ARTICLES

Mediterranean Botany
ISSNe 2603-9109

EDICIONES COMPLUTENSE

http://dx.doi.org/10.5209/MBOT.59574

Trichomes morphology and density analysis in some *Nepeta* species of Iran

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Received: 9 October 2017 / Accepted: 19 February 2018

Abstract. *Nepeta* are widely distributed worldwide and in different parts of Iran where seventy-nine species were registered. The essential oil of these aromatic plants, which accumulates and secrets by trichomes, has been used in medicinal and industrial products. In addition, these epidermal structures can be used as taxonomic traits. We examined the morphology and density of leaves trichomes of twelve *Nepeta* species using the light and scanning electron microscopes. Three types of indumentum are found in these species, which composed of two main types of glandular: peltate and capitate, and non-glandular; branched and unbranched trichomes. The number of the observed hairs differed between the studied species and analyses of variant test (ANOVA) showed significant variations among some of them. CA-joined plot proved that trichomes could be used as distinguishing characteristic. Therefore, we suggested that the type of indumentum and the most abundant glandular and non-glandular trichomes have high taxonomic value in species identification within the genus.
Keywords: *Nepeta* species; trichomes; indumentum; glandular trichomes; non-glandular hairs.

Análisis de la densidad y morfología de los tricomas en especies iraníes de *Nepeta*

Resumen. El género *Nepeta* se distribuye ampliamente en todo el mundo y, más concretamente, en distintas partes de Irán donde se registran setenta y nueve especies. El aceite esencial de estas plantas aromáticas se acumula en tricomas y ha sido utilizado en productos industriales y también medicinales. Además, los tricomas se utilizan como un carácter diferencial taxonómico. Hemos analizado la morfología y densidad de tricomas de las hojas de doce especies de *Nepeta* usando el microscopio óptico y el electrónico de barrido. Se han identificado tres tipos de indumento: se glandular (peltado y capitado), y eglandular; además los tricomas pueden ser ramificados o no. El número de pelos observados difirió entre las especies estudiadas y los análisis de la varianza (ANOVA) mostraron variaciones significativas entre algunos de ellos. El análisis CA sustentó el carácter diferencial de los tricomas en las distintas especies estudiadas.
Palabras clave: Nepeta; tricomas; indumento; tricomas glandulares; pelos no glandulares.

Introduction

The genus *Nepeta* has more than 250 taxa over the world, which naturally distribute in the various parts of central and southern parts of Europe, Asia, and the Middle East (Pojarkova, 1954: Jamzad & *al.*, 2000). It has seventy-nine species in Iran (Jamzad, 2012), some of them are important in traditional medicine and food industry. For example, Adiguzel & *al.* (2009) has reported that *N. cataria* L. is employed in traditional medicine for the treatment of colic, cough, diarrhea, asthma, and bronchosis. Furthermore, this species is used as antispasmodic, antitussive, astringent, carminative, diaphoretic, semmengogue, refrigerant, sedative, stimulant, stomachic, and tonic. *N. cataria* is used for flavoring sauces, juices, soups, and foods. *N. glomerulosa* Boiss. is used in Iranian folk medicine to treat pneumonia (Sajjadi & Ghassemi, 1999). *N. binaludensis* Jamzad, *N. bracteata* Benth., *N. pogonosperma* Jamzad & Assadi, and *N. pungens* (Bunge) Benth. are used as medical herbs in Iranian traditional medicine. Moreover, *Nepeta caesarea* Boiss. is employed for the treatment of gastric disorders. *N. troodi* Holmboe and *N. parnassica* Heldr. & Sart. are used for toothache and for treatment of contusions and rheumatic pain (Formisano & *al.*, 2011). Sonboli & *al.* (2004) stated that an infusion and beverage obtained from *N. crispa* Willd. has been used as a smoothening, sedative, carminative, and for nervous and respiratory disorders. Salehi & *al.* (2007) reported the antibacterial activity of *N. ispahanica* Boiss. against different bacterial species. The antimicrobial and antioxidant activity of essential oils extracted from different *Nepeta* species shows the potential use of these plants in the food industries. These oils may also be valuable for increasing the expiry dates of foodstuffs (Hussain & *al.*, 2016).

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Trichomes are uni- or multi-celled pustule, which originate from the epidermal cells of the aerial organs. They are significantly variable in morphological characters, locations, ability to secrete, and type of secretion (Werker, 2000).

Previous studies proved that the type and density of hairs differs between taxa, therefore they have definite taxonomic values and could be considered for identification of species (Giuliani & *al*., 2008). These variables play a prominent role in the classification of several families such as Adoxaceae, Asteraceae, Convolvulaceae, Labiatae Scrophulariaceae and Verbenaceae as well (Makbul & *al.*, 2006; Krak & Mráz, 2008; Adedeji, 2012; Khokhar & *al.*, 2012; Konarska, 2017). Nevertheless, trichomes type and density may differ on organs of the same plant (Uphof, 1962; Hardin, 1979; Husain & *al.*, 1990; Christophel & *al.*, 1996).

Trichomes have many roles in every plant. For example, non-glandular hairs are thought to decrease the heat load of plants, maximize freezing tolerance, participate in seed dispersal, hold balance of water in plant leaves, deflect intense radiation of the sun, and offer protection from herbivores (Xiao & *al.*, 2017). On the other hand, glandular ones offer chemical protection against different herbivores and pathogens. Moreover, several studies (e.g. van Dam & *al.*, 1999; Wagner, 1991) have shown that these hairs are also connected to pollinator animals' attraction or accumulation of salt.

