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RESEARCH PAPER

Study of floristic-ecologic diversity and essential oil variation in *Stachys lavandolifolia* vahl populations from west of Iran

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Abstract

This study carried out for determination and discrimination of floristic-ecologic and essential oil variation within *Stachys lavandolifolia*Vahl Populations from west of Iran. In order to study diversity in *S. lavandolifolia*, D.S.S. method was used. Different stations of *S. lavandolifolia* were explored and 4 special stations were determined among them. floristic-ecologic data collected from each special station. The aerial parts of different populations *S. lavandulifolia* were collected at full flowering stage from special stations. The essential oils were obtained by hydro-distillation and analysed by gas chromatography-mass spectrometry (GC/MS). Then data obtained were analyzed by Pcord and Canoco software. In the survey of all special stations, 36 plant species were distinguished as associated species. 89 oil components were identified by GC/MS method. As the result of analysis of floristic data, as floristic marker, three groups were determined. Analysis of ecologic data was also confirmed the above mentioned groupment. Essential oil was subjected to determine the level and kind of diversity. 3 groups resulted from essential oil study in special stations. Therefore, in this species, the groupment that introduced by floristic marker, was confirmed by ecological and essential oil data as well. The results of the study have shown that the different populations of *S. lavandolifolia* were adapted to their habitat and the ranges of chemical variation were high between populations which led to creation of chemotypes.

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Introduction

Biodiversity that is result of different ecological factors existence in various stations shows the biological capacity and ability of each area. One of the most important reserve that led to biodiversity is inter and intraspecific diversity (Atri *et al.*, 2007a). The view expressed by Tuxen (1942) that the plant can measure habitat factors better than any instrument is symptomatic of the scepticism with which the sociologist regards intensive ecological investigation, in spite of the fact that the only exact knowledge, which he possesses of the tolerance of species has been obtained by extrapolation (often unjustified) from original instrumental measurements. The knowledge of the floristic composition of an area is a perquisite for any ecological and phytogeographical studies and conservation management activities. In studying any particular piece of vegetation, from an ecological point of view, our first step must be to determine the facts as they exist on the ground: facts regarding the vegetation, on the one hand; facts regarding the habitat, on the other (Nicholes, 1930). If there is any one set of facts, which is more susceptible to direct study and exact characterization than any other, it is the floristic composition of the vegetation. Creation of intraspecific variation is the main origin and storage of speciation and genetic divergence among populations of a species (Briggs *et al.*, 1984; West-Eberhard, 2005). In this order, emergence of intraspecific variation at different levels of each taxon brings richness to an area. Individuals of a species those are able to response appropriately to a tremendous variety of different conditions have wide distribution in the different stations with various ecological conditions. Genetic diversity is essential both for short-term adaptations to environmental changes and for long-term impact on the species and communities (Talebi *et al.*, 2014). The mentioned studies did not use a special method in plant specimen's collection process, while for collecting correct and precise floristic-ecologic data; we must apply an appropriate method that is according to factors governing nature. In this order, we used Determination of Special Station (DSS) method

(Special Station is an area of vegetation that is homogeneous view point of floristic-ecologic) (Atri, 2007b). Some Investigations by using this method show that this method can suitable for plant diversity studies (Atri *et al.*, 2006; Asgari *et al.*, 2007; Atri *et al.*, 2009; Asgari *et al.*, 2010; Chehregani *et al.*, 2011; Atri *et al.*, 2012; Kalvandi *et al.*, 2013; Talebi *et al.*, 2014).The aim of this project, was carried out from two different aspects, the studies of floristic-ecologic variation in these species belong to their stations and investigation on variation of essential oil patterns in their populations is special station.

