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Ecological Factors Regulate Essential Oil Yield, Percent and Compositions of Endemic Yarrow (*Achillea eriophora* DC.) in Southeast Iran

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Abstract
Endemic yarrow (*Achillea eriophora* DC.) has been widely used in folk medicine for centuries. The impact of climatic and edaphic conditions was investigated on essential oil (EO) yield and compositions of different yarrow ecotypes, native to Kerman province in southeast of Iran. The aerial part of plants was collected from 13 natural habitats. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a significant variation in EO yield and compositions among ecotypes. 116 components were identified from 13 regions. EO extraction yields fluctuated from 0.71 to 5.83% (v/w) in KP12 and KP02 regions, respectively. The predominant components were L-borneol (0-45.93%), bornyl acetate (0-22.45%), bornyl ester (0-10.46%), (E)-α-ionol (0-28.77%), 6βBicyclo [4.3.0] nonane5β-iodomethyl-1β-isopropenyl-4α, 5α-dimethyl (0-12.14%) and eucalyptol (0- 3.96%). For the first time, the presence of hexadecenoic acid, 6β-bicyclo [4.3.0] nonane and 5β-iodomethyl-1β-isopropenyl-4α, 5α-dimethyl were reported in *A. eriophora*. EO yields and compositions were affected by the ecological factors. Among them, soil pH and latitude showed the most significant impacts.

Keywords: *Achillea eriophora*, Chemical variability, Correlation analysis, L-borneol.

Introduction

Native medicinal plants have been extensively used in health care and food products. These plants are valuable and rich sources of active biological compounds with antiviral, antifungal, antibacterial, pesticide, and antioxidant effects (Kordali et al., 2005; Abed, 2007). According to the World Health Organization (WHO, 2008) more than 80% of the world population rely on traditional medicine for their basic health care. Also, the growing prevalence of drug-resistant bacteria or viruses and low susceptibility to antibiotics increase the threat of infectious diseases; therefore, they raise the concern for new infection-fighting resources (Vital and Rivera, 2009). *Achillea* species are recommended as effective tonic, tranquilizers, diuretic, carminative remedies and widely recommended for the treatment of stomach illness, hay fever, inflammation, gastrointestinal, hemorrhoid, and wound healing properties in folk medicine. They are also recognized as effective drugs that

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enhance breast-feedings and regulate menstruation (Agar et al., 2015; Jarić et al., 2007; Zargari, 1997).

Achillea eriophora DC. belongs to Asteraceae family. The genus *Achillea* is one of the most important genus in this family, with more than 130 species which 19 of them are growing wild in Iran (Mozaffarian, 2009). *A. eriophora* grows mostly in the south of Iran at the altitude of 700-3000 m (Huber-Morath, 1986).

The first scientific study on *A. eriophora* was reported by Weyerstahl et al. (1997c), in Shiraz Bajgah region, who identified 90 compounds in the essential oil (EO). Jaimand and Rezaee (2004) studied the EO compositions of *A. eriophora* leaf and flower in Bamoo region (25 Km Shiraz, south Iran), which were extracted by different form of distillations (distillation by water or steam). The dominant compounds of *A. eriophora* EO that was collected from the Khabr region (Kerman, Baft) included camphor, 1,8-cineole, α- thujene, camphen, and β-thujene (Saberi-ameli et al. 2007). It has been reported that essential oil yield and its quality are related to climate, genetic, soil conditions, growth stage (vegetative or flowering stage), organogenesis, and harvesting time (Gudaitytė and Venskutonis, 2007; Nemeth, 2005). Variation of yields (from 0.14 to 0.44%) and essential oil compositions (no = 54) were detected from different populations of *Achilla wilhelmsii* in south Iran. The three top commonly components in EOs were chrysanthenone (0.2-38.8%), *trans*-carveol (tr-27.5%) and linalool (0.8–26.1%). It was speculated that the discrepancy among EO of ecotypes was due to geographical and climatic differences (Saeidi et al., 2018). However, essential oils are considered to have high heritability (Sagnard et al., 2002). Franz (1993) showed that chemical variation in EOs can be explained by the following factors: 1) genetic variation in the population; 2) diversity among different parts of each plant and growth stages; 3) environmental factors. Furthermore, it was reported that the medicinal plant substances, specially secondary metabolites, are the reflection of environmental factors, growth stage and plant genetic background (Liu et al., 2015). Weather factors including temperature, precipitation and soil can affect oil content and composition in many aromatic plants (Barra, 2009; Sangwan et al., 2001; Moghaddam and Farhadi, 2015). In some cases, soil pH is the most important environmental factor affecting chemical composition of the EOs. The EO of *Rosmarinus offcinalis* L. varied strongly as a result of exposure to different soil pH (Barra, 2009). While, the essential oil of *Helichrysum italicum* ssp. microphyllum was not affected by pH at the same altitude and climate conditions (Satta et al., 1999). Proximal latitudes influence the chemical composition of the EO of *Citrus bergamia* cv. Risso (Di Giacomo and Mincione, 1995). The EO of *Hyptis suaveolens* L. obtained from low latitudes was higher in sesquiterpenes (Azevedo et al., 2002). Similar results were obtained for the EO of *Satureja montana* L. ssp. montana (Slavkovska et al., 2001). Letchamo et al. (1995) concluded that higher altitude and variation in soil type had profound effects in the spread and amount of volatile constituents of *Thymus serpylloides*. Özguven and Tansi (1998) and Pirbalouti et al. (2011) showed that altitude was the major factor that affect the physiological and chemical characters of *Thymus* species and *Thumys daenensis* cv. Celak. Hassiotis et al. (2014) stated that the EO content of *Lavandula angustifolia* was positively regulated by temperature and flowering stage. The assessment of EO composition pertaining to environmental conditions can provide important insight into the factors that determine chemical polymorphism in EO. Therefore, the aims of this study were to investigate the chemical profiles of *A. eriophora* EOs from Kerman ecotypes and to evaluate the impacts of morphological traits, edaphic and climatic conditions on the yield and compositions of EO.

Materials and methods

Plant materials

In the present study, thirteen habitats of *Achillea eriophora* in Kerman province including Qaleasgar (KP01), Kianshahr (KP02), Pabdana (KP03), Shabjare (KP04), Dehshib (KP05), Zoghalsang (KP06), Chatroud (KP07), Arab Abad (KP08), Mianroodab (KP09), Bondar Kouhbanan (KP10), Bardsir (KP11), Zarand (KP12) and Kuhpayeh (KP13) were selected and climatic and edaphic parameters were recorded (Table 1). From each site, the aerial parts of 100 plants were randomly collected at bloom stage in May 2017. The botanical identification was recognized using the Flora Iranica (Rechinger, 1972). Morphological traits such as plant height, number of inflorescences, inflorescence length, diameter, and leaf length were also recorded. The samples were dried at room temperature and kept in cold room until EOs extraction.

