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Effects of stress on foliar trichomes plasticity in *Mentha piperita*

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Abstract. *Askary M, Talebi SM, Amini F, Bangan ADB. 2016. Effects of stress on foliar trichomes plasticity in* Mentha piperita*. Nusantara Bioscience 8: 32-38. Mentha piperita* is considered as the most economically important aromatic as well as medicinal herb of Labiatae family. This medicinal herb has traditionally been used to treat a variety of digestive complaints. In the present study, effects of twenty treatments of salt and nanoparticles of Iron oxide were examined on the leaf trichomes morphology of *M. piperita* samples. In total twenty solutions, combinations of four salt concentrations with five iron oxide nanoparticles concentrations were used for the treatments. Different kinds of glandular and non-glandular trichomes were found in the studied plant samples. Glandular trichomes were seen in the shapes of peltate, short-stalked capitate and non-stalked and non-glandular ones were unbranched and had one to seven cells. The obtained results showed that the kind and abundant of trichomes differed between the studied treatments. Statistical analyses showed significant positive correlations between salt concentrations with trichomes density. While, negative significant correlations have occurred between concentrations of nanoparticle of iron oxide with some kinds of trichomes.

Keywords: Iron nanoparticles, *Mentha piperita*, trichome morphology, salt, stress

INTRODUCTION

The family Labiatae has many aromatic taxa used as culinary herbs, folk medicines, fragrances, etc**.** Many species of it possess essential oils secreted by glandular trichomes (Richardson 1992). Essential oils consist of a large number of secondary compounds that are utilized as pharmaceuticals, nutriceuticals, natural pesticides, flavorings, fragrances and for other non-food or fiber purposes. Duke (1994) suggested that the mentioned compounds have different biological activities, presumably as a result of the co-evolution of the producing plants with other organisms such as pathogens, herbivores, pollinators.

Mentha piperita is considered as the most economically important aromatic as well as medicinal herb of Labiatae family. Various names have been applied to this species such as Peppermint, Lamb mint, Brandy mint, Balm mint, Candy mint, Vilayati pudina or Paparaminta (Shah and Mello 2004). *M. piperita* is thought to be natural hybrid between *M. spicata* L. and *M. aquatica* L., then itself being a hybrid of *M. longifolia* (L.) Huds. and *M. rotundifolia* (L.) Huds., therefore this species is a triple hybrid (Fleming 1998).

This medicinal herb has traditionally been used to treat a variety of digestive complaints such as infant's colic, indigestion, diarrhea, flatulence, nausea and vomiting, morning sickness and anorexia, and as a spasmolytic to reduce gas and cramping (Tyler 1992; Robbers and Tyler 1999).

Rodriguez et al. (1984) believed that trichomes may be considered as simple hairs which deter herbivores, guide the path of pollinators or affect photosynthesis, temperature of leaf, or water loss through increased light reflectance as seen in many desert species. In addition, trichomes may be more specialized tissues such as glandular secreting trichomes whose main function (s) may be to make pest-or pollinator-interactive chemical components which are stock or volatilized at the surface of plant (Dell and McComb 1978).

Jamil et al. (2006) stated that soil or water salinity is one of major stress obstacles to increase production in plant growing areas all over the world and also certainly in arid as well as semi-arid regions. This factor can intensely bind production of plant.

Although different studies (e.g. McCaskill et al. 1992; Voirin and Bayet 1996; Rohloff 1999) have been done about essential oil contents of trichomes in *M. piperita,* but as far as we could search, no comprehensive study was found on the effect of different salinity treatments on trichomes density and morphology in this species. For this reason, in the present study effects of twenty treatments of salt (NaCl) and nanoparticles of Iron oxide were investigated on the foliar trichomes morphology of *M. piperita* specimens.

