Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2016, 44(1):189-194. DOI:10.15835/nbha44110118
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Original Article

# Variability in *Ziziphora clinopodioides* subsp. *bungeana* (Juz.) Based on Morphological Traits and Essential Oils Profile

Mohammad R. ASGHARIPOUR*, Abuzar AKBARI ABJAHAN, Mahdi DAHMARDE

*University of Zabol, Faculty of Agriculture, Department of Agronomy, Zabol, Iran; m_asgharipour@yahoo.com; (*corresponding author); a_abjahan@yahoo.com; dahmard@yahoo.com*

**Abstract**

Variability among populations of *Ziziphora clinopodioides* Lam. subsp. *bungeana* (Juz.) was analyzed, to evaluate the level and distribution of differentiation among four distant populations from sub-humid, upper semi-arid and semi-arid bioclimates of Khorasan provinces, Iran. Analyses of variances and cluster analysis have been carried out to define the variability and significance of morphological differentiation. Morphological differentiation was correlated with ecological situations at the location of origination and a high variation among populations based on morphological traits was observed between the plants belonging to semi-arid populations vs. the sub-humid ones. Essential oil composition varied among populations. In all of the populations pulegone was the main component, followed by isomentone and thymol. The clustering, based on oil analysis generated two distinct clusters. Essential oils of the upper semi-arid and sub-humid populations were rich in Iso-menthone, while populations from the semi-arid bioclimate were characterized by high amounts of pulegone. The relatively low morpho-chemical diversity in the populations of *Z. clinopodioides* indicates that the maintenance of their evolutionary potential is at risk if population sizes are not maintained and if there is no protection of the habitats.

***Keywords:*** chemical and genetic diversity, environmental parameters, Lamiaceae, medicinal plant, morphology, phytochemical analysis

## Introduction

The genus *Ziziphora* belongs to the Lamiaceae includes four species (*Z. clinopodioides* Lam., *Z. tenuior* L., *Z. persica* Bunge and *Z. capitata* L.) and has a broad distribution all over Iran (Mozaffarian, 1996). Blue Mint Bush with the scientific name of *Ziziphora clinopodioides* Lam., locally known as "*kakuti-e kuhi*" grows wild in Iran, Turkey, Afghanistan, Azerbaijan and Iraq (Sardashti *et al.*, 2012; Schulz *et al.*, 2005). This species is an edible medicinal plant which the dried aerial parts, leaves, flowers and stem of the plant are used for production of tea, condiments or additive in foods and yogurt and other dairy products to offer aroma and flavor (Meral *et al.*, 2002; Ozturk and Ercisli, 2007; Zargari, 1993). *Ziziphora clinopodioides* encompasses nine subspecies native to Iran. *Z. clinopodioides* subsp. *bungeana* (Juz.) Rech. f. grows wild in the eastern parts of Iran (Rechinger, 1982).

Traditionally, *Z. clinopodioides* subsp. *bungeana* has been utilized as infusion for different intention such as anti-fever, wound healing, stomachache, antiseptic, sedative and carminative (Verdian, 2008). As well as, in folklore of Iran, this plant have been often used as culinary and also for treatment of gastrointestinal disorders and common cold (Naghibi *et al.*, 2005).

In recent years, the demand for *Z. clinopodioides* is increased for its use in traditional medicine and pharmaceutical industries. However, raw material of the species used comes from natural populations which are severely affected by low rainfall, low soil quality and anthropogenic activity (overgrazing, clearing, etc.). These factors compounded with an increasing harvest and habitat destruction have led to severely depletion and fragmentation of populations (Yang *et al.*, 2008). The habitat fragmentation and the spatial isolation of populations increase genetic drift and differentiation between them, and reduce their potential adaptation to environmental changes (Ellstrand and Elam, 1993).

The maintenance of populations for sustainable exploitation and the ability of a species to adapt to biotic and abiotic changes is determined by the level of their diversity (Chograni *et al.*, 2008). Therefore, develop strategies is the base for conservation and effectively harnessing the potential of the species. In this context, knowledge about the variation within and among populations of such species will be valuable to an understanding of their future maintenance and to developing improvement and conservation practices of endangered and geographically restricted species (Cole, 2003; Godt *et al.*, 2005). However, nothing is known about the population structure and diversity of this species.

Morphological and phytochemical traits are complementary in determining the similarity of inter- and intra-species and the relationship between the populations (Kohler and Friedt, 1999).

