



Essential Oil Composition of Lavender (*Lavandula angustifolia* Mill.) at Various Plantation Ages and Growth Stages in the Mediterranean Region

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ABSTRACT

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Lavender is a very old herb that has been used for centuries as an antiseptic and relaxant. The demand for its essential oil continues to grow on a global scale. The fact remains that harvesting at the right time of growth is critical to maximizing the rate of active ingredients found in the plant. Thus, in the current study, *L. angustifolia* plants were collected at various growth stages (pre-flowering, mid-flowering, and post-flowering) from Adana, Turkey in order to determine the most suitable harvest time for the highest amount of essential oils and its important compounds. The highest flower essential oil content (7.50 mL 100 g⁻¹) was obtained at mid-flowering of the third year of plantation. The major compounds for *L. angustifolia* were linalyl acetate (25.63-31.63%), linalool (16.33-24.79%), nerol (8.83-13.43%), beta-farnesene (3.67-5.70%), β-cis-ocimene (1.76-8.14%), respectively. The obtained data have been inquired by principal components analysis (PCA), allowing differentiation of plantation ages and growth stages. Compared to the plantation ages, essential oil content and linalool content increased significantly in the third year of cultivation. As a result, the most suitable harvest time may be considered as mid-flowering in terms of essential oil content and in terms of high linalyl acetate and linalool content.

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Introduction

Lavender (*Lavandula angustifolia* Mill.) is a Mediterranean shrub belonging to the *Lamiaceae* family (Cardia et al., 2018). The term lavender comes from the Latin 'lavando' part of the verb 'lavare' to bathe. As a result of their widespread cultivation since antiquity and their familiarity as garden plants, many legends and folklore stories have developed around this plant. There are two species of *Lavandula* and one hybrid that produce aromatic oils of interest to the perfumery industry. Each of the three groups produces individual oils with varying commercial value. Between the ribs of the flowers' hairy calices, small globular glands secrete oil. Immature oil traces can also be found in other parts of the plant, but if the flowers are ripe, these traces have no effect on the perfume; however, with under-ripe flowers, they may contribute to a 'green' note in the oil (Denny, 2002).

Its popularity stems from its therapeutic properties, which have been used in folk medicine since ancient times, as well as the well-known, pleasant floral-woody-

herbaceous fragrance (Buchbauer, 2002; Sevindik et al., 2017; Kina et al., 2021). Essential oils extracted primarily from *L. x intermedia* and *L. angustifolia* are economically significant in the perfumery and fragrance industries. While *L. angustifolia* produces the highest quality oil, lavandin produces the highest yields, the latter is considered to be of lower quality due to its relatively high camphor and low linalyl acetate contents (Lawrence and Reynolds, 1994).

Lavender is a very old herb that has been used as a disinfectant, antiseptic, and relaxant, as well as for culinary, medicinal, and therapeutic purposes since Roman times. *Lavandula* species are mainly cultivated for their essential oils (EO) that are used in cosmetics, food processing, and aromatherapy applications. For centuries, dried flowers have been used in pillows and other products to help people relax and sleep better (Lis-Balchin, 2002). Fifty years ago, a Bulgarian research team reported on the relaxing, calming, and stress-relieving properties of lavender EO (Tasev et al., 1969). Today, lavender essential

oil is one of the most widely used EO in phytotherapy, aromatherapy, and esoteric alternative medicine. For example, compared to the control, patients who received lavender oil aromatherapy had a significantly reduced level of anxiety (Dunn et al., 1995). With another example, atherosclerotic plaque formation in the aorta was reduced by inhalation of lavender oil volatiles, which is to say that these EOs have an angioprotective effect (Nikolaevskii et al., 1990). Furthermore, the effects of inhaling terpenic volatiles on blood pressure in humans were studied, and it was discovered that these EO-volatiles quickly reduced the elevated systolic blood pressure to normal levels after the jogging tour was completed (Romine et al., 1999; Suzuki and Aoki, 1994). Moreover, the extracts and EO of *L. angustifolia* have a variety of pharmacological effects described in the literature, including anxiolytic (Bradley et al., 2007; Woelk and Schläfke, 2010), antioxidant (Spiridon et al., 2011), anticholinesterase (Costa et al., 2011), antimicrobial (Danh et al., 2013), and antifungal (Behmanesh et al., 2015) activities. As an insect repellent, spike lavender is found in a number of veterinary shampoos and other products, particularly for fleas.

