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Effect of environmental factors on the fruit essential oils of *Pistacia terebinthus* L. growing wild in Turkey

Serkan Gülsoy [1✤iD], Kürşad Özkan [1iD], Gülcan Özkan [2iD]

[1] Isparta University of Applied Sciences, Faculty of Forestry, Isparta, Turkey
[2] Suleyman Demirel University, Faculty of Engineering, Isparta, Turkey

SILVICULTURE

ABSTRACT

Background: The aim of this study was to investigate the effect of environmental factors on the essential oil yields (EOYs) and major essential oil components in the ripe fruits of *Pistacia terebinthus* L. (turpentine tree), which is a medicinal and aromatic plant. Fruit samples were collected from 34 different locations of the turpentine tree growing wild in the Lake District in the Mediterranean Region of Turkey. EOYs (%, v/w) and essential oil component ratios (%) were determined in fruit samples by hydrodistillation and GC-MS methods, respectively. The effect of environmental factors on these variables were analyzed using Pearson Correlation and Principal Component Analysis.

Results: The essential oil yield of fruits collected from different localities ranged from 0.05% (v/w) to 0.19% (v/w). In terms of average values, α-pinene with 41.01% and limonene with 14.28% were the major components in the essential oils of ripe fruits. Among the environmental variables, longitude and sand % in 10-30 cm were the variables that made a statistically significant difference ($p < 0.05$) in fruit essential oil yield. According to the results of principal components analysis (PCA), α-pinene and Ocimene components were most affected by silt (%, 10-30 cm) and pH (%, 30-50 cm) ratios. The longitude and total annual precipitation (mm) were the most determining variables in the sabinene and limonene components, respectively. It was seen that the total lime ratios at different depth levels of the soils taken from the localities were the most effective variables for Myrcene and p-Cymene components.

Conclusion: The study findings showed that EOYs and intended volatile component ratios in ripe fruits of turpentine trees grown under appropriate environmental conditions can be increased.

Keywords: Essential oil, limonene, locations, ripe fruits, turpentine tree

HIGHLIGHTS

Essential oil yields of the turpentine tree fruits varied in different locations.
The most abundant component in EOYs of the turpentine tree fruits was α-pinene.
Silt ratio (%) and pH in soil, total annual precipitation (mm) and longitude presented the most affective relations to chemical composition of EOYs of the turpentine tree fruits.
In certain environmental conditions, higher EOYs and targeted chemical compounds can be obtained in plants.

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✤Corresponding author e-mail: serkangulsoy@isparta.edu.tr
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INTRODUCTION

The Anacardiaceae family includes 81 genera and more than 800 species (Amiri and Mohammadi, 2021). The genus *Pistacia* in this family is classified as 11 species in 4 subsections (Zohary, 1952; Whitehouse, 1957). The *Pistacia* species with the widest distribution in forest areas in Turkey is *P. terebinthus* (Davis, 1967). The *P. terebinthus* species, known as the turpentine tree, has many traditional uses in terms of food and medicine in the Mediterranean basin and its distribution areas in Asia (Baytop, 1999; Topcu et al., 2007). In archaeological studies, it has been determined that the fruits of the species have been used as food by humans since 7000 BC (Ozcan, 2004). Today, in rural areas, the fruits of the species are traditionally consumed as a snack or mixed with products such as walnuts and figs as food. Similarly, turpentine tree fruits are consumed as an additive in some special village breads and as a spice material called 'zahter' (Aydin and Ozcan, 2002). The oils obtained from the fruits and nuts of this species, are widely used in the production of soaps called 'Bıttım' as well as for edible use in Turkey (Baytop, 1999; Kafkas et al., 2002). This soap is an important natural product that cleanses the skin from toxins (Tastekin et al., 2014).

Because *P. terebinthus* is rich in tannin and resin contents, it has an aromatic plant quality. In terms of traditional medicine, the fruits of this species have been used directly or externally in the treatment of diseases such as infections, rheumatism, eczema, ulcer, stomach-ache, cough, asthma and sunstroke (Baytop, 1999; Yesilada et al., 1995; Tuzlacı and Eryasar Aymaz, 2001; Flamini et al., 2004; Ozcan, 2004; Ertas et al., 2013). In Turkey, the leaves of the turpentine tree are used in the treatment of burns, and the resin is used in the treatment of antiseptic bronchitis (Topcu et al., 2007). Ripe fruits, crushed by pounding, are used as a mouthwash to prevent toothache after boiling in sugar water or molasses (Senkardes and Tuzlaci, 2014).

Turpentine fruits are rich in protein, fat, fibre, unsaturated fatty acids and minerals, which increases their nutritional value commercially (Ozcan, 2004; Matthäus and Ozcan, 2006; Yilmaz et al., 2010; Senyay-Oncel et al., 2011). Turpentine coffee made of fruits, which had limited use in rural areas in the past, has now become a product of commercial importance. It has been determined that Turpentine coffee, which is completely natural and caffeine-free, is a good source of trace elements and fatty acids (Ciftci et al., 2009; Kizil and Turk, 2010; Ozel et al., 2014). Turpentine coffee has been found to have higher antioxidant activity compared to direct fruit consumption (Orhan et al., 2012). The resin of this species is valuable as a supporting additive to improve the viability of probiotic bacteria in yogurt, cheese and similar dairy products (Schoina et al., 2015; Schoina et al., 2018). Apart from the specified features, it has been stated that Turpentine oils have good performance as biodiesel fuel and can be an environmentally friendly natural fuel mixture (Ozcanlı et al., 2011; Kar et al., 2012). The films in which the leaf, fruit and shoot extracts of the turpentine tree were used in the mixture offered an alternative food packaging material (Kaya et al., 2018).

