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Variability of Essential Oil Composition of *Origanum vulgare* L. subsp. *gracile* Populations from Turkey

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Abstract: The aerial parts of fifteen different populations of *O. vulgare* L. subsp. *gracile* were investigated to determine their variability among essential oil compositions. Thymol, carvacrol, γ-terpinene and *p*-cymene were detected as the major components of all population samples in different amounts. Almost all populations had the same essential oil contents, except for minor differences. The most abundant components were thymol and carvacrol.

Key words: Oregano, *Origanum vulgare*, essential oil, ecotypes.

Introduction

Oregano (*Origanum vulgare* L.), (Lamiaceae family), is an erect, perennial, aromatic plant of 20-80 cm height. It is distributed and cultivated mainly in the Mediterranean region and also in many areas of mild, temperate climates of Europe, Asia, North Africa, and America [1]. Aromatic plants are of great interest for their flavours, medicinal properties and their use as spice and condiments, animal foodstuff and ornamental uses; thus, they are suitable for multifunctional sustainable crop models [2-5]. Despite its economic significance, *Origanum* taxa are often referred to as an under-utilized, in the sense genetic resources and variability, and potential usage that need to be, fully researched [6]. Centre of differentiation of *Origanum* is located in the Mediterranean area [7]. Oregano essential oils have antibacterial, antioxidant, antifungal, carminative, diaphoretic, antispasmodic, antifungal, antimicrobial and analgesic effects [8-10]. Carvacrol and thymol are responsible for major biological activities [11,12]. It is important to consider that the essential oil yield and composition of *Origanum* L. taxa are the result of different factors; including genotype, environment, ecological conditions and developmental stage [13-16]. *Origanum* taxa are characterized by a wide range of secondary metabolites [17].

Origanum vulgare L. commonly known as `oregano' in most European countries [18]. Distinguishes four subspecies in flora of Turkey on the basis of morphological characters: *gracile*, *hirtum*, *vulgare* and *viride*. The subspecies *gracile* is widespread in Flora of eastern Turkey especially, whereas other subspecies are generally found in the south, west and northern parts of the country [19]. There are several studies on the essential oil composition of *O. vulgare* subsp. *gracile* [20] and Lamiaceae taxa [21-24], whereas variability of essential oil composition of *O. vulgare* subsp. *gracile* has never been studied.

This study aimed to conduce to the characterization of the geographical and biochemical variability of the *Origanum* taxa; to dedicate the stud-

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ies on the content, qualitative and quantitative analysis of the essential oil extracted from the different populations of *O. vulgare* subsp. *gracile*; to characterize as an important source of essential oil, in order to contribute to the conservation and exploitation of genetic resources in eastern part of Turkey.

Materials and methods

Plant material source

The aerial parts of fifteen samples of *O. vulgare* subsp. *gracile* were collected from naturally grown populations of the plants from locations given in Table 1.

Plant materials were identified with keys mentioned in the Flora of Turkey and East Aegean Islands [19]. Voucher specimens were deposited in Department of Park and Garden Plants of Bingol University and in Yildirimli Herbarium from Ankara, Turkey. All plant samples were air-dried at room temperature in a shady place and kept away from direct light.

HS-SPME method

Aerial parts of dry samples powdered with a blender. 5 g powder plant sample were analyzed head space solid phase microextraction method using polydimethyl siloxane fiber. Before analysis fiber was preconditioned in the injection port of the gas chromatography. 5 g plant samples were weighed in a 40 ml vial. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C [25]. Then fiber was introduced into the gas chromatography injector, and was left for 3 min to allow the analyses thermal desorption. In order to optimize method, sample volume, headspace volume, heating temperature and extraction time were studied on the extraction efficiency as previously reported [25].

GC-MS analysis

Gas-chromatography and mass spectrometry is an analytical method that combines the features of different compounds within a test sample. A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer was used with 260°C injector temperature.