MATERIALS AND METHODS

The aerial parts of the same samples of *M. piperita*, which had previously been described by Jamzad (2012), were used for this study. The study was set up in a growth chamber maintained at an air temperature of 20-25°C (in night/ day respectively), daily light period was 14-h and 10-h dark period throughout the duration of the experiment. 14 × 12 cm flowerpots were used. At this stage, treatments of plants with salt (sodium chloride: NaCl, MW 58.44 g) and iron solutions (as nanoparticles of iron oxide and iron chalet) commenced. Combinations of four salt concentrations (0, 50, 100, and 150 mM) with five iron concentrations (0, 10, 20 and 30µm of Fe_2O_3 nanoparticles and normal Fe-EDTA), in total twenty solutions, were used for treatments (Table 1). Irrigations were done weekly with 100ml of complete Hoagland solution containing Iron-chelate as Fe-EDTA or without iron-chelate and containing different concentrations of Fe_2O_3 nanoparticles and sodium chloride. Plants under 0 µm (as control treatment) are not received iron in 90-day period. Different concentrations of Fe_2O_3 nanoparticles were prepared by Prasad et al. (2012) method.

At ninety days after the commencement of the salinity treatments, mature leaves were collected for trichomes investigation on both abaxial and adaxial sides using scanning electron microscopy (SEM) and light microscopy (LM). For Light microscopy, the embedded materials were used. Transverse hand sections of the lamina were made from the mid-part of fully-grown leaves. Embedded materials were prepared as follows: adult leaves samples were fixed with F.A.A. solution (formalin 5%: acetic acid 5% and 90% ethanol) for 48hours, then dehydrated in a graded ethanol series and embedded in ethanol 90%. For scanning electron microscopy (SEM), the samples were transferred directly to double-sided tape affixed stubs and vacuum-coated with gold in the Biorad E5200 auto sputter coater and photographed with the Camscan MV2300 scanning electron microscope at 10kV. Trichomes were counted directly on both sides of each leaf from four plants, by means of a light microscope at a magnification of 20x (Olympus CH_2, Japan). Numbers of hair per leaf sides were determined. Density of hairs was presented as the ratio between mean trichome number per leaf pair and the corresponding leaf area.

RESULTS AND DISCUSSION

Results

In this study effects of twenty salt and iron oxide nanoparticles, stresses were examined on the leaf trichomes plasticity of *M. piperita* samples. Two main types of trichomes, glandular and non-glandular, were found in the studied plant samples. Glandular trichomes were seen in the shapes of peltate, short-stalked capitate and non-stalked. Non-glandular hairs were unbranched and had one to seven cells (Table 2).

The kind and abundant of trichomes differed between the studied treatments. Peltate trichomes were short and had a unicellular stalk with a large secretory head consist of 6 to 12 cells arranged in one or two concentric circles. Numbers of peltate hairs differed between the studied treatments, so maximum and minimum numbers of them were seen in treatments no.16 and 4 respectively. This condition holds true for short-stalked trichomes. This type of trichome had a basal cell with one-celled stalk and head. Non-stalked glandular hairs were found in two types one and two-celled (Figure 1.A). The highest numbers of one and two-celled glandular hairs were occurred in treatment no.16, while smallest numbers of one and two-celled were recorded in treatments no. 3 and 4 respectively. The most abundant glandular trichomes in all of studied treatments were short-stalked hairs with the exception of treatment no.2, which non-stalked glandular trichomes were most abundant.

Non-glandular hairs were divided on the bases of their length and cell no. Therefore ten types of these hairs were recorded; short/ long unicelled, short/ long bicelled, short/long tree-celled, four, five, six and seven-celled trichomes. Highest value of unicelled non-glandular hair was registered in treatment no. 16, while its lowest value was found in treatment no. 3. Among non-glandular trichomes, the one-celled type had highest frequency and was the prominent hair within and among treatments with the exception of treatment no. 3, which bicelled trichomes had highest values (Figure1.B). Simple six and seven-celled trichomes had lowest amplitude and were found in treatments no.16, 17, 18 and 20 (Figures 2, 3, 4).

