

ANALYSIS OF TRICHOME MORPHOLOGY AND DENSITY IN *SALVIA NEMOROSA* L. (LAMIACEAE) OF IRAN

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Abstract

Talebi S.M., Mahdiyeh M., Nohooji M.G., Akhani M., 2018: Analysis of trichome morphology and density in *Salvia nemorosa* L. (Lamiaceae) of Iran. – *Botanica*, 24(1): 49–58.

Salvia nemorosa L. is an aromatic herb distributed widely in various regions of Iran, and is used in traditional and modern medicine. In the present study, leaf trichome densities and also type were investigated in six populations of the species, because the effective substances of this species exist in essential oil of glandular trichomes. For this, the mature intact leaves of each population were fixed in FAA solution, and then transverse hand sections were double-stained and studied using light microscopy. Leaf indumentum in all of the populations was dense pilose, with the exception of Tehran population that had loose pilose. Thirteen kinds of trichomes were identified, the main of which were peltate and capitate. PCA-biplot showed that each of these populations had prominent trichome trait(s). Furthermore, significant positive/negative correlations were found between some trichome types with main ecological factors of habitat. The studied populations clustered separately in the UPGMA tree, moreover, PCA and also PCO plots produced similar results. Our findings confirmed that ecological parameters of a habitat have strong effects on trichome morphology and density.

Keywords: correlation, ecological factor, population, *Salvia nemorosa*, trichome.

INTRODUCTION

The genus *Salvia* L. is a member of Nepetoideae subfamily of the family Labiatae (JAMZAD, 2012). It is the most prominent and the largest genus of the family with more than 1000 species (FARIMANI et al., 2015). However, sixty one of these naturally grow in different regions of Iran (JAMZAD, 2012). Investigations showed that the species of this genus are traditionally used in folk medicine all around the world. Moreover, many species of *Salvia* have economic significance, since they are used as food, spices, and flavours (BAHADORI et al., 2016). *Salvia nemorosa* L. (syn. *S. sylvestris*) is commonly known as wood sage naturally growing in different regions of Europe and also Western Asia (BAHADORI et al., 2017). In addition,

this aromatic plant is widely distributed in various parts of Iran (JAMZAD, 2012).

BAHADORI et al. (2017) have demonstrated that leaves of this plant are used in traditional Turkish medicine to stop the bleeding. Besides, in the folk medicine of Bulgaria, *S. nemorosa* is employed mainly for the treatment of different disorders such as stomach ache, diarrhea, hemorrhages, and furuncles. Different studies (e.g. ALBAYRAK & AKSOY, 2013; NIKOLIC et al., 2014) have stated that the essential oils as well as plant extracts have achieved significant interest in food industries. There is a growing fondness for medicinal herbs as remedial agents versus different sickness such as Alzheimer's disease, diabetes mellitus, and also oxidative damages (ZENGIN et al., 2014).

The essential oil synthesis, storage and also release in aromatic plants happen in different specialized secretory structures such as ducts, oil cells, schizolysigenous and lysigenous cavities or glandular hairs conforming to their botanical families (FEIJÓ et al., 2014). KELSEY et al. (1984) have stated that presence and type of pubescence is a very general characteristics in higher plant taxa. Furthermore, the adaptive value of trichome density is generally thought to be related to the plant water economy (EHLERINGER, 1984) and the defense of plant against herbivore taxa as well as various diseases (KELSEY et al., 1984).

In the Lamiaceae family, volatile organic components are yielded and stored in different glandular trichomes that can be used as reliable taxonomic traits for the taxa identification as well as for resolving taxonomic complexity (HAYAT et al., 2009). These structures are widely distributed over the aerial parts of reproductive and vegetative organs in Labiate. Glandular trichomes have different types: capitate, subsessile, clavate, and branched types. While, non-glandular ones are more common than glandular trichomes in this family and can be classified according to different factors as size, thickness, surface and ramification mode (SEYEDI & SALMAKI, 2015). TALEBI et al. (2012) have found that the type of habitat soil has intense influence on trichomes in *Ziziphora tenuior* L. This condition has also been seen in *Acinos graveolens* (M.B.) Link. (TALEBI & SHAYESTEHFAR, 2014). FU et al. (2013) have reported that density of hairs is a plastic adaptive feature to drought, on account of its barrier effect against the influence of carbon dioxide and water exchange that declines extreme transpiration and photoinhibition of plant. The amplified frequency of non-glandular hairs with high irradiation could be possibly related to decreased photoinhibition and loading of heat (SKELTON et al., 2012).

