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ORIGINAL PAPER

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VARIATION AND RELATIONSHIPS OF AERIAL YIELD, MORPHO-AGRONOMIC TRAITS AND ESSENTIAL OIL COMPOSITION IN DOMESTIC POPULATIONS OF *Ziziphora tenuior* IN IRAN

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ABSTRACT

The present study was carried out to evaluate aerial yield, agronomic traits, essential oil production, and their relationships in 21 populations of *Ziziphora tenuior* in Karaj, Iran over two years (2016–2017). The experimental layout was a randomized complete block design with three replications. Data were collected for GDD (Growth Degree Days), plant height, canopy area, stem number, flower number, aerial fresh yield, aerial dry matter (DM) yield, seed yield, essential oil percentage and oil production. The essential oil compositions were detected in accession 2929 (Tassoj) using GC/MS. Result of combined analysis of variance across two years showed significant differences between years, populations and population × year interaction for all traits ($P < 0.01$). In comparisons among populations, the population of Avaj and Ijroud with average values of 13.5 and 14.33 g/p had higher aerial dry yield and other agronomic traits followed by Meshkin2 and 3. For seed yield, Ormieh with average values of 3.11 g/p had higher production. For oil content, the populations of Tassoj, Salmas and Sharkord with average values of 1.25 to 1.56% had higher oil content and essential oil production. Result of correlation analysis showed that DM yield was positively correlated with GDD, plant height, canopy area, stem number, flower number. Oil content was negatively correlated with aerial DM yield. The results of PCA analysis showed that the first four components accounted for 50, 20, 12 and 10% of the total variation, respectively. Plant height, canopy area, stem number, flower number and aerial yield in the PCA1, essential oil content and oil production in the PCA2, seed yield in the PCA3 and GDD in PCA4 components were identified as important traits. Using Ward cluster method, the 21 populations were grouped into 3 clusters. In total, 23 compounds were identified in the essential oil from the aerial parts *Z. tenuior.* The major components in the essential oil were pulegone ranged (47.54 to 65.26%) followed by neo-iso-dihydro carveol, carvacrol, piperitenone, limonene, 1.8-cineole, p-mentha-3,8-dien, alpha-terpinene and terpinen-u-ol. It was concluded that there were wide range of variations for agronomic trait and essential oil in domestic germplasm of *Z. tenuior* to improved breeding new varieties in Iran.

Key words: *Ziziphora tenuior*, aerial part yield, agronomic traits, essential oil

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INTRODUCTION

Lamiaceae family, a large and diverse family of about 200 genera and 3200 species is distributed in world [Zargari 1997]. The *Ziziphora* genus belongs to Lamiaceae family, with more than 15 species, mainly distributed in the Mediterranean mountainous regions of Europe, Asia and Africa. This plant with the Persian name of Kakuti, consists of four species including *Ziziphora clinopodioides*, *Z. tenuior*, *Z. capita* and *Z. persica* is found in mountainous regions of Iran [Rechinger 1982, Mozaffarian 1996]. *Z. capitata* is an annual herb 2n = 16, that grows in Europe, Central Asia, Iraq, Syria, Turkey, Caucasus. In Iran, it is distributed from northern to southwestern parts [Jamzad 2012]. *Ziziphora* species are edible medicinal plant that leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavor [Zargari 1997].

The aerial parts, leaves and flowers of *Ziziphora* species, have been used as antiseptic, carminative and sedative agent to treat cold, flu, cough, stomach ache, diarrhea, antiseptic, migraine, fever and carminative [Salehi 2005, Zargari 1997]. Sarangzai et al. [2013], reviewed traditional uses of *Z. tenuior* in Pakistan as perfume and also used for fever and cold. In relation to the phytochemical qualities of *Ziziphora* genus, many researchers stated that the *Ziziphora* species are rich in monoterpenoides and phenolic compounds such as thymol, pulegone, piperitenone and p-menth-3-en-8-ol, which can have antioxidant and antimicrobial activity against many infectious diseases and pathogens [Thuille et al. 2003, Aliakbarlu and Shameli 2013].

Z. tenuior is very aromatic plants which are used for food and medicine and its aroma is near to thyme and oregano. It is a perennial herb and its bushes are thick and its height is 20–50 cm and has purple flowers [Omidbeygi 2006]. This plant in the mountainous areas of Iran as a spice widely used in foods and cosmeticcs and its aroma is very close to oregano and thyme and a bit too similar to pennyroyal. New shepherds and mountaineers gather *Z. tenuior* and drink it as a tea [Mozaffarian 1996].

There are many published articles for essential oil and its compound in *Ziziphora* species but there less reports for evaluation of species for DM yield and agronomical traits and their relationships with essential oil. Batooli et al. [2012] found the essential oil percentage of *Z. tenuior* ranged 0.2 to 2.3% (w/w). In total, 18 components were identified in it’s essential oil. The main components of *Z. tenuior* essential oil were pulegon (80.01–90.10%), and piperitenone (4.5–7.14%).