Linalyl acetate, a key component of *L. angustifolia* EO, is used for its antiseptic and febrifuge properties (Gabriel et al., 1998). This monoterpene ester has also been linked to anti-inflammatory and peripheral analgesic properties (Moretti et al., 1997). Tests on rabbit conjunctiva and rat phrenic nerve-hemidiaphragm preparations were conducted to determine *L. angustifolia* EO's local anesthetic activity on rabbit conjunctiva (Ghelardini et al., 1999). The EO, as well as its main constituent's linalool and linalyl acetate, were found to reduce electrically evoked contractions of the rat phrenic-hemidiaphragm in a dose-dependent manner. These odorants allow a dose-dependent increase in the number of stimuli required to provoke the reflex in the rabbit conjunctival test, confirming in vivo the local anaesthetic activity.

The importance and therapeutic properties of medicinal and aromatic plants are usually defined by their active ingredients, which have a wide range of applications (Akgul et al., 2020; Barut et al., 2021; Korkmaz et al., 2021). Due to the genetic instability of EO bearing plants as well as variations in temperature, water quantity, altitude, fertilizers, time of year, geographic distribution, and other factors, lavender EO has a very variable composition (Lis-Balchin, 2002). However, when to harvest lavender is a common question. Because of this, a manufacturer should be aware of the variations in active ingredients and harvest plants at the appropriate growth stages when they are rich in active ingredients. Taking these changes into account, the optimal plantation age and growth stage for a high supply of active ingredients can be determined. Thus, this study aimed to assess the quality characteristics of *L. angustifolia* by comparing it with various plantation ages and growth stages in the Mediterranean region where the effects of global warming and climate change are intensively felt.

Material and Method

Plant Material

L. angustifolia Mill. species were harvested from the lavender field in Çürüklü, Kozan/Adana (37°39'23.6"N 35°57'04.1"E) with a sickle. The slope is rocky-scrub covered with *Elymus* sp., *Echinops orientalis* Trautv.,

Taraxacum officinale (L.) Weber ex F.H. Wigg., *Genista albida* Willd. The materials were harvested in June-July 2021 at various growth stages (pre-flowering (12.06.2021), mid-flowering (30.06.2021), and post-flowering (15.07.2021)) after the third year of plantation (January, 2018) and first years of plantation (January, 2021). The distance between rows was 1 m and intra row spaces were 50 cm.

Essential Oil Extraction

For hydrodistillation of EO, 25 g dry stem flower sample from each treatment was weighed and placed in a glass balloon with 250 mL distilled water then placed in the Clevenger apparatus for 2 hours. In order to measure the amount of EO extracted from the samples, the graduated section was used to record the amount of oil that had accumulated on the water. The samples were stored at 4°C until further analysis. The EO content was expressed as a percentage of dry tissue weight. The chemical analyses were carried out in triplicate.

Gas Chromatography±Mass Spectrometry (GC MS) Analysis

GC MS analyses were performed in the Department of Biology at Kahramanmaraş Sutcu Imam University. 10 µL EO was mixed with 0.25 mL dichloromethane and 1 µL mixture was injected into the column. An Agilent 5975C mass spectrometer in conjunction with an Agilent GC 6890 II series was used to investigate EO compounds. The GC was equipped with an HP-88 capillary column (100 m x 250 µm x 0.20 µm film thickness) and the carrier gas flow rate was 1.0 mL/min. The oven temperature was held at 70°C for 1 min and then increased from 70 to 220°C at a rate of 10°C/min and waited for 10 min. Then increased to 230°C at a rate of 10°C/min and held at 10 min. The temperature of the injection part was 250°C. The mass spectrometer was operated in EI mode at 70 eV. The split ratio was 20:1. Mass range 35–400 m/z; scan speed (amu/s): 1000. The compounds were identified by mass spectra using Flavor2, W10N14 and Wiley7Nist05 libraries as reference compounds.

Statistical Analysis

Analysis of variance (one-way) was used to analyze the experimental data pertaining to the results of the chemical analysis. Using the Least Significant Difference (LSD) test, significantly different means were separated at P=0.05. JMP® (version 14.0, SAS Institute Inc., Cary, NC, 1989-2019) statistical software was used to perform ANOVA and principal components analysis on correlations. Correlation analysis was performed by R software. Flourish Studio was used to create the heat map.