Table 1. *Achilla eriophora* ecotypes, environmental conditions, and soil properties of wild growing regions

Regions	Altitude (m)	Latitude (°N)	Longitude (°E)	Precipitation (mm)	Average temperature (°C)	pH	EC	Organic matters (%)	Clay	Silt	Sand	Lime percentage	P (ppm)	K (ppm)
KP01	2615.7	29.530	56.666	270.6	10.03	7.1	0.81	2.8	6.8	21.1	72.1	9.0	48.33	412.24
KP02	2293.86	31.161	56.430	140	16.00	7.26	1.13	0.61	5.7	17.65	76.65	13	46.67	315.62
KP03	2208.46	31.189	56.446	142	16.20	8.12	1.313	0.55	2.2	11	86.8	13	57.65	204.15
KP04	1689.9	31.050	56.205	109	18.82	7.81	1.534	0.67	1.16	5.74	93.1	11.75	29.49	318.52
KP05	2190.03	30.675	56.991	42.6	22.05	7.8	27.6	0.82	8.7	20.5	70.8	22.5	24.80	376.89
KP06	2592.94	31.179	56.510	129.2	17.78	7.46	0.87	3.06	8.2	19	72.8	6.25	87.60	496.13
KP07	1837	30.543	56.970	88	18.53	7.65	3.11	0.97	9.2	18.65	72.15	23.25	53.04	284.44
KP08	2566.4	29.927	57.523	108.2	16.18	8.4	0.911	1.17	6.29	19.91	73.8	26.75	60.36	450.51
KP09	2720	29.417	56.625	265	11.03	8.07	13.2	0.99	15.29	16.91	67.8	8	48.06	645.57
KP10	1946.63	31.401	56.412	149.4	16.75	8.27	2.31	2.44	4.16	9.3	86.54	45	48.60	312.74
KP11	2033.1	29.94	56.565	25.3	18.12	8.19	4.66	0.62	8.2	15.65	76.15	10.5	32.98	726.44
KP12	1699.96	30.749	56.649	109	18.82	8.25	1.012	-	3	7.65	89.35	-	19.83	-
KP13	1882.1	30.491	57.302	88.9	18.43	8.34	1.014	0.53	6.79	12.41	80.8	15.5	55.38	224.33

- : missing data

Soil parameters

Soil reaction and soil electrical conductivity was measured using pH meter and EC meter on soil saturation extracts. Hydrometric method was used to determine soil texture. Lime percentage was evaluated using titration method. The Walkley and Black (1934) method used to determine soil Organic Matters (OM). Available phosphorus content was evaluated using the Olsen method (1982). Potassium content was determined using Flame Photometric method.

Extraction of essential oil

Dried pulverized samples (100 g) were hydro-distilled using a Clevenger-type apparatus until no more condensing EO was seen (4 h). All experiments were conducted in triplicate. EOs were stored at 4 °C until analyzed by GC-MS. The amount of EO was measured and the yield was calculated as percent of the ratio between extracted EO and the plant dry weight.

GC-MS detected EOs percentages. The yield was calculated using the following equation, where W1 is the mass of the extracted oil (g) and W2 is the plant dried biomass (g).

$$\text{Essential oil yield (\%)} = W1 / W2 \times 100$$

Gas Chromatography - Mass Spectrometry Analysis (GC-MS)

The EOs were analyzed using GC (Agilent Technologies, USA) coupled with MS (Varian Saturn 2000, USA). The apparatus equipped with a HP- 5MS column (60 m × 0.25 mm i.d., film thickness 0.25 m). Helium was used as carrier gas with a flow rate of 1.5 mL/min, 0.1 µL of each EO was injected and ionization potential was 70 ev. The initial temperature of oven was 40 °C (held for 2 min) then heated up to 240 °C with a

rate of 5 °C/min, and kept constant for 5 min at 240 °C. The identification of compounds was done by mass spectral data, literature and National Institute of Standards and Technology (NIST) computer library.

Results

Essential oil yield, percent and compositions of 13 Achillea eriophora ecotypes

The EOs yield showed significant variation among the 13 *A. eriophora* ecotypes. The maximum content was reported in KP02 ecotype (5.83 %) and the lowest was observed in KP12 ecotype (0.71 %) (Table 2). EO extraction percent fluctuated from 68.21% to 96.92 % in KP05 and KP09 regions, respectively (Table 2). Results showed positive correlation between yield and latitude of plant growing sites (Table 3).

Table 2. Essential oil compositions of *Achilla eriophora* ecotypes from southeast Iran analyzed by GC-MS

Compounds	R.T. (min)	R.I.	KP01	KP02	KP03	KP04	KP05	KP06	KP07	KP08	KP09	KP10	KP11	KP12	KP13
Santolina triene	12.7	807	-	0.18	-	-	-	-	-	-	-	-	-	-	-
L-α-Pinene	13.3	832	-	-	-	-	-	-	0.14	-	-	0.3	-	-	-
Artemisia triene	13.4	848	-	0.33	-	-	-	-	-	-	-	-	-	-	-
β-Thujenee	13.5	925	-	-	-	-	-	-	-	-	-	0.44	-	-	-
1R-α-Pinene	13.8	934	1.06	2.19	-	-	-	1.61	-	-	0.52	-	-	-	-
Cyclofenchene	13.8	946	-	-	2.15	-	-	-	2.49	-	-	-	-	-	2.76
Camphene	14.3	948	1.78	1.16	0.91	-	-	-	-	1.93	-	-	0.58	-	-
Dicyclohexyl ethyl phosphate	14.4	952	-	-	-	-	2.21	-	-	-	-	-	-	-	-
β-Phellandrene	15.1	954	-	0.515	-	-	-	-	-	-	-	-	-	-	-
Cyclooctane, 1,5-dimethyl-2,6-bis(methylene)	15.1	962	-	-	-	-	-	-	2.77	-	-	-	-	-	-
d-Camphene	15.1	964	-	0.48	-	-	-	0.46	-	-	-	-	-	1.19	-
β-Terpinene	15.1	973	-	-	0.28	-	-	0.34	-	-	-	-	-	-	-
L-β-Pinene	15.3	974	-	-	0.38	2.52	-	-	0.67	0.56	-	1.26	-	-	0.77
β-Pinene	15.3	975	-	-	-	0.78	-	-	-	-	-	-	-	-	-
Yomogi alcohol	15.3	988	-	-	-	-	-	0.29	-	-	0.18	-	-	-	-
3-Carene	16.1	1007	-	-	-	1.97	0.7	-	-	2.4	-	7.29	0.81	-	-
L-limonene	16.2	1023	-	-	-	-	-	-	-	-	-	1.74	-	-	-
7-Propylidene-bicyclo[4.1.0]heptane	16.3	1025	-	-	-	-	-	1.84	-	-	1.06	6.04	-	-	2.89
(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	16.3	1029	-	16.63	4.84	-	-	-	0.56	-	-	-	-	-	-
4-δ-carene	16.4	1032	-	-	-	-	-	2.2	-	-	-	1.91	-	-	-
Propanoic acid, 2-methyl-, pentyl ester	16.4	1034	-	-	-	0.61	-	-	-	-	-	-	-	-	-
Butanoic acid, 3-methylbutyl ester	16.6	1042	-	-	-	-	-	-	-	-	-	0.81	-	-	0.46
1,3-Cyclopentadiene, 1,2,3,4,5-pentamethyl	16.6	1046	-	1.48	1.41	-	-	1.55	-	-	-	-	-	-	-
3-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl-, (E)	16.7	1047	-	-	4.06	-	-	-	-	-	-	-	-	-	-
3-Methylene-1,5,5-trimethylcyclohexene	16.8	1053	-	-	-	-	-	-	-	-	-	1.24	-	-	-
Alpha-terpinyl isovalerate	16.9	1063	-	-	-	-	-	1.54	-	-	-	-	-	-	-
m-Cymene	16.9	1068	-	1.9	1.48	0.7	-	3.14	0.42	-	-	-	0.32	-	1.1
Terpinolene	17.1	1079	-	-	-	-	-	-	-	-	-	0.54	-	-	-
Eucalyptol	17.1	1081	0.88	2.13	2.87	-	-	2.42	1.76	-	2.0	-	3.96	1.35	1.95
Terrein	17.2	1095	-	-	-	3.96	-	-	-	6.62	-	-	-	-	-
α-Campholenal	18	1107	-	-	-	-	-	-	1.08	-	-	-	-	0.48	-
cis-p-Menth-2-en-1-ol	18.2	1115	-	0.49	0.41	-	-	0.8	-	-	-	-	-	-	-
n-Amyl isovalerate	18.4	1118	-	-	-	-	-	-	-	-	-	-	-	-	0.63
Acetic acid, 1-methyl-3-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl ester	18.6	1125	-	-	-	-	0.97	-	-	-	-	8.23	-	-	-
γ-Terpinolene	18.9	1128	-	-	-	0.59	-	-	-	0.69	0.32	-	-	-	-
Terpineol, cis-β-	18.9	1129	-	0.74	-	-	-	1.42	-	-	-	-	-	-	-
3-Benzylsulfonyl-2,6,6-trimethylbicyclo(3.1.1)heptane	19	1135	-	2.11	2.22	-	-	-	-	-	-	-	-	3.67	-
trans-Z-α-Bisabolene epoxide	19.1	1138	0.13	-	-	-	-	-	-	-	-	-	-	-	-
Pentanoic acid, pentyl ester	19.2	1144	0.49	-	-	0.72	-	-	0.33	-	-	0.77	-	-	0.62
Anethofuran	19.4	1147	-	-	-	-	-	-	-	-	-	-	-	0.56	-
Isoborneol	19.6	1148	-	-	0.86	1.2	-	-	-	-	-	-	-	-	-
1,5,5-Trimethyl-6-methylene-cyclohexene	19.7	1152	-	-	-	-	-	-	-	-	-	-	-	4.09	-
Borneol	19.96	1153	1.31	-	-	-	-	-	1.90	2.7	-	-	1.37	-	2.01
	20.1	1158	-	9.52	-	-	-	-	-	-	-	-	-	-	-