EJJI & SALMAKI (2016) have studied the trichome features in 46 species of *Salvia* in Iran. They have found that glandular trichomes are composed of stalked, subsessile or sessile types. Their investigation has revealed the usefulness of trichome characters in providing fundamental taxonomic criteria for taxon delimitation in these genera at various levels, especially at the specific rank. LEMBERKOVICS and MARCZAL (2003) have observed glandular and non-glandular trichomes on the leaves of *Salvia nemorosa*.

The non-glandular trichomes were conical unicellular or multicellular. The glandular types were peltate, capitate type I and II, similar to the other species of this family. Recent study on trichome morphology in *Salvia nemorosa* subsp. *tesquicola* has shown that numerous glandular and non-glandular trichomes are present, vary in structure, shape and size (BERCU et al., 2012).

Although, different examinations exist on the phytochemistry characteristics of *S. nemorosa* (COISIN et al., 2012; MESHKATALSADAT & NORANI, 2015; BAHADORI et al., 2017), much less is known about the variation in morphology and density of glandular and non-glandular trichomes among the populations of this species. To date, knowledge about the trichome morphology and infraspecific variations in trichomes of this plant is absent. Therefore, we carried out a study on morphology and distribution of glandular and non-glandular trichomes present on the leaves of *S. nemorosa*. The main purposes of the study were: identification of the structures responsible for production and secretion of various compounds such as essential oil (FEIJÓ et al., 2014) and understanding of the effect of ecological factors on trichome morphology as well as density.

MATERIALS AND METHODS

In this investigation, the type and frequency of glandular and non-glandular trichomes were examined on both leaf sides of six populations of *S. nemorosa*. These populations were collected from different parts of Iran in spring 2016. Each of the collected population had special ecological and phytogeographical conditions. Tehran, Sangak and Kerman samples were found in Atropatenian, Atropatenian and Farso-Kermanian sub-provinces, respectively. Amir Kabir and Shazand plants were belonging to Kurdo-Zagrosian sub-province. All of these sub-provinces belong to Irano-Turanian sub-region. However, Polor population was selected from Hyrcanian district from Euxino-Hyrcanian province. Eleven ecological factors were examined from each habitat: longitude, latitude, altitude, mean temperature of growth season, percentages of clay, silt and sand of soil and its texture, pH and EC of soil (Table 1). Plant samples were identified on the base of the descrip-

Table 1. Localities and habitats of the studied *Salvia nemorosa* populations

Populations	Localities	Longitude (E)	Latitude (N)	Altitude (m)	Average temperature (C°)	Precipitation (mm)	Soil pH	Soil EC	Clay (%)	Silt (%)	Sand (%)	Soil texture
Amir kabir	Markazi province, Amir Kabir	49°54'	34°53'	1670	22	17.30	7.4	1.9	16	28	56	Sand
Kerman	Kerman province, Rabor, Naniz	56°54'	29°19'	2418	30.52	7.75	7.5	0.82	20	38	42	Sand-Loam
Sangak	Tehran province, Lashkarak, Sangak	51°45'	35°15'	2100	23.7	13.61	7.6	0.63	26	38	36	Loam
Shazand	Markazi province, Shahzand	49°35'	33°05'	2000	21.67	12.27	7.6	0.74	26	54	20	Silt
Tehran	Tehran province, Gajroud	51°06'	35°34'	1700	18	18.6	7.7	0.80	28	46	28	Silt
Polor	Mazandaran province, Amol, Polor	52°13'	34°48'	2623	16	23.62	7.3	0.93	23.1	39.4	37.5	Loam

tions provided in flora of Iran (JAMZAD, 2012). From each population, five flowering stems and of each stem one mature leaf were randomly selected.