Results and Discussion

Chemical Composition of Essential Oil

The chemical composition of EO for *L. angustifolia* was analyzed by GC MS, and obtained results were summarized (Table 1). A plant's EO content varies greatly depending on its stage of development (Barut et al., 2021). Mid-flowering of the third year of plantation resulted in the highest EO content (7.50 mL 100g⁻¹) which was 86 percent higher than the latter growth stages of pre-flowering of the

third year of plantation (Figure 1). When we compared these EO content findings with the earlier studies, diverse results were found; 0.19-0.42 mL 100g⁻¹ (Tarakemeh et al., 2012), 0.88 mL 100g⁻¹ (Chrysargyris et al., 2016), 3.91-4.95 mL 100g⁻¹ (Cordovilla et al., 2014), 0.05-4.58 mL 100g⁻¹ (Sönmez et al., 2018), 0.70-0.85 mL 100g⁻¹ (Fascella et al., 2020). Baydar and Erbaş (2007) reported that from the first harvest (8.25 mL 100g⁻¹) to the last harvest (7.30 mL 100g⁻¹), the EO content decreased. Similar to what we detected in our study; EO content was decreased with post-flowering at the third year of plantation.

The chemical composition of EO varied according to the plantation ages and growth stages. Representative GC MS chromatograms of the EO were given in Figure 2.

Forty-two compounds were found, representing 97.48-99.81% of the total EO. The major compounds for *L. angustifolia* were linalyl acetate (25.63-31.63%), linalool (16.33-24.79%), nerol (8.83-13.43%), beta-farnesene (3.67-5.70%), β -cis-ocimene (1.76-8.14%), respectively. Compared to the growth stages for linalyl acetate, higher amounts were found in the pre- and mid-flowering stages. When we compared the linalyl acetate content with the earlier studies, diverse results were found; 10.40% (Caputo et al., 2016), 6.90% (Germinara et al., 2017), 52.10% (López et al., 2017), 46.25% (Adaszyńska-Skwirzyńska and Szczubińska, 2019), 20.00% (Rai et al., 2020), 42.03% (Lari et al., 2020), 25.20% (Golubkina et al., 2020), 15.76% (Sałata et al., 2020), 20.36% (Donatello et al., 2020).

Table 1. Essential oil compounds of *L. angustifolia* from different growth stages and plantation ages