Table 2. (continued) Essential oil compositions of *Achilla eriophora* ecotypes from southeast Iran analyzed by GC-MS

Compounds	R.T. (min)	R.I.	KP01	KP02	KP03	KP04	KP05	KP06	KP07	KP08	KP09	KP10	KP11	KP12	KP13
4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	20.3	1159	-	-	-	-	-	-	4.80	-	-	-	-	-	-
Furan, 2,5-dibutyl	20.4	1160	-	-	1.51	-	-	-	-	-	-	-	-	-	-
(E)-α-Ionol	21.4	1161	-	-	-	-	11.7	-	-	-	28.77	-	-	-	-
L-Borneol	21.4	1162	38.41	18.78	14.85	43.04	-	41.84	45.16	35.71	3.08	34.16	16.59	0.4	45.93
5-(1-Bromo-1-methylethyl)-2-methylcyclohex-2-enone	21.4	1163	-	-	-	-	-	-	-	1.54	-	-	-	-	-
Cyclopentanecarboxylic acid, 3-isopropylidene-, bornyl ester	21.4	1164	-	7.49	-	2.53	-	10.46	1.9	2.4	-	5.39	-	-	-
Terpinen-4-ol	21.5	1165	-	-	0.75	-	-	0.67	-	-	-	-	1.39	-	-
Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene	21.5	1166	0.22	-	-	0.76	-	-	-	-	-	-	-	-	0.55
Naphthalene, 1,1'-(1,10-decanediyl)bis[decahydro	21.5	1170	-	-	7.51	-	-	-	-	-	-	-	-	-	-
Terpinyl acetate	21.7	1173	0.75	-	1.70	1.82	-	-	0.72	3.02	2.03	1.76	3.07	-	0.85
α-Terpineol	21.8	1175	-	-	-	-	-	1.72	-	-	-	-	-	0.29	-
Isogeraniol	22.1	1181	-	-	0.49	-	-	-	-	0.38	-	-	0.85	0.35	-
Geraniol	22.1	1186	0.25	-	-	-	-	-	-	-	-	-	-	0.37	-
(4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	22.5	1195	-	5.22	-	-	-	-	-	-	-	-	-	-	-
Bornyl bromide	22.6	1195	-	-	1.548	-	-	-	-	-	-	-	2	-	-
Fenchyl acetate	23.5	1209	-	1.9	-	-	-	-	-	-	-	-	-	-	-
Farnesol	24	1215	-	-	-	-	-	-	-	-	0.37	-	-	-	-
Longifolene-(V4)	24.1	1235	-	-	-	-	1.47	-	-	-	-	-	-	-	-
Cyclopentanecarboxylic acid, 3-methylene-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester(bornyl acetate)	24.2	1251	8.42	-	10.8	4.74	1.2	7.16	3.64	4.68	2.04	3.74	22.45	2.46	4.70
Isocyclocitral	24.3	1264	-	0.74	0.71	-	-	-	-	-	-	-	-	-	-
Bornyl acetate	24.5	1270	0.57	-	-	-	-	-	0.32	-	3.9	-	-	31.27	0.41
Thymol	24.6	1273	2.19	-	3.23	2.01	-	2.02	2.44	3.13	2.28	-	3.02	2.94	2.07
Geraniol butyrate	25.3	1287	-	-	-	-	0.32	-	-	-	-	-	-	-	-
Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl	25.7	1290		-	-	0.65	-	-	0.41	-	-	-	-	-	-
Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	26.6	1323		-	-	-	-	-	-	0.63	-	-	-	-	-
«delta»-Elemene	26.7	1340	-	-	-	-	-	-	-	-	-	-	-	0.29	-
Geranyle acetat	26.8	1354		0.45	-	-	-	-	-	0.26	-	0.77	-	-	0.46
β-Cubebene	27.2	1383	-	-	-	-	-	-	-	-	0.54	-	-	-	-
Geranyl butyrate	27.4	1384		-	2.15	-	-	-	-	-	-	-	-	-	0.48
Fenchyl acetate	27.4	1390	-	-	-	-	-	0.64	-	0.72	-	-	-	-	-
Bicyclo[2.2.1]heptane, 2-[9-borabicyclo[3.3.1]non-9-yloxy]-, 1,7,7-trimethyl	27.5	1412	-	-	-	-	-	-	-	-	-	-	4.2	-	-
Caryophyllene	27.5	1419	1.71	0.68	1.86	0.73	-	0.70	0.72	-	0.3	0.61	-	1.05	-
Isocaryophillene	27.6	1422	-	-	-	-	-	-	-	-	2.86	-	-	-	-
Isoaromadendrene epoxide	27.7	1435	5.35			4.891									
Alloaromadendrene oxide-(2)	28.8	1435	0.46	-	-	0.1	-	-	-	-	-	-	-	-	-
Aromadendrene	28.8	1439	-	-	-	-	-	-	-	1.42	-	-	-	-	-
γ-Elemene	29.8	1449	-	-	0.62	0.55	-	-	0.25	0.42	0.26	0.61	1.08	-	-
Benzenepropanoic acid, pentyl ester	30.8	1455	0.71	-	-	-	-	-	-	-	-	-	-	-	-
3-Phenyl-propionic acid, isopropyl ester	31.1	1469	-	-	-	-	-	-	0.09	-	-	-	-	-	-
γ-HIMACHALENE	31.3	1471	-	-	-	2.84	-	-	3.74	-	-	-	1.37	-	-
β-Guaiene	31.9	1478	-	-	2.69	-	-	1.18	-	-	-	-	3.47	-	-
Urs-12-en-28-al, 3-(acetyloxy)-, (3β)-	32.2	1509	-	-	-	-	7.11	-	-	-	-	-	-	-	-
Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	32.2	1526	1.79	-	-	-	-	-	-	4.41	-	1.25	-	4.25	2.46
(E)Nerolidol	32.3	1550	-	1.33	-	-	-	2.92	1.63	1.33	-	-	-	0.05	-
Aromadendrene oxide-(2)	32.5	1550	-	-	1.17	-	-	-	-	2.18	1.16	0.56	-	1.16	1.22
Geranylgeraniol	32.8	1553	-	-	-	-	-	-	-	-	-	2.39	-	-	2.97
6ββBicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-,	32.8	1557	8.69	-	6.43	4.18	2.03	-	-	-	12.14	-	8.09	-	-
L-Spathulenol	33	1566	0.44	-	-	-	-	-	-	-	-	-	-	-	-
Fenretinide	33.1	1568	-	-	-	-	10.44	-	-	-	-	-	-	-	-
Longipinocarvone	33.3	1569	-	-	-	-	-	-	0.98	-	-	-	-	-	-
Caryophyllene oxide	33.4	1570	-	1.73	-	-	-	2.78	-	-	-	4.79	-	7.83	-
Gitoxigenin	33.8	1571	-	-	-	-	10.15	-	-	-	-	-	-	-	-
2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)	34.4	1574	-	-	6.63	-	1.79	-	7.29	8.16	8.89	-	9.09	-	5.36
iPropyl 9tetradecenoate	34.4	1576	-	1.03	-	-	-	1.35	-	-	-	1.07	-	-	-
13-Bromotetradecanoic acid	34.5	1583	2.22	-	2.07	2.58	-	-	2.34	-	2.58	-	1.24	-	1.97
Betulin	34.6	1592	-	-	-	-	2.63	-	-	-	-	-	-	-	-
Butyl 9-tetradecenoate	34.6	1598	-	-	-	-	-	-	-	-	-	-	-	2.06	-
Isoaromadendrene epoxide	35.2	1634	-	-	-	-	-	-	1.16	-	-	-	1.67	-	-
Geranyllinalool	35.2	1653	0.22	-	-	-	-	-	-	-	-	-	-	-	-
β-Santanol acetate	35.2	1676	-	-	-	-	-	-	-	-	-	-	-	-	2.83
Pentadecanal-	35.9	1695	-	-	-	-	-	-	0.19	-	-	-	-	0.62	-

