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Variation of Yield, Morphological Traits, and Essential Oil in Populations of Five Species of *Stachys* in Iran

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ABSTRACT

The genus *Stachys*, (Lamiaceae family) has several spicy and aromatic species. In order to investigate the diversity of aerial biomass, morphological traits, and essential oil yield, 47 populations out of five species including S. *lavandulifolia, S. laxa, S. inflata, S. germanica* and *S. byzantina* were evaluated during two years, at a research farm in Khorramabad, Iran. The traits such as plant height, leaf length, leaf width, stem number, stem diameter, vegetation cover, 1,000 seed weight, and aerial dry (DM) yield in all 47 populations were measured. The essential oil was extracted using the hydrodistillation method with a Clevenger-type apparatus. The essential oil compounds were detected using GC and GC/MS. Data were analyzed using nested ANOVA with Minitab$_{16}$ software. The results showed that both *S. germanica* and *S. byzantina* had higher aerial biomass than the other species. The Saveh and Tehran populations of *S. inflata*, the populations of Qazvin and Qorveh in *S. lavandulifolia*, and the populations of Ardebil and Semirom in *S. Byzantine* were superior to the others for the measured traits. For essential oil yields (w/w), the highest and lowest values were 2.0 and 0.8%, obtained in *S. byzantina* and *S. lavandulifolia*, respectively. Phytochemical analyses of *S. lavandulifolia* identified 43 compounds. The main compounds were β-eudesmol (3.48%), germacrene-D (4.59%), δ-cadinol (4.69%), bicyclogermacrene (6.85%), δ-cadinene (9.69%), spathulenol (10.08%) and α-cadinol (12.86%). *S. lavandulifolia,* with early maturity and higher essential oil, was recommended for domestication and breeding of improved varieties.

Keywords: Phytochemical trait, S. byzantine, S. germanica, S. inflata, S. lavandulifolia, S. laxa.

INTRODUCTION

The genus of *Stachys* belongs to the family Lamiaceae, includes about 300 species. More than 39 species of this genus are grown and distributed in various regions of Iran (Rechinger, 1982). The species of this genus grow in Iran, in Central Asia, Turkmenistan, Afghanistan, Caucasus, Anatolia, and Iraq (Mozaffarian, 1996). There are two centers of origin for this plant. The first is in East Anatolia, Caucasus, northwestern Iran and northern Iraq, and the second is the Balkan island (Bhattacharjee, 1980).

Several species of this genus are extensively used in various traditional medicines. They are consumed as herbal preparations to treat stress, skin inflammations, gastrointestinal disorders, asthma, and genitalia (Tomou *et al.*, 2020). *S. lavandulifolia* Vahl is called “Chaaye Koohi” in Iran. Most research conducted on medicinal plants focused on the extraction of active ingredients and secondary compounds. Without access to genetic diversity, the breeder has no chance of

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improving breeding varieties. Native medicinal plant cultivars and their wild relatives are accounted for most of the valuable flora in every country. Nowadays, researchers are trying to present new strategies to breed medicinal plants (Rahimmalek *et al.*, 2009). A medicinal plant yield is cost-effective when its essences is at the desired limit coupled with high biomass production. Thus, managing the environmental factors can lead to maximum production (Kusano *et al.*, 2008). According to Rakover *et al.* (2008), the essence of different *Stachys* species can contribute to healing the sinusitis headache and preventing tooth decay due to the disinfection property.

The *Stachys* species have vast distribution in Iran. Their vegetative form is an annual, biennial, perennial shrub and semi-shrub that grows in the rock and mountainous steppes (Rechinger 1982). Martin *et al*. (2011) found that in all the Stachys taxa, the chromosome number of the somatic cells was 2n= 30.

Some studies show the effects of environmental parameters such as temperature, moisture and precipitation, and altitude on the essential oil content and composition of *S. lavandulifolia* (Aghaei Noroozloo *et al.,* 2015; Chorli, *et al.* 2017). Both of them concluded that by decreasing the altitude and rainfall, the essential oil in *S. lavandulifolia* was increased. In study of relationship between morphological traits in four *Salvia* species, Yousefi Azar (2017) found that leaf number was negatively correlated with the leaf length, leaf width and flower length.

Aghaei Noroozloo *et al.,* (2015) in assessment of eight populations of *Stachys lavandulifolia* Vahl. in four provinces of Iran reported the Azarshahr population had higher mean values for aerial yield and essential oil content than other genotypes in 8 regions of Iran. Mirzaee Nodoshen *et al.* (2006), using path analysis in three *Thymus* L. species, found negative effects of chromosome length on the essential oil content, but the chromosome length through increasing leaf length had a positive indirect effect on essential oil yield. Tabaei-Aghdaei *et al.* (2018) studied the diversity of two *Satureja spicigera* and *S. sahendica* species and found important traits of dry shoot weight, plant height, plant canopy, and days to flowering as selection indices for breeding improved varieties. Arabsalehi *et al.* (2016), in the assessment of the genetic variation of 41 accessions in *Stachys lavandulifolia,* reported Damaneh 20 had the highest amount of essential oil (3.37%), while Damaneh 17 had the lowest essential oil content (0.092%). Cluster analysis classified 41 accessions into five major groups and, using factor analysis, found aerial weight, plant height, and floret number showed the highest values in factor 1 and leaves number and branch number had the highest value in factor 2.

