

## Morpho-chemical Diversity among Iranian *Teucrium polium* L. (Lamiaceae) Populations in Fars Province

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### ABSTRACT

Differentiation among populations of the Iranian *Teucrium polium* L. was analyzed on the basis of morphological and phytochemical variability, to evaluate the level and distribution of diversity among four distant populations from arid, semi-arid, and sub-humid regions of Fars Province. Morphological analysis included 11 characters related to the plant, leaf, and stem morphology. Analyses of variances and clustering were done to establish the variability and significance of morphological differentiation. The morphological analysis of plants from the studied populations confirmed that the species belonged to malacophyllous xeromorphic species and were distinguished by stable conservative xeromorphic characteristics. Morphological variation was correlated with ecological conditions at the site of origin and there was a small difference between the plants belonging to arid and semi-arid populations and the sub-humid ones. Chemical analysis was performed using combination of capillary GC, GC-MS after fractionation on column chromatography. The chemical composition of their oil differed qualitatively and quantitatively between the populations.  $\beta$ -Caryophyllene was the major oil compound in the sub-humid and semi-arid populations, while the main compound of arid populations were farnesene-cis-b and linalool. In addition, oil samples from semi-arid and sub-humid populations contained  $\beta$ -bisabolene (1.6-2.2%), myrcene (0.9-1.1%), bornyl acetate (0.7-0.8%), and 3-octanol (0.6-0.8%), which were not detected in oil samples from arid populations. All oil samples, however, were dominated by hydrocarbon compounds. The relatively low morpho-chemical diversity in the populations 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:** Essential oil, Medicinal plant, Morphology.

### INTRODUCTION

Plant morphological and phytochemical variation is fundamentally involved in the ability of a species to adapt to biotic and abiotic changes and the maintenance of populations for sustainable use. Therefore, recognition of the levels and distribution of the variation within and among populations of a species is crucial for an understanding of their future maintenance and developing improvement and conservation programs and preservation of endangered and geographically restricted species (Cole,

2003; Godt *et al.*, 2005; Escudero *et al.*, 2003; Shah *et al.*, 2008).

*Teucrium* is a genus of perennial plants, the largest of the Labiatae (Lamiaceae) family in the Mediterranean area, which constitute more than 300 species widespread all around the world and comprises about 12 species in Iran (Tutin *et al.*, 1972; Sadeghia *et al.*, 2014). Plants belonging to the genus *Teucrium* have evolved in nature through natural hybridization and selection, showing substantial variation in terms of their natural habitats, growth characteristics, and aromas (Lakušić *et al.*, 2006; Tutin *et al.*, 1972).

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*Teucrium polium* L., locally called "Kalpooreh", is found mainly in the hills and deserts of Mediterranean and Western Irano-Turanian phytogeographical region. The leaves, 1–3 cm long, are sessile, oblong or linear; the stems are ending in shortly pediculate or corymbose inflorescences, corolla is white or pale cream colored (Lakušić *et al.*, 2010). Phytochemical investigations have shown that *T. polium* contains various compounds, such as iridoids, flavonoids, and diterpenoids (Piozzi *et al.*, 2005). Several researchers have evaluated the composition of the essential oil of *T. polium* grown in different geographic areas (Moghtader, 2009; Djabou *et al.*, 2012a; Sadeghia *et al.*, 2014). These studies revealed some chemical differences in the oil compositions, probably related to the different subspecies and/or the geographical origin of the plants.

Traditionally, *T. polium* has been utilized in Mediterranean countries for its antispasmodic and hypoglycemic activities (Abu-Irmaileh and Afifi, 2003). In addition, the plant possesses insulinotropic, anti-inflammatory and antimicrobial activities, hypolipidemic, antinociceptive and antioxidant properties (Esmaeili and Yazdanparast, 2004). The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (Dixon *et al.*, 2005). Essential oils of the plant have been the subject of several investigations and diverse chemical compositions were reviewed. It appears that *Teucrium polium* essential oils are characterized by mono and sesquiterpenes hydrocarbon compounds (Kovačević *et al.*, 2001; Ali *et al.*, 2008; Mitić *et al.*, 2012). The quantitative prevalence of sesquiterpene and monoterpene hydrocarbon have been reported as chemical characteristics of the essential oil of the plant which grow wild in Khuzestan Province, Iran (Sadeghia *et al.*, 2014).

