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Effect of Nano Iron Chelate Fertilizer on Iron Absorption and Saffron (*Crocus sativus* L.) Quantitative and Qualitative Characteristics

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ABSTRACT

In order to investigate the effect of nano iron chelate and compare to EDDHSA chelate on saffron, a factorial experiment with six treatments and three replications was conducted in saffron research farm of Shahed University in crop year of 2013-2014. Treatments included iron fertilizer at two levels of application of nano iron chelate fertilizer and iron chelate fertilizer with base of EDDHSA and the second agent was amount of fertilizer at three levels of 0,5 and 10 kg ha^{-1}. Number of flowers, flowers performance, yield of wet and dry stigmas, amount of chlorophyll a and b, total chlorophyll, leaf area index, yield of dry leaf, concentration of leaf iron and total iron were investigated in this study. Qualitative features of Saffron including secondary metabolites of crocin (color agent), picrocrocin (taste agent) and safranal (scent) were measured. The results showed that all traits under study except picrocrocin, safranal, crocin, chlorophyll b, total chlorophyll and leaf area were affected by type, amount or interaction of type in amount of fertilizer. With increase of iron nano fertilizer content by 10 kg of flower number and yield of wet flower increased compared to the control. But application of 5 kg nano chelate led to increased yield of dry stigma, yield of dry leaf, concentration of leaf iron and total iron, compared to control. The overall results showed that a nano-based iron fertilizer is more effective than micro and 5 kg application of this fertilizer is superior to 10 kg.

Key words: Dry stigma, iron chelate, nano chelate, saffron, yield

INTRODUCTION

Saffron with scientific name *Crocus sativus* L. belongs to the family of the lily and it is one the most valuable and precious farming plant species in the world that are often planted areas with an arid climate (Abdullaev *et al*., 2003; Melnyk *et al*., 2010; Molina *et al*., 2005), yield and quality of saffron are affected by different aspects of economic, social, cultural, educational of saffron planting (Aghaee and Rezagholizadeh, 2011). Better farming and improvement operations are process of production and supplying product and finally creating necessary changes for qualitative and quantitative improvement of product as a result of research, education and promotion of new methods of planting, growing and harvesting product. Application of new techniques to saffron could help Iran to compete in global markets with saffron produced in other countries (Aghaee and Rezagholizadeh, 2011). Currently 85% of world production of saffron cultivation is allocated to Iran and its annual under cultivation area is permanently increasing, so that, the area under cultivation in the Khorasan province has reached from 2950 ha in 1974 to about 65,000 ha in 2013. Despite the precedence of saffron cultivation, this plant, in comparison with many of the current crop products in country, has lower portion of the new technologies and its production is heavily dependent on indigenous knowledge (Koocheki, 2004). Nano is gradually moving from the experimental stage to applied and operational phase and this will lead more sensitive stage of this technology in the agricultural sector (Baruah and Dutta, 2009). In this regard, the use of nano fertilizers for accurate control of releasing nutrients can be an effective step towards achieving sustainable agriculture compatible with the environment (Cui *et al*., 2006). Using nano fertilizers as an alternative to conventional fertilizers, fertilizers nutrients are released gradually in a controlled way into soil (Chinnamuthu and Boopathi, 2009). Saffron cultivation is common in arid and semi-arid regions and these regions, because of high pH, alkaline soil and high electrical conductivity and free carbonates in soil, face iron chlorosis problem in a way that affects yield of the crop (Zuo and Zhang, 2011). The importances of iron in plant nutrition, considering strategies used by plants to absorb this element under stress, have been emphasized. Iron may be absorbed as ferric ion (Fe^{3+}) or ferrous (Fe^{2+}), however, because the higher solubility of ferrous ions, it is more absorbent. Generally, plants use two distinct strategies for solution and absorption of iron from soil: The first strategy that is observed in the non-grasses cotyledons and monocotyledon in response to iron deficiency.

