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Original Article

INVESTIGATION OF VOLATILE OIL CONTENTS AND SOME ECOLOGICAL CHARACTERISTICS OF WILD AND CULTIVATED *SIDERITIS STRICTA* BOISS. & HELDR.

Orhan Ünal*¹, Canan Dülgeroğlu¹

¹ Department of Biology, Faculty of Science, Akdeniz University, 07-070 Antalya, Türkiye.

* Correspondence, e-mail: ounal@akdeniz.edu.tr

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ABSTRACT

The indiscriminate removal of medicinal and herbal plants from their natural habitats, the colonization of alien and invasive species in the habitats where these valuable plant species grow, the decrease in the purity rates of plants, and the extinction of plant species make these types of plants vulnerable to threats. The cultivation of such valuable plant species can prevent such situations. However, it remains a subject of interest for ecologists whether cultivated plants undergo any loss of characteristics due to these factors. In this study, the endemic medicinal plant *Sideritis stricta* Boiss. & Heldr. was utilized to shed light on this matter. The volatile oil contents of two wild forms (Kemer: W1 and Serik: W2) and one cultivated form (Kaş: C) of the species were compared, and environmental factors believed to influence volatile oil content were examined. The analysis results revealed that the major components (α -pinene, β -pinene, and caryophyllene) were mostly present in similar amounts. β -pinene was found to be the predominant compound in C, W1, and W2 samples, with percentages of 32.28%, 27.33%, and 40.61%, respectively. All volatile oils were found to be rich in monoterpenes. The humidity and soil pH values in the cultivation area differed from the natural habitats of the wild samples, and these factors had an impact on volatile oil yield and composition. Consequently, when conducting cultivation studies on a species, it is essential to adapt the natural form of the species to cultivation conditions by closely observing the environmental factors.

KEYWORDS: *Sideritis stricta*, volatile oil content, ecology, endemic

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1. Introduction

Approximately 393 thousand plant species belonging to 452 vascular plant families are introduced to the scientific world and an average of 2000 new plant species are described each year [1-2]. Turkey's flora is represented by approximately 12,000 plant taxa, 33.73% of which are endemic species. [3-4]. It is known that 28,187 plant species in the world are used for medicinal purposes [2]. *Sideritis* (Mountain tea) belongs to the Lamiaceae family and is one of the genera with the highest rate of endemism in Turkey. Although there are 45 species (60 taxa) of the *Sideritis* genus in Turkey, 80% of it consists of endemic species [5]. *Sideritis* consists of three sections (*Hesiodia*, *Burgsdorfia* and *Empedoclia*) in Turkey. The *Empedoclia* section has all 42 endemic species and its endemism rate is 80%. Moreover, Turkey is the gene center of this section [6-9]. *Sideritis stricta* Boiss. & Heldr. is an endemic Eastern Mediterranean element. It blooms from May to August and grows in *Pinus brutia* openings, *Quercus maquis*, cliffs at the seaside and serpentine areas, at altitudes of 0-915 m. The distribution

area of the plant is the Western Taurus Mountains. *S. stricta* is in the category of species (VU) with a great danger of extinction in its natural environment [8]. Medicinal plants have been used for many different purposes in the historical process [10]. It has been widely used in the treatment of diseases in human history [11]. In many studies, it has been reported that plants have activities such as anticancer, antimicrobial, antioxidant, DNA protective and cytotoxic effects [12-14]. Properties with biological activities are realized by secondary metabolites produced in many natural materials [15-19]. In this context, it is very important to determine the compounds that have biological activities in different plants. The herbal tea, which has been prepared from *S. stricta* has been used as a folk remedy since ancient times [20]. Recent studies have shown that it has pharmacological effects, such as carminative, analgesic, anticancer, anti-inflammatory and antinociceptive activities, treatment of flu and cold, treatment of Parkinson's and Alzheimer's disease [21-24]. The species are collected from nature for herbal use and trade, so the plant's presence in nature is under pressure due to over-