Table 1. The locations of *O. vulgare* subsp. *gracile*

1	Elazig: Baskil district, vicinity of Kürsatlar hamlet, 1250-1300 m., 30.05.2015
2	Elazig: Keban, vicinity of Aslankaþý village, woodlands and wetlands, 850-1000 m, 31.05.2015
3	Elazig: Keban, North of Güneytepe transmitting station, wet and stony areas, 1250-1300 m, 30.05.2015
4	Elazig: Center, vicinity of Dilek village, edge of stream, 1150-1200 m, 20.06.2015
5	Tunceli: Ovacik, Munzur mountains, Yilanli mountain, rocky and stony place, 1800-2000 m, 12.06.2015.
6	Tunceli: Between Tunceli and Ovacik, 20 km, left of road, edge of Munzur river, 1300-1400 m, 13.06.2015
7	Bingöl: Vicinity of new ski center, stony areas, 1750-1800 m., 27.06.2015.
8	Bingöl: Center, Dikme village, volcanic cliff and stony, *Quercus* openings, 1750-1800 m, 21.06.2014
9	Bingöl: South of Yelesen village, stony areas, 1600-1700 m, 25.05.2015
10	Bingöl: East of Direkli village, cliff, stony, slopes, 1650-1700 m, 09.06.2014
11	Bingöl: North of Haserek mountain slopes, wet areas, 1900-2000 m, 10.06.2015
12	Bingöl: Solhan, Hazarþah village, Aksakal Göl hamlet, stony areas of edge of river, 1700-1750 m, 22.06.2015
13	Sanliurfa: Siverek to sanliurfa 15. Km, stony and bushes areas, 850-1000 m, 04.07.2015
14	Adiyaman: Between Turus village and Atatürk Dam, rocky areas, 600-700 m, 28.06.2015
15	Malatya: Akçadað, vicinity of Sultansuyu Dam, stony areas, 27.06.2015

Injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 µm thickness. The oven temperature was adjusted as: 45°C held for 5 min, then increased to 80°C at the rate of 10°C/min, and to 240°C at 2°C/min. Helium was the carrier gas and used at a constant pressure of 10 psi; the transfer line temperature 250°C; acquisit ion range, 40 to 200 m/z; scan rate, 1 us^{-1} [26].

Essential oil constituents were detected using the NIST and mass spectral library as described by Van den Dool and Kratz [26]. The relative amounts were computed on the basis of peak-area ratios. The major essential oil compounds of fifteen different populations of *O. vulgare* subsp. *gracile* are listed in Table 2.

Results

An examination of Table 2 revealed definite chemotaxonomic similarities and differences among the collections from fifteen different locations; the percentage of constituents falls into two groups: thymol and carvacrol type; γ-terpinene and *p*-cymene type. The *O. vulgare* subsp. *gracile* specimens of fifteen populations from different regions of Turkey were found to contain between 33 and 36 compounds in their essential oils, making up between 87 % and 94 % of the total compounds present (Table 2). The yield of 15 sample oils are between ca. 0.25 - 0.50 mL/100 g. γ-terpinene (15.12 % - 30.21 %) and thymol (31.46 % - 35.75 %) in Elazig populations; thymol (27.95 % - 40.04 %) and carvacrol (25.05 % -29.84 %) in Tunceli populations; *p*-cymene (18.02 % - 23.52 %) and carvacrol (30.45 % - 33.21 %) in Bingöl populations; *p*-cymene (21.05 %) and γ-terpinene (23.41 %) in Sanliurfa population; thymol (26.24 %) and γ-terpinene (27.95 %) in Adiyaman population; thymol (30.54 %) and γ-terpinene (27.82 %) in Malatya population; were determined major compounds (Table 2). The identification of particular chemotypes of *O. vulgare* subsp. *gracile* in this study, displaying a dominant production of thymol, carvacrol, *p*-cymene, γ-terpinene has led to the development of a hypothesis that particular populations (or chemotypes) of this subpecies are rich especially in thymol and carvacrol, but they did not produce both groups of constituents in higher amounts simultaneously (Table 2). Thymol, carvacrol, γ-terpinene, *p*-cymene were determined in the essential oils of all investigated populations its proportion ranging from 7 to 40 %, 8 to 33 %, 9 to 30 % and 3 to 23 %, respectively (Table 2). Carvacrol and γ-terpinene had previously also been identified as a major components [27,28]. Regarding the qualitative pattern of the essential oils of some *Origanum* taxa, there are similar results for thymol and carvacrol, major/high component reported [29]. Nevertheless a large differences occured in the amounts of some compounds. It is noteworthy that in the composition of Elazig, Tunceli, Adiyaman and Malatya pupulations thymol was determined as major compound; this compound showed little amounts, (Table 2). Carvacrol was detected highest in Tunceli and Bingöl populations; also *p*-cymene was determined highest in Sanliurfa and Bingöl populations; γ-terpinene was found highest in Elazig, Sanliurfa, Adiyaman and Malatya populations (Table 2). Carvacrol (74.86 %) and γ-terpinene (13.83 %) were found as major components in the essential oils of *O. scabrum* and *Origanum microphyllum* as well [28].