A series of reactions and changes in form and physiological functions happen in roots, these changes increase the ability of plants to absorb and dissolve the iron from environment. In order to compensate lack of iron, Plants of this group create proton inside their root (Regenerative) that is pumped out the root and so ferric iron turns into ferrous iron which is more readily absorbed by the plant. The second strategy is observed in plants such as grasses and poaceae it is Secretion and release of regenerating compounds in roots such as citric acid and Malic acid and also release of organic ligands with low molecular weight, called siderophores that is an amino acid protein that causes conversion and restoration of Fe^{3+} and Fe^{2+} and increasing solubility (Marschner, 1986). According to researchers' recommendation (Ahmadi and Jabbari, 2009; Zuo and Zhang, 2011) chelated form of micronutrients have higher efficiency than conventional compounds, therefore, iron deficiency can be compensated by directly use of iron chelated in the root zone or spraying the herb foliage (Ahmadi and Jabbari, 2009; Khoshgophtarmanesh *et al*., 2012) chelating factor EDDHA, keep the trivalent iron with a lot of power and prevents its deposition in the soil. Thus, concentration of dissolved iron in the soil significantly increases and near the roots iron is reduced as bivalent after secretion of acidic material. Since the stability of the chelating agent with bivalent iron is not much, in the vicinity of the roots iron is separated from chelate iron and easily absorbed by the roots (Malakouti and Samar, 1998). Nano iron chelated fertilizer, with hydrocarbon base without ethylene bond without hormones, with high stability and a gradual release of iron in a wide pH 3-11 can be a rich and certain source of bivalent iron supply for plant (Baghai and Farahani, 2013). Studies by Baghai *et al*. (2012) on green cumin showed that in limited irrigation, by applying 6 kg nano iron fertilizer per hectare, yield decline due to increase of irrigation cycle can be compensated (Moghadam *et al*., 2012). In an study on spinach, it was found that the use of 4 kg nano iron chelated kilograms per hectare could lead to 7.3 t ha^{-1} yield in the Virofly cultivar that was 76% better than the control. Research by Baghai and Farahani (2013) was as factorial experiment conducted in farm cultivation. Experiment factors include two types of conventional and nano iron chelated fertilizer at three levels: 0, 5 and 10 kg ha^{-1}. Traits such as leaf length, leaf number, flower number, total corm weight, wet flower weight, main corm weight, number of corms, yield of dried stigmas, iron concentration in the shoot and main corm diameter were significantly (at level 15%) affected by the treatments. By increasing amount of each type of fertilizer, yieldof saffron increased and application of 10 kg of nano chelate increases weight of dry stigma by (59%), wet flower weight (69%), number of flowers (51%), number of leaves (62%), leaf length (14%), main corm diameter (33%) and total weight of corms (42%), compared to control. Application of 5 kg nano chelate lead to an increase of 56% in corm number compared to the control. Total iron uptake in shoot in nano fertilizer treatments significantly increased by 11% compared to the micro fertilizer. The overall results indicate that iron fertilizer with the basis of nano is more effective than micro. After investigating and comparing different types chelate including EDDHA, N-ethylenediamine and BIS'S (hydroxyphenylacetio-O) in the sandy clay soil with a pH 7.7, researchers reported that soya plant treated with iron chelate Fe-EDDHA, increased total iron by 50% (Schenkeveld *et al*., 2008). Hamzehpour *et al*. (2010) conducted greenhouse study in order to investigate the interaction of zinc, iron and manganese, iron and the effect of concentration of these elements in different organs of plants, the treatments consisted of three levels of zinc (0, 40 and 80 mg kg^{-1} soil), three levels of iron and three levels of manganese (0, 15 and 30 mg kg^{-1} of soil) and concluded that soil application of Fe-EDDHA chelate decreased zinc and manganese concentration in cluster (in the order of 6.46 and 31.43-18.44 and 76.33 mg kg^{-1} dry matter) and shoot (35.34 and 43.24-97.25 and 42.20 mg kg^{-1} of dry matter, respectively) and an increase in their concentration roots. Iron deficiency always causes loss of chlorophyll and distraction of chloroplast structure (Ahmadi and Jabbari, 2009).

This study aimed to evaluate the effect of nano-iron chelate and conventional iron chelate on the yield and quality of saffron.

MATERIALS AND METHODS

To investigate the effect of type and amount of iron fertilization on the growth and yield of saffron, the present study was conducted as factorial in a randomized complete block design with three replications in the Saffron Research Farm of Shahed University in crop year of 2013-2014. Geographic and climatic characteristics of the farm are brought in Table 1.

The first factor of study included two types of nano-iron chelate fertilizer (produced by knowledge-based company of Sudur Ahrar Shargh) and normal chelate EDDHSA and the second factor was the amount of fertilizer at three levels: 0, 5 and 10 kg ha^{-1}.