Plantation ages	First-year of plantation		Third-year of plantation			LSD (%5)	
	Growth stages		Pre-flowering	Post-flowering	Pre-flowering		Mid-flowering
Essential oil content (ml 100g ⁻¹)**	1.02D	3.1C	4.03B	7.5A	3.06C	0.82	
Compounds	RT (min)	Relative Peak Area (%)					
Myrcene	11.146	0.75	0.42	0.73	0.71	0.49	-
Limonene	11.621	0.49	0.24	0.27	0.32	0.22	-
β -cis-Ocimene**	11.882	4.46BC	8.14A	6.22AB	1.76C	2.45C	2.7
Δ -3-carene	12.173	1.52	3.56	2.3	2.43	2.10	-
Terpinolene	12.511	0.16	0.14	0.2	0.14	0.08	-
Eucalyptol	12.66	1.42	3.18	1.08	1.30	3.38	-
p-Cymene	13.063	0.57	0.44	0.25	0.12	0.37	-
Hexyl acetate	13.164	0.36	0.56	0.27	0.98	1.02	-
3-Octanone	13.965	0.16	0.29	0.28	1.96	1.26	-
Alloocimene	14.048	0.20	0.36	0.34	-	-	-
1-Octen-3-ol, acetate	14.286	1.40	0.72	1.22	1.46	0.86	-
Hexyl butyrate	15.028	0.41	0.40	0.21	0.63	0.79	-
Linaloloxide	15.746	0.43	-	1.60	0.25	0.26	-
α -Santalene	15.817	0.74	0.51	0.42	0.25	0.23	-
Camphene	15.977	0.41	0.33	0.30	0.22	0.28	-
Linalool**	16.316	17.11C	16.33C	19.89B	24.79A	24.77A	2.24
Linalyl acetate**	16.553	30.58A	28.19B	30.09A	31.63A	25.63C	1.8
beta-Farnesene**	16.868	4.20B	5.17A	3.67B	5.70A	5.49A	0.75
Nerol**	17.265	10.10B	13.43A	9.01C	8.83C	9.41BC	0.96
4-Carvomenthenol	17.895	4.81	4.01	4.91	3.11	3.26	-
γ -Terpinene	18.126	1.19	1.49	1.48	1.52	2.24	-
γ -Cadinene	18.322	0.59	0.59	-	-	-	-
Germacrene D	18.607	0.19	0.23	0.22	0.47	0.33	-
Neryl acetate	18.737	1.38	0.39	1.21	1.10	-	-
Camphor	18.832	-	0.81	-	-	1.30	-
α -Terpineol	19.028	4.05	2.74	5.28	4.58	3.23	-
Isoborneol	19.444	1.47	1.85	1.05	0.80	2.32	-
Geranyl butyrate	19.687	0.77	0.63	0.94	0.73	0.82	-
Geraniol	20.197	1.24	0.79	1.86	1.40	0.92	-
Cosmene	20.512	0.29	0.21	0.42	0.14	-	-
Chrysanthenyl acetate	20.880	0.07	0.05	0.06	0.04	0.06	-
Thymol	21.129	0.37	0.25	0.22	0.13	0.30	-
Crypton	21.414	0.39	0.24	0.19	0.31	0.32	-
Cuminaldehyde	21.521	0.71	0.40	0.77	0.53	0.56	-
Isoterpinolene	22.174	0.54	-	0.41	0.11	0.31	-
Aromadendrene	23.035	0.39	0.05	-	-	0.21	-
Caryophyllene oxide	23.147	0.38	0.15	0.23	0.07	0.31	-
α -Farnesene	23.735	0.17	0.05	0.02	-	0.09	-
τ -Cadinol	23.937	0.90	0.44	0.29	0.20	0.47	-
Longifolene	24.156	3.85	1.60	1.69	0.60	1.34	-
β -Sesquiphellandrene	24.792	0.34	0.19	0.21	0.09	-	-
γ -Pyrone	25.243	0.16	-	-	-	-	-
Total		99.72	99.57	99.81	99.41	97.48	-

RT: Retention time, *: P < 0.05, **: P < 0.01

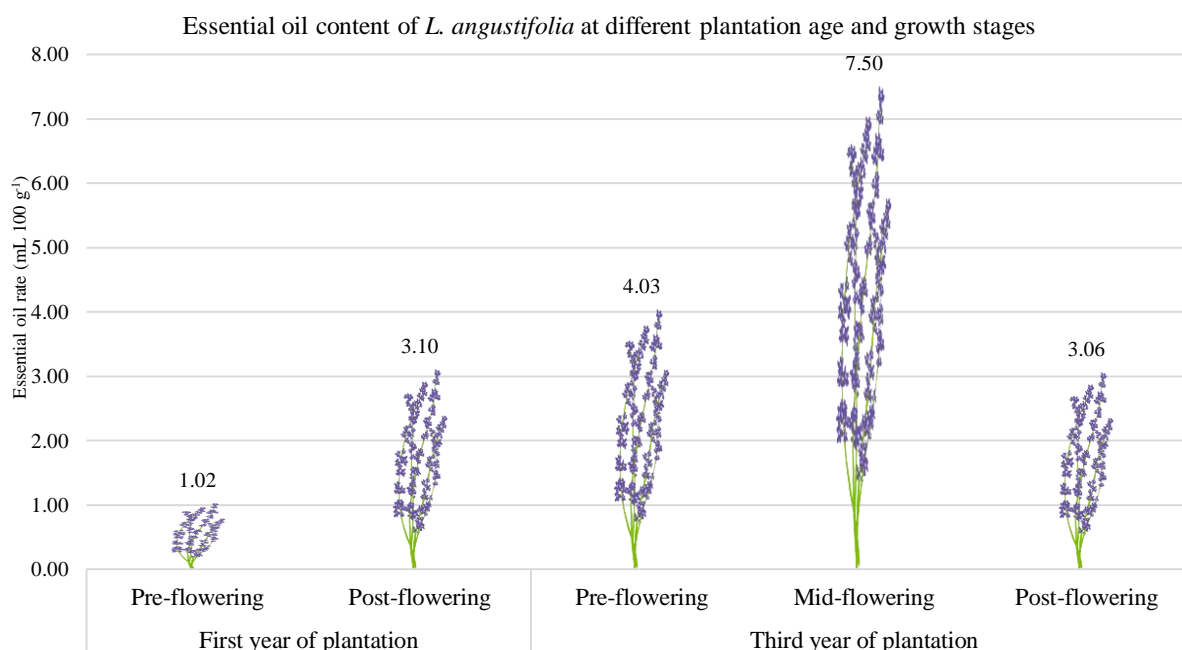


Figure 1. Essential oil content of *L. angustifolia* at different plantation ages and growth stages.

