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ENVIRONMENT EFFECT ON DIVERSITY IN QUALITY AND QUANTITY OF ESSENTIAL OIL OF DIFFERENT WILD POPULATIONS OF KERMAN THYME

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Thymus (thyme) is one of the most important genera with regard to the number of species within the family Lamiaceae. Kerman thyme (*Thymus carmanicus* Jalas) is an endemic Iranian species, intensively utilized because of its wide ranging medicinal and culinary properties. Aerial parts of *T. carmanicus* collected from various altitudes including 2000-2500, 2500-3000, and 3000-3500 m above sea level in Zagros Mountains, Kerman province, South Iran. The yellow oil yields ranged between 0.80 to 1.10% (v/w) for populations collected from various elevations and for the populations collected from various regions ranged between 0.55-1.61% (v/w). GC-MS analyses revealed compounds, constituting 92.2-99.9% of total essential oils. The major constituents of essential oils were carvacrol (47.6-57.9%), thymol (8.3-19.0%), □-terpinene (7.3-7.9%) and *p*-cymene (4.4-7.6%), that monoterpenes, especially oxygenated monoterpenes was the main constituent group in essential oil from the aerial parts of *T. carmanicus*. The results of current study indicated that increasing elevation decreased thymol content in essential oils of the wild populations of *T. carmanicus*.

Key words: *Thymus carmanicus* Jalas., essential oil, high altitude, thymol, carvacrol

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INTRODUCTION

The genus *Thymus* L. belongs to the mint family (Lamiaceae), and consists of about 215 species of herbaceous perennials and small shrubs in the world. The Mediterranean region can be described as the center of the genus (GHASEMI PIRBALOUTI *et al*. 2013a). *Thymus*, with the common Persian name of "Avishan or Azorbe", consists of 14 species which are found wild in many regions of Iran, which are endemic; *T. carmanicus* Jalas, *T. daenensis* Celak subsp. *daenensis* Celak, *T. daenensis* Celak subsp. *lancifolius* (Celak.) Jalas, *T. persicus* (Roniger ex Reach. F.), and *T. trautvetteri* Klokov & Desj.-Shost (MOZAFFARIAN, 2008).

Thymus caramanicus Jalas (Kermani thyme) is an endemic species to Iran. The areal parts and volatile constitutes of *T.* caramanicus are commonly used as medicinal and aromatic herb. *T. caramanicus i*s used as herbal tea, flavoring agents (condiments and spices), food preservation and medicinal purpose such as for treatment of rheumatism, skin disorders and as an antibacterial agent (NEJAD EBRAHIMI *et al*. 2008). The published results reveal that the major volatile constituents obtained from the aerial parts of *T. caramanicus* ***a***re oxygenated monoterpenes (RUSTAIYAN *et al*. 2000; NEJAD EBRAHIMI *et al*. 2008; MAKIZADEH TAFTI *et al*. 2010). The essential oil and extracts isolated from *T.* caramanicus have biological properties, including antibacterial (NEJAD EBRAHIMI *et al*. 2008; NARIMAN *et al*. 2009; DADFAR *et al*. 2012), antifungal (NABIGOL & MORSHEDI, 2011), and antioxidant (SAFAEI-GHOMI *et al*. 2009).

It has been reported that yield and its components in plants in general is primarily related to their genetic (SHAFIE *et al.* 2009), climate, edaphic, elevation and topography (POUROHIT & VYAS, 2004; LOZIENE & VENSKUTONIS, 2005; RAHIMMALEK *et al*. 2009; GHASEMI PIRBALOUTI *et al*. 2013a,b), and their interaction (BASU *et al*. 2009). Recent findings indicated that some of the medicinal plant characteristics can be affected by genetic and ecological factors, including precipitation, temperature, plant competition, and nitrogen content in the soil (GHASEMI PIRBALOUTI *et al*. 2013b). GHASEMI PIRBALOUTI *et al*. (2011a) reported that the altitude showed be considered as a major factor influencing the chemical of *Thumys daenensis* Celak. Altitude seems affecting essential oil content of only oil rich and oil-intermediate aromatic plants.