The pubescence degree of adaxial and abaxial leaf surfaces was determined under binocular on the base of description provided by ESAU (1965). The embedded leaf samples were used for microscopic studies. For this, fresh adult plant leaves of each sample were fixed in a FAA solution (formalin 7.5%: acetic acid 7.5% and ethanol 85%) for 48h. Transverse hand sections of the lamina were made from the mid-part of fully-grown leaves, sections were then immersed in two consecutive treatments of 5% KOH (18 h per treatment), rinsed in deionized water, treated for 2 minutes in glacial acetic acid, rinsed in deionized water, cleared in bleach solution (5% sodium hypochlorite) and rinsed in deionized water again. Following clearing, leaves were dehydrated in a series of ethanol, stained in 1% carmine (in 99% water) for 45 min. and in 0.01 methylene blue for 10 s, and mounted onto microscope slides. At the final stage, suitable thin slices were placed on glass lamina, observed and photographed using a light microscope (Olympus CH₂). The used terminology in respect of trichome types followed ESAU (1965).

The total hair number of each type on the abaxial and adaxial surfaces of the leaf was counted using a light microscope. The areas of leaves were measured from digital images and were analysed by Image Tool ver. 2.0. Trichome density was calculated by

dividing the hair number by the leaf area (GONZALES et al., 2008).

Statistical analyses

The mean as well as standard deviation of the observed trichome number were calculated. For grouping the studied populations on the basis of trichome traits, the data were standardized (mean = 0, variance = 1), then various multivariate analyses such as the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Principal Coordinate Ordination (PCO) and Principal Coordinate Analysis (PCA) were performed (PODANI, 2000). Moreover, the one-way analyses of variances test (ANOVA) was used to assess the significant trichome number differences among the studied populations and also the Pearson correlation coefficient was used to show significant correlations between different hair types. This was done, because GLOVER (2000) has suggested that leaf epidermal cells may differentiate into either glandular or non-glandular trichomes. The used softwares for statistical analyses were SPSS ver. 9 (1998) and MVSP ver. 2.

RESULTS

The leaf surfaces of all plants in the studied populations were dense pilose, except for Tehran population that had loose pilose leaves. A total of thirteen

Table 2. Number (mean and STDEV) of the observed trichomes on leaves of the studied plants from different populations

Trichome types	Populations					
	Tehran	Sangak	Kerman	Shahzand	Amir kabir	Polor
One-celled*	1.33 ± 1.15	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Conidial	4.67 ± 3.21	77.00 ± 62.23	28.00 ± 29.05	144.33 ± 56.58	124.33 ± 22.36	146.00 ± 8.18
Two-celled*	41.33 ± 0.21	171.00 ± 143.04	181.67 ± 111.54	305.67 ± 1.01	296.33 ± 43.15	384.00 ± 6.77
Three-celled**	61.33 ± 1.50	105.67 ± 8.50	82.67 ± 34.26	142.67 ± 8.32	155.67 ± 8.50	106.00 ± 8.48
Four-celled*	6.33 ± 6.80	38.33 ± 12.66	39.67 ± 4.16	20.33 ± 1.52	16.33 ± 3.05	10.00 ± 4.24
Five-celled*	2.33 ± 3.21	6.33 ± 2.08	9.33 ± 3.21	2.67 ± 0.57	0.00 ± 0.00	0.00 ± 0.00
Six-celled	0.00 ± 0.00	1.00 ± 0.57	1.33 ± 0.57	1.00 ± 1.15	0.33 ± 0.57	0.00 ± 0.00
Peltate	5.33 ± 6.65	1.00 ± 0.00	43.00 ± 74.47	2.00 ± 3.46	2.00 ± 1.73	1.50 ± 0.70
Digitate	1.00 ± 1.15	1.00 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
One-celled glandular sessile*	21.0 ± 12.76	1.00 ± 1.00	4.33 ± 4.16	2.00 ± 0.00	1.33 ± 0.57	4.00 ± 1.41
Two-celled glandular sessile*	6.33 ± 1.52	2.33 ± 2.08	0.33 ± 0.57	2.33 ± 1.52	1.67 ± 1.15	1.00 ± 1.414
Short-stalked capitate*	9.00 ± 8.71	17.00 ± 16.82	268.33 ± 52.17	185.67 ± 7.00	71.00 ± 20.07	124.00 ± 33.94
Long-stalked capitate*	2.00 ± 3.46	7.67 ± 3.78	52.33 ± 4.50	24.00 ± 21.93	34.67 ± 24.78	29.50 ± 10.60

* Significance level differences ($p \leq 0.05$) among the populations according to the results of ANOVA.

different types of hairs were observed on the surface of leaves of the studied populations, which were divided into two main groups: glandular and non-glandular (simple). The glandular ones had capitate, peltate, digitate and sessile hairs. Furthermore, simple trichomes were seen as conidial and one of these was ascribed to six-celled type (Table 2).

