BIODIVERSITAS
Volume 18, Number 3, July 2017
Pages: 964-970

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180314

Infraspecific variations in essential oil and glandular trichomes in *Nepeta heliotropifolia*

MAHBOBEH YARMOOHAMMADI[1], SEYED MEHDI TALEBI[1,♥], MAJID GHORBANI NOHOOJI[2]
[1]Department of Biology, Faculty of Sciences, Arak University, Arak, 38156-8-8349 Iran. Tel.: +98-863-4173317. ♥email: seyedmehdi_talebi@yahoo.com
[2]Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

Manuscript received: 4 April 2016. Revision accepted: 1 June 2017.

Abstract. *Yarmoohammadi M, Talebi SM, Nohooji MG. 2017. Infraspecific variations in essential oil and glandular trichomes in* Nepeta heliotropifolia*. Biodiversitas 18: 964-970. Nepeta heliotropifolia* Lam. (Labiatae) is one of the perennial medicinal and aromatic plants, naturally grows in different regions of Iran. The essential oil composition of aerial parts and also the micromorphology and distribution of foliar trichomes of *N. heliotropifolia* from two different regions of Iran were studied. The extracted essential oils were significantly composed of caryophyllene oxide and phytol, which were quantified by GC and identified based on their GC/MS spectra. The essential oil compositions differed greatly with habitat as well as former investigations. Phytol was quantified between 0.75-10. 29% of the total essential oil, but caryophyllene oxide was quantified between 3.90-12.17% of the total essential oil. Trichomes investigations showed that leaves bear an indumentum of glandular and non-glandular hairs. Two main kinds of glandular trichomes were observed; peltate and capitate. The numbers of capitate and peltate trichomes varied between populations. Such great differences in the essential oil composition may be attributed to chemotype creation and also an adaptation of these populations to particular habitats.

Keywords; *Nepeta heliotropifolia*, essential oil, trichomes, infraspecific variations

INTRODUCTION

Investigations confirmed that various agents, for example, genetic, physiological or environmental variables, cause the chemical differences in essential oil composition. For this reason, although the essential oil chemical composition was genetically determined it can suffer influence as a result of many ecological parameters, especially when referring to vegetal material used in pharmacological, chemical and agronomic examinations, which their aims are obtain herbal medicines. Because, pharmacological qualities can vary due to changes in the compositions of essential oil (Lima et al. 2006; Paula et al. 2011).

Former investigations on different medicinal plant species, such as sage (Russo et al. 2013) and chamomile (Formisano et al. 2015), proved that environmental factors strongly influence the phytochemical compositions as well as biological activities of plants. Peltonen-Sainio et al. (2010) stated that the plant yield is the result of a complex interaction between weather, genotype, soil condition and management parameters on growth as well as development processes. Furthermore, other ecological factors such as irradiation, temperature, and also precipitation can directly and indirectly influence plant's yield (Takashima et al., 2013). Manukyan and Schnitzler (2006) explained temperature mainly determine the quality and productivity of yield. In addition, the availability of water is the main factor to manufacture assimilates during various phases of growth used both for creation of vegetative dry matter and also for reserve carbohydrates in the vegetative tissues (Delfine et al. 2001).

Nepeta is a genus of the Labiatae family and comprises of approximately 250 species. Many species of it are used for pharmaceutical purposes. These aromatic plants naturally grow in different regions of Iran and until now seventy nine species of *Nepeta* were listed by the country. *Nepeta heliotropifolia* Lam. is a perennial herb of the genus and grows naturally in Iran (Jamzad 2012). It is important to know that aromatic species of Lamiaceae are economically very important due to the production of essential oils (Mc Caskill and Croteau 1995).

Glandular and non-glandular hairs are widely distributed over the aerial parts of both reproductive as well as vegetative organs of Lamiaceae taxa, and the secretions of glandular trichomes contribute largely to their great importance. Glandular hairs are the primary secretory structures of these species, and their morphological traits can differ widely between taxa (Kaya et al., 2003). Werker (1993) demonstrated these trichomes are often microscopic and secrete different kinds of constitutes, such as essential oil. It may act to protect the plant aerial parts against pathogens and also herbivores. Moreover, the biological activity of the secondary metabolites in the essential oil can be used in different industries, for example; pesticide, flavoring and fragrance and pharmaceutical (Werker 1993; Bisio et al. 1999).