Fertilizer treatments along with water of first irrigation were applied to plots according to plan that had been determined based on factorial in form of randomized complete block design. The area of each plot was flatted and boundary marked as 10 m. Corms were planted in 8 rows with 20 cm row spacing of 5 cm at a depth of 15 cm. According to calculation, for per plot as 5 g (5 kg ha^{-1}) and 10 g (10 kg ha^{-1}) of nano-chelate was added to the related plots and in conventional chelated treatments for compensation of low iron concentration 15.5 and 10.30 g EDDHSA chelate were added to the plots for three years and the data were collected in forth year. The fertilizers were

Table 1: Geographic and climatic characteristics of saffron research farm of Shahed University

Rainfall (mm)	Climate	Minimum temperature (°C)	Maximum temperature (°C)	Altitude (m)	Latitude	Longitude
259	Semi-arid	-8.5	40	1050	36'.°31”	53'.°48”

Table 2: Physical and chemical properties of soil (0-20 cm depth)

Properties	Values
Fe (mg kg^{-1})	2.2
Zn (mg kg^{-1})	0.98
K (mg kg^{-1})	507
P (mg kg^{-1})	25
N (%)	0.07
Organic carbon (%)	0.73
Saturation extract (pH)	7.5
EC (dS m^{-1})	6.85
Soil texture	Silty loam

completely dissolved in bottle and then were added to related plots during the irrigation. Soil analysis conducted by the Soil and Water Research Institute (Table 2) showed that the soil of farm is deficient in nitrogen and based on calculations 100 kg ha^{-1} of urea (the split) was added to the plots.

All cultivation practices such as irrigation, weeding, crust breaking, pest and disease control were applied in all treatments as equally. Irrigation of plots was done every 15 days during the months of October to April by irrigation tube and as plot.

Crust breaking operations was conducted with a trowel after full harvest of flowers in autumn and after leaf harvest in May. Combating weeds was done by hand during the growing period and summer sleeping time. Fortunately, due to health of corms, no disease was observed in the plots. But there was tick of saffron corms and ant in some plots, whose number was below the threshold of economic losses and chemical control was not done with them. To evaluate the effect of treatments on qualitative and quantitative characteristics of saffron, sampling was done in flowering period for one week from 1 m^2 area using quadrate. Number of flower, weight of flower, wet stigma weight and dry stigma weight were measured. At the time full leaf production in May, to determine the dry and wet weight of shoot, chlorophyll a and b, number of leaves per corm and shoot iron concentration, samples were taken from 1 m^2 and recorded. In order to assess iron concentration, leaves were prepared by atomic absorption device by Jones *et al*. (1991) method and were read by atomic absorption.

To measure the total absorption of iron, iron concentration multiplied by biological yield and expressed in kilograms per hectare. Qualitative features of saffron include secondary metabolites of crocin (color agent), Picrocrocin (taste agent), crocin (scent agent) were measured according to ISO/TS 3632-1/2 (2003). Statistical analysis of data was performed using MSTAT-C software. Mean comparison was done Duncan's multiple range test at 5% significance level. Then tables and graphs were plotted using Excel software.

RESULTS AND DISCUSSION

The interaction between type of iron fertilizer and amount of iron fertilizer were significant on all features except the dried yield of stigma, picrocrocin, safarnal, crocin, chlorophyll a, chlorophyll b, total chlorophyll, leaf area index, iron concentration of leaf (Table 3 and 4).

Analysis of variance (Table 3) showed that the type and amount of iron fertilizer and their interactions at the level of 1% affected number of flowers. Increasing trend of the graph related to comparison of means in trait of flower number indicates that response of saffron to fertilizer treatments in these trait was positive and by increasing amount of fertilizer, number of saffron flower increased, so that the number of flowers in the treatment of 10 kg nano-chelate per hectare was 125% more than control. Although the number of flowers produced in saffron produced with conventional fertilizer was less than treatment with nano-chelated fertilizer. According to the

Table 3: Analysis of variance of the effect of amount and type of iron fertilization on characteristics measured in Saffron flower

