surface of leaves and flowers. The essential oils of these species are very valuable. Essential oils of lavender are used in perfumes and aromatherapy (Lis-Balchin, 2002).

The essential oils chemical composition is known by the availability of terpenes (e.g. *linalyl acetate* and *linalool*) and terpenoids (e.g. *1,8-cineole*). These components have revealing the characteristic flavor of essential oil and its biological and therapeutic properties (Lesage-Meesen et al., 2015). The essential oil contains more than 100 components with *camphor, linalool, 1,8-cineol* and *linalyl acetate* as the main components.

Lavender has been used for a long time as a medicinal plant (Castle and Lis-Balchin, 2002), and it was used as antiseptic, disinfectant and relaxant since Roman times. It was used as a culinary herb, for medicinal and therapeutic purposes (Holmes, 2002). Today, lavender is mainly used as a source of the essential oil for use in cosmetics, perfumes, soaps, some veterinary shampoos and in the manufacture of other products like insect repellents (Castle and Lis-Balchin, 2002). Their extracts are also used as additives to expand shelf-life of beverages, foods, cosmetics and their antimicrobial and antioxidant characteristics (Deans, 2002).

Amount of *linalyl acetate* and *linalool* increase commercial value of the lavender essential oil, but the essential oil quality decrease with increase of camphor contents (Adam, 2006). Although the effect of genetic factors on the essential oil content is high, some applications such as geographical distribution, drying techniques, climatic and seasonal conditions, distillation method (Sefidkon et al., 2006; Kara and Baydar, 2013), distillation time (Cannon et al., 2013; Kara and Baydar, 2013), harvesting season, harvest periods and time affect essential oil yield and components (Lammerink et al., 1989). In addition, plant growth regulators and plant hormones are among the factors that affect the essential oil production (İzmirli and Yildirim, 2018). They were determined that different doses of GA_3 and harvest times had different effects on essential oil content and components. Therefore, studies on determining essential oil of lavender have gained importantance. High and the best essential oil yield and quality must be determined in locations, where the plant is cultivated.

Some studies report essential oil content and components from lavender in Turkey (Arabacı ve Ceylan, 1990; Başer, 1993; Arabaci and Bayram, 2005; Baydar, 2007; Atalay, 2008; Kara and Baydar, 2011; Kara and Baydar, 2013; Karık et al., 2017 etc.). However, there are no adequate studies on the comparison in changes of lavender essential oil content and components along with their chemical composition under diurnal and nocturnal conditions.

In view of the above information, the study aimed to make a comprehensive comparison in the effects of both diurnal and nocturnal variations (06:00, 09:00, 12:00, 15:00, 18:00, 21:00, 00:00, 03:00) on the essential oil contents and components of lavender under hot-summer Mediterranean (Csa type) climatic conditions of Uşak province (Turkey).

MATERIAL and METHODS

This study was conducted on the hot summer ecological conditions on the Mediterranean climate (Csa- Köpen Geiger classification) at Uşak province of Turkey (Anonymous 2018a). Lavender plants growing for 4 years at the Aromatic and Medicinal Plants Garden of the Faculty of Agriculture and Natural Sciences of Uşak University.

Uşak province (Aegean region of Turkey) lies 911 meters above sea level (located at a 38° 40' N latitude and 29° 24' E longitude) that is surrounded by Afyon, Denizli, İzmir and Kütahya provinces. It has total precipitation of 547.3 kg m^{-2} with annual mean maximum and minimum temperatures of 12.5 °C, 23.4 °C (July, August) and 2.3°C (January) in the same order with a mean precipitation of 14.9 kg m^{-2} in July 2016. The average temperature was 25.2 °C, average relative humidity was 40.9%, and monthly total precipitation of 6.4 kg m^{-2} during July 2016 when the plants were harvested. Total recorded rainfall during 2016 is 456.3 kg m^{-2}. The total amount of rainfall recorded in July 2016 is lower than the monthly average of a long-time average. The data was taken from the Directorate General of Meteorology, Ankara showed day length of 14.37 (Sunrise at 05.50 hours, sunset 20.27 hours) hours (Anonymous 2018b).

The experiment determined both diurnal and nocturnal variabilities in essential oil content using a completely randomized block design technique with 3 replicates. The samples of flowers, peduncles and leaves were harvested at full blooming (14 July) using eight different

times (06:00, 09:00, 12:00, 15:00, 18:00, 21:00, 00:00, 03:00) in 24 hours. The samples were dried under shade in a cool, dry ventilated place (24±1 °C).

Essential Oil Isolation

The air-dried 100 g each of flowers, peduncles and leaves were taken from the plants and water distilled extraction for 3 h with the usage of a Clevenger-type apparatus as per the standard protocol described in the European Pharmacopoeia to determine the oil content (v/w %). The extracted essential oils were collected and dried with (anhydrous) sodium sulfate (Na_2SO_4) and stored in amber vials at +4 °C before analysis of components. Thereafter, the essential oil samples were subjected to GC-MS analysis.

GC-MS Analysis Conditions

Essential oil components were analyzed in the Central Laboratories of Uşak University (UBATAM). Agilent 7890 A GC, equipped with an HP-5 MS capillary column (30 m × 0.250 mm, 0.25 Mm) and an HP 5975 C mass selective detector was used for the analysis of essential oil. The electron ionization energy of 70 eV was maintained for GC-MS detection. Helium was used as a carrier gas and used at a flow rate of 1 mL/min. the injector and MS transfer line temperatures were set at 220 and 290 °C, in the same order. Column temperature remained 50°C for the first 3 min, followed by a gradual increase to 150°C at a 3°C/min rate. Finally, the temperature was raised to 250°C at the increase rate of 10°C/min in 10 min. The samples were diluted (1/100 in hexane, v/v) of 1.0 µL and were injected automatically in splitless mode. The components identification depended on comparison among their relative retention time and mass spectra with the predetermined standards, NIST05a, Wiley library data of GC-MS system and literature on the subject (Basalma et al., 2007).

