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## GROWTH CHARACTERS AND YIELD OF DRAGONHEAD IN RELATION TO $\text{Fe}_2\text{O}_3$ NANO-SCALE FERTILIZER AND SOWING DENSITY

### SUMMARY

Dragonhead (*Dracocephalum moldavica* L.) is an annual, herbaceous, balm-scented and spicy medicinal plant of Lamiaceae family. An experiment was carried out during successive season to investigate the response of dragonhead to various plant sowing densities (10, 15, 20 and 40 cm) and iron nano-fertilizer (0, 1, 2 and 3 g  $\text{lit}^{-1}$ ) applications. Iron is a necessary element for plant's growth and plants deficiency or deactivation of iron show with chlorosis of their leaves. Results of ANOVA indicated there is not any significant interaction between nano-fertilizer and sowing density. Iron nano-fertilizer levels had a promoting influence on most of growth traits and accelerated essential oil accumulation. Similarly, wider plant spacing indicated the greatest effect on some growth components. Generally, the third rate of iron nano-fertilizer (2 g  $\text{lit}^{-1}$ ) combined with moderate distance between plants (15 cm) had a favorable effect on most of growth traits. The forth level of sowing density (40 cm) was the best in number of flowering branches, number of secondary branches and stem diameter traits while the second level of sowing density (15 cm) was the best in essential oil yield. Three levels of iron nano-fertilizer (0, 1 and 2 g  $\text{lit}^{-1}$ ) produced long height of first flowering branch while two levels of nano-fertilizer (1 and 3 g  $\text{lit}^{-1}$ ) had high number of secondary branches. The third level of iron nano-fertilizer (2 g  $\text{lit}^{-1}$ ) had high values of total anthocyanins, chlorophyll b and total flavonoid traits. Two levels of iron nano-fertilizer (1 and 2 g  $\text{lit}^{-1}$ ) indicated high magnitudes of chlorophyll a, total chlorophyll, flavonoid 270 nm, flavonoid 300 nm and flavonoid 330 nm traits. It can be advised that medium distance (15 cm) and the second iron nano-fertilizer level (2 g  $\text{lit}^{-1}$ ), could be used for achieving high yield and essential oil performance.

**Keywords:** *Dracocephalum moldavica*; Essential oil; Nano-scale ferric oxide; yield component.

### INTRODUCTION

The dragonhead (*Dracocephalum moldavica*), a member of the *Lamiaceae* family, is a grassy annual plant up to 80 cm tall. The origin of the dragonhead is

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reported from southern Siberia and the Himalaya and naturally grows in temperate areas of Europe and Asia (Galambosi and Holm 1989; Galambosi et al. 1989). It contains terpenoids with the odor of molasses, the major constituents of which are citral isomers, flavonoids, iridoids, tannins, and hydroxycinnamic and carboxylic acids (Popova et al. 2008). Dragonhead has some therapeutic properties such as sedative, tonic, antimicrobial, and wound healer and it is widely used in folk medicine as a painkiller and for the treatment of kidney complaints (Hassani, 2006). Extracts of the dragonhead are used against toothache and colds as a poultice against rheumatism, also, it acts as stimulated evolution in female animals (Chachoyan and Oganessian, 1996). The essential oil of dragonhead ranged from 0.20 to 0.62% (Racz et al. 1978), while Hornok et al. (1990) reported that the essential oil at flowering stage reached 0.74%. Aziz and El-Sherbeny (2003) reported that the essential oil of dragonhead was characterized by a high percentage of oxygenated monoterpenes and the major components were geranial, geranyl acetate, neral and geraniol.

Environmental and agronomical factors have an important effect on the product yield and its components derived from herbs, but it was not possible to fully control these factors but can managed environmental impact with a certain methods. Among the agronomical factors, sowing density has an important role in achieving the proper conditions during the growth period of medicinal plants to achieve optimum yield; also, it is also an important factor in determining productivity (Hassani, 2006). If the planting density is greater, the environmental factors will not be enough for the plant growth and development, while, if it is less, environmental factors are not used more effectively, and leading to a decrease in the performance (Galambosi and Holm, 1991). Hussein et al. (2006) indicated that wider plant distances of dragonhead increased the herbage biomass because individual plants in wider distances had more branches and herbage yield compared to the narrow distances. Sarvari et al. (2013) reported the highest plant height, number of primary shoot, fresh weight and dry weight as well as the highest herbage yield ( $10.4 \text{ ton ha}^{-1}$ ), essential oil content (0.46 %) and essential oil yield ( $44.9 \text{ kg ha}^{-1}$ ) obtained from 40 cm planting distance.