Few researchers have evaluated essential oil composition of *Ziziphora* species and it was found *Ziziphora species* contains

*Received: 24 Sep 2015. Received in revised form: 05 Mar 2016. Accepted: 08 Mar 2016. Published online: 14 June 2016.*

secondary metabolites such as pulegone, isomenthone, limonene, 1,8-cineole and piperitenone (Dembistikii *et al.,* 1995; Meral *et al.*, 2002). Sajadi *et al.* (2003) explored the composition of the essential oil from *Z. clinopodioides* in Bakhteyari province and twenty-two components were identified in which pulegone was 53.2%, ρ-methalenalol was 21.4%, 1,8-cineole was 10.3% and β-pinene was 1.6%. In another research Verdian (2008) studied *Z. clinopodioides* composition of essential oil Lam. collected from North of Iran and 26 components were recognized including 97.62% of total essential oil. The main constituents were pulegone (36.45%), piperitenone (19.12%), and menth-2-lenalol (5.31%). Menthol, (+)-pulegone, 1,8-cineole and limonene were found to be the main components of the essential oils of *Z. clinopodioides* (Ozturk and Ercisli, 2007). Moreover, the species is able to produce some phenolic compounds such as caffeic acid or flavonoid derivatives including luteolin, 7-methylsudachitin or thymonin (Yang *et al.*, 2008).

Investigations on *Ziziphora clinopodioides* mainly focused on antibacterial activity of the essential oil and its essential oil composition (Meral *et al.*, 2002; Ozturk and Ercisli, 2007). A few studies described population structure and diversity of this species. To provide further scientific basis for development methods for appropriate conservation strategies of this endangered medicinal species, the present study was designed to investigate morpho-chemical differentiation and population structure among 4 populations of *Ziziphora clinopodioides* subsp. *bungeana* from Iran which has not been examined previously.

## Materials and Methods

### *Surveyed populations and plant material*

Populations of Z. *clinopodioides* were collected from four different localities of Razavi Khorasan and North Khorasan Provinces, Iran, during June 2014 when plants were at late flowering stage. Population 1 was collected from Abjahan, Bajgiran (37.2215° N, 58.2621° E, 2120 m above sea level), population 2 from Aliabad, Quchan (37.0358° N, 52.0011° E, 1450 m above sea level), population 3 from Bashmahale, Farouj (36.5943° N, 58.0726° E, 2060 m above sea level) and population 4 from Tudeh, Shirvan (37.2421° N, 57.4425° E, 1200 m above sea level). Geographic distances between populations vary from 58 km (between Aliabad and Bashmahale) to 165 km (between Aabjahan and Tudeh). The average of the annual rainfall ranged from 415.2 to 241.1 mm. The altitudes of the harvest ranged from 1208 to 1350 m in Tudeh and from 2110 to 2494 m in Aabjahan. The majority of populations were mixed with species such as *Achillea millefolium, Hyssopus officinalis, Borago officinalis, Thymus vulgaris, Origanum majorana* and *Hypericum perforatum.* Site characteristics of identified populations are presented in Table 1. Bioclimatic zones were defined according to Emberger's (1966) Q2 pluviothermic coefficient.

$$Q^2 = 2000P/M^2{-}m^2$$

where $Q^2$ is pluviothermic coefficient, $P$ is the average of annual rainfall (mm), $M$ is the mean of maximal temperature (K: Kelvin) for the warmest month (July) and $m$ is the average of minimal temperature (K) for the coldest month (February).

In each sampling site, 10 individuals were gathered at random with a minimum distances exceeding 50 m from each other to avoid collecting multiple plants from the same parent. The limited number of samples analysed was due to the small size of the existing populations. From each individual, branches with young leaves were taken by hand. Samples were placed on ice in plastic bags and transported to the laboratory for morphological and chemical analyses. Voucher specimens are kept in herbarium of the University of Zabol.

### *Morphological analysis*

In morphological analysis 14 quantitative features were studied from vegetative and reproductive organs of *Z. clinopodioides* subsp. *bungeana* plants. Morphological characters were; The height of the shrub, Stem diameter, Fresh weight, Dry weight, leaf length, Leaf width, Bract length, Pedicle length, Number of branches, Number of inflorescence, Inflorescence leaf length, Inflorescence leaf width, Calyx length, Calyx width, Corolla length, Internodes distance, Inflorescence length, Inflorescence width, Leaf length/leaf width, Leaf length/ Inflorescence leaf length, leaf width/ Inflorescence leaf width.

### *Preparation and phytochemical analysis of essential oil*

Dried aerial parts (500-600 g) of the plant was put into the distillation unit along with 250 ml water and the oil separated by hydrodistillation method for 2.5 hours using a Clevenger type apparatus. The obtained oils were dried over anhydrous sodium sulfate and stored at 4-6 °C.

GC analyses were carried out using a Konic gas chromatograph (model 2000 C) equipped with a flame ionization detector (FID) and a Spectra Physic (model 4290) electronic integrator. An OV-17 (60 m × 0.22 mm, film thickness 0.40 μm) capillary column was employed. The oven temperature was programmed with different stationary phases: 60 °C for 6 min, then increased by 5 °C/min to 150 °C and held isothermally for 10 min; injector and detector temperatures were 225 and 250 °C respectively. The carrier gas was hydrogen and the samples were injected using the splitless technique. The percentages of the components were calculated from the GC peak areas, using the normalization method.