The means of essential oil contents of harvests during diurnal and nocturnal times were subjected to ANOVA (Analysis of variance) using the IBM SPSS version 24 computer software at the α=0.01 level. Comparison among the means was made using DMRT (Duncan Multiple Range Test) following Düzgüneş et al., (1983).

RESULTS and DISCUSSION

Essential Oil Content (%)

Diurnal and nocturnal changes of lavender essential oil contents were determined in this study. Analysis of variance data shows significant (p<0.01) differences among means of the essential oil contents in all treatments obtained from three different parts of the plants.

Essential oil contents changed between 6.73 - 10.27% in dry flowers, 0.29 - 0.76% in peduncles and 0.08 - 0.42% in leaves. The highest (10.27%) and lowest (6.73%) essential oil content were noted from flowers harvested in-between 15:00 and 06:00 using the same order (Table 1). The highest (0.76% and 0.42%) and the lowest (0.29% and 0.08%) essential oil contents in peduncle and leaves were determined at 06:00 and 15:00 and 03:00 and 21:00 respectively. The essential oil content of dry flowers at 15:00 harvest were significantly (p<0.01) different and higher compared to other harvest times. Generally, essential oil content means of fowers were 7.92%; in the peduncle & leaves as 0.51% & as 0.17% respectively.

Table 1. Essential oil contents (%) obtained at different harvest times from different parts of the lavender plant.

Harvest Time	Flowers **	Peduncle**	Leaves**
06: 00	6.73d	0.76a	0.20b
09: 00	8.33b	0.51d	0.20b
12: 00	6.87d	0.40e	0.13c
15: 00	10.27a	0.50d	0.42a
18: 00	8.33b	0.42e	0.10c
21: 00	7.53c	0.57c	0.08c
00: 00	7.67c	0.63b	0.10c
03: 00	7.60c	0.29f	0.10c
Means	**7.92**	**0.51**	**0.17**

*** Means shown in the same columns using different letter (s) show that they are significantly different at the 0.01 level of significance using Duncan Multiple Range Test.*

The results of this study make a comparison among diurnal and nocturnal harvest times and recommend harvest of flowers either at 15:00 or 18:00 and 09:00 during the full blooming stage to get the maximum amount of essential oil contents. These results indicated that the harvesting time is very important to obtain the

desired essential oil contents and rates of components in the essential oils of lavender. Hassiotis et al. (2010) emphasized that the diurnal essential oil yield of lavender presented non-significant differences. Contrarily, the major or main compounds showed diurnal fluctuations. They mentioned that at 12:00 and 15:00 harvests are suitable times for lavender harvesting. In terms of dry flower essential oil contents, the esults of this study are in agreement with the findings of the previous research.

Ceylan et al. (1988) observed the essential oil content of lavender varied between 1.26 and 3.14%. Renaud et al. (2001) from flower essential oil content ranged 2.8 - 5.0% in lavender and 7.1 - 9.9% in lavandin samples in the same order. Atalay (2008) and Arabaci and Bayram (2005) noted that the lavender essential oil contents varied between 2.1-2.6% and 1.54 - 2.34% respectively. Kara ve Baydar (2011) emphazied that in dry flower samples of *L. intermedia* var. Super A, essential oil contents flower ranged 7.50 - 8.60% at Isparta province hot-summer Mediterranean (Csa type) climatic conditions.

Kara and Baydar (2013) reported that agricultural properties of the lavandin and lavender cultivars during 2009 and 2010. The highest dry floral yield was obtained from -lavandinr cultivar Super A (first year 1083 kg ha^{-1} and second year 1463 kg ha^{-1}). The highest essential oil content in dry flowers 9.62% during the first year and 8.87% during the second year from lavender cultivar Silver. The minimum yield of dry flower essential oil contents were noted from lavender cultivar Munstead (first year 2.10% and second year 2.30%).

Essential Oil Components of Flowers

The essential oil content of lavender flowers is much higher compared to the essential oils obtained from peduncle and leaves in terms of quality and quantity. The changes under the experimental conditions of this experiment in some major components of lavender essential oils are presented in Figure 1 below. Linalool was the main component of lavender essential oil from flowers with the highest percentage at all harvest times. *Linalool* contents of the essential oils of the flowers increased from 39.91% to45.45% at 21:00 to 03:00 respectively.

Linalool contents of the essential oils was ranged between 21.2% and 36.9%. In the peduncle, *Linalool* content of the essential oils was ranged between 0.9% and 13.2% at 12.00 and 03.00 respectively (Figure 1). Hassiotis et al. (2010) emphasized that *Linalool* contents changed from 34.38% in the morning to 28.76% in the evening. According to the findings in this study, Linalool contents are lower at 21:00 and at 06:00, but the contents of linalool increases at 03:00 (Figure 1). The results obtained in general terms were higher than the values obtained by Hassiotis et al. (2010).

The changes among the other main components (*1,8-Cineole, Camphor, Borneol,*) are presented in Figure 1. The results show *camphor* contents in the essential oil of the flowers decreased from 12.52% at 09:00 to 8% at 15:00. Thereafter, it incresed again to 12.13% at 00:00, but the amount of camphor in leaves varied during the day (Figure 1). The findings of this research are not in agreement with Hassiotis et al. (2010), who mentioned that *camphor* contents changed from 3.5% in the morning to 2.48% in the evening. This could be due to variable cultivation and climatic conditions in the two experiments. Although camphor rich lavender oil is not desired for use in the perfume industry, it is characteristically highly antibacterial, antifungal and antiseptic, therefore it can be used both as a preservative and in therapeutic industry (Lis-Balchin, 2002). The essential oils of Lavandula cultivars that have high camphor contents should be used in pharmaceutical industry for the preparation of therapeutics and preservatives (Silva et al., 2017).