Table 2. (continued) Essential oil compositions of *Achilla eriophora* ecotypes from southeast Iran analyzed by GC-MS

Compounds	R.T. (mn)	R.I.	KP01	KP02	KP03	KP04	KP05	KP06	KP07	KP08	KP09	KP10	KP11	KP12	KP13
2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	36	1698	0.51	-	-	-	0.99	-	-	0.18	0.72	-	-	-	-
Hexahydrofarnesyl acetone	36.1	1832	-	-	-	-	-	-	0.32	-	-	-	-	1.12	-
L-Isolongifolol, acetate	36.8	1857	-	-	-	-	0.86	-	-	-	-	-	-	-	-
Methyl hinokiate	37.2	1865	0.34	-	-	-	-	-	-	-	-	-	-	-	-
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	37.3	1909	0.13	-	0.82	-	-	-	-	-	0.67	-	-	-	-
3Alpha,5-cyclo-6beta,19-epoxy-5alpha-androstan-17-one	38.6	2367	-	-	-	-	4.60	-	-	-	-	-	-	-	-
Hexadecenoic acid	38.8	2438	1.74	0.68	2.17	2.14	-	1.19	1.67	1.91	2.27	0.95	2.2	3.78	1.99
2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-,	38.9	2454	0.46	-	-	-	-	-	-	-	-	-	-	-	-
1-Tricosanol	39.2	2468	0.43	-	-	-	-	-	-	-	-	-	-	-	-
1-Decanol, 2-hexyl-	40.3	2478	-	-	-	-	-	-	-	-	-	-	-	-	4.44
7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene	40.5	2540	-	0.56	-	1.65	-	-	1.63	2.08	-	-	-	1.74	-
Total			91.7	69.01	96.14	90.00	68.21	95.59	95.51	91.42	96.92	92.05	95.09	76.79	94.12

R.T. = Retention Time
R.I. = Retention Index

Table 3. Correlations among some of climatic, edaphic conditions and morphological traits with *Achilla eriophora* essential oil yield and main compositions