The GC±MS analyses were done on a Thermo mass spectrometer (model Trio 1000), combined with a Thermo gas chromatograph (model 8000) using an OV-17 column (25 m × 0.25 mm film thickness 0.40 μm). Other conditions of GC±MS were the same as set out above. Oil injected volume: 0.1 l, fraction

Table 1. Main ecological and soil physic-chemical characteristics for *Ziziphora clinopodioides* subsp. *bungeana* populations analysed

| Parameters | Population | | | |
|---|---|---|---|---|
| | Aabjahan | Aliabad | Bashmahale | Tudeh |
| Altitude (m) | 2120 | 1450 | 2060 | 1200 |
| Latitude | 37.2215 | 37.0358 | 36.5943 | 37.2421 |
| Longitude | 58.2621 | 52.0011 | 58.0726 | 57.4425 |
| Rainfall ($mmyr^{-1}$) | 415.2 | 300.8 | 351.4 | 241.1 |
| Mean tem (°C) | 11.1 | 12.5 | 15.7 | 15.8 |
| Mean RH | 62 | 55 | 51 | 53 |
| $Q^2$ coefficient[a] | 3034.2 | 2521.9 | 1880.9 | 1434.5 |
| Bioclimates[b] | Sub-humid | Upper semi-arid | Semi-arid | Semi-arid |
| Nitrogen (%) | 0.12 | 0.13 | 0.14 | 0.10 |
| Phosphorus (ppm) | 19 | 14 | 5 | 10 |
| Potassium (ppm) | 233 | 116 | 176 | 84 |
| EC (dSm-1) | 1.02 | 0.96 | 0.83 | 0.56 |
| Soil pH | 8.0 | 8.4 | 8.2 | 8.4 |
| Soil texture | Sandy loam | Sandy loam | Sandy loam | Sandy loam |

[a] Bioclimatic zones were defined according to Emberger's (1966) Q2 pluviothermic coefficient climate classification
[b] Q2 was calculated for each site using *P*, *M* and *m* average values for the period 1981-2011 from data provided by the Iranian National Institute of Meteorology

injected volume: 0.2 l. Identification of the components was based on the comparison of their GC retention indices (RI) on non-polar and polaSr columns, determined to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literatures data (Djabou *et al.,* 2012a). To estimate the concentrations of the constituents of the oils, we calculated response factors for all chemical groups relative to tridecane used as internal standard.

*Statistical analysis*

In order to indicate significant differences in studied morphological characters among locations, analysis of variance (ANOVA) followed by the Least Significant Differences (LSD) tests were carried out. To display degree of separation or similarity among populations the cluster analysis (CA; dendrograms) was conducted using the unweighted pair-group method with arithmetic average (UPGMA) and the Euclidean distance as the similarity coefficient. SAS ver. 9.1 and NTSYSpc Ver. 2.02 was used for statistical analysis.

## Results and Discussion

*Plant morphology*

The data of different morphological parameters in different populations of *Z. clinopodioides* are presented in Table 2. *Z. clinopodioides* is mat-forming perennial with prostrate to erect stems generally much-branched at base. Its leaves display a high alteration in shape and size (Keshavarzi *et al.,* 2008; Hatri *et al.,* 2013). The plants are hairless to hairy with hairs up to 0.25 mm length. Hair distributed very sparsely and infrequent. The hair are direct and white colored (Keshavarzi *et al.,* 2008).

In all the studied populations, the height of the shrubs was between 218.1 and 305.1 mm, and a basal woody part is obviously developed and reaches the length of up to 250 mm. Range of fresh and dry weight span was from 58.4 to 80.1 g plant$^{-1}$ and from 19.4 to 24.9 g plant$^{-1}$, respectively. However, slight differences could be observed between the populations growing in different ecological conditions. In general, in all the studied populations the leaf length was between 13.6 and 20.3 mm, whiles the leaf width changed between 5.5 and 8.2 and mm. Some differences were observed in inflorescence leaf length and Inflorescence leaf width of different populations. The inflorescence leaf size in Abjahan were generally larger than those found in other locations. The average bract length varied between 0.8 and 1.2 mm. The panicle length usually varies in between 0.7 and 0.8 mm. In the individuals from Aliabad, Bashmahale and Tudeh populations, the internodes distance usually varies in between 8.6 and 15.7 mm. The internode distances are very small and compact, so that the leaves overlap each other. Contrary to them, the internodes distance of the Aabjahan population reach a height of over 28 mm, having longer internode distance; accordingly their leaves often do not overlap. The stem morphology in all the investigated populations is more or less uniform. The herbaceous stem of *Z. clinopodioides* specimens is close to square-shaped (in the cross section). The stem diameter ranges from 1.7 to 2.3 mm, which is within the normal amounts found in xeromorphic plants (Fahn and Cutler, 1992; Gorgini Shabankar *et al.,* 2015). There is a little difference regarding the morphology of inflorescence between populations. Inflorescence length and width of all plants studied ranged between 42.1 and 55.3 and 5.7 and 7.8 mm, respectively, being the longest in the plants from the Abjahan. The calyx length was between 5.3 and 7.4 mm, whereas the calyx width ranged between 1.6 and 1.7 mm. Collar length was 6.9 to 8.2 mm and the longest collar, on average, were observed in Abjahan population and the shortest collars were observed in Bashmahale population.