Paired samples test showed significant variations ($p \leq 0.05$) between all types of trichomes with salt and iron oxide nanoparticles concentrations, with the exception of short-stalked capitate, three-celled simple and sessile two-celled glandular hairs (Table 3).

Results showed that increasing the amount of salt, maximize the trichomes number. Statistical analyses by SPSS software (version 16) showed significant positive correlations ($p<0.01$) between salt concentrations with trichomes density. It means that increment of environmental salinity maximized all kinds of trichomes on the leaf surfaces. While, negative significant correlations ($p<0.05$) have occurred between concentrations of nanoparticle of iron oxide with some kinds of trichomes such as one, four, five and six-celled non-glandular hairs, peltate, one-celled and short-stalked glandular trichomes.

Table 1. Concentrations of NaCl and nanoparticles of iron in the studied treatments

Treatment	NaCl	Iron	Treatment	NaCl	Iron
1 (control)	0	0	11	100 mM	0
2	0	10 µm Fe-nano	12	100 mM	10 µm Fe-nano
3	0	20 µm Fe-nano	13	100 mM	20 µm Fe-nano
4	0	30 µm Fe-nano	14	100 mM	30 µm Fe-nano
5	0	Fe-chelat	15	100 mM	Fe-chelat
6	50 mM	0	16	150 mM	0
7	50 mM	10 µm Fe-nano	17	150 mM	10 µm Fe-nano
8	50 mM	20 µm Fe-nano	18	150 mM	20 µm Fe-nano
9	50 mM	30 µm Fe-nano	19	150 mM	30 µm Fe-nano
10	50 mM	Fe-chelat	20	150 mM	Fe-chelat

Table 2. Mean numbers of hair per each leaf in the studied samples

Treatment	Glandular trichomes				Non-glandular trichomes						
	Peltate	Short-stalked capitate	Sessile one-celled	Sessile two-celled	One-celled	Two-celled	Tree-celled	Four-celled	Five-celled	Six-celled	Seven-celled
1	3	8	4	3	2	2	1	2	0	0	0
2	4	5	3	2	2	1	2	1	0	0	0
3	2	7	3	2	1	1	2	0	0	0	0
4	2	4	3	2	1	1	1	0	0	0	0
5	4	9	5	3	2	2	2	0	0	0	0
6	5	14	7	5	3	2	2	3	2	0	0
7	4	11	7	4	2	2	1	0	0	0	0
8	4	10	6	4	2	1	2	1	2	0	0
9	4	10	5	3	2	2	2	1	1	0	0
10	5	12	7	4	2	2	2	2	0	0	0
11	7	19	12	6	4	3	3	5	2	0	0
12	6	17	10	5	4	3	2	2	3	0	0
13	6	16	9	5	4	4	3	5	4	0	0
14	6	14	8	5	3	3	3	4	2	0	0
15	6	22	11	6	3	1	4	5	2	0	0
16	11	30	17	8	6	4	8	7	8	8	9
17	9	25	14	7	5	3	5	8	4	6	2
18	9	23	13	6	5	3	4	4	3	1	2
19	8	20	12	6	4	3	3	2	3	0	0
20	10	26	15	7	6	4	6	11	7	6	3

Table 3. Paired samples test analyses of iron and salt concentrations with trichomes densities