The aims of this study were to analyze the composition of essential oil of the aerial parts and trichomes morphology and densities of *N. heliotropifolia*, growing at two various habitats, in a quest to investigate the effects of ecological factors on trichomes and also their essential oil compositions. We observed that phytol was the major component in the essential oil of one population in the study that is never reported so far from *N. heliotropifolia*.

MATERIALS AND METHODS

In the study, the kind and frequency of glandular and non-glandular hairs were examined on both leaf sides of two populations of *N. heliotropifolia*. These populations were collected during spring 2016 from two different regions of Iran, i.e. Sefidkhani (Markazi Province) and Qazvin (Qazvin Province) (Table 1), and were identified on the basis of the descriptions provided in valuable references such as; Flora Iranica (Rechinger 1982) and Flora of Iran (Jamzad 2012).

Isolation and analysis of essential oils

At each population site, forty plant samples were selected depending on the size of the population with a minimum distance of 50 m. Then samples were mixed for homogenization, and used for isolation of essential oil. The essential oil was extracted from each population by hydrodistillation in the Clevenger apparatus during 2.5 h (European Pharmacopoeia 2008). Then it was dried with a salt (hydroussodium sulfate) and freezed until analysis. The identification of the essential oil compositions were relying on a GC-2010 Plus instrument equipped with a GC-QP 2010 Plus (Shimadzu) series mass selective detector in the electron impact ionization mode at 70 eV. Constitutes separation was done on fused silica column (30 m × 0.25 mm ID) with film thickness0.25mm. Helium was carrier gas and its flow rate was 1.6 mL/min. The injector and detector temperatures was 250◦C. Initially, the temperature of GC oven was 50◦C (isothermal for 7 min), then maximized to 250◦C at the rate 4◦C/min to (isothermal for 5 min) and further amplified at the rate 30◦C/min to 300◦C, the final temperature kept for 2 min. The identification of studied components was based on the retention indices (RIs) comparison (Adams, 2007), library of computer mass spectra and analytical standards of compounds. The quantitative analysis of the main components were carried out using gas chromatograph with a flame ionization detector on the silica capillary column TR-5MS (30 m × 0.25 mm ID × 0.25 _m film thickness) (Thermo Electron Corporation, USA) under the same chromatographic conditions. The percentage quantities of the studied constituents were recounted pursuant to the FID chromatographic peaks areas assuming that all the essential oil compounds comprise 100%.

Trichomes investigations

From each population, five flowering stems and of each stem one mature leaf were selected, randomly. The samples of embedded leaves were used for microscopic studies. For this, a fresh adult leaf of each plant was fixed in an F.A.A. solution (formalin 7.5%: acetic acid 7.5% and 85% ethanol) for 48h. Transverse hand sections of the leaf lamina were made from the mid-part of a fully-grown leaf, then plant sections were immersed in two consecutive treatments of 5% KOH (18 h per treatment), rinsed in deionized water, treated for 1.5 minutes in glacial acetic acid, rinsed in deionized water, cleared in bleach solution (6% sodium hypochlorite) and rinsed in deionized water again. Following clearing, a series of ethanol were used for leaf dehydration. After these stages, sections stained in 1% carmine (in 99% water) for 40 min. and in 0.01% methylene blue for 10s, and mounted into microscope slides. Then, suitable thin slices were placed on the glass lamina, observed and photographed with Olympus (CH_2) light microscope. The used terminology in respect of trichomes kinds followed Esau (1965).

The total number of each observed trichomes on the surfaces of leaves was counted. The leaves areas were measured from digital images and were analyzed by Image Tool ver. 2.0. The density of hairs was calculated by dividing the trichome number by the area of leaf (Gonzales et al. 2008).

RESULTS AND DISCUSSION

Essential oil investigation

Essential oil content differed between 0.09-0.2% being minimum 0.09% in Qazvin population and maximum 0.2% in Sefidkhani population. Present investigation showed that, caryophyllene oxide and phytol were the major constituents of the essential oil in the samples of *N. heliotropifolia*. The results were listed in table 2, more that >97% of the total content of essential oils was determined by use of GC-MS analysis. This table presents the retention index (RI) of each compound. The compound numbers were nearly equal between the studied populations, 32 and 33 in Sefidkhani and Qazvin populations, respectively (Table 2).