Although, there are several investigations about the chemical compounds of essential oil in many species of *Nepeta* (e.g. Sajjadi & Ghassemi, 1999; Salehi & *al.*, 2007; Adiguzel & *al*., 2009; Formisano & *al.*, 2011; Hussain & *al.*, 2016), the morphology and structure of the trichomes, that are secretory tissues in this genus, have been scarcely studied. The trichomes morphology was previously studied for *N. sibthorpii* Benth. (Rapisarda & *al.*, 2001), *N. racemosa* Lam. (Bourett & *al.*, 1994), and *N. congesta* Fisch. & Mey. (Kaya & *al.*, 2007). However, data on trichomes morphology of many *Nepeta* species is not available.

Due to wide use of *Nepeta* taxa essential oil in traditional medicine and its applications in plant taxonomy, the aims of the current research were to determine the type and density of glandular and non-glandular trichomes and their importance in species identification. Furthermore, trichomes morphology of most species was studied for the first time.

Material and Methods

Plant samples

The sampling locations of *Nepeta* twelve species were selected from habitats suggested by Flora Iranica (Rechinger, 1982) and Flora of Iran (Jamzad, 2012) (Table 1, Figure 1). Based on the geographical distribution, one or two populations were collected of each species. Plant samples were identified according to the descriptions of above mentioned references. The voucher samples were deposited in Herbarium of Research Center of Agricultural and Natural Resources of Mashhad (MRCH) and Institute of Medicinal Plants Herbarium (IMPH).

Light and Scanning electron microscopy (LM, SEM)

Five mature leaves were selected from five plant samples from each population. The leaves were fixed in a FAA solution (formaldehyde 90%, ethanol 5%, and acetic acid 5%). The plant samples were dehydrated in an ethanol series (Johansen, 1940). The anatomical analysis of the hairs was based on the semi-thin sections made from transects obtained from the central part of the leaf blade. The leaves slices were stained with a 1% aqueous methylene blue and carmine solutions. The observations were made and microphotographs were shot using an Olympus CH_2 light microscope.

For SEM observations small parts (6 mm×10 mm) of each selected leaf were fixed in a 5% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.0) for 10 h at 24°C. Then, the plant samples were washed in the same buffer three times at 15-min intervals and after that dehydrated in different series of ethanol (30, 50, 70, 90, and 95%) followed by the usage of absolute alcohol two times. When slices dehydrated, they were transferred to acetone. In this time, plant slices dried at a critical point in liquid CO_2 and coated with gold using the Polaron SC 7640 sputter coater (Robards, 1978). The leaves surfaces were investigated and imaged under a SU 3500 scanning electron microscope, at an accelerating voltage of 5-10 kV.

Statistical analyses

The mean and standard deviation of the recorded hairs number were calculated. Data were standardized (mean=0, variance=1) for correlation analyses (CA; Higgs, 1991). One-way analysis of variance (ANOVA) was used to compare the trichomes numbers among the studied species. We used SPSS 9.0 (1998) and MVSP 2.0 (1998).

Trichomes densities of four slices per leaf were counted under a light microscope. The areas of leaves on the digital images were measured by Image Tool ver. 2.0. Trichomes density was calculated by dividing the hair number per mm^2 by the leaf area (Gonzales & *al*., 2008).

Results

Morphology and distribution of trichomes

Various types of indumentum existed on leaf surfaces of the *Nepeta* studied species. Densely-pubescent is observed in *N. cataria* (both populations), *N. menthoides*, *N. crassifolia, N. pogonosperma* and *N. glomerulosa,* (both populations), while the sparse type was found in *N. saccharata*, *N. satureioides*, *N. bracteate* and *N. ispahanica* (both populations). Other species had semi-dense indumentum. All studied leaves of these species had different types of non-glandular and glandular trichomes (Table 2).

Table 1. Location and herbarium voucher number (V.N.) of studied *Nepeta* species.

Taxa	Locality	Latitude	Longitude	V.N.
N. bracteata Benth.	South Khorasan province, Ferdous, Karghaneh altitudes, 1800 m asl.	33°21’42.09”N	58°28’34.51”E	6497-MRCH
N. cataria L. (Mazandaran population)	Mazandaran province, Siahbisheh, 2300 m asl.	36°14’33.0”N	51°15’04.11”E	7046-IMPH
N. cataria L. (Khorasan population)	Khorasan Razavi province, Kalat, Gharesoo, 1100 m asl.	37°00’05.20”N	59°45’27.01”E	11116-MRCH
N. crassifolia Boiss. & Buhse.	Semnan province, Semnan to Damghan road, Ahovan, 1300 m asl.	37°20’24.30”N	57°03’13.9”E	0243-IMPH
N. glomerulosa Boiss. (Jiroft population)	Kerman province, Jirof, 42 km to Kerman, Cal. Deh-bakri, 2260 m asl.	28°93’85.04”N	57°46’27.27”E	5907-MRCH
N. glomerulosa Boiss. (Gachsaran population)	Kohkiloyeh and boier Ahmad, Ghachsaran, Lar, 1400 m asl.	30°72’46.29”N	50°84’56.22”E	9087-MRCH
N. ispahanica Boiss. (Birjand population)	South Khorasan Province, Birjand, 4km in Shooshood Roud, 1600 m asl.	32°09’03.13”N	58º73’74.54”E	1971-MRCH
N. ispahanica Boiss. (Sero population)	West Azerbaijan, 60 km Salmas to Urmia, 1580m asl.	38°19’73.13”N	44°76’53.34”E	7047-IMPH
N. kotschyi var. *persica* (Boiss.) Jamzad	South Khorasan Province, Kashmar, Norh Baharieh, 1350 m asl.	35°25’28.09”N	58°50’45.13”E	7049-IMPH
N. menthoides Boiss. & Buhse.	East Azerbaijan, Khalkhal, 2500 m asl.	37°37’60.00”N	48°32’59.99”E	1575-IMPH
N. pogonosperma Jamzad & Assadi	Qazvin province, Alamout, 3000 m asl.	36°08’81.16”N	49°85’47.34”E	7050-IMPH
N. saccharata Bunge	Khorasan Razavi province, Torbat heydarieh, Khomari, 1700 m asl.	35°23’08.02”N	59°12’23.00”E	3033-MRCH
N. satureioides Bioss.	South Khorasan province, Ferdous, Galekooh slopes, 1600 m asl.	33°22’51.07”N	58°28’34.51”E	4454-MRCH
N. sessilifolia Bunge	Isfahan province, Golpaygan, Alvand, 2000 m asl.	33°56’08.22”N	50°43’08.33”E	7048-IMPH
N. ucranica subsp. *kopetdaghensis* (Pojark.) Rech.f.	Khorasan Razavi province, Ghoochan- Dargaz.70 km to Tandooreh, 1600 m asl.	37°26’39.98”N	59°06’29.02”E	5523-MRCH