collecting. Collection of medicinal plants from nature may bring along some negativities such as the admixture of foreign substances, deterioration of the purity of the product, accidental collection of plants, as well as the destruction of the natural populations of the species [25]. It is possible to prevent such problems by cultivating medicinal plants [26]. However, it is a matter of concern whether the cultivated plants will suffer any loss of properties. The present study aims to clarify this subject. The essential oil contents of *S. stricta*'s two wild (Kemer: W1 and Serik: W2) and cultivated (Kaş: C) populations were compared and environmental factors thought to affect the essential oil were investigated.

2. Materials and Methods

2.1. Ecological properties

The material of the study is *S. stricta* which is an endemic plant to the south-west of Turkey. The wild and cultivated samples of the plant and soil samples from the areas where they grow were used (Figure 1, Table 1). The plant samples used as cultivated form were propagated from the seeds taken from the wild population in Kemer (W1) and were grown in open-air conditions by using a drip irrigation system. The plant materials used in the study were collected in the flowering stage of the species (May/June). Furthermore, after bringing the plant and soil samples to the laboratory, they were dried at room temperature.



Fig 1. A) Turkey and Antalya province, B) *S. stricta* and C) W1, W2, C2 populations

Table 1. The populations used in the study

Form	Code	Localities	Voucher	Coordinates
Nature	W1	C3/Kemer/Antalya a/Turkey: near Tekirova, <i>Pinus brutia</i> forest openings, calcareous slopes, 184-400 m asl.	O. Ünal 2170	N=36° 30' 29.0" E=30° 29' 29.8"
		C3/Serik/Antalya/ Turkey: near Sağirin village, 50-100 m asl.	O. Ünal 2192	N=37° 04' 18.9" E=31° 14' 00.5"
Culture	C	C3/Kaş/Antalya/ Turkey: from Kaş to Demre 18 th km, Evrenler Medicinal herb farm, 481 m asl.	O. Ünal 2185	N=36° 14' 21.5" E=29° 48' 07.6"

2.2. Climate

To compare the climatic characteristics, the climate data of the study areas were taken from the Turkish State Meteorological Service as a long period of data. In light of these data, a climate diagram and climatogram have been prepared for the Kemer, Kaş and Serik populations.

2.3. Soil Analysis

Analyses were carried out on soils taken from three different places at a depth of 0-30 cm in the region where the species are grown. The soils taken from the field were put in plastic bags and brought to the laboratory. The soils were dried in the shade and at room temperature in the laboratory, the dried soils were placed in numbered paper bags after sifting through a 2 mm sieve. According to Bouyoucos, the soil texture analysis was conducted based on the sedimentation principle by hydrometer method and sand %, silt %, and clay % amounts were determined [27]. The obtained results were applied to the soil texture triangle, which was prepared according to the international particle size scale so that the soil texture was determined [28]. Soil acidity was determined by a pH meter (Hanna Instruments 8521 brand). The electrical conductivity was determined according to Jackson [29]. The calcium carbonate amount was determined by Scheibler Calcimeter according to Çağlar [30]. The quantity of the organic matter in the soil was determined according to the modified Walkley-Black method [31] and the total nitrogen was measured by using the modified Kjeldahl method [32]. The available phosphorus in the soil was found with 0.5 M NaHCO₃ extract according to the Olsen method [33]. The quantities of available potassium, calcium, and magnesium were ascertained by utilizing the 1 N ammonium acetate (pH 7) method [32]. The available iron, manganese, and zinc amounts were determined according to Lindsay and Norvell [34].

2.4. Essential oil extraction

The plant samples were collected from the field and dried in the shade. After drying, the essential oils of the plant samples were obtained by the hydrodistillation method in the Clevenger apparatus. In this method, 150 g of each of the shredded plant samples were placed in the glass balloon chambers of the Clevenger apparatus and 300 mL of pure water was added to them. After the device reached the boiling temperature, its temperature was lowered and distillation was performed with a low boiling pace for 2 hours. The essential oil quantities obtained in the Clevenger apparatus were recorded in mL. The extract have been stored in airtight bottles at +4 °C until their usage time [35].