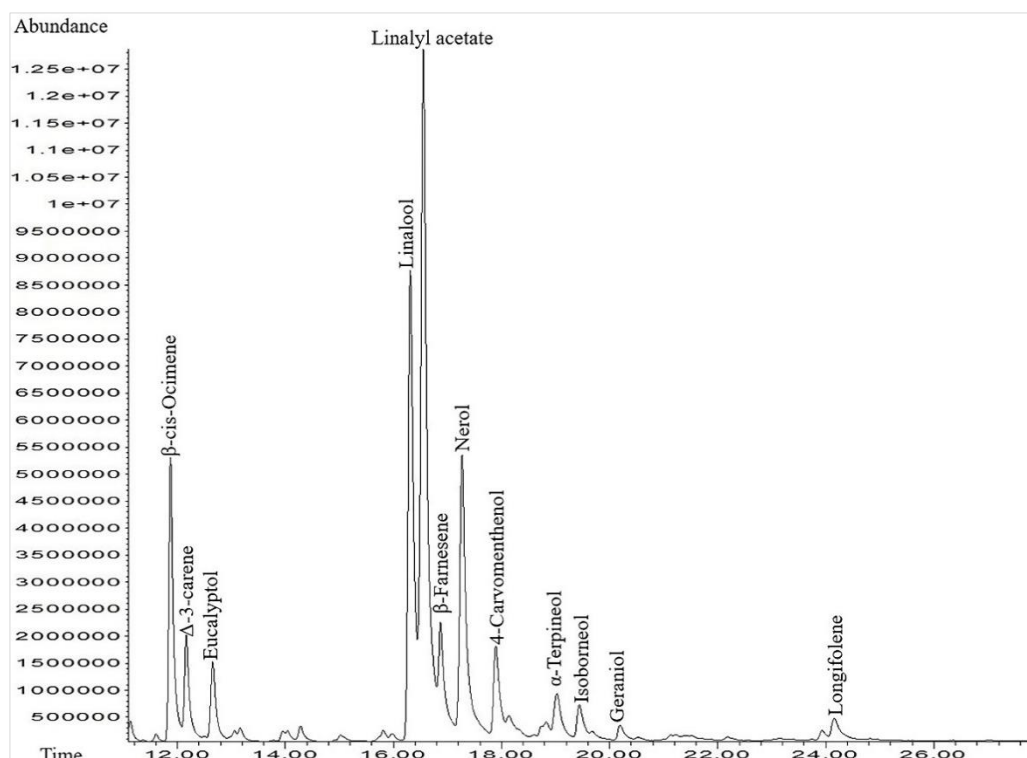


Figure 2. The representative GC MS chromatogram of *L. angustifolia* essential oil.

The EO composition of *Lavandula* varies from oil to oil, country to country, and with the age of the plant (Harborne and Williams, 2002). The International Organization for Standardization (ISO 3515, 2002) defines *L. angustifolia* EO, expressed as linalyl acetate, with a minimum of 25 percent and a maximum of 45 percent with a maximum camphor content of 0.5 percent.

Compared to linalyl acetate content of some other plants from the *Lavandula* genus, it was reported in *L. intermedia*; 11.75-23.32% (Périno-Issartier et al., 2013), 9.83% (Blažeković et al., 2018), 23.00% (Garzoli et al., 2019); in *L. multifida* 7.30% (Msaada et al., 2012); in *L. latifolia* 0.05-0.83% (Herraiz-Peñalver et al., 2013), 0.10-

0.70% (Carrasco et al., 2016); in *L. dentata* 28.65% (Msaada et al., 2012); in *L. stoechas* 15.26% (Malika et al., 2012); in *L. stoechas*; in *L. rejdalii* 9.02% (Gharby et al., 2020), *L. vera* 17.08-25.62% (Bruni et al., 2006). In the findings, it can be observed that the linalyl acetate content of reported some *Lavandula* species does not reach the linalyl acetate content of *L. angustifolia* except for reported by Msaada et al. (2012). The composition of EO is an important marker in medicinal and aromatic plants that has been influenced by a number of factors such as plant species, plant age, climatic conditions, agricultural practices, plant development stages, soil properties, post-harvest processing (Barut et al., 2021).

