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TIME-BASED CHANGES IN ESSENTIAL OIL CONTENTS AND COMPONENTS OF ENGLISH LAVENDER (*Lavandula angustifolia* MILL.) AFTER FOLIAR APPLICATION OF GIBBERELLIC ACID (GA_3)

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ABSTRACT

Lavender (*Lavandula angustifolia* Mill. family Lamiaceae) is a perennial and highly drought tolerant plant species. It grows well on calcareous slopes, and produces a fragrant essential oil. The aim of study was to evaluate foliar application of gibberellic acid (GA_3) and delaying harvesting for different durations on essential oil and components of English lavender after foliar treatment with 0, 200, 400 and 600 mg l^{-1} gibberellic acid (GA_3). Clevenger apparatus-based water-vapor distillation method was used to determine essential oil and its components by GC-MS. The experiment was established in split plots in randomized complete block design with two factors and 3 replications. The main plots were formed at harvest time and the sub-plots were created by GA_3 applications. Statistical analyzes were made using MSTAT-C package program. When the GA_3 doses and harvest times interaction were evaluated together, the average essential oil yield of the harvested lavender flowers ranged 6.20 to 8.20 % showing the prominent influence of the concentration of GA_3 and delay in harvesting (in hours) on concentrartration of alkaloids. More than 50 components were identified, the most important among these was 1.8-cineole, linalool, camphor, borneol, lavandulol, and terpinene-4-ol. The amount of linalool, which improves the quality of the essential oil ranged 34.49-38.75%. The minimum and the maximum essential oil values were noted from the flowers harvested after 30 hours (control treatments), and 54 hours from 400 mg l-1 GA_3 treated plants in the same order. However, essential oil contents showed a slight decrease at higher GA_3 treatments and prolonging duration of harvest after respective GA_3 treatments. GA_3 treatments enhanced the quantity of essential oil substantially, and the optimal dose was determined as 400 mg l^{-1} GA_3 on samples harvested after 54 hours following application. The results further showed that the quantities of essential oil's primary components varied according to harvest time.

Keywords: Aromatic plant, camphor, flower, linalool, plant growth regulator

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INTRODUCTION

Lamiaceae is an important family of flowering plants which includes 236 genera and 6900-7200 species. All species stand out with their distinguished spicy flavors or medicinal properties (Tamokou, 2017). The genus *Lavandula* constitute 41 species (Anonymous, 2021), and many registered varieties are obtained by crossing these species (Upson and Andrews, 2004; Benabdelkader *et al.* 2011; Sahin, 2017). Lavender is commonly grown in the Balkans and Mediterranean coastal countries (Beetham and Entwistle, 1982; Ilieva-Stoilova *et al.* 2002; Kara and Baydar, 2013), with naturalisation in the other parts of the world (Boelens, 1995). The dried flowers of the lavender plant and the essential oils are being used for centuries to give a pleasant fragrance to medicines and cosmetics (Cavanagh and Wilkinson, 2002). The Lavender flower is used to treat headaches, jaundice, liver, gall bladder disorders, and general visual impairment (Ilisulu, 1992). Its antimicrobial and antioxidant compounds are used as additives that extend the shelf life of beverages, foods, and cosmetics (Secmen *et al.* 2000).

Lavender essential oil is found in the leaves, stalks, and most of the flowers of the plant. It is among the top 15 essential oils in the global trade. Linalool, linalyl acetate, and camphor are the most important essential oil components. The other important components of the essentil oil include terpinene-4-ol, borneol, β-pinene, lavandulol, cineol and eucalyptol (Baser, 1993). These components are responsible for determining the characteristic aroma and biological properties (Lesage-Meessen *et al.* 2015). Essential oils in plants and their amounts can vary according to environmental factors (abiotic and biotic environmental factors), applied agricultural practices, and the genetic background of the plant. Variation in the essential oils of the plants may also depend on the morphogenetic,

ontogenic, and diurnal or nocturnal stage of the plant at the time of harvest (Yıldırım *et al.* 2019). External applications of plant growth regulators influence the amount of essential oil contents, and their components (Prins *et al.* 2010; Sharafzadeh and Zare, 2011; Yıldırım *et al.* 2019). Even trace amounts of plant growth regulators applied externally; affect physiological events in plants, as they influence physiological activities in dissimilar parts of the plant (Dobrev *et al.* 2005). The basic functions of plant growth regulators within the cell are to regulate the basic stages of development such as cell, and organ differentiation along with cell division (Davies, 1995). Generally known and commercially available plant growth regulators include Auxins, Gibberellins, Cytokinins, Ethylene, and Abscisic acid. Gibberellins (GA) are endogenous plant growth regulators that control many physiological events, such as seed germination, leaf shaping, flowering, flower development, corm propagule regeneration, root, shoot, and fruit growth, etc. (Olszewski, 2002; Tyler *et al.* 2004; Alabadi *et al.* 2004; Swaın and Sıngh, 2005; Yıldırım *et al.* 2016; Yıldırım and Hajyzadeh, 2018). Therefore, gibberellins are widely used in various plant and seed-based studies in laboratories, greenhouses, and fields.

