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DETERMINATION OF CHEMOTAXONOMIC INDICES OF ARTEMISIA SIEBERI BESSER BASED ON ENVIRONMENTAL PARAMETERS IN IRAN

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Artemisia sieberi Besser is the most common species in Iran. In this research, yield and chemical composition of the essential oil of *A. sieberi* are studied from 34 populations in Semi-arid, Arid and Hyper-arid climate conditions. Also, plant seeds were collected from nature and planted in homogeneity condition of farm. The essential oils of harvested plants (including natural and farms plants) were isolated. The components of oils were identified by GC and GC-MS. T-student test showed significant difference between average of oil yields in both natural and farm plants. Correlation between yields of essential oil in natural habitats with ecological factors showed that days of precipitation, nitrogen, carbon and phosphor of soil were the most effective parameters in quantity of yields. Among chemical components, camphene, 1, 8-cineole, trans-thujone, camphor, borneol didn't have significant difference between natural and farm plants. The results showed that chemical components of essential oil in *A. sieberi* can be valuable for recognizing of the species as chemotaxonomic indices. Also PCA analysis revealed that camphor, cis-thujone and trans-thujone are principal chemical components for separating of *A. sieberi* climatic groups.

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Key words. Artemisia sieberi, Chemotaxonomy, Environmental parameters, Essential oil, PCA, T-student, Iran.

تعیین شاخص‌های کموتاکسونومیک بر پایه پارامترهای محیطی درمنه دشتی در ایران

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Artemisia sieberi Besser رایج‌ترین گونه در ایران می‌باشد. در این تحقیق بازده و ترکیب‌های شیمیایی اسانس ٣٤ جمعیت *A. sieberi* در سه ناحیه اقلیمی نیمه خشک، خشک و فراخشک مورد مطالعه قرار گرفت. همچنین بذور جمع‌آوری شده از این جمعیت‌ها در شرایط یکنواخت مزرعه مورد کشت قرار گرفت. اسانس گیاهان طبیعی و مزرعه‌ای استخراج گردید و ترکیب‌های اسانس توسط دستگاه‌های GC و GC-MS مورد تجزیه و تحلیل قرار گرفت. آزمون T-student بین متوسط بازده اسانس در مزرعه و طبیعت تفاوت معنی‌داری را نشان داد. همبستگی بین عوامل محیطی و بازده اسانس بررسی شد که از میان کلیه عوامل مورد بررسی، تعداد روزهای بارندگی، نیتروژن، کربن و فسفر خاک مؤثرترین پارامترها در کمیت اسانس بودند. ترکیب‌های شیمیایی اصلی اسانس مانند camphene، 1, 8-cineole، trans-thujone، camphor و borneol تفاوت معنی‌داری بین مزرعه و طبیعت نشان ندادند. نتایج نشان داد که ترکیب‌های شیمیایی اسانس در *Artemisia sieberi* می‌توانند به عنوان شاخص‌های کموتاکسونومیک مورد استفاده قرار گیرند. همچنین آنالیز PCA براساس ترکیب‌های شیمیایی در سه گروه اقلیمی مشخص نمود که ترکیب‌های camphor، cis-thujone و trans-thujone ترکیب‌های اصلی تفکیک‌کننده این گروه‌های اقلیمی می‌باشند.

INTRODUCTION

The genus *Artemisia* is one of the largest and most widely distributed of the nearly 100 genera in the tribe *Anthemideae* of the *Asteraceae* (*Compositae*) (Mucciarelli & Maffei, 2002). As reported by Tutin and Presson (1976) 400 *Artemisia* species share the following common morphological characters: Herbs or small shrubs, frequently aromatic, leaves alternate, capitula small, usually pendent, racemose, paniculate or capitate inflorescences, rarely solitary. Involucral bracts in few rows. Receptacle flat to hemispherical, without scales, sometimes hirsute. Florets all tubular. Achenes obovoid, subterete or compressed, smooth, finely striate or 2-ribbed; pappus absent or sometimes a small scarious ring (Tutin and Presson, 1976).