On both leaf surfaces of the studied species, there were glandular hairs that were classified into two main kinds, i.e. peltate and capitate.

Peltate trichomes. The trichomes were outgrowths of leaf epidermal cells and had large size. The scanning electron microscopy micrographs of the heads of peltate hairs proved they smooth surface. These hairs had basal and a stalk cell and a head that was consisted of four secretory cells arranged in a single layer disc (Figures 2, 3a). Analyses of variance confirmed significant variations for peltate hairs, its highest number was recorded in *N. pogonosperma* (Table 3).

Capitate trichomes. We registered capitate trichomes on abaxial and adaxial leaf surfaces. Based on the morphology and dimensions of stalk and head cells, the capitate hairs were divided into two types. Type I had two basal cells, a long unicellular stalk, a neck cell, and a bulbiform 1-celled head (Figures 2, 3b). This trichome was absent in many species like *N. cataria* (Mazandaran population), *N. pogonosperma*,

N. menthoides, *N. crassifolia*, *N. glomerulosa* (both populations), while it was a prominent type of glandular trichomes in *N. sessilifolia N. saccharata*, and *N. kotschyi* var. *persica.*

The trichomes of type II possessed a basal cell, a short unicellular stalk, and an oval 1-celled head (Figure 2c). These were typical for all the studied species except for *N. ispahanica* (Birjand population); however, it was found abundantly on the leaves of *N. pogonosperma*. The results of ANOVA proved significant variations for both types of these trichomes among the species (Table 3).

Figure 1. Distribution map of the studied *Nepeta* taxa in Iran (numbers are the name of *Nepeta* species and their populations as presented in Table 1).

Non-glandular trichomes. The studied *Nepeta* species have two types of non-glandular hairs. The first type comprised single, multicellular, non-branched, and pointed trichomes. These trichomes were mainly observed on both leaf surfaces of the studied species. We registered different shapes of them, namely: one, two, three, four, five, six, seven, and eight-celled (Figures 2d and 3c). The first four trichomes were common and observed in all of the studied species. The three-celled one was the prominent type in *N. saccharata*, *N. ucranica* subsp. *kopetdaghensis*, *N. glomerulosa* (Gachsaran population), *N. ispahanica* (both populations), *N. kotschyi* var. *persica,* and *N. cataria* (both populations). But bi-celled type was considered as the main non-glandular hair in *N. crassifolia* and *N. satureioides*. The five and six-celled hairs were less common and only existed in *N. satureioides*, while seven and eight-celled types were only seen in *N. crassifolia* and *N. cataria* (Khorasan population; Figure 2e). ANOVA results proved significant variations only for one, two, three, and eight-cell trichomes (Table 3). These hairs had ornamentations on their outer cell surfaces (Figure 2f).

The second type includes branched multicellular hairs (Figure 3d). These hairs were recorded in *N. menthoides*, *N. crassifolia* and *N. glomerulosa* (both populations). The mentioned species had various shapes of branched trichome, like two-branched, tree-like, and star-shaped. However, branched- type was the most frequent type of trichomes in *N. menthoides*, *N. crassifolia*, and *N. glomerulosa* (both populations).

Table 2. Number of observed glandular and non-glandular trichomes in studied taxa. Abbreviations are: 1-c.-6-c.: one-six celled; Pelt.: peltate; Cap.II: capitate type II; Cap.I: capitate type I; N: Number of samples; Std.dev: Standard deviation.