The main goals of this study were to (i) determine the variation of chemical constitutes of of different populations of *T. caramanicus* collected from three geographical regions of Iran, and (ii) evaluate the effects of various elevations and environmental conditions on qualities and quantities of the essential oil of *T. caramanicus.*

MATERIALS AND METHODS

Plant material

The aerial parts of wild populations of *Thymus caramanicus* Jalas were collected from natural habitats in Dehbakri (N 29°04´/W 57°55´, ~ 350 to 400 mm annual rainfall, temperature range ~ 14 to 16 °C), Bardsir (N 29°59´/W 56°20´, ~ 250 to 300 mm annual rainfall, temperature range ~ 16 to 18 °C), and Kuhsha (N 29°24´/W 50°45´, ~ 250 to 300 mm annual rainfall, temperature range ~ 14 to 16 °C) in Kerman province, South Iran. The voucher specimen was identified at the Herbarium, Agricultural and Natural Resources Research Center of Kerman province, Iran.

EO extraction

The aerial parts were dried at room temperature for once week. One hundred gram of powdered plants was distillated with 1000 ml water for 3 h using a Clevenger-type apparatus. The separated oil was dried over anhydrous sodium sulfate (Merck, Germany) stored in dark glass bottles at 4 °C prior to use.

Identification of the Oil Components

The essential oils were analyzed using an Agilent 7890 A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30.00 m x 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60 °C for 4 min initially, and then raised at the rate of 4 °C/min to 260 °C. Injector and detector temperatures were set at 290 °C and 300 °C, respectively. Helium was used as carrier gas at a flow rate of 2 mL/min, and 0.1 µL samples were injected manually in the split mode. Peaks area percents were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200 °C. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY /ChemStation data system (ADAMS, 2007).

Statistical analyses

The data was statistically analyzed using a completely randomized design (CRD) using SPSS (19.0) software. Means of the main constituents of the essential oils were compared by Duncan's multiple range test at $p \leq 0.05$ level. Analytical data for hierarchical cluster analysis were treated by means of the SPSS statistical software.

RESULTS AND DISCUSSION

Chemical composition

The chemical constituents identified by GC-MS, are presented in Table 1. GC-MS analysis resulted in identification of 35 constituents of the oil. Their sum constituted the bulk of the oils and ranged from 92.18% to 99.97% of total oil. The analysis of essential oils detected four major compounds, viz. carvacrol (47.60 ± 4.86 to 57.90 ± 7.94%), thymol (8.30 ± 4.61 to 19.00 ± 0.36%), □-terpinene (7.30 ± 0.63 to 7.90 ± 1.68%), and *p*-cymene (4.40 ± 0.59 to 7.60 ± 2.97%) for *T. caramanicus* populations collected from different elevations (Table 2). Carvacrol (47.60 ± 4.86 to 65.31±2.54 %), thymol (5.07 ± 2.24 to19.04 ± 0.36%), α-terpinene (7.09 ± 1.51 to 8.19 ± 1.21%) and *p*-cymene (4.41 ± 0.59 to 7.59 ± 2.79%) were the main constitutes of *T. caramanicus* populations collected from various regions (Table 3). The results indicated the essential oils obtained from *T. caramanicus* populations contained monoterpenes, including oxygenated monoterpenes (65.3-78.1%) and hydrocarbons monoterpenes (14.6-31.8%). The published results reveal the major volatile constituents obtained from the aerial parts of *T. caramanicus* are thymol, carvacrol, *p*-cymene, γ-terpinene, and borneol (RUSTAIYAN *et al*. 2000;

NEJAD EBRAHIMI *et al.* 2008; SAFAEI-GHOMI *et al.* 2009; EFTEKHAR *et al.* 2009; MAKIZADEH TAFTI *et al.* 2010).

Table 1 The essential oil components of *T. caramanicus* from different natural habitates