The most abundant chemical category in the essential oil of Qazvin population was sesquiterpene hydrocarbons (53.53%), followed by oxygenated sesquiterpenes (22.93%), oxygenated monoterpenes (15.15%), monoterpene hydrocarbons (2.2%) and other components (2.98%). However, in Sefidkhani population the most abundant chemical category were oxygenated monoterpenes (28.4%), sesquiterpene hydrocarbons (25.19%), oxygenated sesquiterpenes (19.15%), monoterpene hydrocarbons (14.19%) and other constituents (9.14%), respectively.

The main components of Qazvin population essential oil were; phytol (12.79%), α-copaene (11.96%), spathulenol (10.64%), germacrene D (10.48%), β-bourbonene (8.04%) and caryophyllene oxide (4.9%), respectively. While, in Sefidkhani population caryophyllene oxide (14.17%), caryophyllene (12.07%), 1, 8-cineole (11.57%), n-tetracosane (9. 14%)*, 4* a-α,7-β,*7* a-α-nepetalactone (8.84%)*,* β-pinene (6.8%), (Z)-β-farnesene (4.48%) and germacrene D (3.31%) were existed as the prominent constitutes.

Trichomes study

Both surfaces of leaves in the studied populations were covered by trichomes. The indumentum of Sefidkhani population had more trichomes and was denser than Qazvin population. In the other hand, the total trichome number of Sefidkhani populations was 1.78 times larger than other population. Two main types of trichomes were recorded on both leaf surfaces of the studied populations; glandular and non-glandular.

The non-glandular ones were simple and unbranched and were observed as one to five-celled. The number of one-celled hairs was the same between two populations, while the other trichomes number varied between these populations. The highest number of two-celled (42) was found in Qazvin population (Figure 1. A), but the largest value of three (111) (Figure 1. B), four-celled (9) (Figure 1. C) as well as five-celled (2) (Figure 1. D) were found in Sefidkhani population. The most frequent non-glandular hair in both populations was three-celled type.

The glandular hairs were divided into three types; peltate, capitate and sessile. The sessile glandular trichomes had two shapes uni and bi-celled. Both trichomes numbers were stable between the studied populations. While, the number of capitate and also peltate hairs differed between these populations. The peltate trichomes had a single basal cell and a large secretory head composed of four to six cells (Figure 1 E). Highest value of peltate type (2) belonged to Sefidkhani population. This condition held true for capitate type. These hairs had a basal cell, a stalk cell, and a head that was uni/bi-celled (Figure 1 F,G). All of the observed capitate hairs had short stalk and the largest number of it with different shapes was reported from Sefidkhani population. The most frequent glandular hair among these populations were short-stalked capitate. The ANOVA test showed significant variations ($p \leq 0.05$) for non-glandular three and four-celled hairs and also short-stalked capitate trichomes (Table 3).

Discussion

In this work, trichomes morphology along with essential oils composition was compared in two populations of *N. heliotropifolia*. The essential oil amounts varied greatly among these populations. Although, it's content in Sefidkhani population was equal to essential oil amount of previous study in this species (Sajjadi and Khatamsaz 2001), the amount of essential oil in Qazvin population was very low in comparison to the mentioned previous study. These findings were in agreement with trichomes densities. We observed that, the total number of glandular trichomes in Sefidkhani population were more than Qazvin population. The infraspecific variations in trichomes were reported in different species (for instance, Rezakhanlou and Talebi 2010). Mc Caskill and Croteau, (1995) considered the glandular hairs as exclusive localities for production of essential oil. Glandular trichomes are epidermal structures covering the plants aerial organs. The density of these trichomes kinds of the leaves sides has been found to be positively correlated with the essential oil amount of the plants (Bosabalidis 2002).

Table 1. Localities address of the studied populations

Population	Habitat address
Sefidkhani	Markazi Province of Iran, Arak, Sefidkhani mountain, 2200 m
Qazvin	Qazvin Province of Iran, Alamout, 1700 m

Table 2. Essential oil composition of the studied populations of *N. heliotropifolia*

