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Original Article

Cultivation Site Influence of Essential Oils Component in Purple and Green Basil

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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) is an herbaceous annual aromatic herb belongs to the Lamiaceae family. The compositions of essential oil are the main parameters for assessing quality of basil for different food, pharmaceutical, and chemical industries. On the other hand, secondary metabolites in basil are affected by the interaction of location and genetics. In this study essential oil composition of two basil cultivars (green and purple) in different cultivation site were determined. The experiment took place in Isfahan and Marand cities with different climate, edaphic and elevation factors. Plants were harvested at flowering stage and transported to the laboratory and samples dried at shade condition. Essential oils were obtained by hydro-distillation and analysis of essential oils was carried out by GC and GC-MS technics. There were differences among constituents in the essential oil content from the basil cultivars at two locations. For green basil, the major constituent of the essential oil from aerial parts were Geraniol (36.21%, in Marand region), nerol (27.02%) and methyl-chavicol (18.79%) in Isfahan region. Green basil, grown in the Isfahan region had higher concentrations of essential oils component than in the Marand region. For purple basil, methyl-chavicol (54.54%) and linalool (26.10%) in Marand region and (E)-β- ocimene (3.86%) in Isfahan regain were the highest essential oil components. According to our results, location could affect the efficacy of production for use of basil in drug industries.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is one of the most famous and annual herbs belonging to the Lamiaceae family, which is growing in several regions around the world for different purposes [1,2]. The genus *Ocimum* contains more than 150 species and is considered as one of the largest genera of the Lamiaceae family [3]. Sweet basil is a popular culinary herb and widely used in food industry, dental and oral products and in fragrance [4,5].

There are many cultivars of basil, which differ in flower color (purple, white and red), leaf color and aroma [6]. Antioxidant, antimicrobial, anticancer, antifungal and insect-repelling properties of basil essential oils have been reported are likely due to its phenolic and aromatic constituents [7-9].

Essential oils are volatile and liquid aroma compounds. The major components of essential oils of different sweet basil varieties are methyl chavicol, linalool, methyl cinnamate, methyl eugenol, eugenol and geraniol [10-12]. The compositions of basil essential oil are varying due to the leaf and flower colors, aroma, plant origin, and variation in chemotypes [13]. The main factor influencing composition of an essential oil is plant genotype [14]. However, Climate, edaphic, elevation and topography may affect essential oil content of medicinal plants [15,16].

One of the differences between the two main types of basil is color. The green and purple basil leaves have a corresponding color, and therefore it is easy to distinguish one variety from another. Furthermore, in terms of aroma, green basil has a delicate smell. Especially distinguished lemon

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variety, known for its pleasant aroma and used because of this in a variety of refreshing drinks. Also, Green basil has a mild flavor and violet has a rich spicy taste and is well suited for Caucasian and Asian cuisine, where exactly the sharpness is preferred [17]. The compositions of essential oil are the main parameters for assessing quality of basil for different food, pharmaceutical, and chemical industries.

There is little information on the influence of location on essential oil percent and compositions in basil cultivars. The study was undertaken to evaluation of essential oil content of two basil cultivars in different regions of Iran.

MATERIAL AND METHODS

The experiment took place in Isfahan province in Agricultural Research Farm of Islamic Azad University, which located at Khatoon Abad village, at 32° 38' N latitude, 51° 39' E longitude and 1570 altitude, and the second area is located in a privative field in Marand city in Azerbaijan province with 38° 26' N latitude, 45° 46' E longitude and 1334 m elevation in sea level. Data related to climatic conditions were collected from the nearest monitor climatic station of each region.

Soil properties of the two regions including Organic matter, total nitrogen, electrical conductivity, pH, Potassium, Phosphorous content and soil texture were taken from the regions at a depth of 30 cm were determined according to standard methods [18].

Seeds of basil cultivars were obtained from Pakan-Bazr Company (Isfahan, Iran). The soil was plowed to 30 cm deep, disked and leveled. Before planting, cow manure fertilizer was applied at the rate of 3 $kg \cdot m^{-2}$. Seed were sown at a depth of 1.5 to 2 cm in 5 rows 1 m in length separated by 20 cm. The distance between plots was 1 m. The soil was flood irrigated twice weekly after sowing, and weekly after emergence of shoots. Thinning was 4 times to achieve a final distance of 10 cm between plants. During the growth period (approximately 2 months), no fertilizer was applied. Weeds were controlled by hand twice and no herbicide was used. The growing period of plants in Isfahan and Marand regions was from 17 June to 25 August 2018.