Few researchers have evaluated diversity among the populations of *Teucrium* species and, to our knowledge, only two studies investigated the diversity of *T. polium*.

Boulila *et al.* (2010) explored the genetic diversity in Tunisian populations of *T. polium* based on RAPD markers and El Oualidi *et al.* (1999) studied the utility of internal transcribed spacer sequences analysis for resolving relationships in *T. polium*.

Raw material of the species used for pharmaceutical purposes is being obtained from the wild, which exerts a huge pressure on its natural populations (CAMP, 2003). Conservation of a minimum level of intra- and inter-population diversity is essential for the establishment of effective maintenance practices for rare and endangered medicinal plants (Barret and Kohn, 1991). However, nothing is known about the population structure and diversity of this species. In the present study, we investigated morpho-chemical differentiation and population structure among 4 populations of Iranian *T. polium*. The main objectives of our study were: (1) to reveal the degree of morpho-chemical diversity and how the variation was distributed within and among the populations, and (2) to describe the morphological characteristics of leaves and stems and identification of chemical composition.

## MATERIALS AND METHODS

### Surveyed Populations and Plant Material

The aerial parts of *T. polium* was collected from plants naturally growing in 4 distantly located populations with different geographic origins in Fars Province during 20-30 August 2013 when plants were at late flowering stage. Population 1 was collected from Zibashahr, population 2 from Sepidan, population 3 from Komehr, and population 4 from Kakan. Geographic distances between populations varied from 15 km (between Kakan and Komehr) to 70 km (between Kakan and Zibashahr). The average annual rainfall ranged from 133.4 to 652.9 mm. The majority of populations were mixed with

species such as *Thymus vulgaris* L., *Origanum majorana* L., *Achillea millefolium* L., *Borago officinalis* L., *Hyssopus officinalis* L. and *Hypericum perforatum* L. Site characteristics of identified populations are presented in Table 1 and Figure 1. Bioclimatic zones were defined according to Emberger's (1966)  $Q_2$  pluviothermic coefficient.

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

Where,  $Q^2$  is pluviothermic coefficient,  $P$  is the average 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 population, 20 individuals were sampled at random with the minimum distances exceeding 50 m from each other to avoid collecting multiple plants from the same parent. The limited number of samples analyzed 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

A morphological analysis was done on plant samples from the collected populations of the species *T. polium*. Morphological characters were: (1) The height of the shrub; (2) diameter of the stem; (3); internodes distance; (4) leaf length; (5) the largest width of the leaf; (6) inflorescence length, and (7) inflorescence dry and fresh weight ( $\text{plant}^{-1}$ ).

### Gas Chromatography (GC)

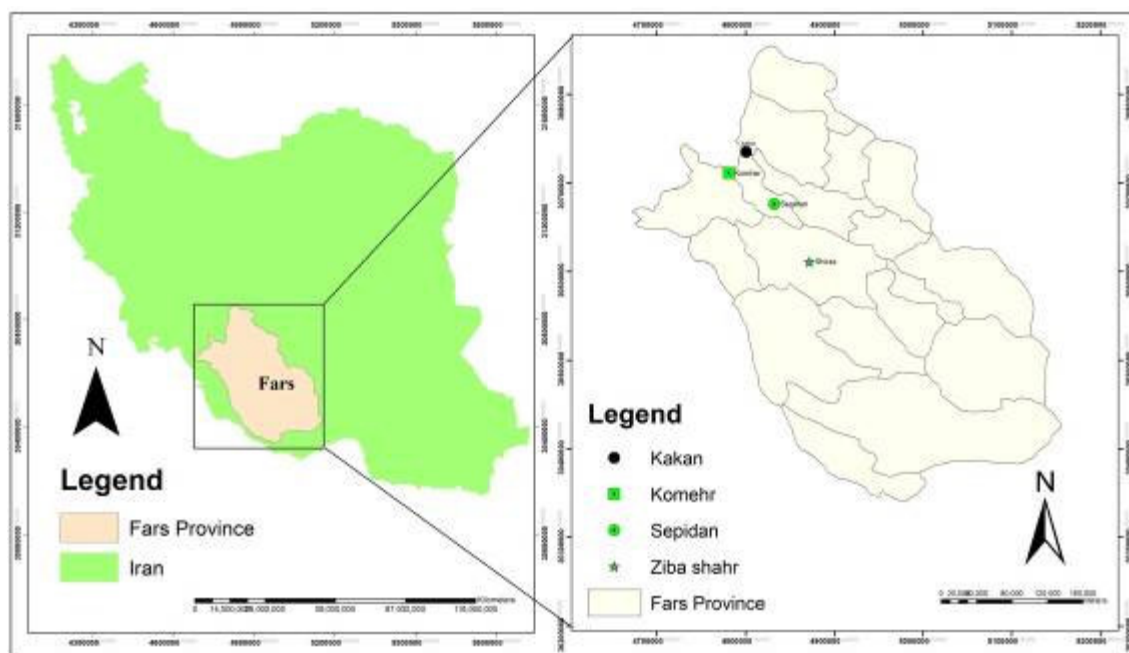
To obtain essential oils, the fresh aerial parts of plants (400–700 g) were subjected to hydro-distillation for 2.5 hours. The