International Organization for Standardization (ISO 3515: 2002; TR ISO 3515: 2004), recommend that lavender essential oil composition quality standards must be between 25.0 - 38.0% for *linalool,* and, 25.0 - 45.0% for *linalyl acetate,* 4.0 - 10.0% for *cymene,* 2.0 - 6.0% for *terpinene-4-ol* and 0 - 0.5% for *camphor* if the oil has to be used in the perfume industry (Anonymous, 2002; Anonymous, 2004). Baser (1993) has also evaluated the quality of essential oil of lavender and reported *linalyl acetate* and *linalool* in the oil along *β-pinene, linalool, terpineol, camphor,* and *borneol* etc,. In this study; the *linalool* contents are higher compared to the amounts which are emphasized in ISO 3515: 2002 and TR ISO 3515: 2004; the results suggest that harvest should strictly perform around 21:00. It is well established that lavender essential oil with high *linalool* and *linalyl acetate* has a sedative activity post inhalative absorption after subjecting it to laboratory animals like goldfish, rats or mice that make their use highly desirable in the therapeutic industry (Buchbauer et al., 1991).

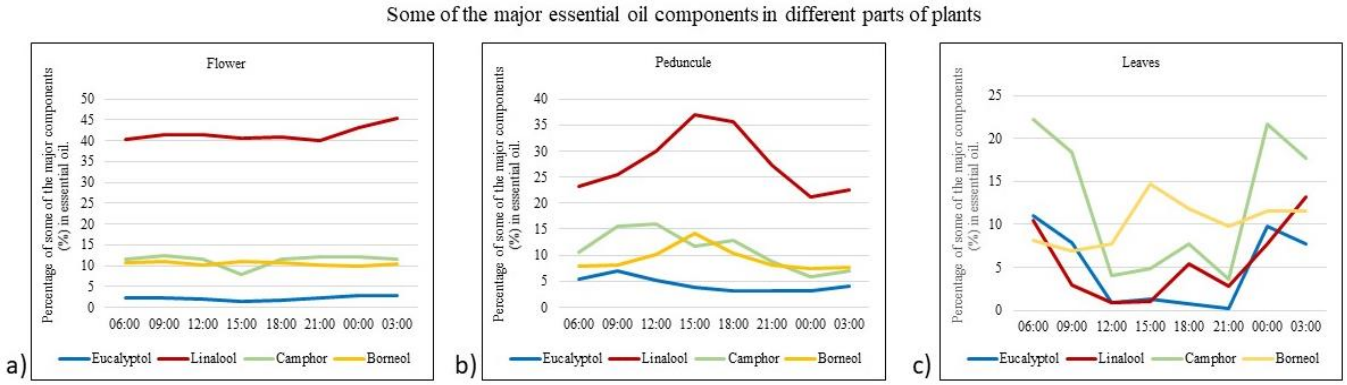

Figure 1. Diurnal and nocturnal variations of four main components of essential oil obtained from different plant parts.

When examined Figure 1; it is seen that the changes in the amount of essential oil components in lavender essential oil obtained from flowers are much more regular than the other plant parts. The amounts of the basic components in the peduncle are not as regular as in the flower, it is seen that the components in the peduncle are more regular than the changes in the leaves. The main components in the essential oil in the leaf show more complex variations. Linalool is the most common constituent of flowers, camphor and borneol are the most common constituents in the leaves.

The camphor contents were higher than the recommended amounts as described in ISO 3515: 2002 and TR ISO 3515: 2004. High camphor contents degrade the quality of the essential oil for their use in the perfume industry. However, the oil can be used for the other purposes such as antibacterial, antifungal and antiseptic, purposes (Lis-Balchin 2002). Kara and Baydar (2013) mentioned that camphor contents of lavender cultivars change between 4.11% - 6.99% in cultivar. Vera of lavender, and 8.35% - 14.3% in cultivar Silver of lavender. The highest camphor contents were obtained from these lavender cultivars.

Therefore, plants must be harvested for lowest camphor value at around 15:00 hours (afternoon). In terms of the essential oils obtained from different parts of the plant different tendencies were identified among major components (Figure 1).

The main components of lavender essential oil were *linalyl acetate* and *linalool*, and their contents ranged 30.0% - 50.0% and 15.0% - 35.0% (Wichtl 1984), and 25.82% - 54.76% and 25.1% - 59.8% (Arabaci and Bayram, 2005), and 24.0% - 29.0% and 34.3% - 54.6% (Kara and Baydar, 2011) in the same order. Camen et al. 2016, emphasized that *linalool* (30.39%) and *linalyl acetate* (23.60%) exceeded half of the total content of essential oil components in the lavender essential oil. They also obtained 8.84% camphor.