This study aimed to determine the best harvest time for obtaining maximum essential oil and components yield of lavender (*L. angustifolia*) after treatment with dissimilar concentrations of gibberellic acid (GA_3).

MATERIALS AND METHODS

The material in the study consisted of 4 years old *L. angustifolia* collection plot (300 m^2 area) located in the medicinal plants parcel of Uşak University Faculty of Agriculture in Türkiye. The experiment was established as split plots in Randomized Complete Block Design (RCBD). The different harvest times were evaluated as main factors. Spray of 0, 200, 400 and 600 mg l^{-1} gibberellic acid (GA_3) were evaluated as sub factors. The lavender flowers were harvested after delay of 24 (morning), 30 (noon), 36 (evening), 48 (morning), 54 (noon), 60 (evening), 72 (morning), 78 (noon), and 84 (evening) hours after the gibberellic acid treatment. They were evaluated after drying them in shade at 25 °C. Nocturnal harvest was avoided. First, 100 g of dried flowers were placed in the distillation flask of Clevenger apparatus, containing 500 ml of distilled water to obtain the essential oil. These were boiled using a mantle heater for 3 hours followed by cooling them for 30 minutes. After the percentage (%) of volatile oil was collected in the fractionated pipe; the essential oil components were analyzed by GC/MS (Unal *et al.* 2005).

GC/MS device used an electron ionization energy of 70 eV during analysis. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector and MS transfer line temperatures were set at 220 and 290 ºC, respectively. The temperature of the column was initially kept at 50 ºC for 3 minutes, then gradually increased by 3 ºC to 150 ºC and lastly by 10 ºC to reach 250 ºC. The diluted samples (v/v in 1/100 hexane) were injected at 1.0 µL per minute (Basalma *et al.* 2007). Analysis of variance (split plots in randomized complete block design with two factors) of essential oil data was performed using MSTAT-C computer software. The differences between the averages of the measured characters were determined by Duncan's Multiple Range Test (Duzgunes *et al.* 1983).

RESULTS AND DISCUSSION

Amount of essential oil: GA_3 concentrations and harvest times interaction showed essential oil collected from lavender flowers ranged 6.20% to 8.20% (percent) (Table 1). The lowest value was obtained after 30 hours from control treatments. The maximum amount of essential oil was obtained from the plots that were treated with 400 mg l^{-1} GA_3 and harvested after 54 hours. The amount of essential oil increased in general as compared to the non-treated plants, which served as control treatment. Similarly, concentrations of GA_3 higher than 400 mg/l were inhibitory showing a decrease in essential oil contents after treatment with respective concentration of GA_3. The amount of essential oil obtained from harvests made after 24 (morning of first day) and 48 (morning of 2nd day) hours was significantly higher compared to that obtained from the harvests done at noon and evenings of the 1st, 2nd or 3rd days.

One of the factors affecting the yield of essential oil from lavender is harvest time. Kaya et al. (2012), evaluated *L. stoechas* (Spanish or topped lavender) in his study before flowering and full bloom, three times a day (7.00, 12.00, and 17:00). Harvested lavender essential oil amounted in the range of 0.71% to1.97%. They also noted that the maximum yield of essential oil was obtained in the morning harvest.

Renaud *et al.* (2001) has reported that the amount of essential oil in dried flower buds of lavender varieties varied between 7.1-9.9% and the amount of essential oil in dried flower buds of dissimilar lavender varieties varied between 2.8-5.0%. Kara and Baydar (2011), evaluated Super A lavender in at Kuyucak village of Isparta province and noted that dried peduncle less flowers showed an essential oil percentage of 7.50 - 8.60%. Kara and Baydar (2013), in another study with different genotypes of lavender; obtained the maximum amount of 9.62% essential oil from dried flowers in the first year and 8.87% in the second year.

Although genetic factors (plant species, development of different plant parts, dissimilar stresses) have a high effect on the rate of essential oils percentage (Atalay, 2008), abiotic (plant age, temperature,

precipitation, moisture, day length, wind, soil), and biotic factors (insect and fungal pathogens, etc.) also contribute the essential oil yield (Arabacı and Bayram, 2005; Hassiotis *et al.* 2010a; Kara and Baydar, 2011; Kaya *et al.* 2012; Kara and Baydar, 2013). Plant growth regulators administration (Hajisamadi *et al.* 2011) and chemicals added to distillation water (Shamspur *et al.* 2012) also influence the essential oil composition of plants.