Compound	RT	Qazvin Population	Sefidkhani Population
2*E*-Hexanal	8.23	0.75	0
Sabinene	13.76	0.49	2.28
β-Pinene	14.03	1.22	6.80
1,8-Cineole	16.95	1.89	11.57
n-Nonanal	20.84	0.47	0
α-Copaene	33.52	11.96	0
β-Bourbonene	33.89	8.04	0.43
β-Cubebene	34.03	3.49	0
Caryophyllene	35.47	3.44	12.07
β-Copaene	35.91	0.56	0
Aromadendrene	36.50	0.24	0
(Z)- β-Farnesene	36.68	0.23	4.48
α-Humulene	37.03	0.42	1.45
allo-Aromadendrene	37.21	0.46	0.51
Germacrene D	38.12	10.48	3.31
Bicylcogermacrene	38.72	4.42	1.69
δ-Cadinene	39.57	4.19	0.62
trans-Calamenene	39.82	0.49	0
α-Calacorene	40.66	0.57	0
1-nor-Bourbonanone	41.50	1.10	0
Spathulenol	42.19	10.64	2.87
Caryophyllene oxide	42.35	4.90	14.17
Hexadecane	42.48	0.61	0
β-Copaen-4-alpha-ol	42.64	1.93	0
cis-Isolongifolanone	43.15	0.75	0
Humulene epoxide II	43.44	0.80	0.72
trans-Isolongifolanone	44.07	0.91	0
Germacrone	45.95	3.66	0
Tetradecanoic acid	48.84	1.31	0
Octadecane	49.81	0.34	0
6,10,14-*trimethyl*-2-Pentadecanone	51.40	3.17	0
Farnesyl acetone	53.81	0.47	0
Phytol	60.03	12.79	0.75
α-Pinene	11.69	0	3.52
Camphene	12.59	0	0.88
Myrcene	14.61	0	0.50
Para-Cymene	16.60	0	0.43
Limonene	16.75	0	1.40
trans-Pinocarveol	22.75	0	1.07
Comphor	23.16	0	0.69
Pinocarvone	23.91	0	0.70
Borneol	24.37	0	1.37
Terpinen-4-ol	24.71	0	0.59
cis-Pinocarveol	25.51	0	1.01
Myrtenal	25.58	0	1.20
Isobornyl acetate	29.52	0	0.98
α-Cubebene	33.50	0	1.63
4 a – α,7-β,7 a – α-Nepetalactone	34.79	0	8.84
Viridifloral	42.83	0	1.75
n-tetracosane	68.89	0	9.14

Table 3. Results of ANOVA test in trichomes numbers among the *N. heliotropifolia* populations

Trichome types		Sum of Squares	df	Mean Square	F	Sig.
Simple uni-celled non-glandular	Between Groups	0.667	1	0.667	1.000	0.374
	Within Groups	2.667	8	0.667		
	Total	3.333	9			
Simple bi-celled non-glandular	Between Groups	504.167	1	504.167	4.007	0.116
	Within Groups	503.333	8	125.833		
	Total	1007.500	9			
Simple three-celled non-glandular	Between Groups	5400.000	1	5400.000	148.624	0.000
	Within Groups	145.333	8	36.333		
	Total	5545.333	9			
Simple four-celled non-glandular	Between Groups	66.667	1	66.667	14.286	0.019
	Within Groups	18.667	8	4.667		
	Total	85.333	9			
Peltate	Between Groups	0.667	1	0.667	1.000	0.374
	Within Groups	2.667	8	0.667		
	Total	3.333	9			
Short-stalked capitate	Between Groups	1944.000	1	1944.000	69.429	0.001
	Within Groups	112.000	8	28.000		
	Total	2056.000	9			
Sessile one-celled glandular	Between Groups	0.667	1	.667	.400	0.561
	Within Groups	6.667	8	1.667		
	Total	7.333	9			
Sessile two-celled glandular	Between Groups	0.167	1	0.167	1.000	0.374
	Within Groups	0.667	8	0.167		
	Total	0.833	9			