Number of branches and number of inflorescences in plants collected from Abjahan was significantly higher than other locations (5.2 branches, 6.7 inflorescence plant$^{-1}$), while plants collected from Aliabad had the least number of branches and number of inflorescences.

The Cluster analysis of *Z. clinopodioides*, based on morphological characteristics defined two major clusters (Fig. 1). The sub-humid and semi-arid populations has shown two morphologically almost completely separate groups and the populations from Bashmahale and Tudeh stand completely separated from the sub-humid populations (Abjahan), which was characterized by the highest amount of vegetative growth. The population from Aliabad shows transitional characteristics between the semi-arid and sub-humid populations, as it was the case also in the ANOVA analysis.

In all studied locations, sub-humid and semi-arid ones, the plant populations which have been studied, to a certain extent pronounced, longer or shorter periods of summer drought stress prevail. Inter-population differences refer to low discrepancy in quantitative and morphological parameters. While all these traits are of the same pattern, they are obviously more represented in the sub-humid than in the semi-arid populations.

As no one has studied *Z. clinopodioides* morphological features before, the results could not be compared with others observations. The studied parameters are assumed to be most closely related to different species of *Mentha* and *Lycopus* in the tribe Mentheae (Briquet 1986). According to the present results, the morphology parameters in *Z. clinopodioides* may be helpful in clarifying the infra specific relationships. As with any morphological survey, the more complete the data, the more persuasive the subsequent analyses regarding relationships and phylogeny.

Table 2. Morphological variables examined in 4 populations of *Z. clinopodioides*

| Morphological variables | Population | | | |
|---|---|---|---|---|
| | Aabjahan | Aliabad | Bashmahale | Tudeh |
| Shrub Height (mm) | 305.1a | 218.1b | 303.7a | 236.5b |
| Stem diameter (mm) | 2.3a | 1.7c | 2.0b | 1.7c |
| Fresh weight (g plant$^{-1}$) | 80.1a | 68.5b | 79.1a | 58.4c |
| Dry weight (g plant$^{-1}$) | 24.9a | 22.0ab | 23.7a | 19.4b |
| Leaf length (mm) | 20.3a | 13.6b | 18.7a | 15.2b |
| Leaf width (mm) | 8.2a | 5.5b | 7.8a | 5.9b |
| Bract length (mm) | 1.2a | 0.8c | 1.0b | 0.9b |
| Pedicle length (mm) | 0.8a | 0.7b | 0.7b | 0.7b |
| Number of branches | 5.2a | 3.1c | 4.7b | 4.4b |
| Number of inflorescence | 6.7a | 3.2c | 3.9c | 4.8b |
| Internodes distance (mm) | 36.2a | 25.7b | 23.7b | 18.6c |
| Inflorescence leaf length (mm) | 11.9a | 12.0a | 10.3b | 9.8b |
| Inflorescence leaf width (mm) | 4.3a | 2.8c | 3.1b | 3.6b |
| Calyx length (mm) | 7.4a | 5.3c | 5.9b | 6.3b |
| Calyx width (mm) | 1.7a | 1.6a | 1.6a | 1.7a |
| Corolla length (mm) | 8.2a | 7.3b | 6.9b | 7.2b |
| Inflorescence length (mm) | 55.3a | 42.1c | 45.7bc | 48.2b |
| Inflorescence width (mm) | 7.8a | 6.1b | 5.7b | 6.2b |
| Leaf length / Leaf width | 2.5a | 2.5a | 2.4a | 2.6a |
| Leaf length / Inflorescence leaf length | 1.7a | 1.1b | 1.8a | 1.6a |
| Leaf width / Inflorescence leaf width | 1.9b | 2.0b | 2.5a | 1.6c |

Means followed by a similar letter within a column are not significantly different at the 0.05 level probability by LSD Test.

