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# EFFECT OF DEVELOPMENT OF DIFFERENT DOSES OF PHOSPHORUS AND SULFUR ON ESSENTIAL OIL AMOUNT AND BIOACTIVE SUBSTANCES IN *Lavandula angustifolia* (Mill.) PLANT

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## ABSTRACT

Lavender (*Lavandula angustifolia* Mill) is a perennial, semi-shrub-like valuable essential oil (EO) plant. There are more than 50 varieties of the Lavandula genus belonging to Lamiaceae family. It is known that phosphorus (P) increases the yield of lavender EO and sulfur (S) is accepted as the fourth main element for plants. This study was conducted to determine the effects of P and S fertilization on the EO amount and bioactive substances of *L. angustifolia* Mill. (LA). The trial was set up with *Sevtapolis* variety of LA. Fertilization was applied with triple superphosphate (42-44% $P_2O_5$) and 80% elemental liquid S fertilizer before planting the seedlings and the study was conducted for consecutive two years 2022-2023. The P doses were applied as $P_0$: 0 kg $P_2O_5$/ha, $P_{60}$: 60 kg $P_2O_5$/ha, $P_{120}$: 120 kg $P_2O_5$/ha, $P_{180}$: 180 kg $P_2O_5$/ha, $P_{240}$: 240 kg $P_2O_5$/ha, and S doses were applied as $S_0$: 0 L S/ha, $S_{30}$: 30 L S/ha, $S_{60}$: 60 L S/ha. EO amounts and bioactive substances analyses were performed for each year. In the second year of the experiment, Lavandula EO ratio was found to be 3.37% and EO yield was 37.6 kg/ha. Effective doses for the highest EO yield were calculated as 117.9 kg/ha P and 47.5 kg/ha S, while for the highest antioxidant activity, effective doses were 92.4 kg/ha P and 30.1 kg/ha S. Essential oil amount and Bioactive substances: Linalool, camphor, cis-β-ocimene, trans-β-ocimene, terpinen-4-ol, lavandulyl acetate, lavandulol, 1,8-cineole, limonene, 3-octanone and β-phellandrene ratios of Lavandula EO were found to comply with the International Organization for Standardization and the European Pharmacopoeia standards. Linalyl acetate ratios remained below the desired values in the standards. α-terpineol ratio was measured above the value determined in the standards. The ratios of both components approached the value range specified in the standards in the second year. Although different doses of P application had effect on linalyl acetate and α-terpineol, it did not affect the ratios of other EO components. However, different doses of S applications did not affect the ratios of any component. In crux, it seemed possible to increase EO yield and antioxidant activity of LA without comporomizing oil quality with P and S fertilization.

**Key words**: *Angustifolia Sevtapolis*, amount of essential oil, bioactive substance, Lavender, phosphorus fertilization, sulfur fertilization.



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## INTRODUCTION

Lavandula (*Lavandula angustifolia* Mill.), also known as English lavender or true lavender, is a variety of Lavandula genus has more than 50 varieties belong to *Lamiaceae* family, wich consists of aromatic plants such as mint, rosemary and basil, and is native to the Mediterranean region. (Erland and Mahmoud, 2016). Lavender, a valuable essential oil plant, is a perennial and semi-shrub-like plant (Uskutoğlu *et al*., 2023). Lavandula angustifolia Mill. (LA), which can grow in any soil that is not overly acidic and well-drained, obtains the highest EO yield in light sandy soils and its odor is more pronounced in calcareous soils (Lim, 2014). It is also known that applying lime to the soil improves the soil by providing neutralization and positively affects the development of lavender (Valcheva, 2020). This shrub is frequently preferred in lavender cultivation for medical applications due to the antisedative, antidepressant and anti-inflammatory effects of the EO it contains (Denner, 2009; Jianu *et al*., 2013). Various studies have shown that the EO yield of lavender is between 0.2-4.5% (Peçanha *et al*., 2021).

Phosphorus (P) is essential for the production of phospholipids, which form the structure of the endoplasmic reticulum, golgi apparatus, nuclear, plasma and mitochondrial membranes in plants. These phospholipids also play an important role in plant development and stress response. In addition to phospholipids, 40-60% of the organic phosphorus in the leaf is found in nucleic acids (Lambers, 2022). Even if there is an inorganic phosphorus ($P_i$) pool present in plant leaves, plants generally protect the $P_i$ pool when they switch to a P-poor environment and stop plant growth within hours (Veneklaas *et al*., 2012). In brief, continued P uptake is very important for the sustainability of plant growth. In studies on the effects of P application on LA plants, it has been determined that P fertilization increases the EO yield of lavender (Peçanha *et al*., 2021). Sulfur (S) is considered the fourth main element for plants after nitrogen (N), P, and potassium (K) (Shah *et al*., 2022). Plants needs S between 0.1% and 0.5% of their dry weight to complete their development healthfully (Beaton *et al*., 2005; Shah *et al*., 2013). Sulfor is involved in the composition of amino acids (Kertesz *et al*., 2007) and is necessary for chlorophyll synthesis and ferredoxin (Marschner, 2011). It directly or indirectly affects the fertility traits as well as the metabolic traits of plants. It has a key role in the formation of the photosynthetic apparatus and electron transport system, and in its deficiency, roots degrowth and yellowish/pale green, short, small and narrow leaves occur in plants due to the decrease in chlorophyll synthesis (Skwierawska *et al*., 2016; Shah *et al*., 2022). In addition, low S concentrations are known to cause a decrease in protein synthesis in plants (Zenda *et al*., 2021). Another important effect of S is that it provides protective and antioxidant mechanisms against many abiotic stresses (Zenda *et al*., 2021).

