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The effect of altitude and soil properties on the essential oil components of Turkish sage (*Salvia fruticosa* Mill.)

[El efecto de la altitud y las propiedades del suelo sobre los componentes de aceites esenciales en la salvia Turca (*Salvia fruticosa* Mill.)]

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Abstract: In this study, the effects of altitude and soil properties on the essential oil amounts and essential oil components of the Turkish sage (*Salvia fruticosa* Mill.) plant were investigated. A location in the south of Turkey in the Kaş district of Antalya with altitude ranges of 0-200 m, 300-500 m, and 600-800 m was defined as the study area. During the full flowering period of S. fruticosa, plants and soil samples were collected. The contents of the essential oils of the plants were analyzed using GC-MS. According to the results obtained, the essential oil content of plants varied between 1.91% and 5.10%. The main component of the essential oils of the plants collected from all study areas was 1,8-cineol, at 44.93%. It was concluded that variations in the proportions of the components limonene and linalool, which are essential oil components of plants, are not only dependent on altitude but also on changes in soil texture and the total $CaCO_3$ content of the soil.

Keywords: *Salvia fruticosa* Mill.; Altitude; Essential oil components; Soil parameters; Turkey

Resumen: En este estudio, se investigaron los efectos de la altitud y las propiedades del suelo sobre las cantidades de aceite esencial y los componentes del aceite esencial de la planta de salvia turca (*Salvia fruticosa* Mill.). Se definió como área de estudio una ubicación de 300-500 m a 600-800 m en el sur de Turquía en el distrito de Kaş de Antalya con rangos de altitud de 0-200 m. Durante el período de plena floración de S. fruticosa, se recolectaron plantas y muestras de suelo. El contenido de los aceites esenciales de las plantas se analizó mediante GC-MS. Según los resultados obtenidos, el contenido de aceite esencial de las plantas varió entre el 1,91% y el 5,10%. El componente principal de los aceites esenciales de las plantas recolectadas de todas las áreas de estudio fue el 1,8-cineol, al 44,93%. Se concluyó que las variaciones en las proporciones de los componentes limoneno y linalol, que son componentes del aceite esencial de las plantas, no solo dependen de la altitud sino también de los cambios en la textura del suelo y el contenido total de $CaCO_3$ del suelo.

Palabras clave: *Salvia fruticosa* Mill.; Altitud; Componentes de aceites esenciales; Propiedades del suelo; Turquía

INTRODUCTION

The *Salvia* genus is the largest in the *Lamiaceae* family. In the world in general, 1000 *Salvia* species are mostly found in three regions: Central and South America (500 spp.), West Asia (200 spp.) and East Asia (100 spp) (Kahraman *et al*., 2012; Martin *et al.*, 2015; Anwar *et al*., 2017). In the flora of Turkey, the number of known species belonging to the *Salvia* genus is 100 (Temel *et al*., 2016).

Salvia species are widely used for their antioxidant properties, because they contain phenolic compounds (phenolic acids, flavonoids, tannins, etc.) and terpenoids (karetonoids, essential oils) (Poulios *et al*., 2020). The components and concentrations of the essential oils of plants belonging to the genus *Salvia* show significant variations specific to the species (Rajabi *et al*., 2014). The main components of the essential oils of *Salvia officinalis*, *Salvia fruticosa* and *Salvia lavandulifolia*, species native to the Mediterranean region, are usually 1,8 cineol, α - β thujone, α - β pinene, camphor, camphene and borneol (Raal *et al*., 2007; Kocabas *et al*., 2010; Peñalver *et al*., 2010).

S. fruticosa Miller (Syn. *S. triloba* L.), generally known as Eastern Mediterranean Sage, grows mostly in calcareous soils where maquis vegetation is predominant. The species is found from sea level to an altitude of 1000 m (Karabacak, 2009).