	Treatments	Paired differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence interval of the difference				
					Lower	Upper			
	Iron nanoparticles treatments								
Pair 1	Peltate-iron nanoparticles	9.765	18.606	4.513	.198	19.331	2.164	16	0.046
Pair 2	Short-stalked capitate-iron nanoparticles	47.647	37.212	9.025	28.514	66.780	5.279	16	0.000
Pair 3	One-celled glandular-iron nanoparticles	20.824	24.063	5.836	8.451	33.196	3.568	16	0.003
Pair 4	Two-celled glandular-iron nanoparticles	4.294	15.579	3.779	-3.716	12.304	1.136	16	0.273
Pair 5	two-celled simple-iron nanoparticles	25.118	23.073	5.596	13.255	36.981	4.488	16	0.000
Pair 6	One-celled simple-iron nanoparticles	36.471	29.829	7.235	21.134	51.807	5.041	16	0.000
Pair 7	Three-celled simple-iron nanoparticles	-2.176	15.828	3.839	-10.315	5.962	-.567	16	0.579
Pair 8	Four-celled simple-iron nanoparticles	-10.824	13.483	3.270	-17.756	-3.891	-3.310	16	0.004
Pair 9	Five-celled simple-iron nanoparticles	-11.706	12.746	3.091	-18.259	-5.152	-3.787	16	0.002
Pair10	Six-celled simple-iron nanoparticles	-12.882	13.124	3.183	-19.630	-6.135	-4.047	16	0.001
Pair 11	Seven-celled simple-iron nanoparticles	-13.176	12.812	3.107	-19.764	-6.589	-4.240	16	0.001
	Salt treatments								
Pair 1	Peltate-salt	-51.650	47.715	10.670	-73.982	-29.318	-4.841	19	0.000
Pair 2	Short-stalked capitate-salt	-13.650	30.991	6.930	-28.154	.854	-1.970	19	0.064
Pair 3	One-celled glandular-salt	-40.750	41.692	9.323	-60.262	-21.238	-4.371	19	0.000
Pair 4	Two-celled glandular-salt	-56.850	50.761	11.350	-80.607	-33.093	-5.009	19	0.000
Pair 5	One-celled simple-salt	-25.400	36.820	8.233	-42.632	-8.168	-3.085	19	0.006
Pair 6	Two-celled simple-salt	-37.400	45.275	10.124	-58.589	-16.211	-3.694	19	0.002
Pair 7	Three-celled simple-salt	-63.300	51.913	11.608	-87.596	-39.004	-5.453	19	0.000
Pair 8	Four-celled simple-salt	-71.850	55.070	12.314	-97.623	-46.077	-5.835	19	0.000
Pair 9	Five-celled simple-salt	-72.850	55.465	12.402	-98.808	-46.892	-5.874	19	0.000
Pair 10	Six-celled simple-salt	-73.950	55.944	12.509	-100.133	-47.767	-5.912	19	0.000
Pair 11	Seven-celled simple-salt	-74.200	56.282	12.585	-100.541	-47.859	-5.896	19	0.000

