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Effects of Phenological Stage and Elevation on Phytochemical Characteristics of Essential Oil of *Teucrium polium* L. and *Teucrium orientale* L.

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Abstract
This study aimed to evaluate effects of elevation and phenological stage on essential oil components of *Teucrium polium* L. and *Teucrium orientale* L. belonging to the Lamiaceae family. The aerial parts of the grown plants in two ranges of elevation including 2000-2500 and 2500-3000 m above sea level were harvested at three time-points, including end of vegetative, flowering, and seed filling stages. The main components in the *T. polium* oil were α-pinene (40.52 to 54.05%), β-pinene (17.36 to 23.3%), and limonene (10.10 to 15.19%) and the major constituents in the essential oil of *T. orientale* were α-pinene (0.00 to 25.10%), and β-caryophyllene (18.18 to 56.01%). Analysis of results confirmed the significant effects of phenological stage and elevation on the percentages of essential oil components. High elevation above sea level caused increase in aromatic compounds such as α-pinene and limonene from monoterpene hydrocarbons but decrease in sesquiterpene compounds such as β-caryophyllene contents. There was significant correlation between essential oil constituents under different elevations and phenological stages. In addition, harvesting time at different phenological stages affected the chemical compositions in the essential oils, in a way that the highest concentrations of α-pinene and β-pinene as volatile components (monoterpene hydrocarbons) and β-caryophyllene (sesquiterpene hydrocarbons) as important constitutes in pharmaceutical industry were obtained from flowering stage.

Keywords: α-pinene, β-caryophyllene, β-pinene, Growth stage, Medicinal Plant.

Introduction

Essential oils, odorous, and volatile products coming from plant secondary metabolism are used in pharmaceutical, chemical, cosmetic, and food industries. In recent years, there has been an increasing interest in the use of natural substances due to concern about the safety of some synthetic compounds, which have encouraged more detailed studies on substances of natural origin (Baczek et al., 2016; Figuieredo et al., 2008).

Teucerium polium L. (family; Lamiaceae) is a hairy perennial herb commonly known as 'Kalpoureh' in Iran and is the most famous species of *Teucrium* belonging to the subfamily Lamioideae and among the largest genera of the entire Lamiaceae family (Mozaffarian, 2008). Another species is *T. orientale* L. that possesses resin, tannin, scutellarin, caryophyllene oxide, linalool and β-caryophyllene in shoots. Both species are erect, rhizomatous perennial thistle that are usually 0.5-1.0 m tall and change their morphology in response to environmental

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conditions (Harley et al., 2004; Bruno and Arnold, 2004; Javidnia and Miri, 2003). These species grow in mountainous area between 2000 to 3000 m above sea level and sometimes higher. The main habitats of this plant in Iran, are relatively wet spots and areas near rivers, springs around the mountainous provinces of Chaharmahal and Bakhtiari, Lorestan, Isfahan, Tehran, Yazd, Markazi, Fars, Kohgiloyeh and Boyerahmad (Koocheki et al., 2011; Javidnia and Miri; 2003; Mirza, 2002). Variations in environmental cues can influence the phytochemical properties of plants. For instance, the artemisin content of *Artemisia annua* (Omer, 2008), antioxidant content of vegetables (Kalt, 2005), essential oil composition and antimicrobial properties of wild mint (Viljoen et al., 2006), *Cynara scolymus* L. (Yousefi and Yadegari, 2016), Stachys (Alimohammadi et al., 2017), *Cirsium arvense* L. (Amiri et al., 2018) and essential oil content of *Origanum vulgare* (Gonuz and Özrgucu, 1999) increase with decrease in latitude. Altitude affects the morphology, epidermis, and anatomy of *Pinus roxburghii* needles (Tiwari et al., 2013). On the other hand, phenological stages of plants have significant effect on essential oil content and composition such as Bakhtiari Savory (Ghasemi Pirbalouti et al., 2017), Thyme (Ghasemi Pirbalouti et al., 2013), *Artemisia aucheri* (Badrabadi et al., 2015), *Ocimum ciliatum* (Moghaddam et al., 2015), *Fumaria vaillantii* (Moghaddam et al., 2018), *Hibiscus sabdariffa* (Parsa Motlagh et al., 2018) and *Rosmarinus officinalis* (Hassanzadeh et al., 2017).

In the present study, GC/FID and GC/MS analysis has been carried out on the essential oil composition of *T. polium* L. and *T. orientale* L. originating from several growth stages and altitude levels in Chaharmahal and Bakhtiari province. The aim of this research was to determine the effects of elevation and phenological stage on essential oil content and composition in *T. polium* L. and *T. orientale* L.

Materials and Methods

To study the essential oil content and composition of *T. polium* L. and *T. orientale* L., a randomized complete design was done in April-July 2017, 2018. Aerial parts of both species of *Teucrium* were collected from natural populations from Shirmard region in the Chaharmahal and Bakhtiari province, Iran. Treatments were three phenological stages (vegetative, flowering and seed filling) and two elevations (2000-2500 m and 2500-3000 m above the see level). Voucher specimens (20077-TUH) of the specie have been deposited in the Herbarium of the Center of Agricultural and Natural Resources of Chaharmahal and Bakhtiari Province, Shahrekord, Iran. Plant species were previously identified by Mozaffarian (2008). The soil and climatic properties of sampling zones obtained from Meteorological Organization of Iran and Chaharmahal & Bakhtiari Agriculture and Natural Resources Research Center (Tables 1 and 2).

