

Essential Oil Composition of *Cirsium arvense* L. Produced in Different Climate and Soil Properties

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(Received June 28, 2017; Revised September 25, 2017; Accepted September 29, 2017)

Abstract: This study investigated the quantitative and qualitative effects of climate and soil properties on essential oil content and composition of *Cirsium arvense* L. (Asteraceae), which is an important medicinal plant. Root, stem, and leaf tissues were collected from plants found in four regions (Shahrekord, Farsan, Chelgerd and Ardal) of the Chaharmahal and Bakhtiari Province, Iran. GC/MS analysis revealed that the main constituents of essential oils from the various populations were nonadecane, β -citronellol, camphor, heneicosane and phytol. The highest levels of nonadecane (40.1-42%) and camphor (18.1-18.4%) were obtained from roots collected from the Chelgerd region, the most levels of β -citronellol (24.9-25.01%) were obtained from leaves from the Chelgerd region, and the most levels of heneicosane (14.4-15.6%) and phytol (11.8-12.58%) were obtained from stems from the Shahrekord region. The most amount of essential oil (0.34-0.33%) was obtained from roots growing in clay soil in the Chelgerd region. Both climate and soil properties had significant effects on the essential oil of *C. arvense*. The highest essential oil contents were obtained from plants growing in clay soil, which seemed to have a greater capacity to hold water and nutrients, both of which promote plant growth and essential oil production.

Keywords: β -citronellol; camphor; *Cirsium arvense*; nonadecane. © 2018 ACG Publications. All rights reserved.

1. Introduction

Cirsium arvense L. (Asteraceae) is a perennial plant that grows in Iran and other countries. The genus *Cirsium* has been used as a flavouring agent, especially for dairy foods (yoghurt and cheese) and meat, by the indigenous people of the Chaharmahal and Bakhtiari province of southwestern Iran [1]. The plant is also used as food and for its medicinal properties. Extracts of *Cirsium arvense* produce a variety of pharmacological effects, including the inhibition of cholesterol biosynthesis [2-5]. *Cirsium arvense* is an erect, rhizomatous perennial thistle that is usually 0.5-1.0 m tall and changes its morphology in response to environmental conditions [6]. The species is invasive in prairies and other grasslands in the Midwest and Great Plains, as well as in riparian areas in the intermountain west, and is particularly troublesome in the northwest and north-central states of the USA [2, 3]. The use of plants for treating ailments dates back several centuries. However, herbal medicine usually relies on tradition and may or may not be supported by empirical data. The belief that natural medicines are safer than synthetic drugs has recently increased and has induced tremendous growth in phyto-pharmaceutical usage [4]. *C. arvense* needs lower temperatures and a

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specific photoperiod to form generative stems and bloom, and though the plant is drought tolerant after establishment, moisture and soil nutrition are necessary for better yields [7]. Herb and spice products are widely used in folk medicine, food flavoring, and in the food industry [8]. In recent years, concern about the safety of synthetic compounds has increased interest in the use of natural substances and has encouraged more detailed studies on plant-derived substances. The quality and quantity of essential oils from medicinal plants are mainly influenced by genetic and environmental factors [9]. Environmental variations can influence the phytochemical properties of plants. For example, the artemisinin content of *Artemisia annua* [10], antioxidant content of vegetables [11], essential oil composition and antimicrobial properties of wild mint [12], and essential oil content of *Origanum vulgare* [13] increase with decreasing latitude. Altitude affects the morphology, epidermis, and anatomy of *Pinus roxburghii* needles [14], and significantly affects variation between durum wheat landraces [15]. Plants of the genus *Cirsium* are characterized by a high content of flavonoid compounds. Previously high content of linarin (5,7-dihydroxy-4-methoxyflavone, 7-O-[0- α -L-rhamnopyranosyl- β -D-glucopyranoside) from leaves [16], apigenin, luteolin, 3-O-methyl kaempferol, cosmosiin, and acacetin 7- β -D-glucopyranosid uronic acid from flowers [17] have been detected. “Recently, some volatiles that attract both floral herbivores and pollinators were detected including 2-phenylethanol, methyl salicylate, p-anisaldehyde, benzaldehyde, benzyl alcohol, phenylacetaldehyde, linalool, furanoid linalool oxides (E and Z), and dimethyl salicylate. Single compounds (and one isomer) set out in scent-baited water-bowl traps trapped over 10 species of pollinators and 16 species of floral herbivores. The two dominant components of the fragrance blend of *C. arvense*, benzaldehyde and phenylacetaldehyde, trapped both pollinators and florivores. Other compounds attracted either pollinators or florivores. Florivores of *C. arvense* appear to use floral scent compounds as kairomones, by advertising to pollinators” [18].