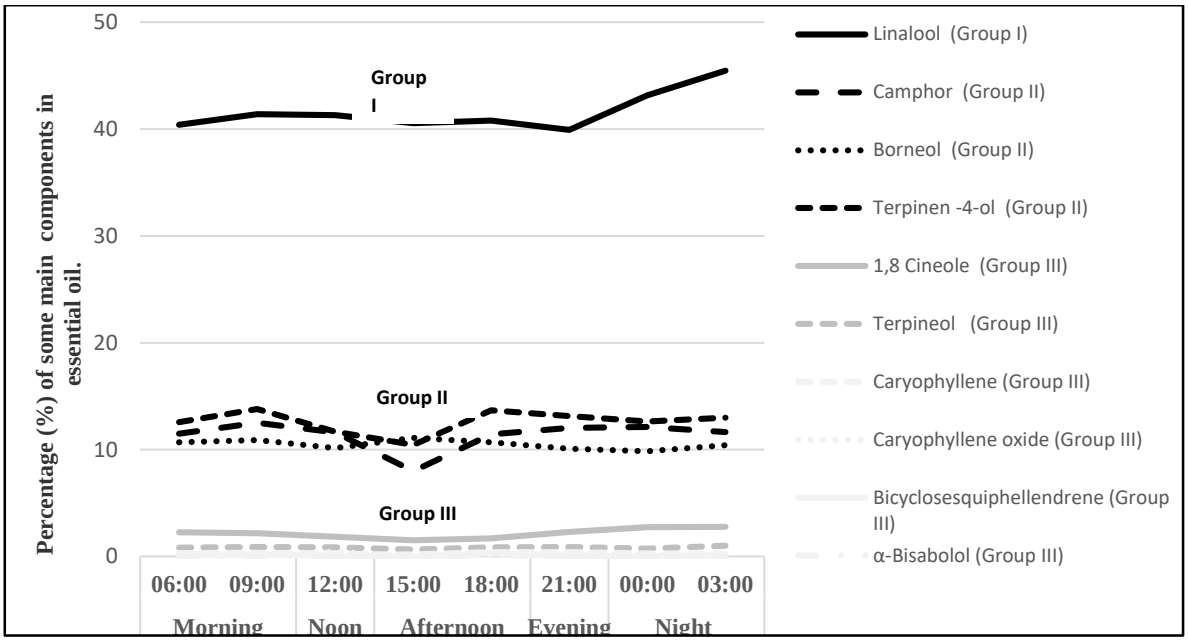

Figure 2. Variations in contents of the main components of essential oil obtained from dry flowers during a day.

Atalay (2008), noted that the lavender essential oil content varied between 2.1% - 2.6% under Konya ecological conditions in Turkey. Also, the essential oil yield was reported in a range of 1.49 - 2.53 kg/da. *Linalool* content of essential oil was noted in a range of 25.93% - 46.04%, *linalyl acetate* ranged 12.97 - 25.71% and *4-terpineol* 0.00 - 9.23%. Kara and Baydar (2011) emphasized that Lavandula intermedia var. Super A had linalool (34.3 - 54.6%), *linalyl acetate* (24.0 - 29.0%), *borneol* (1.6 - 6.7%) and *camphor* (1.2 - 6.0%) as important components of the essential oils.

The harvest times affected the major compounds of essential oil in this study; such that *Linalool* increased from 45.45% at 03:00 while decreased to 39.91% at 21:00. Besides *linalool, terpinene 4-ol, borneol, camphor 1,8 cineole, terpineol, caryophyllene* and *caryophyllene oxide* have been found as main components in the essential oil of dry flowers. Daily changes of these main components of dry flowers are illustrated in Figure 2. Comparing the amounts, the main components are shown in three different groups as shown in Figure 2. The first group consisted of *linalool,* the second group is *camphor, borneol, terpinene-4-ol,* and the others are in the third group (Figure 2). Especially, decreasing amounts of the components *camphor*, *terpinen-4-ol,* etc. which degrade essential oil quality in the afternoon are also significant. The results of this study are in line with previous literature in terms of dry flower essential oil components.

Hassiotis et al. (2010) emphasized that information about changes in compound composition during daytime are advantageous for determining of the harvest time. The percentage composition of *camphor, Llinalyl acetate*, *linalool,* and *1,8-cineole* strongly affects the aroma of the lavender essential oils. Quality of aroma is improved by the abundance of *linalyl acetate* and *linalool* in the oil. The lesser contents of *1,8-cineole* and *camphor* have wide acceptance in general.

The composition of essential oil of lavender are affected by genetic and number of abiotic and biotic factors (plant species, plant parts, plant age, temperature, rainfall, humidity, day length, winds, location, altitude attack of insect and fungal pathogens etc.) (Wichtl, 1984; Harborne and Williams, 2002; Arabaci and Bayram, 2005; Atalay, 2008; Hassiotis, et al., 2010; Kara and Baydar, 2011; Kaya et al., 2012; Kara and Baydar, 2013). Although the effect of genetic factors on the essential oil content is high, some applications such as distillation method (Sefidkon et al., 2006; Kara and Baydar, 2013), distillation time (Cannon et al., 2013; Kara and Baydar, 2013) and harvest periods affect essential oil yield and components. These contents also vary in components and depend on changes in climatic, seasonal conditions and geographical distribution or harvesting seasons, drying techniques and the harvesting time. At the same time, it is possible to influence the production and chemical composition of plant essential oils by external applications as well as the levels of plant growth regulators and hormones in the plant (İzmirli and Yildirim, 2018). According to their findings, the highest amount of essential oil was obtained from the flowers (% 8.2) which were applied 400 mg/l GA_3 and harvested on the second day (54th hour). Some important essential oil components such as *linalool, terpinene-4-ol, camphor,*

borneol, 1,8-cineole, lavandulol, and more than 50 components have been identified.

Table 2. Diurnal and nocturnal variation of essential oil components in dry flowers of lavender (*Lavandula angustifolia* Mill).

