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Effect of different natural habitats on the variation in essential oil components of *Origanum onites* L.

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ABSTRACT

Origanum onites L., which is native to the Mediterranean region, is among one of the economically significant medicinal and aromatic plants that are not only gathered in the wild, but also cultivated in our country. The purpose of this study was to determine how the region in which *Origanum onites* grows affects the amount of essential oil and essential oil components in the plant's flowers and leaves. In this context, the effect of the plant's growing location on the essential oil content and essential oil components of the flowers and leaves of the *Origanum onites*, which grows naturally between 0-100 m in the Serik-Aksu, Demre, and Kaş regions of Antalya, was investigated together with the soil properties. The essential oils were extracted from the plant samples' leaves and flowers using the hydrodistillation method. The analysis of essential oils was done using gas chromatography-mass spectrometry (GC-MS) systems. Soil samples were analyzed for their pH, EC, $CaCO_3$, texture, organic matter, total N, available P, and K values. The organic matter and exchangeable potassium contents of the soils, as well as the content of essential oil in the flowers and leaves of the plants, were found to increase gradually towards the west of Antalya. It has been demonstrated that the amounts of essential oil components of the plants varied depending on the region in each of the three different study locations.

1. Introduction

The genus *Origanum* L. (*Lamiaceae*) with 21 species (13 endemics out of 24 taxa) and 13 hybrid taxa has a wide distribution in Turkey flora (Arabaci et al. 2020). Species in *Origanum* are plants that have commercial prominence and are exported in substantial amounts. Its cultivation is widely done along the shores of the Aegean and the Western Mediterranean (Balıkesir, İzmir, Aydın, Muğla, Antalya). Turkey leads exportation worldwide, with a 19957 hectares cultivation area and 21174 tons of production per year in 2021–(TUIK 2022). *Origanum onites* L. which is colloquially known as İzmir kekiği, bilyalı kekik, eşek kekiği as regional names, and is called Turkish thyme (Türk kekiği) in Europe, has the largest thyme export share (approximately 80%) in the world (TRGM 2020).

Since antiquity, *O. onites* L. has been used as a spice, flavoring, herbal tea, and essential oil. The essential oil in the plant's leaves and flowers is the most important secondary metabolite, and the quantity of essential oil varies from 0.2 percent to 8.0 percent (Can et al. 2021). Carvacrol, thymol, linalool, ɣ-terpinene, α-terpinene, terpinen-4-ol, cymene, β-bisabolene, α-pinene, borneol, myrcene, α-thujene, β-caryophyllene, β-pinene, camphene, α-terpineol, caryophyllene oxide, terpinene, limonene and α-phellendran are the primary components of *O. onites* essential oil (Demirci et al. 2004; Tepe et al. 2016; Ozdemir et al. 2018). Essential oil extracted from *O. onites* possesses features such as antibacterial and antifungal (Vanti et al. 2021), antimicrobial (Tepe et al. 2016), antiviral, insecticidal (Ayvaz et al. 2010), anti-inflammatory (Aykac et al. 2020), anticancer (Spyridopoulou et al. 2019), analgesic activities (Aydın et al. 1996) and a source of natural antioxidant (Ozdemir et al. 2018). The composition and quantity of essential oils extracted from medicinal and aromatic plants may vary based on the kind of plant, plant organs, production technique, harvest time, drying process, geographical structure, and climate of the location in which they are cultivated (Kpoviessi et al 2016, Ozer et al 2018, Masoudi 2018, Mehalaine and Çencuni 2021).

In this study, the effect of the location where the plant grows on the essential oil yield and essential oil components in the flowers and leaves of the *O. onites* plant, which grows naturally in the Antalya districts of Serik-Aksu, Demre, and Kaş, as well as the soil properties was researched.

2. Materials and Methods

This research was carried out in June 2011 on plant and soil samples in Serik-Aksu, Demre and Kaş districts where *Origanum onites* naturally spreads. Samples were taken during the plant's full blooming periods when the essential oil components of the plant are at their highest. Between 0-100 meter altitude, 10 from Serik-Aksu district, 10 from Demre district, with the inclusion of 10 from Kaş district, a total of 30 soil and 30 plant samples were taken. The GPS coordinates were determined, and the locations of the samples taken are shown in Figure 1.