Essential oil components: The components in lavender flowers have the greatest impact on the essential oil quality. There are many components obtained from the essential oils of lavender flowers. The results of the analysis as observed through GC/MS, showed 6 main components namely 1,8 cineole, linalool, camphor, borneol, lavandulol, and terpinen-4-ol which are important for cosmetic, perfumery, and pharmaceutical industries.

The highest amount of 1.8-cineole of 5.10% was obtained at 600 mg l^{-1} GA_3 treatment after 78 hours of treatment and the minimum amount of 2.82% of the same compound was obtained using 200 mg l^{-1} GA_3 after 48 hours (Table 2).

According to gibberellic acid concentrations, the maximum linalool of 37.27% was noted from the plants using 600 mg l^{-1} GA_3 treatments after 54 hour with a minimum value of 34.49% from the plants using 200 mg l^{-1} GA_3 treatments after the 48 hours. In the control group, it varied between 35.99%-38.75% on the samples after 30 and 54 hours respectively. It was observed that the linalool content in GA_3 treatments was generally lower compared to the control treatment first two days (Table 2). Hassiotis et. al., (2010b), noted the morning and evening hours linalool contents of 34.38% and 28.76% respectively. According to the International Organization for Standardization, lavender oil quality standards, the percentage of linalool and linalyl acetate should be at least 25% (Anonymous, 2002). According to the ISO 8902:2009a and ISO 3515:2002 linalool amount changed 25-38% in lavender essential oil (Kuş and Duru, 2021). Linalool ratio meets desired quality specifications.

Another component that affected lavender essential oil quality is camphor. The highest and lowest amount of camphor was obtained using control treatments with 12.50% and 10.65% camphor noted in between 30th and 48th hour in the same order. The maximum camphor value of 12.14% and the minimum value of 11.13% were noted using 200 mg l^{-1} GA_3 treatment. Camphor values between 12.29% and 10.77% were realized at 400 mg l^{-1} GA_3 treatment. The maximum (11.79%) and the minimum (10.75%) camphor amounts were noted using 600 mg l^{-1} GA_3 treatments (Table 2). Although the amount of camphor in lavender essential oil varied depending on many factors, the most important factor remained the variety (genotype). According to the ISO 8902:2009a and ISO 3515:2002 camphor amount changed 0.5-1.0% in lavender and 6-8% in lavandin essential oil (Kuş and Duru, 2021). The camphor rates obtained in the study are above the desired rates and affect the quality negatively. Chatzopoulou and Golaris (2003) found a significant difference between 5.03% - 11.35% in 'Super' and 'Special' hybrids in their experiments. Lavender essential oil quality decreased with an increase in the amount of camphor (Renaud *et al.* 2001). However, due to the antiseptic effect of camphor on the lungs and respiratory tract (Ayral, 1997), this is the desired level of lavender essential oil allowed for medicinal use. The high level of camphor in essential oil is very important in terms of antifungal, antibacterial, and antiseptic usage (Ilieva-Stoilova *et al*, 2002; Yıldırım *et al.* 2019).

In control treatment, the maximum (7.57% and 7.44%) amount of borneol in lavender essential oil components was harvested after 36 and 24 hours. After 54 and 84 hours of treatment with 400 mg l^{-1} GA_3, the minimum value was determined as 6.57%. The highest borneol percentage of 8.06% was obtained after 24 hours of 600 mg l^{-1} GA_3 treatment (Table 2). The average borneol percentage of 7.08%, 7.42%, 6.89%, and 7.52% was found depending on the treatment with gibberellic acid concentration. The average maximum borneol concentration was noted using 600 and 200 mg l^{-1} GA_3 treatments in the same order. It is important that the amount of borneol tended to decrease from day 1 to day 3 (Table 2).

Minimum amount of 1.19% and maximum amount of 1.88% lavandulol was obtained using 400 mg and 200 mg l^{-1} GA_3 treatment after 84 and 24 hour treated samples in the same order (Table 2). The previous study by Kara and Baydar (2011) confirm minimum and maximum lavandulol concentration of 0.33% and 2.28% in the dry peduncle less flowers of lavender and lavandin cultivars. The highest and the minimum amount of terpinene-4-ol was 12.07% and 9.82% was obtained 24 hours using 200 mg l^{-1} and 78 hours using 600 mg l^{-1} GA_3 treatments. In general, current study showed higher values for terpinene-4-ol after treatment with 200 and 400 mg l^{-1} GA_3 applications (Table 2).