Sources of variation	df	No. of flowers	Fresh weight of flowers	Wet yield of stigma	Dry yield of stigma	Picrocrocin	Safranal	Crocin
		Mean Squares (MS)						
Block	2	11222263888.889[ns]	1461.556[ns]	5.065[ns]	1.711[ns]	0.588[ns]	5.493[ns]	0.042[ns]
Types of fertilizer	1	650560222222.222**	123317.195**	189.216**	2.598**	0.038[ns]	0.249[ns]	0.001[ns]
Amount of fertilizer	2	294807055555.556**	45190.951**	131.144**	8.536**	0.037[ns]	1.757[ns]	0.043[ns]
Type×amount fertilizer	2	170130722222.223**	31627.809**	87.750**	0.908[ns]	0.172[ns]	0.295[ns]	0.018[ns]
Error	10	15703113888.889	3041.184	6.159	0.330	0.110	0.940	0.043
Coefficient of variation		19.37	21.31	17.79	18.73	3.79	21.74	3.17

[ns]: Non significant, * and **Significant at $p = 0.05$ and 0.01, df: Degree of freedom

Table 4: Analysis of variance of the effect of amount and type of iron fertilization on characteristics measured in Saffron leaf

Sources of variation	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Leaf area index	Dry yield of leaf	Fe concentration	Total Fe
		Mean Squares (MS)						
Block	2	410.028[ns]	20395.093[ns]	109807.105[ns]	0.079[ns]	45999.264[ns]	338.042[ns]	4861154481.556[ns]
Types of fertilizer	1	188.238[ns]	23895.748[ns]	37125.298[ns]	1.628[ns]	319333.681**	1974.014[ns]	72448480088.889**
Amount of fertilizer	2	1392.139*	44686.070[ns]	112123.873[ns]	3.346[ns]	1036372.056**	6972.042**	204545351661.014[ns]
Type×amount fertilizer	2	1122.120[ns]	42393.590[ns]	20582.469[ns]	0.862[ns]	538087.056**	672.264[ns]	99468036306.430**
Error	10	367.605	17275.000	77329.363	1.483	37369.264	710.842	79489144.022
Coefficient of variation		2.70	18.35	2.62	32.56	19.91	7.59	25.38

[ns]: Non significant, * and **Significant at $p = 0.05$ and 0.01, df: Degree of freedom

researchers (Sadeghi *et al*., 1997; Omidi *et al*., 2010) the number of flowers in saffron was highly correlated with the cultivated corm yield and also metals effectively increase the number of flowers (Koocheki *et al*., 2011), so that, unlike the little fertilizer need of this plant, about 16-80% of the flower yield change is dependent to soil variables (Temperini *et al*., 2009).

Investigating number of flowers indicated the difference between the treatment of nano-chelate and conventional chelate that is most likely due to the conductive and metal ability of element by this nano-chelate to saffron (Fig. 1a).

Results of variance analysis (Table 3) showed that the simple and interaction effect of type and amount of fertilizer on wet yield of flower was significant. Investigating trend of changes showed that with the addition of iron fertilization, wet yield flower has had increasing trend, so that by increasing fertilizer wet yield of flower shows, this increase in nano fertilizer treatments had more gradient.

Comparing the average interaction of these factors indicate the superiority of treatment of applying 10 kg nano-chelate as 200% than the control and the lowest wet yield of flower was in control treatment.

The results show that application of nano-chelate affected wet yield of flower, so that, application of 5 and 10 kg iron fertilizer increased wet yield of flower but the results show that application of nano-chelated fertilizer was more effective than conventional fertilizer. Sadeghi (1992), in a research reported increase of wet yield of flower under the influence of appropriate nutrition (Fig. 1b). The effect of iron fertilizer on this trait indicates that metal of this element is effective in flower production process and can be agent of significant difference between treatments (Fig. 1b). The interaction between the type and amount of iron fertilizer affect wet yield of saffron stigma at level 1% (Table 3). The results showed that the use of nano iron fertilizer was effective on the wet yield of the stigma and had increasing trend. For application of nano-chelated iron fertilizer, increased wet yield of stigam in nano chelate is seen but application of conventional chelated fertilizer, although increased the wet yield of the stigma but it was significant compared to the control. All treatments applied increased the wet yield of stigma that may be due to the effects of iron element on more floral of lily family.