This study used gas chromatograph with flame ionization detector (GC/FID) and gas chromatography linked to a mass spectrometer show the main components (GC/MS) to analyse the effects of climate and soil on the phytochemical and morphological characteristics of *C. arvense* in Chaharmahal and Bakhtiari Province of Iran over two seasons.

2. Materials and Methods

2.1. Sample Collection

To determine the best environmental conditions and tissue for producing high-quality *C. arvense*, three types of tissue (root, stem, and leaf) were collected from plants found in four regions (Shahrekord, Farsan, Chelgerd, and Ardal) of Chaharmahal and Bakhtiari Province of southwest Iran in April-July 2016-2017, using a randomized complete design with three replicates per region. Plant material was collected from natural populations in each region. The climatic and soil characteristics of the sampling regions are listed in Tables 1 and 2. After sampling plants from each region, the root length, stem length, leaf area indices and total dry matter of the samples were measured.

Table 1. Climatic properties of regions of Chaharmahal and Bakhtiari Province, Iran

Characters	Region			
	Shahrekord	Farsan	Chelgerd	Ardal
Average Annual precipitation (mm)	380	516.9	1480	500
Average of annual temperature (C°)	11.7	12.1	9.5	18.4
Average Maximum temperature (C°)	23.9	24.5	22.1	27.8
Average Minimum temperature (C°)	-1.8	-1.4	-5.1	4
Height (m)	2060	2072	2390	1388
Latitude and Longitude	32°19'N-50°51'E	32°26'N-50°56'E	32°40'N-50°26'E	31°99'N-50°66'E

Table 2. Physico-chemical properties of experimental soil (0-30 cm) in four regions of Chaharmahal and Bakhtiari Province, Iran

Year	Characters	Region			
		Shahrekord	Farsan	Chelgerd	Ardal
2016	N _{total} (%)	0.153	0.119	0.186	0.046
	Organic Mat (%)	0.66	0.31	0.78	0.23
	pH	8.03	7.49	7.66	7.71
	P (mg/kg)	15.3	27.1	35.7	17.6
	K (mg/kg)	328	288	621	162
	Ca (mg/kg)	4.74	4.11	3.81	4.3
	Mn (mg/kg)	9.23	10.27	12.36	8.81
	Fe (mg/kg)	3.1	4.17	6.55	2.96
	Cu (mg/kg)	0.63	0.77	0.86	0.55
	Zn (mg/kg)	0.72	0.86	0.96	0.79
	EC (ds/m)	0.77	1.7	0.45	0.45
	Texture	Clay Loam	Clay Loam	Clay	Clay
2017	N _{total} (%)	0.14	0.12	0.191	0.036
	Organic Mat (%)	0.59	0.34	0.91	0.21
	pH	7.9	7.2	7.52	7.61
	P (mg/kg)	14.7	25.3	32.6	16.9
	K (mg/kg)	311	273	614	172
	Ca (mg/kg)	4.9	4.2	3.7	4.5
	Mn (mg/kg)	8.14	9.3	10.1	7.9
	Fe (mg/kg)	2.8	3.91	6.12	2.81
	Cu (mg/kg)	0.61	0.62	0.77	0.49
	Zn (mg/kg)	0.69	0.82	0.91	0.71
	EC (ds(m)	0.65	0.93	0.42	0.41
	Texture	Clay Loam	Clay Loam	Clay	Clay

2.2 Treatments

Fresh aerial *C. arvensis* tissues were dried for 10 days at room temperature (25±5°C), and then ground to fine a powder using a Moulinex food processor. The essential oil was extracted by heating 50 g of each tissue (root, stem or leaf) in a 2 L flask with 1 L water using a heating jacket at 100 °C for 3 h in a Clevenger-type apparatus, according to the procedures outlined by the British Pharmacopoeia. Voucher specimens (20078-TUH) were those described by Mozaffarian [19] and were deposited in Herbarium of the Center of Agricultural and Natural Resources of Chaharmahal and Bakhtiari Province, Shahrekord, Iran.