For linalool content, the highest values were obtained from the mid- and post-flowering stages at the third year of plantation (24.77-24.79%). Similar to the linalyl acetate content, the linalool content in the previous studies was found different; 33.10% (Caputo et al., 2016), 23.80% (Germinara et al., 2017), 37.40% (López et al., 2017), 35.17% (Adaszyńska-Skwirzyńska and Szczerbińska, 2019), 14.20% (Rai et al., 2020), 31.42% (Lari et al., 2020), 36.50% (Golubkina et al., 2020), 7.87% (Sařata et al., 2020), 30.61% (Donatello et al., 2020).

Correlation Analysis, Principal Component Analysis (Pcabiplot) on Correlations, and Heatmap According to Essential Oil Compounds

When the correlation analysis of EO compounds is interpreted, the colors indicate the negative or positive correlation and the size of the circles indicates the degree of importance (Figure 3). Moreover, PCAbiplot is critical for representing a data source in a way that multiple components accurately reflect the data's variance (Barut et al., 2021). It allows you to focus on the data and show the relationships between the variables. Positive and negative

correlations are easily discernible through this analysis. Lines in the same direction and close together have a positive correlation, while lines in opposite directions and far apart have a negative correlation. Through this analysis, one can clearly see the positive and negative correlations. There is a positive correlation between the lines that are in the same direction and close to each other, while there is a negative correlation between the opposite and distant lines. In order to visualize the influence of plantation ages and growth stages on correlations, PCAbiplot was performed (Figure 4).

The experimental groups were separately discriminated using PCAbiplot. Clear discrimination was revealed on the plotted scores, where component 1 and component 2 accounted for 80.70% of the total variance in terms of the EO components. The first axis and second axis explained 53.10% and 27.60% of the total variance, respectively. As seen in the Figure 4, PCAbiplot indicates that linalyl acetate was positively correlated with neryl acetate, geraniol, 1-Octen-3-ol, acetate, 4-Carvomenthenol, α -Terpineol, while Camphor, eucalyptol, isorborneol were negatively correlated.