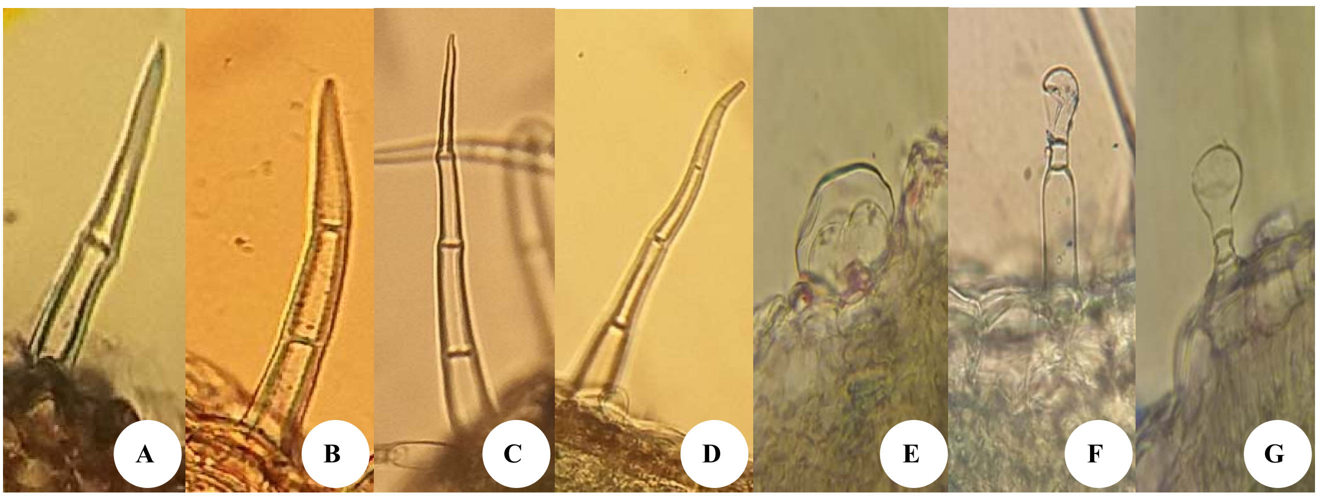

Figure 1. Different types of trichomes in *Nepeta heliotropifolia.* A. two-celled, B. three-celled, C. four-celled, D. five-celled, E. peltate, F, G. short-stalked capitate

Three different kinds of glandular trichomes were seen on the leaf epidermal surfaces, but the main of them were peltate and capitate. These types of trichomes were observed in other species of the Lamiaceae family. For instance, the peltate trichomes are also observed in *Salvia blepharophylla* (Bisio et al. 1999), *Ocimum basilicum*, *Teucrium chamaedrys, Teucrium siculum* (Servetazz et al. 1994), *Sideritis syriaca*, *Lagochilus macracanthus* Fisch. & C.A. Mey (Talebi and Rezakhanlou 2012) and *Pogostemon cablin* (Werker et al. 1993). Moreover, species like *Nepeta* *racemosa* (Bourett et al. 1994), *Acinos graveolens* (Talebi and Shayestehfar 2014), *Ziziphora tenuior* (Talebi et al. 2012), *Mentha piperita* (Askari et al. 2016a) and *Lallemantia royleana* (Askari et al. 2016b) had different types of capitate hairs.

In addition to morphological variations (Fahn 2000), peltate and capitate hairs have different secretion mechanisms. Siebert (2004) stated that the compounds secreted by capitate hairs are mostly excreted to the surrounding environment, apparently through pores in the cuticle of the head cell (s). While, in peltate hairs the secretions accumulate in a capacious subcuticular space shaped 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 phytochemicals that they secrete.

Not only the major components of the essential oil differed between the studied populations, but also many of the minor constitutes of Qazvin population essential oil were absent in Sefidkhani population. However, it is important to know that the types and also amounts of the essential oil main compounds of Sefidkhani population were similar to those were reported by Sajjadi and Khatamsaz (2001). They found that 1, 8-cineole (19.0%), caryophyllene oxide (14.2%), P-caryophyllene (11.3%), spathulenol (8.3%), myrtenol (5.9%), *trans* p-farnesene (5.1%), *trans*-pinocarveol (4.2%) and α-terpineol (4.1%) were the main compounds of essential oil in Hamadan population of this species. Although, one of the major compounds of Sefidkhani population, 4 a-α, 7-β, 7 a-α-Nepetalactone (8.84%) didn't present in Hamadan population. Former studies (e.g. Handjieva et al. 1996; Sharma and Cannoo 2013) showed nepetalactones, as one of the principal constituents of *N. angustifoliates, N. cataria, N. mahanensis* and *N. nepetella*. The common name of these terpenoids is catmints. They have antimicrobial and insect repellent activities, while nepetalactones are also strong feline attractants. The components are reported to accumulate in the subcuticular space of the peltate glandular trichomes (Clark et al. 1997).

Although, the change in peltate trichomes number not so great between these populations, the number of short-stalked capitate and most types of non-glandular hairs widely varied between two populations, there are many possible reasons for it. It seems that nearly a 500m variation in habitat altitude between these populations is the main factor. Former studies confirmed that plants grow at a higher altitude have special morphological and also anatomical traits, which help them to tolerate hard ecological conditions (Kofidis et al. 2003). The change in indumentum density was one of them that were clearly observed between the studied populations. These conditions were explained for other *Nepeta* species. For example, significant differences in density of trichomes were found between populations grow at different altitudes (Kofidis et al. 2007). Karabourniotis et al. (1998) stated that plants at higher altitudes have to be more protected from irradiance of excessive UV-B, and the flavonoid-containing trichomes help it. Apart from the protection of UV-B, the dense leaves covered with non-glandular trichomes at higher altitudes may further contribute to protection from low temperatures (Kofidis and Bosabalidis 2008). Therefore, changes in trichomes number influence the manufacture as well as the secretion of essential oil.