The domestication and cultivation of *Stachys* is high priority in Iran. However, many of the researchers have examined genetic diversity in *Stachys lavandulifolia* and there are fewer reports regarding other species of this genus. Therefore, there is less information on the genetic structure, genetic diversity, and morphological variability *Stachys* species in Iran.

This research aimed to study the pattern of variation and correlation between yield and morphological traits in 47 populations out of five species to introduce promising cultivars with appreciable yield and agronomic traits and detection of essential oil and its compounds in *S. lavandulifolia* as an important medicinal plant

MATERIALS AND METHODS

This research was conducted in 2017 and 2018, in a research farm of Lurestan Agricultural and Natural Resources Research and Education Center, Khoramabad, Iran, at 48° 34′ 88″ E latitude and 33° 50′ 11″ N longitude at 1,208 m asl.

Seeds of five species and 47 populations of *Stachys* as *S. lavandulifolia*, *S. byzanthina*, *S. laxa*, *S. inflata,* and *S.*

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germanica originated from different regions of Iran and were provided from the natural resource gene bank, Tehran, Iran. The information on populations, such as origins, gene bank codes, altitude and 1,000 seed weight is presented in Table 1. The species distribution map was prepared using Diva-Gis software. Thus, the latitude and longitude of the site were recorded using GPS at the time of seed sample collection and entered into the database in two separate fields. Then, the recorded data points were plotted on the map of Iran (Figure 1).

Since the seeds of *Stachys* species require pre chilling treatment to break dormancy, the seeds were moistened and kept at fridge at 4°C for four weeks before the cultivation. Afterward, the three seeds were cultivated in jiffy pots in a greenhouse. Then, 60 seedlings from each population were cultivated. The seedlings were transferred to the field at 4 to 5 leaved stages and cultivated as spaced plants based on a randomized complete block design with three replications. In each replication, plots consisted of 20 plants in two spaced rows. The distance between plants was 50 cm and between rows 75 cm. It should be noted that before transferring the seedlings to the field, the seedlings were gradually aerated in order to be adapted to the field conditions. Afterward, the field was irrigated regularly, and the weeds were controlled manually. During the establishment year (2017), no data were collected. In the second year (2018), at the flowering stage, morphological traits were assessed as follows:

- The plant height was measured in 5 stems and averaged for each plot (cm).
- Stems number was determined by counting stems number in 5 individual plants and averaged for each plant
- The stem diameter was measured in 5 stems (five plants) and averaged for each plot (mm).
- The leaf length and leaf width were measured in 5 plants and averaged for each plot (cm).
- The vegetation cover area was calculated by the mean diameters of 5 plants and averaged for each plant (cm^2).
- Aerial part dry weight was determined by cutting and weighing all plants of each plot and, after drying at room temperature, the subsamples were oven-dried at 75°C for 48 hours and averaged as g $plant^{-1}$.
- The 1,000 seed weight was determined by counting and weighing the 1000 seeds (g).

Essential Oil

For extraction and measuring essential oil, from each species, one population was selected as *S. lavandulifolia* (code 29861), *S. laxa* (code 40610), *S. inflata* (code 39860), *S. germanica* (code 36632), and *S. byzantina* (code 28316) (Table 1). In the full flowering stage, aerial parts of the herb were harvested, then, 80 g dried material was grounded for measuring essential oil by Clevenger Instrument. About 80 g of each dried samples (aerial parts) was separated, triturated and steam-hydro distillation for 2 hours. The extraction of oil was carried out according to the method of Hungarian pharmacopeia (1984) as follows:

$$\text{Essential oil\%} = \frac{\text{Essential oil weight g}}{\text{Aerial biomass yield g}} \times 100$$

Since *S. lavandulifolia* had high oil content (2%) and higher medicinal property compared to other species, phytochemical analysis was performed on this species. The essential oil of *S. lavandulifolia* was analyzed using Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC-MS). GC analysis was performed using a Thermo-UFM gas chromatograph equipped with a Ph-5 fused silica column (10 m× 0.1 mm id, film thickness 0.40 µm). Then, a Varian 3400 GC-MS system equipped with a DB-5 column, 30 m in length, 0.25 mm in diameter and 0.25 µm in thickness, was used for essential oil identification (Adams, 2007

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Table 1. List of seeds received from Iran's natural resource gene bank and their characteristics as code, origin, 1000 seed weight and altitudes.