No.	Compound	RI[a]	%[c]								
			B-I[b]	B-II	D-I	D-II	D-III	K-I	K-II	K-III	K-IV
	Hydrocarbons monoterpenes										
1.	α-Thujene	929	1.11	0.96	1.30	0.83	1.40	1.75	1.84	0.91	1.01
2.	α-Pinene	936	2.11	2.86	0.83	0.71	1.07	2.93	3.82	1.90	2.81
3.	Camphene	950	0.11	0.12	0.36	0.19	0.51	0.21	0.23	0.11	0.12
4.	Sabinene	974	tr	-	tr	-	0	0.05	tr	-	-
5.	β-Pinene	978	0.27	0.36	0.23	0.15	0.28	0.86	0.79	0.32	0.39
6.	3-Octanone	987	tr	0.05	0.07	0.11	0.06	0.23	0.22	0.04	0.07
7.	Myrcene	992	1.11	1.09	1.25	0.78	1.28	1.88	1.95	1.11	1.15
8.	α-Phellandrene	1006	0.11	0.22	0.25	0.14	0.24	0.35	0.37	0.21	0.23
9.	Δ-3-Carene	1011	0.12	0.07	0.11	0.07	0.11	0.11	0.11	0.07	0.07
10.	α-Terpinene	1017	1.71	1.86	2.02	1.19	1.85	2.58	2.69	1.65	1.89
11.	*p*-Cymene	1026	3.99	4.83	6.83	4.53	6.61	10.89	8.91	5.19	5.37
12.	Limonene	1029	0.45	0.55	0.45	0.20	0.43	0.82	0.82	0.51	0.56
13.	(E)-β-Ocimene	1046	0.11	0.07	0.09	-	0.07	0.12	0.11	0.07	0.07
14.	γ-Terpinene	1059	6.89	7.78	8.56	5.54	7.16	8.76	9.47	6.66	7.86
15.	Terpinolene	1087	0.11	0.18	0.23	0.17	0.25	0.21	0.16	0.16	0.17
	Oxygenated monoterpenes										
16.	1,8-Cineol	1031	0.08	0.06	1.01	0.55	1.68	0.27	0.34	0.09	0.08
17.	(E)-Sabinene hydrate	1066	-	-	0.42	0.63	0.92	0.63	0.51	0.09	0.09
18.	Linalool	1099	1.11	1.05	0.33	0.57	0.59	1.01	0.86	0.11	0.10
19.	Camphor	1142	-	-	0.06	-	0.05	-	-	-	-
20.	Borneol	1162	-	-	1.2	0.60	1.53	-	-	-	-
21.	Terpin-4-ol	1174	0.11	0.30	0.84	0.43	1.02	0.96	0.80	0.51	0.32
22.	*α*-Terpineol	1190	0.21	0.17	-	-	-	0.14	0.33	0.12	0.18
23.	Thymol methyl ether	1231	0.38	0.23	0.52	-	0.05	0.64	0.33	1.04	1.13
24	Carvacrol methyl ether	1240	0.11	0.30	-	-	1.05	-	-	-	-
25.	Neral	1245	-	-	0.12	0.10	-	-	-	-	-
26.	Geraniol	1278	-	-	-	0.20	-	-	-	-	-
27.	Thymol	1290	19.29	18.78	7.55	4.44	3.21	9.54	8.72	12.19	18.34
28.	Carvacrol	1304	44.11	50.99	62.37	66.88	66.67	49.29	51.21	61.05	51.29
29.	Thymyl acetate	1349	0.29	0.43	0.06	1.54	tr	0.41	0.26	0.34	0.44
30.	Carvacryl acetate	1368	2.91	1.69	0.05	0.11	0.06	2.38	2.45	2.54	1.76

Continued Table 1.

No.	Compound	RI	B-I[b]	B-II	D-I	D-II	D-III	K-I	K-II	K-III	K-IV
	Sesquiterpenes										
31.	β-Caryophyllene	1412	1.81	2.27	1.76	0.12	0.13	0.11	1.11	0.95	1.16
32.	Aromadendrene	1432	-	0.05	0.05	tr	-	-	0.35	-	-
33.	α-Humulene	1444	-	-	-	-	-	-	0.05	-	-
34.	(E)-α-Bisabolene	1496	-	-	-	0.07	0.47	0.39	-	-	-
35.	Caryophyllene oxide	1574	0.21	-	-	0.14	0.21	-	0.21	-	-
	Total		92.18	99.81	99.97	93.55	99.65	99.74	99.28	99.14	98.51

[a]RI: Retention indices determined on HP-5MS capillary column.
[b]Bardsir-I (Above Sea Level, 2000-2500 m); Bardsir-II (ASL, 2000-2500 m); Dehbakri-I (ASL, 3000-3500 m); Dehbakri-II (ASL, 3000-3500 m);
Dehbakri-III (ASL, 2500-3000 m); Kuhshah-I (ASL, 2500-3000 m); Kushah-II (ASL, 3000-3500 m); Kushah-III (ASL, 2500-3000 m); Kushah-IV (ASL, 3000-3500 m)
[c]Calculated from TIC data
[d]trace (<0.05%).