They are mostly perennial herbs and shrubs dominating the vast steppe communities of Asia, the New World and South Africa. *Artemisia* is a highly involved genus with a wide range of life forms, from tall shrubs to dwarf herbaceous alpine plants, occurring in a variety of habitats between Arctic Alpine and Montana environments to the dry deserts (Heywood et al., 1977).

Several secondary metabolites characterize the chemical composition of the genus *Artemisia*. A survey of the literature indicates that almost all classes of compounds are present in the genus, with particular reference to terpenoids and flavonoids. The wide array of molecules present in the genus and the distribution of plants in several different habitats provide the opportunity for the study of genotypic and phenotypic variations as well as chemotaxonomic relationships among species (Mucciarelli and Maffei, 2002).

The terpenoids present in *Artemisia* species are representative of all classes of compounds, from monoterpenes up to triterpenes. Most of the species are characterized by the typical fragrance of lower terpenoids, such as monoterpenes and sesquiterpenes. These volatile molecules are present in the essential oils, which are important strong aromatic odors in the plants. Among the various compounds, lower terpenoids such as camphor, thujone, borneol and 1, 8-cineole are the most representative (Ravid et al., 1992). As for many aromatic plants, the oil content of *Artemisia* is affected by environmental factors (Cedarleaf et al., 1983).

34 species of *Artemisia* has been reported in Iran (Mozaffarian, 2008). *Artemisia sieberi* is the most widely distributed species in Iran. For determination of chemotaxonomic characters, we studied several populations of *A. sieberi* in different habitats in Iran. Also, seeds of each population were collected and planted in farm. With comparision of chemical components between plant specimens of nature and farm, would be recognized constant components as chemotaxonomic indices.

MATERIALS AND METHODS

Aerial parts of *Artemisia sieberi* were collected from 34 populations in Iran at full flowering stage. The exact date and place of each locality have been shown in table1. The collected plant speciments were dried at room temperature and after 48 hours hydro-distilled for their essential oils. The herbarium specimens have been deposited in the herbarium of Research Institute of Forests and Rangelands (TARI). Also, farm plant speciments were harvested and hydro-distilled as well as natural plants.

The essential oils of all harvested plants (nature and farm) were isolated by hydro-distillation in Clevenger-type apparatus for 3.5 hours. The oils were stored in sealed vials at low temperature before analysis.

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40 ºC for 5 min and then programmed to 250 ºC at a rate of 4 ºC / min; for all of oils. Injector and detector (FID) temperature were 260 ºC; carrier gas, helium with a linear velocity of 32 cm/s.

GC-MS analyses were carried out on a Varian 3400 GC-MS. The column was same as GC, oven temperature was at 40 ºC to 270 ºC at a rate of 4 ºC, transfer line temperature 280 ºC, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 e V; scan time 1 s; mass range 40-300 amu.

The components of oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Adams, 1989; Shibamoto, 1987; Davies, 1990).

Correlation between yield of essential oil in natural habitats with climate, altitude and soil characteristics was studied. Also, total of main chemical components in nature and farm plants were compared by T-student test after equal variances test (Levin's test) and normality test (Anderson – Darling normality test).

Main components were determined. Also, the populations placed in three climates condition: 1- Semi-arid, 2- Arid, 3- Hyper-arid. Chemical components in 3 groups were analyzed by PCA method.

Table 1. Habitat characteristics and yield of essential oil of *Artemisia sieberi* collections.