Apart from its traditional uses, the turpentine species is also important in terms of modern medicine and pharmacy (Bozorgi et al., 2013). This plant species has various bioactivities, such as antioxidant, antimicrobial and cytotoxic, with its flavonoid, phenolic and alkaloid contents in parts such as flowers, leaves, fruits and shoots (Topcu et al., 2007; Durak and Ucak, 2015; Hacibekiroglu et al., 2015; Çoban et al., 2017). It has been determined that the high antioxidant capacity of the extracts obtained from the leaves of the species has a possible protective role in cancer risks (Lampronti et al., 2005; Kavak et al., 2010). The antioxidant activities of the flowers collected in spring have been found to have preventive or retarding effects against the oxidative stress that causes aging and some chronic diseases (heart, diabetes, cancer, etc.) (Turkoglu et al., 2017). It has been stated that the fruit essential oils of the turpentine tree are promising for the treatment of colorectal cancer cells (Awwad et al., 2020). Turpentine tree oils have phytochemical properties as they are rich in unsaturated fatty acids, tocopherols, polyphenols and carotenoids (Durmaz and Gokmen, 2011). It has been determined that extracts obtained from dried fruits of the turpentine tree have a hypolipidemic effect on rabbits (Bakirel et al., 2003), and their antioxidant properties have a cholesterol reduction (about 1%) effect (Riemersma et al., 1991). This species has shown potential effects for obesity and diabetes prevention and phytotherapy (Foddai et al., 2015).

In all the beneficial forms mentioned above, the essential oil characteristics of this aromatic plant are particularly important. Especially, the young shoots, flowers, raw and ripe fruits of the turpentine tree contain essential oil (Couladis et al., 2003). Recently, the *P. terebinthus* species has started to be the focus of attention in sectors such as pharmacology, cosmetics, agriculture and food because of its essential oil properties (Ulukanli et al., 2014). In the literature, it is seen that different results have been obtained in many studies on the amount, quality and composition of essential oil in certain organs of the turpentine tree (Couladis et al., 2003; Flamini et al., 2004; Ozcan et al., 2009; Pulaj et al., 2016; Piras et al., 2017). In these studies, it has been mentioned that these differences, which cause changes in the aromatic and medicinal properties of the plant, are caused by variables such as sample collection time and method, essential oil analysis method and climatic characteristics of the locations where the samples are harvested. Our hypothesis is that apart from the variables mentioned above and the genetic characteristics of the plant, various environmental factors can cause significant changes in the essential oil characteristics of the Turpentine species. In this study, the effects of environmental factors (elevation, slope, aspect, climate, etc.) on the essential oil yield and component ratios of *P. terebinthus* fruits were investigated.

MATERIAL AND METHODS

Study area

The study area, the Lake District (38°25′-36°06′ N, 29°30′-32°34′ E), is located in the Mediterranean phytogeographic region of Turkey. The Lake District covering the provinces of Antalya, Isparta and Burdur has coastlands, rugged terrains, plains and mountainous parts up to 3,000 m. Although typical Mediterranean climate prevails near the coastal parts of Antalya in the district, there is a transition to a continental climate in mountainous areas and parts extending towards central Anatolia in Isparta and Burdur. Mostly carbonated rocks, red Mediterranean soils and brown forest soils are dominant in the study area (Sandler et al., 2015). In this area, *P. terebinthus* is located in red pine forests at different heights, in maquis or preudomaquis vegetation types where the forest structure is degraded and on arid, rocky and stony slopes up to the altitude of 1500-1600 m in the Taurus Mountains (Davis, 1967). Fruit samples of *P. terebinthus* were collected from 34 wild populations in different locations of the district (Figure 1). At each location, elevation (m), slope (%), surface stoniness (%), mean annual temperature (° C) and total annual precipitation (mm) have been recorded as environmental variables. Location aspects were converted into radiation index values (McCune and Keon, 2002). In addition, sand (%), silt (%), clay (%), pH, total lime (%), organic matter (%) and soil skeleton content (%) were determined in soil samples taken from 0-10 cm, 10-30 cm and 30-50 cm depth levels at each location.

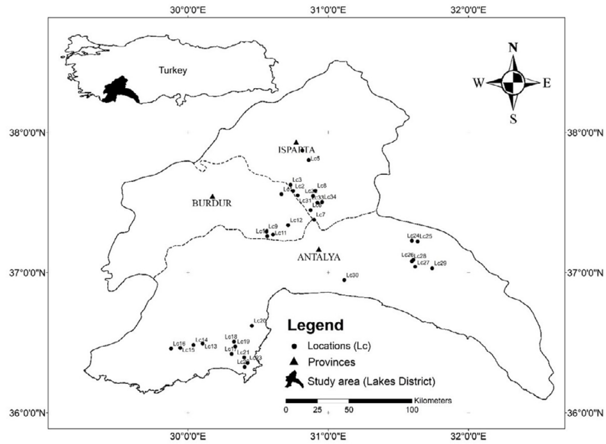

Figure 1. Map of the study area showing the sampling locations in the Lake District, Turkey.