Discussion

Thymol and carvacrol followed by their precursors γ-terpinene, *p*-cymene that was present in high percentages in all studied samples of *O. vulgare* subsp. *gracile* populations. Similar results have been previously reported in *Origanum syriacum* L. populations growing in Syria [29]. It is noteworthy that thymoquinone was not determined in all studied populations of this research (Table 2). The average population values of γ-terpinene and *p*-cymene varied between 9.15 % and 30.21 % and between 3.07 % and 23.52 %, respectively. The value of 23.52 % for *p*-cymene in population eleven was an extreme value based on one sample with an extraordinarily low value of 3.07 % in population fifteen; all other samples showed a continuous variation for *p*-cymene of up to 22.85 % (Table 2). The major components of the essential oil extracted from *O. vulgaris* subsp. *hirtum* in Sicilia included thymol (24.0 % - 54.4 %), γ- terpinene (9.8 % - 30.5 %), *p*-cymene (5.2 %) [30]. Another research suggest that major components is thymol along with carvacrol [31], as

Table 2. Relative composition of essential oils of *Origanum vulgare* subsp. *gracile* populations

Compounds	RRI*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
α-Thujene	1015	0.82	0.35	0.47	1.25	0.53	0.87	1.50	2.01	0.90	0.25	1.02	0.33	0.41	1.21	0.40
α-pinene	1020	0.21	0.32	0.46	0.62	0.78	0.65	0.90	0.40	0.56	0.63	0.88	0.62	0.49	0.56	0.53
Camphene	1032	-	0.08	0.21	0.16	0.05	0.21	-	0.09	0.07	0.11	-	0.04	0.02	-	0.11
Sabinene	1045	1.15	0.16	0.17	-	0.15	0.08	0.21	0.52	0.23	0.09	0.35	0.24	-	1.05	0.07
β-pinene	1050	0.05	-	0.02	0.30	-	-	0.18	0.14	-	0.18	0.08	-	0.13	0.19	-
Octanon	1055	0.12	0.02	-	0.10	0.08	0.20	0.21	-	0.45	-	0.03	0.02	0.12	0.05	0.03
β-myrcene	1062	1.05	1.25	2.10	1.65	0.85	1.38	2.09	1.73	2.22	1.32	2.01	2.12	1.25	3.20	0.95
α-Phellandrene	1075	-	0.17	0.30	0.21	0.31	0.15	-	0.41	0.35	0.28	0.19	-	0.39	0.37	0.23
δ-3-carene	1080	0.02	-	0.04	0.06	-	0.04	0.02	0.10	0.07	0.03	-	0.11	-	0.40	0.12
α-terpinene	1085	5.01	1.45	3.02	1.25	0.98	3.01	2.23	1.87	1.52	3.07	1.75	2.80	1.56	0.78	1.13
p-cymene	1093	3.45	3.12	5.05	8.45	6.25	3.70	20.41	18.75	18.02	22.85	23.52	19.87	21.05	4.12	3.07
Limonene	1096	-	0.12	0.45	0.05	0.43	0.37	-	0.44	0.12	-	0.47	-	0.25	-	0.74
1-8-cineole	1098	0.15	0.14	-	-	1.12	-	0.21	-	0.45	1.05	-	0.07	0.18	0.04	-
β-ocimene	1102	0.32	-	0.12	0.05	0.75	0.23	0.15	0.08	-	0.52	0.99	1.02	-	0.54	0.19
γ-terpinene	1115	15.12	30.21	22.89	18.78	9.45	10.12	14.58	13.31	10.86	9.15	10.54	14.10	23.41	26.24	27.82