Although the effects of P fertilization on the EO and antioxidant effect of LA have been investigated in the literature, the studies lack a clear P dose recommendation. Furthermore, although the EO quality of Lavandula has been compared with other lavender varieties in previous studies, the impact of P fertilization on the EO quality of Lavandula has not been clearly investigated. Another important deficiency in the literature is the lack of studies investigating the effects of S fertilization on LA. In order to fill these gaps, this study aimed to determine the effects of P and S fertilization on the EO yield, oil quality and antioxidant activity of LA.

## MATERİALS AND METHODS

**Plant materials:** *Sevtapolis* variety of *L. angustifolia* Mill. was used as plant material. The seedlings used in the study were cut from clonal rootstocks and produced at the Fruit Research Institute Directorate (Isparta/Türkiye). These seedlings were provided as 1+1 year old bare-root seedlings.

**Experimental design:** The research was established in the medicinal plants application area of the Faculty of Agricultural Sciences and Technologies of Niğde Ömer Halisdemir University (37.94" North and 34.62" West, Turkey). The region has typical continental climate characteristics. According to the analysis carried out in the Niğde Provincial Special Administration Soil and Water Analysis Laboratory, the saturation of the experimental area soil was determined as 41.8%, pH value as 7.8, salt ratio as 0.01%, lime ($CaCO_3$) ratio as 42.1%, organic matter ratio as 0.2% and available phosphorus ($P_2O_5$) level as 24 kg/ha. Phosphorus fertilization was mixed into the soil close to the root zone using triple superphosphate (42-44%). Phosphorus doses were applied as 0 kg/ha ($P_0$), 60 kg/ha ($P_{60}$), 120 kg/ha ($P_{120}$), 180 kg/ha ($P_{180}$) and 240 kg/ha ($P_{240}$). Sulfur fertilization was applied to the soil with 80% sulfur fertilizer in liquid form. Sulfur doses were applied as 0 L/ha ($S_0$), 30 L/ha ($S_{30}$) and 60 L/ha ($S_{60}$). The experimental was laidout following randomized complete block design under factorial arreangement with 3 replications. Irrigation was applied homogeneously with drip irrigation method.

**Measurements:** Lavandula plants were harvested in August 2022 and August 2023. Since the first harvest was made in the establishment year of lavender bushes, the data obtained from the second harvest of lavandula, a perennial plant, more suitable for evaluating the yield potential (Giannoulis *et al*., 2020). Morphological measurements were made before the second harvest harvested by cutting 10 cm above the ground. Fresh weight yield (kg/ha) was measured quickly after the harvest. The plants were left to dry in a dark place at room temperature. Dry weight (kg/ha) was measured after drying. These data were used to calculate EO yield. EO yield per hectare was calculated by multiplying the dry weight yield by the EO percentage.

For EO analysis, 25 g of dried flower sample was taken from each plot and the samples were sent to the Western Mediterranean Agricultural Research Institute (Antalya/Turkey). The oil of the samples was obtained by water vapor distillation method using Clevenger device. For EO analysis, Gas chromatography/Mass spectrometry (GC-MS) and Gas chromatography (GC-FID) methods were used as suggested by Özek *et al*. (2010). The samples were diluted with hexane at a ratio of 1:100. After dilution, 1 µL was injected into the GC device (Shıadzu GC-2030) with a split ratio of 40:1. For the separation of components, a capillary column (Stabilwax) with dimensions of 60 m × 0.25 mm id × 0.25 µm was used and at the end of the column the flow was divided into the FID and mass spectrometer detector (SHİMADZU GCMS-QP2020 NX) with a ratio of 1:1 by a splitter. Helium (0.8 ml/min flow rate) was used as the carrier gas. The column temperature program was set as 60 °C for 5 min, from 60 °C to 210 °C at 3 °C/min (50 min) and at 210 °C for 5 min - a total of 60 minutes, the injector temperature was kept at 250 °C. The scan range (m/z) for the mass detector was 35-450 atomic mass units and the electron bombardment ionization energy was 70 eV. The identification of essential oil components was based on data from OIL ADAMS, WILEY and NIST libraries. The relative percentages of EO components were determined using FID detector data.

Total phenolic substance (TPS) measurements of the samples were determined by making some modifications to the method suggested by Spanos and Wrolstad (1990). 900 µL of pure water ($H_2O$), 5 mL of 0.1 N Folin-Ciocalteau solution and 4 mL of 7.5% sodium bicarbonate ($Na_2CO_3$) solution were added to 100 µL of sample extract. The resulting mixture was kept in the dark (90 min). The absorbance of the mixture was measured at 765 nm wavelength in a spectrophotometer (Shimadzu UV 1800, Japan). The results were calculated as mg GAE (Gallic Acid Equivalent) /100g.

The 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activities of the samples were determined by using the method suggested by Cemreoğlu (2010) in calculating the half maximal effective concentrations ($EC_{50}$). Different volumes of sample extract were added to 600 µL of 1 mM DPPH radical solution, the volume was completed to 6 mL with pure methanol and incubated for 15 min. At the end of the period, the absorbance values of the mixtures were read at 515 nm wavelength on a spectrophotometer (Shimadzu UV1800, Japan). The % inhibition values corresponding to the sample volumes were calculated. $EC_{50}$ values were calculated with the help of the equation obtained from the % inhibition rates of the mixtures to which different volumes of sample extract were added and expressed in µL.