Plant material

The *P. terebinthus* is a deciduous tree that can grow up to 6 metres or a shrub that can grow to 2-3 metres in height. The globose and broadly obovate fruits of the species are drupe. Although the fruits are reddish in raw form, they gradually darken in shades of green during the ripening phase. Ripe fruits are 4-6 mm wide and 5-6 mm long (Davis, 1967). The ripe fruit samples were collected from 34 wild populations from September to the end of October in 2010. The fruit samples taken from the field were brought to the laboratory in polyethylene packages on the same day. The fruits, which were dried until they became air-dried, were first ground in an electronic mixer. Essential oils were obtained by the hydro distillation method for 3 h in a Clevenger-type apparatus by adding 4 litres of water to 350 grams of samples. As a result of this process with 2 repetitions, the average of essential oil yields (EOYs; %, v/w) was recorded for each of the 34 different locations. The samples, which were transferred to Eppendorf by means of micropipettes without water, were stored at −20 °C for use in essential oil component analyses after being covered with parafilm.

Gas chromatography–mass spectrometry (GC/MS) analyses

Analyses were made using a Shimadzu QP 5050 GC/MS spectrometer system in the EI mode at 70 eV ionization energy. The separation was conducted on a CP-Wax 52CB column (50 m x 0.32 mm, 1.2 μm film thickness). For the Carrier gas Helium was used with an inlet pressure of 10 psi and the injection volume of 10 μL. The oven temperature increased from 60 °C to 220°C at a rate of 2 °C/min, then held isothermal at 220 °C for 20 min with an injector temperature of 250 °C and a detector temperature of 240 °C. Essential oil components were identified by comparing the retention indices with Wiley, NIST, Tutor and private libraries online as well as published articles (Adams, 2007).

Statistical analysis

Principal component analysis (PCA) was applied to determine the relationships between major essential oil components (higher than 5%) and environmental variables in different locations. '*FactoMineR*' and '*ggplot2*' packages were used in R Studio for PCA analysis (Le et al., 2008; Wickham, 2009). Pearson correlation coefficients (using a 5% significance level) between EOYs and environmental variables was also calculated using '*corrplot*' package in RStudio (Wei et al., 2017).

RESULTS AND DISCUSSION

Just like other secondary metabolites (phenolics, flavonoids etc.,), essential oils are important indicators in terms of medicinal and aromatic qualities of plants. EOYs from ripe fruits of the turpentine tree and the chemical compositions of these essential oils obtained from 34 different locations (Lc) in the Lake District of Turkey are given in Table 1. EOYs in ripe fruits of the turpentine tree ranged from 0.05% (v/w) to 0.19% (v/w) in the populations of the different locations. The highest EOYs (0.19%) was obtained from the fruits collected from Lc19 at an elevation of 551 m in Antalya province. In total, 21 volatile components, 94.5% and 99.7% of the total essential oils, were identified. The number of components and their ratios vary in different populations. The major components of the turpentine tree essential oil were *α*-pinene, limonene, Ocimene, *p*-Cymene, myrcene and sabinene. The most abundant component was *α*-pinene (an average rate of 41.01%), which ranged from 20.87% in Lc4 (Isparta) to 60.98 % in Lc14 (Antalya). Limonene is the second major component, with an average rate of 14.28% in 34 populations. Ocimene, *p*-Cymene, myrcene and sabinene component ratios were determined as 10.68, 7.73, 5.97 and 5.17%, respectively.

In a similar study previously conducted in Turkey, EOYs were found between 0.06% and 0.16% in fruit samples of the turpentine tree collected from different locations, such as Antalya, Izmir and Hatay. *α*-pinene (9.5-51.3%), limonene (<0.1-39.0%) and caryophyllene oxide (<0.1-51.5) were identified as the most abundant volatile components in essential oils (Ozcan et al., 2009). It has also been stated that EOYs and volatile component ratios vary in fruit samples collected from different localities. EOYs were determined as 0.02% and 0.12% in fruit samples of the turpentine tree harvested from Prizren and Ura e Shejtë in Kosovo, respectively. Although *α*-pinene ratios were between 12.58–21.43%, limonene ratios were 15.65-23.87% in fruits taken from different locations. The ratios of (Z)-β-Ocimene (2.23-44.85%) and (E)-β-Ocimene (9.59-40.49 %) differed significantly according to the locations (Pulaj et al., 2016). Apart from these studies in the literature, EOYs were found as 0.54% and 0.73% in the raw and ripe fruits, respectively, of the Turpentine tree origins from İçel province in Turkey. In the same study, the major volatile components in the essential oils of ripe fruits were limonene (32.8%), β-pinene (22.5%), α-pinene (5.3%) and germacrene D (4.6%), respectively (Couladis et al., 2003). In the ripe fruits of turpentine trees originating in Jordan, (E) -ocimene (33.8%), sabinene (24.1%), (Z) -ocimene (13.0%) and α pinene (6.5%) were determined as the major components, respectively (Flamini et al., 2004). In another recently completed study in Turkey, EOYs was determined as 0.082% in the ripe fruits of the species, and the major components were α-pinene (37.23%) and limonene (19.89%) (Inan, 2021).