No.	*Species name*	Code	Province	County	1000-seed weight (g)	Altitude (m asl)	Year collection
1	*Stachys germanica* L.	11034	Markazi	Arak	2.4	1850	2002
2	*S. germanica*	30554a	Ardabil	Namin	1.7	1547	2009
3	*S. germanica*	36632	Azarbaijan E	Kalibar	1.8	1032	2010
4	*Stachys byzantina* C.Koch.	639	Azarbaijan E	Arak	2.2	1250	1994
5	*S. byzantina*	1404	Khorasan		1.9		1995
6	*S. byzantina*	2678	Golestan	Gorgan	2.6	1715	1999
7	*S. byzantina*	3837	Semnan	Shahrood	1.9	2514	2005
8	*S. byzantina*	21553	Semnan	Semnan	1.7	2265	2006
9	*S. byzantina*	28316	Isfahan	Semirom	1.1	1547	2009
10	*S. byzantina*	30554b	Ardabil	Namin	1.7	1547	2009
11	*S. byzantina*	31460	Semnan	Damqan	1.4	3783	2010
12	*S. byzantina*	34124	Chahar Mahal-	Borojen	2.8	1989	2010
13	*S. byzantina*	35610	Mazandaran	Amol	1.3	1715	2010
14	*S. byzantina*	37985	Ardabil	Ardabil	1.3	1325	2010
15	*S. byzantina*	37992	Ardabil	Ardabil	1.3	1325	2010
16	*S. byzantina*	38408	Azarbaijan W	Uramia	1.7	1210	1999
17	*Stachys inflata* Bht.	2735	Hamadan	Hamadan	2.2	1750	1998
18	*S. inflata*	9438	Lorestan	Khorramabad	2.0	1687	2002
19	*S. inflata*	10121	Isfahan	Meimeh	4.3	1920	2002
20	*S. inflata*	11025	Markazi	Ashtian	4.1	2150	2002
21	*S. inflata*	17451	Ilam	Ilam	0.8		2004
22	*S. inflata*	17471	Ilam	Ilam	0.7		2004
23	*S. inflata*	17536	Ilam	Ilam	0.7		2004
24	*S. inflata*	17609	Qom	Qom	4.9	1210	2004
25	*S. inflata*	17981	Azarbaijan E	Urmia	2.5		2004
26	*S. inflata*	18067	Azarbaijan E	Mako	1884		2004
27	*S. inflata*	30604	Semnan	Damqan	1800	1884	2009
28	*S. inflata*	31099	Lorestan	Aleshtar	1303	1800	2010
29	*S. inflata*	34834	Gilan		1618	1303	2010
30	*S. inflata*	34929	Tehran	Tehran	1884	1618	2010
31	*S. inflata*	35019	Isfahan	Semirom	4.2	2604	2010
32	*S. inflata*	35083	Isfahan	Semirom	3.3	2513	2010
33	*S. inflata*	35452	Markazi	Tafresh	3.6	2850	2011
34	*S. inflata*	35483	Markazi	Saveh	3.4	1550	2011
35	*S. inflata*	38218	Khorasan N	Maneh	1.9	1678	2011
36	*S. inflata*	38430	Azarbaijan E	Khoy	3.7		2010
37	*S. inflata*	39858	Markazi	Mahalat	3.3	1620	2012
38	*S. inflata*	39860	Markazi	Saveh	3.2	2350	2012
39	*Stachys lavandulifolia* Vahl.	10663	Lorestan	Borujerd	4.5	2250	2002
40	*S. lavandulifolia*	10674	Lorestan	Khorramabad	4.2	1394	2002
41	*S. lavandulifolia*	14074	Isfahan	Fariden	5.4		2002
42	*S. lavandulifolia*	29861	Kordestan	Qorveh	2.8		2009
43	*S. lavandulifolia*	33241	Qazvin	Qazvin	3.2	2152	2010
44	*S. lavandulifolia*	38150	Khorasan N	Bojnord	2.8	1950	2011
45	*S. lavandulifolia*	40709a	Khorasan N	Bojnord	2.9	1970	2012
46	*Stachys laxa* Boiss.	40610	Khorasan N	Asfarayen	3.5	2198	2012
47	*S. laxa*	40709b	Khorasan N	Bojnord	2.9	1970	2012