**Statistical analyzes:** In the analysis, morphological measurements and chemical analysis results obtained from plots divided into random blocks in accordance with the field trial design are given as mean and standard deviation. Differences between years were tested with T-test. Differences between treatments applied at different doses of P and S and random blocks were tested with univariate analysis of variance. Tukey test was used for the analysis of differences between treatments. As a result of the variance analysis, the relationships of the parameters showing statistically significant differences with different P and S doses were examined with quadratic regression analysis and effective P and S fertilization doses were calculated. Correlation analyzes were performed to determine whether there was a relationship between the data obtained in the research. All data were analyzed with 95% confidence intervals using IBM SPSS Statistics for Windows (Ver22.0, IBM Corp., Armonk, NY: USA, 2013).

## RESULTS

Soil analysis was repeated after all fertilization treatments were applied in both study years. In the soil analysis tested in 2nd year, saturation was 36.45%, pH was 7.67, salt content was 0.01%, lime ($CaCO_3$) content was 37.91%, organic matter content was 1.97%, available phosphorus ($P_2O_5$) content was 312.6 kg/ha and sulfur ratio was 36.44%.

The average EO content was 2.54±0.44% in the first trial year and 3.37±0.38% in the second trial year and the difference between the years was found to be statistically significant ($p<0.001$). The average EO yield was 12.8±4.0 kg/ha in the first trial year and 37.6±5.9 kg/ha in the second trial year and the yield increase in the second study year was found to be statistically significant ($p<0.001$). The average TPS was 1.97±0.42 mg GAE/100g in the first trial year and 0.93±0.15 mg GAE/100g in the second trial year. The average $EC_{50}$ values were found to be 1.19±0.35 µL in the first trial year and 1.94±0.40 µL in the second trial year. The difference between the trial years was found to be significant at the level of $p<0.001$ for both TPS and $EC_{50}$ values.

The EO component ratios measured in the study are given in Table 1 together with the International Standards Organization (ISO, ISO3515:2002) and European Pharmacopoeia (Ph. Eur.) standards. According to these results, although the Linalool ratio decreased in the second study year, it was found to be within the range determined in the standards in both study years. Linalyl acetate ratios remained below the desired values in the standards in both years of the study, but approached the standard value range at a statistically significant level in the second year of the study ($p<0.001$). Camphor ratio showed a statistically significant decrease in the second year of the study ($p<0.001$). α-terpineol ratio was measured above the value specified in the standards in both study years, and a statistically significant decrease was found in the α-terpineol ratio in the second year of the study ($p<0.001$). It was found that the EO component ratios became more compatible with the ISO and Ph. Eur. standards in the second year of the study. The distribution diagrams of the EO components are shown in Figure 1.

**Essential oil ratio and yield:** EO ratios showed statistically significant differences between the interactions of treatments applied with different P and S doses in 2022 ($p=0.016$). The highest lavender EO percentage was obtained in the $P_0 \times S_{30}$ interaction as 3.08%, followed by $P_{180} \times S_0$ (3.00%) and $P_{240} \times S_{30}$ (3.00%) interactions. The lowest EO percentage was measured in the $P_{60} \times S_{30}$ interaction as 2.00%, followed by $P_{180} \times S_{30}$ (2.17%) interaction . In 2023, a statistically significant differences were found between the treatments applied at different doses of P, between the treatments applied at different doses of S and between the interactions of these treatments ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). Among the treatments applied with different doses of P, the highest EO ratio was measured in the $P_{60}$ treatment with 3.74%, followed by the $P_{120}$ (3.52%), $P_{180}$ (3.31%), $P_{240}$ (3.19%) and $P_0$ (3.19) treatments. In the treatments applied with different doses of S, the highest EO ratio was measured in the $S_{30}$ (3.53%) treatment. This was followed by the $S_{60}$ (3.43%) and $S_0$ (3.15%) groups. In the 2nd year of the study, the highest EO ratio was found in the $P_{60} \times S_{30}$ (3.88%), $P_{60} \times S_{60}$ (3.69%) and $P_0 \times S_{30}$ (3.68%) interactions and the lowest in the $P_0 \times S_0$ (2.48%), $P_{240} \times S_{30}$ (2.94%) and $P_{180} \times S_0$ (3.04%) interactions.

The effects of different doses of P and S application on EO percentage and yield were investigated by creating quadratic regression models for 2nd study year (Figure 2). As a result of the modeling, the effective fertilization doses required to obtain the highest percentage of EO were calculated, the effective dose for P was determined as 111.9 kg/ha ($y=-0.0031x^2+0.0694x+3.1985$, $R^2=0.2516$) and the effective dose for S 38.4 kg/ha ($y=-0.0273x^2+0.2096x+3.1507$, $R^2=0.1869$). The effective P dose to obtain the highest EO yield was calculated as 117.9 kg/ha ($y=-0.0047x^2+0.1108x+3.4474$, $R^2=0.2365$) and the effective S dose to obtain the highest EO yield was calculated as 47.5 kg/ha ($y=-0.0236x^2+0.2243x+3.438$, $R^2=0.1485$).

**Table 1. The essential oil (EO) component ratios measured in the study together with the International Standards Organization (ISO, ISO3515:2002) and European Pharmacopoeia (Ph. Eur.) reference ranges.**