When the results obtained from our research and other studies completed in the literature are interpreted together, although there are some partial differences in EOYs and components, the results generally overlap. It was stated that occurring partial differences may depend on variables such as the time and shape of the samples that were collected and the analysis methods (Gogus et al., 2011; Ozel et al., 2014; Inan, 2021). On the other hand, in some studies, it was determined that although standard methods were applied to the samples collected at the same ripening time from different localities, there were variations in EOYs and component ratios (Table 1)(Ozcan et al., 2009; Pulaj et al., 2016).

Table 1. Essential oil yields and the chemical composition of the essential oils obtained from ripe fruits of the turpentine tree in different locations.

	Lc1	Lc2	Lc3	Lc4	Lc5	Lc6	Lc7	Lc8	Lc9	Lc10	Lc11	Lc12	Lc13	Lc14	Lc15	Lc16	Lc17	ID
α-Pinene	54.99	44.16	48.11	20.87	36.09	46.16	59.69	39.26	46.10	41.38	52.99	33.42	51.02	60.98	27.01	52.51	44.02	a, b
Camphene	1.64	2.47	1.03	1.26	0.99	1.65	1.50	1.86	1.63	0.94	1.35	1.07	1.76	2.80	1.21	2.05	1.75	a, b
β-Pinene	1.16	5.42	6.42	6.00	2.42	7.71	2.59	9.73	9.32	2.11	5.75	5.97	3.59	6.69	2.00	3.09	2.87	a, b
Sabinene	3.21	0.74	1.27	7.78	1.40	6.46	1.25	11.05	4.84	9.63	4.95	4.75	6.12	5.03	9.13	2.99	3.03	a
Myrcene	1.94	26.48	2.29	13.46	4.90	10.82	2.80	2.19	3.39	1.37	7.69	2.25	1.78	1.69	12.32	2.92	6.36	a, b
Phellandrene	n	n	n	n	n	n	2.93	1.27	2.31	0.94	2.44	1.53	0.44	n	0.46	n	n	a, b
Limonene	26.95	15.99	12.22	3.23	22.68	8.28	9.43	7.49	16.90	8.07	9.23	8.37	28.45	6.03	14.02	4.08	18.23	a, b
Ocimene	0.46	0.42	0.82	29.39	8.13	5.73	5.35	12.26	3.76	1.01	3.33	23.18	0.63	8.68	9.18	2.16	17.14	a
p-Cymene	0.92	0.47	0.66	11.41	6.49	2.19	1.02	4.27	2.35	26.16	1.42	10.71	0.69	2.60	17.50	0.81	4.19	a, b
β-Caryophyllene	4.48	1.34	11.90	1.32	4.47	1.81	3.38	3.13	3.14	1.47	1.87	2.71	1.46	1.25	1.14	n	0.89	a, b
Terpinene	n	n	n	1.42	4.23	n	3.36	3.53	3.73	n	n	1.09	n	n	0.19	1.11	0.21	a, b
Terpinolene	n	n	n	n	1.86	6.14	n	n	n	3.34	5.77	2.38	0.39	0.62	n	23.95	0.24	a, b
D-carene	n	n	n	n	4.66	n	2.52	n	n	n	n	n	n	n	3.39	2.98	0.16	a
α-Copaene	0.58	0.15	0.16	n	n	n	0.14	n	n	n	n	n	n	n	n	n	n	a
Terpineol-4	n	n	n	n	n	n	n	n	n	n	n	n	0.82	n	n	n	n	a, b
α–Humulene	n	n	1.58	n	n	n	0.41	n	n	n	n	n	n	n	n	n	n	a
Germacrene D	n	n	1.40	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
5-Ethenyl undecane	n	n	2.12	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Ylangene	n	n	1.10	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Naphthalene	1.98	1.75	0.38	n	0.35	n	n	n	n	n	n	n	n	0.36	n	n	n	a
Codinene	n	n	1.77	n	n	n	0.27	n	n	n	n	n	n	n	n	n	n	a
Muurolol	n	n	1.29	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Total (%)	98.29	99.38	94.50	96.12	98.64	96.92	96.62	96.02	97.47	96.41	96.76	97.40	97.14	96.71	97.53	98.63	99.06	
EOYs	0.07	0.09	0.08	0.09	0.05	0.08	0.14	0.08	0.14	0.09	0.13	0.14	0.14	0.09	0.10	0.09	0.12	

EOYs: Yields of Essential Oil (%, v/w), ID: Identification, Lc: Locations, a: Identification by retention time and comparison with GC-MS spectra. b: Identification by retention time and comparison with GC-MS spectra and comparison with authentic standards prepared by co – chromatography, n: Not detected

Table 1.(continue) Essential oil yields and the chemical composition of the essential oils obtained from ripe fruits of the turpentine tree in different locations.