**Table 1.** Main ecological and soil physicochemical characteristics for the 4 Iranian *Teucrium polium* populations studied.

Population	Altitude (m)	Latitude	Longitude	Rainfall ( $\text{mm yr}^{-1}$ )	Mean tem ( $^{\circ}\text{C}$ )	Mean RH	$Q^2$ coefficient <sup>a</sup>	Bio-climate <sup>b</sup>	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Organic matter (%)	Soil pH	Soil texture
Zibashahr	1600	52.33	29.39	324.2	18	41	70.91	semi-arid	0.265	38.80	29.12	0.75	7.3	Loamy
Sepidan	2500	52.00	30.16	652.9	14.8	40	224.46	Sub-humid	0.139	43.22	22.75	0.14	7.7	Loamy
Komehr	2800	51.57	30.19	133.4	13.75	34.5	32.07	Arid	0.142	39.60	30.00	0.09	7.9	Loamy
Kakan	2250	51.48	30.34	227.2	11.8	38	46.36	Arid	0.138	48.55	21.44	0.09	7.5	Loamy

<sup>a</sup>  $Q_2$  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.

<sup>b</sup> Bioclimatic zones were defined according to Emberger's (1966)  $Q_2$  pluviothermic coefficient climate classification.



**Figure 1.** The four sampling regions, namely, Zibashahr, Sepidan, Kakan, and Komehr in Fars Province, Iran.

distillate was dried over anhydrous sodium sulfate and stored at  $4\pm 6^{\circ}\text{C}$ .

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 $\times$ 0.22 mm, film thickness 0.40  $\mu\text{m}$ ) capillary column was employed. The oven temperature was programmed with different stationary phases:  $60^{\circ}\text{C}$  for 6 minutes, then increased by  $5^{\circ}\text{C min}^{-1}$  to  $150^{\circ}\text{C}$  and held isothermally for 10 min; injector and detector temperatures were 225 and  $250^{\circ}\text{C}$ , 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.

### Gas Chromatography $\pm$ Mass Spectrometry

The GC $\pm$ 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 $\times$ 0.25 mm film thickness 0.40  $\mu\text{m}$ ). Other conditions of GC $\pm$ MS were the same as set out above. Oil injected volume: 0.1  $\mu\text{L}$ , Fraction injected volume: 0.2  $\mu\text{L}$ . Identification of the components was based on the comparison of their GC retention indices (RI) on non-polar and polar 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.

### Data Analysis

Morphological variables analysis of variance was used to assess the degree of separation or similarity among the populations. The cluster analysis (CA; dendrograms) was conducted using the un-

weighted pair-group method with arithmetic average (UPGMA) and the Euclidean distance as the similarity coefficient, in the NTSYSpc ver. 2.02 software. In the discriminant analysis, 8 measurements were used for each population, and in the cluster analyses the score for each character was the mean value of the 8 measurements.