Code	Habitat	Altitude (m)	Latitude Longitude	Date of collection	Climate	Voucher	Rainfall (mm)	Temperature (°C)	Yield % (nature)	Yield % (farm)
1	Esfahan province, Kashan, Selkhak	1600	34 16 55 N 51 04 31 E	Nov. 2006	Arid	85834	198.5	16.56	1.58	0.89
2	Esfahan province, Kashan, Keh village	2010	34 07 48 N 51 04 53 E	Nov. 2006	Semi-arid	85825	237.2	12.55	3.27	0.67
3	Esfahan province, 65 km Kashan to Ardestan	1700	33 42 24 N 51 53 31 E	Nov. 2006	Arid	85822	198.5	16.56	2.31	1.14
4	Esfahan province, Kashan, 5 km AghaAli Abbas to Siahkuh	1020	33 44 50 N 52 06 12 E	Nov. 2006	Hyper-arid	85833	111	19.45	2.98	1.27
5	Esfahan province, 5 km Ardestan to Zafarghand	1350	33 19 21 N 52 22 31 E	Nov. 2006	Hyper-arid	85832	115.1	19.95	2.10	2.15
6	Esfahan province, 4 km Zafarghand to Naeen	1900	33 09 47 N 52 30 49 E	Nov. 2006	Hyper-arid	84446	115.1	19.95	2.40	1.32
7	Esfahan province, Kashan, Maranjab	830	34 17 01 N 51 43 04 E	Nov. 2006	Hyper-arid	84445	133.6	19.38	2.35	1.52
8	Qom province, Qom lake beside	870	35 02 21 N 50 51 56 E	Dec. 2006	Arid	85823	144	18.06	0.47	0.46
9	Qom province, 60 km Qom to Tehran	1300	35 10 43 N 50 59 24 E	Dec. 2006	Arid	84434	223.6	19.42	1.15	1.14
10	Khorasan province, 10 km Fariman to Torbate Jam	1390	35 38 59 N 59 56 16 E	Nov. 2006	Arid	85836	212.1	12.78	1.30	1.36
11	Khorasan province, 130 km Torbate Heydarieh to Bejestan	1160	34 34 16 N 58 12 25 E	Nov. 2006	Arid	85835	159.2	17.97	1.47	1.12
12	Yazd province, 40 km Aliabad to Marvast	1900	30 57 29 N 54 13 03 E	Dec. 2006	Hyper-arid	85827	88.8	18.3	1.25	1.56
13	Yazd province, 60 km Taft to Nodushan	2350	31 52 55 N 53 39 04 E	Dec. 2006	Arid	85826	176.7	14.34	0.86	0.65
14	Yazd province, 42 km Yazd to Bafgh	1370	31 44 51 N 54 43 48 E	Dec. 2006	Hyper-arid	85830	59.6	19.5	1.51	1.02
15	Yazd province, 10 km Bahabad to Bafgh	1610	31 53 20 N 55 54 56 E	Dec. 2006	Hyper-arid	85829	79	19.64	1.88	0.95
16	Yazd province, 5 km Robate Poshte Badam to Yazd	1340	32 59 14 N 55 32 34 E	Dec. 2006	Hyper-arid	85828	107.5	20.79	1.99	2.27
17	Yazd province, 13 km Ardakan to Naeen	1120	32 21 37 N 53 52 27 E	Dec. 2006	Hyper-arid	85831	63.8	20.21	1.61	1.31
18	Yazd province, 90 km Ardakan to Naeen	1390	32 43 18 N 53 16 29 E	Dec. 2006	Hyper-arid	84447	101.9	13.65	1.05	1.29
19	Kerman province, 85 km Jiroft to Kerman	1570	29 16 27 N 57 58 20 E	Dec. 2006	Arid	84448	141.1	16.55	1.28	1.52
20	Kerman province, 45 km Kerman to Jiroft	2240	29 56 52 N 57 23 51 E	Dec. 2006	Arid	84449	141.1	16.55	1.72	0.66
21	Kerman province, 15 km Kerman to Zarand	1835	30 26 49 N 57 00 31 E	Dec. 2006	Arid	84450	141.1	16.55	1.52	1.33
22	Fars province, 25 km Abadehtashk to Arsanjaan	1680	29 47 14 N 53 32 52 E	Dec. 2006	Semi-arid	84433	350.1	18.53	2.23	0.50
23	Fars province, 100 km Sirjan to Neyriz	1660	29 10 39 N 54 50 59 E	Dec. 2006	Arid	84432	185.4	18.84	1.64	1.80
24	Fars province, 6 km Harabarjan to Tutak	1650	30 19 23 N 54 08 46 E	Dec. 2006	Arid	84435	162.7	16.29	1.04	0.86
25	Markazi province, Anjilavand saveh	1000	34 59 08 N 50 35 11 E	Dec. 2006	Arid	84436	223.6	19.42	0.84	0.85