There are more than 100 components in lavender essential oil, the most important of which are linalool, camphor, 1.8-cineole, terpinene-4-ol and linalyl acetate (Koulivand *et al.* 2013; Yıldırım *et al.* 2019). Lavender is generally used as an antidepressant, anticonvulsant, anxiolytic, and tranquilizer (Cavanagh and Wilkinson 2002; Koulivand *et al.* 2013). Lavender is mostly used internally, although it is also used in aromatherapy (Setzer, 2009; Sasannejad 2012;). The essential oil is quickly absorbed by the skin. It is mentioned that linalool and linalyl acetate are detected rapidly in plasma shortly after administration (Jager *et al.* 1992; Koulivand *et al.* 2013). It is well established that amount of linalool helps

Table 1. Essential oil content (%) of lavender according to different treatments of GA_3 concentrations and times of harvest.

GA_3 concentrations (mg l^{-1})	Time of harvest after GA_3 treatments (hours) **								
	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening
	24.	30.	36.	48.	54.	60.	72.	78.	84.
Control	6.60^{ijkl}	**6.20^{l}**	6.77 ^{hijkl}	7.20^{defgh}	6.60^{ijkl}	7.00 ^{efghij}	6.87^{ghijk}	7.00^{efghij}	7.33^{cdefg}
200	7.60^{bcd}	7.17^{defgh}	6.83^{ghijk}	7.50^{cde}	7.60^{bcd}	7.10^{defghi}	6.60^{ijkl}	6.80^{hijk}	6.90^{fghijk}
400	7.60^{bcd}	7.57^{bcd}	7.00^{efghij}	8.00 ^{ab}	**8.20^{a}**	8.03 ^{ab}	8.03 ^{ab}	7.23^{defgh}	7.80^{abc}
600	6.97^{fghij}	6.40^{kl}	7.40^{cdef}	7.60^{bcd}	6.50^{jkl}	7.00^{efghij}	7.40^{cdef}	6.40^{kl}	7.40^{cdef}

LSD for doses (0.05): 0,1433; LSD for hours: 0,2582; Dose x Hour Interaction for LSD (0,05): 0,430
** Differences between the averages in the block indicated by different letters are significantly different at $p \leq 0.01$ level

Table 2. The effect of disimilar harvest times and GA_3 doses on lavender essential oil components