To identify possible differentiation between the chemical oil compositions, cluster analysis were applied to essential oil compositions in different populations. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

## RESULTS AND DISCUSSION

### Plant Morphology

The data of different morphological parameters in different populations of *T. polium* are presented in Table 2.

*T. polium* is an evergreen, branchy, and semi-ligneous shrub. In all the populations studied, the height of the shrubs was between 12.2 and 34.1 cm, and a basal woody part was clearly developed and reached the length of up to 20 cm. Range of fresh and dry weight span was from 524.3 to 721.6 g plant<sup>-1</sup> and from 45.5 to 66.6 g plant<sup>-1</sup>, respectively. However, slight differences could be observed between the populations growing in different ecological conditions. In the individuals from Komehr, Kakan, and Sepidan populations, the height of the shrubs usually varied between 12 and 22 cm. The internode distances were very short and dense, so that the leaves overlapped each other. Contrary to them, the shrubs of the Zibashahr population reached a height of over 30 cm, having longer internode distance; therefore, their leaves mostly did not overlap. In general, in all the studied populations, the leaf length was between 13.4 and 28.7 mm, whereas the leaf width ranged between 4.1 and 30.0 mm. The

**Table 2.** Morphological variables examined in 4 populations of *T. polium*.<sup>a</sup>

Locations	ShH (cm)	SD (mm)	FW (g plant <sup>-1</sup> )	DW (g plant <sup>-1</sup> )	LL (mm)	LW (mm)	ID (mm)	LSA (m <sup>2</sup> plant <sup>-1</sup> )	IL (mm)	IFW (g plant <sup>-1</sup> )	IDW (g plant <sup>-1</sup> )
Zibashahr	34.1a <sup>b</sup>	4.0a	721.6a	66.6a	2.9a	3.0a	3.0a	78.6a	3.0a	50.0a	18.7a
Kakan	14.3c	2.0b	524.3b	47.9b	2.5a	0.4b	2.0a	43.1b	1.5c	32.2b	9.0b
Kouhmar	12.2c	1.0c	597.2b	55.6ab	1.3b	0.4b	2.1a	41.2b	1.4c	33.3b	10.5b
Sepidan	21.2b	2.5b	527.7b	45.5b	2.6a	0.8b	2.1a	50.4b	2.0b	44.5ab	12.8b

<sup>a</sup> ShH: The height of the shrub; SD: Stem Diameter; FW: Fresh Weight; DW: Dry Weight; LL: Leaf Length; LW: The Largest Width of the leaf; ID: Internodes Distance; LSA: Leaf Surface Area; IL: Inflorescence Length; IFW: Inflorescence Fresh Weight; IDW: Inflorescence Dry Weight.

<sup>b</sup> Means followed by a similar letter within a column are not significantly different at the 0.05 level probability by Duncan's Multiple Range Test.