	Lc18	Lc19	Lc20	Lc21	Lc22	Lc23	Lc24	Lc25	Lc26	Lc27	Lc28	Lc29	Lc30	Lc31	Lc32	Lc33	Lc34	ID
α-Pinene	30.82	47.61	28.90	27.82	25.90	24.45	43.26	44.10	40.49	40.73	24.43	44.14	29.81	28.42	60.21	49.30	45.38	a, b
Camphene	0.92	1.54	0.94	1.03	0.41	n	1.34	1.38	1.05	1.14	n	0.52	1.79	1.48	2.60	1.50	1.46	a, b
β-Pinene	4.84	3.44	3.46	3.85	4.01	3.50	4.26	3.23	4.37	4.69	1.80	2.98	14.84	2.92	4.42	2.65	5.10	a, b
Sabinene	2.35	0.57	4.84	2.44	2.35	0.88	6.25	3.32	9.40	6.44	3.14	4.31	16.13	12.32	3.49	8.02	6.09	a
Myrcene	3.37	1.06	3.93	4.93	3.37	3.69	6.05	3.27	3.03	6.81	2.29	4.53	1.45	31.80	2.05	11.53	5.10	a, b
Phellandrene	n	n	n	n	n	n	2.18	2.09	3.11	2.51	0.41	4.42	n	2.08	0.76	0.25	1.33	a, b
Limonene	12.50	29.35	10.67	20.10	13.93	18.61	13.50	17.62	12.28	16.38	51.28	13.20	2.51	9.40	14.60	2.91	6.94	a, b
Ocimene	22.27	0.90	27.34	24.42	29.34	29.50	7.91	11.23	7.43	7.04	9.94	13.99	24.51	5.26	4.90	3.50	2.07	a
p-Cymene	15.39	13.85	8.00	7.10	9.86	9.71	10.08	8.78	8.42	7.93	3.61	9.86	6.03	1.65	4.70	19.28	24.70	a, b
β-Caryophyllene	1.12	0.40	3.50	0.46	n	n	2.64	2.98	2.65	2.74	2.64	0.82	0.76	3.64	1.47	0.42	1.17	a, b
Terpinene	0.89	n	1.21	0.80	1.27	0.60	n	n	n	n	n	n	0.40	n	n	n	n	a, b
Terpinolene	4.02	0.55	4.77	3.26	5.59	7.57	2.07	1.73	6.61	0.76	n	0.50	n	n	n	n	n	a, b
D-carene	n	n	n	2.83	3.38	0.99	n	n	n	n	n	n	n	n	n	n	0.33	a
α-Copaene	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Terpineol-4	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a, b
α–Humulene	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Germacrene D	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
5-Ethenyl undecane	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Ylangene	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Naphthalene	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Codinene	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Muurolol	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	a
Total (%)	98.45	99.25	97.55	99.00	99.40	99.47	99.51	99.70	98.82	97.14	99.52	99.24	98.20	98.95	99.19	99.33	99.65	
EOYs	0.11	0.19	0.09	0.12	0.10	0.12	0.07	0.08	0.09	0.09	0.11	0.13	0.08	0.10	0.07	0.14	0.12	

EOYs: Yields of Essential Oil (%, v/w), ID: Identification, Lc: Locations, a: Identification by retention time and comparison with GC-MS spectra. b: Identification by retention time and comparison with GC-MS spectra and comparison with authentic standards prepared by co – chromatography, n: Not detected

Only longitude (r:−0.344) and sand % in 10-30 cm (r:0.346) from the environmental variables indicated statistically significant correlations with EOYs at the level $p < 0.05$ (Figure 2). Similar to the results of this study, the increase in the amount of sand (%) in the soil was also positively correlated with the EOYs of *Juniper* cones collected from the Lake District (Gulsoy et al., 2019). In Mediterranean-type arid or semi-arid ecosystems, the increase in the amount of sand in the soil is one of the most important factors that cause a decrease in plant nutrients and an imbalance in the water economy of the soil. It has been determined that the turpentine tree, which spreads by closing its stomata in arid environments, is a water-spender plant, and accordingly, the relative humidity content of the plant changes in different environments (Sakcali and Ozturk, 2004). It is possible that the changes in the nutrient and water economy in the soil depending on the sand content increased the drought stress in the plant and changed the amounts of the secondary metabolites, such as essential oil and phenolics.

PCA is a suitable technique for associating the major essential oil components from fruits in different geographical origins with numerous environmental variables. The eigenvalues and percentages of variance explanation for the first 3 PCA axes were 1.98 and 32.9 for PC1, 1.48 and 24.7 for PC2 and 1.09 and 18.3 for PC3, respectively. The first 3 PCA axes explained 75.9% of the total variance in the essential oil components data set (Table 2).

Table 2. Eigenvalues and variances (%) of the first 3 PCA axes according to the Essential Oil Components dataset.

PCA Axes	Eigenvalue	Variance (%)	Cumulative Variance (%)
PC1	1.98	32.9	32.9
PC2	1.48	24.7	57.6
PC3	1.10	18.3	75.9