2.3 Essential Oil Extraction

The essential oil content was determined by distilling tissues in Clevenger type apparatus. One thousand gram of plant tissue were placed in 6 L Clevenger-type distillation apparatuses and distilled for 5 h in 3 L of pure water. The quantities of plant oils obtained at the end of distillation were measured in mL and ratios (% w/w) were determined by multiplying oil content with oil density (i.e., 0.858 g.cm³). All the essential oil samples were dried over hydrous sodium sulphate, and stored at 4 °C until GC and GC–MS analysis.

2.3 Essential Oil Analysis

Ground GC analysis was done using an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column. The carrier gas was helium at a flow rate 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 composition of the essential oil were identified by comparing retention indices relative to a series of *n*-alkanes (C₇–C₂₄); retention times and mass spectra were those of authentic samples from Wiley library [20].

3. Results and Discussion

The morphology, essential oil content, and essential oil composition of the populations differed significantly ($p \leq 0.01$) (Tables 3 and 4). Twenty-five compounds, accounted for 84.2–91.9% and 82.89–99.9% of the total oil content, (Table 5). The five most abundant components, which composed 65.2–81.1% and 64.1–79.5% of the essential oil content in first and second years, respectively, were camphor, β -citronellol, heneicosane, nonadecane and phytol. Furthermore, based on dry weight, the yield (w/w) of the obtained essential oils ranged from 0.24–0.34% to 0.28–0.33% in the first and second years, respectively (Table 5). Meanwhile, the least abundant components were 3-buten-2-one, 2-pentadecanone, bicyclo[5.2.0]nonane, 1,6-octadien-3-ol, 6-octen-1-ol, 2-naphthalenemethanol and cyclohexanone (0–6.6% to 0–7.8%). The highest levels of nonadecane (40.1–42%) and camphor (18.1–18.4%) were obtained from the roots of plants found in the Chelgerd region, the highest levels of β -citronellol (24.9–25.01%) were obtained from the leaves of plants found in the Chelgerd region, and the highest levels of heneicosane (14.4–15.6%) and phytol (11.8–12.58%) were obtained from stems of plants found in the Shahrekord region. The greatest essential oil content (0.34–0.33%) was obtained from plant roots plants growing in clay soil in the Chelgerd region (Table 5). The greatest levels of camphor, β -citronellol, and nonadecane were observed for plants growing in clay soil in the Chelgerd region at elevations > 2300 m. At higher elevations, the most abundant essential oil components were camphor, β -citronellol, heneicosane, nonadecane, cis-2,6-dimethyl-2,6-octadiene and phytol. Both climate and plant tissue affect the yield of essential oils from medicinal plants. In this study, the percentages of organic matter, phosphorus, potassium, manganese, iron, copper and zinc increased with increasing altitude. At higher elevation, greater precipitation also increased plant growth (Tables 1 and 2). There were significant differences among the essential oil compositions of the various samples, and there was a positive correlation between the essential oil content and components contained in each of the three analysed plant tissues. Contents of the main essential oil components (camphor, β -citronellol, heneicosane, nonadecane, and phytol) were significantly correlated with the essential oil content of each plant tissue. Correlation coefficients between the essential oil content and composition of each plant tissue suggested that the essential oil content of each plant tissue usually had a negative effect on the essential oil content of the other tissues, and that the main essential oil components of each plant tissue negatively affected the main essential oil components of the other plant tissues (Table 6).

In this study, nonadecane was the most abundant essential oil component of *C. arvense*, even though this was not reported previously. Other compounds such as β -citronellol, camphor, heneicosane and phytol. β -citronellol is used in perfumes and insect repellents and as a mite attractant [21]. Camphor is believed to be toxic to insects and is sometimes used as a repellent [22]. Benzaldehyde and phenylacetaldehyde in *C. arvense* have previously been reported previously, to trap pollinators and florivores. Other compounds such as 2-phenylethanol, methyl salicylate, p-anisaldehyde, benzaldehyde, benzyl alcohol, phenylacetaldehyde, linalool, furanoid linalool oxides (E and Z), and dimethyl salicylate attracted either pollinators or florivores [18]. The oils of *Cirsium creticum* (Lam.) (4-ethyl guaiacol, hexadecanoic acid, (E)-b-damascenone, dihydroactinidiolide, 4-vinyl guaiacol) and *Carduus nutans* L. (hexadecanoic acid, hexahydrofarnesylacetone, heptacosane, 4-vinyl guaiacol, pentacosane and eugenol) have weak inhibitory activity against seed germination and radical elongation of *Raphanus sativus* L. (radish), *Lactuca sativa* L. (lettuce), and *Lepidium sativum* L. (garden cress) [23].