Code	Habitat	Altitude (m)	Latitude Longitude	Date of collection	Climate	Voucher	Rainfall (mm)	Temperature (°C)	Yield % (nature)	Yield % (farm)
25	Markazi province, Anjilavand saveh	1000	34 59 08 N 50 35 11 E	Dec. 2006	Arid	84436	223.6	19.42	0.84	0.85
26	Markazi province, Gheshlaghe nemati, Ghate 4 Zarand Saveh	1390	35 27 33 N 50 39 58 E	Dec. 2006	Arid	84437	223.6	19.42	0.94	1.15
27	Tehran province, Kavir National Park	1050	34 45 55 N 52 10 32 E	Dec. 2006	Hyper-arid	84444	126.4	18.61	2.68	0.68
28	Semnan province, 55 km Semnan to Damghan	1550	35 51 06 N 53 54 06 E	Jan. 2007	Hyper-arid	84439	138.4	18	1.53	1.71
29	Semnan province, 53 km army road Semnan to Anjilo	1400	35 26 12 N 53 52 49 E	Jan. 2007	Hyper-arid	84440	138.4	18	1.84	0.87
30	Semnan province, Turan, 4 km Delbar to Ahmadabad	1050	35 58 30 N 56 02 25 E	Jan. 2007	Hyper-arid	84441	126.7	16.47	2.13	0.88
31	Semnan province, 33 km Shahroud to Sabzevar	1400	36 26 30 N 55 17 07 E	Jan. 2007	Arid	85824	160.6	14.56	0.65	0.81
32	Semnan province, 8 km Semnan to Sorkheh	1200	35 29 32 N 53 15 30 E	Jan. 2007	Hyper-arid	84442	138.4	18	1.72	2.03
33	Semnan province, 5 km Eivanekei to Garmsar	1050	35 19 11 N 52 07 09 E	Jan. 2007	Hyper-arid	84443	126.4	18.61	3.50	2.05
34	Hormozgan province, 25 km Hajiabad to sirjan	1388	28 30 897 N 55 47 537 E	Jan. 2007	Arid	84438	140.6	17.66	1.77	1.68

RESULTS AND DISCUSSION

Yields of essential oils isolated from the aerial parts of *Artemisia sieberi* at full flowering stage in nature and farm are shown in table 1 and figure 1. Yield of essential oil in most natural habitats are higher than the farm. It can be because of drought stress in natural habitats (the farm were regularly irrigated). Average of essential oil yields in nature and farm were compared by T-student test: P-value= 0.001, difference was significant at 99.9 % level.

Correlation between yield of essential oil in natural habitats with climate, altitude and soil characteristics showed that the most effective parameters were number of days with precipitation, nitrogen, carbon and phosphor of soil (Table 2). Comparison of chemical components mean in nature and farm are given in figure 2.

Comparison of main chemical components between nature and farm plants by T-student test have presented in table 3. Camphene, 1, 8-cineole, trans-thujone, camphor, borneol did not have significant difference between nature and farm. But (Z)-β-ocimene and chrysanthenyl acetate at 95% level, p-cymene, cis-thujone, pinocarvone, terpinen-4-ol at 99% level, (E)-β-ocimene, γ-terpinene, myrtenol and bornyl acetate at 99.9% level had significant difference. Probably, non-significant components are influenced by environmental factors lower than significant components.

Average of chemical components in climatic groups is shown in table 4. Populations in drier habitats have cis-thujone more than the others. Trans-thujone in semi-arid populations is more than the others. In fact, principle components cis-thujone and trans-thujone were succeeded together. In drier condition cis-thujone increases but trans-thujone decreases.

Three climatic groups of populations completely were separated by PCA analysis (Figure 3). Camphor, cis-thujone and trans-thujone were recognized as principal components for separating climatic groups (Figure 4).