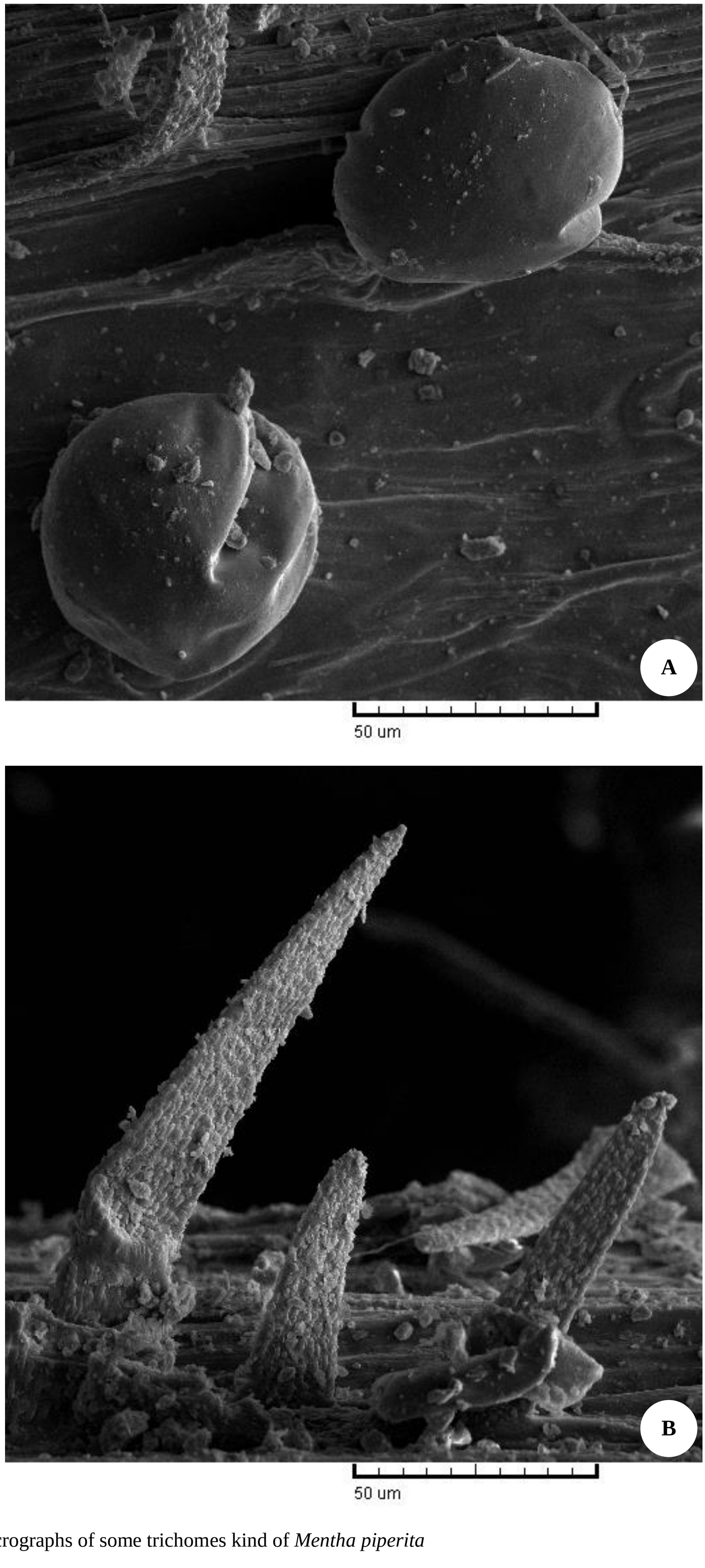

Figure 1. Electronic micrographs of some trichomes kind of *Mentha piperita*

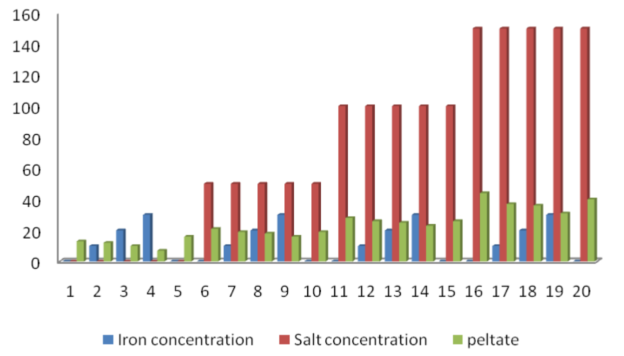

Figure 2. Number of peltate trichomes of *Mentha piperita* in different concentrations

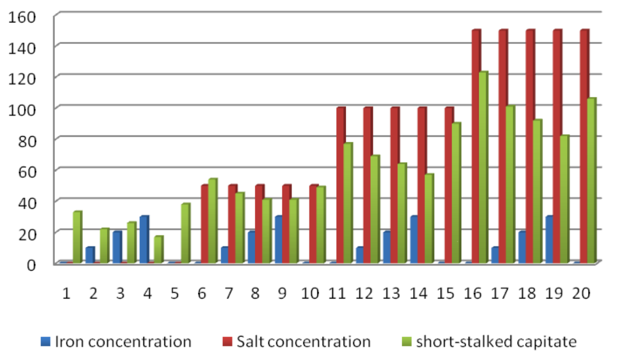

Figure 3. Effect of different treatments on the number of short-stalked trichomes of *Mentha piperita*