Table 1. Climatic and geographical properties of Shirmard region

Average Annual precipitation(mm)	Average of annual temperature(C°)	Average Maximum temperature(C°)	Average Minimum temperature(C°)	Height (m)	Latitude and Longitude
600	14.7	16	6.1	2820	$31^0 18^{/}$N-$51^0 15^{/}$E

Table 2. Some physical and chemical properties of the soil (0-30 cm)

Elevation (m)	Texture	E.C (ds.m^{-1})	N_{total} %	O.C %	pH	K_{ava} mg kg^{-1} (ppm)	P_{ava}	Zn_{ava}	Mn_{ava}	Fe_{ava}	Cu_{ava}
Year 1											
2000-2500	Loam	0.612	0.175	1.7	7.66	222	12.5	0.97	11.2	8.1	1.3
2500-3000	Loam	0.63	0.251	1.78	7.12	272.5	8.4	1	11.3	8.03	1.2
Year 2											
2000-2500	Loam	0.62	0.181	1.5	7.5	201	11.7	0.99	12.8	8.4	1.1
2500-3000	Loam	0.62	0.244	1.6	7.2	233.2	9.9	1.2	12.4	7.93	1.4

The essential oil content was determined by distilling flowers in Clevenger type apparatus. For each sample in each replicate, a 1000 g of *Teucrium* shoot was placed in 6 L Clevenger type distillation apparatus and subjected to distillation for 3 h with 3 L of pure water. The quantities of *Teucrium* oils obtained at the end of distillation were measured in ml and percentage ratios (w/w) were determined by multiplying oil content with oil density i.e., 0.858. The unit of the oil content is g/100 g dry weight basis.

Ground GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column. The carrier gas was helium at a flow of 0.8 ml/min. Initial column temperature was 60 °C and programmed to increase at 4°C/min to 280 °C. The split ratio was 40:1. The injector temperature was set at 300 °C. The purity of helium gas was 99.99% and 0.1 ml samples were injected manually in the split mode. GC–MS analyses were carried out on a Thermo Finnigan Trace 2000 GC/MS system equipped with a HP-5MS capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 120°C for 5 min and then programmed to reach 280 °C at a rate of 10 °C/min. Detector temperature was 260 °C and injector temperature was 260 °C. The compositions of the essential oil were identified by comparison of their retention indices relative to a series of *n*-alkanes (C7-C24), retention times and mass spectra with those of authentic samples in Wiley library (Adams, 2007).

Statistical analysis

All data were subjected to ANOVA using the statistical computer package SAS (version 8). Treatment means were separated using Least Significant Difference test and Pearson correlation at the significance level ($P<0.05, 0.01$).

Results

Species and phenological stage affected the morphological traits such as leaf width, leaf length and plant height (Table 3 and 4). *T. orientale* L. had more height than the other species. Increase in elevation caused an increase in leaf width, leaf length and plant height (Table 5 and 6). Results of phytochemical essential oil analysis in *T. orientale* L. and *T. polium* L. showed diversity in the phytochemical properties of the essential oil in the two studied species.
The results of phytochemical analysis are shown in Tables 3-8. The analysis of mean squares variation in essential oil compositions in two species showed significant effectiveness of phenological stages and elevations. Elevation and phenological stage caused difference in morphology, essential oil content, and essential oil composition of the populations ($p\leq0.01$) (Table 3 and 4). The main constituents of essential oil from *T. orientale* L. in elevation of 2500-3000 m and at the end of vegetative, flowering and seed filling period stages were β-cubebene (55.64%-55.52%); β-caryophyllene (45.74%-43.2%) and β-caryophyllene (56.01%-55.5%), respectively. The main essential oil constituents in *T. orientale* in elevation of 2000-2500 m and at the end of vegetative, flowering and seed filling period stages were β-cubebene (54.74%-53.8%); β-caryophyllene (50.01%-49.51%) and β-caryophyllene (52%-51.1%), respectively (Table 5 and 6). The main detected essential oil constituents in *T. polium* L. in elevation of 2000-2500 m or 2500-3000 m and at the end of vegetative, flowering and seed filling period stages were α- pinene (monoterpene hydrocarbons). Most of α- pinene (59.82%-58.4%) were obtained from elevation of 2000-2500 m and at the end of vegetative stage (Table 6, 7).

Eleven compounds, accounted for 88.87-99.75% to 87.8-99.81% in the total oil content of *T. orientale* L. and seven compounds (97.8-98.1% to 93.7-98.9%) in the total oil content of *T. polium* L. (Tables 5-8). The most abundant components, which ranged from 18.18-56.01% to 17.98-55.5% in *T. orientale* L. and 40.52-59.82% to 39.3-58.4% in *T. polium* L. of the essential oil content under several vegetative stages and elevations were β-caryophyllene and α-pinene, respectively. Furthermore, based on dry weight, the yield (w/w) of the obtained essential oils ranged from 0.034-0.051% to 0.032-0.053% in the first year and 0.034-0.055 to 0.037-0.055% in the *T. orientale* L. and *T. polium* L., respectively (Tables 5 and 6). Meanwhile, the least abundant components were cis-β-ocimene and β-ocimene (2-1.8 to 13.1-14.2%) in *T. orientale* L., sabinene and β-caryophyllene in *T. polium* L. (1.35-1.85 to 6.32-6.5%). The highest levels of β-caryophyllene (56.01-55.5%) in *T. orientale* L. were obtained from the elevation of 2500-3000 m and seed filling period stage. The highest levels of α- pinene (59.82-58.4%) in *T. polium* L. were obtained from elevation of 2000-2500m and in vegetative stage. The highest essential oil contents in *T. polium* L. (0.053-0.055%) and in *T. orientale* L. (0.051-0.055%) were obtained from plants grown at elevation of 2500-3000 m and in the flowering stage (Tables 7 and 8). At higher elevations, the most abundant essential oil components were β-caryophyllene and β- cubebene in *T. orientale* L. and α-pinene and β-pinene in *T. polium* L. Contents of the main essential oil components (α-pinene, sabinene, β-pinene, myrcene, limonene, α-cubebene and β-caryophyllene) were positively correlated with the essential oil content of *T. orientale* L and on other hand, the main essential oil components (α-pinene, sabinene, β-pinene, myrcene, limonene and β-caryophyllene) in *T. polium* L. were positively correlated (Tables 9 and 10). Results of simple Pearson correlation between traits studied in *T. orientale* showed positive correlation between sabinene and α- pinene; β-pinene with α- pinene and sabinene; myrcene with α-pinene, β-pinene and sabinene; limonene with α-pinene, β-pinene, myrcene and sabinene (Table 9). In *T. polium* L., there were positive correlations between β-pinene with α- pinene; α- pinene with sabinene, myrcene, limonene and β-caryophyllene (Table 10).