In medicinal plants it is important to study the response to different fertilizers, since these elements may influence the essential oil content of these species such as dragonhead. Fe deficiency results in substantial yield loss due to stunted plants with pale green or yellow symptoms; also it has negative effect on other physiological activates (Wiersma, 2005). Therefore, most studies have been performed to correct Fe deficiency by applying various seed, soil or foliar Fe chelates or fertilizers. Soils of Mediterranean-type areas generally have high pH and low organic matter, and in consequence, Fe deficiency is one of the most important micronutrient disorders in such areas and Fe status of the soil affects uptake and use efficiency of macronutrients (Fageria, 2001; Rashid and Ryan, 2004; Malakouti, 2008). The employment of nanoparticles in agriculture involves the using of these particles imparting specific beneficial effects to the crops due to potential of nanotechnology for increasing the value of agricultural products

and environmental problems. Using of nanoparticles and nano-powders, researchers can produce controlled or delayed release fertilizers as well as beneficial effect on seedling growth and development (Sheykhbaglou et al. 2010; Kottegoda, et al 2011). Azarpour et al. (2013) reported foliar application of nano-iron had significant effects on saffron yield. Recent studies on nano-particles and nano-fertilizers in most crops has evidenced for enhanced germination and crop performance showing their potential for application in agriculture (Kole et al. 2013). Using of nano-fertilizer causes an increase in nutrients use efficiency, reduces soil toxicity, minimizes the potential negative effects associated with over dosage and reduces the frequency of the application so, nanotechnology has a high potential for achieving sustainable agriculture (Naderi and Danesh-Shahraki, 2013). The aim of this study was to evaluate effects of planting density and iron nano-fertilizer on essential oil percentage and some morphological characteristics of dragonhead.

### MATERIAL AND METHODS

The experiment was carried out during successive season of 2014/2015 in the experimental farm, to investigate the response of *D. moldavica* to various plant densities and iron nano-fertilizer application. The experimental design was randomized complete block design with three replicates. Four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit<sup>-1</sup>) and four plant spacing (10, 15, 20 and 40 cm) were arranged in experimental plots. Synthesized nano particles had been characterized morphologically by scanning electron microscope (Figure 1).

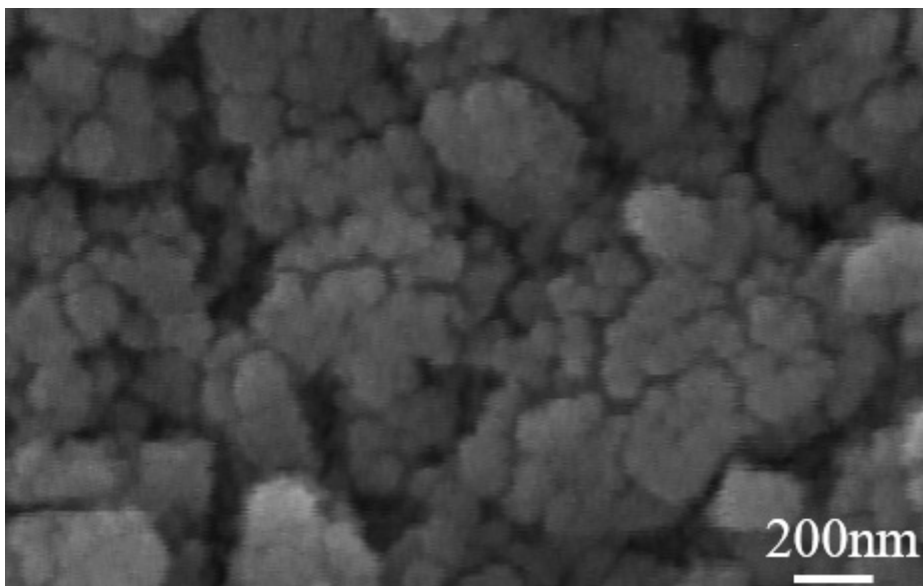


Figure 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles ferric oxide for iron nano-fertilizer.