In Turkey, the leaves and stems of *S. fruticosa* are used as a herbal tea, which is drunk hot. At the same time, essential oils obtained from *S. fruticosa* are used in the treatment of flu (colds, cough) (Ugurlu & Secmen, 2008), tonsillitis, digestive problems (stomachache, flatulence, constipation), skin disease, and wounds (Gurdal & Kultur, 2013)

The essential oil of *S. fruticosa* is commonly called "apple oil". This is because the galls of the plant look like apples. Studies indicate that *S. fruticosa* extracts and essential oils carry antioxidants (Selcuk *et al*., 2021), antimycobacterials (Askun *et al*., 2010), antifungals (Pitarokili *et al*., 2003), antimicrobials (Kulaksız *et al*., 2018) and antivirals (Pirintsos *et al*., 2020). The leaves of *S. fruticosa* contain the essential oil 1,8-cineole in the range of 0.5-5%. This component, which is found in large amounts in the essential oil of *S. fruticosa*, makes this species a valuable medicinal plant (Topcu *et al*., 2013).

Many studies have shown that altitude differences in locations where plants grow affect the plants' yield of fresh or dry seeds, inflorescences and essential oil (Katar *et al*., 2017; Ascrizzi *et al*., 2019) and their chemical contents (phenol, polyphenol, flavonoid, antioxidant, vitamin C, essential oil components, etc.) (Maric *et al*., 2006; Schnitzler *et al*., 2008; Gupta *et al*., 2011; Bidgoli *et al*., 2013; Dhanik *et al*., 2017; Amiri *et al*., 2018).

It is thought that in addition to the effect of altitude differences on the essential oil amounts and component proportions of the plants, soil differences also have an important effect on these (Intrigliolo *et al.*, 1999; Kazemi *et al.*, 2017). Therefore, it is of great importance for its cultivation and trade sustainability to determine the growing area where the amount of essential oil of the *S. fruticosa* plant and the proportions of the major components forming the essential oil are high, and to evaluate the essential oil components of this plant in relation to soil properties, since it is collected from the wild and its export has commercial importance.

In this study, an examination was made of the effects of altitude and soil properties on the amount of essential oil and the essential oil components of *S. fruticosa*, which is part of the natural flora of the Mediterranean.

MATERIAL AND METHODS

Plant material

This research was conducted in the Kaş district of Antalya province in the south of Turkey in 2012-2013 during the full flowering period of *S. fruticosa* when essential oil components are high. Three different altitude ranges were determined considering the distance from the sea of habitats where the plants grow naturally: 0-200m (36°12'21.20"N - 29°38'13.87"E), 300-500 m (36°12'11.94"N - 29°39'21.51" E) and 600-800 m (36°14'48.07"N - 29°40'43.69"E). Most of the natural habitats of the plants were mountainous areas. Plant and soil samples were taken from a depth of 0-30 cm from a total of 30 different points at three different altitudes. GPS coordinates were determined, and the locations of the samples taken are shown in Figure No. 1. Ten different plant and soil samples were taken from each altitude; that is, 30 plants and 30 soil samples were collected in total.