Materials and methods

Plant material

At the first phase, ubiquiste species were selected. Different stations of *S. lavandolifolia* were determined in the Touyserkan city, Hamedan province in Iran, by using the accessible reference, herbarium and available information. Then we referred to different stations in study area, in growth seasonal for determine general stations. In each general station, location of special station determined on base of presence of individual study species. Then for determination of special station of individual study species, minimal area determined by using the area-species method with area-species curve and Cain (1959) method. All floristic–ecologic data (the study species & companion species as floristic marker, longitude (E°), latitude (N°), altitude (in meters), soil factors (pH, EC, OC, texture and TNV)) were collected from each special station. Plant specimens deposited in the Herbarium of Payam Noor University in Touyserkan, Hamedan province, Iran. Total of 4 special stations were selected or investigation in study area.

Essential oil preparation

The aerial parts of the plant were air-dried. The oil was obtained by hydrodistillation using a Clevenger-type apparatus for 3 hours, with water as solvent. The oils were stored at 4 °C for further analyses. Volatile components were identified by GC-MS using a Finnigan TRACE GC-MS.

Statistical analysis

Data obtain from floristic-ecologic and rate and components of essential oil in each special habitat were analyzed and compared by using Pcord and Canoco software by means of TWINSPAN, DCA, DC and PCA methods.

Results and discussion

Different populations of *S. lavandolifolia* were selected in different localities of of Touyserkan city, Hamedan province in Iran and special stations were identified for each population on the basis of the presence of individual of *S. lavandolifolia*. Four special stations were set in and a total of 36 associated species or infraspecific taxa were distinguished (Table 1).

Table 1. Floristical composition following with *S. lavandulifolia* in special stations.

Plant species	sample 1	sample 2	sample 3	sample 4
Acantholimon bromifolium	1	0	0	0
Alium sp	0	1	0	0
Alyssum sp	0	1	1	1
Amygdalus lysioides	0	1	1	0
Anthemis sp	0	1	0	0
Astragalus compactus	0	1	0	0
Astragalus verus	1	1	1	1
Bromus danthoniae	1	1	0	1
Bromus tectorum	1	0	1	1
Ceratocephalus sp.	1	0	0	0
Cosinia cylenderica	1	1	1	0
Dactylis glomerata	1	0	1	0
Echinaria capitata	1	0	0	0
Echinophora platyloba	1	0	0	0
Echinophs chiardiana	0	0	1	1
Festuca ovina	1	0	0	0
Gondelia torenfortia	1	1	1	1
Heteranthelium piliferum	1	0	1	0
Hordeum glaucanum	1	0	0	0
phlomis olivieri	1	0	1	1
Poa bolbusa	1	0	1	1
Scariola orientalis	0	1	1	1
Senecio glaucus	1	1	1	1
Sillen conoidea	0	1	0	0
Stachys lavandifolia	1	1	1	1
Stipa barbata	1	1	0	1
Taeniatherum crinitum	1	0	0	0
Thymus eriocalyx	1	0	0	0
Zizphora tenuior	0	1	1	0
trigonella	0	0	1	0
Centura virgata	0	0	1	0
Taraxacum	0	0	1	0
Fibijia	0	0	1	0
pterocephalus cunus	0	0	1	0
Astragalus cyclophyllus	0	0	1	0
Zizphora clynopoides	0	0	0	1

The number of associated taxa varied between the stations. On the Basis of the floristic marker, the studied stations of *S. lavandolifolia* were divided into three main groups. These groups were classified on the basis of similarity and dissimilarity of the associated taxa of each *S. lavandolifolia* special

station (fig. 1-4). The above-mentioned groups showed high degree of intraspecific variations in *S. lavandolifolia*. Since floristic composition in each environment reflects the ecological conditions that influence plant variation, the special stations with similar floristic composition have similar ecological characteristics. The special stations were selected in different environments confronting various ecological factors, which affected the chemical features of *S. lavandolifolia* populations and the floristic composition of their associated taxa. Ten different ecological factors were examined among the stations (Table 2).

Table 2. Ecological factors in special stations.