The seeds of dragonhead were sown directly in field on 23<sup>rd</sup> of May. The physical and chemical properties of experimental soil were determined using the methods of Chapman and Pratt (1978) and the data are shown in Table 2. Four levels of iron nano-fertilizer were applied in 23<sup>rd</sup> of June at vegetative growth period. After two weeks from sowing, the plants were thinned twice, leaving one plant in hills. The plants were collected at full flowering stage in 24<sup>th</sup> of July, and the following data were recorded for plant growth characters: number of flowering branches (NFB), height of first flowering branch (HFB), Number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY), total anthocyanins (TA), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), and total flavonoid (TF). Photosynthetic pigments (chlorophyll a and b and total carotenoids mg g<sup>-1</sup>) of the leaves were determined by AOAC (1990). The resulted essential oil from each treatment was dehydrated over anhydrous sodium sulfate and then subjected to GLC analysis with Varian VISTA 6000 FID model. All data obtained based on two-factor experiment in randomized complete block design was analyzed according to Snedecor and Cochran (1990), means of the traits were compared by least significant difference (LSD) test of Duncan's multiple range test (DMRT) at  $p < 0.05$  level by means of the SAS 9.1 statistical software.

## RESULTS AND DISCUSSION

According to analysis of variance (Table 1), sowing density was significant for number of flowering branches (NFB), essential oil content (EOC), dry weight kg/ha (DW) and essential oil yield (EOY) at 0.01% probability level while it was significant for height of first flowering branch (HFB), number of secondary branches (NSB) and stem diameter (SD) at 0.05% probability level.

Table 1. Analysis of variance for the measured morphological traits of dragonhead (*Dracocephalum moldavica*).

SOV†	DF‡	NFB	HFB	NSB	SD	EOC	DW	EOY
Block	2	0.2788 <sup>ns</sup>	0.29 <sup>ns</sup>	7.310 <sup>**</sup>	0.314 <sup>ns</sup>	0.0013 <sup>ns</sup>	3775956.8 <sup>**</sup>	17.86 <sup>ns</sup>
Density (D)	3	5.0167 <sup>**</sup>	44.52 <sup>*</sup>	4.427 <sup>*</sup>	4.515 <sup>*</sup>	0.0078 <sup>**</sup>	17656819.8 <sup>**</sup>	193.52 <sup>**</sup>
Fertilizer (F)	3	1.7273 <sup>ns</sup>	36.25 <sup>ns</sup>	5.837 <sup>**</sup>	0.454 <sup>ns</sup>	0.0060 <sup>*</sup>	238818.0 <sup>ns</sup>	20.14 <sup>ns</sup>
D×F	9	1.2420 <sup>ns</sup>	16.30 <sup>ns</sup>	2.531 <sup>ns</sup>	1.248 <sup>ns</sup>	0.0030 <sup>ns</sup>	864817.5 <sup>ns</sup>	11.86 <sup>ns</sup>
Error	30	0.8312	12.69	1.302	1.047	0.0017	599931.9	8.10
CV¶		14.7	24.4	11.8	14.2	15.3	16.2	22.1

† SOV, Source of variation; ‡DF, Degrees of freedom; ¶CV, Coefficient of variation.

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup> are significant at 1 and 5% probability level and non-significant, respectively.

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

The nano-fertilizer application had significant effect on number of secondary branches (at 0.01% probability level) and essential oil content (at 0.05% probability level). The sowing density  $\times$  nano-fertilizer interaction was not significant for all of the traits of Table 1, therefore, the mean comparison can be performed on main effects of the factors (sowing density as well as nano-fertilizer). The analysis of variance for the other remained traits (Table 2), showed that sowing density was significant for total anthocyanins (TA) at 0.01% probability level and for flavonoid 330 nm (F330) at 0.05% probability level.

Table 2. Analysis of variance for the pigment flavonoid traits of dragonhead (*Dracocephalum moldavica*).