Bakhshi Khaniki et al. [2010] showed that ecological factors had significant effect ($P < 0.01$) on oil yields of *Z. clinopodioides* ranged 0.37 to 1.0%. They identified the main oil compounds as pulegone (ranged 30 to 60%) and followed by 1,8-cineole as main compounds. Dembitskii et al. [1994] using hydro distillation in *Z. clinopodioides* found 1.4% w/w essential oil content. The major components of the oil were pulegon (30.1%) and thymol (21.3%). Asgharipour et al. [2016] in study of variability in *Z. clinopodioides* population based of essential oil found the essential oils of population ranged from 0.23 to 0.61% and the pulegone was the main oil component.

There are few reports for variability of DM yield and agronomical traits in *Ziziphora* species. Karimi et al. [2017] in application of organic fertilizers on morphological traits of *Z. clinopodioides* found positive effects of fertilizers on morphological traits but negative effects on some biochemical characteristics such as phenols, flavonoids and antioxidants. So that by increasing the application of organic fertilizers, the amount of morphological parameters were increased and biochemical parameters decreased.

Asaadi [2016], for *Z. clinopodioides* found plant height ranged from 21 to 40 cm with a crown diameter of 39 to 42 cm. The density of plants in habitat was counted as 11 500 per ha. Dry matter production of this plant in the areas was 241 kg/ha.

Despite the unique role of different species of *Ziziphora* in the production of pharmaceutical species, unfortunately little information on the genetic diversity among populations of the *Ziziphora* species exists for shoot and essential oil yield here in Iran. There are many reports for essential oil production of *Ziziphora* wild population in their natural habitat, but there is lack of enough information about their domestication in the field condition for both DM and essential oil yield.

This study aimed to study variation and relationships of aerial yield, agronomic traits and essential oil production in domestic populations of *Z. tenuior* via multivariate statistical analysis.

MATERIALS AND METHODS

In this research, 21 populations of *Z. tenuior* originated from different parts of Iran were provided from the Natural Resource Gene Bank of Research Institute of Forest and Rangelands, Tehran, Iran. The geographical characteristics of 21 populations is presented in Table 1. Seeds of populations were sown in Jiffy pots in March, 2015. Then, after seed emergence, seedlings were transferred into field in Alborz research station in Karaj, Iran in April 2015. Alborz research station has a longitude of 51°31' and latitude of 42°3' with a altitude of 1291 m above sea level. The average annual rainfall is 248 mm and the average temperature is 21.16°C, with an absolute maximum of 44°C and an absolute minimum of 8°C. Most of annual precipitation is occur in winter and the most rainy month in March. The average relative humidity is 40–50% annually and the climate of research station is semi-arid. The soil of Alborz research station has a loamy texture with a pH of 5.5–7.5.

The experiment layout was Randomized Completely Blocks Design (RCBD) with three replications. Each unit of experiment consists of six rows with 50 cm distance between rows and 40 cm between plants within rows. The experiment was conducted in irrigation condition and the plots were irrigated once per week. In establishment year no data were collected and data were collected in the next year of planting. The following traits were analyzed: plant canopy cover area (cm^2), plant height (cm), flower number per plant, time required for flowering (days), aerial biomass yield (g/p), seed yield (g/p), growing degree days to flowering (GDD), essential oil concentration as percent and essential oil yield (mg/p).

Table 1. The address and geographical characteristics of 21 populations

Populations origin	Province	Accessions code	Longitude	Latitude	Altitude (m)
Tassoj	Azerbaijan E	2929	47°45'30"	38°48'62"	1450
Daran	Esfahan	8633	50°29'00"	33°02'00"	2378
Mimah	Esfahan	10096	54°38'00"	32°27'00"	1920
Khalkhal	Ardebil	13321	48°43'35"	27°23'39"	2257
Meshkin1	Ardebil	13322	47°03'42"	38°45'55"	2291
Khosbijan	Markazi	13495	49°23'17"	34°09'07"	2700
Arak	Markazi	13560	49°37'27"	33°56'55"	2190
Najafabad	Esfahan	14026	60°18'33"	57°17'61"	1950
Salmas	Azerbaijan W	14231	49°23'17"	34°9'07"	1980
Ormieh	Azerbaijan W	17983	52°12'21"	35°24'21"	2350
Yazd	Yazd	18650	63°29'53"	14°56'31"	2235
Taft	Yazd	23378	42°06'54"	09°06'31"	2496
Sharkord	Chormahal	29580	46°56'50"	17°18'32"	2173
Avaj	Gazvin	29627	88°32'49"	37°34'35"	2000
Golpaygan	Esfahan	35016	58°16'20"	47°30'37"	1942
Shazand	Markazi	35393	30°05'49"	39°58'33"	2350
Meshkin2	Ardebil	36842	54°26'47"	29°21'38"	1236
Meshkin3	Ardebil	37953	39°11'65"	23°32'32"	1640
Gazvin	Gazvin	39369	51°00'51"	23°37'21"	2075
Ijroud	Zanjan	40371	84°58'59"	61°53'36"	1676
Kerman	Kerman	41200	95°04'46"	36°35'34"	1219

The canopy cover area was estimated using the means of width and length diameters of the plant canopy. The aerial plant parts were harvested in full flowering stage, weighed, air dried in room temperature for one week and weighed again to obtain as dry aerial biomass yield.