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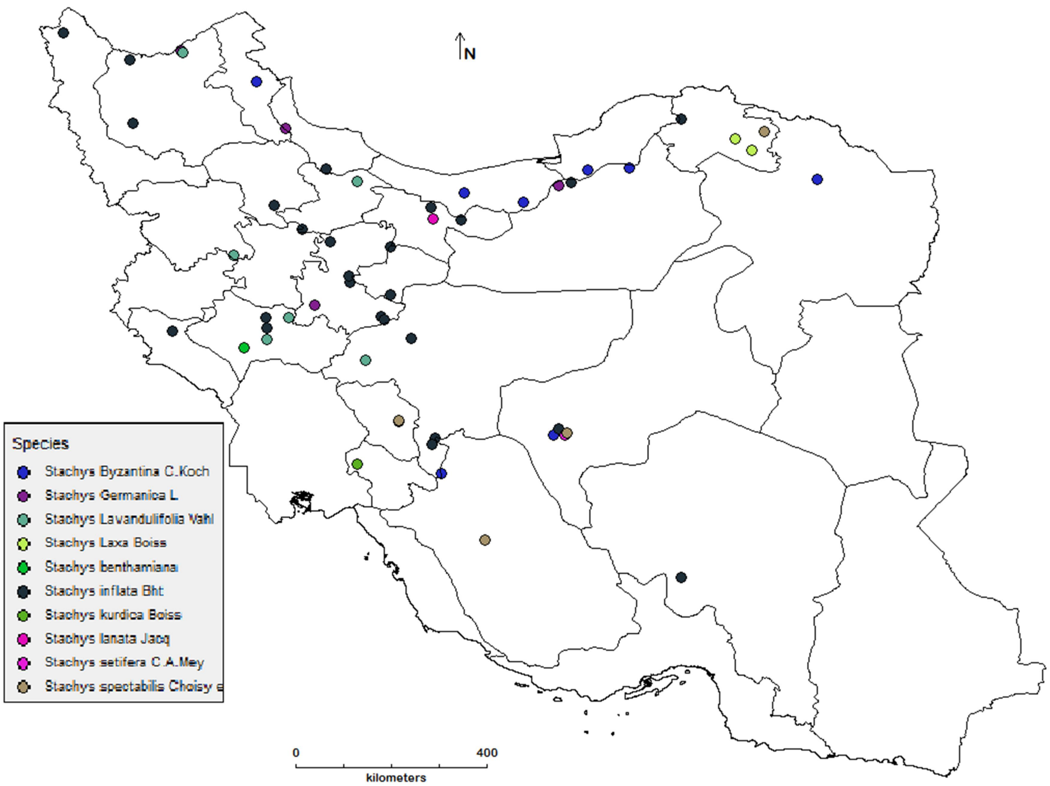

Figure 1. Distribution of various populations of *Stachys* species available in natural resources gene bank of Iran.

Sefidkon *et al*., 2006). The percentage and number of different chemical classes of *S. lavandulifolia* essential oil was determined.

The collected data were analyzed using nested ANOVA between five species and between populations within each species. Tukey's test was used to make mean comparisons among the species and among the populations within each species. The phenotypic correlation between traits was determined. Principal Component Analysis (PCA) and cluster analysis were carried out using the UPGMA method. All statistical analyses were conducted using Minitab16 software.

RESULTS

Analysis of Variance and Mean Comparison between Species

Results of ANOVA (Table 2) showed significant difference between species for all of the traits ($P< 0.01$), with similar results for significant difference between populations within each species ($P< 0.05$, 0.01).

Mean comparisons between species were made using Tukey's test (Table 3). Results of plant height showed that *S. germanica* and *S. lavandulifolia,* with average values of 110 cm and 8.71 cm, had the highest and lowest plant height, respectively. For leaf length and leaf width, the highest and lowest leaf length with average values of (8.97 and 2.71 cm) and (3.87 and 8.6 cm) were obtained in *S. germanica* and *S. Laxa*, respectively. For stems number, there was no significant differences between *S. byzantina, S. germanica, S. inflata* and *S.*

Table 2. Analysis of variance for aerial DM yield and morphological traits between five species and between 47 populations of *Stachys*.

Source	DF	MS							
		Plant height	Leaf Length	leaf width	Stem number	Stem Diameter	Vegetation cover	DM yield	1000 seed weight
Replication	2	31.12	0.91	0.27	14.29	0.36	2936.3	657.3	0.14
Species	4	26631**	104.1**	34.2**	122.8**	105.9**	4961.8**	418341**	11.16**
Populations (Species)	42	297.1**	2.11*	0.25**	46.9**	1.82**	4439.8*	5524**	1.40**
Error	92	59.42	0.63	0.63	15.41	0.27	3032.7	1177	0.21
Total	140								

**, *= Significant at 1 and 5% probability levels, respectively.

Table 3. Means of Aerial DM yield and morphological traits in five species of *Stachys.*[a]

Traits	Species				
	S. byzantina	*S. germanica*	*S. inflata*	*S. lavandulifolia*	*S. Laxa*
Plant height (cm)	89.49b	110.11a	26.68c	8.71 d	33.95c
Leaf Length (cm)	6.72 b	8.97 a	2.72d	3.27 d	2.71 d
leaf width (cm)	3.55a	3.87 a	1.03c	1.08 c	0.86 c
Main Stems number	12.69a	11.22 ab	13.26 a	6.48 b	13.60 a
Stem Diameter (mm)	6.48 a	6.02 a	1.73c	1.81bc	1.87 bc
Vegetation cover (cm^2)	3339.7 b	4567.3 a	524.7d	262.4d	1262.3 c
Aerial DM yield (g/plant)	283.9 b	423.9 a	36.7 d	13.0 d	118.4c
1000 seed weight (g)	1.90c	1.88c	2.87b	3.57 a	2.63 b
Essential oil yield (%)	0.8 c	1.0 c	1.6 b	2.0 a	1.8 ab

[a] (a-d) The means of data in each row with the same letter are not significantly different based on Tukey's method.