| EO ingredient | Ph. Eur. | ISO | | Mean | SD | p-value |
|---|---|---|---|---|---|---|
| Linalool (%) | 20-45% | 20-43% | 2022 | 40.00 | 1.73 | <0.001 |
| | | | 2023 | 36.65 | 1.54 | |
| Linalyl acetate (%) | 25-47% | 25-47% | 2022 | 18.49 | 2.77 | <0.001 |
| | | | 2023 | 24.11 | 1.52 | |
| Camphor (%) | ≤1.2% | ≤1.5% | 2022 | 0.45 | 0.10 | <0.001 |
| | | | 2023 | 0.28 | 0.06 | |
| Cis-β-ocimene (%) | - | 1-10% | 2022 | 2.81 | 0.49 | <0.001 |
| | | | 2023 | 8.79 | 0.49 | |
| Trans-β-ocimene (%) | - | 0,5-6% | 2022 | 1.02 | 0.17 | <0.001 |
| | | | 2023 | 2.93 | 0.20 | |
| Terpinen-4-ol (%) | 0.1-8% | ≤8% | 2022 | 5.80 | 0.24 | <0.001 |
| | | | 2023 | 7.14 | 0.45 | |
| Lavandulyl acetate (%) | ≥0.2% | ≤8% | 2022 | 6.45 | 0.45 | <0.001 |
| | | | 2023 | 3.43 | 0.21 | |
| Lavandulol (%) | ≥0.1% | ≤3% | 2022 | 0.81 | 0.09 | <0.001 |
| | | | 2023 | 1.15 | 0.18 | |
| 1,8-Cineole (%) | ≤2.5% | ≤3% | 2022 | 2.19 | 0.40 | <0.001 |
| | | | 2023 | 0.53 | 0.12 | |
| α-Terpineol (%) | ≤%2 | ≤%2 | 2022 | 4.48 | 0.33 | <0.001 |
| | | | 2023 | 3.11 | 0.26 | |
| Limonen (%) | ≤%1 | ≤%1 | 2022 | 0.20 | 0.16 | <0.00.1 |
| | | | 2023 | 0.17 | 0.14 | |
| 3-Octanone (%) | %0,1-5 | ≤%3 | 2022 | 0.68 | 0.14 | - |
| | | | 2023 | | | |
| β-phellandrene (%) | - | ≤%1 | 2022 | | - | |
| | | | 2023 | | | |
| Other essential oil components (%) | - | - | 2022 | 16.54 | 0.69 | - |
| | | | 2023 | 11.71 | 0.56 | |

**Phenolic substances:** In 2022, the amount of TPS showed a statistically significant difference between the treatments applied at different doses of P ($p<0.001$), the amount of TPS in the $P_{60}$ treatment was measured as 2.37 mg GAE/100g and was found to be statistically significantly higher than all other treatments. The lowest amount of TPS was found in the $P_{240}$ treatment as 1.68 mg GAE/100g. In addition, a statistically significant difference was found between the interactions of the treatments applied in 2022 ($p <0.001$). The highest TPS amount was found in the $P_{60} \times S_0$ (2.84 mg GAE/100g) and $P_{180} \times S_{60}$ (2.48 mg GAE/100g) interactions, while the lowest TPS amount was found in the $P_{180} \times S_{30}$ (1.48 mg GAE/100g) interaction. In 2023, a statistically significant difference was found between the treatments applied with different doses of P and different doses of S and the interactions of these treatments ($p = 0.007$, $p = 0.003$, $p = 0.002$, respectively). Among the treatments applied with different doses of P, the highest amount of TPS was measured in the $P_{120}$ (1.00 mg GAE/100g) and $P_0$ (0.99 mg GAE/100g), while the lowest amount of TPS was measured in the $P_{60}$ (0.83 mg GAE/100g) treatment.

In the treatments applied with different doses of S, the highest amount of TPS was measured as 1.01 mg GAE/100g in the $S_{30}$ treatment and the lowest amount f TPS was measured as 0.87 mg GAE100g in the $S_0$ treatment. Again, among the interactions of treatments applied with different doses of P and S in 2023, the highest TPS amount was measured in the $P_{120}$ x $S_{30}$ (1.14 mg GAE/100g) interaction, and the lowest TPS amount was measured in the $P_{60}$ x $S_0$ (0.67 mg GAE/100g) interaction.