The ANOVA test confirmed that significant differences ($p \leq 0.05$) in the number were presented between all observed types of trichomes, except for six-celled, conical, peltate and digitate.

Capitate glandular trichomes had two different types: long-stalked and short-stalked. The short-stalked type had one to two basal cell(s) with a one/two-celled head (Fig. 1 A), whereas in another type, two to three long cells with a short-celled neck were seen associated with a head that consisted of one to two cells (Fig. 1 B). The lowest and largest amounts of these trichomes were recorded in leaves of Tehran and Kerman populations, respectively. Short- and long-stalked capitate hairs were found in the leaves of the studied samples, but their numbers varied between the leaves of populations. The main differences were related to stalk length as well as cell number. In long-stalked ones, the basal cells were long and consisted of more than two cells. The capitate glandular trichomes commonly have rounded to pear-

shaped heads of one to two cells supported by stalks of changing length.

Peltate trichomes consisted of a short-celled stalk with a broad head that had four cells (Fig. 1 D). The highest number of this kind of trichomes was seen in the leaves of Kerman population and its lowest amount was found in the leaves of Sangak population. Sessile hairs were found in two shapes, one-celled (Fig. 1 D) and two-celled (Fig. 1 E). The largest numbers of one- and two-celled trichomes occurred in the leaves of Tehran population, however, their lowest amounts were recorded in the leaves of Sangak and Kerman populations, respectively. Digitate trichomes had restricted distribution and were only seen in the leaves of Tehran and Sangak populations (Fig. 1 C). Non-glandular trichomes had no branch and the constituted cells were linearly arranged with thin cell wall. The main difference of these trichomes was related to the cell numbers.

The lowest numbers of simple uni- and bi-celled trichomes (Fig. 1 G, H) were observed in the leaves of Tehran population, while the leaves of Polor population had the highest amounts of these trichomes. Not only maximum value of five-celled non-glandular hairs (Fig. 1 K) was seen in the leaves of Kerman population, but also in these leaves the largest number of four-celled type (Fig. 1 J) was recorded. In