SOV†	DF‡	TA	CA	CB	TC	F270	F300	F330	TF
Block	2	0.2223 <sup>ns</sup>	0.0054 <sup>ns</sup>	0.0004 <sup>ns</sup>	0.0041 <sup>ns</sup>	1314.4 <sup>ns</sup>	8099.3 <sup>ns</sup>	1937.2 <sup>ns</sup>	27687.3 <sup>ns</sup>
Density (D)	3	2.6075 <sup>**</sup>	0.0044 <sup>ns</sup>	0.0011 <sup>ns</sup>	0.0094 <sup>ns</sup>	707.0 <sup>ns</sup>	8697.4 <sup>ns</sup>	7234.3 <sup>*</sup>	17952.5 <sup>ns</sup>
Fertilizer (F)	3	1.3721 <sup>**</sup>	0.0302 <sup>**</sup>	0.0131 <sup>**</sup>	0.0858 <sup>**</sup>	11452.4 <sup>**</sup>	20632.1 <sup>**</sup>	11251.9 <sup>**</sup>	126263.7 <sup>**</sup>
D $\times$ F	9	0.2188 <sup>ns</sup>	0.0062 <sup>ns</sup>	0.0028 <sup>ns</sup>	0.0166 <sup>ns</sup>	3986.5 <sup>ns</sup>	3112.7 <sup>ns</sup>	693.0 <sup>ns</sup>	13257.5 <sup>ns</sup>
Error	30	0.2402	0.0035	0.0017	0.0080	2446.9	3909.4	1916.3	13954.5
CV¶		6.9	10.6	11.8	9.8	19.6	25.3	14.8	14.8

† SOV, Source of variation; ‡ DF, Degrees of freedom; ¶ CV, Coefficient of variation.

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup> are significant at 1 and 5% probability level and non-significant, respectively.

Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF)

Also, application of nano-fertilizer was significant for total anthocyanins (TA), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330) and total Flavonoid (TF) traits (Table 2). Similar to Table 1, the sowing density  $\times$  nano-fertilizer interaction was not significant for all of the traits of Table 2, thus, the main effects of the sowing density and nano-fertilizer factors can be compared with mean comparison tools.

Mean comparisons were performed via least significant difference (LSD) test for significant effects and via Duncan's multiple range test (DMRT) for non-significant effects. The forth level of sowing density (40 cm) was the best in number of flowering branches, number of secondary branches and stem diameter traits while the second level of sowing density (15 cm) was the best in essential oil yield (Table 3). The height of first flowering branch and essential oil content were high in the first (10 cm), second (15 cm) and third (20 cm) sowing density

while dry weight was high in the first (10 cm) and second (15 cm) sowing density (Table 3).

Table 3. Mean comparison of the measured morphological traits of dragonhead (*Dracocephalum moldavica*) for four levels of sowing density (10, 15, 20 and 40cm)

Density	NFB		HFB		NSB		SD		EOC		DW		EOY	
D1	5.95	B	16.07	A	9.17	B	6.73	B	0.265	AB	5364.3	A	14.22	B
D2	6.21	B	15.54	A	9.39	B	6.89	B	0.282	A	6003.1	A	16.90	A
D3	5.61	B	15.03	A	9.50	B	7.17	B	0.289	A	4533.8	B	12.99	B
D4	7.12	A	11.79	B	10.54	A	8.10	A	0.232	B	3193.7	C	7.36	C

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

There is not any significant differences among four levels of sowing density in chlorophyll a, chlorophyll b, total chlorophyll, flavonoid 270 nm and total flavonoid (Table 4). The first (10 cm), second (15 cm) and third (20 cm) sowing density had the high values for flavonoid 300 nm, while the first (10 cm) and third (20 cm) sowing density had the high values for total anthocyanins and the first (10 cm) and second (15 cm) sowing density had the high values for flavonoid 330 nm (Table 4).

Table 4. Mean comparison of the measured pigment and flavonoid traits of dragonhead (*Dracocephalum moldavica*) for four levels of sowing density (10, 15, 20 and 40 cm).