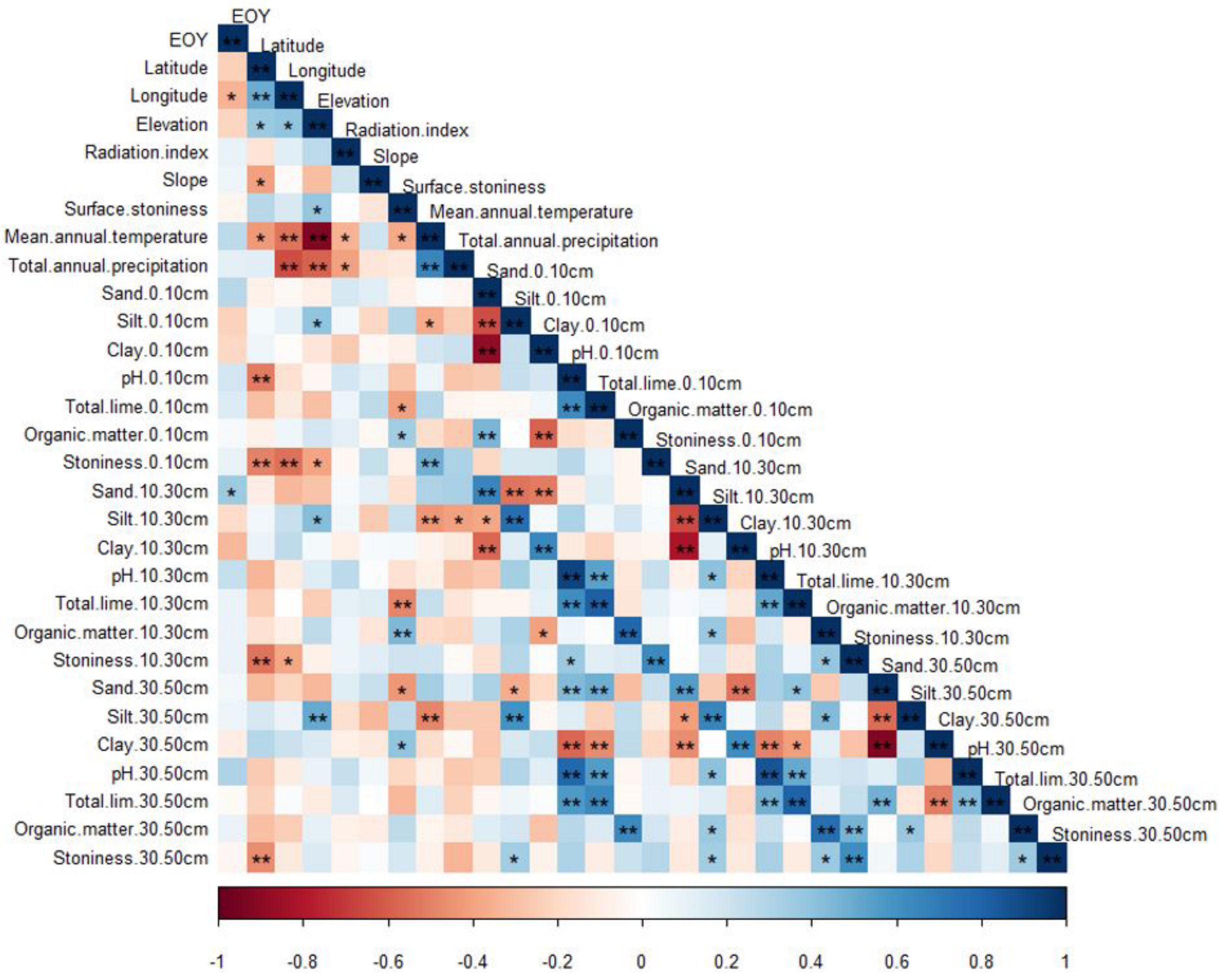

Figure 2. Correlation coefficients (* p < 0:05, ** p < 0.01) between essential oil yield and environmental variables.

The correlation coefficients of the first 3 PCA axes with essential oil components and environmental variables are listed in Table 3. Although PC1 axis (with a 32.9% variance) had the highest negative correlation with α-Pinene (r: −0.816) among 3 PCA axes, this axis provided the highest positive contribution to Ocimene (r: 0.719). PC2 (with a 24.7% variance) was the axis with the highest positive contribution to Sabinene (r: 0.593) and the highest negative contribution to Limonene (r: -0.685). PC3 (with a 18.3% variance), the 3rd axis, had the highest negative correlation with p-Cymene (r: −0.591) and the highest positive correlation with Myrcene (r: 0.776).

When environmental variables and essential oil components are linearly correlated according to the highest correlation rankings on the same PCA axis, silt (%, 10-30 cm) and pH (30-50 cm) were the variables with the highest positive contribution to α-Pinene and the highest negative relation to Ocimene according to PC1. In

Table 3. The correlation coefficients (r) of the first 3 PCA axes with essential oil components and environmental variables.

Major Essential Oil Components			
PCA Axes	PC1	PC2	PC3
Alfa Pinene	-0.816	0.440	-0.288
Sabinene	0.553	0.593	-0.024
Myrcene	0.238	0.439	0.776
Limonene	-0.407	-0.685	0.251
Ocimene	0.719	-0.523	0.000
pCymene	0.514	0.047	-0.591
Environmental Factors			
PCA Axes	PC1	PC2	PC3
Latitude	0.040	0.029	0.023
Longitude	-0.098	0.432	0.161
Elevation (m)	-0.061	0.018	-0.289
Radiation index	-0.235	0.051	-0.045
Slope (%)	-0.337	-0.142	0.148
Surface stoniness (%)	-0.112	-0.162	-0.348
Mean annual temperature (°C)	0.130	-0.132	0.198
Total annual precipitation (mm)	0.243	-0.388	0.034
Sand (%, 0-10cm)	0.193	-0.127	-0.073
Silt (%, 0-10cm)	-0.252	0.175	-0.153
Clay (%, 0-10cm)	-0.094	0.056	0.186
pH (0-10cm)	-0.147	0.332	0.011
Total lime (%, 0-10cm)	-0.018	0.276	0.413
Organic matter (%, 0-10cm)	0.069	-0.152	-0.263
Stoniness (%, 0-10cm)	-0.115	-0.052	-0.162
Sand (%, 10-30cm)	0.239	-0.176	0.012
Silt (%, 10-30cm)	-0.341	0.218	-0.122
Clay (%, 10-30cm)	-0.054	0.066	0.078
pH (10-30cm)	-0.287	0.191	-0.009
Total lime (%, 10-30cm)	-0.067	0.315	0.590
Organic matter (%, 10-30cm)	-0.024	-0.210	-0.291
Stoniness (%, 10-30cm)	-0.121	0.148	-0.358
Sand (%, 30-50cm)	0.116	0.175	0.007
Silt (%, 30-50cm)	-0.153	0.040	-0.137
Clay (%, 30-50cm)	-0.068	-0.224	0.054
pH (30-50cm)	-0.388	0.210	0.046
Total lime (%, 30-50cm)	0.040	0.311	0.257
Organic matter (%, 30-50cm)	0.047	0.012	-0.361
Stoniness (%, 30-50cm)	-0.206	0.138	-0.232