Table 3. Analysis of variance of content and composition of main essential oils in *Teucrium* species as affected by different phoenological stages and elevations.

Year 1									
S.O.V†	**D.F**	**Content**	**Trans-beta-ocimene**	**Cis-beta-ocimene**	**Limonene**	**Myrcene**	**Beta pinene**	**Sabinene**	**Aalpha pinene**
Species (A)	1	77.1**	45.5**	46.09**	102.9**	158.15**	2384.5**	5.05**	1666.3**
Phenological stage (B)	2	1.1**	44.9**	27.5**	148.4**	1.12**	160.7**	10.85**	175.2**
Elevation (C)	1	4.4**	44.4**	21.6**	2.27*	3.71**	0.84ns	35.96**	212.4**
A×B	2	6.4**	45.3**	27.5**	102.4**	6.01**	176.1**	1.14**	641.8**
A×C	1	5.5**	44.2**	21.6**	32.1**	2.42**	212.7**	4.51**	65.3**
B×C	2	4.1**	42.4**	39.2**	1.44ns	0.78**	129.2**	17.02**	153.1**
A×B×C	2	9.7**	39.7**	39.2**	55.5**	6.78**	29.5**	4.43**	246.5**
Error	24	0.04	0.052	0.038	0.51	0.051	1.2	0.018	5.28
C.V		3.3	11.8	20.1	17.4	7.2	7.6	7.15	9.4
Year 2									
S.O.V	**D.F**	**Content**	**Trans-beta-ocimene**	**Cis-beta-ocimene**	**Limonene**	**Myrcene**	**Beta pinene**	**Sabinene**	**Aalpha pinene**
Species (A)	1	81.5**	47.6**	72.5**	214.1**	177.9**	1721.7**	12.2**	2124.7**
Phenological stage (B)	2	4.5**	49.1**	33.4**	159.9**	5.5**	321.8**	24.5**	243.7**
Elevation (C)	1	8.5**	112.1**	52.8**	18.9*	8.9**	10.9ns	66.9**	333.7**
A×B	2	7.7**	77.1**	35.8**	114.9**	16.9**	333.5**	8.8**	789.6**
A×C	1	9.7**	82.6**	44.8**	66.9**	14.4**	414.4**	15.7**	124.8**
B×C	2	7.6**	55.9**	55.9**	178.9**	4.9**	246.9**	25.9**	226.9**
A×B×C	2	11.1**	66.7**	44.6**	89.1**	23.6**	55.9**	81.3**	332.9**
Error	24	0.051	0.066	0.045	0.63	0.089	2.9	0.043	4.9
C.V		4.5	12.5	20.1	12.2	9.2	6.5	9.9	8.8

†S.O.V: Source Of Variation, D.F: Degree of Freedom, CV: Coefficient of Variation
ns,* and **: Non significant, significant at the 5% and 1% levels of probability, respectively.

Table 4. Analysis of variance of morphological treats and main essential oils composition in *Teucrium* species as affected by different phoenological stages and elevations..

Year 1								
S.O.V	**D.F**	**Leaf length**	**Leaf width**	**Height of plant**	**Beta caryophyllene**	**Beta cubebene**	**Copaene**	**Alpha cubebene**
Species (A)	1	83.05**	30.4**	2466.3**	12507.7**	4482.9**	180.6**	305.6**
Phenological stage (B)	2	187.3**	67.9**	520.1*	905.2**	2753.6**	4.45**	303.5**
Elevation (C)	1	0.87^{ns}	1.22^{ns}	21.7^{ns}	16.1*	113.3**	7.33**	0.19^{ns}
A×B	2	0.05^{ns}	0.02^{ns}	60.7^{ns}	813.6**	2753.9**	4.45**	303.4**
A×C	1	0.02^{ns}	0.003^{ns}	40.1^{ns}	31.5**	113.3**	7.33**	0.19^{ns}
B×C	2	2.33^{ns}	6.87^{ns}	33.4^{ns}	51.4**	98.1**	91.1**	0.18^{ns}
A×B×C	2	0.06^{ns}	0.01^{ns}	28.4^{ns}	33.1**	98.1**	91.1**	0.19^{ns}
Error	24	5.74	4.28	83.8	3.61	1.99	0.07	0.17
C.V		13.1	16.6	21.9	7.8	12.6	12.1	14.4
Year 2								
S.O.V	**D.F**	**Leaf length**	**Leaf width**	**Height of plant**	**Beta caryophyllene**	**Beta cubebene**	**Copaene**	**Alpha cubebene**
Species (A)	1	99.4**	126.8**	3245.8**	9823.1**	2457.1**	222.7**	532.1**
Phenological stage (B)	2	243.6**	251.1**	550.6*	887.5**	2431.6**	78.9**	432.9**
Elevation (C)	1	111.4**	0.99^{ns}	33.7^{ns}	25.5*	114.9**	11.5**	0.89^{ns}
A×B	2	0.14^{ns}	0.14^{ns}	9.2^{ns}	923.4**	2131.1**	12.2**	438.1**
A×C	1	0.17^{ns}	0.17^{ns}	94.8^{ns}	77.7**	212.4**	9.9**	0.22^{ns}
B×C	2	4.6^{ns}	8.8^{ns}	44.8^{ns}	56.9**	111.6**	121.6**	0.51^{ns}
A×B×C	2	1.4^{ns}	0.9^{ns}	33.5^{ns}	4.7^{ns}	145.8**	88.8**	0.11^{ns}
Error	24	2.9	5.58	77.9	3.9	2.2	0.09	0.72
C.V		14.4	14.1	16.3	10.1	11.9	14.4	8.9

†S.O.V: Source Of Variation, D.F: Degree of Freedom, CV: Coefficient of Variation

ns,* and **: Non significant, significant at the 5% and 1% levels of probability, respectively

Table 5. Means comparisons of essential oil constituents of *T. orientale* L. and *T. polium* L. as affected by phenological stages and elevation.