At the flowering stage in both regions, the above ground parts of cultivated basil were harvested. The plants were carefully cleaned manually to remove dirt and damaged leaves discarded. Samples were kept in polyethylene bags, kept cool, for transport to the laboratory and dried in the shade under open-air condition (25 °C) for about 5 days. Essential oils were obtained by hydro-distillation technique. A sample of 100 g of powdered tissue was extracted over 4 h in an all-glass Clevenger type apparatus [16]. The extracted crude essential oil was dried over anhydrous sodium sulphate (Na_2SO_4), stored in sealed glass vials immediately after extraction and kept in amber vials in the dark at 4 °C until further analysis. The essential oil content expressed as v/w based on dry weight [19].

Essential oil samples were determined by gas chromatography (GC) using a Thermo-UFM (Ultra-Fast Module, Milan, Italy) gas chromatograph equipped with a ph-5 fused silica column (10 m × 0.10 mm, film thickness 0.40 μm). The gas chromatography/mass spectrometry (GC/MS) analyses of essential oil were carried out on a Varian (model 3400-GC/MS, (Cridersville, OH) system equipped with a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm). Essential oil samples (0.1 μL) were injected manually. The initial temperature of the column was 60 °C which was increased to 285 °C at 5 °C /min (kept constant for 10 min), and injector temperature of helium gas was 230 °C. After injection, complexes were compared with compounds in the chromatogram library. Retention indices (RI) were calculated at the same conditions using retention times of injected-alkenes (C_6-C_{24}). The mass spectra of compounds were obtained using Agilent MSD Chemstation Libraries according to installation of the national institute of standards and technology. Compound percent was calculated using the area normalization method, excluding the response factors [20, 21].

Statistical analysis the experiment was arranged in a randomized complete block design with 3 replications. Data were analyzed by SPSS (ver. 19.0, SPSS, Irvine**,** CA) software. Means were compared by Duncan's multiple range test.

RESULTS

The environmental factors in two cultivation sites during the growth period presented in Table 1. The study of environmental factors indicated that the cultivation sites are different in evaluated factors. The average of temperature, wind speed and sunshine hours in Isfahan region was higher than Marand region.

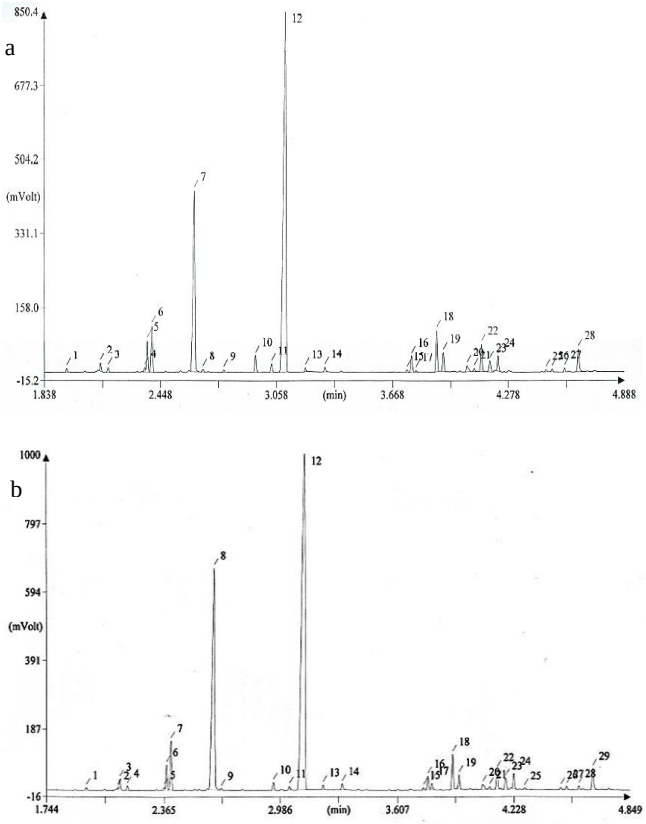

Fig. 1 The chromatograms of profile the essential oils from *O. basilicum* cultivated in Isfahan region (a and b related to purple and green cultivars, respectively).