Table 3. ANOVA (mean of squares) results of regional effects on morphological characteristics of *Cirsium arvense*

S.O.V [†]	D.F	First Year				Second Year			
		T.D.M	S.L	R.L	L.A.I	T.D.M	S.L	R.L	L.A.I
Treatment	3	151.55**	61.13**	3.79 ^{ns}	13.91**	148.2**	44.17**	11.82**	24.8**
Error	8	45.18	3.47	6.56	2.41	35.2	9.9	4.2	4.5
C.V		12.42	11.25	8.63	3.25	4.8	7.7	5.5	6.63

[†]S.O.V: Source Of Variation, D.F: Degree of Freedom, T.D.M: Total Dry Matter, S.L: Stem Length, R.L: Root Length, L.A.I: Leaf Area Indices
 ns: Not Significant, *and** : Significant at $P \leq 0.05$ and $P \leq 0.01$ levels respectively.

Table 4. ANOVA (mean of squares) of regional effects on the main essential oil component in *Cirsium arvense* roots, stem, and leaves.

Year	Part of Plant	Compound	Treatment	Error	C.V
2016	Root	Heneicosane	25.17**	0.82	1.7
		β -citronellol	6.3**	0.5	4.6
		Phytol	1.35**	0.119	5.9
		Nonadecane	12.4**	0.36	3.3
		Camphor	26.38**	0.015	0.77
	Stem	Heneicosane	71.7**	0.07	5.3
		β -citronellol	12.5**	1.1	4.3
		Phytol	18.16**	0.034	1.6
		Nonadecane	15.97**	0.07	2.4
		Camphor	5.92**	0.24	1.9
	Leaf	Heneicosane	0.034**	0.0023	6.5
		β -citronellol	3.3**	0.3	1.2
		Phytol	1.13**	0.0043	4.4
		Nonadecane	0.37**	0.02	5.4
		Camphor	7.82**	0.01	1.44
2017	Root	Heneicosane	11.5**	0.33	1.04
		β -citronellol	2.7**	0.09	1.1
		Phytol	2.6**	0.93	4.1
		Nonadecane	9.9**	0.45	1.6
		Camphor	11.7**	0.95	4.8
	Stem	Heneicosane	55.2**	7.1	6.7
		β -citronellol	10.1**	2.4	1.9
		Phytol	16.1**	0.3	2.2
		Nonadecane	12.4**	0.8	4.1
		Camphor	10.3**	1.1	3.6
	Leaf	Heneicosane	0.67**	0.031	7.3
		β -citronellol	11.6**	3.2	5.2
		Phytol	4.8**	0.55	3.8
		Nonadecane	0.45**	0.5	4.2
		Camphor	8.8**	0.2	2.2

ns: Not Significant, *and** : Significant at $P \leq 0.05$ and $P \leq 0.01$ levels respectively.

Table 5. Chemical composition (%) of the essential oils of *Cirsium arvense* in two years (in each row, numbers have same word, are same group).