Species		1-c.	2-c.	3-c.	4-c.	5-c.	6-c.	Pelt.	Cap.II	Cap.I
N. bracteata	Mean	2.33	18.00	18.66	1.33	0.00	0.00	6.33	3.00	1.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	4.04	3.60	2.51	2.30	0.00	0.00	2.08	3.00	0.57
N. cataria	Mean	3.00	5.33	17.00	23.33	15.00	1.66	8.00	29.66	22.00
(Khorasan population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.73	2.30	6.55	4.72	1.73	1.15	1.00	9.29	2.64
N. cataria	Mean	6.00	42.00	1.04	17.33	0.00	0.00	15.66	23.66	0.00
(Mazandaran population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	2.64	8.18	9.84	4.04	0.00	0.00	3.51	1.13	0.00
N. crassifolia	Mean	1.00	25.33	10.00	2.66	0.00	0.00	4.66	65.00	0.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	0.57	2.55	1.40	4.61	0.00	0.00	4.16	6.76	0.00
N. glomerulosa	Mean	0.66	35.66	49.00	23.33	7.66	0.00	11.00	5.00	0.00
(Gachsaran population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.15	3.26	4.24	3.53	1.32	0.00	1.27	5.56	0.00
N. glomerulosa	Mean	1.00	19.66	22.00	20.66	11.33	1.00	13.33	5.33	0.00
(Jiroft population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	0.57	2.58	2.48	2.99	1.05	1.15	1.25	5.50	0.00
N. ispahanica	Mean	0.00	1.00	3.33	2.66	1.00	1.00	2.00	0.00	1.00
(Birjand population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	0.00	1.73	5.77	4.61	1.73	1.15	.00	.00	1.73
N. ispahanica	Mean	1.66	9.00	19.66	4.00	0.00	1.66	3.00	1.66	15.00
(Sero population)	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.15	0.00	8.14	3.60	0.57	1.52	2.64	.57	3.60
N. kotschyi var. *persica*	Mean	1.66	7.00	9.66	4.66	4.66	3.66	8.00	47.33	58.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.15	3.46	6.02	4.72	6.42	4.72	6.08	1.95	1.32
N. menthoides	Mean	1.00	19.00	20.00	1.33	1.00	0.00	8.66	61.33	0.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	.55	9.84	1.80	1.52	0.57	0.00	6.02	2.38	0.00
N. pogonosperma	Mean	0.00	0.00	0.00	0.00	0.00	0.00	24.50	1.58	0.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	0.00	0.00	0.00	0.00	0.00	0.00	9.19	6.71	0.00
N. satureioides	Mean	3.00	18.66	13.33	4.66	0.00	1.00	1.00	1.00	4.00
	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.73	5.77	8.08	2.51	0.00	0.57	0.00	1.15	3.60
	Mean	2.00	4.66	11.00	5.66	2.66	1.33	6.33	1.33	16.00
N. saccharata	N	5	5	5	5	5	5	5	5	5
	Std.dev.	2.00	2.51	4.58	5.03	3.05	1.52	1.15	0.57	3.46
	Mean	3.33	1.00	0.00	0.00	0.00	0.00	1.00	60.66	90.33
N. sessilifolia	N	5	5	5	5	5	5	5	5	5
	Std.dev.	3.05	0.57	0.00	0.00	0.00	0.00	0.00	1.05	7.95
	Mean	1.00	20.00	39.33	14.33	0.00	0.00	1.33	15.00	0.67
N. ucranica subsp. *kopetdaghensis*	N	5	5	5	5	5	5	5	5	5
	Std.dev.	1.00	3.46	1.92	9.01	0.00	0.00	1.52	6.08	1.15

CA-joined plot showed that some of species had significant type(s) of trichome(s) that were used for identification of species (Figure 4). For example, the number of capitate trichomes of type II was a relevant trait for the identification of *N. pogonosperma* and capitate hair of type II was relevant trait for *N. sessilifolia* and *N. kotschyi* var. *persica*. Moreover, presence of six- and eight-celled hairs is a prominent trait for *N. saccharata* whereas branched-type is for identification of *N. crassifolia* and *N. menthoides*.

To investigate trichomes types and density infra-specifically, two populations of *N. glomerulosa*, *N. ispahanica,* and *N. cataria* were considered. Although, the type of indumentum was rather stable between populations of each species, the type and density of glandular and non-glandular hairs varied significantly between. In addition, the populations of each species did not cluster closely in CA-joined plot (Figure 4) that confirmed high infraspecific variations in trichomes characters.