Species of the genus *Artemisia* produce many eudesmanolides, the group of sesquiterpene lactones that exhibit the highest antibacterial activity and anti-inflammatory properties, while the insecticidal properties of many of the species is due to the presence of 1,8-cineole (Aggarwal et al., 2001). Cis- and trans-thujone are the active ingredients in herbal medicines and seasoning for food and drinks. The oils of *Artemisia* species can be classified according to their primary constituents, suggesting the use of essential oils for taxonomic classification and clarification: *A. sieberi* (camphor), *A. annua* (artemisia ketone, α-

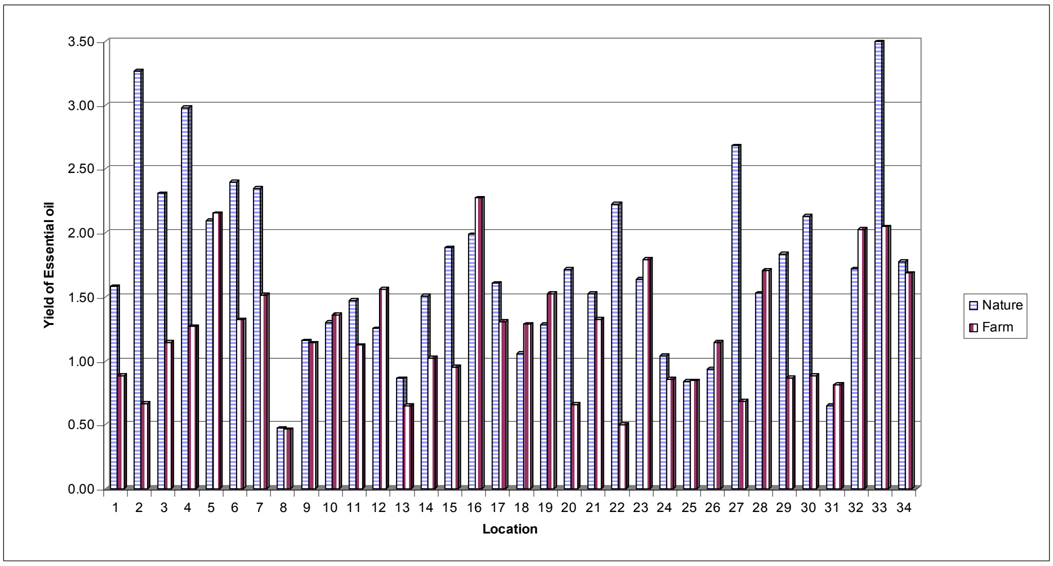

Fig. 1. Comparison between yield of essential oils in nature and farm plants.