Year	Compound	RI	Shahrekdor ×Root	Shahrekdor ×Stem	Shahrekdor ×Leaf	Farsan ×Root	Farsan ×Stem	Farsan ×Leaf	Chelgerd ×Root	Chelgerd ×Stem	Chelgerd ×Leaf	Ardal ×Root	Ardal ×Stem	Ardal ×Leaf
2016	Camphor	1145	15.1±0.5b	7.4±0.3e	5.5±0.4f	15.2±0.2b	9.1±0.1d	7.7±0.1e	18.1±0.5a	9.1±0.2d	7.7±0.2e	11.5±0.5c	7.6±0.1e	5.1±0.1f
	Cyclohexanone, 5-methyl	1191	0.0001±0.00001	0.001±0.0001	0.0001±0.00001	1.1±0.001	1.2±0.01	0.65±0.01	3.2±0.002	1.1±0.003	1.2±0.01	0.0001±0.00002	0.0001±0.00002	0.0001±0.00002
	β-Citronellol	1210	1.4±0.4f	12.2±0.5d	15.2±0.5c	9.1±0.2e	18.1±0.2b	24.8±0.2a	9.1±0.1e	18.2±0.5b	24.9±0.8a	2.3±0.5f	14.4±0.5c	15.1±0.2c
	1,6-Octadien-3-ol	1234	0.12±0.02	0.00003±0.00006	0.00001±0.000001	4.2±0.2	2.1±0.02	1.1±0.1	1.1±0.3	1.8±0.03	2.5±0.01	0.13±0.001	0.0001±0.00002	0.0001±0.00001
	6-Octen-1-ol	1248	0.11±0.01	0.0006±0.00001	0.00001±0.000001	0.11±0.01	0.001±0.0001	0.0002±0.00001	0.0001±0.00001	0.0001±0.00001	0.0002±0.00001	0.11±0.01	0.00001±0.000001	0.00001±0.00001
	2,6-Octadien-1-ol	1355	0.2±0.01	0.1±0.01	0.04±0.001	0.11±0.01	0.002±0.0001	0.001±0.0001	0.1±0.001	0.001±0.0001	0.002±0.0004	0.11±0.003	0.11±0.001	0.08±0.006
	Bicyclo[5.2.0]nonane	1394	0.02±0.001	0.00005±0.000002	0.00001±0.000005	0.02±0.003	0.001±0.0002	0.0002±0.00002	0.001±0.0005	0.003±0.0001	0.0004±0.00001	0.03±0.001	0.00002±0.000002	0.00002±0.00008
	2-Naphthalenemethanol	1468	0.02±0.005	0.00008±0.000006	0.00006±0.000003	0.1±0.002	0.001±0.0001	0.0001±0.00005	0.02±0.003	0.003±0.0005	0.0002±0.00004	0.014±0.001	0.00001±0.000005	0.00001±0.000007
	Pentadecane	1500	0.01±0.001	0.001±0.0004	0.0003±0.00003	0.77±0.07	0.88±0.1	0.55±0.005	1.8±0.003	1.01±0.1	1.1±0.02	0.01±0.003	0.006±0.0003	0.0002±0.00003
	α-Terpinolen	1510	0.9±0.01	0.8±0.03	0.8±0.01	0.9±0.02	0.82±0.011	0.01±0.001	0.83±0.01	0.6±0.01	0.009±0.0001	1.01±0.008	0.05±0.001	0.81±0.001
	Cyclohexanone	1597	0.001±0.0001	0.00005±0.000001	0.00002±0.000003	0.9±0.001	2.2±0.01	2.61±0.01	1.1±0.01	3.3±0.011	2.2±0.03	0.001±0.0001	0.00002±0.000001	0.00001±0.000005
	Cis-2,6-Dimethyl-2,6-octadiene	1611	2.2±0.1	1.4±0.5	14.4±0.5	1.2±0.1	8.4±0.5	14.8±0.6	1.01±0.02	9.01±0.1	11.1±0.1	3.9±0.2	1.7±0.1	3.5±0.05
	Trans-Caryophyllene	1632	1.1±0.01	1.3±0.04	0.94±0.04	0.11±0.02	0.88±0.02	0.45±0.03	0.11±0.04	0.89±0.01	0.4±0.002	1.4±0.02	0.8±0.02	0.8±0.1
	Trans-Geraniol	1645	1.45±0.1	1.2±0.02	0.96±0.01	0.86±0.02	4.4±0.02	1.1±0.1	0.14±0.002	5.5±0.1	1.4±0.1	1.5±0.1	0.98±0.02	1.2±0.2
	β-Lonone	1672	1.4±0.1	0.94±0.1	0.87±0.1	0.1±0.01	0.001±0.001	0.00002±0.000001	0.12±0.02	0.002±0.0001	0.01±0.001	0.87±0.02	0.78±0.1	0.91±0.02
	Heptadecane	1701	2.3±0.1	0.85±0.01	1.2±0.1	0.02±0.002	0.04±0.001	0.00002±0.000001	0.02±0.001	0.05±0.001	0.03±0.004	2.7±0.1	0.94±0.1	1.1±0.1
	9-Nonadecene	1811	1.4±0.1	0.9±0.01	0.89±0.01	0.03±0.005	0.002±0.0001	0.00008±0.000001	0.03±0.001	0.0001±0.00002	0.0002±0.00002	1.1±0.1	0.9±0.01	0.9±0.01
	n-Nonadecane	1901	2.1±0.02	1.4±0.01	1.1±0.1	2.2±0.4	2.1±0.1	2.1±0.03	2.2±0.1	2.2±0.01	2.4±0.01	1.9±0.01	1.6±0.04	1.4±0.04
	Heneicosane	2109	2.1±0.1d	14.4±0.02a	0.99±0.05e	6.82±0.6b	1.92±0.1d	1.1±0.1e	7.7±0.3b	2.2±0.2d	1.1±0.1e	4.9±0.2c	5.5±0.4c	0.9±0.2c