GDD for flowering stage GDD were recorded by Frank et al. [1993] as follows:

$$GDD = \frac{Tmax + Tmin}{2} - Tb$$

where: GDD – growing degree days, Tmax – maximum daily temperature °C (mean for days from March 21 till full bloom), Tmin – minimum daily temperature C (as above), Tb – the base temperature physiological zero for germination, for those species as (6°C).

The maximum and minimum daily temperatures were provided from Karaj, Iran Metrological Station [IRIMO 2011].

The essential oil was extracted using the hydro distillation method with a Clevenger type apparatus. Each sample containing 10 plants per plots were harvested at full flowering stage and dried and ground. The materials of every species (80 g in three replications) were placed in a three liter round-bottomed flask containing 1.5 l of water and refluxed for 3 h. After completion, the oil volume (ml) was recorded according to the Hungarian plant pharmocopy letter [Hungarian Pharamacopoeia VII 1986] as follow:

$$\text{Essential oil \%} = \frac{\text{Essential oil weight (g)}}{\text{Areal dry biomas weight (g)}}$$

Essential oil yield was calculated by essential oil % × aerial biomass yield (g/p).

The essential oil compositions in accession 2929 (Tassoj) were detected using GC/MS based on method of [Mirza et al. 1996].

STATISTICAL ANALYSIS

Data was combined analyzed of variance over two years using and means comparisons were made using Tukey's method. Phenotypic correlations among characteristics were estimated via pair-wise combinations. All variables were used in principal components and cluster analysis, whereby the variables were standardized for cluster analysis (Ward method) using Minitab 16 and SAS9 software.

RESULTS AND DISCUSSION

The results of analysis of variance (ANOVA) showed significant differences between years and populations for all of traits ($P < 0.01$) – Table 2. Population by year interaction effects were also significant for all of traits indicating difference response of populations for each year.

In comparisons among 21 populations, in the first year, result showed the populations of Tassoj, Arak, Shazand and Ijroud with GDD values of 1916 to 2370°C and Kerman with GDD values of 1012 to 1194°C were considered as late and early maturity populations, respectively. In overall the highest and lowest values of GDD (2263°C and 1103°C) were obtained for Tassoj and Kerman population over two years, respectively (Tab. 3). Therefore populations having lower GDD that goes to flower sooner than other populations. This result was in agreement with result of Adeli et al. [2013] who reported effect of temperature on development of phenological stage in species of *Matricaria* genus.

For agronomical traits, the higher values of plant height were obtained in Avaj and Ijroud (18.33 and 16.11 cm) in the first year and Najafabad and Meshkin3 (24.01 and 25.67 cm) in the second year, respectively. For mean of two years the Avaj and Meshkin3 with average values of 19.67 and 19.42 cm had higher plant height, respectively (Tab. 3).

For canopy cover, Avaj and Meshkin3 with average values of 428.89 and 481.27 cm^2 had higher canopy cover in the first and second year, respectively. For mean of two years, Avaj and Meshkin3 with average values of (367.33 and 356.76 cm^2) had higher canopy area than other populations, respectively (Tab. 3).

For stem density, Avaj and Ijroud with average value of 31.67 and 42.00 No./p had the higher stems number, in the first and second year, respectively. For means of two years Avaj with (34 No./p) had higher stem number than other populations (Tab. 3).

For flower number Avaj and Meshkin3 with average values of 3126 and 2980 No./p had high flower number per plant in the first and second year, respectively. For

Table 2. ANOVA of traits for 21 populations of *Z. tenuior* evaluated in Karaj, Iran over two years (2016–2017)

Source	DF	MS								
		Plant height (cm)	Canopy area (cm^2)	Stem No./p	Flower No./p	Aerial fresh yield (g/p)	Aerial dry yield (g/p)	Seed yield (g/p)	Oil content (%)	Oil yield (mg/p)
Year (Y)	1	2165.3**	624165**	3651.8**	17683518**	409.25*	349.3**	24.96**	0.157*	167.48**
Error1	4	11.48	12478	126.26	1170668	33.25	15.74	0.171	0.012	1.21
Population (P)	20	45.27**	42899**	213.85**	1724920**	477.95**	57.05**	3.85**	1.258**	30.85**
Y × P	20	47.60**	34280**	156.81**	1409358**	531.45**	50.50**	2.79**	0.219**	14.25**
Error2	80	4.46	3418	20.95	181323	33.90	4.89	0.08	0.009	0.59

* significant at the 0.05 probability level, ** significant at the 0.01 probability level

means of two years Avaj with 2596 No./p had higher flower number than other populations (Tab. 3).

For Aerial fresh yield Meshkin2 with (60.88 g/p) in the first year and Khalkhal, Ormieh, Meshkin3, Ijroud with (32 to 40 g/p) in the second year had high production, respectively.