Year 1								
		Cis beta ocimene	**Trans cis ocimene**	**Limonene**	**Myrcene**	**Beta pinene**	**Sabinene**	**Alpha pinene**
Species	*T. polium*	0.000b	0.000b	15.19a	5.06a	23.45a	1.81a	51.95a
	T. orientale	2.2a	2.2a	4.5b	0.87b	7.17b	1.06b	8.92b
	LSD	0.15	0.13	0.49	0.15	0.75	0.09	1.5
Phenological stages	Vegetative	0.00b	0.00c	5.7b	2.6c	11.08b	0.34c	29.1b
	Flowering	3.37a	2.84a	12.08a	2.9b	17.36a	1.91b	27.43b
	Seed filling period	0.00b	0.51b	11.6a	3.2a	17.4a	2.06a	34.7a
	LSD	0.19	0.16	0.6	0.1	0.92	0.11	1.93
Elevations	2500-3000m	2.2a	1.8a	9.5b	2.6b	15.4a	0.44b	28.01b
	2000-2500m	0.00b	0.34b	10.1a	3.2a	15.1a	2.44a	32.8a
	LSD	0.15	0.13	0.49	0.15	0.75	0.09	1.58
Year 2								
		Cis beta ocimene	**Trans cis ocimene**	**Limonene**	**Myrcene**	**Beta pinene**	**Sabinene**	**Alpha pinene**
Species	*T. polium*	0.000b	0.000b	14.4a	5.4a	24.1a	2.2a	52.3a
	T. orientale	2.2a	2.4a	4.2b	0.91b	8.3b	1.2b	9.1b
	LSD	0.44	0.14	0.87	0.18	0.66	0.08	1.7
Phenological stages	Vegetative	0.00b	0.00c	6.1b	3.1c	12.3b	1.5b	33.1b
	Flowering	3.5a	2.7a	11.1a	3.5b	18.1a	1.96b	28.2b
	Seed filling period	0.00b	0.6b	11.8a	3.8a	17.7a	2.2a	35.4a
	LSD	0.4	0.22	0.8	0.1	0.9	0.14	1.1
Elevations	2500-3000m	2.5a	1.9a	9.2b	2.6b	14.9a	0.48b	29.2b
	2000-2500m	0.00b	0.44b	10.5a	3.1a	14.8a	2.6a	35.2a
	LSD	0.24	0.14	0.51	0.1	0.8	0.1	1.4

Means in each column followed by the same letters are not significantly different (P<0.05).

Table 6. Means comparisons of essential oil constituents and morphological properties of *T. orientale* L. and *T. polium* L. as affected by phenological stages and elevation.

		Leaf length	Leaf width	Height of plant	Beta caryophyllene	Beta cubebene	Copaene	Alpha cubebene
Year 1								
Species	*T. polium*	2.73 a	11.7 a	33.5 b	5.58 b	0.00 b	0.00 b	0.00 b
	T. orientale	3.03 a	13.08 a	50.05 a	42.8 a	22.3 a	4.4 a	5.8 a
	LSD	1.6	1.4	6.2	1.3	0.97	0.18	0.29
Phenological stages	Vegetative	3.3 a	11.4 b	42.8 a	14.3 c	28.4 a	2.49 a	8.7 a
	Flowering	2.7 a	12.7 ab	42.16 a	27.7 b	5.07 b	2.68 a	0.00 b
	Seed filling period	2.5 a	14.7 a	40.3 a	30.6 a	0.00 c	1.54 b	0.00 b
	LSD	2.01	1.4	7.71	1.6	1.19	0.22	0.35
Elevations	2500-3000m	3.4 a	12.6 a	42.5 a	24.8 a	12.9 a	1.7 b	2.8 a
	2000-2500m	2.7 a	12.24 a	41 a	23.5 b	9.3 b	2.6 a	2.9 a
	LSD	1.64	1.42	6.2	0.13	0.9	0.18	0.29
Year 2								
		Leaf length	**Leaf width**	**Height of plant**	**Beta caryophyllene**	**Beta cubebene**	**Copaene**	**Alpha cubebene**
Species	*T. polium*	2.8 a	12.2 a	32.2 b	6.3 b	0.00 b	0.00 b	0.00 b
	T. orientale	3.2 a	12.5 a	51.1 a	40.4 a	24.1 a	4.8 a	6.1 a
	LSD	1.1	1.1	4.4	1.4	1.1	0.22	0.3
Phenological stages	Vegetative	3.5 a	12.1 b	41.1 a	15.2 c	26.6 a	2.55 a	8.9 a
	Flowering	2.9 a	12.5 b	43.3 a	28.2 b	6.1 b	2.77 a	0.00 b
	Seed filling period	2.8 a	14.9 a	41.4 a	31.5 a	0.00 c	1.2 b	0.00 b
	LSD	1.9	1.1	4.4	1.4	1.1	0.31	0.44
Elevations	2500-3000m	3.2 a	12.2 a	42.1 a	25.1 a	14.2 a	1.8 b	2.9 a
	2000-2500m	2.8 a	12.4 a	40.9 a	23.1 b	9.5 b	2.8 a	3.1 a
	LSD	1.9	1.1	3.3	1.2	1.1	0.22	0.4

Means in each column followed by the same letters are not significantly different (P<0.05).

Table 7. Essential oil constituents in *T. orientale* L. as affected by phenological stages and elevation.