	Nonadecane	2112	38.2±2.3a	33.2±3.3b	25.1±1.4d	38.1±3.2a	30.2±1.2c	18.4±1.1f	40.1±1.1a	30.1±1.1c	17.4±1.2f	37.7±1.5b	35.5±2.4b	30.2±1.4c
	3-Buten-2-one	2111	0.01±0.001	0.9±0.1	0.89±0.1	0.11±0.001	0.00001±0.000001	0.00002±0.000002	0.1±0.002	0.00001±0.000001	0.00001±0.000002	0.02±0.001	0.85±0.001	0.8±0.002
	2-Pentadecanone	2115	0.02±0.001	0.002±0.0002	0.0004±0.00006	0.01±0.001	0.00001±0.000001	0.00003±0.000001	0.01±0.001	0.00001±0.000001	0.00001±0.000001	0.02±0.001	0.002±0.0002	0.0003±0.00001
	1,2-Benzenedicarboxylic acid	2117	3.3±0.2	2.2±0.1	3.8±0.04	0.02±0.004	0.0002±0.00001	0.00002±0.000003	0.03±0.001	0.0002±0.00001	0.0001±0.00002	3.9±0.1	5.5±0.1	4.4±0.04
Z-5-Nonadecene	2118	4.4±0.1	3.5±0.05	4.1±0.1	0.87±0.1	0.92±0.02	0.77±0.1	1.1±0.1	0.0005±0.00001	1.3±0.03	4.9±0.1	3.8±0.1	4.4±0.03	
Phytol	2120	9.9±0.1b	4.9±0.1c	11.8±0.7a	1.4±0.3d	1.1±0.1d	9.9±0.4b	0.66±0.3d	0.88±0.2d	9.9±0.3b	11.9±0.3a	4.4±0.1c	11.4±0.2a	
Total		87.76	87.59	88.58	84.36	84.36	86.05	88.6	85.95	84.6	91.9	85.42	84.2	
Essential oil content (%)			0.28±0.02b	0.29±0.01b	0.25±0.01c	0.3±0.01b	0.28±0.02b	0.27±0.01c	0.34±0.01a	0.29±0.01b	0.31±0.02a	0.29±0.01b	0.28±0.02b	0.24±0.01c
2017	Camphor	1146	14.2±0.9b	6.9±0.8f	5.9±0.3f	18.3±0.3a	9.2±0.1d	8.2±0.2e	18.4±0.6a	9.3±0.3d	8.4±0.4e	12.5±0.6c	6.9±0.1f	5.2±0.1g
	Cyclohexanone, 5-methyl	1190	0.00012±0.00001	0.001±0.0001	0.0005±0.00001	4.1±0.001	1.9±0.01	0.95±0.001	4.2±0.002	2±0.003	1.02±0.001	0.00001±0.000002	0.00001±0.000004	0.00001±0.000005
	β-Citronellol	1210	1.8±0.3g	14±0.8e	16.7±0.5d	9.5±0.3f	19.2±0.3c	24.01±0.4a	9.6±0.2f	20.1±0.7b	25.01±0.9a	1.23±0.3g	13±0.9e	16.2±0.9d
	1,6-Octadien-3-ol	1234	0.14±0.01	0.00004±0.00005	0.00002±0.000001	5.2±0.1	2.3±0.01	2.1±0.1	5.3±0.03	2.9±0.04	2.6±0.02	0.13±0.001	0.00001±0.000002	0.00001±0.000001
	6-Octen-1-ol	1247	0.17±0.001	0.00007±0.000005	0.00002±0.000001	0.33±0.01	0.002±0.0001	0.0001±0.00001	0.0003±0.00001	0.0003±0.00002	0.0004±0.00001	0.15±0.01	0.00001±0.000001	0.00001±0.000002
	2,6-Octadien-1-ol	1356	0.26±0.01	0.17±0.01	0.1±0.01	0.21±0.03	0.004±0.0001	0.001±0.0002	0.22±0.001	0.005±0.0001	0.0022±0.0004	0.24±0.003	0.16±0.05	0.09±0.006
	Bicyclo[5.2.0]nonane	1393	0.03±0.002	0.00008±0.000005	0.00002±0.000007	0.01±0.004	0.001±0.0006	0.0003±0.00007	0.002±0.0005	0.0032±0.0005	0.00045±0.00006	0.022±0.001	0.00001±0.000007	0.00001±0.000008
	2-Naphthalenemethanol	1468	0.03±0.006	0.00009±0.000007	0.00009±0.000008	0.11±0.004	0.002±0.0001	0.0003±0.00005	0.12±0.003	0.0034±0.0006	0.0004±0.00008	0.024±0.004	0.00001±0.000007	0.00001±0.000009
	Pentadecane	1500	0.02±0.008	0.007±0.0008	0.0004±0.00005	1.1±0.09	1.01±0.1	0.81±0.009	1.2±0.008	1.02±0.1	1.23±0.08	0.012±0.007	0.0065±0.0005	0.00025±0.00004
	α-Terpinolen	1510	1.02±0.02	1.1±0.06	1.2±0.08	1.02±0.09	0.99±0.01	0.012±0.001	1.03±0.05	1.01±0.07	0.014±0.005	1.01±0.008	0.99±0.006	1.1±0.004
	Cyclohexanone	1595	0.002±0.0001	0.00007±0.000002	0.00004±0.000008	1.2±0.004	4.8±0.01	4.1±0.01	1.3±0.04	4.99±0.05	4.8±0.09	0.001±0.0005	0.00003±0.000001	0.00002±0.000007