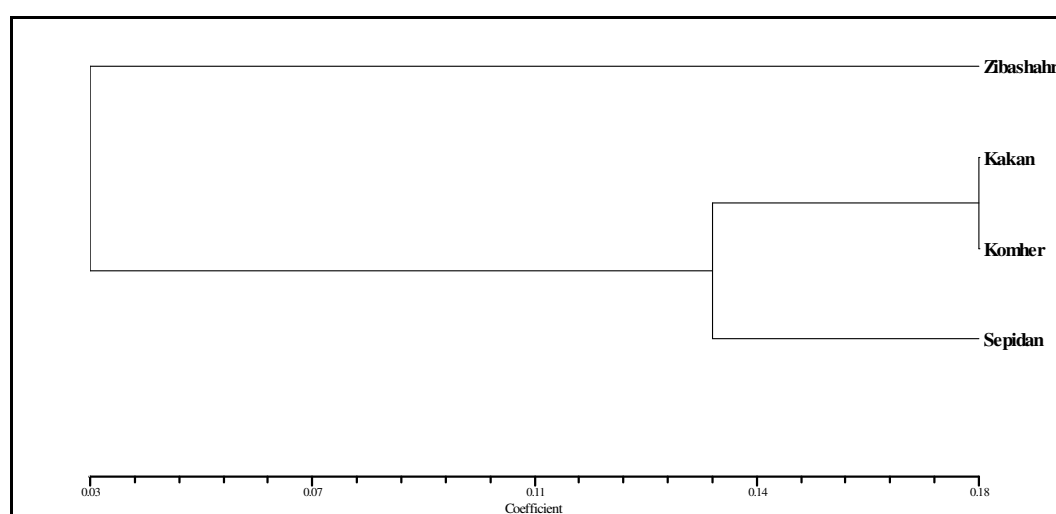


average leaf surface area varied between 41.2 and 78.6 mm<sup>2</sup>. The stem morphology in all the investigated populations was more or less uniform. The herbaceous stem of *T. polium* specimens was close to square-shaped (in the cross section). The stem diameter ranged from 1.0 to 4.0 mm, which was within the usual values found in xeromorphic plants (Fahn and Cutler, 1992). There was little difference regarding the morphology of inflorescence between the populations. Inflorescence length of all plants studied ranged between 14.4 and 30.4 mm, being the longest in the plants from the Zibashahr. The inflorescence fresh weight was between 32.0 and 50.0 g plant<sup>-1</sup>, whereas the inflorescence dry weight ranged between 9.0 and 18.7 g plant<sup>-1</sup>.

By ANOVA, statistically significant differences between all populations were established. It should be pointed out that the most important characters in structural differentiation are those related to leaf width, leaf length, and stem diameter features, in this order of significance (Table 3). Cluster analysis of the studied populations of *T. polium* showed that there were 3 groups (Figure 2). The sub-humid and arid populations represented 2 groups that were morphologically almost completely separate, and the populations from Kakan and Komehr stood completely separated from the sub-humid populations (Zibashahr), which was characterized by the highest amount of vegetative growth. The population from Sepidan showed transitional

**Table 3.** Analysis of variances on the level of morphological characters in 4 population of *T. polium*.

Dependent variable	Mean sq. effect	Mean sq. error	F (3)	p-Level
The height of the shrub	398.94	29.5	12.84811	0.047835
Diameter of the stem	2088.61	268.3	10.43215	0.036789
Fresh weight	11172321.87	983102.91	1.120391	0.041341
Dry weight	931673.32	75381.02	2.304139	0.039810
Leaf length	17.57	4.7	3.451313	0.011239
Leaf surface area	817381.02	67309.91	5.987123	0.037813
the largest width of the leaf	12.67	2.4	10.12373	0.000005
Internodes distance	0.74	0.2	2.331428	0.067631
Inflorescence length	4.28	1.8	2.568301	0.031341
Inflorescence dry weight	798158.93	48112	2.718360	0.048321
Inflorescence fresh weight	444773.86	2774	3.578932	0.041241



**Figure 2.** Cluster analysis of morphological characteristics of *T. polium* from Fars Province.

characteristics between the arid and sub-humid populations, as it was the case also in the ANOVA analysis.

In all studied locations, i.e. sub-humid, semi-arid, and arid sites, the plant populations studied, had longer or shorter intervals of summer drought stress. Inter-population differences refer to small variations in shrub height, leaf size, fresh and dry weight of shrub, as well as inflorescence fresh and dry weight. While all these morphological features are of the same pattern, they are clearly more expressed in the sub-humid than in the arid and semi-arid populations (data not provided).

### Chemical Essential Oil Composition

A comparative study of the essential oils of *T. polium* was carried out. The yields of essential oils were in the range of 2.06% for Kakan, 2.31% for Komehr, 2.69% for Sepidan, and 3.06% for Zibashahr. Since the protrusions of capitate secretory cells have only a small storing space, there is a continuous evaporation of essential oils (Werker *et al.*, 1985) and thus reducing essential oil of the leaves. It is well known that all species of the genus *Teucrium* are distinguished by only a low quantity of essential oils (Kovačević *et al.*, 2001; Djabou *et al.*, 2011; Djabou *et al.*, 2012a, b).