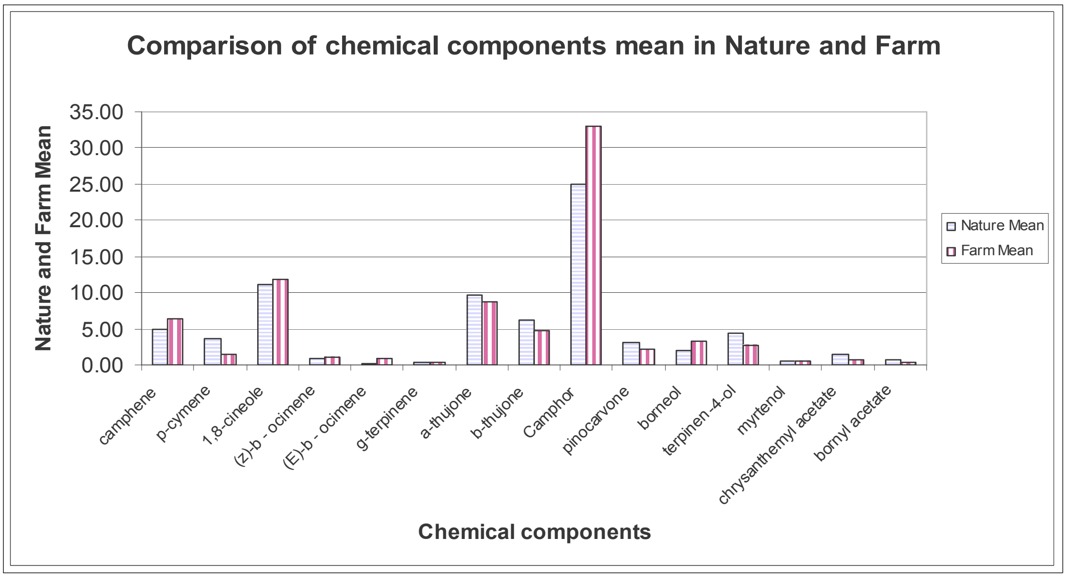

Fig. 2. Comparison of chemical compounds mean in nature and farm.

pinene and camphor), *A. aucheri* (verbenone), etc (Jaime A., 2004).

The quality and yield of essential oils from *Anthemideae* plants is influenced by the harvesting season (Cornu et al., 2001), fertilizer and pH (ideal in acidic, pH 4.5-5.4) of soils (Alvarez-Castellanos & Pascual-Villalobos, 2003), the choice and stage of drying conditions (Tateo & Riva, 1991), the geographic location (Maffei et al., 1994), chemotype or subspecies (Goren et al., 2001), choice of plant part or genotype (Mishra et al., 1999; Nori-Shargh et al., 1999; Keskitalo et al., 2001) or extraction method (Scalia et al., 1999).

Weyerstahl et al. (1993) showed that camphor, 1, 8-cineole, camphene, terpinen-4-ol and α-terpineol are the main oil components of *A. sieberi*. Sefidkon et al. (2002) introduced camphor, 1,8-cineole and bornyl acetate as main oil components of *A. sieberi*.

In this research, main components were camphor (25.01 % in nature and 32.98 % in farm), 1, 8-cineole (11.08 % in nature and 11.91 % in farm), cis-thujone

Table 2. Effects of environmental parameters on yield of essential oil.

Parameters	Pearson Correlation	P-value
Average annual temperature	0.088	0.621
Average minimum temperature	0.043	0.810
Average maximum temperature	0.017	0.923
Absolute minimum temperature	0.011	0.949
Absolute maximum temperature	0.157	0.376
Annual precipitation	-0.024	0.895
Number of days with precipitation	-0.260	0.137
Altitude	-0.055	0.758
pH	0.004	0.984
EC (ds/m)	0.116	0.514
SP (%)	0.138	0.435
N (%)	-0.238	0.176
Lime (%)	0.007	0.970
Gyps (%)	0.134	0.451
C (%)	-0.216	0.220
Sand (%)	0.012	0.948
Silt (%)	-0.007	0.969
Clay (%)	-0.014	0.937
Na (mg/kg)	-0.068	0.703
Mg (mg/kg)	0.049	0.784
Ca (mg/kg)	0.166	0.349
K (mg/kg)	-0.094	0.596
P (mg/kg)	-0.188	0.286

Table 3. T- Student test of chemical components in nature and farm plants.