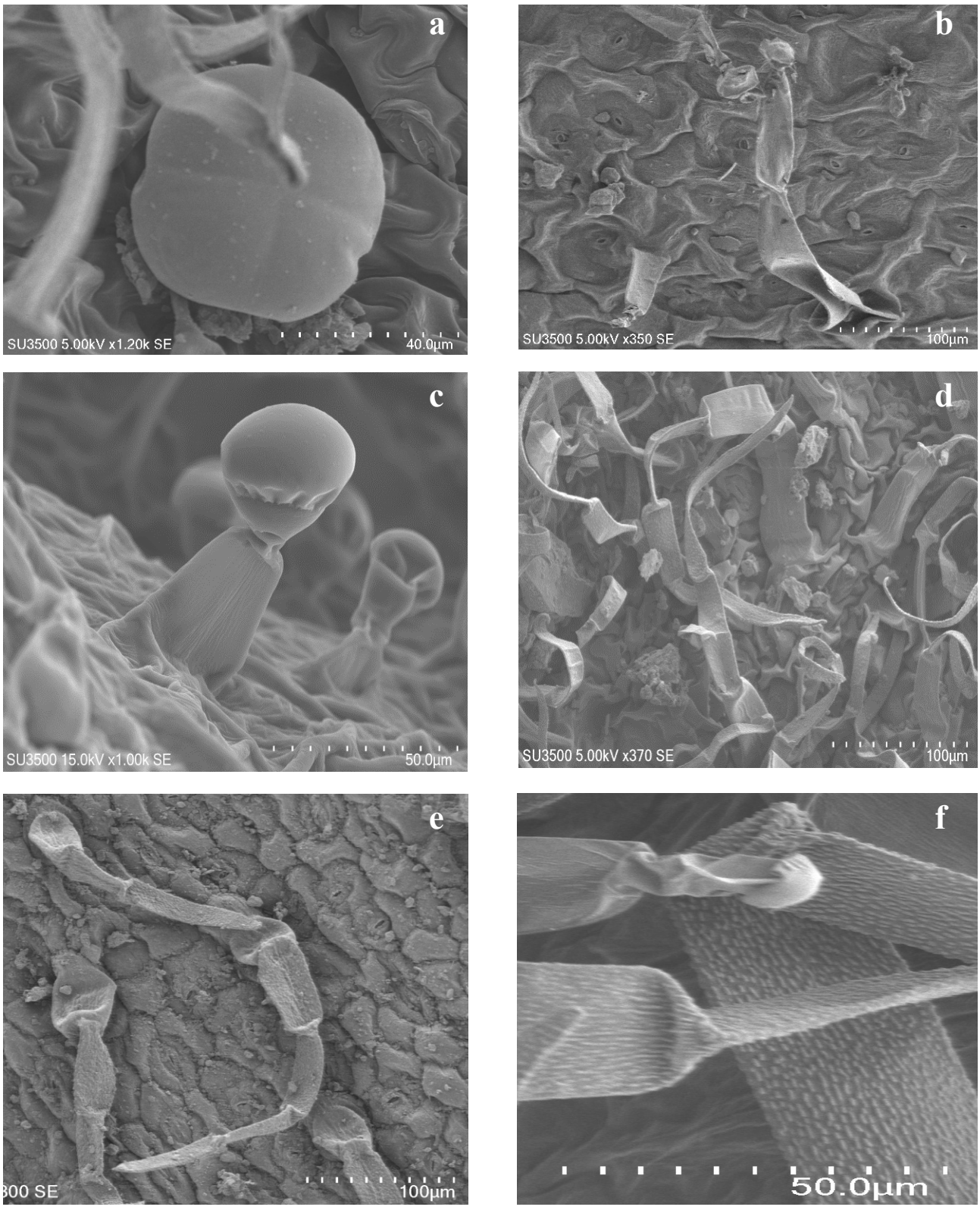

Figure 2. Electronic micrographs of some observed types of trichomes within *Nepeta* species. a) peltate (*N. pogonosperma*); b) capitate type I (*N. sessilifolia*); c) capitate type II (*N. crassifolia*); d) four and five-celled trichomes (*N. cataria*); e) eight-celled hairs (*N. crassifolia*); f) micro-ornamentation.

Brief description of the trichomes in the studied taxa

Both populations of *N. cataria* had densely-pubescent indumentum and their dominant glandular trichomes were capitate of type II. In Khorasan population, the dominant non-glandular trichome was four-celled, while in Mazandaran population it was two-celled with larger number of peltate hairs. In *N. crassifolia* densely-pubescent indumentum had branched non-glandular hairs with highest numbers of non-branched two-celled and capitate type II hairs. Both populations of *N. glomerulosa* had densely-pubescent indumentum with branched non-glandular hairs. Their most frequent glandular and simple non-glandular

hairs were peltate and three-celled, respectively, with equal numbers of capitate type I and II. The main differences of them were related to the number of non-glandular two- and three-celled trichomes. The indumentum in *N. menthoides* was densely-pubescent with two- and three-branched non-glandular hairs. Capitate type II and simple three-celled trichomes were more frequent.

Table 3. Results on the ANOVA analysis to assess for differences in trichome number in *Nepeta* species. Abbreviations are: df: degrees of freedom; F: F-statistic; P: probability.

Trichomes type		Sum of Squares	df	Mean Square	F	P
Non-glandular One-celled	Between Groups	110.917	14	7.923	2.361	0.025
	Within Groups	97.333	60	3.356		
	Total	208.250	74			
Non-glandular Two-celled	Between Groups	6621.765	14	472.983	2.603	0.014
	Within Groups	5268.667	60	181.678		
	Total	11890.432	74			
Non-glandular Three-celled	Between Groups	28296.977	14	2021.213	7.962	0.000
	Within Groups	7362.000	60	253.862		
	Total	35658.977	74			
Non-glandular Four-celled	Between Groups	3146.636	14	224.760	1.368	0.231
	Within Groups	4766.000	60	164.345		
	Total	7912.636	74			
Non-glandular Five-celled	Between Groups	943.909	14	67.422	1.518	0.166
	Within Groups	1288.000	60	44.414		
	Total	2231.909	74			
Non-glandular Six-celled	Between Groups	44.879	14	3.206	1.483	0.179
	Within Groups	62.667	60	2.161		
	Total	107.545	74			
Non-glandular Seven-celled	Between Groups	5.098	14	0.364	.932	0.539
	Within Groups	11.333	60	0.391		
	Total	16.432	74			
Non-glandular Eight-celled	Between Groups	1.242	14	0.089	3.860	0.001
	Within Groups	.667	60	0.023		
	Total	1.909	74			
Non-glandular Two-branched	Between Groups	27581.644	14	1970.117	15.271	0.000
	Within Groups	3741.333	60	129.011		
	Total	31322.977	74			
Non-glandular Tree-shaped	Between Groups	1200.129	14	85.723	5.966	0.000
	Within Groups	416.667	60	14.368		
	Total	1616.795	74			
Glandular peltate	Between Groups	1585.629	14	113.259	3.403	0.003
	Within Groups	965.167	60	33.282		
	Total	2550.795	74			
Glandular Capitate type I	Between Groups	60161.500	14	4297.250	3.254	0.004
	Within Groups	38298.500	60	1320.638		
	Total	98460.000	74			
Glandular Capitate type II	Between Groups	28727.182	14	2051.942	4.539	0.000
	Within Groups	13110.000	60	452.069		
	Total	41837.182	74			

Indumentum of *N. pogonosperma* had different types of branched non-glandular with very small number of simple non-glandular trichomes. The dominant glandular hair was peltate. *N. saccharata* and *N. satureioides* had sparse pubescent indumentum. The dominant glandular trichome was capitate type I, the more frequent non-glandular hairs were three and two-celled, respectively. In *N. bracteata* sparse pubescent indumentum was characterized by peltate and three-celled hair without any five and six-celled simple hair.