The results indicated that there no were significant differences between for *T. caramanicus* populations collected from various elevations for contents of thymol, carvacrol, *p*-cymene, ᵞ-terpinene, *α*-thujene, *α*-pinene, myrcene, and *α*-terpinene in oils (Table 2). On the other hand, the results of analysis variance among *T. caramanicus* populations collected from three natural habitats indicated that there were significant differences between for carvacrol ($p \leq 0.01$), thymol ($p \leq 0.01$), and *α*-pinene ($p \leq 0.05$) contents in oils (Table 3). The highest value of carvacrol (65.31 ± 2.54%) was related to the plants collected from the Dehbakri population while the lowest value of carvacrol (47.55 ± 4.86%) was found in *T. caramanicus* population collected from the Kushah population. The highest of thymol amount (12.20 ± 4.35%) in oils obtained from the plants growing in the Dehbakri population. The variability of carvacrol and thymol contents in the essential oils obtained from *T. caramanicus* wild growing attributed mainly to environmental conditions and different chemotypes (LOZIENE & VENSKUTONIS, 2005; RAHIMMALEK *et al*. 2009; GHASEMI PIRBALOUTI *et al*. 2011a; GHASEMI PIRBALOUTI *et al*. 2013a). The physiological situation of plants, time of collection, and different ecological conditions has a great effect on both quality and quantity of essential metabolites in medicinal and aromatic plant (IBAÑEZ & USUBILLAGA, 2006). Results of a study by NEJAD EBRAHIMI *et al*. (2008) indicted that oxygenated monoterpenes were the major portion of the essential oils of *T. caramanicus* collected from Baft, Kerman province (at an altitude of 2300 m). They were reported that the highest content of carvacrol as major component (68.9%) was observed at full flowering stage, and the lowest content of carvacrol was observed at vegetative stage. They also were suggested at vegetative and seed set stages the amount of phenolic (carvacrol + thymol) portion was decreased, but the amount of their precursors (*p*-cymene + γ-terpinene) increased.

Table 2 The main constitutes of essential oil and oil yield of T. caramanicus from three altitudes

Character	ASL, 2000-2500 m	ASL, 2500-3000 m	ASL, 3000-3500 m	*ANOVA*
α-Thujene	1.0±0.11†	1.35±0.42	1.25±0.44	n.s††
α-Pinene	2.49±0.53	1.97±0.93	2.04±1.53	n.s
Myrcene	1.10±0.01	1.41±0.41	1.28±0.49	n.s
α-Terpinene	1.79±0.11	2.03±0.49	1.95±0.61	n.s
p-Cymene	4.41±0.59	7.56±2.97	6.41±1.91	n.s
γ-Terpinene	7.34±0.63	7.53±1.00	7.86±1.68	n.s
Thymol	19.03±0.36	8.31±4.61	9.76±5.99	n.s
Carvacrol	47.55±4.86	59.00±8.87	57.94±7.94	n.s
Essential oil content	0.86±0.04	0.80±0.43	1.10±0.52	n.s

†Values of major compounds are given as means ± SD.
††ns: not significant

Table 3 The main constitutes of essential oil and oil yield of T. caramanicus from three natural habitates

Character	Bardsir	Dehbakri	Kushah	*ANOVA*
α-Thujene	1.38±0.49†	1.18±0.30	1.04±0.11	n.s††
α-Pinene	2.87±0.79 a	0.87±0.18 b	2.49±0.53 a	*
Myrcene	1.52±0.45	1.10±0.28	1.10±0.01	n.s
α-Terpinene	2.21±0.51	1.69±0.44	1.79±0.11	n.s
p-Cymene	7.59±2.79	5.99±1.27	4.41±0.59	n.s
γ-Terpinene	8.19±1.21	7.09±1.51	7.33±0.63	n.s
Thymol	12.20±4.35 a	5.07±2.24 b	19.04±0.36 b	**
Carvacrol	53.21±5.31 b	65.31±2.54 a	47.55±4.86 b	**
Essential oil content	0.86±0.04 b	1.47±0.15 a	0.60±0.06 c	**

†Values of major compounds are given as means ± SD.
††ns: not significant, * at $p \leq 0.05$; **: significant at $p \leq 0.01$.

In current study, the populations collected from three altitudes but the same region showed different compositions, especially carvacrol and thymol, reflecting the influence of local environment or genetic variability among the samples. TALEBI KOUYOKHI *et al.* (2008) reported that phytochemical variations were not only found among samples of different regions but also among samples of the same region with different altitude reflecting the effect of environment on essential oil components. In this study, correlation analysis of the main compounds was done to determine the relationship between chemical components of *T. caramanicus* oil and environmental conditions. The highest negative correlation ($p \leq 0.05$) was between elevation and thymol (-0.685) (data not shown). KIZIL *et al.* (2010) reported that populations of *Thymbra spicata* var. *spicata* L. belonging to low altitudes indicated higher oil content when compared to the plant samples collected from higher altitudes. Results of a study by GHASEMI PIRBALOUTI *et* *al*. (2011b) indicated that carvacrol, as the main constituent in the essential oil of *Satureja khuzestanica* Jamzad., had a positive relationship with altitude.