According to Voirin *et al.* (1990), the oil yield is favored with higher temperatures, water deficit, and higher summer sunshine, which is the case in the Zibashahr and Sepidan, but not so much in Kakan and Komehr, which may explain the difference found in the yields. The essential oil yield, in the 10 wild populations of two Mediterranean subspecies of *T. scorodonia* collected at full flowering, ranged from 1.4 to 2.6% (essential oil weight per plant dry weight; w dw<sup>-1</sup>), averaging 2.1% (w dw<sup>-1</sup>) (Djabou *et al.*, 2012b). These values are in accordance with the reported oil yield studied 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 identification of 25 compounds from population of Zibashahr and Sepidan, 20 compounds from population of Kakan, and 18 compounds from population of Komehr, ranging from 89.2 to 98.8% of the total oil composition. Among them, 8 to 10 sesquiterpenes, 5 to 8 monoterpenes, 1 to 3 aromatics, 1 to 2 alcohols, and 2 ester compounds were identified. The concentrations (%) of all the identified oil components are listed in Table 4 in order of their elution on column.

The main components of *T. polium* oil collected from population of Zibashahr were  $\beta$ -Caryophyllene (28.4%),  $\beta$ -pinene (10.9%), farnesene-*cis-b* (10.7%), carvacrol (8.6%),  $\alpha$ -pinene (6.4%), bicyclogermacrene (6.2%) and *p*-Cymene (2.9%). The main components of *T. polium* oil collected from population of Kakan were Farnesene-*cis-b* (18.4%),  $\beta$ -Caryophyllene (12.8%), bicyclogermacrene (12.0%), germacrene D (11.8%), linalool (7.8%),  $\alpha$ -camphene (6.1%) and  $\gamma$ -cadinene (3.2%). In the case of population of Komehr, the main components of *T. polium* oil were linalool (15.6%),  $\beta$ -Caryophyllene (15.3%), germacrene D (13.4%), carvacrol (7.1%), farnesene-*cis-b* (5.6%), valenene (5.4%) and spathulenol (4.2%). The major components of *T. polium* oil collected from population of Sepidan were  $\beta$ -Caryophyllene (20.6%), farnesene-*cis-b* (9.8%), germacrene D (9.3%),  $\beta$ -pinene (7.3%), carvacrol (6.5%),  $\alpha$ -pinene (5.1%), and linalool (4.4%).

It is noticeable that the chemical composition of *T. polium* population studied here was in accordance with those previously reported in literature (Djabou *et al.*, 2012a, Cozzani *et al.*, 2005; Tomi and Casanova, 2006).

All oils were dominated by hydrocarbon compounds (Table 5): 80.7, 75.4, 61.8 and 74.4% in populations of Zibashahr, Kakan, Komehr and Sepidan, respectively; however, the population of Zibashahr and Sepidan revealed higher amounts of sesquiterpenes

**Table 4.** Chemical compositions of *T. polium* essential oils collected from different location.

Retention Index <sup>a</sup>	Composition of essential compounds	Rt <sup>b</sup>	Zibashahr	Kakan	Komehr	Sepidan
1	$\alpha$ -Thujene	936	0.10	0.80	-	0.10
2	$\alpha$ -Pinene	942	6.40	2.70	3.40	5.10
3	$\alpha$ -Camphene	954	2.60	6.10	2.22	1.80
4	Sabinen	972	2.40	1.80	4.10	3.20
5	$\beta$ -pinene	977	10.90	0.87	2.40	7.30
6	3-octanol	982	0.84	-	-	0.60
7	Myrcene	986	1.10	-	-	0.90
8	Limonene	1023	1.40	0.10	-	0.80
9	p-Cymene	1025	2.90	1.90	-	1.70
10	1,8-cineole	1032	0.78	1.60	0.80	0.46
11	Linalool	1127	1.80	7.80	15.65	4.40
12	Bornyl acetate	1267	0.80	-	-	0.70
13	Carvacrol	1272	8.60	1.70	7.10	6.50
14	Camphene	1385	0.10	0.80	0.30	0.50
15	$\beta$ -Caryophyllene	1417	28.40	12.80	15.30	20.60
16	Farnesene-cis-b	1445	10.70	18.40	5.60	9.80
17	$\alpha$ -humulene	1450	2.30	-	2.32	3.40
18	Germacrene D	1475	2.10	11.80	13.40	9.30
19	Cyperene	1484	2.40	0.90	0.76	1.20
20	Bicyclogermacrene	1491	6.20	12.00	3.40	4.00
21	$\beta$ -bisabolene	1500	1.60	-	-	2.20
22	Valenene	1503	2.40	0.80	5.40	4.10
23	$\gamma$ -cadinen	1506	0.20	3.20	2.60	1.30
24	Spathulenol	1564	0.98	2.70	4.20	0.30
25	Caryophyllen oxide	1570	0.80	0.40	1.80	2.60

<sup>a</sup> Order of elution is given on column, <sup>b</sup> Retention time on the column (Second).