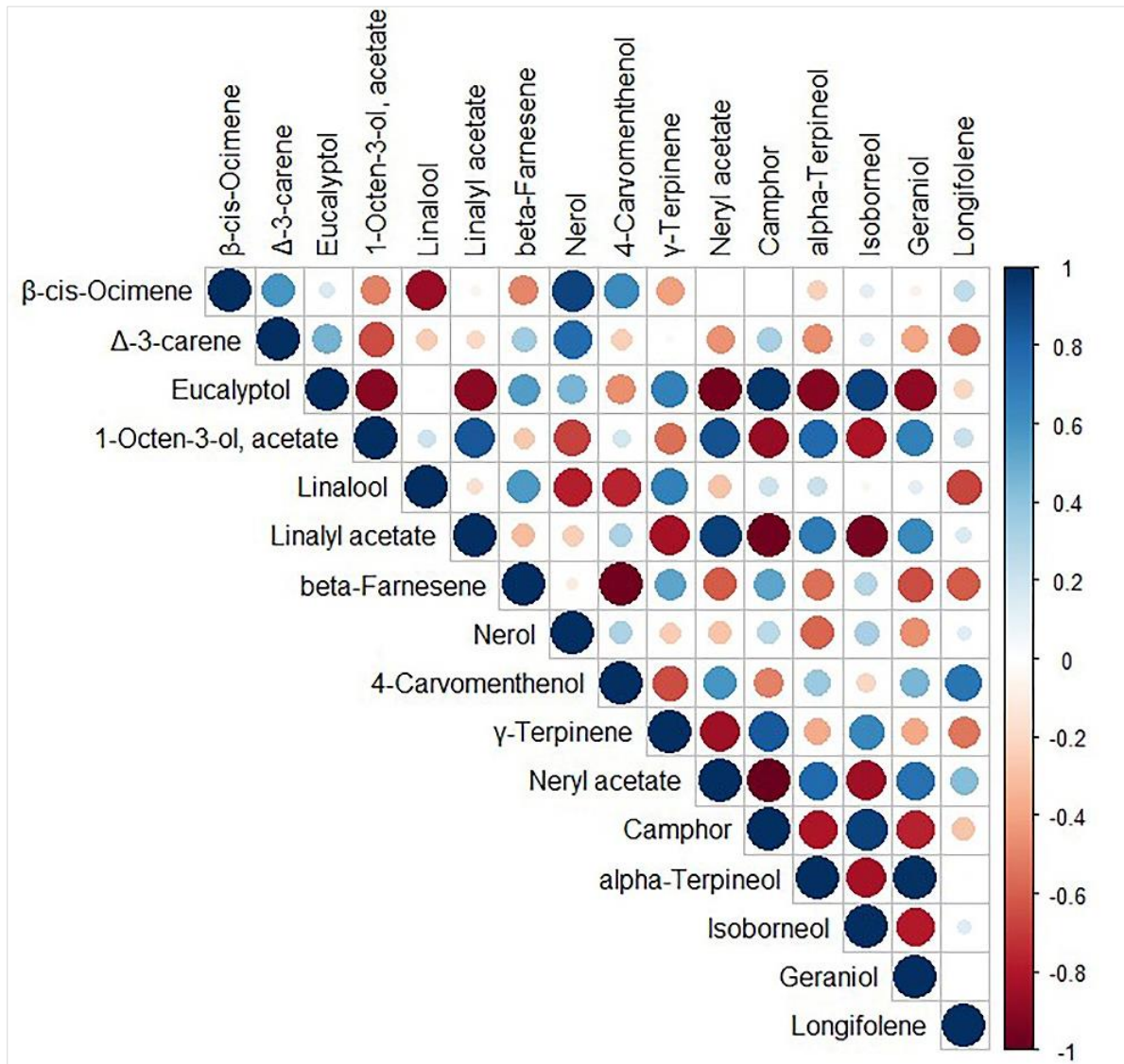


Figure 3. Correlation analysis of essential oil compounds

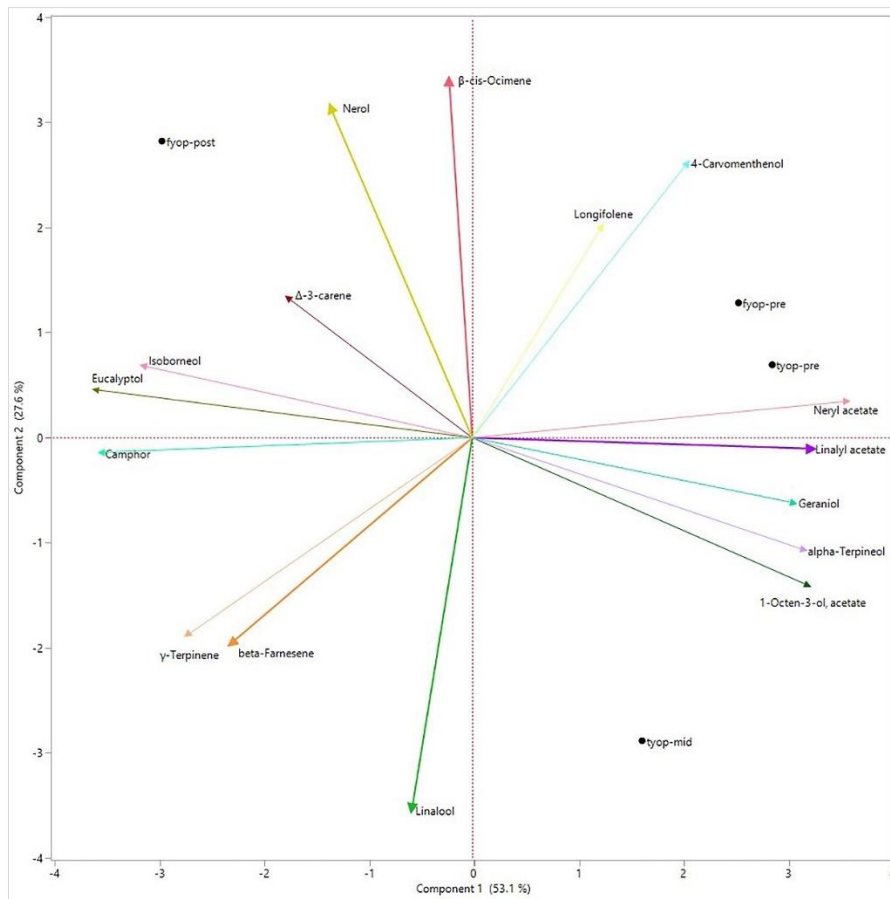


Figure 4. Principal component analysis on correlations of essential oil compounds (fyop: first year of plantation, tyop: third year of plantation; pre: pre-flowering, mid: mid-flowering, post: post-flowering).