Essential oil yield

The yellow oil yields ranged between 0.80-1.10% (v/w) for the populations of *T. caramanicus* collected from various elevations, and 0.55-1.61% (v/w) for the populations of *T. caramanicus* collected from various natural habitats (Tables 2 and 3). Results of analysis of variance indicated that there no were significant differences among various populations collected from three elevations (Table 2), while there were significant differences ($p \leq 0.01$) between various populations collected from three natural habitats. The highest oil yield was obtained from the Dehbakri population (1.47 ± 0.15), whereas the Kohshah population produced the lowest oil yield (0.60±0.06). The yield of the oils extracted from other ecotype that have been reported by other researchers were 0.99% (w/w) from Kashan region (SAFAEI-GHOMI *et al*. 2009), 2.5% (v/w) from Tehran (EFTEKHAR *et al*. 2009), 2.0, 1.9, 2.5, 1.8, and 1.5% (v/w) from Yazd, Sirch (Kerman), Rayn (Kerman), Shahrood and Karkas (Isfahan), respectively (MAKIZADEH-TAFTI *et al*. 2010). NEJAD EBRAHIMI *et al.* (2008) reported that yields of *T. caramanicus* oils (w/w) obtained from at different stages were 2.5, 2.1, 2.0, and 1.9% at flowering, floral budding, seed set and vegetative stages, respectively. Higher elevation and colder temperature provides a better growing condition resulting in a higher accumulation of oil in the leaves of *T. caramanicus*. Essential oil productivity is eco-physiologically and environmentally friendly. These and other aspects of the modulation of essential oil production are presented, along with a brief outline of the current concept of the relevant biosynthetic mechanisms (SANGWAN *et al*. 2001). Results of previous studies indicated that altitude is the most important environmental factor influencing on oil content in *Origanum vulgare* ssp. *hirtum* and *Thymbra spicata* var. *spicata* L. (KIZIL, 2010) and, the highest values of essential oils were recorded at low altitudes. Unsuitable environmental conditions may limit photosynthesis in plants and alter nutrient uptake and carbon, sugar, amino acid and inorganic ion fluxes.

CONCLUSION

T. caramanicus is not cultivated under semi-arid climatic conditions of Mediterranean regions of Iran, where it grows naturally at high altitude of 2000 to 3500 m above sea level on sandy, loamy and medium clay soils having pH in range of 7.11 and 8.01, annual rainfall of 250 to 400 mm/year and average temperature of 14 to 18 °C. Variable environmental conditions affect on the essential oil content that could be utilized in a variety of ways including its use as herbal tea due to presence of many important mineral elements, necessary for human health. The essential oil yields of *T. caramanicus* collected from three elevations ranged from 0.55 to 1.61% (v/w). The essential oil of *T. caramanicus* is rich in carvacrol carvacrol (47.6-57.9%). For production of the essential oil and carvacrol from *T. caramanicus* in future, the preference should be given to the Dehbakri population. The farmers can be encouraged and given incentive to grow the plants with high carvacrol rich in essential oil. Since essential oils are the product of a predominantly biological process further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under different soil and climatic conditions.

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UTICAJ SPOLJNE SREDINE NA DIVERZITET KVALITETA I KOLIČINE ULJA RAZLIČITIH POPULACIJA *Thymus carmanicus*

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Izvod

Thymus carmanicus je jedan od najznačajnijih rodova u odnosu na broj vrsta unutar familije Lamiaceae. Kerman (jalas) je endemska iranska vrsta intenzivno korišćena u medicini i kulinarstvu. Utvrđeno je da su glavne komponente esencijalna ulja carvacrol (47.6-57.9%), timol (8.3-19.0%), αterpinen (7.3-7.9%) i *p*-cymen (4.4-7.6%), a grupa monoterpena, posebno oksidovani monoterpeni su glavna sastavna grupa esencijalnih ulja *T. carmanicus*. Rezultati ukazuju na smanjenje timola u esencijalnim uljima divljih populacija *T. carmanicus*.

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