2.1. Soil analysis

Soil samples were taken to define edaphic characteristics from a depth of 0-30 cm by considering *O. onites'* root system. The samples were sifted through a 2 mm sieve after being dehydrated in room conditions. Suspension of soil samples' pH and EC at 1:2.5 soil: water mixed rates were defined with a WTW pH meter (Jackson 1967) and WT-720 EC meter. The content of $CaCO_3$ was measured by the Scheibler calcimeter (Çağlar 1949). The analysis of soil texture was defined with the hydrometer method (Black 1957). The organic matter content was determined according to the modified Walkley-Black method (Black 1965). The total amount of nitrogen in the soil was defined using the modified Kjeldahl method (Kacar 1995), the amounts of available phosphorus were defined using the Olsen method (Olsen and Sommers 1982), and the contents of exchangeable potassium were defined with the 1N Amonyum Asetat method (Kacar 1995). An ICP-OES Perkin Elmer 7000 DV device was used for the analysis of phosphorus and potassium.. While the obtained values were classified, the soil pH was determined according to Kellog (1952) and the electrical conductivity was determined by the U.S. According to Soil Survey Staff (1951), lime levels according to Evliya (1964), texture classification according to Black (1957), organic matter contents according to Black (1965), total nitrogen according to Loue (1968), available phosphorus was determined according to Olsen and Sommers (1982) and exchangeable potassium according to Pizer (1967).

2.2. Essential oil amount

Plant samples taken from three different distribution areas were dried in the shade at room temperature. Essential oils of dried flower and leaf samples were obtained by distillation in a Clevenger type hydro-distillation device. The method described in the ICS 11.120.10.67, 120.10 (TSE 1991) report of the Turkish Standards Institute was used to determine the essential oil content in dried leaf and flower samples. 20±0.001 g of plant materials were mixed with 300 ml of distilled water, and the samples were then distilled in the Neoclevenger apparatus for five hours. The amounts of essential oils obtained were measured in ml and their percentages (v w^{-1}) after taking their average were calculated.

2.3. GC-MS Analysis

O. onites' essential oil component rates (%) that were obtained from dried plant materials were obtained with an Agilent 7890A 5975C GC-MS device. As GC-MS capillary columns HP Innowax Capillar columns 60.0 m X 0.25 mm X 0.25 µm were used. Capillary column heat program: 60ºC It was increased by 20ºC in 10 minutes to 250ºC and kept at 250ºC for 8 minutes. Injection block's heat 250ºC, Rate of Split: 50:1, Volume of Injection: 1 µL, Carrier Gas: Helium, Rate of Flow: 1 mL min^{-1}. In defining essential oil components, Wiley, Nist and Flovor mass spectral library data were used.

2.4. Statistical analysis

In this study, a licensed SPSS 23 statistical program was used for obtaining digital data. An analysis of variance (ANOVA) and a Duncan test were applied to the data of the study.

3. Results and Discussion

3.1. Soil properties

The pH of the soils ranged from 6.84 to 7.98, with 96.67 percent exhibiting mildly alkaline and alkaline responses. The $CaCO_3$ level of the samples ranged from 1.75 to 34.30 percent and was determined to be in generally the category of high to very high calcareous soils. The EC analysis results were between 0.14-0.91 dS m^{-1} and it was determined that all of the soils were salt-free. Despite the fact that soil textures vary greatly, 93.3 percent of the soil samples are loamy. The quantity of organic matter in the soils varies between 0.41 and 11.80 percent, according to the findings. The quantity of organic materials rose