Compound	RI[z]	2000-2500m×seed filling period stage	2500-3000m×seed filling period stage	2000-2500m×flowering stage	2500-3000m×flowering stage	2000-2500m× vegetative stage	2500-3000m×vegetative stage
Year 1							
Alpha pinene	938[y]	12.01±0.82 c	15.01±0.99 b	25±1.2 a	–	–	–
Sabinene	971	2.63±0.2 a	1.23±0.12 b	2.36±0.2 a	–	–	–
Beta pinene	978	12.3±0.99 a	14.26±1.4 a	13.23±1.2 a	–	–	–
Myrcene	988	1.47±0.05 b	2.36±0.08 a	1.25±0.02 b	–	–	–
Limonene	1028	8.7±0.22 a	–	7.9±0.12 b	–	–	–
Cis-beta-ocimene	1045	2±0.1 b	–	–	9.04±0.22 a	–	–
Beta ocimene	1053	2±0.01 b	–	–	13.1±0.8 a	–	–
Copaene	1356	6±1.1 b	–	–	10.42±1.2 a	9.68±0.9 a	–
Beta cubebene	1392	–	–	–	19.7±1.1 b	54.74±2.1 a	55.64±3.4 a
Alpha cubebene	1398	–	–	–	–	15.3±1.4 a	15.55±1.1 a
Beta caryophyllene	1420	52±1.9 b	56.01±2.1 a	50.01±3.1 bc	45.74±2.6 c	18.18±1.1 e	27.8±1.2 d
Total		99.11	88.87	99.75	98	97.9	98.99
E.O		0.034±0.002 c	0.048±0.002 a	0.041±0.002 b	0.051±0.002 a	0.039±0.002 b	0.049±0.002 a
Year 2							
Compound	**RI[z]**	**2000-2500m×seed filling period stage**	**2500-3000m×seed filling period stage**	**2000-2500m×flowering stage**	**2500-3000m×flowering stage**	**2000-2500m× vegetative stage**	**2500-3000m×vegetative stage**
Alpha pinene	938[y]	14.1±0.5 b	14.4±1.1 b	24.2±1.1 a	–	–	–
Sabinene	971	2.7±0.4 a	1.2±0.14 b	2.5±0.4 a	–	–	–
Beta pinene	978	9.1±1.1 b	14.3±1.1 a	14.1±0.9 a	–	–	–
Myrcene	988	1.6±0.08 b	2.4±0.09 a	1.4±0.1 c	–	–	–
Limonene	1028	8.9±0.3 a	–	8.1±0.1 b	–	–	–
Cis-beta-ocimene	1045	1.8±0.3 b	–	–	10.1±0.1 a	–	–
Beta ocimene	1053	2.3±0.04 b	–	–	14.2±0.5 a	–	–
Copaene	1356	6.1±0.8 b	–	–	9.92±1.1 a	9.9±0.8 a	–
Beta cubebene	1392	–	–	–	18.2±0.8 b	53.8±1.9 a	55.52±2.8 a
Alpha cubebene	1398	–	–	–	–	14.4±1.1 a	16.1±0.9 a
Beta caryophyllene	1420	51.1±1.5 b	55.5±1.1 a	49.51±2.1 b	43.2±1.8 c	17.98±1.4 e	26.6±1.1 d
Total		97.7	87.8	99.81	95.62	96.08	98.22
E.O		0.037±0.005 bc	0.049±0.004 a	0.04±0.001 b	0.055±0.005 a	0.034±0.004 c	0.051±0.001 a

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes.

[y] Values are means of triplicates ± standard deviation (p <0.05). E.O: Essential oil content (w/w%, g/100g fresh)

Table 8. Essential oil constituents in *T. polium* L. as affected by phenological stages and elevation.

Year 1							
Compound	**RI**	**2000-2500m×seed filling period stage**	**2500-3000m×seed filling period stage**	**2000-2500m×flowering stage**	**2500-3000m×flowering stage**	**2000-2500m× vegetative stage**	**2500-3000m×vegetative stage**
Alpha pinene	938	52.0±2.1 b	51.61±2.7 b	40.52±1.8 c	40.99±2.2 c	59.82±0.002a	53.29±2.4 b
Sabinene	971	4.17±0.3 a	--	5.06±0.9 a	--	--	1.35±1.1 b
Beta pinene	978	17.24±0.32 c	24.09±0.55 b	25.51±0.72 a	26.65±0.66 a	17.99±0.15 c	25.04±0.2 a
Myrcene	988	6.07±0.18 a	2.81±0.11 d	4.95±0.12 c	5.32±0.15 b	5.41±0.11 b	4.92±0.12 c
Limonene	1028	13.89±1.5 bc	12.97±0.9 c	14.62±0.7 b	20.09±1.1 a	9.3±0.7 d	9.19±0.8 d
Beta cubebene	1392	--	--	7.15±0.14	--	--	--
Beta caryophyllene	1420	4.52±0.1 c	6.32±0.11 a	--	4.86±0.6 bc	5.38±0.14 b	4.31±0.2 c
Total		97.89	97.8	97.81	97.91	97.9	98.1
E.O		0.032±0.002c	0.05±0.002 a	0.043±0.002 b	0.053±0.002 a	0.04±0.002 b	0.051±0.002 a
Year 2							
Compound	**RI**	**2000-2500m×seed filling period stage**	**2500-3000m×seed filling period stage**	**2000-2500m×flowering stage**	**2500-3000m×flowering stage**	**2000-2500m× vegetative stage**	**2500-3000m×vegetative stage**
Alpha pinene	938	51.1±1.1 b	52.2±1.8 b	39.3±0.82 d	42.2±1.1 c	58.4±0.01 a	52.2±1.1 b
Sabinene	971	4.5±0.5 a	--	4.4±1.4 a	--	--	1.85±0.91 b
Beta pinene	978	16.6±0.6 d	23.5±0.7 b	24.4±1.1 b	25.5±0.8 ab	18.2±0.1 c	26.6±1.1 a
Myrcene	988	7.2±0.3 a	2.5±0.5 d	5.1±0.4 b	5.5±0.8 b	5.2±0.3 b	4.92±0.12 c
Limonene	1028	14.2±2.2 b	14.2±0.5 b	12.4±0.5 b	22.2±0.9 a	8.67±0.9 c	8.6±0.91 c
Beta cubebene	1392	--	--	8.1±0.5	--	--	--
Beta caryophyllene	1420	4.8±0.4 b	6.5±0.5 a	--	3.5±0.2 c	6.6±0.2 a	4.6±0.5 b
Total		98.4	98.9	93.7	98.9	97.07	98.77
E.O		0.037±0.003c	0.055±0.02 a	0.048±0.003 b	0.051±0.02 ab	0.044±0.003b	0.054±0.004 ab

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes.
[y] Values are means of triplicates ± standard deviation (p <0.05). E.O: Essential oil content (w/w%, g/100g fresh)

Table 9. Results of correlation between characters in *T. orientale* L. as affected by phenological stages and elevation.