It seems that infraspecific variations in the essential oil compositions have adaptive value for plants. Ding et al. (2010) stated the compositions of essential oil are also involved in a variety of ecological interactions, such as allelopathy. It is a mechanism of biochemical interaction between plant-plant, plant-microbe, plant-insect or plant-herbivore (Alipour and Saharkhiz 2016). Allelopathic products can be volatile and release from the different organs such as leaves, stem as well as roots. Scrivanti et al. (2003) demonstrated allelopathy is generally accepted as a prominent environmental parameter in determining the structure and also composition of plant communities. Studies (e.g. Talebi et al. 2014) confirmed that the populations of a species that grow in the different habitats face with different ecological conditions. Therefore, they interact with various plant taxa and for their survives and better growth must compete with a wide range of accompanied plants. For this reason, like phenotype plasticity that occurred for plant fitness with the niche ecological condition, chemical composition of essential oil of each population co-evaluated.

Franzios et al. (1997) believed that the essential oil biological effects are often related to the presence of some main compounds. While, the activity of the prominent constituents of the essential oil can be modulated by the minor molecules. Therefore, each population had its unique essential oil compositions for better establishment in its habitat and makes the molecules that needs for its allelopathic interactions. For example, terpinen-4-ol completely inhibited the germination of seeds and also the growth of seedlings in different species, like *Amaranthus retroflexus*, *Rumex crispus* and *Chenopodium album* (Kordali et al. 2007). Moreover, thymol, carvacrol and carvone showed high inhibition even at low concentrations against weed seeds such as *Amaranthus retroflexus*, *Centaurea solstitialis*, *Raphanus raphanistrum*, *Rumex nepalensis*, *Sinapis arvensis* and *Sonchus oleraceus* (Azirak and Karaman 2008).

Wang et al. (2010) found that β-caryophyllene inhibited the germination of seed, growth of the root as well as shoot in various taxa such as *Raphanus sativus*, *Mikania micrantha*, *Brassica campestris*, *Lactuca sativa*. Moreover, δ-cadinene had phytotoxic activity on *Isolepis nodosa* (Ens et al. 2009).

Since, the essential oil major compound were different between the studied populations, the medicinal properties of these population are different. Phytol is an important unsaturated terpene and is a product of chlorophyll metabolism. The compound has high antimicrobial activity, high stability, and also low toxicity (Ghaneian et al. 2015). Alpha-copaene is a tricyclic sesquiterpene. Former investigations indicated it has anticarcinogenic, antioxidant, hepatoprotective and antiinflammatory activities (Turkez et al. 2014).

While, β-caryophyllene and β-caryophyllene oxide are Natural bicyclic sesquiterpenes and both of them have significant anticancer activities, affecting growth and proliferation of many cancer cells (Fidyt et al. 2016).

In total, the obtained results proved that the morphology and densities of trichomes with essential oil compositions differ between populations of the same species. In some cases, these variations are not so great, while sometimes high infraspecific variations in trichomes as well as essential oil amount and compositions are observed between populations and lead to the creation of chemotypes as seen in Qazvin population.

REFERENCES

Adams RP. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, fourth ed. Allured Publishing Corp., Carol Stream, IL.

Alipour M, Saharkhiz MJ. 2016. Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phonological growth stages. Biocatal Agric Biotechnol 7: 271-278.

Askary M, Talebi SM, Amini F, Dousti Balout Bangan A.2016a. Effect of NaCl and iron oxide nanoparticles on *Mentha piperita* essential oil composition. Environmental and Experimental Biology 14: 27-32.

Askary M, Talebi SM, Amini F, Sarabadani B. 2016b. Trichomes plasticity in *Lallemantia royleana* under different stress. Nusantara Bioscie 8: 128-133.

Azirak S, Karaman S. 2008. Allelopathic effect of some essential oils and components on germination of weed species, Acta Agr Scand B S P 58 (1): 88-92.