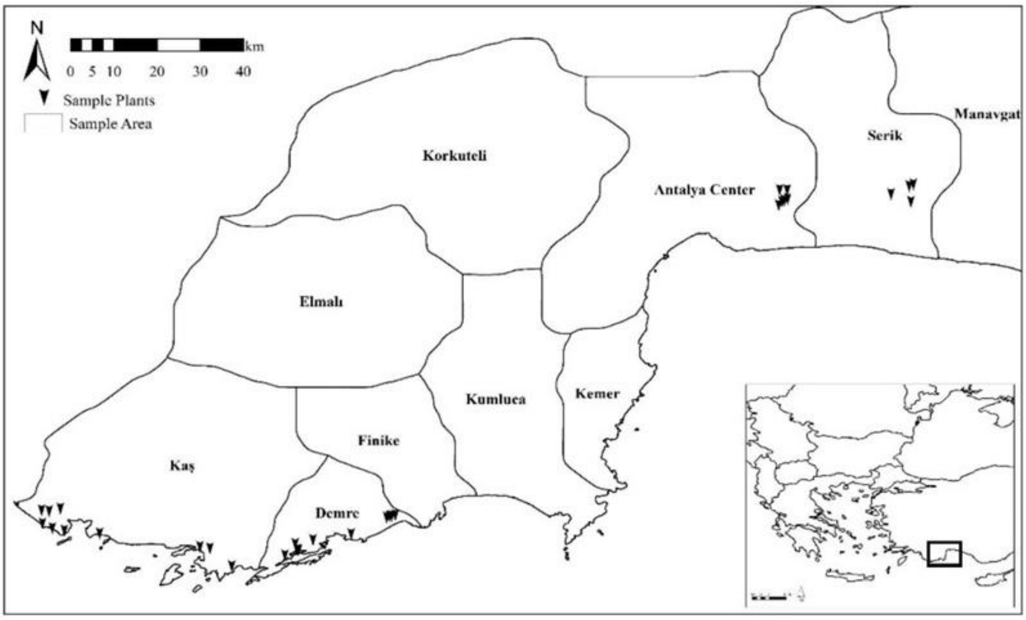

Figure 1. Location of the study area and sampling points.

from Serik-Aksu to Kaş. As shown in Table 1, the soil sample from Kaş had the maximum quantity of organic matter (8.52 percent), which is statistically significant at the 5 percent level.

According to Thun (1955)'s classification of the organic matter content of soil samples, all of the soils from Kaş and 60% of the soils from Demre are categorized as highly humus soil. In Serik-Aksu, 70% of the soil is classified as humus-poor or low-humus.

Soils with nitrogen contents ranging from 0.06 to 1.28 percent (on average 0.48 percent), phosphorus contents ranging from 44.71 to 68.95 mg kg^{-1} (on average 55.78 mg kg^{-1}), and potassium contents ranging from 0.19 to 2.69 me 100 g^{-1} (on average 0.77 me 100 g^{-1}) were found. In a general assessment of the nitrogen, phosphorus, and potassium contents of the studied soils, it was established that 90 percent are in good condition in terms of nitrogen content, 100 percent are high in terms of phosphorus content, and 83.33 percent are adequate in terms of potassium content. The phosphorus content in soil samples from Demre (61.39 mg kg^{-1} P) and the potassium content in soil samples from Kaş (1.14 me 100 g^{-1} K) were higher than in other locations, and these differences were statistically significant at the 1% level (Table 1).