Laxa with higher values (from 11.22 to 13.60). The lower stems number with average value of 6.48 was obtained from *S. lavandulifolia.*

For stem diameter, using Tukey's test for mean comparison between species, the species were divided into two groups. The first group, including *S. byzantina, S. germanica*, with average values of 6.48 and 6.02 cm, had higher stem diameters. The stem diameters of the other group with three species ranged from 1.73 to 1.87 mm, which were lower.

For vegetation cover, *S. germanica* and *S. lavandulifolia,* with average values of 4567.3 and 262.4 cm^2 had higher and lower vegetation cover, respectively. In terms of aerial DM weight, *S. germanica* and *S. lavandulifolia* with average values of 423 and 13 g per plant, had the highest and lowest aerial production, respectively. For 1,000-seed weight, results indicated that *S. lavandulifolia* and *S. germanica,* with average values of 3.57 and 1.88 g, had the highest and lowest seed weights (Table 3).

Correlation between Traits

Correlation analysis showed strong and significant correlations between plant height, leaf length, leaf width, stem diameter, vegetation cover, and aerial DM weight ($P< 0.01$) while all of these traits had weak and insignificant correlations with stem number. In contrast, 1,000-seed weight had a negatively strong and significant correlation with all the traits, except stem number (Table 4).

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Table 4. Correlation coefficients of morphological traits in five *stachys* species.

Traits	Plant height	Leaf length	Leaf width	Stem number	Stem diameter	Plant coverage	DM yield	1000 seed weight
Plant height	1							
Leaf length	0.92**	1						
Leaf width	0.92**	0.93**	1					
Stem number	0.19	0.03	0.02	1				
Stem diameter	0.90**	0.92**	0.96**	0.03	1			
Plant coverage	0.96**	0.94**	0.93**	0.13	0.92**	1		
DM yield	0.93**	0.92**	0.90**	0.13	0.90**	0.98**	1	
1000 seed weight	-0.78**	-0.66**	-0.72**	-0.15	-0.71**	-0.73**	-0.71**	1

**, *= Significant at 1 and 5% probability levels, respectively

Principle Component Analysis (PCA)

The results of PCA analysis showed that the first two components accounted for 79% and 13% of the total variation. Plant height, leaf length, leaf width, stem diameter, vegetation cover, aerial DM weight positively, and 1,000 seed weight negatively, had high Eigenvector coefficients in the first component and stems number was identified as the important traits in the PCA2 (Table 5). The 47 populations were scattered on PCA1 *vs*. PCA2 (Figure 2). It shows that, in the right side of the diagram, the populations of (*S. byzantina, S. germanica,*) are associated with the first component variables as yield and morphological. In other words, species *of* the *S. inflata.* In the left side of the diagram, *S. lavandulifolia* and *S. Laxa* had lower values for yield and morphological traits, but higher values of 1,000 seed weight (Figure 2). The second component separated populations according to the vertical axis. The populations on the upper side of the diagram had lower stem number. However, a subgroup of *S. inflata* populations, scattered in lower side of the diagram, had higher stems number.

Cluster Analysis

Based on UPGMA cluster analysis with the cut at a distance of 3.6, the 47 entries were divided into two big groups. The first group (Cluster 1) had two subgroups. The 13 populations of *S. byzantina* were allocated to subgroup 1 and the three populations of *S. germanica* were placed in subgroup 2. The other big group (Cluster 2) included all populations belonging to species of *S. inflata,* S. *lavandulifolia,* and *S. laxa*. In other words, many of the populations belonging to the same species were placed in the same cluster (Figure 3). Clusters 1 (populations of S. *byzantina and S. germanica*) averaged well above the overall mean of yield and morphological traits, except 1000 seed weight. In contrast, the populations in cluster 2 had lower values of yield and morphological traits, but higher value of 1,000 seed weight. The five species were split into 2 clusters and scattered on PCA1 *vs.* PCA2 (Figure 2). The cluster 1 *(S.*

Table 5. Matrix of coefficients eigenvectors and variance proportion of the first two principal component axes in five *Stachys* species.[a]

Variable	PC1	PC2
Plant height	**0.39**	-0.08
Leaf length	**0.38**	0.10
Leaf width	**0.38**	0.11
Stem diameter	**0.38**	0.08
Plant coverage	**0.39**	-0.01
DM yield	**0.39**	0.00
1000 seed weight	**-0.33**	0.12
Stem number	0.04	**-0.98**
Eigenvalue	6.32	1.03
Proportion	0.79	0.13
Cumulative	0.79	0.92

[a] The bold and underline coefficients have significant correlation with the relevant axes.

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byzantina) and *(S. germanica,)* are distributed in the right side of the diagram. The cluster 2 (*S. inflata*, *S. lavandulifolia* and *S. Laxa*) are distributed in the left side of the diagram. This results indicate that the distribution of populations based on the first two-component scores agree with cluster analysis (Figures 2 and 3).