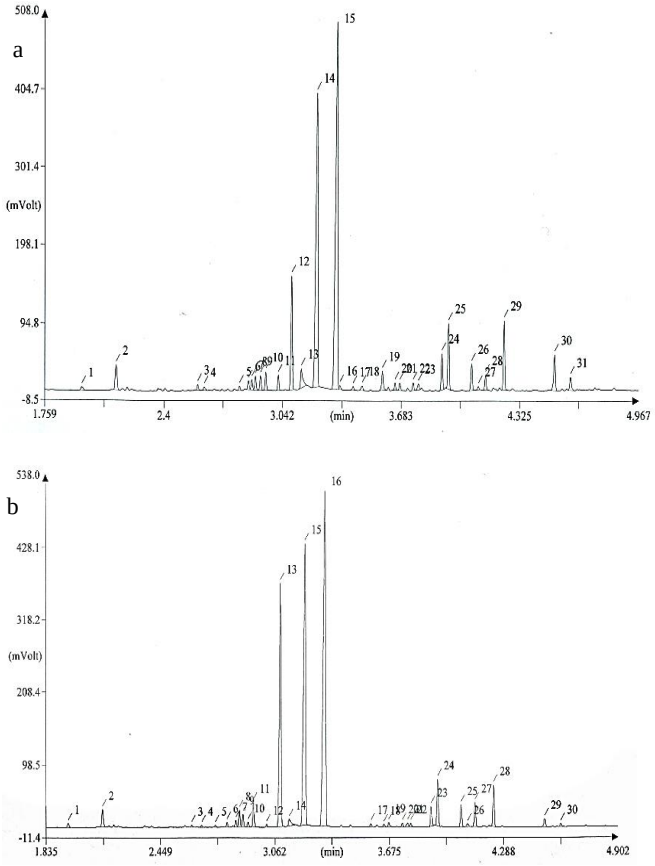

Fig. 2 The chromatograms of profile the essential oils from *O. basilicum* cultivated in Marand region (a and b related to purple and green cultivars, respectively).

During the summer, Isfahan remains hot with maxima temperature around 35 °C and low humidity and moderate temperatures at night. On the other hand, the relative humidity and precipitation in studied growth period in Marand were higher than another region (Table 1).

The soil from the two locations differed in some respects (Table 2). Soil acidity (pH), nitrogen, organic matter and potassium value in Marand was higher than Isfahan; electrical conductivity (EC) and phosphorus in soil in the Isfahan was higher than in Marand.

Total essential oil amounts in the basil cultivars were different in studied regions (Table 3, 4). Essential oil in purple basil in both regions contained several compounds. The essential oils in both regions found in the highest concentrations were methyl-chavicol, linalool, E -β- ocsimene and α-trans-bergamotene.

Region affected essential oil composition of purple basil; in Isfahan and Marand. Amounts of ethyl-chavicol; linalool; and β-osmium were substantial. Camphor, limonene and α-pinene had the lowest concentrations in both regions (Table 2, Fig. 1).

In green basil, 19 compounds were identified with high levels of geraniol, nerol, and methyl-chavicol compounds in both regions. Plants in the Isfahan had high amounts of geraniol, nerol, and methyl-chavicol. The essential oils geraniol, nerol, and methyl-chavicol in green basil had the highest concentration in the Marand region. The essential oils with the lowest concentration were for α-cocaine and limonene oxide (Table 3, Fig. 1).

DISCUSSION

In this study, the responses for methyl chavicol, linalool, and β- ocimene in purple basil which was found in the highest concentrations, agree with Martins *et al*. [22]; Keita *et al*. [23] and Sajjadi [3]. In green basil, the highest concentration of essential oils; geraniol, nerol, and methyl-cavicol compounds had levels which agreed with Ozcan and Chalchat [5] and Sajadi [3]. The various basils have different scents because the herb has a number of different essential oils. The main essential oils of basil cultivars, which were detected in this study, belong to terpenoids compounds. Terpenoids are the most important secondary metabolites compounds. They are non-nutrient constitute while they are considered as biochemically active materials affecting human health.

Table 1 Climate condition parameters during the growth period in Isfahan region (2018)

	Isfahan			Marand		
Parameter	June	July	August	June	July	August
The average of temperature (°C)	24.3	27.2	24.7	20.1	28.3	27.1
RH (%)	32	17	19	50	34	41
Precipitation (mm)	11.3	0	0	38.5	10	0.7
The average wind speed (m/s)	25	12	11	20	11	12
The average sunshine hours (h)	344.1	376.1	352.8	297.3	389.4	361.1

Table 2 Characteristics of soils from Isfahan and Marand regions

Location	Soil texture	pH	EC (ds/m)	Nitrogen (%)	Organic matter (%)	Potassium (mg/kg)	Phosphorus (mg/kg)
Isfahan	Loamy Clay	7.55	1.97	0.06	1.18	200	116
Marand	Loamy Clay	7.83	0.75	0.08	1.80	880	103

Table 3 Chemical composition of essential oils of *O. basilicum*, cv. Purple, from Isfahan and Marand regions