2.5. Gas chromatography (GC) / Mass Spectrometry (MS) Analysis

The essential oil composition of the plant samples was determined by using GC and GC-MS systems. The samples were diluted 1:50 with acetone for analysis and the essential oil composition analysis of the samples was performed by using a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm) and gas chromatography (Agilent 7890A) with mass detector (Agilent 5975C). Also, helium was used as a carrier gas in the analysis at a flow rate of 1 mL/min. Samples were injected into the device with a split ratio of 50:1 as 1 µL. The column temperature program was set at 60 °C (10 minutes), 20 °C/minute increment from 60 °C to 250 °C

and 250 °C (8 minutes), injector temperature was kept at 250 °C. In line with this temperature program, the total analysis time was 27.5 minutes and scanning range (m/z) for mass detector 35-500, atomic mass unit and electron ionization (EI) 70 eV were used, and the data in OIL ADAMS, WILEY, and NIST libraries were taken as the basis for the identification of the components of the essential oil [36].

2.6. Statistical Analysis

Compatibility analysis was applied to the obtained results by using IBM SPSS package program (version 21.0) ($\alpha = 0.05$) [37].

3. Results

3.1. Climate

A climatogram was created based on the climate data of the Serik and Kemer districts, where the wild samples of *S. stricta* were collected, and the Kaş district, where the cultivated samples were obtained. In this climatogram, while Kemer and Serik have similar climatic features, Kaş indicated a different characteristic from these areas. The average annual humidity was highest in Serik (67.00%), and lowest in Kaş (54.92%) (Figure 2). The average annual temperature was highest in Kaş at 19.70 °C and similar in the other areas Kemer (18.40 °C), and Serik (17.86 °C). When considering the annual rainfall amounts in the respective areas, it was observed that the total annual rainfall in the Serik district (1073 mm) is higher compared to Kemer (887.1 mm) and Kaş (750.3 mm) (Figure 3).

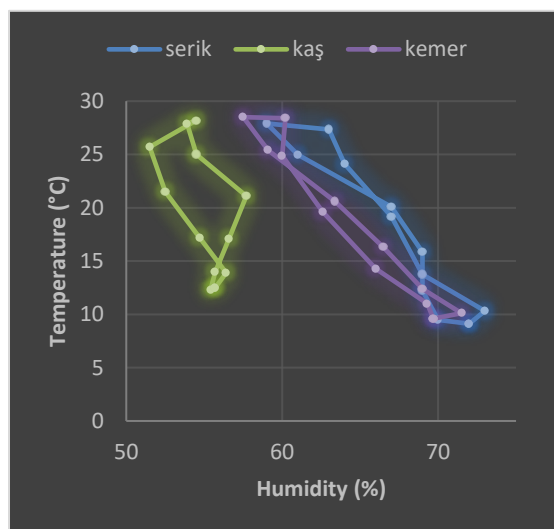


Fig 2. Climate diagram of the areas where plant samples are collected

When all climate data were taken into consideration, it was seen that the winter months were mild and rainy, the summer months were hot and dry, and the typical Mediterranean climate prevailed in these areas. However, it was determined that the temperatures in Kaş were higher the amount of precipitation was lower in the winter months, the average annual humidity was lower, and the driest period was the longest in a year, compared to other areas (Serik and Kemer).

3.2. Soil characteristics

The chemical and physical analysis results of the soils belonging to different localities are given in Table 2. Accordingly, the soil was determined to be alkaline in all

regions, and the pH value in the cultivation area was lower than the pH value in the wild areas. It was found that the lime content was higher in the Serik samples. The texture of the soil was rough. Since fertilizing processes were carried out in the soil of the cultivated area, the soil nutrients were higher. The rate of sand in the soils occupied by wild populations was higher.