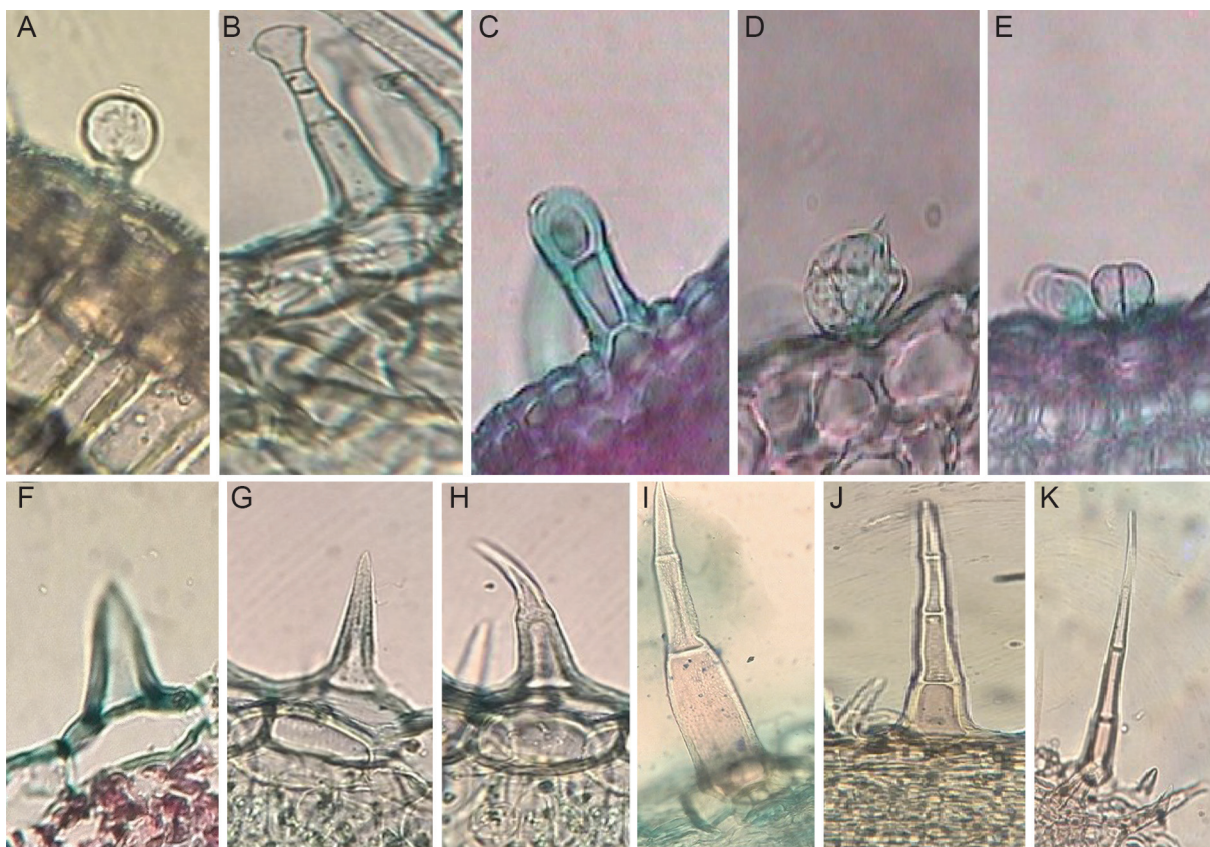


Fig. 1. Different types of trichomes in *Salvia nemorosa*. Glandular trichomes: A – short-stalked capitate, B – long-stalked capitate, C – Digitate glandular, D – peltate, E – sessile two-celled. Non-glandular trichomes: F – conidial, G – one-celled, H – two-celled, I – three-celled, J – four-celled, K – five-celled.

addition, the lowest number of four-celled trichomes was found in the leaves of Tehran population. Furthermore, the leaves of Amir Kabir and Tehran populations had maximum and minimum amounts of three-celled simple trichomes, respectively (Fig. 1 I). The most numerous glandular hair in the leaves of all studied populations was short-stalked capitate. Among the non-glandular trichomes, two-celled type was dominant in the leaves of all studied populations, except for the leaves of Tehran population, in which non-glandular three-celled trichomes were dominant.

PCA-biplot showed that leaves of each studied populations had distinct feature(s), which were useful in identification of populations. For example, the number of a simple two-celled trichome is a distinguishing trait for the leaves of Polor population, however, the leaves of Amir Kabir population were identified by the highest number of three-celled hair. In addition, the leaves of Kerman population were

characterized by the highest number of peltate hair (Fig. 2). Significant negative and positive correlations were observed between trichome density and some ecological features of the habitats. For example, significant negative correlations were reported between the northern distribution of populations and five- and six-celled ($p \leq 0.05$) and also long/short-stalked capitate trichomes, while uni-celled trichome had significant positive correlation ($p \leq 0.05$, $r = 0.54$) with latitude. A significant negative correlation ($p \leq 0.05$, $r = -0.4$) was found between bi-celled sessile glandular hair and altitude, contrarily, this ecological feature had a positive significant correlation ($p \leq 0.01$, $r = 0.71$) with short-stalked trichome. Moreover, significant negative/positive correlations were observed between different types of glandular and non-glandular trichomes. For example, peltate hair had a significant positive correlation ($p \leq 0.05$, $r = 0.5$) with a short-stalked capitate. Significant negative correlations ($p \leq 0.05$) were found between