Table 5. Continued..

Cis-2,6-Dimethyl-2,6-octadiene	1610	4.3±0.1	2.2±0.5	14.9±0.8	1.3±0.2	9.2±0.3	15.2±0.2	1.4±0.04	9.99±0.3	15.9±0.2	4.2±0.3	2.1±0.1	4.3±0.09
Trans-Caryophyllene	1632	2.84±0.08	1±0.09	1.01±0.07	0.12±0.04	0.98±0.01	0.21±0.05	0.13±0.07	0.99±0.03	0.3±0.001	2.33±0.06	0.95±0.06	0.99±0.03
Trans-Geraniol	1645	2.25±0.1	1.7±0.09	2±0.08	0.14±0.04	6.2±0.03	2.1±0.1	0.16±0.001	6.1±0.2	2.99±0.2	2.11±0.1	1.68±0.09	1.97±0.2
β-Lonone	1672	1.35±0.2	1±0.1	1.01±0.2	0.12±0.04	0.001±0.003	0.00002±0.000005	0.13±0.07	0.001±0.005	0.012±0.005	1.1±0.04	1±0.2	0.99±0.03
Heptadecane	1700	2.6±0.1	1±0.1	1.36±0.1	0.023±0.005	0.048±0.005	0.00003±0.000008	0.025±0.005	0.051±0.007	0.032±0.007	3.2±0.2	1±0.1	1.25±0.1
9-Nonadecene	1811	1.65±0.2	1±0.1	1±0.2	0.02±0.004	0.001±0.001	0.00009±0.000007	0.04±0.002	0.00012±0.00005	0.0001±0.00005	1.23±0.2	0.92±0.1	1±0.1
n-Nonadecane	1900	2.3±0.09	2±0.09	2±0.2	3.01±0.2	3.02±0.2	2.22±0.09	3.02±0.2	3.03±0.1	2.8±0.1	2.23±0.1	1.99±0.09	1.99±0.08
Heneicosane	2109	2.9±0.3d	15.6±0.2a	1±0.09e	7.2±0.4b	2.2±0.4d	0.98±0.1e	8.1±0.5b	2.4±0.5d	1.2±0.1e	5.53±0.2c	5.2±0.1c	0.97±0.2e
Nonadecane	2110	41±0.9a	34.2±3.3c	28.1±1.8d	40±3.7a	31.1±1.4c	17.1±2.3e	42±1.2a	32.1±1.5c	18.2±1.3e	38.2±0.9b	33.2±2.6c	27.2±2.8d
3-Buten-2-one	2111	0.02±0.001	1±0.2	1±0.1	0.123±0.003	0.00001±0.000002	0.00002±0.000007	0.16±0.004	0.00001±0.000006	0.00001±0.000009	0.022±0.001	0.99±0.001	0.98±0.003
2-Pentadecanone	2115	0.03±0.001	0.004±0.0005	0.0006±0.00008	0.014±0.0009	0.00001±0.000007	0.00006±0.000003	0.02±0.001	0.00001±0.000002	0.00001±0.000002	0.025±0.001	0.0026±0.0005	0.00034±0.00002
1,2-Benzenedicarboxylic acid	2117	4.9±0.2	4.3±0.1	3.98±0.09	0.024±0.005	0.0003±0.00001	0.00004±0.000008	0.033±0.001	0.0004±0.00002	0.0002±0.00001	4.3±0.2	4.2±0.1	3.8±0.07
Z-5-Nonadecene	2118	5.7±0.1	3.8±0.06	4.12±0.1	1.2±0.1	1±0.06	1±0.1	1.6±0.1	0.0006±0.00004	1.9±0.09	5.2±0.1	3.4±0.1	3.84±0.09
Phytol	2120	10.2±0.2b	5.36±0.1c	12.58±0.7a	1.2±0.1d	1.02±0.1d	11.2±0.9a	1.4±0.6d	1.03±0.5d	11.89±0.8a	12±0.4a	5.2±0.1c	12.36±0.3a
Total		99.9	96.5	98.1	95.5	94.18	90.19	99.5	97.02	98.3	97.03	82.89	84.32
Essential oil content (%)		0.29±0.03ab	0.3±0.02ab	0.295±0.01ab	0.28±0.01b	0.3±0.05ab	0.29±0.02b	0.33±0.02a	0.285±0.02b	0.3±0.02ab	0.29±0.01b	0.29±0.01b	0.25±0.02c