Table 1. Chemical and physical properties of the soils

Soil parameters	Critical level /Mean value	Evaluation	Serik- Aksu		Demre		Kaş		Total	
			N[1]	%	N	%	N	%	N	%
pH	6.6-7.3	Neutral	-	-	1	10	-	-	1	3.33
	7.4-7.8	Less alkaline	6	60	8	80	10	100	24	80
	7.9-8.4	Alkaline	4	40	1	10	-	-	5	16.67
Total $CaCO_3$(%)	0-2.5	Low	1	10	-		-	-	1	3.33
	2.6-5.0	Lime	2	20	5	50	3	30	10	33.34
	5.1-10.0	High	1	10	1	10	2	20	4	13.33
	10.1-20.0	Very high	-	-	1	10	2	20	3	10
	20.0<	Extremely high	6	60	3	30	3	30	12	40
EC (dS m^{-1})	2.5>	Saltless	10	100	10	100	10	100	30	100
Structure		Sandy Loam	4	40	3	30	2	20	9	30
		Loam	1	10	1	10	2	20	4	13.33
		Sandy-Clay Loam	4	40	-	-	2	20	6	20
		Clay Loam	1	10	4	40	4	40	9	30
		Clay	-	-	2	20	-	-	2	6.67
Organic matter (%)	0-2	Very low	4	40	2	20	-	-	6	20
	2-5	Low	3	30	2	20	-	-	5	16.67
	>4.0	High	3	30	6	60	10	100	19	63.33
Total N (%)	0.070>	Very low	1	10	-	-	-	-	1	3.33
	0.09-0.11	Medium	1	10	1	10	-	-	2	6.67
	0.11-0.13	High	2	10	1	10	-	-	3	10
	0.13<	Very high	6	60	8	80	10	100	24	80
Available P (mg kg^{-1})	>10	High	10	100	10	100	10	100	30	100
Exchangeable K (Meq $100g^{-1}$)	0.26>	Very low	-	-	1	10	-	-	1	3.33
	0.26-0.39	Low	3	30	1	10			4	13.33
	0.39-0.51	Medium	3	30	2	20			5	16.67
	0.51-0.64	Good	3	30	1	10	2	20	6	20
	0.64-0.82	High	1	10	1	10	4	40	6	20
	0.82<	Very high	-	-	4	40	4	40	8	26.67
									Anova	
pH			7.84[2]		7.60		7.62		ns	
$CaCO_3$(%)			20.12		12.97		12.78		ns	
EC (dS m^{-1})			0.24		0.27		0.24		ns	
Structure		Sand (%)	55.68		42.14		42.84		ns	
		Clay (%)	19.23		30.15		24.40		ns	
		Silt (%)	25.03		27.71		32.76		ns	
Organic matter (%)			3.59 b		4.89 b		8.52 a		*	
Total N (%)			0.44		0.37		0.62		ns	
Available P (mg kg^{-1})			53.45		61.30		52.60		**	
Exchangeable K (Meq 100 g^{-1})			0.50 b		0.67 b		1.14 a		**	

[1]: Number of samples, [2]: Means with the same letter are not significantly different from each other (*P*>0.05 ANOVA followed by Duncan's multiple range tests).
*, **: Statistically significant at 0.05 and 0.01 probability levels, respectively. The letters represent different groups at 0.05 probability level. ns: Not significant.

3.2. Amount of plants' essential oil and components

The essential oil content of *O. onites* leaves and flowers range from 1.21% to 6.55 % (v w^{-1}), 2.61% to 8.13% (v w^{-1}), with an average of 3.09% and 5.79% (v w^{-1}), respectively. In every three locations where the sampling was made, the content of essential oil in flowers was higher than in leaves (Table 2). The essential oil content in the flowers is substantially greater than in the leaves because the blooms have more glandular hairs (Werker et al. 1985; Karamanos and Sotiropoulou 2013). Kacar et al. (2010) determined that the average essential oil content of *O. onites* leaves and flowers is between 1.88 and 3.06 percent (v w^{-1}) and 2.85 and 4.08 percent (v w^{-1}), respectively. In his research on *O. onites*, Özer (2020) estimated the essential oil content to be 1.75 percent (v w^{-1}) in the leaves and 4.25 percent (v w^{-1}) in the flowers. Our research determined that the average essential oil content of the leaves and flowers of *O. onites* was greater than that of the other two studies. The differences in results among studies can be explained by the physiological variation of the plant, the time, the harvest, and environmental conditions such as the geographical location of the place where the plant grows, climate, and soil characteristics (Öner and Sonkaya 2020; Efendi et al. 2021; Mehalaine and Çencuni 2021).

As seen in Figure 2, it was determined that the amount of essential oil in the leaves and flowers of the plants increased as the locations go from Serik-Aksu towards Kaş. While the change in the amount of essential oil in the leaves was statistically insignificant, the change in the amount of essential oil contained in the flowers was statistically significant at the 5% level. The highest amount of essential oil contained in the flowers was obtained from plants collected from Kaş, with 6.58%, followed by Demre with 5.38% and Serik-Aksu with 4.64.

Components of *O. onites*' essential oil define its quality and aroma. In leaves and flowers, that were collected from the research areas, a minimum of 14 and a maximum of 43 essential oil components have been defined in leaves and flowers that were collected from the research areas. In a statistical analysis of plants' essential oil components, 11 common components that are contained by leaf samples that were taken from each of three locations and 10 common components that are contained by flowers were assessed. Data for *O. onites*' essential oil components in its leaves and flowers is presented in Table 2. Carvacrol was found to be the most abundant component of essential oils in both leaves and flowers from all three places, followed by thymol in Serik-Aksu and Kaş, and cymene in Demre. The effects of research areas on the carvacrol and thymol parts of plant essential oils did not differ in a way that was statistically significant.