For aerial dry yield Avaj and Shazand with (15.05 and 13.63 g/p) in the first year and Taft and Ijroud with (17.83 and 16.33 g/p) had higher aerial dry matter production, respectively. For means of two years, the population of Avaj, Ijroud with (13.5 and 14.33 g/p) had highest aerial dry yield two years. This populations although had high DM production but their oil content value was relatively low (Tab. 4). This result was similar with the result of Alizadeh et al. [2017]. They studied seven species of *Tanacetum* genus for plant canopy cover, plant height, and flower, aerial biomass yield, GDD, essential oil percentage and essential oil yield. Their result showed that the species of *T. balsamita* was superior to other six species for aerial biomass yield and essential oil yield. The two species of *T. tabrisianum* and *T. uniflorum* were nominated as the early maturity because of their lowest value of GDD and time required for flowering.

For seed yield Tassoj, Ormieh and Gazvin with (4.08, 4.53 and 4.59 g/p) in the first year and Khalkhal with (3.25 g/p) in the second year had higher seed production (Tab. 4). For means of two years, Ormieh with average values of 3.11 g/p had higher seed production. For essential oil content, Najafabad and Yazd with (1.33 and 1.65%) in the first year and Salmas and Sharkord with (2.07 and 2.03%) in the second year had higher essential oil percentage. For mean of two years, the populations of Tassoj, Salmas and Sharkord with (1.25, 1.54 and 1.56%) had higher oil content over two years and as a result had higher essential oil production (Tab. 4).

Correlation between traits. Result of correlation analysis showed that GDD had positively correlated with both fresh and dry matter yield, indicating the late maturity species had higher areal production. Plant height was positively correlated with canopy area, stem number, flower number and DM yield. Indicating the taller plant has higher stem and flower number and led to higher production. Similarly, canopy area was positively correlated with stem and flower number and led to higher DM yield production. Oil content had weak negative correlation with aerial DM yield and other agronomic traits, however, there was strong positive correlation between essential oil content and essential oil production (Tab. 5). This result was in agreement with the results of Solouki et al. [2008] and Pirkhezri et al. [2010] in *Matricaria chamomilla* and Hamisi et al. [2012], in *Tanacetum parthenium* that found positive correlation between essential oil %, flower number and aerial biomass yield.

Principal component analysis (PCA). The results of PCA analysis showed that the first four components with Eigen values higher than one had accounted 91% from total variations. The amount of variance explained by each components were 50, 20, 12 and 10% of the total variation, respectively (Tab. 6). In the first

Table 3. Mean of GDD, plant height, canopy area, stem number per plant, flower number per plant in 21 populations of *Z. tenuior* evaluated in Karaj, Iran over two years (2016–2017)

Populations origin	Province	Accessions code	GDD*		Plant height (cm)		Canopy area (cm^2)		Stem No./p		Flower No./p	
			year 1	year 2	year 1	year 2	year 1	year 2	year 1	year 2	year 1	year 2
Tassoj	Azerbaijan E	2929	**2158**	**2370**	13.22 bcde	22.56 bcd	182.77 cd	340.42 bcd	17.33 de	28.00 bcde	790.0 def	1866.7 bcd
Daran	Esfahan	8633	1488	1301	10.17 fgh	22.11 bcd	66.18 ghi	226.67 def	11.33 fghi	18.33 efg	841.3 de	1301.7 bcde
Mimah	Esfahan	10096	1382	1926	10.22 fgh	23.89 abc	76.65 fghi	335.96 bcd	9.33 ghi	23.00 defc	315.0 efg	2334.0 ab
Khalkhal	Ardebil	13321	1424	1423	9.61 fghi	21.78 bcd	110.45 efg	321.22 bcde	12.33 fgh	21.67 defg	373.0 efg	1226.7 cde
Meshkin1	Ardebil	13322	1516	1423	15.78 abc	20.78 cde	243.74 b	191.93 efg	25.00 b	15.00 fg	1656.3 c	933.0 de
Khosbijan	Markazi	13495	1568	2081	8.39 ghij	11.11 i	66.34 ghi	60.57 g	11.00 fghi	11.33 g	394.0 efg	663.7 e
Arak	Markazi	13560	**1992**	**2370**	13.22 bcde	14.56 ghi	135.10 def	201.86 efg	10.67 fghi	24.00 defc	565.3 defg	564.3 e
Najafabad	Esfahan	14026	1012	1955	9.11 ghi	**24.01 ab**	31.97 hi	236.04 def	6.33 i	22.33 def	168.7 g	1898.3 bcd
Salmas	Azerbaijan W	14231	1424	2512	9.55 fghi	18.67 defg	73.75 fghi	372.98 abcd	14.00 efg	35.67 ab	429.3 efg	1985.0 bc
Ormieh	Azerbaijan W	17983	1240	1797	8.11 hij	20.33 cde	27.56 i	388.37 abc	6.67 i	31.33 abcd	158.0 g	2068.0 abc
Yazd	Yazd	18650	1173	1423	8.28 ghij	16.56 fgh	42.32 ih	142.01 fg	7.33 hi	18.00 efg	172.0 g	1136.0 cde
Taft	Yazd	23378	1240	1797	7.33 ij	18.55 efg	25.40 i	417.60 ab	7.00 hi	36.33 ab	399.0 efg	1792.3 bcd
Sharkord	Chormahal	29580	1568	2223	7.33 ij	14.78 ghi	47.48 ih	265.07 cdef	10.00 fghi	25.33 cde	515.0 defg	1740.7 bcd
Avaj	Gazvin	29627	1760	1423	**18.33 a**	21.00 bcde	**428.89 a**	305.77 bcde	**31.67 a**	36.33 ab	**3126.7 a**	2066.0 abc
Golpaygan	Esfahan	35016	991	1848	5.78 j	23.45 abcd	39.76 ih	266.09 cdef	6.33 i	22.33 def	178.7 g	1814.3 bcd
Shazand	Markazi	35393	**1992**	**2370**	12.67 cdef	13.89 hi	113.05 efg	127.73 fg	14.33 efg	20.33 defg	853.0 de	892.3 de
Meshkin2	Ardebil	36842	1382	1423	14.28 bcd	20.45 cde	273.81 b	297.58 bcde	23.67 b	17.67 efg	2205.3 b	1472.0 bcde
Meshkin3	Ardebil	37953	1541	2223	13.17 bcde	**25.67 a**	232.25 bc	**481.27 a**	21.33 bcd	33.33 abc	846.3 de	**2980.0 a**
Gazvin	Gazvin	39369	1382	1797	14.28 bcd	10.45 i	240.51 b	62.47 g	22.67 bcd	14.67 fg	1022.3 d	665.3 e
Ijroud	Zanjan	40371	**1916**	**2168**	**16.11 ab**	19.11 def	259.25 b	422.70 ab	23.00 bc	**42.00 a**	2107.7 bc	2303.3 ab
Kerman	Kerman	41200	1012	1194	5.50 j	20.89 cde	15.33 i	224.31 def	6.33 i	25.33 cde	206.7 g	1354.0 bcde
Average			1483	1859	10.97	19.27	130.12	270.89	14.17	24.87	824.93	1574.17