Year 1														
Characters	**Alpha pinene (1)**	**Sabinene (2)**	**Beta pinene (3)**	**Myrcene (4)**	**Limonene (5)**	**Cis beta ocimene (6)**	**Beta ocimene (7)**	**Alpha cubebene (8)**	**Alpha-Copaene (9)**	**Beta cubebene (10)**	**Beta caryophyllene (11)**	**Height of plant (12)**	**Leaf width (13)**	**Leaf length (14)**
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.86**	1	-	-	-	-	-	-	-	-	-	-	-	-
3	0.94**	0.9**	1	-	-	-	-	-	-	-	-	-	-	-
4	0.77**	0.72**	0.92**	1	-	-	-	-	-	-	-	-	-	-
5	0.87**	0.88**	0.98**	0.96**	1	-	-	-	-	-	-	-	-	-
6	-0.38^{ns}	-0.3^{ns}	-0.3^{ns}	-0.36^{ns}	-0.37^{ns}	1	-	-	-	-	-	-	-	-
7	-0.4^{ns}	-0.41^{ns}	-0.4^{ns}	-0.41^{ns}	-0.44^{ns}	0.98**	1	-	-	-	-	-	-	-
8	0.64**	-0.65**	-0.69**	-0.65**	-0.69**	-0.37^{ns}	-0.31^{ns}	1	-	-	-	-	-	-
9	-0.58**	-0.31^{ns}	-0.55**	-0.52**	-0.51*	0.63**	0.59**	0.15^{ns}	1	-	-	-	-	-
10	-0.79**	-0.8**	-0.86**	-0.81**	-0.86**	-0.1^{ns}	-0.1^{ns}	0.95**	0.25^{ns}	1	-	-	-	-
11	0.69**	0.68**	0.79**	0.78**	0.85**	0.2^{ns}	0.13^{ns}	-0.94**	-0.3^{ns}	-0.94**	1	-	-	-
12	-0.28^{ns}	-0.26^{ns}	-0.1^{ns}	-0.65**	-0.18^{ns}	0.1^{ns}	0.11^{ns}	0.15^{ns}	-0.1^{ns}	0.15^{ns}	-0.1^{ns}	1	-	-
13	0.22^{ns}	0.15^{ns}	0.28^{ns}	0.35^{ns}	0.3^{ns}	0.11^{ns}	0.11^{ns}	-0.37^{ns}	0.01^{ns}	-0.37^{ns}	0.35^{ns}	-0.3^{ns}	1	-
14	-0.25^{ns}	-0.23^{ns}	-0.2^{ns}	-0.22^{ns}	-0.24^{ns}	0.13^{ns}	0.14^{ns}	0.17^{ns}	0.19^{ns}	0.22^{ns}	-0.16^{ns}	0.69**	-0.5**	1
Year 2														
Characters	**Alpha pinene (1)**	**Sabinene (2)**	**Beta pinene (3)**	**Myrcene (4)**	**Limonene (5)**	**Cis beta ocimene (6)**	**Beta ocimene (7)**	**Alpha cubebene (8)**	**Alpha-Copaene (9)**	**Beta cubebene (10)**	**Beta caryophyllene (11)**	**Height of plant (12)**	**Leaf width (13)**	**Leaf length (14)**
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.81**	1	-	-	-	-	-	-	-	-	-	-	-	-
3	0.9**	0.87**	1	-	-	-	-	-	-	-	-	-	-	-
4	0.81**	0.8**	0.88**	1	-	-	-	-	-	-	-	-	-	-
5	0.76**	0.8**	0.77**	0.91**	1	-	-	-	-	-	-	-	-	-
6	-0.2^{ns}	-0.2^{ns}	-0.2^{ns}	-0.3^{ns}	-0.3^{ns}	1	-	-	-	-	-	-	-	-
7	-0.33^{ns}	-0.4^{ns}	-0.3^{ns}	-0.2^{ns}	-0.4^{ns}	0.9**	1	-	-	-	-	-	-	-
8	0.8**	-0.58**	-0.81**	-0.71**	-0.6**	-0.3^{ns}	-0.3^{ns}	1	-	-	-	-	-	-
9	-0.78**	-0.2^{ns}	-0.5**	-0.5**	-0.3*	0.77**	0.81**	0.1^{ns}	1	-	-	-	-	-
10	-0.82**	-0.91**	-0.77**	-0.87**	-0.77**	-0.1^{ns}	-0.1^{ns}	0.76**	0.2^{ns}	1	-	-	-	-
11	0.8**	0.8**	0.88**	0.91**	0.72**	0.12^{ns}	0.1^{ns}	-0.72**	-0.31^{ns}	-0.81**	1	-	-	-
12	-0.1^{ns}	-0.2^{ns}	-0.2^{ns}	-0.86**	-0.1^{ns}	0.3^{ns}	0.22^{ns}	0.2^{ns}	-0.1^{ns}	0.1^{ns}	-0.2^{ns}	1	-	-
13	0.3^{ns}	0.3^{ns}	0.1^{ns}	0.3^{ns}	0.11^{ns}	0.1^{ns}	0.3^{ns}	-0.3^{ns}	0.2^{ns}	-0.3^{ns}	0.4^{ns}	-0.1^{ns}	1	-
14	-01^{ns}	-0.1^{ns}	-0.1^{ns}	-0.31^{ns}	-0.14^{ns}	0.1^{ns}	0.2^{ns}	0.1^{ns}	0.1^{ns}	0.2^{ns}	-0.1^{ns}	0.88**	-0.3**	1

ns,* and ** : Non significant, significant at the 5% and 1% levels of probability, respectively.