Harvest Time (h)	GA_3 Doses (mg l^{-1})	*1,8-cineole*		*Linalool*		*Camphor*		*Borneol*		*Lavandulol*		*Terpinen-4-ol*	
		RT	*%*	*RT*	*%*	*RT*	*%*	*RT*	*%*	*RT*	*%*	*RT*	*%*
	Control	12.80	4.27	16.42	38.49	18.16	12.25	19.16	7.44	19.34	1.75	19.76	11.55
24	**200**	12.81	3.86	16.52	35.96	18.22	11.81	19.25	7.89	19.42	**1.88**	19.83	**12.07**
	400	12.80	3.63	16.45	35.46	18.19	12.29	19.20	7.68	19.37	1.83	19.79	11.60
	600	12.78	3.26	16.29	36.28	18.08	11.17	19.11	**8.06**	19.28	1.69	19.70	11.16
	Control	12.79	4.08	16.33	**38.75**	18.11	**12.50**	19.10	6.36	19.29	1.45	19.70	10.86
30	**200**	12.79	3.23	16.32	36.78	18.11	12.14	19.12	7.67	19.29	1.79	19.71	11.44
	400	12.79	3.68	16.35	36.43	18.11	11.45	19.13	7.29	19.31	1.68	19.73	11.87
	600	12.79	4.32	16.36	35.72	18.13	11.74	19.14	7.63	19.31	1.79	19.73	10.56
	Control	12.79	3.64	16.35	36.05	18.11	11.15	19.13	7.57	19.31	1.75	19.72	10.95
36	**200**	12.78	2.84	16.32	35.90	18.10	11.25	19.13	8.04	19.31	1.84	19.72	11.93
	400	12.78	3.56	16.27	35.72	18.07	11.23	19.09	7.06	19.27	1.46	19.68	11.64
	600	12.78	3.13	16.31	35.77	18.10	11.04	19.11	8.01	19.29	1.77	19.71	11.37
	Control	12.79	3.36	16.25	36.08	18.07	10.65	19.09	7.32	19.26	1.61	19.67	10.48
48	**200**	12.79	2.82	16.29	34.49	18.09	11.13	19.11	7.53	19.28	1.71	19.70	11.84
	400	12.79	3.69	16.22	35.77	18.05	10.77	19.07	6.69	19.25	1.30	19.66	11.05
	600	12.78	3.05	16.21	35.94	18.05	10.89	19.06	7.35	19.24	1.63	19.65	10.45
	Control	12.79	3.70	16.19	35.99	18.04	11.10	19.06	7.16	19.23	1.61	19.64	09.83
54	**200**	12.79	3.08	16.19	35.51	18.04	11.34	19.06	7.42	19.24	1.56	19.64	10.81
	400	12.78	3.83	16.19	36.65	18.04	11.09	19.05	6.57	19.23	1.28	19.64	11.24
	600	12.78	2.93	16.21	37.27	18.04	10.75	19.06	7.59	19.24	1.62	19.65	11.08
	Control	12.79	3.42	16.20	37.16	18.04	10.82	19.06	7.12	19.23	1.62	19.64	10.17
60	**200**	12.78	3.11	16.20	35.85	18.05	11.44	19.06	7.45	19.24	1.65	19.65	11.18
	400	12.79	3.31	16.17	36.09	18.03	10.96	19.05	6.85	19.23	1.27	19.64	11.86
	600	12.78	3.37	16.23	36.62	18.06	11.20	19.08	7.76	19.25	1.67	19.66	10.96
	Control	12.79	4.05	16.17	36.08	18.03	11.43	19.05	6.76	19.22	1.46	19.63	09.87
72	**200**	12.78	3.53	16.19	35.52	18.04	11.40	19.05	6.85	19.23	1.51	19.64	10.77
	400	12.78	3.76	16.19	36.26	18.04	10.95	19.06	6.67	19.23	1.19	19.64	11.83
	600	12.79	3.41	16.20	36.73	18.04	11.03	19.06	7.43	19.23	1.49	19.65	10.81
	Control	12.78	4.23	16.19	36.26	18.04	11.58	19.06	6.93	19.23	1.50	19.64	09.97
78	**200**	12.79	3.99	16.18	35.15	18.04	11.92	19.06	7.08	19.23	1.46	19.64	10.91
	400	12.79	4.08	16.19	35.30	18.04	11.50	19.06	6.67	19.23	1.23	19.65	10.99
	600	12.79	**5.10**	16.19	35.41	18.05	11.79	19.06	6.69	19.24	1.28	19.64	09.82
	Control	12.79	4.11	16.19	36.05	18.05	11.19	19.06	7.10	19.23	1.44	19.64	09.97
84	**200**	12.79	3.75	16.18	36.82	18.04	11.30	19.06	6.82	19.23	1.42	19.64	10.41
	400	12.79	4.51	16.19	37.19	18.04	11.21	19.06	6.57	19.23	1.19	19.64	11.05
	600	12.79	3.87	16.17	34.99	18.03	10.89	19.05	7.20	19.22	1.38	19.63	10.29

RT: Retention time

in maintaining the high quality of lavender products. Karık et al. (2017) has reported variable essential oil components according to the varieties in a study under the Menemen/Turkey ecological conditions. They found that this difference was due to the influence of applied plant growth regulator (GA_3), harvest time difference, environmental conditions, and genotypes. In addition, it has been clearly demonstrated that the applied phytohormone (GA_3) and the harvest time have an effect on the amount and components of essential oil.

Conclusions: Different concentrations of gibberellic acid (GA_3) caused changes in the quality and quantity of lavender. Different GA_3 dosages and harvest periods were shown to have different impacts on essential oil components. Their concentrations could be planned with careful harvesting schedule and rescheduling of the harvest programme to get high essential oil levels in lavender plants. The current study suggested the application of 400 mg l^{-1} GA_3 treatment to be the best practice in terms of essential oil extraction at harvest time after 48, 54, 60 and 72 hours. This approach could increase the efficiency of extracting substantial volumes of essential oil from lavender. The rate of linalool, which positively affects the essential oil quality, was at the desired rates, but the high amount of camphor affected the quality negatively. The high camphor content emphasizes the usage of non-food goods like insect repellants. It is noteworthy that the amounts of borneol and terpinene-4-ol obtained from the first day are higher compared to those obtained on subsequent days. If high levels of 1.8-cineole levels are desired the 78th and 84th hours of the third day would be a good time to harvest. This research is expected to contribute to future research and studies in a variety of ecological conditions. The quality and quantity of lavender essential oil could be improved as per aims of use.

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Authors' contribution: Conceived and designed the research and statistical analysis MUY, performance of the experiment and collected data Aİ, wrote the paper MUY, other contributions Aİ

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