Essential Oil Composition

Means of essential oil contents in five species were 2.0, 1.8, 1.6, 1.0 and 0.8% for *S. lavandulifolia, S. laxa, S. inflata, S. germanica and S. byzantina,* respectively (Table 3). The *S. lavandulifolia* species in early maturity had higher essential oil content (2.0%) than other species (Table 6). The essential oil of *S. lavandulifolia*

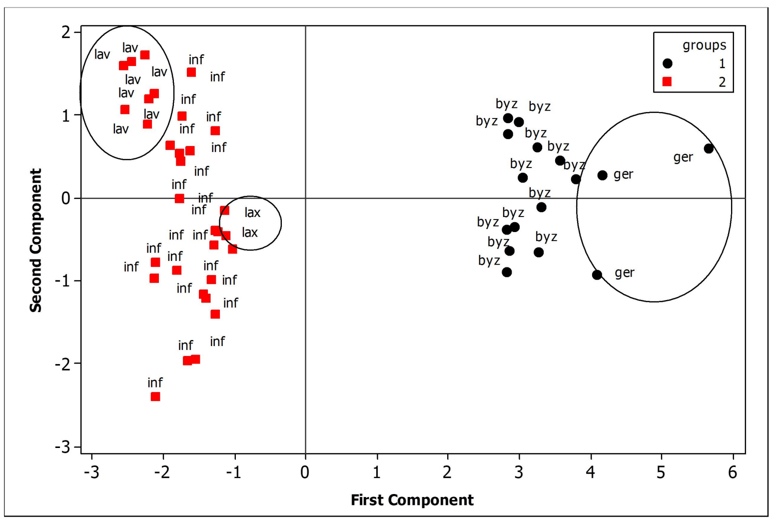

Figure 2. Scatter plot of five species and their (47 populations) for the first two principal components axes and three clusters.

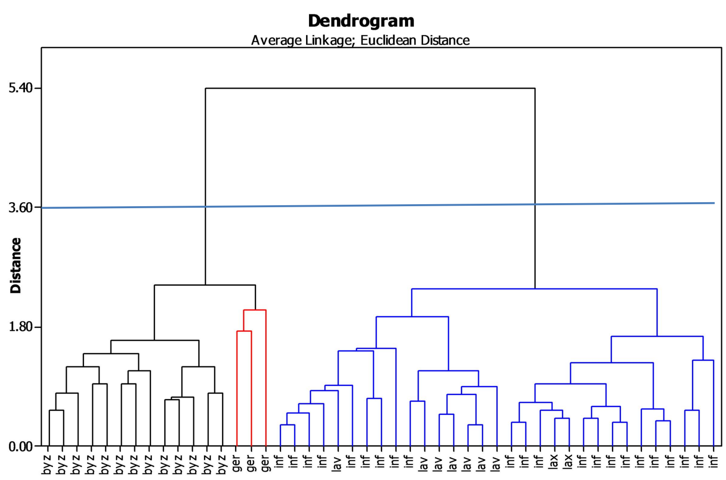

Figure 3. UPGMA dendrogram, based on morphological traits in 47 populations of five species of *Stachys* genus.

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(Accession no 29861, originated from Qorveh in Kordestan Province, Iran) was analyzed using GC and GC-MC and its compositions were identified (Table 6). Totally, 43 compounds were identified**.** The main compounds were β-eudesmol (3.48%), germacrene-D (4.59%), δ-cadinol (4.69%), bicyclogermacrene (6.85%), δ-cadinene (9.69%), spathulenol (10.08%) and α-cadinol (12.86%), respectively (Table 6). The percentage and number of different chemical classes of *S. lavandulifolia* essential oil were also determined. It comprised of 8 monterpene (4.3%), 15 sesquiterpene (39.9%), 3 organic compounds (18.1%), and 16 non- terpene (17.9%).

Table 6. Chemical composition (%) of *Stachys lavandulifolia* oil from Iran.