Bisio A, Corallo A, Gastaldo P, Romussi G, Ciarallo G, Fontana N, De Tommas N, Profumo P. 1999. Glandular hairs and secreted material in *Salvia blepharophylla* Brandegee ex Epling grown in Italy. Ann Bot 83: 441-452.

Bosabalidis AM. 2002. Structural features of *Origanum* sp. In: Kintzios SE (ed.). Oregano. The Genera *Origanum* and *Lippia*. Taylor and Francis, London.

Bourett TM, Howard RJ, O' Keefe DP, Hallahan DL. 1994. Gland development on leaf surfaces of *Nepeta racemosa*. Inter J Plant Sci 155: 623-632.

Clark LJ, Hamilton JGC, Chapman JV, Rhodes MJC, Hallahan DL. 1997. Analysis of monoterpenoids in glandular trichomes of the catmint *Nepeta racemosa*. Plant J 11: 1387-1393.

Delfine S, Loreto F, Alvino A. 2001. Drought-stress effects on physiology, growth and biomass production of rainfed and irrigated bell pepper plants in the Mediterranean region. J Am Soc Hortic Sci 126:297-304.

Ding L, Jing H, Qin B, Qi L, Li J, Wang T, Liu G. 2010. Regulation of cell division and growth in roots of *Lactuca sativa* L. seedlings by the entkaurene diterpenoid rabdosin B. J Chem Ecol 36: 553-563.

Ens EJ, Bremner JB, French K, Korth J. 2009. Identification of volatile compounds released by roots of an invasive plant, bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*), and their inhibition of native seedling growth. Biol Invasions 11: 275-287.

Esau K.1965.Plant Anatomy (4^{th} ed.). Pergamon Press, New York.

European Pharmacopoeia 2008. 6th edition, vol. 1 Directorate for the Quality ofMedicines and HealthCare of the Council of Europe (EDQM), Strasbourg, France.

Fahn A. 2000. Structure and function of secretory cells. In: Hallahon DL, Gray JC (Eds.). Advances in Botanical Research. Incorporating Advances in Plant Pathology. Plant Trichomes, vol. 31. Academic Press, London.

Fidyt K, Fiedorowicz A, Strządała L, Szumny A.2016. *β*-caryophyllene and *β*-caryophyllene oxide—natural compounds of anticancer and analgesic properties. Cancer Med 5 (10): 3007-3017.

Formisano C, Delfine S, Oliviero F, Tenore GC, Rigano D, Senatore F. 2015.Correlation among environmental factors, chemical composition andantioxidative properties of essential oil and extracts of chamomile (*Matricaria chamomilla* L.) collected in Molise (South-central Italy). Ind Crop Prod 63:256-263.

Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P. 1997. Insecticidal and genotoxic activities of mint essential oils. J Agric Food Chem 45: 2690-2694.

Ghaneian MT, Ehrampoush MH, Jebali A, Hekmati Moghaddam SH, Mahmoudi M.2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2 (1): 13-16.

Gonzales WL, Negrittoa MA, Suarez LH, Gianoli E.2008. Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes Acta Oecol 33:128-132.

Handjieva NV, Popov SS, Evstatieva LN. 1996. Constituents of essential oils from *Nepeta cataria* L., *N. grandiflora* M.B. and *N. nuda* L. J Essent Oil Res 8:639-643.

Jamzad Z. 2012. Flora of Iran, no. 76, Lamiaceae. Research Institute of Forest and Rangelands, Tehran, Iran.

Karabourniotis G, Kofidis G, Fasseas C, Liakoura V, Drossopoulos I. 1998. Polyphenol deposition in leaf hairs of *Olea europaea* (Oleaceae) and *Quercus ilex* (Fagaceae).Am J Bot 85: 1007-1012.

Kaya A, Demirci B, Baser KHC. 2003. Glandular trichomes and essential oils of *Salvia glutinosa* L. S Afr J Bot 69: 422-427.

Kofidis G, Bosabalidis A M. 2008. Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda* L. Bot Stud 49: 363-372.

Kofidis G, Bosabalidis A M, Moustakas M .2003. Contemporary seasonal and altitudinal variations of leaf structural features in oregano (*Origanum vulgare* L.). Ann Bot 92:635-645.

Kofidis G, Bosabalidis AM, Moustakas M. 2007. Combined effect s of altitude and season on leaf characteristics of *Clinopodium vulgare* L. (Labiatae). Environ Exp Bot 60: 69-76.