N. ispahanica both populations' leaves were covered by sparse pubescent indumentum. Dominant glandular and non-glandular trichomes in both populations were similar, while Sero population had more trichomes than Birjand population. *N. ucranica* subsp. *kopetdaghensis* was characterized by semi-dense indumentum with more abundant three-celled and capitate type II trichomes. The indumentum of *N. kotschyi* var. *persica* was semi-dense and possessed peltate and three-celled as the most abundant trichomes.

Discussion

Based on our results of trichomes morphology and density from twelve *Nepeta* species, we suggested that the type of indumentum could be used as a good trait for identification of the studied taxa concerning it stable position between studied populations of each species. We proposed to divide the studied taxa into three groups: species with densely-pubescent,

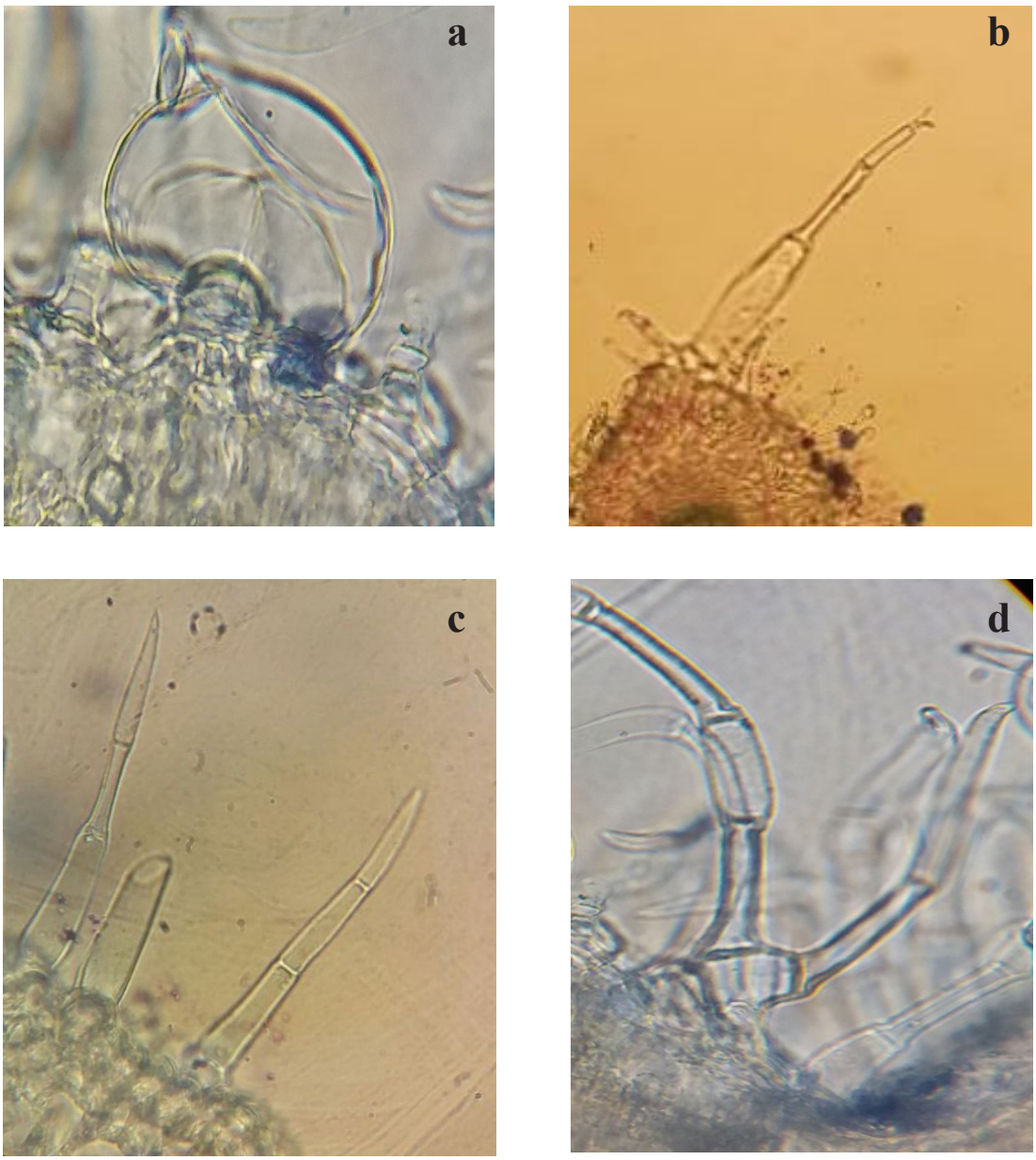

Figure 3. Light microscope images showing different trichomes. a) peltate (*N. pogonosperma*), b) capitate type I (*N. sessilifolia*), c) three-celled non-glandular (*N. glomerulosa*), d) two-branched non-glandular (*N. menthoides*).

sparse-pubescent, and semi-dense indumentum. In each group, the type of dominant glandular or non-glandular hairs differed and therefore could be used as important characteristics for species identification within each group. Referring to previous investigations (e.g. Metcalfe & Chalk, 1950; Gairola & *al*., 2009), the traits of indumentum are very useful in the taxonomical studies of Labiatae at various levels, either infrageneric (Navarro & El Oualidi, 2000), or infraspecific (Giuliani & *al*., 2008).