	Component names	Morning				Noon		Afternoon	
		06:00		09:00		12:00		15:00	
		RT	%	RT	%	RT	%	RT	%
1	α-pinene	8.614	0.24	8.615	0.25	8.616	tr	8.623	0.10
2	campnhene	9.192	0.21	9.187	0.20	9.194	tr	9.201	0.10
3	3-octanone	10.565	0.26	10.566	0.25	10.571	0.23	10.585	0.10
4	β-myrcene	11.063	0.12	11.064	0.10	11.065	tr		nd
5	acetic acid hexyl ester	12.116	0.19	12.116	0.16	12.118	0.15	12.142	0.10
6	o-cymene	12.505	0.13	12.500	0.13	12.507	0.15	12.502	0.13
7	limonene	12.682	0.48	12.683	0.43	12.684	0.19	12.680	0.36
8	1,8-cineole	12.779	2.26	12.780	2.17	12.781	1.86	12.811	1.51
9	(E)β-ocimene	13.191	0.50	13.192	0.46	13.188	tr	13.189	0.29
10	y-terpinene	14.090	0.11	14.090	tr		nd		nd
11	cis-sabinene hydrate	14.484	0.18	14.480	0.20	14.487	0.19		nd
12	trans-linalool oxide	14.776	0.11		nd		nd	14.768	0.26
13	1-octanol	14.834	0.13	14.783	0.21		nd	-	nd
14	cis-linalool oxide		nd		nd	14.756	0.89	15.512	0.26
15	4 -carene	15.451	0.24	15.452	0.19	-	nd	-	nd
16	Linalool	16.373	40.40	16.299	41.39	16.323	41.31	16.061	40.54
17	camphor	18.124	11.51	18.084	12.52	18.103	11.63	18.018	8.00
18	propanoic acid 2-methyl-hexyl ester	18.415	0.12	18.411	0.13	18.412	0.12	-	nd
19	borneol	19.165	10.68	19.109	10.90	19.127	10.14	19.031	11.11
20	lavandulol	19.325	2.23	19.280	2.34	19.293	2.25	19.203	1.77
21	terpinen- 4- ol	19.743	12.58	19.691	13.80	19.711	11.69	19.597	10.46
22	cryptone	20.058	0.27	20.047	0.30	20.048	0.28	-	nd
23	a-terpineol	20.275	0.84	20.259	0.89	20.266	0.87	20.244	0.66
24	butanoic acid hexyl ester	20.441	0.86	20.430	0.91	20.437	0.80	20.421	0.39
25	isoborneol	20.864	0.11	20.854	tr		nd		nd
26	bornyl formate	21.952	0.13	21.941	0.11	21.942	0.12		nd
27	sabinene		nd		nd		nd		nd
28	nerol		nd	22.021	0.10		nd		nd
29	hexyl 2-mehylbutanoate	22.472	0.39	22.467	0.40	22.467	0.32	22.464	0.16
30	hexyl isovalerate		nd		nd	22.692	0.19		nd
31	hexyl n-valerate	22.695	0.21	22.691	0.21		nd		nd
32	β-pinene	23.336	2.44	23.326	2.27	23.327	2.21	23.311	1.05
33	lavandulyl acetate	24.916	1.59	24.911	1.47	24.918	1.60	24.907	0.69
34	hexyl tiglate	26.638	0.17	26.633	0.18	26.634	0.19		nd
35	thymol		nd		nd	27.104	0.22		nd
36	hotrienol		nd		nd	27.887	1.21		nd
37	3-carene	28.097	0.24		nd		nd		nd
38	geranly acetate	28.927	0.28		nd	28.105	0.11		nd
40	hexanoic acid. hexyl ester	29.035	0.29	29.031	0.28	29.032	0.29	29.033	0.13
41	zingiberene	29.167	0.13	29.162	0.13	-	nd		nd
42	caryophyllene	30.311	0.34	30.312	0.36	30.308	0.19	30.303	0.20
43	β-farnesene	31.942	1.53	31.949	1.55	31.944	1.01	31.945	0.66
44	germacrene D		nd	32.858	0.21		nd		nd
45	geranyl butyrate		nd		nd	32.305	0.20		nd
46	β-cubebene	32.858	0.26		nd		nd		nd
47	trans carveol		nd		nd	34.742	0.20		nd
48	caryophyllene oxide	36.863	0.22	36.858	0.21	36.855	0.46		nd
49	β -bisabolene		nd	-	nd	41.798	tr		nd
	Total		92.98		95.41		91.27		79.03

RT: retention time; nd: not-detected; tr: trace (between 0.01-0.09%

...Continued Table 2.

Component names		Afternoon		Evening		Night			
		18:00		21:00		00:00		03:00	
		RT	%	RT	%	RT	%	RT	%
1	α-pinene	8.610	0.17	8.610	0.28	8.619	0.24	8.616	0.30
2	campnhene	9.188	0.15	9.188	0.24	9.197	0.22	9.188	0.26
3	3-octanone	10.561	0.23	10.567	0.25	10.571	0.28	10.567	0.30
4	β-myrcene	11.065	0.10	11.064	0.14	11.068	0.11	11.065	0.15
5	acetic acid hexyl ester	12.127	0.16	12.112	0.20	12.127	0.19	12.123	0.20
6	o-cymene	12.501	0.10	12.506	0.26	12.510	0.14	12.501	0.13
7	limonene	12.684	0.41	12.684	0.56	12.688	0.45	12.684	0.55
8	1,8-cineole	12.781	1.69	12.781	2.30	12.785	2.76	12.781	2.78
9	(E)β-ocimene	13.187	0.50	13.187	0.44	13.191	0.47	13.187	0.62
10	y-terpinene	14.086	0.10		nd	14.095	tr	14.092	0.17
11	cis-sabinene hydrate	14.475	0.26	14.480	0.20	14.484	0.20		nd
12	trans-linalool oxide		nd		nd		nd		nd
13	1-octanol		nd		nd		nd		nd
14	cis-linalool oxide		nd	14.767	0.14		nd		nd
15	4 -carene	15.453	0.21	15.453	0.12	15.457	0.20	15.453	0.29
16	Linalool	16.392	40.80	16.403	39.91	16.287	43.15	16.283	45.45
17	camphor	18.148	11.45	18.142	12.04	18.078	12.13	18.068	11.66
18	propanoic acid 2-methyl-hexyl ester	18.423	0.15	18.417	0.25	18.415	1.01	18.406	0.11
19	borneol	19.201	10.70	19.178	10.08	19.102	9.84	19.104	10.41
20	lavandulol	19.361	2.44	19.350	2.38	19.274	2.00	19.270	2.24
21	terpinen- 4- ol	19.779	13.67	19.762	13.13	19.691	12.62	19.688	12.98
22	cryptone	20.071	0.27	20.065	0.29	20.046	0.29	20.037	0.24
23	α-terpineol	20.288	0.86	20.282	0.89	20.258	0.75	20.254	1.01
24	butanoic acid hexyl ester	20.449	1.01	20.448	0.95	20.430	0.78	20.432	0.75
25	isoborneol	20.872	0.11		nd		nd		nd
26	bornyl formate	21.953	0.10	21.953	0.12	21.946	0.13	21.942	0.10
27	sabinene		nd		nd		nd	22.022	0.15
28	nerol	22.028	0.13	22.028	0.12		nd		nd
29	hexyl 2-mehylbutanoate	22.474	0.47	22.474	0.44	22.467	0.35	22.469	0.32
30	hexyl isovalerate		nd		nd	22.690	0.19	22.692	0.17
31	hexyl n-valerate	22.697	0.27	22.697	0.25		nd	-	nd
32	β-pinene	23.350	2.90	23.344	2.65	23.325	2.14	23.321	1.58
33	lavandulyl acetate	24.929	1.85	24.923	1.53	24.910	1.35	24.906	1.09
34	hexyl tiglate	26.634	0.23	26.634	0.19	26.632	0.15	26.634	0.15
35	thymol		nd		nd		nd		nd
36	hotrienol	-	nd	27.836	0.22		nd		nd
37	3-carene		nd		nd	28.921	0.43	28.923	0.17
38	geranly acetate	28.923	0.29	28.928	0.34		nd		nd
40	hexanoic acid. hexyl ester	29.031	0.32	29.037	0.28	29.030	0.22	29.026	0.15
41	zingiberene	29.163	0.13	29.163	0.17	29.161	0.11		nd
42	caryophyllene	30.313	0.41	30.313	0.41	30.311	0.29	30.308	0.23
43	β-farnesene	31.955	1.87	31.955	1.91	31.942	1.32	31.944	0.98
44	germacrene D	32.859	0.34	32.859	0.25		nd		nd
45	geranyl butyrate		nd	32.304	0.19	32.303	0.14		nd
46	β-cubebene		nd		nd	32.852	0.21	32.854	0.15
47	trans carveol		nd		nd		nd		nd
48	caryophyllene oxide	36.859	0.18	36.859	0.28	36.857	0.20	36.859	0.13
49	β -bisabolene	42.764	0.10	42.764	0.10		nd		nd
	Total		95.13		94.50		95.06		95.97