Table 3. Chemical compositions of *Z. clinopodioides* essential oils collected from different location

| Retention index[a] | Composition of essential compounds | R.t.[b] | Abjahan | Aliabad | Bashmahale | Tudeh |
|---|---|---|---|---|---|---|
| 1 | Hexanal | 878 | 0.9 | 0.8 | 0.6 | - |
| 2 | Tricycllene | 895 | 1.1 | 0.7 | 0.9 | 1.0 |
| 3 | α-Thujene | 917 | 1.3 | 0.9 | 1.1 | 0.8 |
| 4 | 3-methyl-cyclohexane | 928 | 1.0 | 1.2 | 1.3 | 0.9 |
| 5 | α-pinene | 936 | 0.9 | 1.1 | 0.7 | 0.7 |
| 6 | CampheNe | 946 | 2.0 | 1.3 | 0.8 | 1.1 |
| 7 | Sabinene | 969 | 1.1 | 1.0 | 0.7 | 0.8 |
| 8 | β-pinene | 980 | 0.9 | 0.8 | 1.0 | 0.7 |
| 9 | Myrcene | 991 | 1.2 | 1.4 | 0.9 | 1.0 |
| 10 | Para-menta-1 (7),8-dien | 1008 | 0.8 | 1.5 | 1.2 | 1.1 |
| 11 | α-terpinene | 1017 | 1.2 | 1.6 | - | 0.8 |
| 12 | p-cymene | 1025 | 1.0 | 1.0 | 0.8 | 0.7 |
| 13 | 1,8-Cineole | 1033 | 3.7 | 5.7 | 7.5 | 5.0 |
| 14 | β-Ocymen | 1041 | 1.1 | 0.9 | 1.1 | 0.8 |
| 15 | Limonene | 1048 | 6.5 | 5.3 | 4.6 | 1.1 |
| 16 | γ-terpinene | 1057 | 1.2 | 1.1 | 0.9 | 0.8 |
| 17 | Sabinen hydrate | 1063 | 1.4 | 1.5 | 1.3 | 1.0 |
| 18 | Linalool | 1079 | 0.9 | 0.8 | 1.0 | 1.2 |
| 19 | Para-menta-3,8-dien | 1085 | 2.3 | 2.6 | 1.2 | 4.5 |
| 20 | Terpinolene | 1096 | 0.8 | 1.1 | 0.9 | 1.3 |
| 21 | Menthone | 1132 | 1.0 | 1.2 | 0.8 | 4.8 |
| 22 | Iso- Menthone | 1150 | 15.2 | 18.3 | 10.8 | 9.1 |
| 23 | L-(-)- Menthol | 1170 | 1.3 | 1.1 | 4.8 | 1.0 |
| 24 | Isopulegone | 1182 | 1.0 | 1.2 | 1.4 | 1.0 |
| 25 | Terpinen-4-ol | 1202 | 1.2 | 1.0 | 0.9 | 0.8 |
| 26 | Pulegone | 1224 | 25.5 | 20.2 | 30.4 | 34.2 |
| 27 | 2-cyclohexene | 1239 | 0.8 | 1.7 | 1.5 | 0.9 |
| 28 | Piperitone | 1252 | 4.6 | 1.0 | 0.8 | 0.7 |
| 29 | Carvon | 1263 | 0.8 | 0.8 | 0.7 | 1.0 |
| 30 | Bronyl acetate | 1279 | 1.2 | 1.2 | 0.9 | 0.8 |
| 31 | Thymol | 1292 | 6.8 | 3.7 | 4.0 | 4.3 |
| 32 | Carvacrol | 1312 | 1.2 | 1.0 | 0.8 | 0.9 |
| 33 | Piperitenone | 1330 | 1.5 | 4.8 | 7.7 | 8.2 |
| 34 | Ment furanone | 1344 | 0.8 | 0.8 | 1.5 | 1.0 |
| 35 | Piperitenone oxide | 1371 | 1.3 | 5.4 | 0.7 | 1.2 |
| 36 | β-Bourbonene | 1395 | 0.9 | 1.0 | 0.9 | 0.8 |
| 37 | Elmene | 1434 | 0.8 | 0.8 | 1.1 | 1.0 |
| 38 | β-caryophyllene | 1450 | 1.2 | 1.2 | 0.8 | 0.9 |
| 39 | Germacrene D | 1466 | 1.0 | 0.8 | 0.7 | 0.7 |

[a] Order of elution is given on column
[b] Retention time on the column (Second)

*Chemical essential oil composition*

he analysis of hydrodistilled essential oils derived from the aerial parts of *Z. clinopodioides* was carried out. The yields of essential oils ranged from 0.23% for Abjahan, 0.40% for Aliabad and 0.57% for Bashmahale and for 0.61% Tudeh. Since the protrusions of capitate secretory cells have just a little storing space, there is a persistent evaporation of essential oils (Meral *et al.,* 2002; Sonboli *et al.,* 2006) and thus reducing essential oil of leaves. It is well known that all species of the genus *Ziziphora* L. are recognized by only a small quantity of essential oils (Dembistikii *et al.,* 1995; Meral *et al.,* 2002; Ozturk and Ercisli, 2007).