relation to PC2, longitude is the most important variable that contributes positively to the Sabinene component, whereas the Limonene component appears to be positively associated with locations where total annual precipitation (mm) increases. When the relationships were interpreted according to PC3, the increases in total lime (%, 0-10 cm) and the total lime amounts of soils taken from different localities (%, 10-30 cm) had a positive effect on the myrcene component, whereas it had a negative effect on p-Cymene. In addition, the positions of all these variables according to their correlations with the PCA axes are showed in Figure 3 and Figure 4.

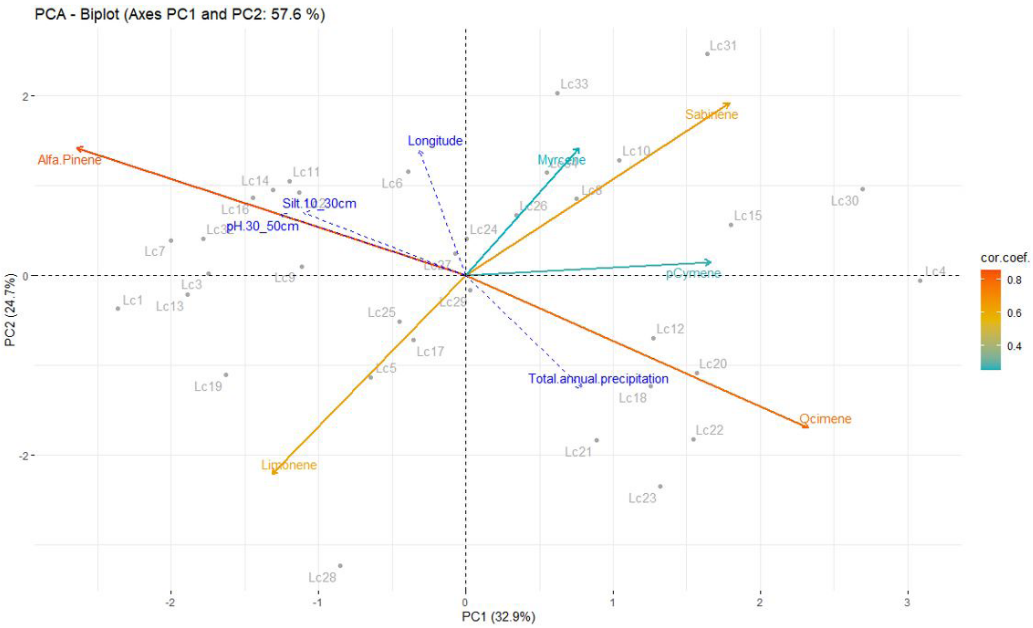

Figure 3. PCA graph of major components (higher than 5%) of *P. terebinthus* L. fruits from 34 different locations and relations with environmental variables in PC1 and PC2.