The means of the column with same letters were not significantly different based on Tukey method $P < 0.05$
* GDD (Growth Degree Days) values were the same for replications, therefore, no mean comparisons were made

Table 4. Mean of aerial fresh yield, aerial dry yield, seed yield, oil content, oil yield in 21 populations of *Z. tenuior* evaluated in Karaj, Iran over two years (2016–2017)

Populations origin	Province	Accessions code	Aerial fresh yield (g/p)		Aerial dry yield (g/p)		Seed yield (g/p)		Oil content (%)		Oil yield (mg/p)	
			year 1	year 2	year 1	year 2	year 1	year 2	year 1	year 2	year 1	year 2
Tassoj	Azerbaijan E	2929	32.64 def	32.00 ab	8.54 def	10.00 cdef	**4.08 a**	1.10 de	0.97 c	**1.53 b**	**82.84 a**	**153.00 a**
Daran	Esfahan	8633	5.50 no	15.17 d	1.44 kl	5.17 fg	0.79 fg	0.65 fgh	0.47 ef	0.48 ef	6.77 j	24.82 ijk
Mimah	Esfahan	10096	13.06 klm	30.50 abc	3.26 ijk	11.33 cde	1.75 e	0.98 def	0.48 ef	0.48 ef	15.65 efgh	54.38 ef
Khalkhal	Ardebil	13321	17.48 ijk	**40.17 a**	4.15 hijk	9.67 cdef	2.32 cd	**3.25 a**	0.47 ef	0.43 fg	19.51 efgh	41.58 fgh
Meshkin1	Ardebil	13322	23.45 fghi	16.00 d	8.26 def	6.50 efg	0.85 fg	0.25 i	0.47 ef	0.51 ef	38.82 bc	33.15 hijk
Khosbijan	Markazi	13495	15.73 ijkl	20.67 bcd	4.10 hijk	6.00 fg	1.78 e	0.78 efgh	0.31 fgh	0.32 gh	12.71 ghij	19.20 jkl
Arak	Markazi	13560	19.76 hij	20.33 bcd	6.09 fgh	7.33 defg	0.35 g	0.42 ih	0.40 efgh	0.37 fgh	24.36 efg	27.12 hij
Najafabad	Esfahan	14026	7.75 lmno	18.00 d	1.76 jkl	6.67 efg	1.12 f	0.41 ih	**1.33 b**	1.03 c	23.41 efgh	68.70 de
Salmas	Azerbaijan W	14231	11.21 jklm	31.67 ab	2.53 ijkl	13.00 bc	1.85 de	0.68 fgh	1.07 c	**2.07 a**	27.07 efg	**229.10 a**
Ormieh	Azerbaijan W	17983	25.62 fgh	**37.17 a**	6.46 efgh	13.33 bc	**4.53 a**	1.69 c	0.79 d	1.03 c	**51.03 b**	**137.30 b**
Yazd	Yazd	18650	5.37 no	**37.17 a**	1.39 kl	11.67 bcd	1.03 f	0.89 defg	**1.65 a**	0.97 c	22.94 efgh	113.20 c
Taft	Yazd	23378	6.82 mno	**38.00 a**	1.89 jkl	**17.83 a**	0.69 fg	1.22 d	0.78 d	0.38 fgh	14.74 efgh	67.75 d
Sharkord	Chormahal	29580	14.55 jklm	16.67 d	3.28 ijk	5.33 fg	1.01 f	0.50 ghi	1.10 c	**2.03 a**	36.08 bcd	108.20 c
Avaj	Gazvin	29627	43.65 bc	30.67 abc	**15.05 a**	12.00 bcd	0.89 fg	0.80 efgh	0.23 gh	0.25 hi	34.62 bcde	30.00 ijk
Golpaygan	Esfahan	35016	10.59 lmn	20.33 bcd	2.57 ijkl	7.33 defg	2.98 b	0.78 efgh	0.37 efgh	0.61 de	9.51 ij	44.71 efg
Shazand	Markazi	35393	48.58 b	18.67 d	**13.63 ab**	6.33 efg	1.63 e	0.71 efgh	0.23 gh	0.24 hi	31.35 cdef	15.19 kl
Meshkin2	Ardebil	36842	**60.88 a**	19.17 dc	11.85 bc	7.83 defg	2.87 b	0.67 fgh	0.24 gh	0.26 hi	28.44 defg	20.36 ijkl
Meshkin3	Ardebil	37953	23.63 fghi	**32.67 a**	7.53 efg	14.17 abc	2.05 cde	0.69 fgh	0.21 h	0.15 i	15.81 ghi	21.26 ijkl
Gazvin	Gazvin	39369	35.22 cde	11.67 d	11.09 bcd	3.92 g	**4.59 a**	0.49 ghi	0.26 gh	0.26 ih	28.83 def	10.19 l
Ijroud	Zanjan	40371	37.03 cd	**34.17 a**	12.11 bc	**16.33 ab**	2.54 bc	2.49 b	0.27 gh	0.23 ih	32.70 cdef	37.56 fghi
Kerman	Kerman	41200	2.18 o	15.50 d	0.52 l	5.67 fg	0.68 fg	2.24 b	0.73 d	0.69 d	3.80 hij	39.12 fghi
Average			21.94	25.54	6.07	9.40	1.92	1.03	0.61	0.68	26.71	60.61