terpinolene	1130	0.02	-	0.14	0.12	0.09	0.15	-	0.21	0.05	-	0.02	-	0.07	-	0.10
Linalool	1142	-	0.41	0.21	0.25	-	0.30	1.05	0.25	1.45	0.72	0.24	0.09	0.32	0.18	0.97
Borneol	1185	0.32	0.15	0.17	0.10	0.08	0.01	0.30	0.13	0.29	0.09	0.22	0.14	0.08	0.45	0.75
α-terpinolen	1205	1.12	1.04	-	0.25	0.13	0.06	0.28	1.24	0.21	0.45	1.04	1.03	5.26	1.04	1.02
Thymol methyl ether	1235	0.81	1.02	2.41	0.09	0.33	0.57	3.05	0.07	0.46	2.08	1.09	0.52	0.45	1.09	1.00
Carvacrol methyl ether	1240	1.25	3.41	0.87	3.17	11.45	12.63	3.15	4.05	5.85	2.87	4.87	0.56	3.75	1.94	2.45
Thymol	1290	35.25	35.75	31.46	34.05	40.04	27.95	7.02	8.05	9.52	14.09	11.15	9.05	9.56	27.95	30.54
Carvacrol	1305	8.69	8.21	12.15	15.45	25.05	29.84	30.45	32.54	31.00	31.45	33.10	33.21	15.25	10.21	15.12
α-cubebene	1332	-	0.02	0.15	-	0.12	0.08	0.01	0.10	0.14	0.08	0.12	0.08	0.12	-	0.10
α-copaene	1358	0.03	-	0.12	0.02	-	-	-	-	0.12	-	-	-	0.12	0.03	-
β-bourbenene	1368	-	0.10	-	0.12	0.01	0.02	0.10	0.02	-	0.04	0.12	0.05	0.02	0.12	0.03
β-caryophyllene	1380	1.02	2.01	0.12	1.85	0.75	1.25	0.85	1.17	2.13	2.02	1.45	0.87	1.25	0.45	0.12
β-cubebene	1395	0.01	-	0.12	0.02	0.01	-	0.12	-	0.03	0.01	-	0.10	-	0.10	-
Aromadendrene	1402	-	0.01	-	-	-	0.24	-	0.02	0.24	-	0.08	-	0.14	-	0.21
α-humulene	1415	0.12	0.35	0.21	0.14	0.25	0.41	0.50	0.36	0.32	0.25	0.44	0.27	0.52	0.65	0.71
Naphthalene	1430	-	0.02	0.12	0.25	-	0.21	0.45	-	0.25	0.05	-	0.27	0.38	0.56	0.24

table 2. (continued).

Compounds	**RRI***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
γ-muurolene	1435	0.10	0.05	0.12	0.14	0.27	0.23	0.12	0.54	0.33	0.61	0.25	0.13	0.08	0.09	0.02
Germacrene D	1440	0.41	0.07	0.82	1.25	0.32	0.63	1.02	0.51	0.11	0.64	1.04	1.15	0.12	0.52	0.27
α-amorphene	1452	0.02	-	-	0.10	0.07	-	0.02	0.14	-	-	0.41	-	0.25	-	-
Valencene	1460	0.01	0.05	0.12	0.06	0.13	0.14	0.12	0.05	0.04	0.12	0.05	0.01	0.03	0.01	0.10
β-bisabolene	1468	2.02	0.56	4.25	2.54	3.02	1.37	0.25	2.09	1.56	0.52	1.21	0.56	0.12	4.45	2.02
δ-cadinene	1470	0.01	0.12	0.05	0.13	0.03	0.16	0.13	0.04	0.07	0.29	0.33	0.10	0.12	0.07	0.25
Spathulenol	1490	0.41	0.15	0.32	0.23	0.14	0.30	0.41	0.21	0.10	0.24	0.05	-	0.10	0.05	-
Caryophylleneoxide	1497	-	0.12	-	0.04	-	0.02	0.21	-	-	0.05	-	0.22	-	0.13	0.04
α-cadinol	1552	0.10	0.02	0.21	-	0.12	-	0.05	0.11	0.03	-	0.05	0.28	0.12	-	0.42
Ericosane	1690	0.01	-	0.10	-	-	0.01	-	0.02	-	0.04	-	-	0.04	-	0.01
Total		89.20	91.03	89.54	91.80	92.69	94.49	91.85	89.88	90.12	93.17	92.29	90.03	87.53	88.84	91.64