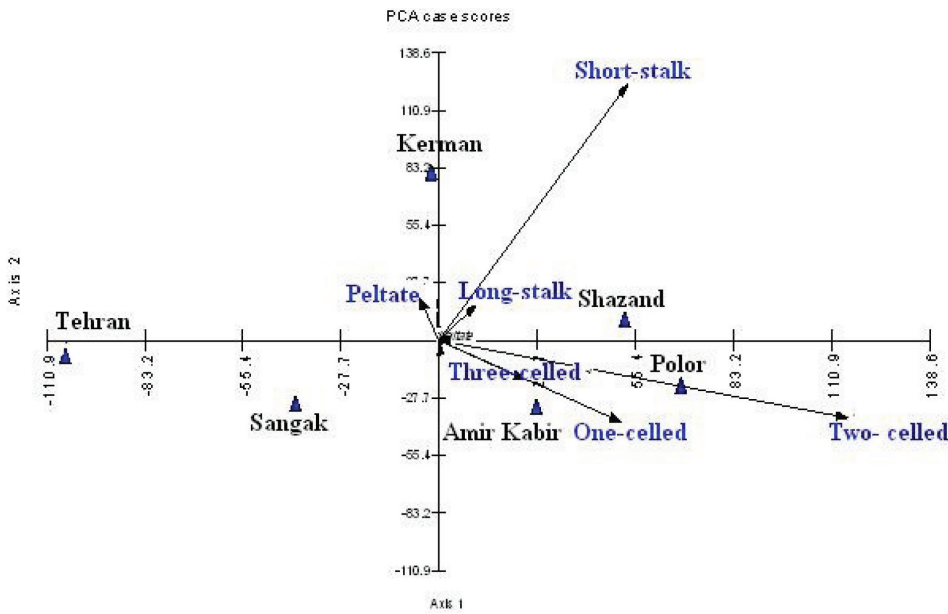


Fig. 2. PCA-biplot of the studied populations and their trichome features

sessile uni-celled glandular trichomes with one, two- and three-celled simple trichomes. However, it had positive significant correlation with digitate as well as sessile bi-celled glandular ones.

DISCUSSION

The type of indumentum was stable among the leaves of all populations, except for Tehran samples. The presence of indumentum on the epidermal surfaces of aerial organs is controlled genetically, while our findings proved that the indumentum density of this species is a semi-variable characteristic, which varies under different ecological conditions. It agreed with the previous studies (TALEBI et al., 2012; TALEBI & SHAYESTEHFAR, 2014). Various types of glandular trichomes were seen on the surface of leaves, but two main types were capitate and peltate. ASCENSÃO et al. (1999) have reported that the attendance of capitate and also peltate glandular hairs is a specific trait of Labiatae taxa. WERKER et al. (1985a) have suggested that three types of glandular trichomes (conforming to their morphology and secretion processes) exist on the vegetative and also reproductive organs of different Labiatae species. For example, short- and long-stalked capitate hairs, which have four-celled heads and thin sub-cuticular area, have been found in

Siderites syriaca (KAROUSOU et al., 1992), *Leonotis leonurus* (ASCENSÃO et al., 1995) and many species of *Teucrium* (MALECI & SERVETTAZ, 1991).

Distribution of peltate trichome not only differed between and within population, but also varied between two surfaces of each leaf. The distribution of this trichome in the abaxial surface was more than adaxial surface. Based on the previous studies (ASCENSÃO et al., 1995, 1999; SERRATO-VALENTI et al., 1997), peltate hairs consist of short uni-celled stalk with a large broad head. These trichomes have four or eight cells in a single disc or consist of 12–18 cells ordered in two concentric circles. Four of these are central and others circumferential cells (WERKER et al., 1985a, b; TELEPOVA et al., 1992). Since essential oil is produced and stored in glandular trichomes, therefore, difference in trichome number and type between various populations of each plant species may lead to infraspecific variation in the essential compositions. This subject has been reported in other species. For instance, ASKARY et al. (2016) have observed difference in the compositions of *Mentha piperita* essential oil, which associated with variations in trichomes diversity. Seven kinds of non-glandular hairs were found on both sides of a leaf. The number of these trichomes varied between the studied populations; however, the numbers of non-glandular were larger than those of glandular ones. Several roles

have been recognized for non-glandular trichomes. For example, these trichomes operate as a mechanical barrier, filter as well as isolation of leaf surface. In different plant taxa, the trichomes of a leaf maximized reflection of radiation and made the micro layer of more humid air circa the leaf epidermis, therefore, decreased the temperature of leaf, transpiration as well as water usage (PETER & SHANOWER, 1998). NAYDENOVA & GEORGIEV (2013) have demonstrated that the non-glandular trichomes in *Trifolium pratense* L. are morphological structures that support the operation of leaf stoma apparatus.