Factor	Essential oil yield	Cyclopentanecarboxylic acid, 3-methylene-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester(Bornyl acetate)	Bicyclo[2.2.1]heptan-2-ol, 2-allyl-1,7,7-trimethyl-	Cyclopentanecarboxylic acid, 3-isopropylidene-, bornyl ester	Terpinyl acetate	Thymol	Caryophyllene	γ-Elemene	6ββicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4aα,5α-dimethyl-,	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	13-Bromotetradecanoic acid	Aromadendrene oxide-(2)	*i*-Propyl 9-hexadecenoate	Eucalyptol
Precipitation (mm)	-0.13^{ns}	-0.25^{ns}	0.01^{ns}	0.01^{ns}	-0.03^{ns}	0.02^{ns}	0.50*	-0.28^{ns}	0.52*	-0.13^{ns}	0.35^{ns}	0.12^{ns}	0.12^{ns}	-0.11^{ns}
Average temperature (°C)	0.11^{ns}	-0.04^{ns}	-0.04^{ns}	0.08^{ns}	-0.23^{ns}	-0.19^{ns}	-0.41^{ns}	0.05^{ns}	-0.63*	-0.12^{ns}	-0.39^{ns}	-0.17^{ns}	-0.18^{ns}	-0.10^{ns}
Latitude (°N)	0.51*	-0.23^{ns}	0.12^{ns}	0.56*	-0.39^{ns}	-0.42^{ns}	0.20^{ns}	-0.02^{ns}	-0.63*	-0.54*	-0.39^{ns}	-0.23^{ns}	-0.27^{ns}	-0.09^{ns}
Longitude (°E)	-0.26^{ns}	-0.16^{ns}	0.16^{ns}	-0.31^{ns}	0.10^{ns}	0.20^{ns}	-0.49^{ns}	-0.26^{ns}	-0.31^{ns}	0.46^{ns}	-0.12^{ns}	0.55*	-0.03^{ns}	-0.21^{ns}
Altitude (m)	0.26^{ns}	0.00^{ns}	-0.09^{ns}	0.21^{ns}	0.10^{ns}	0.03^{ns}	0.04^{ns}	-0.21^{ns}	0.41^{ns}	0.17^{ns}	-0.07^{ns}	0.16^{ns}	-0.28^{ns}	0.09^{ns}
pH	-0.57*	0.27^{ns}	-0.09^{ns}	-0.63*	0.60*	0.44^{ns}	-0.17^{ns}	0.40^{ns}	0.18^{ns}	0.43^{ns}	0.09^{ns}	0.73*	0.51*	-0.13^{ns}
Organic Matters (%OM)	0.19^{ns}	-0.10^{ns}	0.31^{ns}	0.73**	-0.20^{ns}	-0.21^{ns}	0.05^{ns}	-0.15^{ns}	-0.34^{ns}	-0.43^{ns}	-0.50^{ns}	-0.12^{ns}	-0.30^{ns}	-0.16^{ns}
Lime percentage	-0.08^{ns}	-0.27^{ns}	0.18^{ns}	0.02^{ns}	0.12^{ns}	-0.42^{ns}	-0.11^{ns}	0.13^{ns}	-0.48^{ns}	-0.17^{ns}	-0.39^{ns}	0.18^{ns}	-0.37^{ns}	-0.62*
P	0.36^{ns}	0.04^{ns}	0.55*	0.60*	0.00^{ns}	0.14^{ns}	0.11^{ns}	-0.14^{ns}	-0.18^{ns}	0.14^{ns}	-0.03^{ns}	0.15^{ns}	-0.23^{ns}	0.24^{ns}
K	-0.18^{ns}	0.47^{ns}	-0.38^{ns}	-0.05^{ns}	0.44^{ns}	0.25^{ns}	-0.49^{ns}	0.35^{ns}	0.58*	0.40^{ns}	-0.07^{ns}	-0.06^{ns}	0.22^{ns}	0.35^{ns}
Plant height	-0.07^{ns}	0.38^{ns}	0.10^{ns}	-0.30^{ns}	0.12^{ns}	0.15^{ns}	-0.21^{ns}	0.23^{ns}	0.04^{ns}	-0.01^{ns}	0.23^{ns}	-0.27^{ns}	0.19^{ns}	0.16^{ns}
Inflorescence diameter	-0.14^{ns}	-0.18^{ns}	0.19^{ns}	-0.29^{ns}	-0.11^{ns}	0.05^{ns}	-0.07^{ns}	-0.03^{ns}	-0.37^{ns}	0.00^{ns}	0.21^{ns}	-0.02^{ns}	0.04^{ns}	-0.23^{ns}
Inflorescence number	-0.10^{ns}	0.03^{ns}	-0.17^{ns}	-0.42^{ns}	-0.04^{ns}	-0.07^{ns}	-0.11^{ns}	0.09^{ns}	0.08^{ns}	-0.07^{ns}	0.25^{ns}	-0.26^{ns}	-0.09^{ns}	-0.20^{ns}
Inflorescence length	-0.04^{ns}	0.43^{ns}	-0.29^{ns}	-0.40^{ns}	0.10^{ns}	-0.01^{ns}	-0.39^{ns}	0.29^{ns}	0.14^{ns}	0.10^{ns}	0.00^{ns}	-0.37^{ns}	-0.05^{ns}	0.13^{ns}
Leaf length	0.35^{ns}	-0.39^{ns}	-0.01^{ns}	0.13^{ns}	-0.13^{ns}	-0.08^{ns}	-0.05^{ns}	-0.28^{ns}	-0.40^{ns}	0.00^{ns}	-0.28^{ns}	0.30^{ns}	-0.32^{ns}	-0.30^{ns}
Clay	-0.11^{ns}	-0.05^{ns}	-0.21^{ns}	-0.08^{ns}	0.05^{ns}	-0.03^{ns}	-0.53^{ns}	-0.17^{ns}	0.42^{ns}	0.51*	0.16^{ns}	-0.03^{ns}	-0.20^{ns}	0.25^{ns}
Silt	0.33^{ns}	-0.05^{ns}	-0.06^{ns}	0.21^{ns}	-0.09^{ns}	-0.13^{ns}	-0.47^{ns}	-0.26^{ns}	-0.04^{ns}	0.37^{ns}	-0.26^{ns}	-0.07^{ns}	-0.54*	0.14^{ns}
Sand	-0.16^{ns}	0.06^{ns}	0.13^{ns}	-0.09^{ns}	0.03^{ns}	0.09^{ns}	0.54*	0.24^{ns}	-0.17^{ns}	-0.46^{ns}	0.09^{ns}	0.06^{ns}	0.43^{ns}	-0.20^{ns}

ns: not significant *:significant at 5% level **: significant at 1% level

GC-MS analysis of the composition of EOs revealed very interesting profiles (Fig. 2-7). The composition of EOs, the percentage of each composition and their retention times are summarized in Table 2. The major and minor 116 components representing an average of 88.7% of the EO compounds were identified.

In the present study, the main components of EO were L-borneol, cyclopentanecarboxylic acid, bornyl acetate and thymol, which were presented in almost all samples (Table 2). L- borneol, which constitutes 26% of the EO was presented as the main component in all regions except KP05, KP09 and KP12. Whereas (E)-α-ionol was the main component in EOs of KP05 and KP09 and it was just found in these regions. Bornyl acetate emerged as a major composition in

KP12. 1, 8-cineol, thymol, bornyl acetate *m*-cymene, caryophyllene, bornyl ester were presented as the main components of the EO of *Achillea* species while in the present study, hexadecenoic acid and 6βbicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl were identified for the first time in *Achillea*.

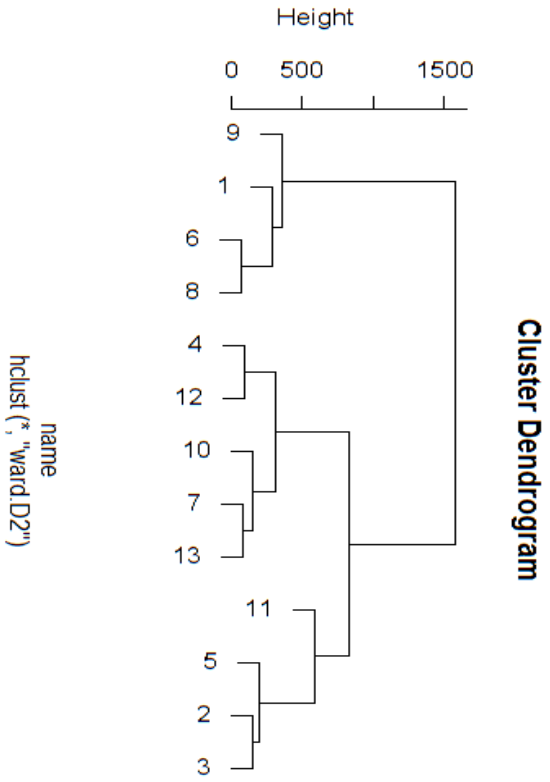

Fig. 1. Dendrogram of the 13 ecotypes of *A. eriophora* resulting from the cluster analysis of all traits and components based on Ward method.