RT: retention time; nd: not-detected; tr: trace (between 0.01-0.09%

Plant essential oils are variable mixtures of principal terpenoids, specifically hemiterpenes (C5), monoterpenes and sesquiterpenes, although diterpenes may also be present. Terpenes are among the chemicals responsible for the medicinal, culinary and fragrant usage. Most terpenes like monoterpenes (C10), sesquiterpenes (C15), diterpene (C20), sester-, tri-, and tetraterpenes (C25, C30, C40, respectively) are derived from the condensation of branched five-carbon isoprene units and are categorised according to the number of these units in the carbon skeleton (Dorman, 1999; Boeckelmann, 2008). Generally, 40-50 different monoterpenes can be identified with *linalool, linalool acetate, 1,8-cineol, ß-ocimene* (usually both cis- and trans-), *terpinene-4-ol* and *camphor* as the main components in lavender essential oil (Kreis and Mosandl, 1992; Flores et al., 2005; Boeckelmann, 2008). Lavender essential oil is a complex mixture of monoterpenes (and

to a lesser extent sesquiterpenes), which give the plant its distinctive aroma. *Linalool* is one of the most abundant monoterpenes found in its oil such that the ratio between *linalool* and *camphor* suggests oil quality. High *linalool* concentrations combined with trace amounts of *camphor* are associated with high quality oil, extracted from lavender (Clark and Menary, 1980a; Clark and Menary, 1980b; Hussein et al., 1996; Turner et al., 2000a; Turner et al., 2000b; Dudareva et al., 2003).

A total number of 49 components were recognized after GC-MS analysis from the essential oil of dry flowers during day time (Table 2). Well over 150 chemicals (many among them are trace components) have been identified in *L. angustifolia* and *L. latifolia*, (Harborne and Williams, 2002). In this study; the total amounts of the components obtained are presented at the Table 2. whereas, the rest of the essential oil amounts have some other components, but these are not shown in this table due to their presence in trace amounts. Linalool content is determined higher than ISO standards.

In this study, *1-octanol*, *α- terpineol* and *limonene* contents were accordance with ISO 3515: 2002 lavender oil standards. Content of *o-cymene* was lower than ISO 3515: 2002 lavender oil standards. *Camphor, 1,8 cineol, terpinene-4-ol* content of the cultivars are high and do not meet ISO 3515: 2002 lavender oil standards (Table 2).

CONCLUSION

Comparing flowers, peduncle, and leaves, flowers were the best organ of the plant to extract the essential oil from lavender. In order to obtain a high amount of essential oil; the harvest of lavandula plant flowers must be done during 15:00 or alternatively at 09:00 and at 18:00. The period of day affected the quality of essential oil in terms of linalool and camphor contents. The most appropriate harvest time for obtaining high values of linalool is at 03:00. Harvest times with high camphor level in the essential oil components of lavender that have a usage in preservative and therapeutic industry. The results of the study show harvest time significantly affect the quality of the essential oil components. Therefore, the harvest time should be given importance based on use/purpose, by of the end product. Review of literature shows very meager data about nocturnal studies in lavender essential oils. This study will help to improve missing information about the effects of nocturnal lavender harvestings on essential oil contents and components.

ÖZET

Amaç: Türkiye'nin Akdeniz iklim kuşağında yer alan Uşak ili ekolojik koşullarında, sıcak yaz Akdeniz ikliminde (Csa tipi) yetiştirilen lavanta bitkisinin tam çiçeklenme döneminde hasat edilen; çiçek, çiçek sapı (peduncle) ve yapraklarındaki uçucu yağ miktarı ve bileşenleri ile bu bileşenlerin diurnal ve nocturnal varyabiliteleri incelenmiştir..

Yöntem ve Bulgular: Çiçek, çiçek sapı ve yaprak örnekleri tam çiçeklenme döneminde (14 Temmuz) 24 saat içerisinde sekiz farklı (06:00, 09:00, 12:00, 15:00, 18:00, 21:00, 00:00; 03:00) zamanda hasat edilmiştir. Gölgede kurutulan yüzer gramlık çiçek, çiçek sapı ve yaprak örnekleri 3 saat süre ile Clevenger tipi aparat kullanılarak su buharı distilasyon ile uçucu yağları elde edilmiş ve elde edilen uçucu yağların bileşenlerine GC-MS ile bakılmıştır.