As seen above, one of the main roles of non-glandular trichomes is to reduce water loss by transpiration. Various investigations have confirmed that declined water accessibility is associated with an increment in leaf pubescence (CANO-SANTANA & OYAMA, 1992; PÉREZ-ESTRADA et al., 2000). Furthermore; in the studied populations, trichomes diminish radiation absorbance, therefore, heat load over surface of leaf (EHLERINGER et al., 1976; VOGELMANN, 1993). This condition leads to declined water dispersal by transpiration, because the comparative humidity nearby the surface of leaf is added, therefore, water potential variation between the leaf tissue and the circumambient air layer is minimized (EHLERINGER, 1984). GONZALES et al. (2008) have found the interactive effect of injury as well as water access on the glandular and non-glandular trichome presentation in *Madia sativa* (Asteraceae). They have suggested that water shortage amplifies the trichome density in this species. In most of the populations studied, bi-celled non-glandular and short-stalked capitate glandular trichomes were the prominent trichome types and these trichomes had significant correlations with habitat elevation. With elevation increment, the number of capitate trichomes increased, while the number of bi-celled ones decreased. It is very important to know that no significant correlation was observed between these trichomes; therefore, it seems that difference in habitat elevation is one of the main ecological factors that afford variations in trichome number. With altitude increment, the wind rate, air aridity and ultraviolet radiation maximize and these conditions are very harmful for plant, so plants amplify their trichomes, especially capitate ones to increase their tolerance (SLETVOLD & ÅGREN, 2012; EHLERINGER, 1984). Similar to altitude, some types of observed trichomes had

significant correlations with longitude; some kinds (e.g. one- and three-celled) had negative correlations, but the remaining trichomes (five-celled, sessile unicelled, short- and long-stalked capitate) had positive significant correlations. Most of glandular ones had significant positive correlations with eastern distribution. The air humidity decreases in Iran from west to east, therefore, with the increase of longitude, the air aridity amplified. So, plant individuals maximized their glandular trichomes for the prevention of water loss. Water economy is one of the most important factors for plants and they use different strategies for it.

CONCLUSION

Presence of indumentum is the main trait of the aerial organ epidermis in the Lamiaceae family. In all of the populations studied, leaf indumentum was similar, except for Tehran population. Indumentum in these populations consisted of glandular (peltate, capitate and digitate) and simple non-glandular (conical, one- to six-celled) hairs. Our obtained results proved that the trichome density and the type of prominent glandular and non-glandular trichomes varied among the studied populations of *S. nemorosa*. Some of the populations were characterized by special number of glandular and non-glandular trichomes. Likewise, significant correlations were observed between some types of trichomes with main traits of population's habitat. Although bearing of trichomes is determined genetically, it seems that ecological conditions of a habitat is one of the main factors that has strong effect on trichome type and density, therefore, some degree of plasticity in indumentum occur for adaption with environmental conditions of a habitat.

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LIAUKINIŲ PLAUKELIŲ MORFOLOGIJOS IR TANKIO ANALIZĖ *SALVIA NEMOROSA* L. (LAMIACEAE) IŠ IRANO

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Santrauka

Salvia nemorosa L., aromatinis augalas, plačiai paplitęs įvairiuose Irano regionuose, naudojamas tradicinėje ir liaudies medicinoje. Šio tyrimo metu šešiose šios rūšies populiacijose buvo tiriami lapų liaukinių plaukelių tankis ir tipas, kadangi eteriniai aliejai kaupiasi šiose struktūrose. Suaugę, nepažeisti augalų lapai iš kiekvienos populiacijos buvo fiksuojami FAA tirpale, o skersiniai dvigubai dažyti jų pjūviai tirti naudojant šviesos mikroskopiją. Visose populiacijose lapai buvo tankiai padengti plaukeliais, išskyrus Teherano populiaciją. Buvo identifikuota trylika liaukinių plau-

kelių tipų, tarp kurių dominavo skydiškos ir buožiškos formos plaukeliai. Principinė komponentų analizė (PCA) parodė, kad kiekviena tirta populiacija skyrėsi liaukinių plaukelių požymiais. Be to, nustatyta, kad kai kurie plaukelių tipai teigiamai ar neigiamai koreliuoja su buveinių ekologiniais veiksniais. Tirtos populiacijos papildomai buvo sugrupuotos, naudojant UPGMA metodą, tačiau gauti rezultatai panašūs į PCA. Tyrimų rezultatai patvirtino, kad buveinės ekologiniai veiksniai turi didelį poveikį liaukinių plaukelių morfologijai ir jų tankumui.