According to Voirin *et al.* (1990), the oil yield is favoured with higher temperatures, water deficit and higher summer sunshine, which is the case in the Tudeh and Bashmahale, but not so much in Abjahan and Aliabad, which may explain the difference in the yields found. The essential oil yield, in the ten wild populations of two Mediterranean subspecies of *T. scorodonia*, collected at full flowering ranged from 0.7% to 1.3% (essential oil weight per plant dry weight; w/d.w.), averaging 0.9% (w/d.w.) (Djabou *et al.,* 2012b). These values are in accordance with the reported oil yield study by Djabou *et al.* (2011) and with some reported oil yields at full flowering for two Mediterranean subspecies of *T. polium* (Djabou *et al.*, 2012a).

GC and GC/MS analysis of essential oils allowed the identification of 39 compounds from population of Abjahan and Aliabad, 38 compounds from population of Bashmahale and Tudeh, representing from 98.6 to 99.7% of the total essential oil composition. Among them 18 sesquiterpenes, 13 monoterpenes, 4 aromatics, 2 alcohols and 2 esters compounds were identified. The concentrations (%) of the components are listed in Table 3 in order of their elution on the HP-1 column.

The main components of *Z. clinopodioides* oil collected from population of Abjahan were pulegone (25.5%), Iso-menthone (15.2%), thymol (6.8%), limonene (6.5%), piperitone (4.6%), 1, 8-Cineole (3.7%) and Para-menta-3, 8-dien (2.3%). The main components of *Z. clinopodioides* oil collected from population of Aliabad were pulegone (20.2%), Iso- menthone (18.3%), 1,8-Cineole (5.7%), piperitone oxide (5.4%), piperitone (4.8%) and thymol (3.7%). The main components of *Z. clinopodioides* oil collected from population of Bashmahale were pulegone (30.4%), Iso- menthone (10.8%), piperitone (7.7%), 1,8-Cineole (7.5%), limonene (4.6%) and thymol (4.0%). The main components of *Z. clinopodioides* oil collected from population of Tudeh were pulegone (34.2%), Iso- menthone (9.1%), piperitone (8.2%), 1, 8-Cineole (5.0%), menthone (4.8%) and Para-menta-3, 8-dien (4.5%). It is noticeable that the chemical composition of *Z. clinopodioides* population studied here was in accordance with those previously reported in literature (Baytop, 1996; Dembistikii *et al.,* 1995; Meral *et al.*, 2002). The present study and a huge body of literature showed that pulegone is the major component of essential oil of *Ziziphora* spp. (Dembistikii *et al.*, 1995; Meral *et al.*, 2002). Pulegone has been known as hepatotoxin that lead to some kind of internal haemorrhage, pulmonary oedema and massive necrosis (Sonboli *et al.*, 2006). All oils were dominated by hydrocarbon compounds (Table 4): 85.3, 81.5, 71.2 and 75.7% in populations of Abjahan, Aliabad, Bashmahale and Tudeh, respectively; however the population of Abjahan and Aliabad revealed higher amounts of sesquiterpenes than those of Bashmahale and Tudeh, while the latter displayed higher amounts of monoterpenes than the first. The aromatic, alcohols and esters fraction is present in oil samples at low values.

Table 4. Concentration (%) of identified oil components arranged according to the five types of chemical groups

| Different chemical groups | Abjahan | Aliabad | Bashmahale | Tudeh |
|---|---|---|---|---|
| Monoterpence hydrocarbons | 43.2 | 41.2 | 42.1 | 45.4 |
| Sesquiterpene hydrocarbons | 42.1 | 40.3 | 29.1 | 30.3 |
| Aromatic | 5.3 | 5.4 | 12.4 | 11.1 |
| Alcohols | 4 | 5.7 | 7.8 | 3.4 |
| Esters | 4.8 | 6.9 | 8.3 | 9.4 |
| Total | 99.4 | 99.5 | 99.7 | 98.6 |

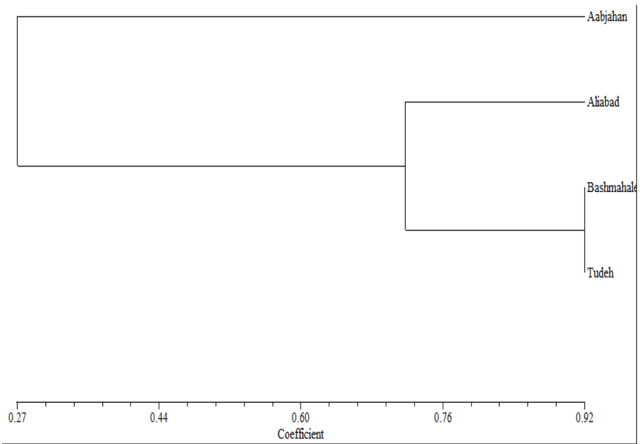


Fig. 1. Cluster analysis of morphological characteristics of *Z. clinopodioides* from Razavi Khorasan and North Khorasan provinces province