**Table 5.** Concentration (%) of identified oil components arranged according to the five types of chemical groups.

Different chemical groups	Sepidan	Komehr	Kakan	Zibashahr
Monoterpence hydrocarbons	21	15.02	16.37	25.2
Sesquiterpene hydrocarbons	53.4	46.78	59	55.48
Aromatic	6.5	5.4	2.7	6.1
Alcohols	5	15.65	7.8	2.64
Esters	6.96	7.9	3.3	9.38
Total	98.8	89.17	90.75	92.86

than those of Kakan and Komehr, while the latter displayed higher amounts of monoterpenes than the former. The aromatic, alcohols, and esters fraction was present in oil samples at low levels. Morphological 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 types of variations that were already seen in *T. polium* 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 (CA) was applied. The data presented in Figure 3 was obtained from the standardized matrix. The dendrogram obtained from the CA suggests that there were two main groups correlated with the climatic condition of geographic origin of the oil samples. Group I included oil samples from Zibashahr and Sepidan and group II included oil samples from Kakan and Komehr. Group I was characterized by a higher amount of  $\beta$ -Caryophyllene (20.6.5–28.4%) and  $\beta$ -pinene (7.3–10.9%) than group II (12.8–15.3% and 0.9–2.4%, respectively). Oil samples in group I contained  $\beta$ -bisabolene (1.6-2.2%), myrcene (0.9-1.1%), and 3-octanol (0.6-0.8%), which were not detected in the oil samples of group II. Group II was characterized by the occurrence of monoterpene compounds such as  $\beta$ -Caryophyllene (12.8-15.3%), Farnesene-*cis-b* (5.6-18.4%) and Linalool (7.8-15.6%) as the main components.

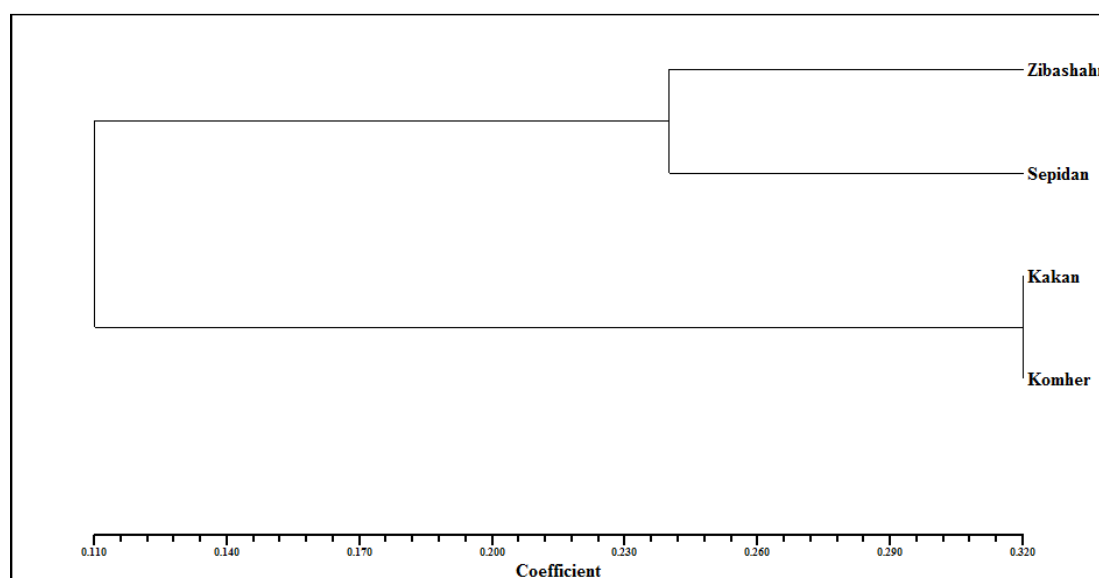
It appears that differences in oil compositions between the oil samples from the three harvest areas could be caused by environmental conditions. The chemical variability could be linked to the presence of divalent metal ions such as  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Ni^{2+}$  and  $Co^{2+}$ , which improve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

plants (Duarte *et al.*, 2010).