Genel Yorum: Elde edilen bileşenlerin yüzdesi; hasat zamanına ve alınan numunenin türüne göre değişim göstermiştir. Elde edilen sonuçlar; uçucu yağ içeriğinin çiçeklerde %6.73-10.27, çiçek sapında (pedinkül) %0.29-0.76 ve yapraklarda ise %0.08-0.42 arasında değiştiğini göstermiştir. GC-MS sonuçlarına göre; tam çiçeklenme döneminde ana bileşenler olarak *Linalool, terpinene-4-ol, camphor, borneol, 1,8 cineole, lavandulol, β-pinene* ve *β-farnesene* belirlenmiştir. En yüksek uçucu yağ (%10.27) ile saat 15:00'de toplanan çiçeklerden elde edilmiş, ana bileşen olarak ise *linalool* belirlenmiştir.

Çalışmanın Önemi ve Etkisi: Lavanta uçucu yağında, yüksek oranda kafur (*camphor*) için saat 09:00'da yapılacak hasadın, *linalool* için ise gece saat 03:00'de yapılacak hasadın uygun olduğu belirlenmiştir. Sonuçlar, çiçeklerde ve çiçek saplarında *linalool* miktarının, yapraklarda ise kafurun daha fazla olduğunu göstermiştir.

Anahtar Kelimeler: Hasat zamanı, uçucu yağ, bileşimler, Akdeniz (Csa tipi) iklimi.

ACKNOWLEDGEMENT

The authors acknowledge the support of ''Usak University, Scientific Analysis and Technological Application and Research Center (UBATAM)'' for analysis of essential oils.

DISCLOSURE STATEMENT

No potential conflict of interest was reported among authors.

KAYNAKLAR

Adam KL (2006) Lavender production, products, markets, and entertainment farms. Retrieved on November 5, 2006 from http:// Composition of lavenders and lavandins cultivated inTurkey 679 attra.ncat.org/attra-pub/lavender.html.

Anonymous (2002) ISO 3515: 2002: Oil of Lavender (*Lavandula angustifolia* Mill.).

Anonymous (2004) TR ISO 3515: 2004. Lavanta yağı (*Lavandula angustifolia* Mill.), Türk Standardı; TSE Ankara.

Anonymous (2018a) https://en.climate-data.org/asia/turkey/usak-576/

(Accessed on June 20, 2019).

Anonymous (2018b) Meteoroloji Genel Müdürlüğü,

https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=USAK

(Accessed on June 05, 2019).

Arabacı O, Bayram E (2005) Aydın ekolojik koşullarında lavanta (*Lavandula angustifolia* Mill.)'nın bazı agronomik ve kalite özellikleri üzerine bitki sıklığı ve azotlu gübrenin etkisi. *ADÜ Ziraat Derg.* 2(2): 13-19.

Arabacı O, Ceylan A (1990) Bazı parfüm bitkilerinde (*Lavandula angustifolia* Mill., *Melissa officinalis* L., *Salvia sclerea* L.) verim ve ontogenetik varyabilite üzerine araştırmalar. *E.Ü. Fen Bil. Ens. Dergisi*, 1(1): 233-236.

Atalay AT (2008) Konya ekolojik şartlarında yetiştirilen lavanta (*Lavandula angustifolia* Mill.)'da farklı dozlarda uygulanan organik ve inorganik azotlu gübrelerin verim ve kalite özellikleri üzerine etkileri. Yüksek Lisans Tezi, Selçuk Üniversitesi, Fen Bil. Ens., Tarla Bitkileri ABD, 46 s. (Turkish)

Basalma D, Gürbüz B, Sarıhan EO, İpek A, Arslan N, Duran A, Kendir H (2007) "Essential Oil Composition of Salvia heldreichiana Boiss", Ex Bentham Described Endemic Species from Turkey. *Asian J. Chem.* 19(3): 2130-2134.

Başer KHC (1993) Essential Oils of Anatolian Lamiaceae: A. Profile. Acta Horticulturae, 333: 217-238.

Baydar H (2007) Tıbbi Aromatik ve Keyf Bitkileri Bilimi ve Teknolojisi. (2. Baskı), Süleyman Demirel Üniversitesi Yayın No: 51. Isparta.

Beetham J, Entwistle T (1982) The Cultivated Lavenders. Royal Botanic Gardens, Melbourne.

Boeckelmann A (2008) Monoterpene production and regulation in Lavenders (*Lavandula angustifolia* and *Lavandula x intermedia*), Master thesis, University of British Columbia, Okanagan 96 p.

Buchbauer G, Jirovetz L, Jäger W, Dietrich H, Plank C (1991) Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. Z Naturforsch C. 1991 Nov Dec, 46(11-12): 1067-1072.

Camen D, Hadaruga N, Luca R, Dobrei A, Nistor E, Posta D, Dobrei A, Velicevici G, Petcov A, Sala F (2016) Research concerning the ınfluence of Fertilization on some Physiological Process and Biochemical composition of Lavender (*Lavandula angustifolia* L.), *Agric. and Agric. Sci. Procedia* 10: 198-205.

Cannon JB, Cantrella CL, Astatkieb T, Zheljazkovc VD (2013) Modification of Yield and Composition of Essential Oils by Distillation Time. *Ind **Crop** Prod*, 41: 214–220.

Castle J, Lis-Balchin M (2002) History of usage of *Lavandula* species. Ed: Maria Lis-Balchin Lavender; the genus *Lavandula* 283 p. Taylor &Francis, London.

Ceylan A, Vomel A, Kaya N, Celik N, Nigdeli E (1988) An investigation on effects to yield and quality of plant space in lavender*. Ege Üniv. Ziraat Fak. Derg*. 25(2): 135-145. (in Turkish).

Clark RJ, Menary RC (1980a) Environmental effects on peppermint (*Mentha piperita* L.). II. Effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition. *Aust J Plant Physiol*. 7: 693-697.

Clark RJ, Menary RC (1980b) Environmental effects on peppermint (*Mentha piperita* L.). I. Effect of day length, photon flux density, night temperature and day temperature on the yield and composition of peppermint oil. *Aust J Plant Physiol.* 7: 685-692.

Deans SG (2002) Antimicrobial properties of lavender volatile oil. Chapter :15, Ed: Maria Lis Balchin: Lavender). 283 p. Taylor&Francis, London.