Water and mineral nutrient contents have been reported to increase with altitude and precipitation, thereby increasing essential oil content [24]. Effects of altitude on soil texture have been reported to affect a variety of physiological properties including the chlorogenic acid content of artichokes [9], thymol and carvacrol contents of thyme [25], artemisin content of *Artemisia annua* [10], antioxidant levels of vegetables [11], essential oil composition and antimicrobial properties of wild mint [12], and essential oil content of *Origanum vulgare* [26]. Of the morphological properties assessed by the present study, total dry matter, and leaf area indices, exhibited the greatest correlation with essential oil content, regardless of region and plant tissue (Table 6). Although many studies have compared the volatile oil contents of plants from various sites, none have investigated the effects of altitude on volatile oil content [1, 27]. Here, we conclude that many essential oil components, (e.g., nonadecane), decrease with altitude, likely owing to two related factors. First many environmental factors (e.g., precipitation) are affected by altitude, wind, daily temperatures, and cloudiness. However, the amount of evaporation and mean temperature decreased with increasing altitude and had a better effect on the essential oil content and composition. Furthermore, the roots of *C. arvense* yielded the greatest essential oil content, regardless of region. It seems that soil depth increases with altitude and that plant metabolism, growth, development, and phytochemical levels are affected by the absorption and transition of essential nutrients [28]. However, morphological and physiological characteristics are also affected by altitude [29] and seasonal variation [30]. The depth of soils in Chelgerd region increased with increasing altitude and the content of many of components of essential oil was increased [9,13,27,31-33].

The results of the present study indicate that the essential oil composition of *C. arvense* is significantly affected by differences in climate and soil properties. The main components of *C. arvense* essential oil were nonadecane, β -citronellol, camphor, heneicosane and phytol, and the most levels of nonadecane and camphor were obtained from the roots of plants found in the Chelgerd region, whereas the most levels of β -citronellol were obtained from the leaves of plants found in the Chelgerd region, and the most levels of nonadecane and overall essential oil were obtained from the roots of plants found in the Chelgerd region. In addition, the present study also demonstrates that the essential oil components of *C. arvense* are affected by climate, soil and plant tissue. This study provides useful information regarding the efficacy of regions and plant tissues on the content and composition of essential oil in *C. arvense*.

4. Acknowledgements

This work was supported by the Islamic Azad University Branch of Shahrekord, Iran. We are very grateful to Mrs. Parvin Karimi from Education Center of Shahrekord for helpful advice for secondary reviewer.

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