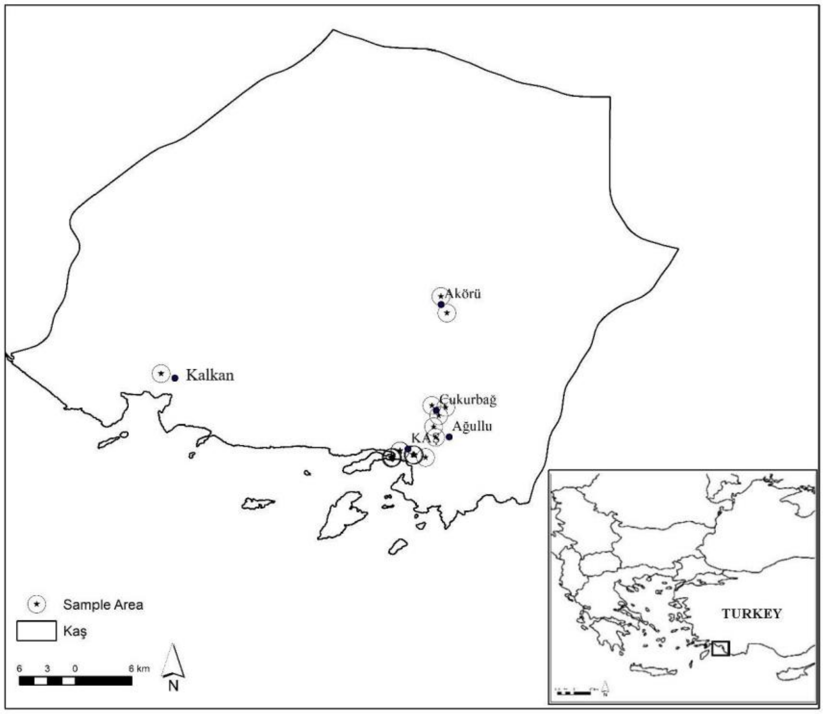

Figure No. 1
Location of the study area and sampling points

Soil analysis

Soil samples obtained from a depth of 0-30 cm were air-dried and passed through a 2 mm sieve. Soil pH and electrical conductivity were determined using the Inolab pH/Cond 720 (WTW, Germany) in a 1:2.5 soil:deionized water (w/v) suspension (Liu *et al.*, 1996). Soil particle size analysis (50 g soil) was performed using the hydrometer method (Bouyoucos, 1951), and soils were classified using the texture triangle (USDA, 2019). A Scheibler calcimeter was used to determine the $CaCO_3$ content of soils by measuring the CO_2 gas produced by the reaction of 0.25g of the soil with HCl (Nelson, 1982). Organic matter was determined by the wet oxidation method (Walkley-Black procedure) using a potassium dichromate ($K_2Cr_2O_7$) solution in 1g of soil sample (Black, 1965). The soil analysis was performed in three replications.

Extraction of essential oils

First, fresh plant samples were collected during the flowering period. Second, the plants were dried in the shade in room conditions. Third, the amount of essential oil in the leaves of the plants was determined after the moisture content of the plants fell below 12% by making frequent measurements using the toluene distillation method (AOAC, 2002). In order to determine the amount of essential oil, 300 ml of distilled water was added to 20 g of dry leaves and distilled in a Clevenger type hydro-distillation (Langenau, 1948) device for 5 hours, and then the amount of essential oil was calculated according to the dry material (v/w). This process was repeated four times in each example. The oils obtained were dried over anhydrous sodium sulfate and stored in a sealed vial at +4°C in the dark until analysis.

GC-MS analysis

The proportions of essential oil components (%) obtained from the dried leaves of *S. fruticosa* collected at different altitudes were obtained according to the method of Ozek *et al*. (2010) using GC/MS equipment (GC Agilent 7890A/MS Agilent 5975C). HP Innowax Capillary columns of 60.0 m x 0.25 mm x 0.25 µm were used as columns. After the column temperature was kept at 60°C for 10 minutes, it was raised to 250°C by increasing by 20°C per minute and finally incubated at 250°C for 8 minutes. Operating conditions were as follows: the temperature of the injection block: 250°C, split ratio:

50:1, injection volume: 1 µL, carrier gas: helium, flow rate: 1 mL/min. Identification of essential oil components is based on data of Wiley 7 and NIST mass spectral library.

Statistical analyses
Variance analysis and Duncan tests were performed using a licensed SPSS Statistics Base v23 program to analyze the study values.

RESULTS AND DISCUSSION

Physical and chemical properties of soils
The average values of the physical and chemical properties of the soil samples according to the altitudes where the *S. fruticosa* plant grows are presented in Table No. 1.