Population names: 1-4 Elazig, 5-6 Tunceli, 7-12 Bingöl, 13 Sanliurfa, 14 Ad-yaman, 15 Malatya. RRI*: Relative Retention Index

was confirmed from *O. vulgare* subsp. *gracile* populations samples (Table 2). In this study, these similarities or differences among essential oil compositions of studied fifteen population samples may be due to local, climatic and seasonal factors. Another research the effects of soil on the yield and characteristics of the oil of *Rosmarinus officinalis* grown in two different areas of Sardinia were investigated. The starting plant material was obtained from cuttings of *R. officinalis* growing spontaneously on granitic silt soil. Cuttings were divided into two homogeneous groups and planted in two different kinds of soil: one predominantly silt with granite and the other with a highly calcareous content. The different character of the soil had a significant effect on the yield and composition of the oil. Plants grown in granitic silt soil appeared more luxuriant and had a more intense aroma than those grown in calcareous soil which, on the contrary, showed evident signs of chlorosis. The main difference in the composition of the oil was a higher percentage of oxygenated components in plants grown in calcareous soil. The main constituent of the oxygenated fraction was 1,8-cineole, reaching up to 31 % in samples from rosemary grown on calcareous soil. This is particularly surprising since the oil obtained from *R. officinalis*, which grows naturally in calcareous soil, usually contains markedly lower levels of 1,8-cineole than oil from plants grown in granitic silt. The high content of 1,8-cineole found in the oil of rosemary grown on calcareous soil could be due to selective adaptation to conditions markedly different from the environment in which the plant originated [32]. So in this study, different soil types in the localities may be effect the yield and characteristics of the oil of *O. vulgare* subsp. *gracile*

Influence of environmental and genetic factors on content and composition of essential oil has been a subject of recent studies of some Lamiaceae taxa [33,34]. Oher studies propose that some changes in the biosynthesis are due to both environmental and ontogenetic causes [35]. Essential oil composition of plant extracts depends also on the harvest time, type of extraction, and processing, *Origanum* taxa display a marked plasticity which allows the adaptation of their phenologies to the growing season in a range of other environments. Researches under controlled conditions have demonstrated the influence of variation of environmental factors such as soil, temperature, irradiance, and photoperiod on essential oil yield, composition and quality. The small variability in the essential oil composition of *O. vulgare* subsp. *gracile* that was found in this study could be attributed to the environmental conditions. The change of environmental conditions such as climatic, geographic, cultivation and other factors had strong influence on an essential oil profile. Thus, qualitative and quantitative comparative investigation of fifteen different populations of *O. vulgare* subsp. *gracile* essential oils allowed us to confirm that the essential oil composition varies substantially, highlighting the importance of environmental and growing conditions. Further investigations of the essential oil compositions of larger number of relative and distant populations of different and close taxa, along with more data about *Origanum* taxa, could be helpful in essential oil composition.

Conclusion

The study clerly shows that essential oil content of *O. vulgare* subsp. *gracile* is significantly influenced and affected by geogrphic and environmental conditions and identifies chemotypes with thymol and carvacrol type; γ-terpinene and *p*-cymene types. Irrespective of the origin, they had 33 to 36 compounds in their essential oils. The results suggest that their concentration is affected by temperature and moisture level of the areas at the time of collection. These conclusions need to be tested under controlled conditions in the greenhouse or fields. Irrespective of the origin of plants they had stable essential oil content that may serve as base to use these plants in commercial extractions for use in aroma, flavour, spice, codiments and pharmaceutical industry favorably.

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