			% GC peak area		
No.	Compound	RI a	Isfahan region	Marand region	ANOVA
1	α-pinene	946.09	0.32	0.16	-
2	β-pinene	978.51	0.58	0.65	-
3	Myrcene	1007.94	0.38	0.29	-
4	Limonene	1055.82	0.32	0.15	-
5	1,8-cineole	1060.64	2.35	1.45	-
6	(E)- β-ocimene	1065.41	3.86 ± 0.1 ab	3.28 ± 0.1 b	$P< 0.01$
7	Linalool	1117.62	19.78 ± 1.5 b	26.1 ± 2.0 a	$P< 0.01$
8	Camphor	1126.23	0.24	0.12	-
9	Terpinene-4-ol	1183.12	1.41	0.52	-
10	α-terpineol	1222.64	0.73	0.25	-
11	Methyl chavicol	1244.7	53.98 ± 4.0 b	54.54 ± 4.0 a	$P< 0.01$
12	E-caryophyllone	1428.13	1.38	0.95	-
13	*Trans*- α - bergamotene	1467.61	3.32 ± 0.4 a	2.32 ± 0.3 b	$P< 0.01$
14	α -humulene	1476.53	1.6	0.97	-
15	Germacene D	1537.15	2.26	1.34	-
16	Bicyclogermacrene	1548.34	1.15	0.97	-
17	α-bulnesene	1559.43	1.23	1.04	-
18	γ-cadinene	1652.53	0.2	0.21	-
19	1,10-di-epi-cubenol	1662.82	0.25	0.28	-
20	Epi- α -cadinol	1709.98	2.15	1.66	-
Total		-	97.49	97.25	-
Essential oil yield		-	0.47 ± 0.17	0.65 ± 0.25	$P\leq 0.05$

[a] RI = Retention indices determined on HP-5MS capillary column.

[b] Bold values represent major compound in purple basil. In a row, values with a different letter are significantly different using DMRT at $P< 0.05$.

The non-nutrient phytochemicals may contribute to normal functioning in humans [24]. Secondary metabolites are responsible for color, flavor and aroma [25]. The diverse compounds identified in the basil cultivars related to responsibility of secondary metabolites present.

Total essential oil amounts in the basil cultivars were different in the regions. Purple basil had the highest amount of oil in both regions.

Table 4 Chemical composition of essential oils of *O. basilicum* L., cv. Green, from Isfahan and Marand regions

No.	Compound	RI[1]	% GC Peak area		ANOVA
			Isfahan Region	Marand Region	
1	α-pinene	946.09	0.26	0.33	-
2	6 methyl-5-hepten-2-one	980.23	1.13	2	-
3	Limonene oxide	1115.44	0.15	0.27	-
4	Citronellal	1163.45	0.38	0.56	-
5	Borneol	1167.44	1.02	0.65	-
6	Terpinene-4-ol	1171.4	0.8	0.91	-
7	*Methyl*-chavicol	1239.24	18.79 ± 0.5 a	7.07 ± 0.5 b	$P< 0.01$
8	Nerol	1276.74	27.02 ± 0.7 a	25.97 ± 0.7 b	$P< 0.01$
9	Geraniol	1302.54	34.92 ± 0.1 b	36.21 ± 0.1 a	$P< 0.01$
10	Neryl acetate	1363.81	0.19	1.38	-
11	α-copaene	1380.19	0.14	0.42	-
12	β-cubebene	1384.82	0.31	0.46	-
13	E-caryophyllone	1467.61	1.28	2.17	-
14	*Trans*- α -bergamotene	1476.53	2.91	3.99	-
15	α- humulene	1514.41	1.4	1.66	-
16	Germacene D	1525.84	0.21	0.2	-
17	α-calacorene	1537.15	1.55	0.92	-
18	Caryophyllone oxide	1564.94	2.52	4.06	-
19	Humulene epoxide	1662.82	0.58	2.53	-
Total			95.56	91.76	
Essential oil yield			0.70 ± 0.12	0.57 ± 0.04	$P\leq 0.05$

[1]RI: Retention indices determined on HP-5MS capillary column.

[b] Bold values represent major compound in green basil. In a row, values with a different letter are significantly different using DMRT at $P< 0.05$.

The essential oil of basil appears to be related to their genetics and cultivation site which agrees with Shafie *et al*. [26] and Ghasemi- Pirbalouti [16].

Environmental conditions where basil is grown is an important factor in expression of biosynthesis of secondary compounds. The process of formation of active ingredients is affected by temperature and humidity [27]. The content of mineral elements of soil plays a role in chemical composition of essential oils [28]. Light intensity, CO_2 level, temperature, fertilization, and biotic and abiotic factors can increase concentration of plant secondary metabolites [29,30].

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