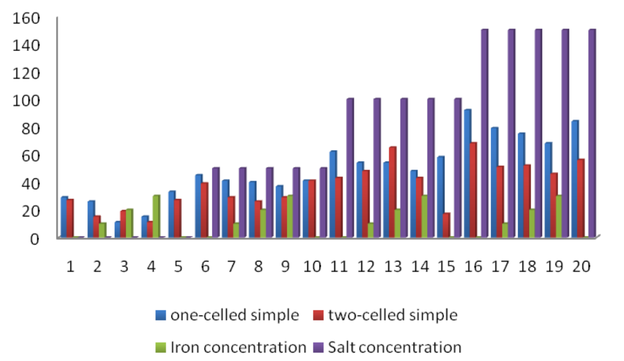

Figure 4. Variation of one and two-celled trichomes number of *Mentha piperita* in the studied treatments

Discussion

In the present study, the effects of different concentrations of NaCl and nanoparticles of iron oxide were examined on the trichomes morphology and density in *M. piperita*. The obtained results showed that different types of glandular and non-glandular trichomes were present on the both leaf surfaces of this species. All of observed kinds of simple hairs were unbranched, while cell number and length differed between them. So that two trichomes with the same cell number may have different length, these conditions were seen in one, two and tree-celled trichomes. The non-glandular trichomes vary in their morphology, anatomy as well as microstructure. These trichomes are classified according to their morphology in two main groups: unicellular and multicellular. Each type can be unbranched and branched (Bosabalidis and Skoula 1998). In addition, different types of glandular trichomes were found in the studied samples, but two main kinds of them, capitate and peltate, were very prominent.

As Fahn (2000) said: in the Labiatae family, the glandular trichomes on the bases of morphological features are generally classified as either capitate (clavate) or peltate (subsessile). Capitate hairs were seen in two different shapes: short and long-stalked, while in the studied samples only short-stalked hairs were present and the length and shape of stalk, as well as head cells of them, were similar among treatments. It was a prominent type of glandular trichomes in almost all of the studied plant samples. This kind of glandular trichomes varied between treated plants, while this difference was not statistically significant. In addition, peltate trichomes were alike between treatments.

Attentive to all of treated plant samples were similar and were propagated from one plant sample by vegetative reproductions, therefore the observed variations in trichomes density, as well as morphology, were related to applied treatments. Therefore, our study confirmed that the mentioned treatments had influences on trichomes morphology and density. The total number of glands produced and their oil content depended on the physiological state of the plant (Maffei et al. 1986). In fact, each of examined treatment is a kind of stress which influenced various physiological and also morphological features of plant. Boughton et al. (2005) studies have shown that stress, exterior circumstances that are suboptimal for plant growth, can affect the of glandular hair`s organization on the leaves epidermal surface. As significant positive/negative correlations have occurred between different treatments with trichomes density. Increase of salt concentration extended all kinds of trichomes in plant samples. With increment of salt concentrations, not only number of glandular and simple hairs accreted, but also type of non-glandular trichomes increased and two new kinds, six and seven-celled hair, were appeared in the treated plants with high concentrations of NaCl. These conditions lead to increment of trichome compression in the aerial parts and appearance of plant became hairy.