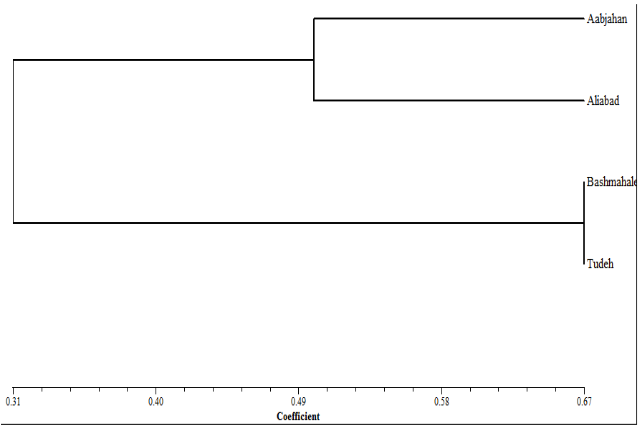


Fig. 2. Cluster analysis of chemical compositions of *Z. clinopodioides* oils from Razavi Khorasan and North Khorasan provinces

Physiological variations (i.e. organ and leaf position), environmental conditions (i.e. soil condition, moisture availability and temperature), geographic variations, genetic factors and evolution are known to affect the biosynthesis of the essential oils (Figueiredo *et al.,* 2008). Thus, these type of variations, that where already seen in *Z. clinopodioides* may be due to the influence of the developmental stage and environmental conditions on the regulation of the essential oil biosynthesis.

Normalized percentage abundances of all identified components were used for statistical analysis. To identify the relationship between the chemical compositions of oils cluster analysis were applied. The data presented in Fig. 2 was obtained from the standardized matrix. The obtained dendrogram suggests that there are two main clusters correlated with the climatic condition of geographic origin of the oil samples. Cluster I included oil samples from Abjahan and Aliabad and Cluster II included oil samples from Bashmahale and Tudeh. Cluster I was characterized by a higher amount of Iso-menthone (15.2-18.3%) and lower amount of pulegone (20.2-25.5%) than group II (9.1-10.8% and 30.4-34.2%, respectively). Oil samples in cluster I contained α-terpinene and hexanal, which were not detected in the oil samples of Bashmahale and Tudeh, respectively.

It appears that differences in oil compositions between the oil samples from the four harvest areas could be caused by environmental conditions. The chemical variability could be linked to the presence of divalent metal ions such as $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$ and $Co^{2+}$ which improve the specific production of hydrocarbon sesquiterpenes in plants (Duarte *et al.*, 2010).

## Conclusion

The use of phytochemical and morphological characters proved to be powerful tools for studying and intraspecific differentiation and for elucidating the influence of environment on chemical and morphology traits. The present study revealed a significant difference in diversity between different population of *Z. clinopodioides* according to chemical and morphology analyses. These differences could be explained by the geographical and genetic separation of the populations. *Z. clinopodioides* samples were clustered in 2 sub-groups characterized by 2 essential oil chemical compositions and an important morphological difference. The chemical and morphological analysis of *Z. clinopodioides* was approximately parallax. The major known components found in essential oil of *Z. clinopodioides* were pulegone, Iso-menthone and thymol. This species were not investigated before and this survey was the first step in Iran to examine variation of this species. The findings of this study will thus facilitate from a conservation perspective, the low genetic and phytochemical diversity observed, within the populations tested is symptomatic and a signal that ecological management of *Z. clinopodioides* habitats is necessary to prevent the consequent decline in population size that could increase the risk of extinction due to demographic and environmental stochasticity. Further studies on polymorphisms and the expression of genes involved in the biosynthesis of essential oil compositions could provide additional information on the structures of plant populations.

**Acknowledgments**

This work was supported by the University of Zabol, Iran.

**References**

Briquet J (1986). Labiatae. In: Engler W, Prantl A (Eds). Die Naturlichen Pflanzenfamilien. Engelmann, Leipzig, Germany pp 231-279.

Chograni H, Messaoud Ch, Boussaid M (2008). Genetic diversity and population structure in Tunisian *Lavandula stoechas* L. and *Lavandula multifida* L. (Lamiaceae). Biochemical Systematic and Ecology 36:349-359.

Cole CT (2003). Genetic variations in rare and common plants. Annual Review of Ecology and Systematics 34:213-237.