The means of the column with same letters were not significantly different based on Tukey method P < 0.05

Table 5. Correlation analysis between yields, agronomical traits, oil production in populations of *Z. tenuior* evaluated in Karaj, Iran over two years (2016–2017)

Traits	GDD	Plant height (cm)	Canopy area (cm^2)	Stem No./p	Flower No./p	Fresh yield (g/p)	Dry yield (g/p)	Seed yield (g/p)	Oil content (%)
Plant height (cm)	0.03								
Canopy area (cm^2)	0.22	0.796**							
Stem No./p	0.34	0.621**	0.911**						
Flower No./p	0.12	0.767**	0.906**	0.877**					
Fresh yield (g/p)	0.40*	0.434*	0.681**	0.663**	0.603**				
Dry yield (g/p)	0.41*	0.507**	0.811**	0.841**	0.743**	0.912**			
Seed yield (g/p)	0.02	0.065	0.228	0.152	0.084	0.47*	0.311		
Oil content (%)	0.05	–0.279	–0.268	–0.189	–0.258	–0.316	–0.357	–0.086	
Oil yield (mg/p)	0.29	–0.022	0.085	0.129	0.002	0.176	0.105	0.272	0.814**

* significant at the 0.05 probability level, ** significant at the 0.01 probability level

Table 6. Eigen vectors from the first three principal component axes for 10 variables used to classify 21 populations of *Z. tenuior* evaluated in Karaj, Iran over two years (2016–2017)

Variable	PC1	PC2	PC3	PC4
GDD	0.15	0.30	0.19	**0.76**
Plant height (cm)	**0.33**	–0.14	–0.36	–0.21
Canopy area (cm^2)	**0.42**	–0.02	–0.18	–0.09
Stem No./p	**0.41**	0.05	–0.17	0.08
Flower No./p	**0.40**	–0.08	–0.29	–0.09
Aerial fresh yield (g/p)	**0.38**	0.11	0.36	0.04
Aerial dry yield (g/p)	**0.41**	0.04	0.19	0.11
Seed yield (g/p)	0.14	0.21	**0.61**	–0.56
Oil content (%)	–0.16	**0.59**	–0.37	–0.11
Oil yield (mg/p)	0.04	**0.69**	–0.12	–0.15
Eigen value	5.00	1.95	1.19	1.00
Proportion	0.50	0.20	0.12	0.10
Cumulative	0.50	0.70	0.81	0.91

The bold and underlined data had higher Eigen values in the relevant factors

Table 7. Means of traits used in classification of the threeclusters

Cluster No.	GDD	Plant height (cm)	Canopy area (cm^2)	Stem No./p	Flower No./p	Fresh yield (g/p)	Dry yield (g/p)	Seed yield (g/p)	Oil content (%)	Oil yield (mg/p)
1(n=4)	1911 a	14.32 b	212.30 b	21.08 b	1194.1 b	25.19 ab	7.81 b	1.93 a	1.32 a	94.45 a
2(n=13)	1579 a	14.32 b	154.66 b	16.27 b	912.4 b	19.75 b	6.37 b	1.29 a	0.57 b	31.16 b
3(n=4)	172 a	18.51 a	337.69 a	28.48 a	2138.4 a	33.87 a	12.11 a	1.63 a	0.23 b	27.59 b

The means of the clusters with same letters were not significantly different based on DMRT P < 0.05 method