Table No. 1
Physical and chemical properties of soils in the different altitude ranges

Soil characteristics	Altitude ranges			Anova
	0-200m	300-500m	600-800m	
Sand (%)	39.41±9.37$^{\theta}$ b	39.93±9.10 b	57.98±14.68 a	**
Clay (%)	24.46±4.62	25.41±8.38	20.40±8.85	ns
Silt (%)	36.13±10.15 a	34.66±8.28 a	21.62±8.80 b	**
pH	7.29±0.19 b	7.48±0.35 ba	7.63±0.18a	*
Total $CaCO_3$ (%)	2.76±2.36 b	9.40±6.51 b	45.92±25.68 a	**
Electrical conductivity ($dS\ m^{-1}$)	0.33±0.078	0.30±0.053	0.34±0.098	ns
Organic matter (%)	8.83±2.60 a	6.55±2.37 b	4.98±2.17 b	**

$^{\theta}$: Means ± standard deviation; a. b: Different letters on the same line indicate statistical difference; ns: Not significant; *: Significant at $p \leq 0.05$; **: Significant at $p \leq 0.01$

It was determined that the sand content of the soils increased and the silt content decreased in parallel with the increase in altitude in the areas where the *S. fruticosa* plant was collected. This change in soil texture was statistically significant at a 1% level. This can be explained by the slow maturation in the soils of high-altitude areas and the emergence of a large number of sand, gravel and stone formations (Charan *et al.,* 2013). The altitude did not cause a significant change in the clay content of the soils. According to the soil texture classification, while soils at 0-200 m and 300-500 m altitudes were in the loam class, soils at 600-800 m altitudes were in the sandy clay loam class.

As the altitude increased, soil pH ($p<0.05$) and total calcium content of soils ($p<0.01$) showed a statistically significant increase. It is known that there is a strong connection between the lime content of the soils and soil pH (Havlin *et al.*, 1999), and this study also supports the link between the lime content of soils and soil pH.

The proportion of organic matter in the soil samples decreased with increasing altitude ($p \leq 0.01$). However, the soil samples of all three regions were in the high organic matter content class. Erosion, which causes the transport of fine organic particles from high altitude areas to low altitude areas, is thought to be the reason for the lower organic matter content of soils in the altitude range of 600-800 m.

Essential oil amount and essential oil components
While the highest amount of essential oil in *S. fruticosa* was obtained from plants which were collected at an altitude of 0-200 m (3.84 ± 0.84%), the amounts of essential oil in the plants which were collected at altitudes of 300-500 m and 600-800 m were found to be similar (3.72 ± 0.78; 3.72 ± 0.35). With increasing altitude, a decrease was determined

in the amounts of essential oil in the plants (Delazar *et al*., 2011; Mohamadi & Rajaei, 2016), but this decrease was not found to be statistically significant.

The *Salvia* taxa of Turkey are classified according to the main components of their essential oils, and are divided into three groups. *Salvia fruticosa* is in the CiCa (1,8-cineole/camphor) group (Baser, 2002). 1,8-cineole is a widely dispersed natural fragrance and complex fluid-structure (Aparicio *et al*., 2007) with a eucalyptus-like scent, which gives the substance its name, eucalyptol (Kirsch & Buettner, 2013).

In this study, between 46 and 81 different essential oil components were identified in the *S. fruticosa* plant, and the statistical analysis of 23 standard components of the plants growing at each of the three altitudes is given in Table No. 2.

Table No. 2
The essential oil components of *Salvia fruticosa* Mill. in different altitude ranges