Different studies on various plant species confirmed our findings. For example, Choi et al. (2004) suggested that when *Nicotiana tabacum* L. was exposed to 0.05 M NaCl, development of hairs were irregular. Furthermore, the findings of Gonzales et al. (2008), investigations on *Madia sativa* Molina (Asteraceae), confirmed that stress induced by drought and deliberate plant damage resulted in overall increase in compression of trichome, and the trichomes kind induced after implantation of stress changed from that of the control plants. Indeed, Perez-Estrada et al. (2000)

showed that deficit of water is closely dependent on an expansion in pubescence of leaf in some plant taxa. Furthermore, other components, which induce stress, create similar results. For example, Boughton et al. (2005) expressed that *Solanum lycopersicum* (Solanaceae) treated with methyl jasmonate developed nine times higher glandular trichomes densities in comparison with control plants. Similar, application of jasmonic acid, gibberellic acid and also benzylaminopurine to *Arabidopsis thaliana* (Brassicaceae) lead to overhead to four times higher glandular hairs aggregations (Maes et al. 2008). In this species, wounding and jasmonic acid considerably amplificated the glandular trichomes numbers, while salicylic acid reduced them (Traw and Bergelson 2003). In *Mimulus guttatus* (Scrophulariaceae), leaves damage on that appeared early in the season enhanced creation of glandular hairs on leaves that developed later. It was also shown that this trait was maternally transmitted to progeny (Holeski 2007). These conditions also were seen in plant natural habitat and different ecological factors act as stress and influence different plant characteristics such as trichomes (Rezakhanlou and Talebi 2010; Talebi et al. 2012; Talebi and Shayestehfar 2014). It appears that bring of trichomes is a way to comparison with environmental suboptimal conditions. For example, investigations show that in some desert taxa the main role of glandular secreting hair is to produce such high levels of exudates that it makes a continuous layer on the surface of plant. It may increase the reflectance of light and thereby decrease temperature of leaf (Dell and McComb 1978).

While, iron oxide nanoparticles had negative effect on trichomes density. It seems that these components had strong effects on the plant structures. Two mechanisms were assumed for reducing trichomes density: (i) perhaps Fe nanoparticles had negative effects on formation of trichomes or (ii) nanoparticles of iron can eliminate the effect of salt stress. The results of Davar Zareii et al. (2014) studies showed that the adverse effects of drought stress can be reduced by application of Fe nanoparticles. Furthermore, the obtained results of different studies (e.g. Eichert et al. 2008; Basiuk et al. 2011), showed that foliar uptake of metal nanoparticles is linked with variations in leaf morphology as well as anatomy, particularly of trichomes, stomata, and hypodermis.

Each kind of trichome, glandular or simple, has a significant role in plant. It seems that glandular ones mainly interfering in secretion of essential oils as well as nectars, therefore are very important in attraction of pollinator and any change in these structures may have a strong affect on plant pollinations. In contrast, non-glandular hairs have key roles in physical protection of plant's aerial parts against biotic and abiotic agents and induced variations in this kind of hairs is very important in plant survival.

Whereas, the mentioned stress effect glandular trichomes density in the studied samples, we can conclude that the amount of essential oils will vary between treatments. Because, glandular trichomes especially peltate trichomes are larger in size and are responsible for most of the peppermint essential oil production (Maffei et al. 1989).

Our findings showed that in the studied samples the most dominate glandular trichomes were short-stalked hairs. So these plants not recommended for the extraction of essential oil, because this kind of trichomes cannot hold its essential oil content and secreted it out.

Siebert (2004) stated that the secreted compounds of capitate glandular trichomes are mostly excreted to the surrounding environment, apparently through pores in the cuticle of the head cell (s). Wile, in peltate glandular hairs these materials accumulate in a space of capacious subcuticular made 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 glandular trichomes operated as repositories for the specialized phytochemical components that they excrete.

In this species, the outer surface of epidermal cells, like other members of Labiatae family were covered by glandular and non-glandular trichomes. Hairs do several tasks in plant, one of the most important of them preparation of plant deal with adverse environmental conditions. Artificial stresses can act as environmental factors and affect different structures of plants. Our findings confirmed that plant, in response to salinity stress, can increase the number and density of its trichomes. In fact, that is a response to drought conditions which were created by salt stress. In contrast, treatment of plant with nanoparticles of iron oxide decreases hairs densities and numbers. It seems that this component removes side effects of drought stress.

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