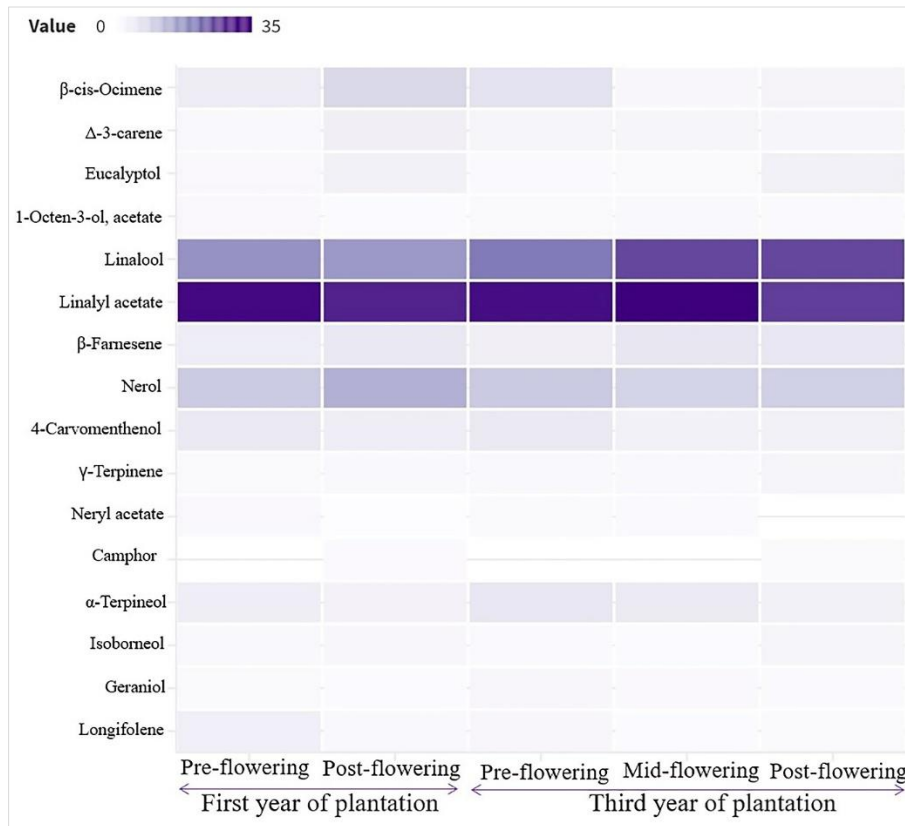


Figure 5. Heatmap based on the plantation ages and growth stages for the main essential oil compounds of *L. angustifolia* (Value=%).

A heat map was used to illustrate the changes in EO compounds (Figure 5). The EO compounds were visualized using the heat map based on the plantation ages and various growth stages. The differences can be observed clearly between growth stages and plantation ages in the EO compounds. For instance, while linalyl acetate production was highest during the pre- and mid-flowering stages, it decreased as harvests were delayed during post-flowering.

Conclusion

The essential oils of are used in a wide variety of ways. The essential oil of *L. angustifolia* has been used in the treatment of various diseases, ailments, disorders, and discomforts, as well as in cosmetic formulations and perfumery, since ancient times. Thus, this oil becomes an extremely valuable and significant treasure in phytotherapy. While farmers benefit economically from lavender cultivation, standardized quality is required for a variety of uses. In terms of essential oil content, as well as high linalyl acetate and linalool content, it is possible to consider mid-flowering as the most appropriate harvest time. Furthermore, it is recommended that additional agronomic studies be carried out, with a particular emphasis on studies to obtain higher quality products that are demanded by the market, as well as studies to examine the yield and quality of *Lavandula* spp. in various geographical locations.

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