No	Compound	RI	%
2	α-Pinene	938	0.32
3	β-pinene	986	0.74
4	Myrcene	991	0.31
5	β-phellandrene	1041	0.22
6	Linalool l	1107	0.72
7	α-Terpineol	1213	0.46
8	Linalyl acetate	1253	1.31
9	Geraniol	1260	0.26
10	1-Decanol	1279	0.37
11	p-Menth-1-en-8-ol, acetate	1354	1.53
12	α-Copaene	1382	1.26
13	β-elemene	1394	1.16
14	Dihydro-α-ionone	1406	0.26
15	α.-Gurjunene	1413	0.53
16	(-)-Aristolene	1428	0.44
17	Caryophyllene	1431	1.28
18	γ-Gurjunene	1454	1.23
19	γ-Cadinene	1484	0.49
20	1-Dodecanol	1484	0.54
21	**Germacrene-D**	**1496**	**4.59**
22	α-Selinene	1496	0.84
23	**Bicyclogermacrene**	**1507**	**6.85**
24	β-selinene	1507	1.41
25	Valencene	1510	0.34
26	**δ-Cadinene**	**1528**	**9.69**
27	Selina-3,7(11)-dien	1537	1.19
28	**Spathulenol**	**1594**	**10.08**
29	Germacrene D-4-ol	1595	0.82
30	Globulol	1603	0.57
31	Caryophyllene oxide	1605	0.31
32	Viridiflorol	1612	0.88
33	Cubenol	1636	0.47
34	Hinesol	1640	0.62
35	Valerenol	1655	0.85
36	**δ-Cadinol**	**1668**	**4.69**
37	**α-Cadinol**	**1673**	**12.86**
38	**β-eudesmol**	**1674**	**3.48**
39	Isolongifolen-5-1	1685	0.98
40	α-Bisabolol	1701	1.93
41	2-Pentadecanone	1847	0.51
42	Manoyl oxide	2015	0.69
43	Thunbergol	2047	2.26
	Total		**80.36**

DISCUSSION

The results showed that the species of *S. germanica* and *S. byzantina* with average values of 110.11 and 89.49 cm had significantly higher plant height than the other species. Also, the vegetation cover areas were 4,567.3 and 3,339.7 cm^2, respectively. In this respect, *S. Lavandulifolia* had the lowest amount of plant height and vegetation cover. Concerning the DM weight, two species of S. *germanica and S. byzantina* had the values of 423.95 and 283.90 g $plant^{-1}$, respectively. In overall, after investigating the *Stachys* species in Khorramabad, Iran, it seems that the two mentioned species were superior in yield and morphological traits. They had a longer flowering stage as compared to the other species, so, they were recommended to be planted in the urban parks in addition to the unique medicinal features. All of the studied species, especially S. *germanica and S. byzantina,* had good potential and capability to attract the honey bees and honey production. Results of *S. inflata* species indicated that the DM weights as well as vegetation cover were high in Tehran and Saveh populations. Similarly, in *S. byzantina*, the populations Namin and Semirom had higher vegetative cover, plant height, and DM weight values. Considering the *S. lavandulifolia,* populations, Qazvin and Qorveh had better morphological traits to improve medicinal plant variety. Aghaei Noroozlo *et al.* (2015) found significant diversities for many

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morphological traits with high values of CV% in S. *lavandulifolia*.

Results of correlation analysis showed strong and significant correlations between plant height, leaf length, leaf width, stem diameter, vegetation cover, and aerial DM weight (P< 0.01). All of these traits had weak and non-significant correlations with stem number. In contrast, 1,000-seed weight negatively correlated strongly with all the traits, except stem number. Yousefi Azar (2017) reported the relationship between morphological traits in four Salvia species. Leaves number was negatively correlated with the leaf length, leaf width, and flower length. Andi *et al.* (2011), in the evaluation of the wild population of *Origanum vulgare* species, reported that plant height was significantly correlated with stem diameter, inflorescence, peduncle length, and the number of stem nodes. Aharizad *et al.* (2013) observed a positive correlation between plant height, nodes in the stem, and DM weight in *Melissa officinalis*.

The results of PCA analysis showed that the first two components accounted for 79% and 13% of the total variation. DM yield and morphological traits were important in the first component, and stem number was identified as the important traits in the PCA2. The first and second components were named as productivity and stem density, respectively.

Using factor analysis in 41 accessions of *Stachys lavandulifolia,* Arabsalehi, *et al.* (2016) found aerial weight, plant height, and floret number showed the highest values in factor 1 and leaves number and branch number had the highest value in factor 2. In two species of *Satureja spicigera* and *S. sahendica*, Tabaei-Aghdaei *et al.* (2018) found important traits of dry shoot weigh, plant height, plant canopy and flowering date as selection indices to improve breeding varieties. In wild populations of *Origanum vulgare* species, Andi *et al.* (2011) found that PCA explained 86% of the variation related characters such as internode length, leaf and peduncle length, number of inflorescence per stem, and petiole length.

Cluster analysis divided five species of *Stachys* into two big groups based on Euclidean genetic distance. The populations of *S. byzantina* and *S. germanica* were allocated to Cluster 1. The other three species of *S. inflata,* S. *lavandulifolia,* and *S. laxa* were placed in Cluster 2 (Figure 3). Many of the populations belonging to the same species were placed in the same cluster. In morphometric study of 6 species of *Salvia* L. in Iran by using cluster analysis, Kharazian (2012) found that the species were divided into two groups based on qualitative and quantitative morphological traits involving *S. nemorosa*, *S. virgate*, and *S. syriaca* in one class and *S. sharifii*, *S. spinosa* and *S. macrosiphon* in another class. In an evaluation of morphological diversity of wild populations of *Satureja mutica* in Iran, Karimi *et al.* (2014) divided savory populations into 3 clusters; plants in class 1 had higher height, leaf width, leaf length, DM weight, and essential oil.