Measured parameters		**Mean**	**Stdev**	**SE Mean**	**Minimum**	**Maximum**	**P-Value**	
camphene	Nature	4.98	3.27	0.56	0.00	11.24	0.1500	ns
	Farm	6.29	4.12	0.71	0.54	15.23		
p-cymene	Nature	3.62	4.57	0.78	0.51	18.52	0.0004	**
	Farm	1.51	1.79	0.31	0.00	7.60		
1,8-cineole	Nature	11.08	8.00	1.37	1.24	32.24	0.5768	n.s
	Farm	11.91	8.32	1.43	0.53	29.06		
(z)-β - ocimene	Nature	0.91	3.33	0.57	0.00	17.94	0.0200	*
	Farm	1.02	2.08	0.36	0.00	8.13		
(E)-β - ocimene	Nature	0.17	0.57	0.10	0.00	3.17	0.0000	***
	Farm	0.90	2.21	0.38	0.09	11.18		
γ-terpinene	Nature	0.28	0.84	0.14	0.00	3.82	0.0000	***
	Farm	0.37	0.34	0.06	0.00	1.53		
cis-thujone	Nature	9.71	14.56	2.50	0.00	66.93	0.0105	**
	Farm	8.72	16.74	2.87	0.00	60.51		
trans-thujone	Nature	6.14	6.59	1.13	0.00	26.00	0.1444	n.s
	Farm	4.76	6.30	1.08	0.00	23.48		
camphor	Nature	25.01	22.70	3.89	0.00	60.60	0.0941	n.s
	Farm	32.98	17.69	3.03	3.56	57.55		
pinocarvone	Nature	3.17	2.97	0.51	0.00	13.24	0.0020	**
	Farm	2.14	2.12	0.36	0.15	9.38		
borneol	Nature	2.01	8.08	1.39	0.00	44.84	0.2630	n.s
	Farm	3.36	6.73	1.15	0.00	28.62		
terpinen-4-ol	Nature	4.45	8.06	1.38	0.00	29.72	0.0032	**
	Farm	2.70	7.46	1.28	0.00	40.00		
myrtenol	Nature	0.58	0.80	0.14	0.00	3.30	0.0000	***
	Farm	0.51	0.71	0.12	0.00	3.28		
chrysanthemyl acetate	Nature	1.46	1.91	0.33	0.00	7.11	0.0252	*
	Farm	0.80	1.73	0.30	0.00	7.81		
bornyl acetate	Nature	0.67	1.13	0.19	0.00	5.30	0.0000	***
	Farm	0.30	0.51	0.09	0.00	2.05		

P-value > 0.05: n.s. (non significant); P-value < 0.05: * (significant at 95%); P-value < 0.01:** (significant at 99%); P-value < 0.001: *** (significant at 99.9%)

Table 4. Average of chemical components in climatic groups.

Compounds	Semi-Arid	Arid	Hyper-Arid
camphene	4.67	5.79	4.21
p-cymene	9.48	2.03	4.47
1,8-cineole	11.94	12.03	10.03
(z)-β - ocimene	0.00	1.21	0.72
(E)-β - ocimene	0.32	0.10	0.23
γ–terpinene	0.00	0.21	0.39
cis-thujone	8.53	5.13	14.43
trans-thujone	16.04	4.51	6.54
trans-pinocarveol	0.53	2.25	4.14
cis-pinocarveol	0.00	7.34	4.61
camphor	27.11	29.66	20.11
pinocarvone	1.21	2.98	3.60
borneol	0.18	2.88	1.36
terpinen-4-ol	0.95	3.44	5.90
myrtenol	0.94	0.75	0.37
chrysanthemyl acetate	0.97	1.60	1.38
bornyl acetate	0.31	0.59	0.80