Among the essential oil components found in the leaves, cymene ranges from 0.32 to 10.50 percent, with an average of 4.82 percent, linalool ranges from 0.27 to 12.29 percent, with an average of 3.12 percent, terpinene-4-ol ranges from 0.18 to 1.35 percent, with an average of 0.85 percent, and β -caryophyllene ranges from 0.57 to 2.56 percent, with an average of 1.39 percent. In previous studies on the essential oil components that the leaves of *O. onites* contained, average rates of between 3.96 and 8.76 percent cymene, between 0.20 and 8.39 percent linalool, between 0.46 and 2.09 percent terpinene-4-ol, and between 0.49 and 1.52 percent β -caryophyllene were reported (Copur et al. 2010; Tonk et al. 2010; Katar and Katar 2020), and the average values of essential oil.

The effect of plant location on the essential oil components cymene, linalool, terpinen-4-ol, and -caryophyllene in the leaves of *O. onites* has been statistically determined to be significant. Serik-Aksu plants have the highest levels of cymene (6.38 percent), terpinene-4-ol (1.06 percent), and -caryophyllene (0.77 percent) in their leaf essential oil components. Linalool content in leaves' essential oil of 6.41% has been defined mostly in samples from Kaş. Among the essential oil components contained in the flowers, β-myrcene between 0.24-1.09%, on average, 0.69%, gamma-terpinene between 0.01-8.17%, on average 2.34%, linalool between 0.34-18.56%, on average 3.57%, and terpinen-4-ol between 0.19-1.85%, on an average 0.78% were identified. In terms of essential oil components in flowers, while the samples taken from Serik-Aksu had the highest amounts of β-myrcene (0.81%), gamma-terpinene (3.49%), and terpinen-4-ol (0.91%), The highest linalool content (6.07%) was detected in the samples from Kaş. These differences in research areas are statistically significant.

Table 2. Average values of essential oil ratios and components in the leaves and flowers of *Origanum onites* L. plants

RI	Essential oil components	SERİK-AKSU (1)			DEMRE (2)			KAŞ (3)			1X2X3	1X2X3
		Leaf	Flower	LXF	Leaf	Flower	LXF	Leaf	Flower	LXF	Leaf	Flower
1155	β-myrcene	0.55[1]B	0.81 A***a***	*	0.44 B	0.76 A***a***	**	0.44	0.50 ***b***	*ns*	ns	*
1238	γ-terpinene	-	3.49 ***a***	-	-	1.20 ***b***	-	-	1.18 ***b***	-	-	**
1264	Cymene	6.38 Aa	3.25 B	**	4.89 Aba	2.65 B	*	3.19 b	2.06	*ns*	*	*ns*
1534	Linalool	2.23 b	2.40 *b*	*ns*	0.71 b	1.06 *b*	*ns*	6.41 a	6.07 *a*	*ns*	**	*
1543	Cis sabinene hydrate	0.70 A	0.39 B	**	0.62 A	0.32 B	**	0.57 A	0.29 B	*	ns	*ns*
1597	Terpinene-4-ol	1.06 a	0.91 ***a***	*ns*	0.88 a	0.81 ***ab***	*ns*	0.63 b	0.56 ***b***	*ns*	**	*
1587	β-caryophyllene	1.77 Aa	1.23 B	**	1.18 b	0.89	*ns*	1.23 b	0.97	*ns*	*	*ns*
1671	α-humulen	0.41	-	-	0.32	-	-	0.28	-	-	ns	-
1694	Borneol	1.04 A	0.65 B	*	1.02	0.77	*ns*	1.21	0.74	*ns*	ns	*ns*
1995	Caryophyllene oxide	0.73	-	-	0.69	-		0.53	-	-	ns	-
2223	Thymol	9.26	5.69	*ns*	0.95	0.67	*ns*	7.78	10.38	*ns*	ns	*ns*
2264	Carvacrol	62.72	76.19	*ns*	78.77 B	85.05 A	**	68.64	73.68	*ns*	ns	*ns*
	Total number of components	35.60 Aa	20.70 B***a***	**	32.40 Aab	22.20 B***a***	**	27.7 Ab	17.40 B***b***	**	**	*
	Essential oil ratios (%)	2.77 B	5.16 A *b*	**	3.04 B	5.56 *ab*	**	3.46 B	6.64 A*a*	**	ns	*

[1]: Means with the same letter are not significantly different from each other $P>0.05$ ANOVA followed by Duncan's multiple range tests.
*, **: Statistically significant at 0.05 and 0.01 probability levels, respectively. The letters represent different groups at 0.05 probability level. ns: Not significant.