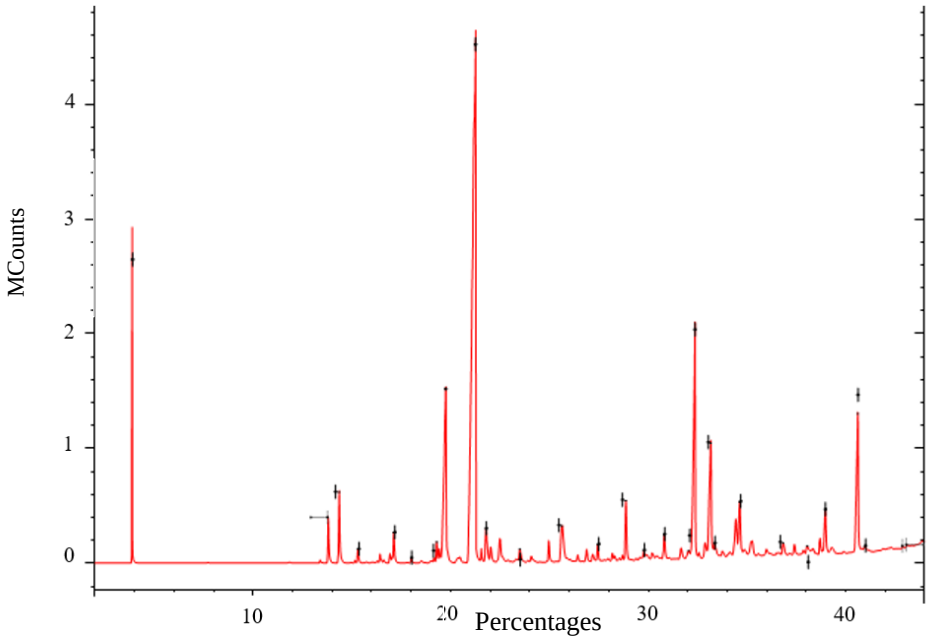

Fig. 2. GC-MS chromatogram of *Achilla eriophora* ecotype from KP01

Relationship between ecological conditions and essential oil

Results showed a significant positive and negative correlations between EO yield with latitude (r = 0.51*) and pH (r = -0.57*), respectively (Table 3). These results showed that the high latitude and low pH increased the EO yield of *A. eriophora*. KP02 had the highest yield and the lowest pH (7.26) among all regions.

Among the 13 peaks, four components have no significant correlation with ecological factors, while the other nine components have significant correlation with up to four ecological factors (Table 3). For example, bornyl ester, is significantly correlated with four ecological factors, and similarly 6βBicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl. Result showed 12 ecological factors are significantly correlated with 1– 4 chemical components.

In this study, the main EO compositions were significantly affected by precipitation, temperature, latitude, longitude, pH, organic matters, lime percentage, available P and K, clay, silt and sand. Meanwhile, main EO components did not significantly changed by longitude and plant morphological traits including plant height, inflorescence diameter, number, length and leaf length (Table 3).

The highest temperature belonged to KP05 (Table 1). Some component such as dicyclohexyl ethyl phosphate, longifolene-(V4), geraniol butyrate, L-isolongifolol, acetate, fenretinide, gitoxigenin, Urs-12-en-28-al, 3-(acetyloxy)-, (3β), betulin and 3α,5-cyclo-6beta,19-epoxy-5alpha-and rostan-17-one only found in this area (Table 2). Meanwhile, 6βBicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-, showed significant negative correlation (r = -0.63*) with temperature (Table 3).

KP09 has the minimum latitude among the studied regions. Farnesol, isocaryophillene and β-cubebene specifically found in KP09. Moreover, there were some components such as 6β-bicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl- and 1,4-methanoazulene, 7-bromodecahydro-4,8,8-trimethyl-9-methylene-, which showed the highest percentage in KP09.

Results of this investigation showed that KP06 had the highest soil organic matters and the lowest lime percentage. Bornyl ester, cis-p-menth-2-en-1-ol and (E) nerolidol were found in the highest amounts in this area. Thujone, a harmful compound for human body, only found at tiny amount in Kuhbanan ecotype. This area has the lowest latitude among studied regions. Among evaluated regions, KP08 and KP04 had the highest and lowest longitude, respectively. Some components such as terrein, borneol, tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4,4-dimethyl-, aromadendrene oxide and 7-oxabicyclo [4.1.0] heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene- were in their highest amount in KP08. The highest plant height and diameter, length and number of inflorescences belonged to KP04. The highest precipitation belonged to Qaleasgar and isoaromadendrene epoxide compound was the highest in this region. Anethofuran, 1,5,5-trimethyl-6-methylene-cyclohexene, butyl 9-tetradecenoate were just found in KP12.

Cluster analysis using all morphological traits and EO components grouped 13 ecotypes of *A. eriophora*, in three clusters (Fig. 1). Ecotypes of KP09, KP01, KP06 and KP08 were grouped into the first cluster. This cluster had the highest phosphorus and potassium content, organic matters, precipitation and EO percentage. Ecotypes of KP04, KP12, Kouhbanan and KP13 were clustered into the second group, which had the highest L-borneol and lime percentage. Ecotypes of KP11, KP05, KP02 and KP03 clustered in the third group that was rich in bornyl acetate and EO yield, while it was poor for L-Borneol and EO percentage.