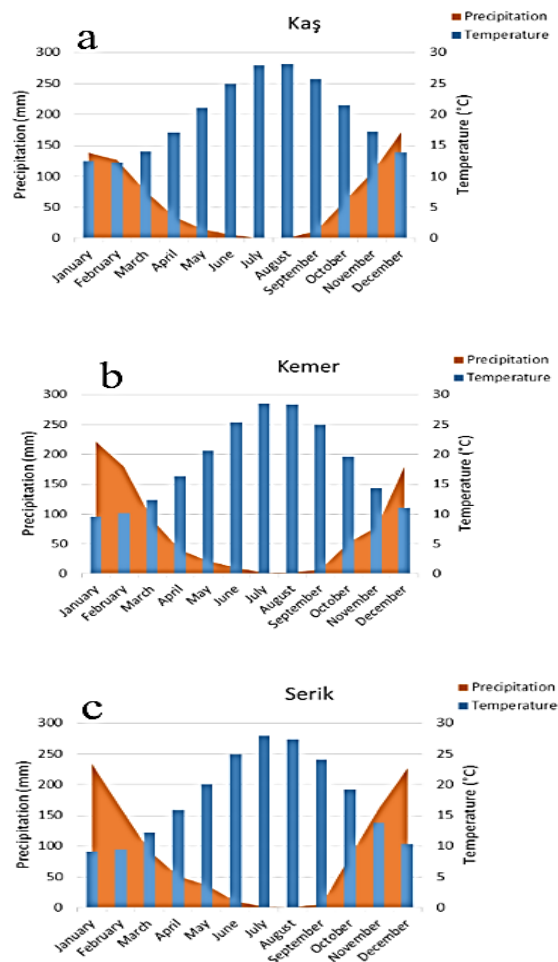


Fig 3. Climatographs of the areas where plant samples were collected: a) Kaş, b) Kemer, c) Serik.

Table 2. Soil analysis results of the soils where *S. stricta* is grown.

Analysis	Kaş (C)	Kemer (W1)	Serik (W2)
pH	7.4	8.6	8.1
Lime (%)	2.0	5.1	18.3
EC micromhos/cm (25°C)	176	165	205
Sand (%)	25	75	51
Mg (ppm)	30	1	18
Clay (%)	45	24	31
Mil (%)	Clayey loam 3.4	Loamy sand 1.0	Loam 5.6
Soil texture org. matter (%)	68	12	12
P (ppm) (Olsen)	429	164	356
K (ppm)	275	1011	175

3.3. Essential oil components

Chromatograms of the wild and cultivated forms of *S. stricta* are given in Figure 4. The percentage of the essential oil yields of C, W1 and W2 samples were 0.20%, 0.60%, and 0.30% v/w on the dry weight basis, respectively (Table 3). These results were compatible with the literature data about the volatile oil yield of this species such as: 0.63% [38], 0.14 - 0.63% [8] and 0.31% [39]. Similarly, the essential oil yields of some *Sideritis* species were 0.05% in *S. condensata*, 0.08% in *S. psidica* and *S. perfoliata* [40], 0.02% in *S. hololeuca* and 0.54% in *S. taurica* [38], 0.83% in *S. congesta* [41], and 0.11% in *S. raeseri* subsp. *raeseri* [42]. The values of the major components, β -pinene, and α -pinene, were different in all samples; however, in the essential oil of the W2 sample, the amount of caryophyllene (16.52%) was higher than that of α -pinene (9.52%). β -pinene was determined to be the most abundant in the C, W1, and W2 samples, with percentages of 32.28%, 27.33%, and 40.61%, respectively. Likewise, Kirimer et al. 2003 (38) reported α -pinene (12.9%), β -pinene (30.0%), and caryophyllene (9.6%) as the major components in the essential oil of wild samples of *S. stricta*. Duman et al. 2005 [8] identified the main components of the essential oil of wild samples of the same species as α -pinene (7-24%) and β -pinene (21-48%). *S. stricta* has a chemotype collected from Muğla province, and its principal components (δ -cadinene 18.3% and cubenol 17.6%) were different from our data and the literature Deveci et al. 2018 [39]. Apart from these studies, β -pinene and α -pinene were found in different amounts in many studies [43- 51].