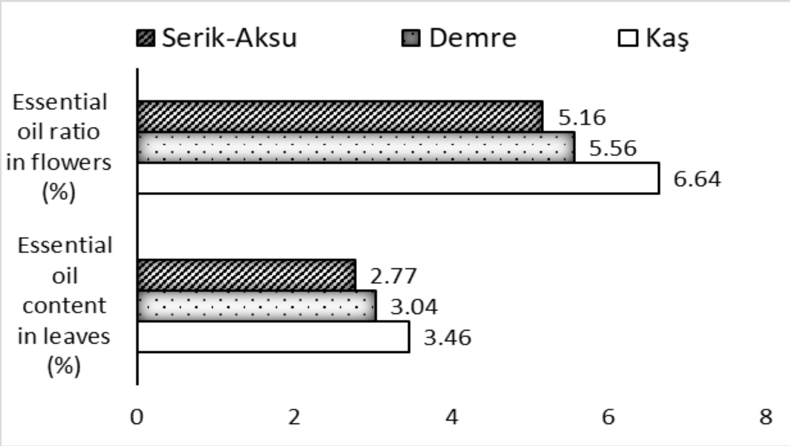

Figure 2. Essential oil ratio of flowers and leaf samples.

When the essential oil components detected in the leaves and flowers of the samples taken from all three locations were evaluated, the amount of cis-sabinene-hydarate was found to be statistically higher in the leaves. In terms of essential oil component numbers, leaves contained more components than flowers and this situation was found to be statistically significant (P<0.001).

The amounts of cymene in the essential oils of the leaves of the plants collected from Demre, and the amounts of β-myrcene and carvacrol in the essential oils of the flowers were found to be high. Cymene, β-caryophyllene and Borneol content were detected to be high in the essential oils of the leaves of the plants collected from Serik-Aksu, while the content of β-myrcene was detected to be higher in the flowers.

According to Kaçar et al. (2006) borneol, from components of *O. onites* essential oil, has been reported more in leaves than in flowers, and carvacrol has been reported more in flowers, and the results are similar to our study. As in another survey that compares essential oil components in leaves and flowers of *O.onites*, the rates of cymene, cis-sabinene hydarate and carvacrol in leaves have been defined higher (Özer 2020). In our survey, cis-sabinene hydarate and carvacrol content weredefined higher in flowers. The situation can be explained by the area's environmental conditions that surveys were conducted in, collection times (ontogenetic and diurnal variabilities), drying, and different analysis methods.

4. Conclusion

In this survey, soil and plant characteristics of *O. onites* L. that grows naturally in Serik-Aksu, Demre, and Kaş locations of Antalya, at an altitude of 0-100 m, are presented. In the meantime, the contribution of plant parts such as leaves and flowers to *O. onites* essential oil, which are used in many fields such as food, chemistry, and pharmaceutical industries, were investigated. The essential oil extracted from flowers is significantly higher. It was observed that, Carvacrol and β-myrcene from essential oil components are high in flowers' essential oils, and cymene, cis-sabinene hydarate, terpinen-4-ol, β-caryophyllene and borneol were high in leaves' essential oils.

In the survey areas, organic matter and potassium content in soils that *O. onites* plant grows have shown a regular and significant increase from the middle regions of Antalya to the west regions, and the highest rates of organic matter and potassium amounts in soil samples were observed in sampling that was done in Kaş. In a similar way, the amount of essential oil in plants' flowers and leaves increased as the location goes to the west of Antalya as well, and the highest amount of essential oil was defined in plants that were collected from Kaş. When all these locations are compared, the Kaş district stands out in terms of soil characteristics and plant essential oil amounts. If production is going to be dependent on the essential oil yield of the *Origanum onites* plant, slightly alkaline, loamy soils with high organic matter may be favored.

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