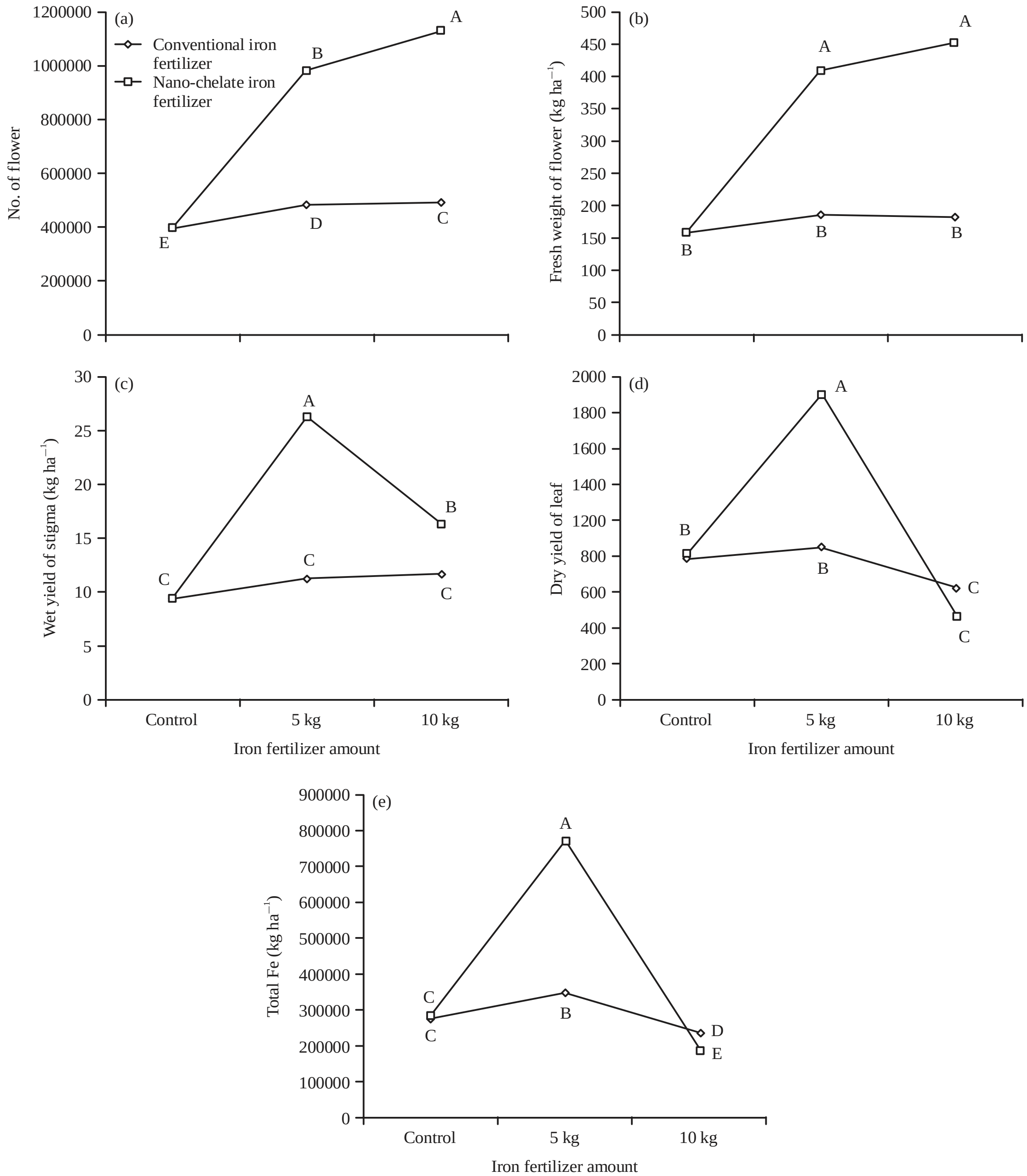

Fig. 1(a-e): Interaction effect of kind and amount of iron fertilizer on (a) Number of flower, (b) Fresh weight of flower, (c) Wet yield of stigma, (d) Dry yield of leaf and (e) Total Fe

Also investigation of the Fig. 1c shows that 5 kg treatment of nano-chelate is the highest compared to other treatments, that by increasing the amount of fertilizer at the rate of 10 kg nano-chelate this trend is the declining. The results of Baghai and Farahani (2013) suggest that the among experiment treatments maximum mean of this trait for 10 kg treatments of Nano chelate as 21 kg ha^{-1} and minimum average weight was found for control treatment was 8 kg ha^{-1}. Yield of wet stigma is one of the most important components of yield that economic value of saffron depends on it. Surveys of researchers (Omidi *et al*., 2010) have shown a high correlation between