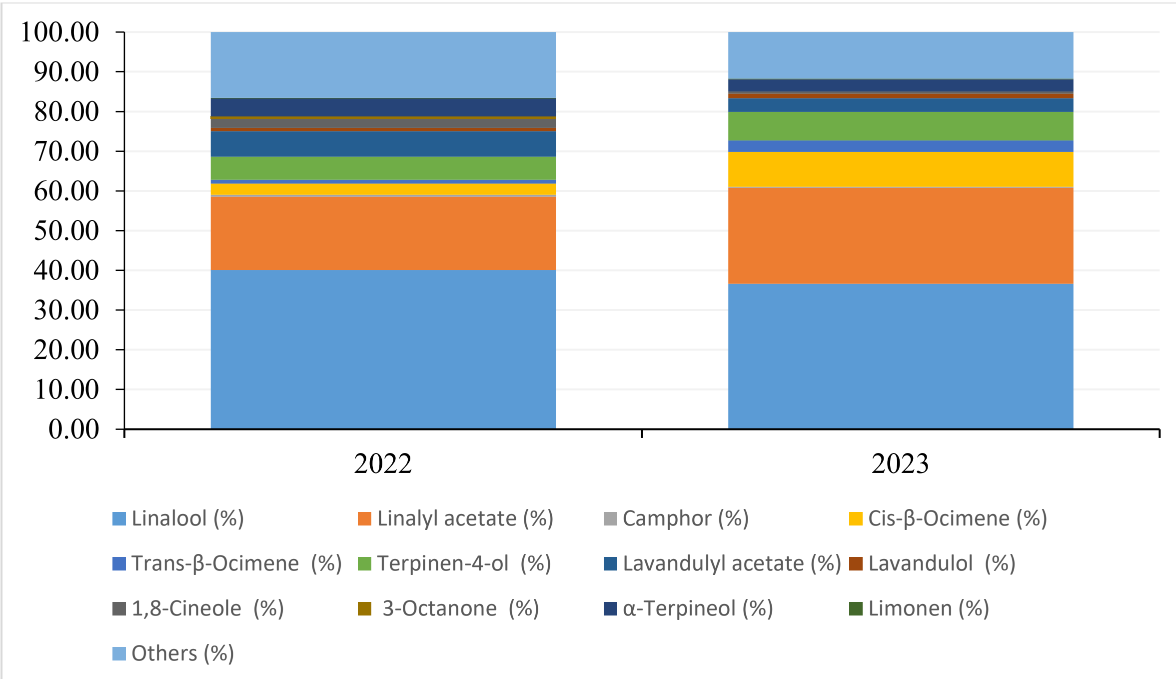


**Figure 1. Diagrams of essential oil components by years.**

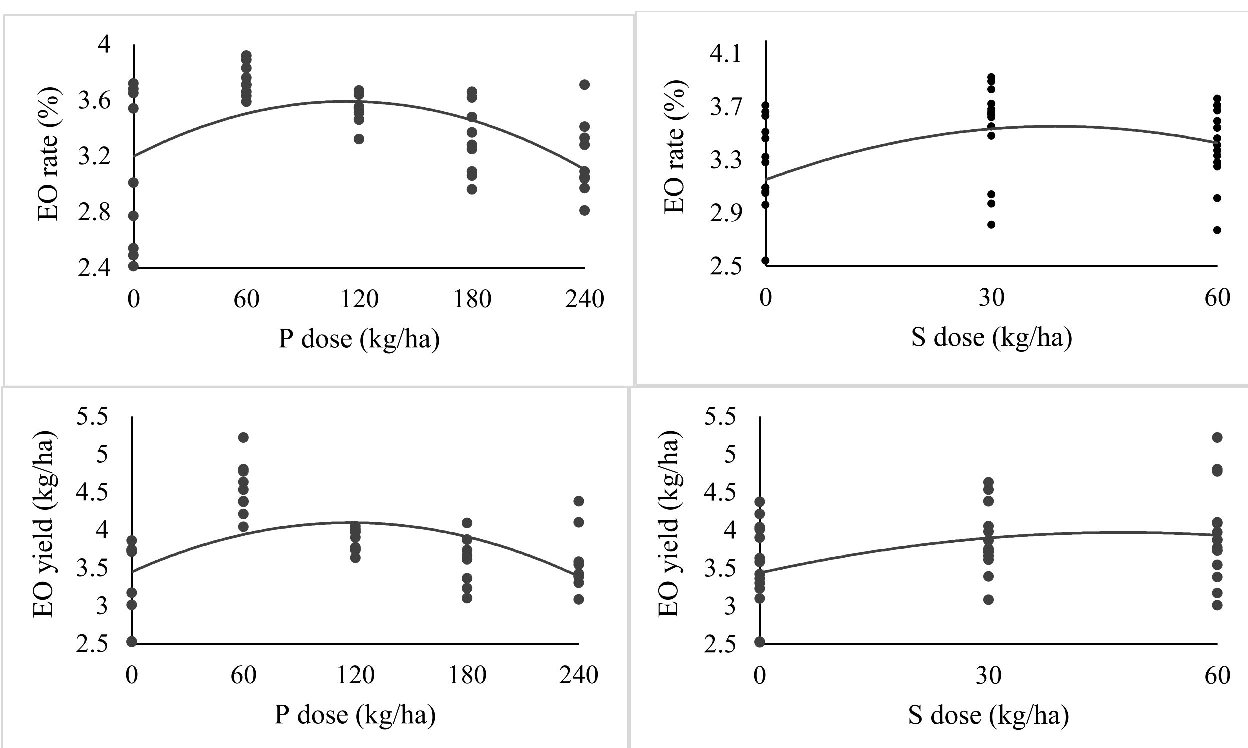


**Figure 2. Regression models for EO rates and yields for different doses of P and S.**

The effects of different doses of P application and different doses of S application on the amount of TPS were examined by creating quadratic regression models for 2023 (Figure 3). As a result of the modeling, it was found that the 160 kg/ha P dose was associated with the lowest TPS amount ($y=0.0002x^2-0.0064x+0.9571$, $R^2=0.0137$). On the other hand, 32 kg/ha S dose was determined as the effective dose for the highest amount of TPS ($y=-0.0133x^2+0.0851x+0.87$, $R^2=0.1541$).