The most abundant compounds in the essential oil of C, W1, and W2 were monoterpene hydrocarbons (75.29%, 73.43%, 49.26%, respectively) and sesquiterpene hydrocarbons (12.63%, 15.05%, 26.50%, respectively). According to Kirimer et al. [38], oxygenated sesquiterpenes (26.6%) and monoterpene hydrocarbons (19.8%) were the main groups of constituents in *S. stricta*, however, Deveci et al. 2018 [39] reported that sesquiterpene hydrocarbons (55.9%) and monoterpene hydrocarbons (37.9%) were the most abundant groups of compounds found in the same species. Fraga 2012 [52], divided the *Sideritis* species of the Mediterranean region into three groups, from the chemotaxonomic point of view, and incorporated *S. stricta* into the third group which was defined by its content in tetracyclic diterpenes of the ent-kaurene type. Literature data showed that there were various predominant terpenoid groups of constituents among *Sideritis* species and chemotypes, for instance, sesquiterpene hydrocarbons [39, 40, 42], oxygenated sesquiterpenes [38], monoterpene hydrocarbons [41, 44, 53], and oxygenated diterpenes [54]. The essential oil composition and the ratio of major components of the volatile oil of *S. stricta* were different from the other *Sideritis* species. These differences may be due to sampling time and sampling location and climatic/seasonal factors particularly genetic features (different chemotypes) [39, 55].

While the most abundant terpene groups of the chemical compounds were the same for the essential oil of wild and cultivated forms, the amount of monoterpene and sesquiterpene hydrocarbons in W2 differed from W1 and C. It may be explained by the genetic relation of C and W1 (C samples were propagated from the seeds taken from the W1 population).