No	Essential oil components	IR	Altitude ranges			Anova
			0-200 m	300-500 m	600-800 m	
1	α-pinene	1016	3.76 ± 0.47⁰	3.72 ± 0.66	4.22 ± 0.56	ns
2	Camphene	1058	1.94 ± 1.68 a	0.55 ± 0.46 b	2.32 ± 1.66 a	*
3	β-pinene	1102	5.25 ± 1.08	5.69 ± 0.85	5.50 ± 0.58	ns
4	Sabinene	1108	0.23 ± 0.33	0.09 ± 0.05	0.27 ± 0.41	ns
5	β-myrcene	1155	2.32 ± 0.67	2.21 ± 0.39	2.13 ± 0.63	ns
6	α-terpinene	1173	0.57 ± 0.24	0.52 ± 0.07	0.49 ± 0.19	ns
7	Limonene	1192	1.29 ± 0.15 b	1.32 ± 0.29 b	1.72 ± 0.49 a	**
8	1.8-cineole	1205	40.82 ± 7.94 b	49.36 ± 4.84 a	44.61 ± 6.07 ba	*
9	ϒ- terpinene	1238	0.99 ± 0.43	0.87 ± 0.12	0.85 ± 0.32	ns
10	Cymene	1264	0.71 ± 0.29	0.65 ± 0.15	0.71 ± 0.25	ns
11	α-terpinolene	1275	0.27 ± 0.08	0.23 ± 0.03	0.26 ± 0.09	ns
12	α-thujone	1420	3.13 ± 2.5	1.75 ± 1.56	3.38 ± 3.99	ns
13	β-thujone	1441	5.01 ± 3.9	7.89 ± 6.16	3.57 ± 2.11	ns
14	Linalool	1534	0.95 ± 0.57 a	0.78 ± 0.35 a	0.33 ± 0.30 b	**
15	Camphor	1519	6.87 ± 4.19 a	2.84 ± 2.43 b	8.38 ± 3.62 a	**
16	Bornyl acetate	1577	1.43 ± 1.63	0.25 ± 0.23	0.99 ± 1.22	ns
17	Terpinene-4-ol	1597	0.77 ± 0.18	0.70 ± 0.11	0.79 ± 0.26	ns
18	β-caryophyllene	1587	3.79 ± 1.12	4.20 ± 1.47	3.79 ± 2.53	ns
19	δ-terpineol	1665	0.88 ± 0.41	0.96 ± 0.41	0.54 ± 0.47	ns
20	α-terpineol	1690	2.19 ± 1.12	3.63 ± 3.08	1.32 ± 1.45	ns
21	δ-cadinene	1752	0.28 ± 0.20	0.24 ± 0.17	0.25 ± 0.25	ns
22	Caryophyllene oxide	1995	0.53 ± 0.23	0.63 ± 0.26	0.43 ± 0.26	ns
23	Viridiflorol	2091	2.20 ± 0.68	2.20 ± 0.77	1.41 ± 0.93	ns

IR: Retention indices; ⁰: Means ± standard deviation; a.b: The different letters on the same line indicate statistical difference; ns: Not significant; *: Significant at p≤0.05; **: Significant at p≤0.01

The effect of altitude on camphene, 1,8-cineole, limonene, linalool, and camphor, which are essential oil components of *S. fruticosa,* were found to be statistically significant. In studies on the essential oil components of *S. fruticosa*, the most important essential oil component is stated to be 1,8-cineole (Bayrak & Akgul, 1987; Leontaritou *et al*., 2020). In our findings also, 1,8-cineole (27.90%-55.03%) was found to be in the highest proportions at all three altitudes. The 1,8 cineol content of essential oils was while similar to that in other studies conducted in Turkey and Greece (Karık *et al.,* 2018; Skoula *et al.,* 2000), but it was found to be much higher than in studies conducted in southern Brazil (Delamare *et al.,* 2007). These changes in essential oil compositions may vary under the influence of the environment (climatic, seasonal, geographical) (Bellomaria *et al.,* 1992), genetic factors (Perry *et al.,* 1999) and agricultural practices (Sorrou *et al.,* 2016). The highest proportion was obtained from plants collected from an altitude of 300-500 m ($p \leq 0.05$). The change in the 1,8-cineole content of essential oils due to the increase in altitude was similar to the results of Mohamadi & Rajaei (2016).