Density	TA		CA		CB		TC		F270		F300		F330		TF	
D1	7.65	A	0.538	A	0.349	A	0.889	A	250.0	A	230.8	AB	331.8	A	812.6	A
D2	6.57	BC	0.573	A	0.360	A	0.933	A	259.8	A	274.0	A	295.5	AB	829.3	A
D3	7.31	AB	0.543	A	0.341	A	0.883	A	256.3	A	265.6	AB	279.4	B	801.4	A
D4	6.94	C	0.574	A	0.362	A	0.935	A	242.4	A	218.1	B	280.1	B	740.6	A

Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF).

Mean comparisons of four levels of iron nano-fertilizer indicated that F4 (3 g lit<sup>-1</sup>) had the high number of flowering branches (6.5), but it had not any significant differences with F1 and F3 (0 and 2 g lit<sup>-1</sup>) treatments (Table 5). Three levels of iron nano-fertilizer (F1, F2 and F4) produced long height of first flowering branch while two levels of nano-fertilizer (F2 and F4) had high number of secondary branches (Table 5).

Table 5. Mean comparison of the measured morphological traits of dragonhead (*Dracocephalum moldavica*) for four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit<sup>-1</sup>)

Fertilizer	NFB		HFB		NSB		SD		EOC		DW		EOY	
F1	6.46	A	15.98	A	9.24	BC	7.28	A	0.258	B	4769.8	A	12.35	AB
F2	5.68	B	14.07	AB	10.17	A	7.44	A	0.296	A	4916.8	A	14.43	A
F3	6.25	AB	12.38	B	8.88	C	6.98	A	0.271	AB	4825.6	A	13.29	AB
F4	6.50	A	16.00	A	10.31	AB	7.18	A	0.243	B	4582.6	A	11.40	B

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

There is not any significant differences among four levels of iron nano-fertilizer in stem diameter and dry weight. Two levels of iron nano-fertilizer (F2 and F3) showed high values of essential oil content while three levels of nano-fertilizer (F1, F2 and F3) had high amounts of essential oil yield (Table 5). The third level of iron nano-fertilizer or F3 (2 g lit<sup>-1</sup>) had high values of total anthocyanins, chlorophyll b and total flavonoid traits (Table 6). Two levels of iron nano-fertilizer (F2: 1 g lit<sup>-1</sup> and F3: 2 g lit<sup>-1</sup>) indicated high magnitudes of chlorophyll a, total chlorophyll, flavonoid 270 nm, flavonoid 300 nm and flavonoid 330 nm traits (Table 6).

Table 6. Mean comparison of the pigment and flavonoid traits of dragonhead (*Dracocephalum moldavica*) for four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit<sup>-1</sup>)

Fertilizer	TA		CA		CB		TC		F270		F300		F330		TF	
F1	6.92	B	0.492	C	0.311	C	0.800	C	224.5	B	210.9	B	271.1	C	706.6	C
F2	7.12	B	0.575	AB	0.357	B	0.934	AB	264.1	AB	252.9	AB	308.5	AB	825.6	B
F3	7.59	A	0.611	A	0.392	A	1.003	A	290.3	A	303.2	A	334.6	A	928.1	A
F4	6.83	B	0.550	B	0.353	B	0.903	B	229.7	B	221.3	B	272.6	BC	723.6	C

Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF).

In this research, the percentage of essential oil ranged from 0.21 to 0.33. The essential oil content of dragonhead show considerable variations due to plant origin, ecological and climatic conditions (Aziz et al. 2010). In Rumania, the percentage of essential oil of dragonhead ranged from 0.20 to 0.62 (Racz et al. 1978), while in Hungary, reported that the essential oil at flowering stage reached 0.74 (Halasz-Zelnik et al. 1988). In Finland, Holm et al. (1978) stated that the maximum percentage of oil of dragonhead was 0.62 during the flowering stage while in Egypt, Aziz and El-Sherbeny (2003) found that the essential oil of this plant, were characterized by a high percentage amounts (0.82 – 0.96). It can be noticed that measured traits of dragonhead were increased with iron nano-fertilizer treatments. These results may be attributed to the role of iron micro-