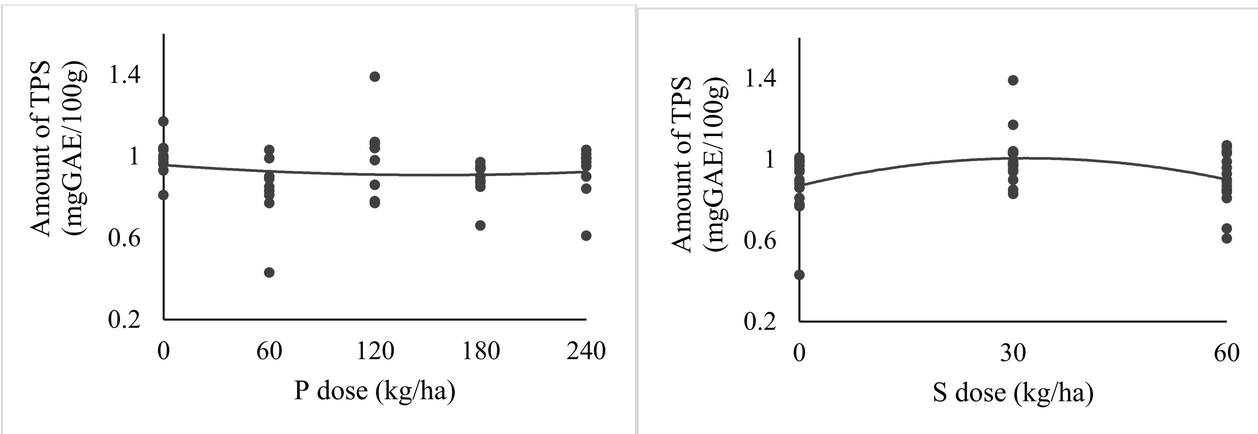


**Figure 3. Regression models for TPS amounts for different doses of P and S.**

**Antioxidant Activity:** In 2022, the antioxidant activities of lavender oils showed a statistically significant difference between the treatments applied with different P and between the interactions of the treatments applied with different P and S doses ($p<0.001$ and $p<0.001$, respectively). Among the treatments applied with different P doses, the highest $EC_{50}$ value was measured in the $P_{240}$ (1.48 µL), while the lowest $EC_{50}$ value was measured in the $P_{60}$ (0.95 µL) and $P_{180}$ (0.97 µL) treatments. In the interactions of treatments, the highest $EC_{50}$ value was measured in the $P_{240} \times S_{30}$ (1.77 µL) interaction and the lowest $EC_{50}$ value was measured in the $P_{120} \times S_{30}$ (0.71 µL). In 2023, a statistically significant difference was found between the treatments applied with different doses of P and S and the interactions of these treatments ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). Among the treatments applied with different P doses, the highest $EC_{50}$ value was measured in the $P_{240}$ (2.24 µL), while the lowest $EC_{50}$ value was measured in the $P_{60}$ (1.72 µL) and $P_{120}$ (1.75 µL) treatments. Among the tratments applied with different S doses, the highest $EC_{50}$ value was measured in the $S_0$ (2.06 µL) while the lowest $EC_{50}$ value was measured in the $S_{30}$ (1.70 µL) treatment. In the interactions of the treatments applied with different P and S doses, the highest $EC_{50}$ value was measured in the $P_{240}$ x $S_{60}$ (2.76 µL) interaction, while the lowest $EC_{50}$ value was measured in the $P_{120}$ x $S_{60}$ (1.22 µL) interaction.

The effects of different doses of P application and different doses of S application on the antioxidant effects of lavender oils were investigated by creating quadratic regression models for the 2023 (Figure 4). As a result of the modeling, it was found that 92.4 kg/ha P dose was associated with the lowest $EC_{50}$ value (highest antioxidant activity) ($y=0.0025x^2-0.0462x+1.9593$, $R^2=0.2221$). Similarly, 30.1 kg/ha S dose was found to be associated with the lowest $EC_{50}$ value ($y=0.0403x^2-0.2428x+2.0607$, $R^2=0.1909$).

**Essential oil components:** In the first year of the study, EO component ratios were not affected by different doses of P and S applications at a statistically significant level. In 2023, different doses of S applied did not affect the EO component ratios, but lavandulyl acetate and α-terpineol levels showed statistically significant differences among the treatments applied with different doses of P. Lavandulyl acetate ratios were found to be highest in the $P_{120}$ (3.64%) treatment and lowest in the $P_{60}$ (3.17%) treatment ($p=0.031$). α-terpineol ratios were found to be highest in the $P_{60}$ (3.44%) treatment and lowest in the $P_0$ (2.88%) treatment ($p=0.031$).

**Correlation analyzes:** Naturally, a strong positive correlation was found between the EO percentage and EO yield in both 2022 and 2023 (2022; R: 0.564; 2023; R: 0825). A strong negative correlation was found between TPS amounts and EO rates in 2022 (R: -0.413), but no statistically significant correlation was found in 2023. A positive correlation (R: 0.296) in 2022 and a negative correlation (R: -0.351) in 2023 was found between the EC50 values and EO ratios of lavender oils. A strong negative correlation was found between the TPS amounts and EC50 values in both years (2022; R: -0.523; 2023; R: -0.367) (Table 2).

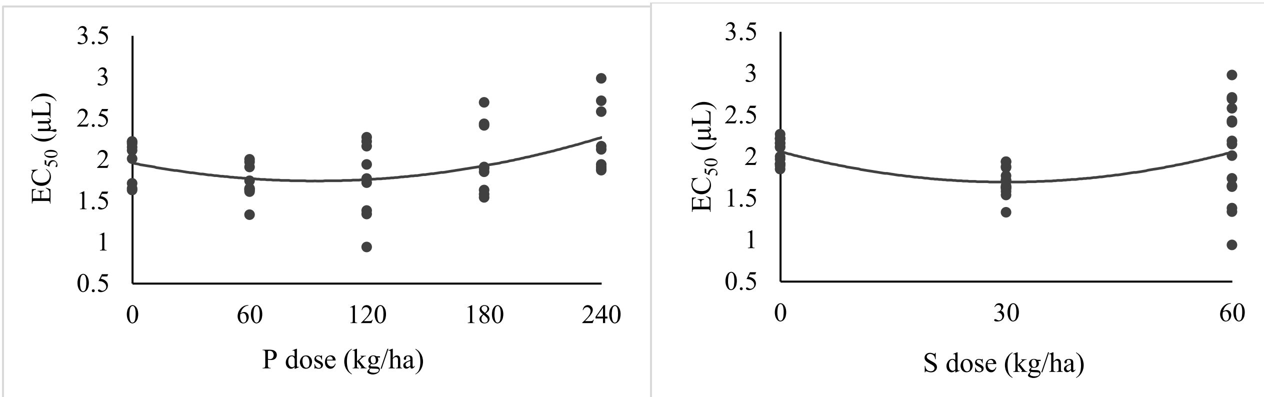


**Figure 4. Regression models for $EC_{50}$ value for different doses of P and S.**

**Table 2. Correlation analysis between EO (%) and yield (kg/ha), TPS amount and antioxidant activity.**

| | | EO (%) | EO yield (kg/ha) | Amount of TPS (mg GAE/100g) |
|---|---|---|---|---|
| 2022 | EO yield (kg/ha) | 0.564$^{**}$ | | |
| | Amount of TPS (mg GAE/100g) | -0.413$^{**}$ | -0.209 | |
| | $EC_{50}$ value (µL) | 0.296$^{*}$ | 0.245 | -0.523$^{**}$ |
| 2023 | EO yield (kg/ha) | 0.825$^{**}$ | | |
| | Amount of TPS (mg GAE/100g) | -0.079 | -0.134 | |
| | $EC_{50}$ value (µL) | -0.351$^{*}$ | -0.278 | -0.367$^{**}$ |