	PH	EC	O.C	T.N.V	Soil texture	longitude	latitude	Altitude
Sample 1	7.2	0.132	1.5	35	SL	268145	3825742	2017
Sample 2	6.9	0.097	1.3	5	SL	269169	3825685	2073
Sample 3	6.9	0.104	1.3	25	CL	244631	3830750	2222
Sample 4	6.9	0.169	1.9	35	CL	244269	3831005	2360

On the basis of ecological factors, the special stations were divided into three groups (fig. 5-6). In order to compare the effect of different environmental factors on the essential oil of these plants, 89 qualitative and quantitative oil characteristics. The means and standard deviations of the studied characteristics are presented in Table 3.

Table 3. Essential oil composition *S. lavandulifolia* in special stations.

Components	Sample2	Sample3	Sample4	Sample1
1,8-Cineol	0.28	0.39	0.3	1.47
2,6,10-trimethyltetradecane	0	0	0	0.03
3-epi-manoyl oxide	0	0	0	0.04
4a-α,7-α,7a-α-Nepetalactone	0.03	0.12	0.03	0
4-Terpineol	0	0	0.01	0.12
6,10,14-trimethyl-2-pentadecanone	0.48	0.66	0.53	1.05
6,9-Guaiadiene	0.24	0.04	0.07	0
7-epi-β-selinene	0	0	0	8.88
ar-Curcumine	0	0.09	0.05	0
bicyclo Elemene	1.1	0.53	0.19	0
bicyclogermacrene	27.6	16.77	6.02	0
carvacrol	0	0	0	0.16
Caryophyllene oxide	1.49	2.47	7.22	16.38
Cembrene	0.16	0.57	0.76	0
cis- verbenol	0	0	0	0.41
cis-caryophyllene	0	0	0	0.01
cis-sabinene hydrate	0.07	0.03	0.03	0.09
cis-sesquisabinene hydrate	0.05	0.06	0.08	0
cis-?-bisabolene	0.13	0.36	0.17	0
cryptone	0	0	0.04	0.04

cumin aldehyde	0.01	0.01	0.05	0.32
d-3-carene	0	0.04	0	0
Dibutylphthalate	4.99	2.65	4.61	4.72
docosane	0	0	0	0.2
E-Citral(Geranial)	0.01	0.02	0	0
Eicosane	0	0	0	0.27
E-Nerolidol	0.12	0.17	0.82	0
epi-α-muurolol	0	0	0	0.36
E-β-Farnesene	2.38	2.66	3.73	0
E-β-Ionone	0.2	0.01	0.22	0
geranyl acetone	0	0	0	0.03
Germacrene D	2.32	12.33	4.85	0.22
β-Selinene	0	0	0	6.88
heptadecane	0	0	0	0.06
humulene epoxide	0.31	0.13	0.19	0.13
Intermedeol	0	0	0.06	1.63
isobutyl phthalate	0.58	4.57	0.78	18.39
isospathulenol	1.44	1.25	1.67	0
limonene	10.23	4.15	1.8	0
linalool	0	0	0.07	0.77
longipinanol	0	0	0	1
manoyl oxide	0	0	0	0.23
Mytenal	0	0	0.1	0.16
nonadecane	0	0	0	0.8
n-tetradecane	0.06	0.4	0.43	0
octadecane	0	0	0	0.48
P-Cymene	0.09	0.03	0	0
phellandrene	0	0.01	0	0
Phytane	0	0	0	0.35
pinocarvone	0	0	0	0.02
pirollene	0	0	0.14	0
pristane	0.19	0.25	1.05	0.63
sabinene	0.37	0.12	0.05	0
Salvia-4(14)-en-1-one	0	0	0	0.3
sclareoloxide	0	0	0	4.87
Sesquisabinene	0	0	0	1.78
spathulenol	9.64	7.7	26.49	0.94
terpinolene	0.66	0.17	0.42	0
tetradecane	0	0	0	0.74
trans- verbenol	0.26	0.25	0.49	0.62
trans-caryophyllene	21.33	26.33	25.11	14.39
trans-pinocarveol	0.52	0.2	0.27	0
trans-α-bergamotene	0.58	0.86	0.77	0.3
trans-α-farnesene	0	0	0	0.53