Non-glandular trichomes of several types were widely observed on both surfaces of the leaves and their number was higher than glandular ones. Some studies showed that this is a common feature in Labiatae family (Metcalfe & Chalk, 1950). Based on the morphology and cell number, the non-glandular trichomes were divided into four types: simple unicellular, simple multicellular, branched unicellular, and branched multicellular (Cantino, 1990). All of the mentioned types of non-glandular hairs were observed on the studied leaves and our findings proved that their number and density could be reliable taxonomic traits.

Specially, three types of observed trichomes were very useful in identification of taxa: multicellular, branched unicellular, and branched multicellular. These trichomes were registered in some studied taxa; nevertheless, they can be used as a taxonomic tool in species identification.

The outer surface of non-glandular ones had small ribbed-shaped projections. Werker (2000) believed that trichomes may be smooth or have micro-ornamentation and these structures are aroused from the wall of cell or its cuticle.

The glandular trichomes are divided into two main types: peltate and capitate. Recent studies have shown that these types of trichomes have been distinguished in

several species of *Nepeta* (Bourett & *al*., 1994; Rapisarda & *al*., 2001). Peltate trichomes have one basal cell with a uni-celled short stalk, and a large multi-celled (usually 4-18) head. Their capitate type consists of a basal cell and a stalk with1-4-celled head (Evert, 2006).

The capitate trichomes are the dominant type of glandular trichomes. Kaya & *al.*, (2007) have recently shown that these trichomes are widespread in the Labiatae, while they differ significantly in their morphology and dimensions.

The studied species had two types of capitate trichomes. The main difference of these trichomes was stalk length. Metcalfe & Chalk (1950) stated that capitate trichomes are very common in *Nepeta* genus and three types of them are recorded in several species of *Nepeta*. For example, the capitate trichomes of *N. congesta* var. *congesta* and *N. racemosa* differed in both stalk and head cells number, and three kinds of it were existed (Kaya & *al.*, 2007; Bourett & *al.*, 1994).

Number of trichome types varied between the studied species and this can be used in the taxonomy of the genus (Figure 4). However, the capitate trichomes were the dominant type of glandular hairs and other types of glandular trichomes distributed among them. This status has been reported in different species. For instance, Marin & *al.*, (2006) observed capitate hairs were densely distributed, but the peltate type was scattered among them in *Marrubium vulgare* L. and *Rosmarinus officinalis* L.

Another type of observed glandular hairs in *Nepeta* species was peltate trichomes, which distributed in the abaxial surface of leaves. The peltate trichomes in the studied taxa were located in deeper depressions of the leaf surfaces, similar to *Dracocephalum moldavia* L., *Origanum vulgare* L. subsp. *vulgare*, *Thymus lykae* Degen & Jav. and *Thymus quinquecostatus* Celak. (Dmitruk & Weryszko-Chmielewska, 2010; Jia & *al.*, 2013; Shafiee-Hajiabad & *al*., 2014). In *M. vulgare* peltate trichomes were located on the surface of the stems and leaves, its secretory head consisted of four cells that arranged in a single circle. Metcalfe & Chalk (1950) pointed out that the peltate trichomes with heads made of four secretory cells are very common in *Nepeta* taxa.