The effect of altitude on the camphor component in the essential oils of *S. fruticosa* was found to be statistically significant at the 1% level. The percentage of camphor in the essential oils of plants varied between 0.55 and 13.40%, and the highest proportion of camphor (6.87-8.38%) was obtained from plants collected at an altitude of 600-800 m. Camphor was the second most important essential oil component of plants collected at altitudes of 600-800 m and 0-200 m, while β-thujone was the second most important essential component in plants which were collected at an altitude of 300-500 m. In many studies, camphor has been reported as the second most important essential oil component of the *S. fruticosa* plant (Giweli *et al.,* 2013). However, the second component varied in some studies (Skoula *et al.,* 2000; Topcu *et al.,* 2013).

Camphene percentages of essential oils of *S. fruticosa* varied between 0.09 - 4.91%. When the Camphene proportions were examined. The highest camphene proportion, an average of 2.32%, was detected from plants which were collected at an altitude of 600-800 m. This was followed in order by plants collected at altitudes of 0-200 m and 300-500 m.

The effect of altitude on the amounts of limonene and linalool in the essential oils of plants was found to be statistically significant ($p \leq 0.05$). As the altitude increased, the amount of limonene in the plants' essential oils increased, while the amount of linalool decreased. Similar to these findings, Gupta *et al*. (2011) reported that as the altitude increased, the amount of limonene in the essential oils of *Zanthoxylum alatum* Roxb. plants increased and the amount of linalool decreased.

The correlation between essential oil components and soil properties

The correlation analysis between the soil properties at different altitudes and the essential oil components of the *S. fruticosa* plant are presented in Table No. 3.

Table No. 3
Correlation between essential oil components and soil characteristics

Soil characteristics (X)	Essential oil components (Y)	Correlation coefficient (r)
pH	limonene	0.590***
	α-terpineol	0.403*
Total $CaCO_3$(%)	limonene	0.417*
	linalool	-0.512**
	viridiflorol	-0.456**
Electrical conductivity (dS/m)	camphene	0.509**
	β-myrcene	-0.591***
	limonene	0.552**
	β-thujone	-0.398*

	camphor	0.475**
	β-caryophyllene	-0.413*
	δ -terpineol	0.418*
	δ -cadinene	0.367*
	Caryophyllene oxide	-0.435*
Sand (%)	limonene	0.463**
	β-thujone	-0.432*
	camphor	0.383*
	linalool	-0.424*
	Caryophyllene oxide	-0.372*
	viridiflorol	-0.388*
Clay (%)	α-pinene	-386*
	limonene	-0.416*
	β-thujone	0.474**
Silt (%)	linalool	0.366*
Organic matter (%)	δ -terpineol	0.391*

***,** or *** indicates significant at *p*≤0.05. 0.01 or 0.001 respectively**

When the correlation analyses between the soil properties at different altitudes and the essential oil components of the *S. fruticosa* plant were examined, a positive relationship was found between the amounts of limonene in the essential oils and pH, EC values, $CaCO_3$ and the sand contents of soils. On the other hand, there was a negative relationship between the amount of limonene and the clay content. A negative correlation was found between the amounts of linalool in the essential oils of plants and the $CaCO_3$ and the sand contents of the soils, and a positive correlation was found between the amounts of linalool and the silt content of the soils. In a study by Intrigliolo *et al*. (1999), which was similar to our study results, it was emphasized that there was a strong relationship between the proportions of linalool and limonene in the essential oils of plants and the soil textures in which the plants grew. A positive correlation was determined between the amount of camphor, which is an important essential oil component of plants, and the EC values and sand content of soils.

CONCLUSION

The 1,8-cineole content of *S. fruticosa* plants growing in an altitude range of 300-500 m in calcareous soils with a loamy texture, slightly alkaline reaction, low salt content, and suitable organic matter content was determined as having the highest value. Therefore, if *S. fruticosa* plant production is planned based on the quantities of essential oil components, the altitude and soil properties where the said component amounts are the highest should be taken into consideration.

At the same time, the variations with altitude in the limonene and linalool contents of the *S. fruticosa* plant and the relationship of these components with the texture and the $CaCO_3$ contents of soils were considered to be statistically significant in this study, and it was concluded that detailed studies on these variations were required.

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