Table 10. Results of correlation between traits in *T. polium* L. as affected by phenological stages and elevation

Characters	Alpha pinene (1)	Sabinene (2)	Beta pinene (3)	Myrcene (4)	Limonene (5)	Cis beta ocimene (6)	Beta ocimene (7)	Alpha cubebene (8)	Alpha-Copaene (9)	Beta cubebene (10)	Beta caryophyllene (11)	Height of plant (12)	Leaf width (13)	Leaf length (14)
Year 1														
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.79^{**}	1	-	-	-	-	-	-	-	-	-	-	-	-
3	0.91^{**}	0.73^{**}	1	-	-	-	-	-	-	-	-	-	-	-
4	0.79^{**}	0.87^{**}	0.88^{**}	1	-	-	-	-	-	-	-	-	-	-
5	0.81^{**}	0.76^{**}	0.77^{**}	0.88^{**}	1	-	-	-	-	-	-	-	-	-
6	-0.38^{ns}	-0.1^{ns}	-0.1^{ns}	-0.1^{ns}	-0.2^{ns}	1	-	-	-	-	-	-	-	-
7	-0.12^{ns}	-0.2^{ns}	-0.1^{ns}	-0.2^{ns}	-0.3^{ns}	0.79^{**}	1	-	-	-	-	-	-	-
8	-0.71^{**}	-0.4^{*}	-0.71^{**}	-0.77^{**}	-0.79^{**}	-0.2^{ns}	-0.3^{ns}	1	-	-	-	-	-	-
9	-0.66^{**}	-0.4^{*}	-0.66^{**}	-0.7^{**}	-0.65^{**}	0.7^{**}	0.66^{**}	0.12^{ns}	1	-	-	-	-	-
10	-0.81^{**}	-0.68^{**}	-0.9^{**}	-0.9^{**}	-0.9^{**}	-0.2^{ns}	-0.1^{ns}	0.87^{**}	0.2^{ns}	1	-	-	-	-
11	0.72^{**}	0.82^{**}	0.8^{**}	0.8^{**}	0.9^{**}	0.3^{ns}	0.2^{ns}	-0.81^{**}	-0.1^{ns}	-0.81^{**}	1	-	-	-
12	-0.72^{**}	-0.1^{ns}	-0.1^{ns}	-0.1^{ns}	-0.1^{ns}	0.1^{ns}	0.3^{ns}	0.2^{ns}	-0.2^{ns}	0.2^{ns}	-0.2^{ns}	1	-	-
13	0.13^{ns}	0.1^{ns}	0.1^{ns}	0.2^{ns}	0.2^{ns}	0.2^{ns}	0.3^{ns}	-0.2^{ns}	0.3^{ns}	-0.3^{ns}	0.1^{ns}	-0.1^{ns}	1	-
14	-0.13^{ns}	-0.1^{ns}	-0.2^{ns}	-0.3^{ns}	-0.2^{ns}	0.2^{ns}	0.3^{ns}	0.1^{ns}	0.3^{ns}	0.3^{ns}	-0.1^{ns}	0.73^{**}	-0.7^{**}	1
Year 2														
Characters	**Alpha pinene (1)**	**Sabinene (2)**	**Beta pinene (3)**	**Myrcene (4)**	**Limonene (5)**	**Cis beta ocimene (6)**	**Beta ocimene (7)**	**Alpha cubebene (8)**	**Alpha-Copaene (9)**	**Beta cubebene (10)**	**Beta caryophyllene (11)**	**Height of plant (12)**	**Leaf width (13)**	**Leaf length (14)**
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.92^{**}	1	-	-	-	-	-	-	-	-	-	-	-	-
3	0.88^{**}	0.7^{**}	1	-	-	-	-	-	-	-	-	-	-	-
4	0.82^{**}	0.8^{**}	0.8^{**}	1	-	-	-	-	-	-	-	-	-	-
5	0.9^{**}	0.7^{**}	0.7^{**}	0.91^{**}	1	-	-	-	-	-	-	-	-	-
6	-0.3^{ns}	-0.2^{ns}	-0.2^{ns}	-0.2^{ns}	-0.1^{ns}	1	-	-	-	-	-	-	-	-
7	-0.1^{ns}	-0.1^{ns}	-0.2^{ns}	-0.1^{ns}	-0.2^{ns}	0.82^{**}	1	-	-	-	-	-	-	-
8	-0.8^{**}	-0.5^{*}	-0.8^{**}	-0.7^{**}	-0.9^{**}	-0.1^{ns}	-0.2^{ns}	1	-	-	-	-	-	-
9	-0.72^{**}	-0.5^{*}	-0.6^{**}	-0.88^{**}	-0.6^{**}	0.88^{**}	0.8^{**}	0.1^{ns}	1	-	-	-	-	-
10	-0.8^{**}	-0.72^{**}	-0.8^{**}	-0.85^{**}	-0.82^{**}	-0.1^{ns}	-0.2^{ns}	0.62^{**}	0.1^{ns}	1	-	-	-	-
11	0.66^{**}	0.8^{**}	0.7^{**}	0.68^{**}	0.81^{**}	0.2^{ns}	0.3^{ns}	-0.77^{**}	-0.2^{ns}	-0.92^{**}	1	-	-	-
12	-0.7^{**}	-0.2^{ns}	-0.2^{ns}	-0.2^{ns}	-0.2^{ns}	0.3^{ns}	0.4^{ns}	0.1^{ns}	-0.3^{ns}	0.1^{ns}	-0.1^{ns}	1	-	-
13	0.1^{ns}	0.2^{ns}	0.2^{ns}	0.3^{ns}	0.1^{ns}	0.1^{ns}	0.4^{ns}	-0.1^{ns}	0.2^{ns}	-0.1^{ns}	0.2^{ns}	-0.2^{ns}	1	-
14	-0.14^{ns}	-0.2^{ns}	-0.1^{ns}	-0.2^{ns}	-0.1^{ns}	0.1^{ns}	0.2^{ns}	0.2^{ns}	0.1^{ns}	0.1^{ns}	-0.2^{ns}	0.91^{**}	-0.81^{**}	1

ns,* and ** : Non significant, significant at the 5% and 1% levels of probability, respectively.