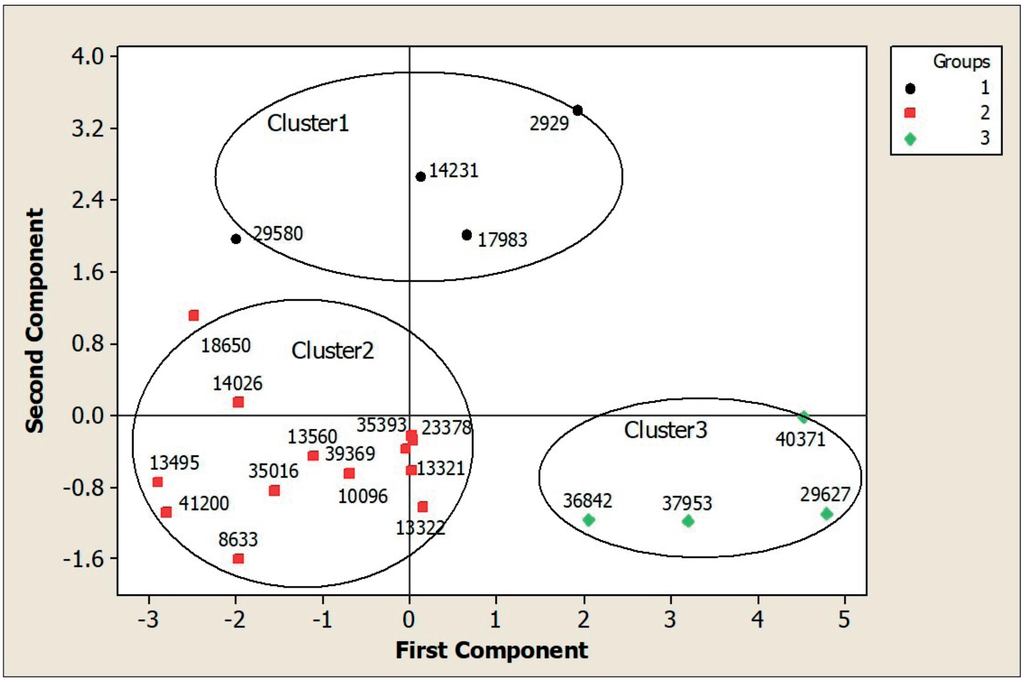

Fig. 1. Scatter plot of 21 populations of *Z. tenuior* for the first two principal components

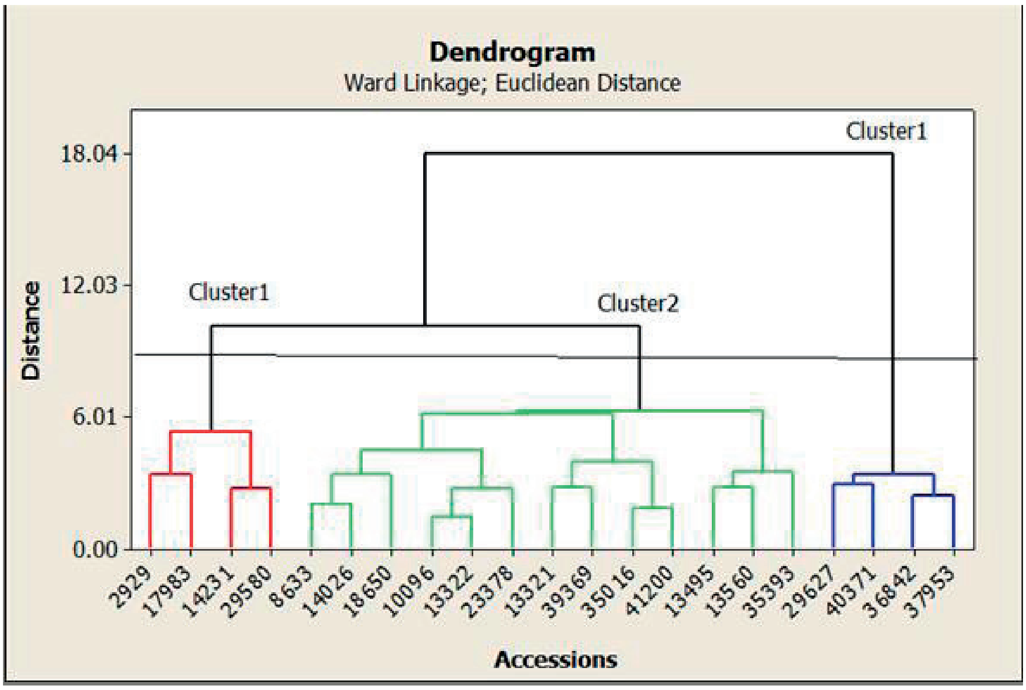

Fig 2. Dendrogram of 21 populations of *Z. tenuior* by analyzing yield, agronomical traits and oil production using Ward cluster analysis method

Table 8. Means of the essential oil components of accession 2929 (Tassoj) in *Z. tenuior* evaluated in Karaj