nutrient, in stimulating metabolic processes, encouraging growth and increasing the synthesis and accumulation of more essential oil in plant tissues. Several investigators mentioned similar results on different plants such as El-Desuki et al. (2001) on sweet fennel, Khalil et al. (2002) on Mexican marigold, Khalil and El-Sherbeny (2003) on mint, and Hussein et al. (2006) on dragonhead, who observed that application of different macro and micro-nutrients significantly improved plant growth characters. This result may be due to effect of iron nano-fertilizer on accelerating metabolism reactions as well as stimulating enzymes. This increment may be due to the effect of iron nano-fertilizer on mass production or/and oil content. Such findings were retrieved by some authors such as El-Masry and Dahab (2001) on pelargonium, and Naguib (2003) on chamomile. Concerning the effect of plant sowing density on essential oil of dragonhead, wider spaces offer ample quantity of nutrients, light and other environmental factors which in turn was reflected on the high amounts of morphological traits and essential oil content.

Concerning the effect of plant sowing density, the obtained results indicated that wide distance decreased most plant characteristics and this might be due to the rapid differentiation of cells in wide spacing than in narrow ones. Wahba and Ezz El-Din (2002) on *Chrysanthemum coronarium* and Hussein et al. (2006) on dragonhead reported similar results, while opposite trends were found by Zayed et al. (2003) on borage plant. Also, number of flowering branches (NFB) was increased with increasing the distance of plants and similar finding was reported by different authors such as Das et al. (1992) on black cumin and Hussein et al. (2006) on dragonhead. Moreover, morphological traits such as dry weight (DW), demonstrated that the widest distance did not result in highest dry weight of herb but some authors found the promotion effect of wider plant spacing on vegetative growth characters such as Belyaonka et al. (1997) on chrysanthemum and Wahba and Ezz El-Din (2002) on *Chrysanthemum coronarium* plants. This promoting effect of the widest distance on growth characters such as number of secondary branches (NSB) and stem diameter (SD) may be due to increment in the amount of nutrients uptake or/and getting more quantity from solar energy for plant.

The effect of the interaction treatments between iron nano-fertilizer levels and plant sowing density was not observed. When calculating the yield performance in a unit area, the narrower spaces will contain high number of plants and consequently more yield will be obtained and the heavier plants obtained in the wider spaces in the same unit area may reach the yield obtained in the narrower distance (Hussein et al. (2006). In the present investigation a positive relationship between increasing plant sowing density (D2 treatment) and essential oil yield was found; the same finding was reported by El-Sherbeny et al. (2005) on ironwort and Sadek et al. (1992) on rosemary (El-Dean and Ahmed, 1997). Data obtained indicated that iron nano-fertilizer application up to 2 g lit<sup>-1</sup> resulted in increasing chlorophyll a and b, and total anthocyanins, so photosynthetic pigments showed almost similar trend of some other growth traits.

This finding might be reasonable since the improvement of growth traits is mainly a result of stimulation in photosynthetic apparatus that leads to more photosynthesis and food reserve which, the beneficial effects of different fertilizer application on the accumulation of photosynthetic pigments were previously observed by El-Ashry et al. (1997), Khalil et al. (2002) and Khalil and El-Sherbeny (2003). Plant spacing had not the same effect of iron nano-fertilizer application on photosynthetic pigments where wider plant distance dose not causes an increase in amounts of pigments but the promoting effect of wider distance on photosynthetic pigments was reported (Mohamed and Wahba, 1993; El-Sherbeny et al. 2005).

Finally, we found that, most of the measured morphological traits as well as biological pigments (chlorophylls and anthocyanins) were decreased with increasing of plant sowing density. This findings could be due to shading of top leaves and early senescence of the shaded leaves. Application of F3 iron nano-fertilizer (2 g lit<sup>-1</sup>) is caused to increase of biosynthesis and stability of biological pigments, but had deterrence effects in higher concentrations (F3 or 3 g lit<sup>-1</sup>). It could be declared that 2 g lit<sup>-1</sup> of iron nano-fertilizer is the critical level of its application in dragonhead. In conclusion, it could be concluded that under Iran environmental condition, the application of iron nano-fertilizer at level of 2 g lit<sup>-1</sup> to dragonhead (*Dracocephalum moldavica*) plants cultivated at 15 cm distance between plants is recommended for good plant growth as well as more essential oil content, which lead at the end to improving the productivity of dragonhead.

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