$^{*}$: Significant correlation at 0.05 level.
$^{**}$: Significant correlation at 0.01 level.

**Table 3. Correlation analysis between EO component ratios and EO percentages, EO yields, TPS amounts, $EC_{50}$ values.**

| | | **Linalool (%)** | **Linalyl acetate (%)** | **Camphor (%)** | **Cis-β-Ocimene (%)** | **Trans-β-Ocimene (%)** | **Terpinen-4-ol (%)** |
|---|---|---|---|---|---|---|---|
| 2022 | EO (%) | -0.476 | 0.427 | -0.427 | 0.448 | 0.126 | -0.140 |
| | EO yield (kg/ha) | -0.632$^{*}$ | 0.690$^{**}$ | -0.474 | -0.179 | -0.493 | -0.140 |
| | Amount of TPS (mg GAE/100g) | 0.041 | -0.190 | 0.311 | -0.105 | 0.051 | -0.088 |
| | $EC_{50}$ value (µL) | -0.126 | 0.057 | 0.049 | 0.328 | 0.371 | -0.193 |
| 2023 | EO (%) | 0.445 | -0.551$^{*}$ | -0.842$^{**}$ | 0.305 | 0.284 | 0.728$^{**}$ |
| | EO yield (kg/ha) | 0.690$^{**}$ | -0.714$^{**}$ | -0.812$^{**}$ | -0.061 | 0.089 | 0.846$^{**}$ |
| | Amount of TPS (mg GAE/100g) | -0.452 | 0.415 | 0.108 | 0.359 | 0.236 | 0.057 |
| | $EC_{50}$ value (µL) | 0.232 | -0.009 | 0.330 | -0.620$^{*}$ | -0.621$^{*}$ | -0.504 |
| | | Lavandulyl acetate (%) | Lavandulol (%) | 1,8-Cineole (%) | 3-Octanone (%) | α-Terpineol (%) | Limonen (%) |
| 2022 | EO (%) | -0.418 | 0.011 | -0.259 | -0.117 | -0.423 | -0.138 |
| | EO yield (kg/ha) | -0.063 | -0.056 | -0.473 | -0.314 | -0.600$^{*}$ | -0.530$^{*}$ |
| | Amount of TPS (mg GAE/100g) | 0.316 | -0.278 | 0.340 | 0.078 | 0.188 | 0.026 |
| | $EC_{50}$ value (µL) | -0.197 | 0.193 | -0.005 | -0.044 | -0.158 | 0.249 |
| 2023 | EO (%) | -0.187 | 0.293 | -0.894$^{**}$ | - | 0.778$^{**}$ | 0.153 |
| | EO yield (kg/ha) | -0.532$^{*}$ | 0.551$^{*}$ | -0.805$^{**}$ | - | 0.858$^{**}$ | 0.119 |
| | Amount of TPS (mg GAE/100g) | 0.396 | -0.357 | -0.060 | - | -0.358 | -0.271 |
| | $EC_{50}$ value (µL) | -0.217 | 0.236 | 0.494 | - | -0.092 | 0.063 |

$^{*}$: Significant correlation at 0.05 level, $^{*}$: Significant correlation at 0.01 level, 3-Octanone was trace amounts for all measurements in 2023, β-phellandrene was not included in the correlation analysis since it was measured in trace amounts in all measurements.

EO component ratios indexed in Ph Eur and ISO standards and EO percentages, EO yields, TPS amounts and $EC_{50}$ values were analysed with correlation analysis and the results are given in Table 3. A negative correlation (R: -0.632) was found between the linalool percentage and the EO yield in 2022, and a positive strong correlation (R: 0.690) was found in 2023. Unlike linalool, a positive strong correlation (R: 0.690) was found between the linaly acetate percentage and the EO yield in 2022 and a negative strong correlation (R: -0.714) was found in 2023. In 2023, a strong negative correlation was found between essential oil (EO) percentages and EO yields for camphor and 1,8-cineole. Conversely, a strong positive correlation was found between EO yields and the abundance of terpinen-4-ol and α-terpineol.

## DISCUSSION

LA, a valuable EO plant, is used in many fields, especially in medical and cosmetic. Although, numerous studies have investigated the effects of various P treatments on Lavandula (Chrysargyris *et al*., 2016; Erbaş *et al*., 2017; Peçanha 2021); research focusing specifically on the impact of P fertilization on EO yield is limited. Nevertheless, it is established that P fertilization enhances Lavandula growth and EO yield. This study, which hypothesized that S fertilization would also increase Lavandula EO yield and antioxidant activity. In a field trial conducted in the Isparta/Turkey, the highest EO yield was obtained in Lavandulas treated with P at 100 kg/ha and 150kg/ha (Erbaş *et al*., 2017). In a study conducted by Peçanha (2021) in Brazil, different P doses (0, 30, 60, 90, 120 mg/dm$^3$) were applied to LA plants grown under greenhouse conditions and in pots, and the highest EO yield was obtained in lavender plants treated with 60 mg/dm$^3$ P. All these studies and the results found in our study showed that the effect of P dose on EO yield is in accordance with the normal distribution. Excess P is associated with conditions such as inhibition of plant growth, acceleration of leaf senescence and micronutrient deficiency (Hawkins *et al*., 2008), and the decrease in EO yield of LA plants applied with high P doses can be explained by these mechanisms.