## CONCLUSIONS

The phytochemical and morphological characters used in this study proved to be powerful tools for studying inter- and intraspecific variations and for elucidating the influence of environment on chemical and morphological profile of *T. polium*. The present study revealed significant differences in diversity between different population of *T. polium* according to chemical and morphological analyses. These differences could be explained by the geographical and genetic separation of the populations.

Iranian *T. polium* samples were clustered in two sub-groups characterized by two essential oil chemical compositions and an important morphological difference. Finally, although essential oils may evolve more rapidly than morphological traits, the rather unusual uniformity found in the essential oils composition in populations with different geographic provenances and at different growing conditions may explain why the morphological traits were more correlated with the genetic variation rather than the phytochemical ones.



**Figure 3.** Cluster analysis of chemical compositions of *T. polium* oils from Fars Province.



From a conservation perspective, the low genetic and phytochemical diversity observed within the populations tested is symptomatic and a signal that ecological management of *T. polium* 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.

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## تنوع مورفو-شیمیایی میان جمعیت‌های *Teucrium polium* L. (از خانواده نعناع)

ایرانی در استان فارس

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### چکیده

در این مطالعه تفاوت‌ها میان جمعیت‌های *Teucrium polium* L. ایرانی بر اساس تغییرات مورفولوژیکی و فیتوشیمیایی برای ارزیابی سطح و چگونگی توزیع تنوع میان چهار جمعیت فاصله دار از نواحی خشک، نیمه خشک و نیمه مرطوب فارس تحلیل شد. تجزیه و تحلیل مورفولوژیکی شامل



بررسی ۱۱ صفت مرتبط با مورفولوژی گیاه، برگ و ساقه بود. تجزیه واریانس و تجزیه کلاستر برای مشخص شدن تنوع و شدت تفاوت‌های مورفولوژیکی انجام شد. تجزیه مورفولوژیکی گیاهان نشان داد این گونه جزء گونه‌های خشکی پسند مالاکوفیلوس می‌باشد و مشخصه اصلی آن همانند سایر گونه‌های خشکی پسند است. تغییرات مورفولوژیکی با شرایط محیطی منشاء جغرافیایی مرتبط بود و تفاوت‌های اندکی بین گونه‌های متعلق به جمعیت‌های خشک و نیمه خشک در مقابل گیاهان متعلق به محیط‌های نیمه مرطوب وجود داشت. تحلیل‌های شیمیایی با استفاده ترکیبی از GC و GC-MS در کروماتوگرافی ستونی اجرا شد. ترکیب شیمیایی اسانس به طور کیفی و کمی بین جمعیت‌ها تفاوت داشت. بتا-کاربوفیلین مهمترین ترکیب اسانس در جمعیت‌های مرطوب و نیمه خشک بود، در حالیکه ترکیب اصلی در جمعیت‌های خشک فارنسن-سیس-بی و لینالول بود. افزون بر این نمونه‌های اسانس جمعیت‌های نیمه-خشک و نیمه-مرطوب حاوی بتا-بیسابولن (۱/۶ تا ۲/۲ درصد)، میرسن (۰/۹ - ۱/۱ درصد)، استات برنیل (۰/۷ - ۰/۸ درصد) و ۳-اکتانول (۰/۶ - ۰/۸ درصد) بود، که در نمونه‌های اسانس جمعیت‌های خشک وجود نداشت. با این حال ترکیب غالب تمام نمونه‌های اسانس ترکیبات هیدروکربنی بود. تنوع مورفو-شیمیایی نسبتاً پایین در جمعیت‌ها نشان می‌دهد چنانچه اندازه جمعیت‌ها حفظ نشود و هیچ حفاظتی از رویشگاه‌های آن صورت نگیرد حفظ پتانسیل تکاملی آن در معرض خطر است.