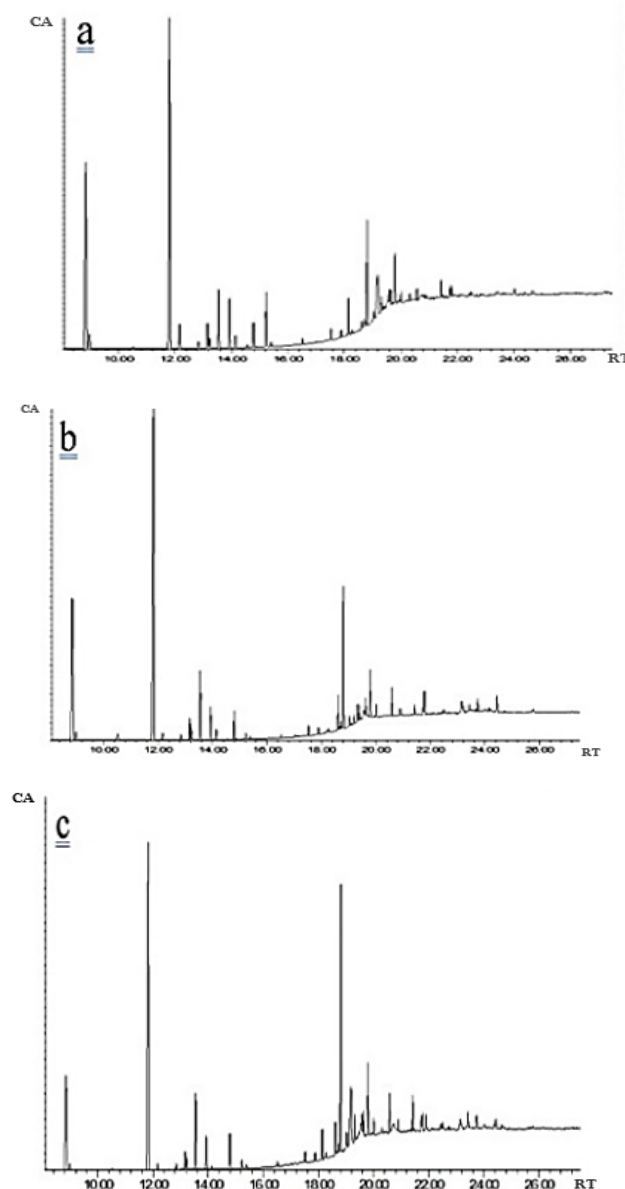


Fig 4. Essential oil chromatograms of samples C(a), W1(b), W2(c) [RT:Retention time, CA: Component amount]

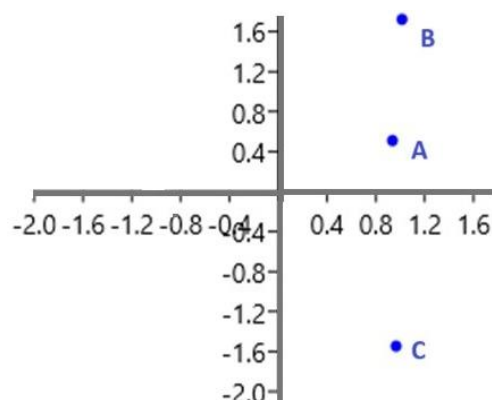


Fig 5. The similarity in the volatile oil content of the samples as a result of the compatibility analysis (A: W1, B: W2, C: C).

The compatibility analysis of the essential oil compositions in the samples is given in Figure 5. As seen in Table 3, the total number of major and minor components that can be determined from C, W2, and W1 essential oil samples are 30, 44, and 30 respectively, and 25 of them are shared components.

Table 3. Essential oil components and proportions of C, W1, and W2 samples.

Terpene type	RT [min]	Component	Component amount (%)		
			Essential Oil/DryMatter%(v/w)	C (0.20)	W1 (0.60)
monoterpene hydrocarbons	8.849	α -pinene	23.17	18.89	9.52
	8.891	α -thujene	1.52	1.04	0.61
	11.824	β -pinene	32.28	40.61	27.33
	12.166	sabinene	2.03	---	0.41
	12.847	δ -3-carene	0.47	---	0.37
	13.164	myrcene	1.65	1.55	1.00
	13.231	α -phellandrene	0.58	0.91	0.79
	13.548	α -terpinene	4.18	5.08	4.56
	13.936	limonene	3.44	2.36	1.88
	14.141	β -phellandrene	0.81	0.69	0.17
	14.794	γ -terpinene	1.66	1.94	1.90
	15.232	p -cymene	3.50	0.36	0.52
	15.402	α -terpinolene	---	---	0.20
	oxygenated monoterpenes	16.523	tyranton	0.39	---
sesquiterpene hydrocarbons	17.528	α -cubebene	0.62	0.57	0.56
	17.889	α -copaene	0.41	0.38	0.41
oxygenated monoterpenes	18.135	linalool	1.96	---	1.34
sesquiterpene hydrocarbons	18.233	α -gurjunene	---	---	0.16
	18.265	β -cubebene	---	---	0.26
oxygenated monoterpenes	18.608	bornyl acetate	0.35	1.98	1.51
	18.716	terpinen-4-ol	0.29	0.43	0.43
sesquiterpene hydrocarbons	18.808	caryophyllene	5.78	8.09	16.52
	19.036	trans-muurolo-3,5-diene	0.27	0.45	0.62
diterpene	19.189	13(16),14-labdien-8-ol	6.62	0.45	6.85
sesquiterpene hydrocarbons	19.324	α -humulene	0.61	1.00	1.15
oxygenated monoterpenes	19.404	borneol	---	0.86	---
sesquiterpene hydrocarbons	19.573	β -copaene	0.71	---	1.17
	19.633	muurolo-4(14),5-diene	0.64	0.90	0.96
	19.791	epi-bicyclosquiphellandrene	3.04	2.93	3.61
	20.001	trans-cadina-1,4-diene	0.55	0.79	0.72
	20.311	trans-calamenene	---	---	0.36
	20.584	cis-muurolo-5-en-4- α -ol	0.62	1.65	1.77
oxygenated sesquiterpene	20.891	cis-muurolo-5-en-4- β -ol	---	0.34	0.51
	21.421	caryophyllene oxide	1.05	0.59	1.75
	21.729	1,10-diepi-cubenol	0.32	0.49	0.63
	21.772	1-epi-cubenol	0.49	1.48	0.94
	21.898	viridiflorol	---	---	0.71
oxygenated monoterpene	22.437	borneol	---	---	0.30