For essential oil yield, *S. lavandulifolia* with an average value of 2% had higher essential oil content than the other species. However, Arabsalehi *et al.* (2016) found lower values of 0.9% essential oil content in the same species. The main compounds of essential oil were β-eudesmol (3.48%), germacrene-D (4.59%), δ-cadinol (4.69%), bicyclogermacrene (6.85%), δ-cadinene (9.69%), spathulenol (10.08%) and α-cadinol (12.86%), respectively (Table 4). Our results were consistent with those reported by other researchers in different parts of Iran (Feizbaksh, *et al.,* 2003, Keshavarzi, *et al.,* 2016, Sarwari, *et al.,* 2015; Hosseini Mazinani *et al.,* 2013). The essential oil of *S. lavandulifolia* were classified into chemical compounds of sesquiterpene (39.9%), organic compounds (18.1%), non- terpene (17.9%) and monterpene (4.3%), respectively. This finding was in agreement with Nejadhabibvash *et al*. (2018) in *S. lavandulifolia* Vahl species.

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CONCLUSIONS

In the present study, we attempted to describe the genetic variation in five species of the genus *Stachys*, as an herbal supplement and medicine. The result of ANOVA and mean comparisons indicated that *S. germanica* and *S. byzantina* had higher mean values for DM yield and morphological traits. According to the results of phenological studies (days number to 50% flowering date), they are late maturity species and had a longer flowering period than other species. Both species also had good potential and the ability to absorb bees, and they are also important in honey industry. So, in addition to having their unique medicinal properties, they could be used for breeding to improve varieties for increasing essential oil and also for amenity plant and cultivation in parks and recreation area.

Overall, among the five species, the populations of *S. lavandulifolia* had useful variation in the studied traits. This species had higher essential oil content coupled with earlier maturity than other species, and is widely used as a traditional medicinal herb for many diseases. It has a high priority for domestication and cultivation under dryland farming for medicinal purposes. For *S. lavandulifolia* 43 compounds were identified. The main compounds were β-eudesmol (3.48%), germacrene-D (4.59%), δ-cadinol (4.69%), bicyclogermacrene (6.85%), δ-cadinene (9.69%), spathulenol (10.08%), and α-cadinol (12.86%). It was concluded that *S. lavandulifolia* with early maturity and higher essential oil could be recommended for domestication and breeding of improved varieties.

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تنوع عملکرد، صفات مورفولوژیکی و اسانس در جمعیت‌های پنج گونه *Stachys* در ایران

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چکیده

جنس *Stachys* L. خانواده (Lamiaceae) دارای گونه‌های تند و معطر متعددی است. به منظور بررسی تنوع زیست توده هوایی، صفات مورفولوژیکی و عملکرد اسانس، ۴۷ جمعیت از پنج گونه *S. lavandulifolia*، *S. laxa*، *S. inflata*، *S. germanica* و *S. byzantina* به مدت دو سال در مزرعه تحقیقاتی در خرم آباد، ایران مورد ارزیابی قرار گرفتند. صفاتی مانند ارتفاع بوته، طول برگ، عرض برگ، تعداد ساقه، قطر ساقه، پوشش گیاهی، وزن هزار دانه و عملکرد خشک هوایی (DM) در هر ۴۷ جمعیت اندازه‌گیری شد. اسانس با استفاده از روش تقطیر با آب با دستگاه کلونجر استخراج شد. ترکیبات اسانس با استفاده از GC و GC/MS شناسایی شدند. داده‌ها با استفاده از تجزیه واریانس آشیانه‌ای با نرم افزار Minitab16 تجزیه و تحلیل شدند. نتایج نشان داد که هر دو گونه *S. germanica* و *S. byzantina* دارای زیست توده هوایی بالاتری نسبت به سایر گونه‌ها بودند. جمعیت‌های ساوه و تهران *S. inflata*، جمعیت‌های قزوین و قروه در *S. lavandulifolia* و جمعیت‌های اردبیل و سمیرم در *S. byzantina* از نظر صفات اندازه‌گیری شده برتری داشتند. برای عملکرد اسانس (وزنی) بیشترین و کمترین مقدار به ترتیب ۲ و ۰/۸ درصد به ترتیب در گیاه *S. byzantina* و *S. lavandulifolia* به دست آمد. با تجزیه و تحلیل فیتوشیمیایی *S. lavandulifolia*، ۴۳ ترکیب شناسایی شد. ترکیبات اصلی عبارت بودند از: بتااودسمول (۳/۴۸%)، ژرماکلین دی (۴/۵۹%)، سیگما کادینول (۴/۶۹%)، بایسیکلوژرماکرین (۶/۸۵%)، سیگماکادینن (۹/۶۹%)، اسپاتولنول (۱۰/۰۸%) و آلفا کادینول (۱۲/۸۶%). نتیجه‌گیری شد که *S. lavandulifolia* با زودرسی و اسانس بالاتر برای اهلی‌سازی و پرورش ارقام اصلاح‌شده، توصیه می‌شود.