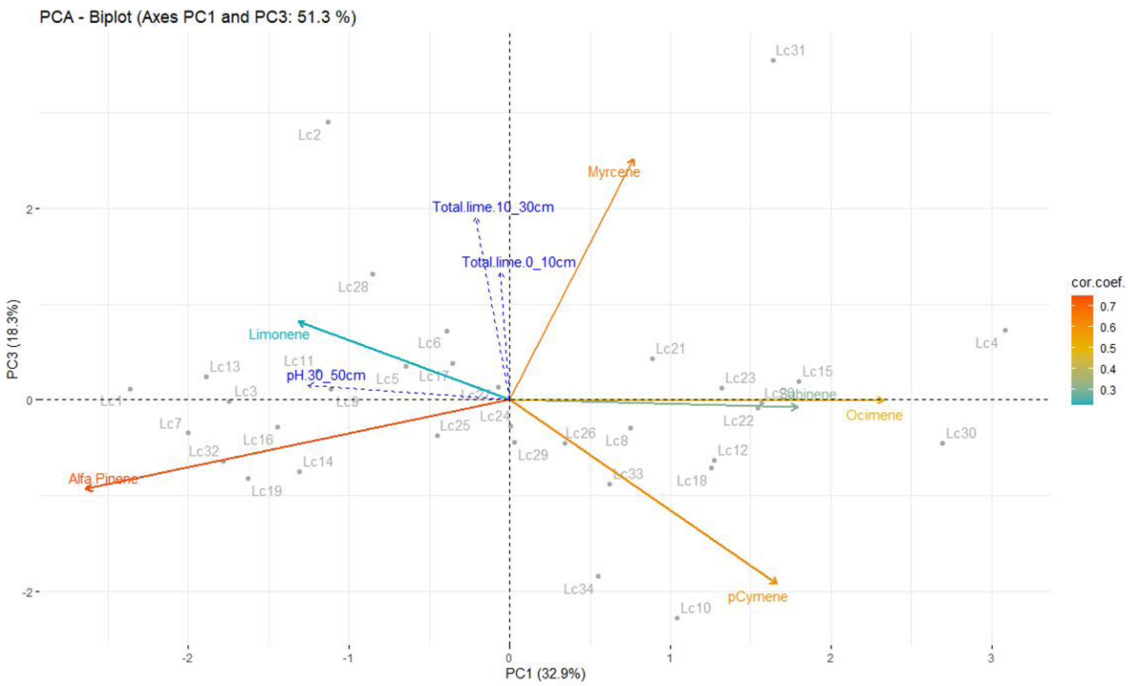

Figure 4. PCA graph of major components (higher than 5 %) of *P. terebinthus* L. fruits from 34 different locations and relations with environmental variables in PC1 and PC3.

It is an important process to obtain high EOYs and desired essential oil component ratios from plants with medicinal and aromatic properties to meet the requirements of the sector. For this, it is necessary to determine the effect factors that cause changes in EOYs and essential oil component ratios. In general, besides genetic, ontogenic and morphogenetic factors, it is stated that another important group of factors that cause changes on various secondary metabolites such as essential oil in plants are environmental variables (Verma and Shukla, 2015). During their growing periods, plants are constantly interacting with biotic and abiyotic environmental factors. Especially changing abiotic environmental factors (climatic, edaphic, and physiographic) can be responsible for the variations in secondary metabolites (phenolics, flavonoids, essential oils etc.,) as an important stress factor in the medicinal plants (Li et al., 2020). As a result of the PCA analysis in this study, it was observed that various abiotic environmental variables caused changes in the essential oil components of the turpentine fruits. In the study, the intensity of annual precipitation, which is one of the climate parameters that vary depending on the location of the sample areas, caused an increase in the Limonene component and a decrease in the Sabinene component in the essential oils of the turpentine fruits. Figueiredo et al., (2008) mentioned that the weather conditions can be crucial determinants for the essential oil properties of various medicinal plant species. In a study, the amount of some essential oil components of the *Flourensia cernua* plant have been detected to increase in shaded environments as a locally climatic factor (Estell et., 2016). Turtola et al., (2003) found that various monoterpene concentrations in the stem wood of two different conifer species (*Pinus sylvestris* L. and *Picea abies* (L.) Karst.) were significantly affected by drought stress. Therefore, it would be correct to interpret the changes in essential oil components due to annual precipitation as drought stress.

On the other hand, in this study, it was observed that some soil properties such as total lime (%), sand (%), pH caused changes in the concentration of essential oil components such as α-pinene, ocimene, myrcene and p-Cymene. In literature, some researchers considered that soil variables may be determinants in the essential oil properties of plants, too. For instance, Hermino and Antonio (1998) reported that p-cymene, which is the major essential oil component of the *Thymus piperella* L. plant, is positively affected by the increasing organic matter in the soil. Also, Pereira et al. (2000) stated that chemical variation in essential oils of Thymus caespititius plant may be affected by genetics and edaphic factors in an environment where many other environmental conditions (slope, altitude etc.) are constant. As a result, although the relationships between essential oil properties and all the factors are very complex, interactions with environmental variables should definitely be considered.

CONCLUSION

Medicinal and aromatic plants containing essential oils provide important ecosystem services to industries such as pharmacology, cosmetics, agriculture and food. It has been stated that the trace element levels of the turpentine tree, which has essential oil properties in different organs, are within the tolerance range prescribed by the World Health Organization and can be evaluated in the category of medicinal and aromatic plants (Hamlat et al., 2019). It is important to determine the factors affecting the EOYs and component ratios, which are decisive for the phytochemical and pharmacological use of the turpentine tree. Many studies mentioned that the EOYs and volatile components of turpentine trees vary depending on the different plant organs, ripening period of plant material, harvest time, locations, climatical conditions and analysis techniques in the laboratory. In this study, it was determined that the EOYs and components extracted from the fruits of the turpentine tree grown in different regions differ according to environmental variables. Soil properties such as sand (%), total lime (%) and pH, annual precipitation from climatic properties and longitude as a location parameter caused changes in the EOYs and component ratios of the turpentine tree. Our findings supported the idea that environmental factors could be a limiting factor for the demands of the essential oil industry. On the other hand, plant essential oil and environmental factor relations are still very complex. Consequently, more research on the bioactivity of the turpentine tree and other similar species is needed to clarify the findings of this study. It is important to strengthen the findings obtained by comparing this study with future research.

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AUTHORSHIP CONTRIBUTION

Project Idea: SG, KÖ, GÖ
Funding: SG, KÖ, GÖ
Database: SG, KÖ, GÖ
Processing: SG, KÖ, GÖ
Analysis: SG, KÖ, GÖ
Writing: SG, KÖ, GÖ
Review: SG, KÖ, GÖ

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