The essential oil yield was higher in the wild samples. In a similar study, [56] found that the total phenolic content of *Salvia fruticosa* Miller was higher in the cultivated form, while the extraction yield, total flavonoid content, and volatile oil quantity were higher in the wild samples. The major components of the essential oils in all samples were found to be the same. Many minor components identified in the W2 sample were not in the C and W1 samples (Table 3). In some cultivated *Mentha* species, the main components of essential oil were found different from wild forms [57]. In another volatile oil research, it was reported that the number of minor components in the volatile oil contents of the cultivated form of *Satureja khuzistanica* Jamzad was higher compared to the wild samples [58]. According to Farsam et al. [58], the reason for the difference in essential oil compositions is the variation in soil composition and growth conditions. *S. stricta* prefers alkaline soils [8]. However, fertilization processes in cultivation areas have decreased soil pH value. For this reason, essential oil yield in cultivated samples was low, while it was higher in wild forms grown in the high pH values soils. It has been stated that this feature is under genetic control because the essential oil composition varies in wild and cultivated samples of the species [59]. In addition, Bilginoğlu [60] concluded that it would be appropriate to cultivate *S. congesta* and *S. stricta* for drug yield in a 10 kg/da nitrogen fertilizer application, but as the nitrogen doses increased, the essential oil yield decreased. The season when the plant was collected [61-63], harvest time [64], phytochrome [65], the amount of N, P and K elements in the soil [66], organic fertilizer applications [67], day length [68-69], and geographical location [70] have been reported to affect the essential oil composition in plants. When studies on medicinal plants' natural and cultivated forms are examined, it has been determined that the natural forms of these plants are richer in terms of bioactive compounds compared to cultivated species [71-74].

4. Conclusions

In this study, it has been determined that the humidity and soil pH values in the cultivation area are lower compared to the natural habitats of the wild samples, and these ecological factors affect the volatile oil yield and composition. As a result of the conducted statistical study, it has been found that natural forms differ from cultivated forms. While natural forms exhibit similarities among themselves in terms of volatile oil content, the cultivated form has shown differences compared to the other samples. Considering the environmental conditions of the natural habitat for the species to be cultured, cultivation fields and cultivation methods should be preferred to be appropriate to the conditions required by the species. Besides, it's important to choose a suitable and qualified origin of the species for cultivation. The variations in the quantities of these bioactive compounds are influenced by abiotic factors such as soil and climate in which the plants grow, as well as biotic factors related to the plants themselves. When considering that the fertilization and irrigation methods applied to the cultivated forms of *Sideritis stricta* are greater compared to the naturally occurring forms of the same plant species, it is believed that the growth, development, and levels of bioactive compounds contained in both the cultivated and natural forms of *Sideritis stricta* are affected. Additionally, it is thought that climatic

conditions and cultivation techniques also form the basis of these differences. The relationships between the volatile oil contents of plant forms and these factors can be elucidated in detail in future studies.

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