Discussion

There were positive correlations between morphological traits with most of the essential constituents in two species. Determination of morphological characteristics such as leaf width, leaf length and height of plant is important for the yield of medicinal and aromatic plants. Species type is one of the important parameters that cause phyto/morpho diversity. The increase in the height of plant in *T. orientale* than *T. polium* can be attributed to genetic factors. Generally, the most important constituents of *T. polium* were monoterpene hydrocarbonate such as α-pinene, β-pinene, myrcene, limonene, sabinene, cis/trans β-ocimene, but the highest constituents in *T. orientale* were sesquiterpenes such as β-caryophyllene, α-cubebene and β-cubebene. Similar research on *T. polium* subsp. *capitatum* in France showed that the most essential oil constituents were α-pinene, β-pinene and para-cymene (Cozzani et al., 2005). The research studies in Iran and other countries showed that the main constituents of *T. orientale* were caryophyllene-oxide, β-caryophyllene and linalool (Bruno and Arnold, 2004; Javidnia and Miri, 2003), α-pinene, camphor, 1,8 cineole, γ-cadinene, delta-cadinene and limonene (Kucuk and Gulec, 2006). The main constituents in sub-species of *glabrescens* were β-cubebene, α-cubebene, α-cupaene and trans-caryophyllene and in sub-species of *taylori*, *orientale* and *puberulens* were β-caryophyllene, hexa-decanoeic acid, germacrene-D, bycyclo-germacrene, caryophyllene-oxide and linalool (Aberumand and Asgarpanah, 2017). The differences in results can be attributed to diversity of genetic and physiological parameters such as ecotype, chemo-type, sub-species and phenological stage of the harvested plant (Ljubuncic, 2006). However, environmental, edaphic, geographic attributes and the interactions between them with heredity factors (Talebi et al., 2018; Amiri et al., 2018; Moghaddam et al., 2015, 2018), and also management factors such as drying, method of extraction and essential oil detection can affect phyto-chemical diversity in medicinal and aromatic plants (Beigi et al., 2018, Moghaddam et al., 2018). In this research, with increase in elevation, the precipitation, nutrients in soil, and essential oil composition were increased in the two studied species. With increase in elevation in *T. orientale*, the constituents of myrcene, limonene, cis-ocimene, β-ocimene and β-caryophyllene were increased but the compositions of α-pinene and sabinene were decreased. Also with increase in elevation in *T. polium*, the constituents of β-pinene and limonene were increased and α-pinene, sabinene and myrcene were decreased. There are various reports about the effectiveness of climatic and edaphic attributes on species of *Teucrium*, for example, in species of *chamaedrys*, the most essential oil constituents were α-pinene, β-pinene and limonene (Kazemizadeh et al., 2008), germacrene-D, trans-β-farnesene, β-caryophyllene, delta-cadinene, and α-pinene (Morteza Semnani and Rostami, 2005). The main essential oil constituents in species of *hyrcanicum* were hexa-hyro-farnesyl acetone, linalool, E-β-farnesene, D-hydro adolan and curcumin. These aforementioned studies showed the important effects of soil and climate on diversity of essential oil composition (Kazemizadeh et al., 2008). In the present study, there were significant correlations between β-pinene and α-pinene. Quality and quantity of essential oil were affected by climatic factors, soil texture, plant tissue, age and phenological stages. The quantity and quality of differences between essential oil can be attributed to ecological properties such as moisture, elevation above sea level and other edaphic or geographic indices (Alimohammadi et al., 2017). In the present study, the highest essential oil constituents were obtained at elevation of 2500-3000 m and at the vegetative or flowering stages in both species. It seems that the environmental stresses in both species increased the amount of constituents. Furthermore, with increase in elevation, the morphological traits were negatively influenced. Environmental factors through influencing metabolism and production can affect type and intensity of chemical compounds (Baczek et al., 2016). Various environmental indices such as amount of soil nutrients, climatic factors in the place of cultivation (e.g. elevation above sea level, temperature and precipitation). Moreover, properties related to place of cultivation and extraction method of essential oil can also affect essential oil content and composition (Parsa Motlagh et al., 2018). In general, indices such as ecology, edaphic, genetic and management factors, essential oil extraction method, and plant characteristics are the important factors influencing quantity and quality of essential oil. In storage tissues of both species, with increase in elevation, the amount of constituents of the essential oil was increased, which is similar to that of *Artemisia annua* L. (Omer, 2008), *Stachys* (Baczek et al., 2016; Ghasemi Pirbalouti and Mohammadi, 2013; Kremer et al., 2016; Alimohammadi et al., 2017), *Mentha longifolia* L.(Viljoen et al., 2006), *Origanum vulgare* ssp (Vokou et al., 1993), *Cirsium arvense* L. (Amiri et al., 2018), *Fumaria vaillantii* (Moghaddam et al., 2018), *Rosmarinus officinalis* (Hassanzadeh et al., 2017), *Hibiscus* *sabdariffa* (Parsa Motlagh et al., 2018) and *Pistacia atlantica* (Gourine et al., 2010).

Conclusions

Phytochemical diversity was detected between two species in this study. Essential oil constituents were influenced by phenological stages, as the highest amount of α-pinene and β-pinene from aromatic compounds and β-caryophyllene from sesquiterpene made in two species especially in seed filling stage. Increase in elevation caused increase in aromatic compounds such as α-pinene and limonene from monoterpene hydrocarbons but decrease in sesquiterpene compounds such as β-caryophyllene. There was significant correlation between essential oil constituents in different elevations and phenological stages. In conclusion, the flowering stage can be selected as the best harvesting time and elevation of 2500-3000 m as the best elevation to obtain the best essential oil content and composition in the two studied species.

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