Dorman HJD (1999) Phytochemistry and Bioactive Properties of Plant Volatile Oils: Antibacterial, Antifungal and Antioxidant Activities. PhD Dissertation, University of Strathclyde.

Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Faldt J, Miller B, Bohlmann J (2003) (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: Function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell.* 15: 1227-1241.

Düzgüneş O, Kesici T, Gürbüz F (1983) "*İstatistik Metodları*", I.A.Ü. Ziraat Fakültesi Yayınları 861, Ders Kitabı: 229, Ankara.

Flores G, Blanch GP, Ruiz del Castillo ML, Herraiz M (2005) Enantiomeric composition studies in *Lavandula* species using supercritical fluids. *J Sep Sci*., 28: 2333-2338.

Harborne JB, Williams CA (2002) Phytochemistry of genus *Lavandula*, Ed: Maria Lis Balchin: Lavender. Taylor & Francis, London. 283 p.

Hassiotis CN, Lazari DM, Vlachonasios, KE (2010) The Effect of Habitat type and Diurnal Harvest on Essential Oil Yield and Composition of *Lavandula angustifolia* Mill. *Fresen Environ Bull*. 19(8): 1491-1498.

Holmes R (2002) Lavender growing in Australia; Ed: Maria Lis-Balchin: Lavender, The genus *Lavandula*

Hussein MS, El-Saeid E, El-Sherbeny S. (1996) Yield and quality of lavender in relation to foliar application of sulphur and phosphorus. *Egypt. J. Hort.,* 23: 167-178.

Ilieva-Stoilova MP, Ivanov Pavlov A, Kovatcheva-Apostolova EG (2002) Further research into *Lavandula* species. Chapter:20, Ed. by: Maria Lis-Balchin: Lavender; the genus *Lavandula*. 283 p. Taylor &Francis, London.

İzmirli A, Yildirim MU (2018) Farkli Giberellik Asit (GA_3) Dozlarinin Lavantada (*Lavandula angustifolia* mill.) Uçucu Yağ Miktarlari Ve Bileşenleri Üzerine Etkisi. Yüksek Lisans Tezi, Uşak Üniversitesi, Fen Bil. Ens., Tarım Bilimleri ABD, 67 p. (Turkish)

Kara N, Baydar H (2011) Essential oil characteristics of lavandins (*Lavandula x intermedıa emeric* ex loisel.) of Isparta province, kuyucak district, where lavender production center of Turkey. *Selcuk J Agr Food Sci*. 25 (4): 41-45. (in Turkish).

Kara N, Baydar H (2013) Determination of lavender and lavandın cultivars (*lavandula* sp.) containing high quality essential oil in Isparta, Turkey. *Turk J Field Crops,* 18(1): 58-65.

Karık Ü, Çiçek F, Çınar O (2017) Menemen ekolojik koşullarında Lavanta (*Lavandula* spp.) tür ve çeşitlerinin morfolojik verim ve kalite özelliklerinin belirlenmesi. *Anadolu J. AARI*; 27(1): 17-28.

Kaya DA, İnan M, Giray ES, Kırıcı S (2012) Diurnal, ontogenetic and morphogenetic variability of *Lavandula stoechas* L. ssp. Stoechas in east Mediterranean region. REV. CHIM (Bucharest) 63(8): 749-753.

Koulivand PH, Ghadiri MK, Gorji A (2013) Lavender and the nervous system. *Evid Based Complement Alternat Med*, ID: 681304. 10 p.

Kreis P, Mosandl A (1992) Chiral compounds of essential oils. Part XI. Simultaneous stereoanalysis of lavandula oil constituents. Flavour Fragr J. 7: 187-193.

Lammerink J, Wallace AR, Porter, NG (1989) Effects ot harvest time and postharvest drying on oil from lavandin (*Lavandula x intermedia*). *New Zeal J. Crop Hort*. 17: 315-326.

Lesage-Meessen L, Bou M, Sigoillot JC (2015) Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in White biotechnology, Appl. Microbiol Biotechnol. DOI 10.1007/s00253-015-6511-7.

Lis-Balchin M (2002) Lavender: the genus Lavandula. 283 p, London, CRC Press.

Prusinowska R, Smigielski KB (2014) Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.), A review, *Herba Pol,* 60(2): 56-66.

Renaud ENC, Charles DJ, Simon JE (2001) Essential oil quantity and composition from 10 cultivars of organically grown lavender and lavandin. *J. Essent. Oil Res*. 13(4): 269-273.

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. *Plant Growth Regul.* 34: 3-21.

Sefidkon F, Abbasi K, Khaniki GB (2006) Influence of Drying and Extraction Methods on Yield and Chemical Composition of the Essential Oil of Satureja hortensis. *Food Chem.* 99: 19-23.

Silva SM, Luz JMQ, Nogueria PAM, Balnk AF, Sampaio TS, Pinto JAO, Junior AW (2017) Organo-mineral Fertilization Effects on Biomass and Essential Oil of Lavender (*Lavandula dendata* L.) *Ind. Crop Prod.* 103: 133-140.

Tucker AO (1985) Lavender, spike and lavandin. The Herbarist, 51: 44-50.

Turner GW, Gershenzon J, Croteau RB (2000a) Development of peltate glandular trichomes of peppermint. *Plant Physiol.* 124: 665-680.

Turner GW, Gershenzon J, Croteau RB (2000b) Distribution of peltate glandular trichomes on developing leaves of peppermint. *Plant Physiol.* 124: 655-664.

Upon T (2002) The taxonomy of the genus *Lavandula* L. Chapter 2; Ed. by: Maria Lis Balchin: Lavender; the genus Lavandula, 283 p. Taylor &Francis, London.

Wesolowska A, Jadczak D, Grzeszczuk M (2010) İnfluence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.), *Herba Pol.* 56(3): 24-36.

Wichtl M (1984) Teedrogen, Wissenschaftliche Verlagsgesellschaft mbH Stuttgard, S.393