trans-α-Ionone	0	0	0	0.05
tricosane	0	0	0	0.58
tridecane	0	0	0	0.17
Viridiflorol	0.8	0.35	0.49	0
Z-citral (Neral)	0.03	0.06	0	0
α-Ambrinol	0	0	0	1.26
α-bisabolol	0.07	0.05	0.09	0
α-Cadinol	0.1	0.25	0.32	0
α-calacorene	0	0	0	0.14
α-campholenal	0.1	0	0.45	0.39
α-copaene	1.1	3.04	0.74	0.38
α-humulene	0.43	0.16	0.21	0.38
β-phellandrene	0.21	0	0.03	0
α-pinene	0.2	0.09	0.05	0.29
α-Selinene	0	0	0	0.15
α-Terpineol	0.07	0	0.3	0.33
α-thujene	0.06	0.02	0	0
β-bourbonene	0.17	0.52	0.3	0
β-elemene	0.35	0.24	0.51	0.05
β-Myrcene	3.74	2.29	1.71	0
β-phellandrene	0	0	1.63	0
α-Pinene	0.66	0.14	0.26	0
α-Selinene	0	0	0	1.17
α-Terpinene	0.23	0.07	0.06	0.11
δ-Cadinene	2.42	5.75	1.34	0

The studied populations differed in their qualitative and quantitative oil characteristics. Classification of the studied populations based on similarity and dissimilarity of their essential oil characteristics set up 3 groups (fig. 7-9). When the results of floristcal, ecological and chemical grouping were compared, we found that: the members of three floristical groups (one group include sample 1and2, second group include sample 3 and three group with sample 4) were absolutely identical with the members of ecological and chemical groups. The fact that members of the chemical groups matched exactly members of the ecological groups led us to the conclusion that in each population, the individuals of *S. lavandolifolia* had changed their chemical characteristics in order to fit into the surrounding environment, and in their stations, there was significant adaptation between chemical characteristics and ecological features. These observations seemed very attractive when were established that; the members of ecological, floristic and chemical groups were absolutely identical. In similar study, Chehregani *et al.* (2011) and Atri *et al* (2012) in the survey of electrophoresis pattern, show existence of differences regarding number and density of the protein bands indicating existence of intraspecific diversity in the populations of *Artemisia incana*. Therefore in this species, the grouping that introduced with floristic marker, confirmed also with ecological and electrophoresis markers. Study by Atri *et al.* (2009) on *Artemisia scoparia* was carried out to determine intraspecific diversity by D.S.S method in the west of Iran. Between obtained results of this study, present 2 Topodemes, 2 Pedodemes and 1 Basodeme for Artemisia scoparia as a medicinal plant from west of Iran. Study on two species of *Achillea* L.

in the west of Iran by Salehi *et al.* (2013) show six distinctive groups that indicated the existence of intraspecific diversity in this species.