Kordali S, Cakir A, Sutay S. 2007. Inhibitory effects of monoterpenes on seed germination and seedling growth.Z Naturforsch C 62: 207-214.

Lima MEL, Cordeiro I, Young MCM, Sobra MEG, Moreno PRH. 2006. Antimicrobial activity of the essential oil from two specimens of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae) native from São Paulo State-Brazil. Pharmacologyonline *3*: 589-593.

Manukyan AE, Schnitzler WH. 2006. Influence of air temperature on productivity and quality of some medicinal plants under controlled environment conditions. Eur J Hortic Sci 71:36-44.

Mc Caskill D, Croteau R. 1995. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha* x *piperita*) rely exclusively on plastid derived 0isopentenyl diphosphate. Planta 197: 49-56.

Paula JAM, Ferri PH, Bara MTF, Tresvenzol LMF, Sá FAS, Paula JR. 2011. Infraspecific chemical variability in the essential oils of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae). Biochem Syst Ecol 39: 643-650.

Peltonen-Sainio P, Jauhiainen L, Trnka M, Olesen JE, Calanca P, Eckersten H, Eitzinger J, Gobin A, Kersebaum KC, Kozyra J, Kumar S, Marta AD, Micale F, Schaap B, Seguin B, Skjelvåg AO, Orlandini S. 2010. Coincidence ofvariation in yield and climate in Europe. Agric Ecosyst Environ139: 483-489.

Rechinger KH.1982. Labiatae in Flora Iranica. Akademische Druckund Verlagsanstalt, Graz, Austria.

Rezakhanlou A, Talebi SM.2010. Trichomes morphology of *Stachys lavandulifolia* Vahl. (Labiatae) of Iran. Procedia Soc Behav Sci 2: 3755-3763.

Russo A, Formisano C, Rigano D, Senatore F, Delfine S, Cardile V, Rosselli S, Bruno M. 2013. Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. Food Chem Toxicol 55: 42-47.

Sajjadi SE, Khatamsaz M. 2001.Volatile Constituents of *Nepeta heliotropifolia* Lam. J Essent Oil Res 13 (3): 204-205.

Scrivanti LR, Zunino MP, Zygadlo JA. 2003. *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochem Sys Ecol 31: 563-572.

Sharma A, Cannoo DS. 2013. Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Labiatae family: A review. Pharmacophore 4 (6): 181-211.

Siebert DJ. 2004. Localization of Salvinorin A and related compounds in glandular trichomes of the psychoactive sage, *Salvia divinorum*. Ann Bot 93: 763-771.

Takashima NE, Rondanini DP, Puhl LE, Miralles DJ. 2013. Environmental factors affecting yield variability in spring and winter rapeseed genotype scultivated in the southeastern Argentine Pampas. Eur J Agron 48:88-100.

Talebi SM, Rezakhanlou A. 2012. Biological study of *Lagochilus maracanthus* Fish. et Mey endemic species of Iran . J Chem Pharm Res 4 (1):633-639.

Talebi SM, Rezakhanlou A, Salahi Isfahani G.2012.Trichomes Plasticity in *Ziziphora tenuior* L. (Labiatae) in Iran: An ecological review. Ann Biol Res 3 (1):668-672.

Talebi SM, Atri M, Sheidai M, Sharifnia F, Noormohammadi Z.2014. Infraspecific variations in *Linum album* based on the Determination of Special Stations approach in Iran. Phytol Balc 20 (1):9-22.

Talebi SM, Shayestehfar AR.2014. Infraspecific trichomes variations in *Acinos graveolens* (M.B.) Link. Annals Biol Sci 2 (2):51-57.

Turkez H, Togar B, Tatar A, Geyıkoglu F, Hacımuftuoglu A.2014. Cytotoxic and cytogenetic effects of *α*-copaene on rat neuron and N2a neuroblastoma cell lines. Biologia 69 (7): 936-942.

Wang R, Staehelin C, Peng S, Wang W, Xie X, Lu H. 2010. Responses of Mikania micrantha, an invasive weed to elevated CO2: induction of β-caryophyllene synthase, changes in emission capability and allelopathic potential of β-caryophyllene. J. Chem. Ecol. 36, 1076-1082.

Werker E. 1993. Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae. Flavour Fragr J. 8: 249-255.

Werker E, Putievsky E, Ravid U. 1993. The essential oils and glandular hairs in different chemotypes of *Origanum vulgare* L. Ann Bot 55: 793-801.