As a result of normal distribution, when applying P fertilization on LA, an appropriate P dose should be calculated by considering the peak of the bell curve. In this context, Peçanha (2021) investigated how P applied at different doses affects Lavandula EO rates using a regression model and a regression model similar to our study was obtained. According to Peçanha's model, it was found that 77 mg/dm$^3$ P dose was the most effective dose to obtain the highest EO percentage. Even if regression models estimate a sharp effective P dose, the P content of the soil under LA cultivation is a very important parameter in determining the P dose to be applied. Moreover, other soil properties such as pH may also affect P uptake by plants through different mechanisms.

This study was a pioneer study examining the effects of S fertilization on LA and added the effective S fertilization doses required to obtain the highest EO rate and EO yield from LA shrub to the literature. The effect of different S doses on Lavandula EO, antioxidant activity and TPS amount was found to be in accordance with normal distribution. While the role of S in plant metabolism is well known, information on the effects of high S concentrations on plants is quite insufficient. It is known that sulfur dioxide causes chlorosis and intervascular necrosis in plants and reduces growth (Chandra and Pandey, 2016). High S level in soil may have caused an increase in toxic S metabolites such as sulfur dioxide in plants, leading to a decrease in EO yield. Likewise, the effects of different doses of P applications on LA antioxidant activity and TPS amount were found to be in accordance with normal distribution. In parallel with this result, in a pot study, P fertilization was applied to Lavandula at doses of 30, 40, 50, 60 and 70 mg/L, and the highest phenolic content and the highest antioxidant activity were obtained in plants treated with 60 mg/L P dose (Chrysargyris *et al*., 2016). The results obtained in our study has made a significant contribution the estimated effective doses of P and S to reach maximum antioxidant activity and TPS amount to the literature.

In the quality criteria published by the European Pharmacopoeia and ISO (European Pharmacopoeia, 2010; ISO 3515:2002) for EO of LA, certain reference intervals have been determined for some EO components. When the EO components were examined, the linalyl acetate ratios were measured below the minimum limit specified in both quality standards (The range of linalyl acetate is 25-47% in both standards), however the linalyl acetate ratio increased in 2023 compared to 2022 and approached the reference value. While the α-terpineol ratio was stated as a maximum of 2% in both standards, it was seen to be above 2% in both years of our study. Similar to linalyl acetate, the α-tarpineol ratio also approached the value determined in the quality standards in 2023. Other EO components indexed in these standards were measured within the intervals specified in the standards. Considering that the quality of LA oil increases with its high linalyl acetate and linalool ratio and that the increase in camphour ratio decrases the oil quality (Biswas *et al*., 2009), the oils obtained in our study became of better quality in 2023 and it was found to comply with all standards except those determined for linalyl acetate and α-terpineol components. Apart from the suboptimal quality of EO, it is highly probable that the lower EO yield, antioxidant activity, and TPS content observed in the first year of lavender cultivation is attributable to the plants' insufficient root length and stem development, which are typical of newly established plants.

It has been found that different P doses have an effect on the levels of lavandulyl acetate and α-terpineol in EO, but no significant effect was found on other EO components. Peçanha (2021) demonstrated that different P doses had a statistically significant effect on all EO components, especially linalyl acetate, linalool, and camphor. However, another study found that while increasing P doses increased linalyl acetate content and decreased linalool content, P dose had no effect on camphor ratio (Erbaş *et al*., 2017). These contradictory results make it impossible to draw a conclusion about the relationship between P dose and the ratios of lavender oil components. Although the study found that S fertilization increased Lavandula EO yield, content, and antioxidant activity, it did not affect the composition of the EO at different S doses. The most likely reason for this is that S plays various roles in the formation of amino acids such as methionine and cysteine, the synthesis of proteins, chlorophyll, and antioxidants (glutathione), the biosynthesis of phytohormones such as ethylene and polyamines, and various physiobiochemical processes in plants (Shah *et al*., 2022), and it can fully enhance the biosynthesis of bioactive compounds without affecting the distribution of EO components. There is no clear study in the literature regarding the application of different doses of S to Lavandula EO.

There were some differences in the LA seedlings provided for the study (root development, height, etc.). Although this situation constitutes a limitation, the seedlings were randomly distributed to the trial plots to prevent this situation from distorting the results obtained from the plots with different doses of fertilization. Since the experiment was set up under field conditions, it was not possible to keep the climate characteristics under control. Nevertheless, since all the trial plots were in the same area, they were affected by different climate conditions at the same time. Another study limitation was that weed control was carried out mechanically (by hand hoeing) throughout the experiment, and to overcome this situation, the hoeing process was repeated at regular intervals.

**Conclusion:** In conclusion, appropriate dozes of P and S fertilizers significantly increased EO yield, TPS amount and antioxidant activity in LA. Regression modeling revealed that the optimal fertilization dozes for maximizing EO yield under continental climatic conditions were 117.9 kg/ha P and 47.5 kg/ha S, while the optimal dozes for maximizing the antioxidant activity of Lavandula EO were 92.4 kg/ha P and 30.1 kg/ha S. Findings of the study clearly demonstrate that both P and S fertilization enhanced EO yield and antioxidant activity in Lavandula without compromising EO quality.

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**Conflict of Interest:** There is no conflict of interest between the authors of this study.

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