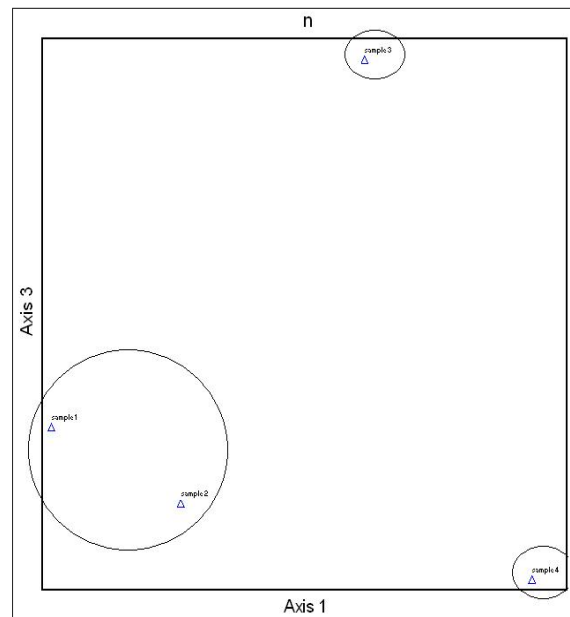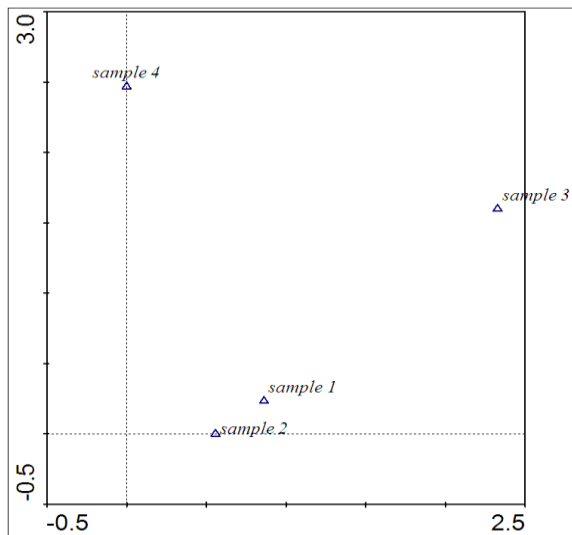**Fig. 1.** Results floristical composition data analysis by TWINSPAN method.

Fig. 2. Results floristical composition data analysis by PCA method.

Fig. 3. Results floristical composition data analysis by CA method.

The result of analysis of ecological data and seed storage proteins for the two species was in accordance with the floristic data. To study the variation of essential oils among population individuals of *Thymus eriocalyx* in Iran D.S.S. method was used by Kalvandi *et al* (2014). In this study, it is noteworthy that the individuals of a population showed variation among themselves in terms of chemical compositions. The results of the study on *Linum album* (Talebi *et al.*, 2014) have shown that the different populations of *Linum album* were adapted to their habitat and the ranges of phenotype plasticity were high between populations which led to creation of ecomorphs. Our studies show that *S. lavandolifolia* has wide distribution in Iran and is endemic. This species is present in many habitats with different ecological conditions in Iran.

According to our results of floristic analyses there are 3 distinctive different groups of *S. lavandulifolia* individuals in study region. Phytochemical studies create 3 kinds of chemotypes which conform and affirm the obtained results of floristic studies. So this study and other studies that done base on this method until to now, show the high efficiency of it in determination and discrimination of inter and intraspecific diversity existence. In the survey of essential oil, existence of differences regarding rate, quantities and qualities were indicating existence of

intraspecific diversity in the *S. lavandulifolia*. Therefore in this species, the grouping that introduced with floristic marker, confirmed also with ecological and phytochemica markers.

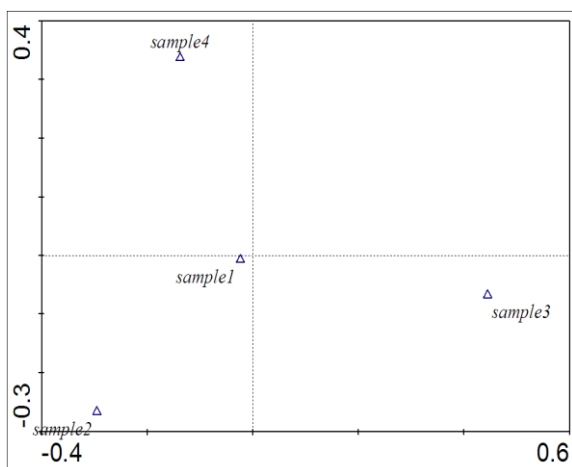**Fig. 4.** Results floristical composition data analysis by DCA method.

Fig. 5. Results ecological factors data analysis by CA method and data table.