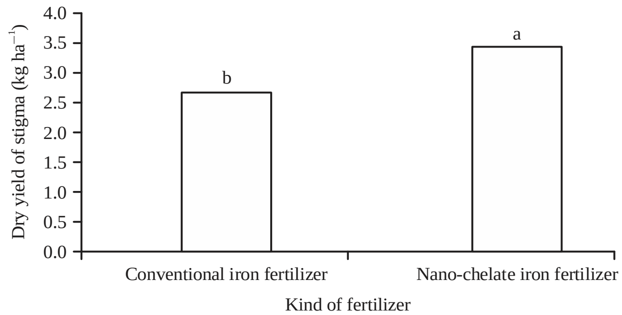

Fig. 2: Effect of kind of fertilizer on dry yield of stigma

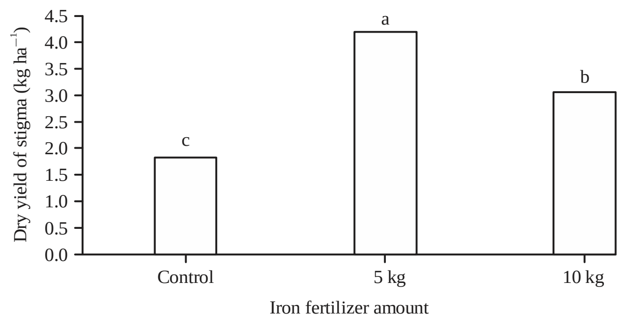

Fig. 3: Effect of iron fertilizer amount on dry yield of stigma

the number of flowers and yield of the stigma, so that, the greater the number of flowers is achieved, the greater yield stigma is obtained according to investigation of flower number trait. Results of variance analysis (Table 3) show that the type and amount of iron fertilizer, each affected dry yield of stigma at level of 1%. Trend of graph shows that effect of nano-chelated fertilizer chelated fertilizer is more than conventional chelate fertilizer (Fig. 2). Also by the use of 5 kg treatment, dry yield of stigma is more than 10 kg treatment and control (Fig. 3). Nano chelate increases the dry yield of stigma, so that the dried yield of stigma (saffron yield) has been significantly influenced by fertilizer treatments that is consistent with traits of wet yield of flower and wet yield of stigma, indicating correlation of this trait with the mentioned traits (Omidi *et al*., 2010). These results are consistent with other researchers' studies on the effectiveness of nutritional elements, along with other factors such as temperature, humidity and corms yield on saffron yield or dried stigma (Koocheki *et al*., 2011). Poorness of farm regarding iron content on one hand, efficiency of nano-chelate in gradual release on the other hand can lead to increase of yield which is indicative of effectiveness of iron found in this plant, like other plants and its role the process of respiration and anabolic (Zuo and Zhang, 2011). But the results show that excessive increase of this element as nano has reverse effect on dry and wet yield of stigma, so that Nano fertilizer treatment that has probably provided more iron to the plant has reduced wet yield of stigma. The results show that the increase over this element as nano adverse effect on dry and wet yieldcan be a stigma. Nano manure so that the plant probably has more iron than the stigma is reduced. Results of variance Analysis (Table 3 and 4) showed that picrocrocin, safarnal, crocin, chlorophyll b, total chlorophyll, leaf area index are not affected by the type and amount of fertilizer, iron and their interactions. Results of variance analysis (Table 4), shows that the chlorophyll a was affected by the amount of iron fertilizer. Comparison of the mean of simple effect of iron content