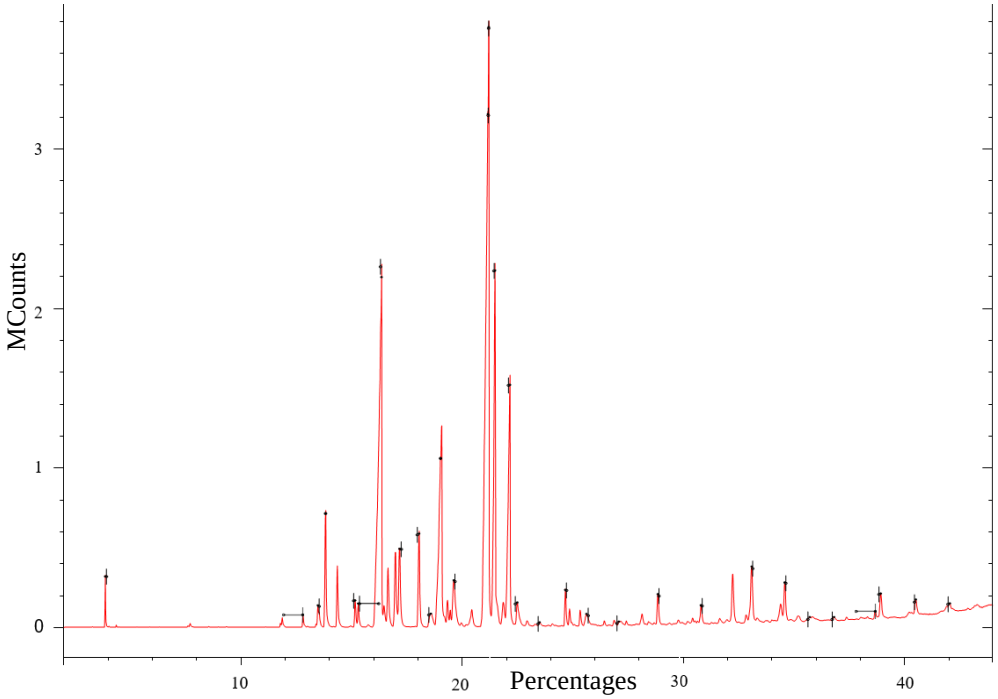

Fig. 3. GC-MS chromatogram of *Achilla eriophora* ecotype from KP02

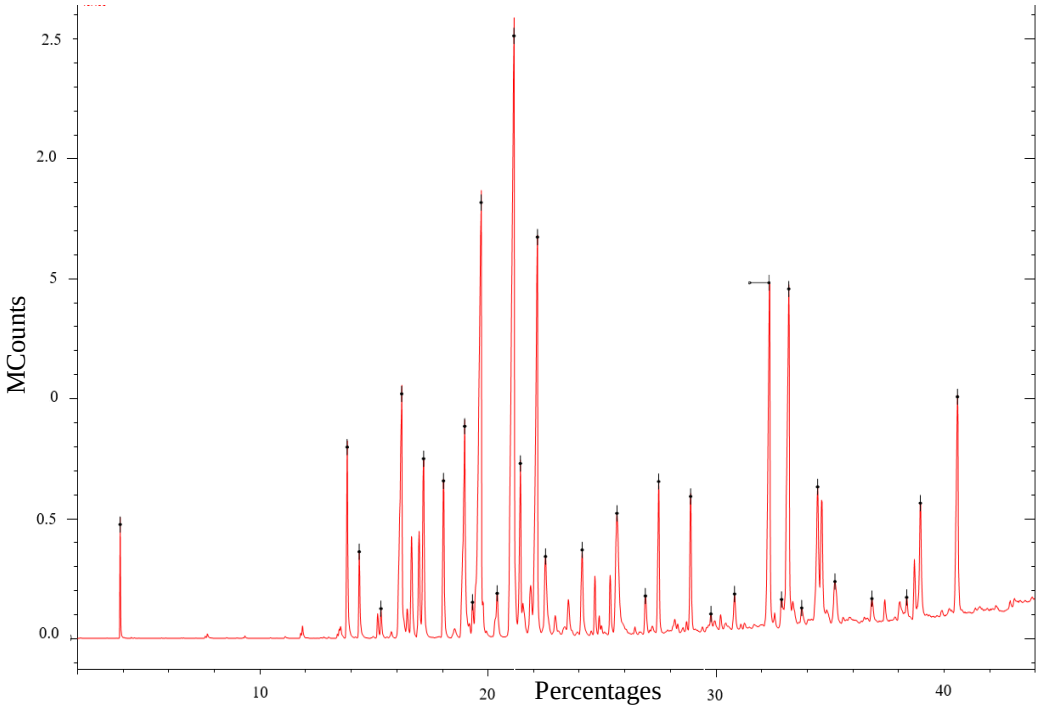

Fig. 4. GC-MS chromatogram of *Achilla eriophora* ecotype from KP03

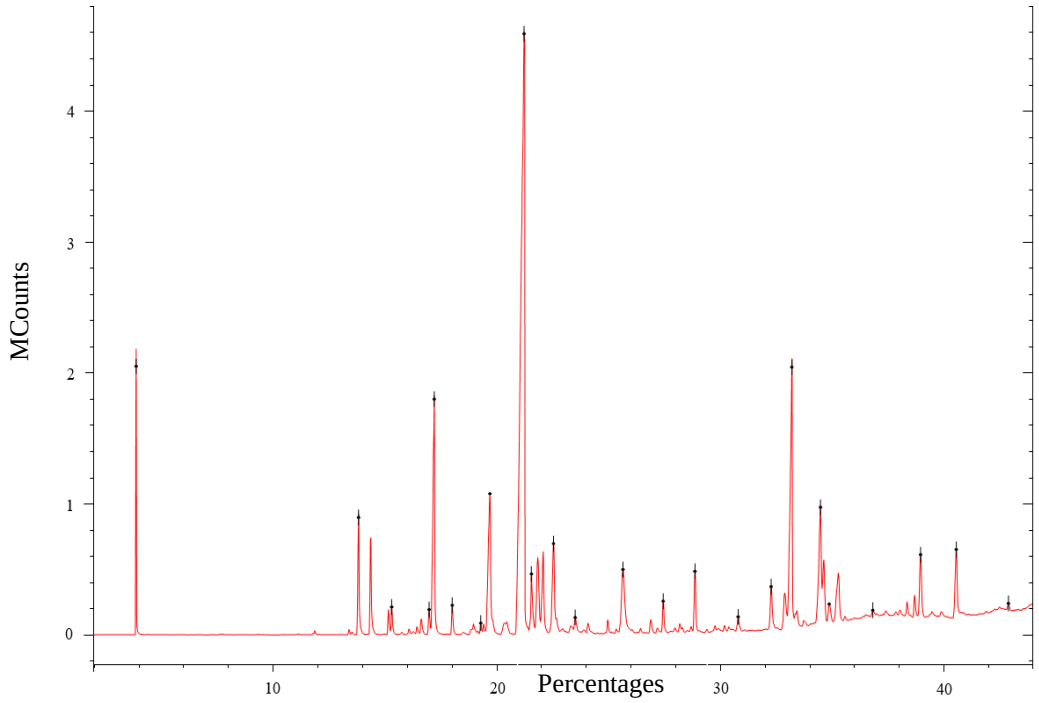

Fig. 5. GC-MS chromatogram of *Achilla eriophora* ecotype from KP08

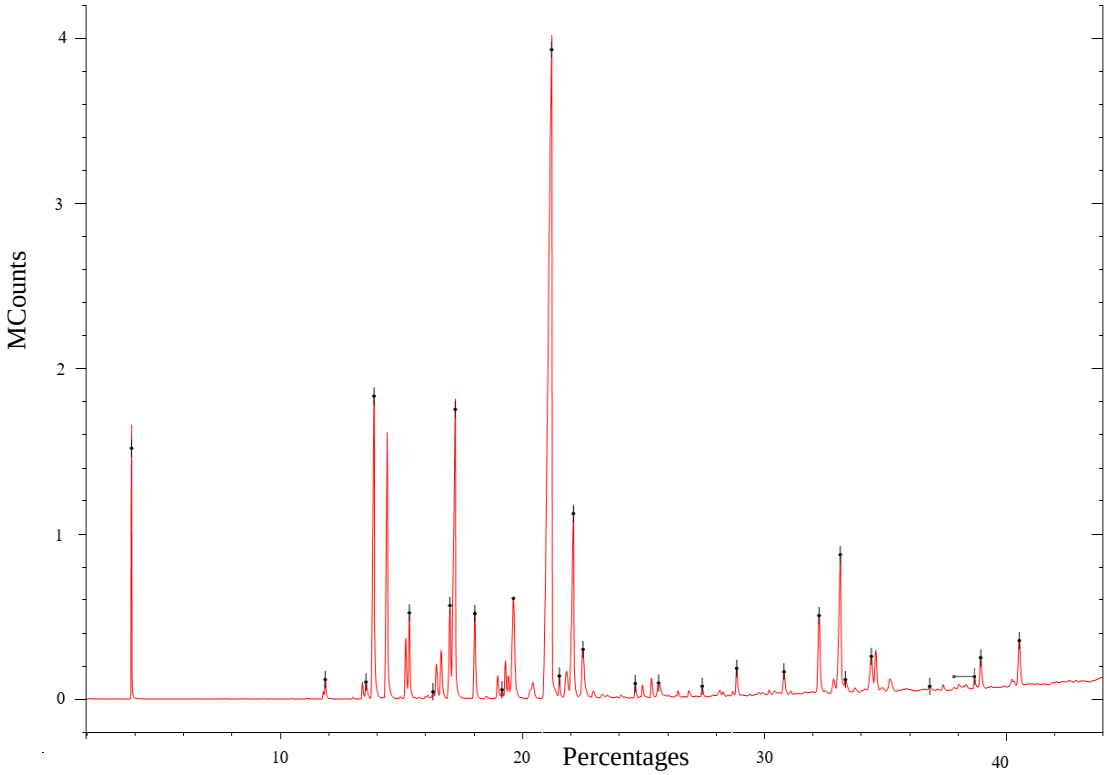

Fig. 6. GC-MS chromatogram of *Achilla eriophora* ecotype from KP10

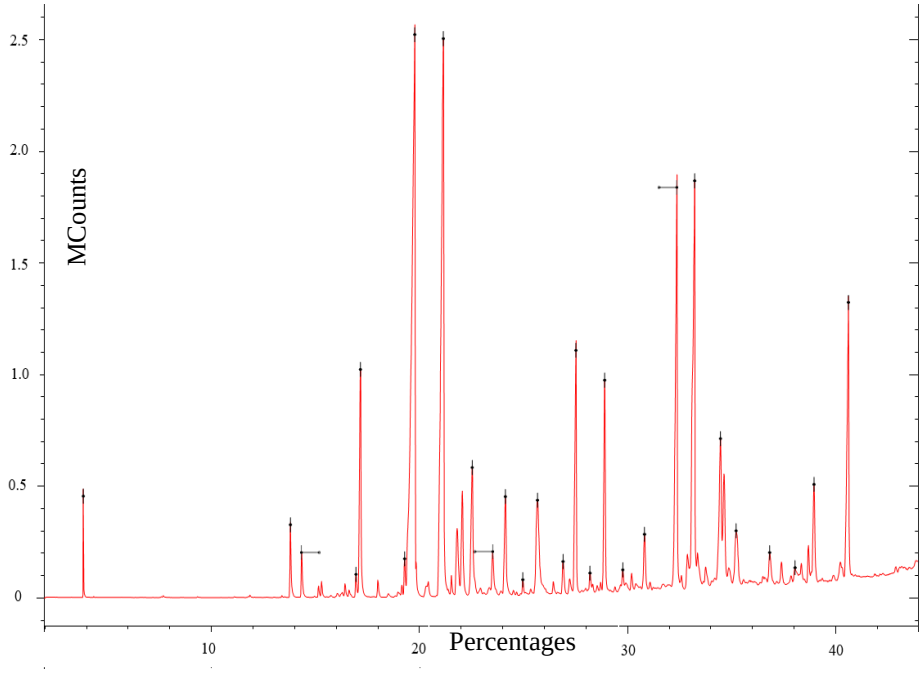

Fig. 7. GC-MS chromatogram of *Achilla eriophora* ecotype from KP11.

Discussion

In the present study, a total of 116 components were identified in *A. eriophora*. These components are more than those reported by Weyerstahl et al. (1997c) who identified 90 different EO compounds in *A. eriophora*. L- borneol was presented as the main composition in almost all studied regions. Whereas (E)-α-ionol was the main component in EOs of KP05 and KP09. These variations may be influenced by genetic background, agroclimatic and geographical conditions. L- borneol has been also reported as the main compound of *Achillea* species such as *A. holosericea*, *A. lingulata* and *A. chrysocoma* from Serbia (Kundakovic et al., 2007; Simic et al., 2000; Stojanović et al., 2005) and *A. ketenoglui* from Turkey (Baser et al., 2001). In the present study for the first time, some compounds such as hexadecenoic acid and 6βbicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl were identified in *Achillea*. These compounds were previously identified in *Viscum album* (Vlad et al., 2016) and *Alstonia scholaris* (Deepa et al., 2014). Compounds like Anethofuran, 1,5,5-trimethyl-6-methylene-cyclohexene, butyl 9-tetradecenoate were just found in KP12. These compounds were reported in jackfruit (Ong et al., 2008), *Anethum gravolens* (Radulescu et al., 2010) and in eucalyptus, respectively.

In the study on *A. eriophora* by Weyerstahl et al. (1997c), they identified 1,8-cineole, α- pinene, β-pinene, α-terpineol and linalool as the main compounds of EO. Furthermore, the EOs of *A. eriophora* in agronomic (Mashhad) and habitat (Fars) conditions showed the difference in the amount of their compounds, but their major compositions were similar (Ghani et al., 2009). Major compounds in Fars were 1,8-cineole, β-Pinene, α-thujene, terpinen-4-ol and (E)-nerolidol (Jaimand and Rezaee, 2004).

Saberi-Ameli et al. (2007) demonstrated the presence of camphor, 1,8-cineole, α-thujene, camphen and β-thujene from Khebr region in Kerman, Iran. Dokhani et al. (2005) reported 1,8-cineole as one of the major compounds of the *A. millefolium*, *A. eriophora* and *A. tenuifolia* EOs. Ghasemi et al. (2008) identified 32 components in EO of *A. eriophora* in which 1,8-cineole (54.93%), linalool (8.92%), α-terpineole (6.66%), and geranyl formate (5.99%) were the main compounds.

EO compounds of *A. eriophora* have been differently reported in the earlier studies. 1,8-cineol was presented in all samples of this plant in the previous studies. Although, in the present study the percentage of this compound was lower and the borneol compound was higher than its percentage in the other studies. In general, these findings showed that EO compositions of plant can vary in quality and quantity in different geographical conditions.