This means that floristic grouping can only be used as a cost-effective and efficient method for studying of diversity. Present results show that *S. lavandolifolia*, from floristic-ecologic and chemical points of view have high variation in the west of Iran. Any vegetation in particular place is influenced by the prevailing environmental factors including: climate, topography, soil, human activities and other biotic factors. Analysis techniques are used in the present study classified the special stations to three groups. At

second phase, phytochemical studies create 3 groups of essential oil that which conform and affirm the obtained results of floristical studies.
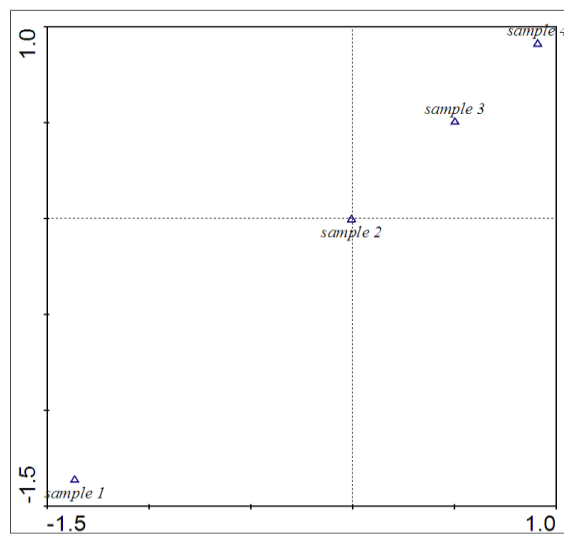
Fig. 6. Results ecological factors data analysis by DCA method and data table.

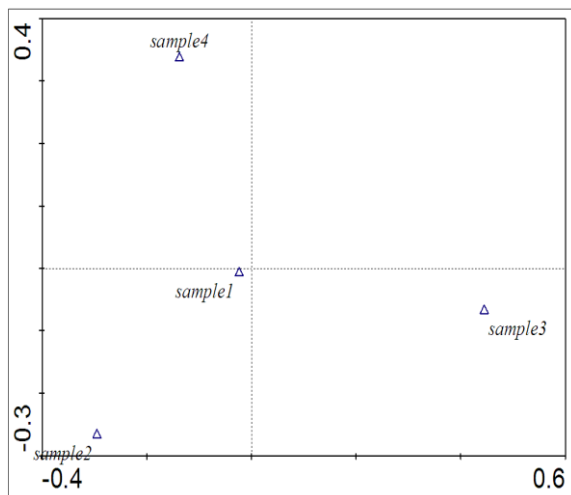**Fig. 7.** Results essential oil composition data analysis by TWINSPAN method.

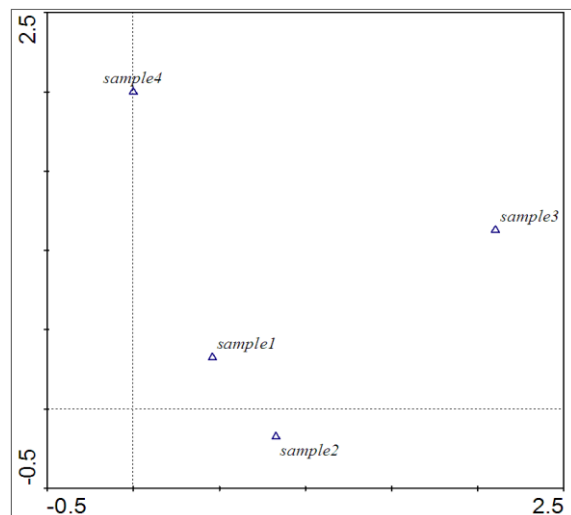**Fig. 8.** Results essential oil composition data analysis by CA method.

Fig. 9. Results essential oil composition data analysis by DCA method.

The results of the study have shown that the different populations of *S. lavandolifolia* were adapted to their habitat and the ranges of chemical variation were high between populations which led to creation of chemotypes.

Conclusion

The results of this study have shown that, in many cases, there were significant correlations between the ecological characteristics of the habitat and the chemical variation of studied populations and their associated taxa. The present study shows that in studying the vegetation and determining ecological factors, employing ecological and floristical criteria as ecophytosociology are not only suitable and exact in the data collection stage to determine the placement of special station, but also it is able to provide results, which conform and agree to the rules that govern the nature in the analysis and result interpretation stage.

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