Dembistikii AD, Bergalier ES, Kyazimer IM (1995). Morphological and phytochemical study of *Ziziphora tenuior L.* Chemistry of Natural Compounds 31:673-675.

Djabou N, Allali H, Battesti MJ, Tabti B, Costa J, Muselli A, Varesi L. (2012a). Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L. Phytochemistry 74:123-132.

Djabou N, Battesti MJ, Allali H, Desjobert JM, Varesi L, Costa J, Muselli A (2011). Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L. Phytochemistry 73:1390-1399.

Djabou N, Muselli A, Allali H, Dib MEA, Tabti B, Varesi L, Costa J (2012b). Chemical and genetic diversity of two Mediterranean subspecies of *Teucrium polium* L. Phytochemistry 74:108-119.

Duarte A, Santos S, Seraphin J, Ferri P (2010). Environmental influence on phenols and essential oils of *Myrciaria cauliflora* leaves. Journal of the Brazilian Chemical Society 21:1672-1680.

Ellstrand NC, Elam DR (1993). Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Emberger L (1966). Une classification biogéographique des climats. Recherches et Travaux des Laboratoires de Géologie, Botanique et Zoologie. Faculté des Sciences Montpellier 7:1-43.

Fahn A, Cutler FD (1992). Xerophytes. Encyclopedia of Plant Anatomy. Borntraeger, Berlin.

Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal 23:213-226.

Godt MJW, Caplow F, Hamrick JL (2005). Allozyme diversity in the federally threatened golden paintbrush, *Castilleja levisecta* (Scrophulariaceae). Conservation Genetics 6:87-99.

Gorgini Shabankare H, Asgharipour MR, Fakheri B (2015). Morpho-chemical Diversity among Iranian *Teucrium polium* L. (Lamiaceae) Populations in Fars Province. Journal of Agricultural Science and Technology 17:705-716.

Hatari Z, Zamani Z, Nazeri V, Tabrizi L (2013). Evaluation of genetic diversity in the medicinal plant Kakooti (*Ziziphora tenuior* L.) using morphological characteristics and RAPD markers. Modern Genetics 8(1):19-28.

Keshavarzi M, Jahandideh R, Nazem Bokaee Z (2008). Morphological and anatomical studies on *Ziziphora clinopodioides* Lam. (Labiatae). Pakistan Journal of Biological Sciences 11(23):2599-2605.

Kohler H, Friedt W (1999). Genetic variability as identified by AP_PCR and reaction to *Sclerotinia sclerotiorum* among interspecific sunflower (*Helianthus annuus* L.) hybrid progenies. Crop Sciences 39:1456-1463.

Meral GE, Konyalioglu S, Ozturk B (2002). Essential oil composition and antioxidant activity of endemic *Ziziphora taurica* subsp. *cleonioides*. Fitoterapia 73:716-8.

Mozaffarian V (1996). A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran.

Naghibi F, Mosaddegh M, Mohammadi Motamed S, Ghorbani A (2005). Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research 2:63-79.

Ozturk S, Ercisli S (2007). Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. Food Control 18:535-540.

Rechinger KH (1982). *Ziziphora*. In: Flora Iranica, No. 150. Akademische Druck u. Verlagsanstalt. Graz. Austria.

Sajadi SA, Ghassemi Dehkordi N, Baluchi M (2003). The study on components of *Z. clinopodioides* plant aerial parts essential oil. Pazhohesh va Sazandegi 16(1):97-100.

Sardashti AR, Valizadeh J, Adhami Y (2012). Chemical composition of the essential oil from *Ziziphora clinopodioides* Lam. from Iran by means of gas chromatography-mass spectrometry (GC-MS). Journal of Horticulture, Forest and Biotechnology 4(10):169-171.

Schulz H, Özkan G, Baransk M, Krüger H, Özcan M (2005). Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy. Vibrational Spectroscopy 39:249-256.

Sonboli A, Mirjalili MH, Hadian J, Nejad Ebrahimi S, Yousefzadie M (2006). Antibacterial activity and composition of the essential oil of *Ziziphora clinopodioides* subsp. *bungeana* (Juz.) Rech. f. from Iran. Zeitschrift Naturforsch 61:112-121.

Verdian MR (2008). Essential oil composition and biological activity of *Ziziphora clinopodioides* Lam. from Iran. American-Eurasian Journal of Sustainable Agriculture 2:69-71.

Voirin B, Brun N, Bayet C (1990). Effect of day length on the monoterpene composition of leaves of *Mentha piperita*. Phytochemistry 29:749-755.

Yang XJ, Li N, Meng DL, Li W, Li X (2008). Isolation and identification of chemical constituents from *Ziziphora clinopodioides* Lam. Journal of Shenyang Pharmacy University 25:456-8.

Zargari A (1993). Iranian medicinal plant. Vol 4, Tehran University Press pp 103-104.