It was showed that caryophyllene oxide, L-borneol, L-bornyl acetate, b-caryophyllene and E-nerolidol have excellent anti-inflammatory effects to suppress nitric oxide production by LPS-stimulated macrophages (Tung et al. (2008). It is also noteworthy that many compounds of EO such as limonene, linalool, α-pinene, 1, 8 -cineole, l-borneol, bornylacetate, have strong antimicrobial activity (Dorman and Deans, 2000; Hendry et al., 2009). L-borneol, which is the highest compound in the EO of *A. eriophora* ecotypes, is frequently used in cosmetics, fine fragrances, shampoos, toilet soaps and other non-cosmetic products such as household cleaners and detergents (Bhatia et al., 2008).

Our results showed significant positive and negative correlations between EO yield with latitude and pH, respectively. KP02 ecotype that grow in high latitude showed the highest yield and the lowest pH in comparison with the other regions that corresponds with the results of Dizajeyekan et al. (2016). They showed almost neutral pH level (7.5), improve soil biological factors including microorganisms and earthworms. These biological factors dissolve soil minerals and nutrients result in improvement of plant growth and metabolites.

This region had moderate temperature (16 °C) in comparison with other regions. In this study, the higher the latitude, the higher the temperature was, while in places with lower latitudes, the temperature was lower due to higher altitude. For example, KP09 and KP01 in lower latitudes had higher altitudes and therefore the lowest temperature was observed in these sites. Therefore, the high yield observed in KP02 ecotype was probably due to the effects of genetic factors and temperature.

Many factors are associated with the difference in EOs. Indeed, chemical compositions are differently influenced by ecological factors. In this study, the main EO components were significantly affected by precipitation, temperature, latitude, longitude, pH, organic matters, lime percentage, available P and K, clay, silt and sand. Meanwhile, main EO compositions did not significantly changed by longitude and plant morphological traits including plant height, inflorescence diameter, number, length and leaf length. The effect of altitude on oil yield is not in agreement with those of Torras et al. (2007) who reported that increased altitude caused a significant decrease in EO yield of *Tymus vulgaris* (Torras et al., 2007). McChesney (1999) reported that the accumulation of secondary metabolites is affected by water availability, exposure to soil microorganisms and changes in soil pH and nutrients. It has been reported that the quality and yield of EOs are affected by environmental factors such as nutrient status and soil pH (Sangwan et al., 2001). Azevedo et al. (2002) stated that the latitude seemed to be the most significant environmental factor affecting the EO content of *Hyptis suaveolens* from Brazilian Cerrado.

Conclusion

Results of the present study revealed that the endemic yarrow (*Achilla eriophora*) ecotypes had considerable variations in EO yield and compositions. These findings help to understand the key roles of environmental factors such as edaphic and climatic conditions on EO quantity and quality. The results have potential application in breeding programs, food, cosmetic and pharmaceutical industries. The constituents L-borneol and bornyl acetate were the main

components in almost all samples, but in different amounts. Strong correlations among latitude and soil pH with EO yield provide scientific foundation to gain higher commercial benefit and to optimize possible environment for domestication.

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