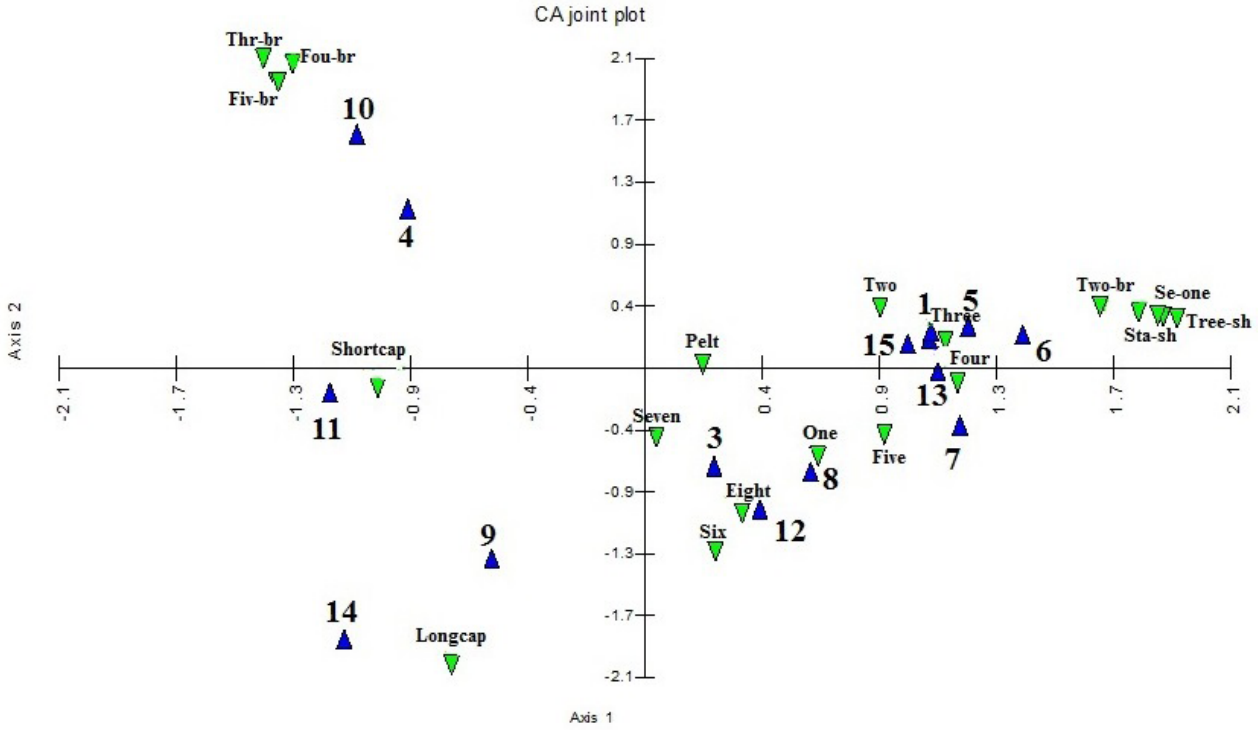

Figure 4. CA-joined plot of species and trichomes in *Nepeta* species. The blue symbols are the name of *Nepeta* species and their populations according to Table 1. The green symbols are abbreviated name of trichomes: longcap: capitate type I; shotcap: capitate type II; pelta: peltate; two-br: two-branched; tree-sh: tree-shaped; thr-br: three-branched; fou-bra: four-branched; fiv-br: five-branched; one to eight are one- to eight-celled non-glandular trichomes, respectively.

Peltate trichomes with four-celled head were also found in other species of Labiatae, as *Ocimum basilicum* L. (Werker & *al*., 1993), *Isodon rubescens* (Hemsl) Hara, or *Ocimum obovatum* E May. ex. Benth. (Naidoo & *al*., 2013). Moreover, high number of head cells have observed in *Leontis leonurus* (L.) R. Br. (Ascensão & *al.,* 1995), *Salvia aurea* L. (Serrato-Valenti & *al.,* 1997), *Mentha* x *piperita* L. (Turner & *al.,* 2000), *Lavandula pinnata* L. (Huang & *al*., 2008), *Origanum vulgare* (Shafiee-Hajiabad & *al.*, 2014) and *Th. quinquecostatus* (Jia & *al*., 2013).

The chemical compositions of accumulated compound are different between these trichomes. Peltate trichomes produce most of the essential oils in

Labiatae taxa (Werker, 1993). According to findings of Jia & *al*. (2012), the capitate types also produce a few lipophilic components. Phytochemical examinations on the essential oil compositions of *Nepeta* taxa (e.g. Yarmoohammadi & *al*., 2017) have shown high chemical variations in the essential oil components inter and intra species. It seems that variation in trichomes type and density is one of possible reason for essential oil differences. For example, significant correlations were found between essential oil variations and type of glandular trichomes in *Nepeta heliotropifolia* Lam. (Yarmoohammadi & *al*., 2017). Askary & *al*. (2016a) have examined trichomes morphology and density in ten NaCl and nanoparticles treated samples of *M. piperita* and observed high infraspecific variations in trichomes types and densities. They predicted that these differences can cause variation in essential oil compositions. Later studies of Askary & *al.* (2016b) on essential oil composition of these plants confirmed these predictions.

The ability of storage of accumulated secondary metabolite, especially essential oil, is different between the capitate and peltate trichomes. Siebert (2004) stated that the chemical compounds secreted by the capitate hairs are mostly excreted to the surrounding environment, apparently through pores in the cuticle of the head cells. But these secretions in peltate trichomes accumulate in a capacious subcuticular space created by the separation of the head cell walls from the cuticular dome that encloses them, and remain there until the cuticle is physically ruptured. Therefore, peltate hairs function as repositories for the specialized phytochemical compounds which they secrete. Hence, the species possessing the higher number of peltate trichomes have more amounts of essential oil.

Infraspecific variation in trichomes types and densities showed that ecological conditions of plant habitat have a strong effect on the trichomes. So, glandular and non-glandular hairs have special roles in plant living patterns and interactions with biotic and abiotic factors. Therefore, the number of trichome types varies among the populations for better adoptions towards the habitat considering the changes in environmental conditions. These variations in trichomes type and densities were seen in different species of the genus e.g. *N. heliotropifolia* (Yarmoohammadi & *al*., 2017) or other genera of the family, such as *Stachys* L. (Rezakhanlou and Talebi, 2010), *Ziziphora* L. (Talebi & *al*., 2012), and *Acinos* Miller (Talebi & Shayestehfar, 2014). Our results confirmed that the different types of trichomes existing on the leaf surfaces of the studied taxa lead to the infrageneric variations in amount and compositions of essential oil.

Conclusion

The leaf epidermal surfaces of the studied taxa have indumentum consisted of glandular and non-glandular hairs. The density of indumentum varies between these taxa and three types of it were observed. The ANOVA test showed significant variations for some types of trichomes. There were two main types of glandular trichomes: peltate and capitate, and non-glandular hairs which were divided into branched and unbranched. CA-joined plot proved that some types of observed hairs such as capitate glandular type I and II, simple non- glandular six and eight-celled as well as branched trichomes could be used as taxonomical keys for some species. Previous research showed that essential oil compositions varied between studied species, and our results proved that essential oil compositions also depended on the frequency of trichomes. It seems that the type of indumentum is more important for species identification than the type and abundance of trichomes.

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