Oil components	Retention index	Percentage
alpha-pinene	947.47	0.15–0.32
sabinene	977.32	0.23–0.37
beta-pinene	979.11	0.27–0.76
myrcene	1005.19	0.65–0.71
p-cymene	1052.23	0.23–0.35
limonene	1061.65	0.75–1.22
1.8-cineole	1077.76	0.10–1.33
p-mentha-3,8-dien	1091.11	0.44–1.12
alpha-terpinene	1091.21	0.52–1.23
linalool	1108.66	0.12–0.20
nonanal	1112.93	0.40
menthofuran	1205.72	0.11
menthol	1214.22	0.25–0.39
terpinen-u-ol	1244.70	0.88–1.52
neo-iso-dihydro carveol	1268.84	2.51
pulegone	1284.56	47.54–65.26
thymol	1315.16	0.47–0.57
carvacrol	1325.12	1.79–2.35
α-elemene	1373.24	0.23
piperitenone	1377.90	0.58–2.16
beta-bourbonene	1425.04	0.12–0.19
E-caryophyllene	1470.59	0.53–0.88
caryophyllene oxide	1650.84	0.20–0.55

component, plant height, canopy area, stem number, flower number and aerial yield had positive Eigen vector and was regarded as productivity factor. In the second component essential oil content and oil production had higher Eigen vectors and trends of these traits were in the same direction. Therefore, this component was named as oil yield factor. In the third component seed yield had higher Eigen vector coefficients and was named as seed production factor and finally in the fourth component GDD had higher Eigen vector coefficients and was named as phonological factor (Tab. 6). Scatter plot of 21 populations of *Z. tenuior* for the first two principal components is presented in Figure 1. There was good agreement between data of PCA analysis in Table 6 and cluster analysis in Figure 2.

Cluster analysis. Cluster analysis of the populations for *Z. tenuior* showed that the populations were divided into three groups according to genetic distance (10.04). The first, second and third clusters had 4, 13 and 4 populations, respectively (Fig. 2).

Analysis of variance between clusters showed that the populations in cluster 1 had higher mean values than that for other two clusters for oil percentage and oil yield (Tab. 7). Cluster 2 with 13 populations had moderate values for most of traits and finally cluster 3 with 4 populations had higher values of aerial yield and agronomical traits. According to the dendrogram (Fig. 2), populations of 2929 (Tassoj), 14231 (Salmas), 17983 (Ormieh) and 29580 (Sharkord) were dropped into cluster 1. The three first populations were originated from West Azerbaijan province, Iran, indicating Genetic variation that is affected by geographic environments. More and less the same trend was observed for populations in cluster 3. In contest, for cluster 2 there were 13 populations that were originated from various part of Iran, indicating no association between genetic and geographic distances for populations. With a similar method, Alizadeh and Jafari [2016] used cluster analysis for the grouping of accession of *Anthemis triumfettii*, *A. tinctoria*, *A. haussknechtii* and *A. pseudocotula*. According of their result, the cluster analysis well separated the accession based on aerial biomass yield, morphological traits and GDD. The distribution of four clusters based on the first two principal components are drawn in Figure 1. There was good agreement between cluster analysis and PCA.

Essential oils compounds in accession 2929 (Tassoj). *Z. tenuior* is found in different regions of Iran, especially in the South East, North and North West, and has numerous health benefits. Of the natural compounds, in total, 23 compounds were identified in the essential oil from the aerial parts *Z. tenuior* (Tab. 8). The results obtained in our study indicated that the major components in the essential oil with highest values were pulegone ranged (47.54 to 65.26%) followed by neo-iso-dihydro carveol (2.51%), carvacrol (1.79 to 2.35), piperitenone (0.58 to 2.16), limonene (0.75 to 1.22), 1.8-cineole (0.10 to 1.33), p-mentha-3,8-dien (0.44 to 1.12), alpha-terpinene (0.52 to 1.23) and terpinen-u-ol (0.88 to 1.52) (Tab. 6). The range of essential oil compound were in agreement with publish data [Ganjali and Pourramazani Harati 2014, Khodaverdi-Samani et al. 2015].

CONCLUSION

The result of this research showed that some populations had higher values of agronomical than other populations, therefore selection of superior populations would be useful for improve breeding new varieties. Remarkable levels of DM yield and essential oil production were obtained whereas effect of year was significant in most traits.

In comparisons among populations, the population of Avaj and Ijroud with average values of 13.5 and 14.33 g/p had higher aerial dry yield and other agronomic traits followed by Meshkin2 and 3. For seed yield, Ormieh with average values of 3.11 g/p had higher production. For oil content, the populations of Tassoj, Salmas and Sharkord with average values of 1.25 to 1.56% had higher oil content and essential oil production. In total, 23 compounds were identified in the essential oil from the aerial parts *Z. tenuior*. The major components in the essential oil was pulegone ranged (47.54 to 65.26%) followed by neo-iso-dihydro carveol, carvacrol, piperitenone, limonene, 1.8-cineole, p-mentha-3,8-dien, alpha-terpinene and terpinen-u-ol.

Based on this conclusion, the best populations were selected for further evaluation in larger plots for their persistence and other agronomic parameters. Increasing the area for cultivation of *Z. tenuior* depends greatly on improvements in seed production, which is still the main cause of the slow development of medicinal plant cultivation.

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