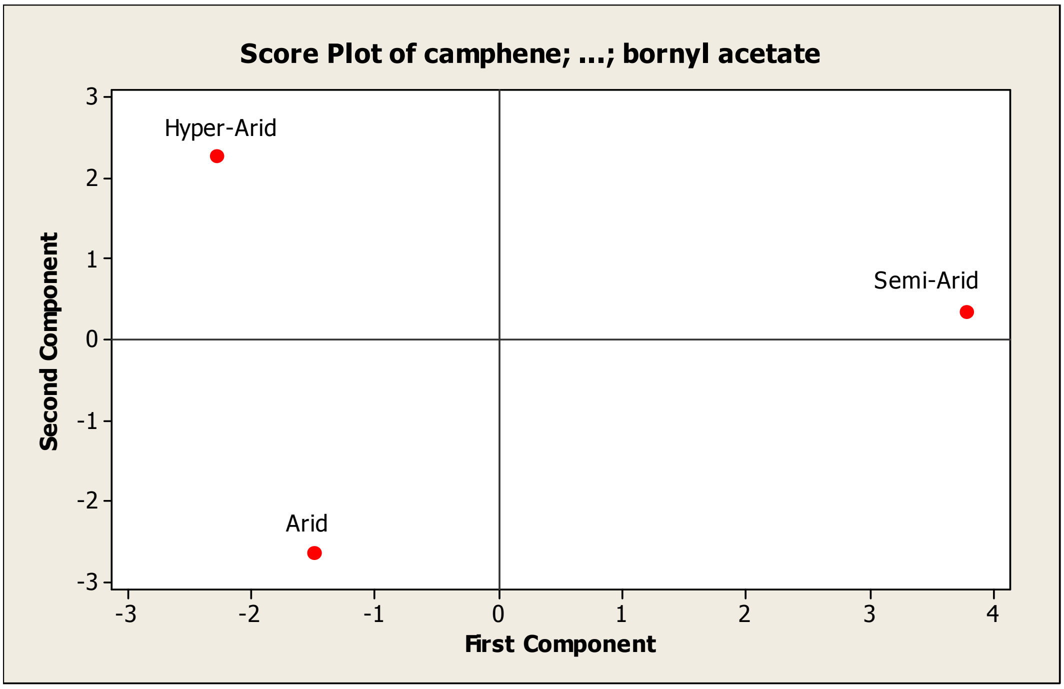

Fig.3. Grouping of populations on the base of climate.

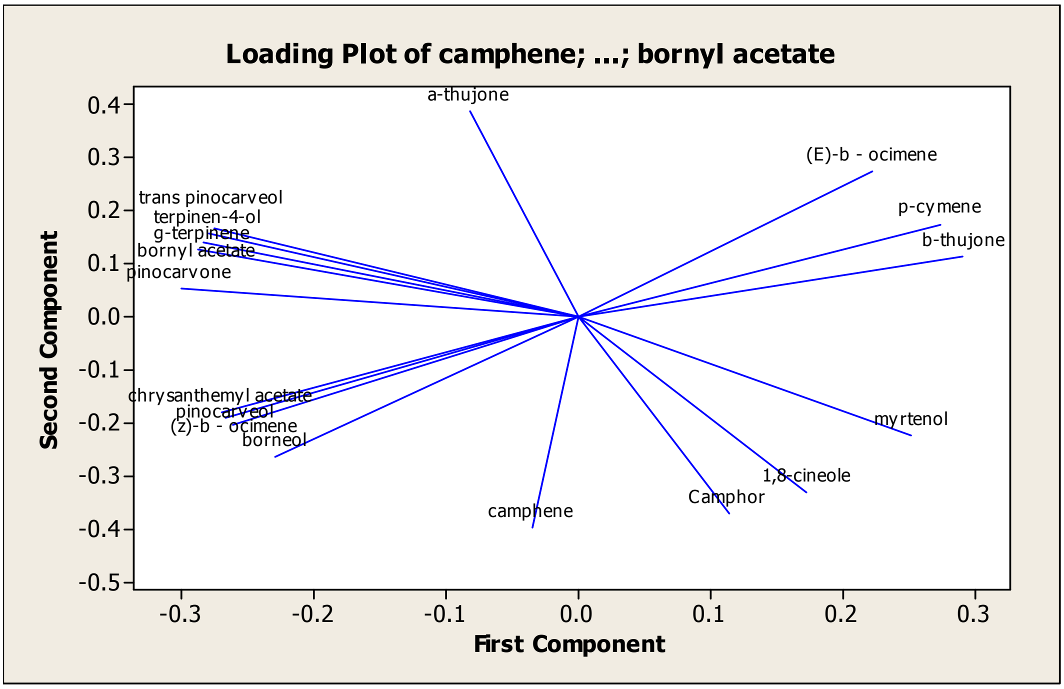

Fig. 4. Effective parameters in populations grouping.

(9.71 % in nature and 8.72 % in farm), trans-thujone (6.14 % in nature and 4.76 % in farm) and camphene (4.98 % in nature and 6.29 % in farm). On the base of the results, chemical components of essential oil in *A. sieberi* were fixed as chemotaxonomic characteristics. These components didn't change when populations were planted in farm condition. In fact, they can be used as indices for recognizing of the species. Also, in spite of significant difference between yield of nature and farm, environmental parameters such as climate, soil and altitude didn't have significant correlation with the yield. Chemical component cis-thujone is the index for dryness of habitat, because this component in hyper-arid habitats is more than arid and semi-arid ones.

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