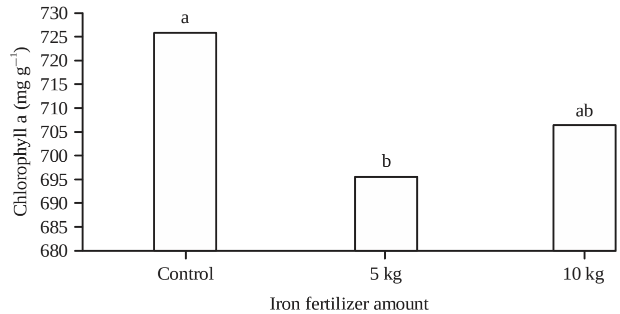

Fig. 4: Effect of iron fertilizer amount on chlorophyll a

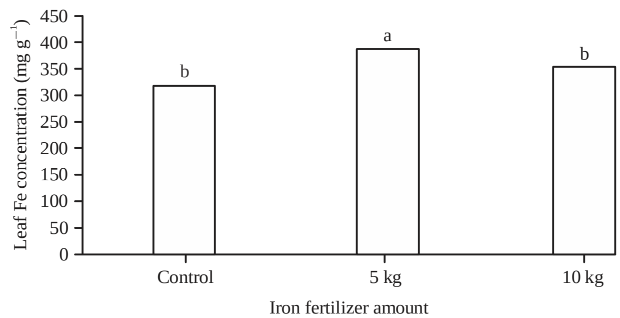

Fig. 5: Effect of iron fertilizer amount on leaf Fe concentration

for chlorophyll a trait shows that control treatment has the highest concentration of chlorophyll a in comparison to the other treatments (Fig. 4). No difference was observed between 5 and 10 kg fertilizer treatments. Dry yield of leaf is influenced by different amount and type of fertilizer was at statistical level of 1% and also its interaction at the level of 1% (Table 4). Investigation of the results of mean comparison for the mentioned trait shows that application of nano chelate increase dry yield of leaf than conventional fertilizers and also application of 5 kg iron significantly increase dry yield of leaf compared to other values of fertilizer. Areal shoot is one of the main characteristics for investigating allocation of photosynthesis material to corm (Fig. 1d). What is clear from these results is that the effect of fertilizer on yield of saffron flowers and stigma is greater than the effect on leaf yield since in both types application of 10 kg of fertilizer reduced leaves yield compared to the control.

Variance analysis table (Table 4) shows that the use of different levels of fertilizer has different effects on the iron concentration in the leaf but the type and interaction of these two factors did not have statistically significant effects. Mean comparisons demonstrate the superiority of 5 kg iron fertilizer application (Fig. 5). Iron is considered an essential micronutrient element for plants whose deficiency leads to yellow leaves and iron change concentration and other metallic elements in the plant’s tissues, these characteristics are closely associated with yield of crop plants (Tabatabai *et al*., 2011). High concentration of iron in 5 kg fertilizer may indicate a high yield of stigma and leaf in this treatment. Findings by Babaeian and Ghanbari (2010) showed that iron spray increase its concentration in sunflower seed. It was reported by the previous study that the use of FEADDHA in soybean increased total iron. Research by Mazahrynya *et al*. (2010) showed that adding iron nano oxide in soil increased iron concentration in a variety of wheat, in this study, this increase in concentration in a real shoots was observed in 5 kg treatment and 10 kg treatment showed a significant increase compared to control. But here, reduction of iron concentration is not a result of dry areal matter production, but may be it is because of treatment of 10 kg was associated with a reduction of leaf yield that shows It was probably due to reaching luxury capacity of saffron for absorption of iron (Malakouti and Samar, 1998). Total iron absorption by plant' areal shoots under the influence of type of fertilizer and also interaction of fertilizer type and amount on total iron content was at statistical level of 1% (Table 4). Investigating results of means comparison shows that 5 kg nano-chelated iron has the highest absorption (Fig. 1e). This characteristic result from iron concentration in the areal shoot and leaf yield and the more is dry yield per unit, the more total nutrients absorption from the soil.

Direct correlation between total iron absorption and iron concentration is desired, that in this study treatment of 5 kg of nano iron fertilizer shows the highest concentrations of iron and total iron with 166%. Results of this study showed that yield of stigma on yield of leaves and iron absorption was affected by chelated iron fertilizers. Since the number of flowers, yield of flowers, dry and wet yield of stigmas, chlorophyll a, dry yield of leaf, iron concentration of leaf and total iron was affected by fertilizer treatments, in a way that iron chelate improved these characteristics and increased iron chelate fertilizer treatment with a higher rate.

It seems that this fertilizer is more effective than conventional iron chelate fertilizers and since most positive on the properties of saffron effect is observed in treatment of 5 kg of this fertilizer, it is recommended that this fertilizer is used in the production of saffron and besides reducing use of other common fertilizers, other benefits of this fertilizers are enjoyed. Using nano fertilizers in order to accurate control of releasing nutrients can be an effective step towards achieving sustainable agriculture and compatible with the environment (Cui *et al*., 2006). Using nano iron fertilizers as a substitute for conventional iron chelate fertilizers, element of iron fertilizer is released gradually and in a controlled way and as a result provides nutrient to plant more effectively (Chinnamuthu and Boopathi, 2009). Since balanced nutrition is one of macro objectives of country that can ensure sustainable production, in this regard, the use of new technologies such as nanotechnology may be an effective step in increasing the quantity and quality of saffron.

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