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Effect of Magnetite Nanoparticles on Vegetative Growth, Physiological Parameters and Iron Uptake in Chrysanthemum (*Chrysanthemum morifolium*) 'Salvador'

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Abstract

Despite the increasing rate of nanoparticles (NPS) production and their application in agriculture, few studies have focused on their effect on plant growth. So, the present research was conducted in laboratory and greenhouse conditions. First, superparamagnetic iron oxide nanoparticles (SPIONS) with a humic acid coating (Fe_3O_4/HA) were synthesized in laboratory conditions by the chemical coprecipitation method. The effectiveness of the synthesized nanoparticles in vegetative growth and nutrients uptake of chrysanthemum cut flower (*Chrysanthemum morifolium*) were evaluated in greenhouse conditions with four replications in a completely randomized design. The treatments consisted of 10, 20 and 40 mg/L of pure Fe from the source of Fe_3O_4/HA NPS and 1.4 mg/L of pure Fe from two sources of iron chelates which contained Fe-EDTA(Fe-Ethylenediaminetetraaceticacid) and Fe-EDDHA [Fe-ethylenediaminedi(O-hydroxy phenylaceticacid)] were considered as control treatments in the open hydroponic cultivation system. The results of the laboratory experiment indicated that the synthesis of Fe_3O_4/HA by the chemical coprecipitation method led to the production of nanoparticles with an average diameter of 8.38 nm and superparamagnetic properties. The greenhouse experiment demonstrated that the application of Fe_3O_4/HA significantly increased Fe uptake, chlorophyll and vegetative growth of the plants versus the control treatments. The highest rates of Fe, N, P, K, Ca, Mg, Mn, and B uptake were observed at the NP rate of 20 mg/L. The branch number per plant, stem height, and total dry weight of the plants were significantly increased by 25, 38, and 39.5% versus the treatment of Fe-EDTA and by 50, 36, and 48% versus the treatment of Fe-EDDHA, respectively. It is concluded that magnetite NPs with a humic acid coating resolved Fe deficiency and increased chrysanthemum growth.

Keywords: Chlorophyll, Fe_3O_4/HA, Humic acid, Iron chelate, Iron oxide.

INTRODUCTION

One of the vital factors for plant growth is iron (Fe), which plays a role in many cellular processes, including photosynthesis, enzymatic systems, N absorption and production, DNA and RNA synthesis, efficiency of photosystems, hormone formation in plants, and the creation and development of chloroplasts (Taiz *et al.,* 2015; Briat and Lobreaux, 1997). About 80% of leaf Fe content is found in chloroplasts (Maathuis, 2013). Despite high amounts of Fe in soils as the fourth most abundant element, its availability in soils is affected by the physical and chemical properties of the environment surrounding roots in the rhizosphere (Guerinot, 2001). Fe deficiency is observed as Fe-induced chlorosis resulting from lime in most calcareous soils in arid and semi-arid regions. The high concentration of bicarbonate in soil solution make Fe ions inaccessible in the rhizosphere and in plant (Marshner, 2012). Therefore, despite the existence of sufficient and even excessive levels of Fe in plants, it is unavailable for the physiological functions of the plants due to the increase in the pH of cell apoplasts (Kosegarten *et al.,* 1999).

A solution to Fe deficiency is to use organic Fe chelating agents. The beneficial effects of natural organic matter such as humic acid and the use of synthetic organic compounds of Fe chelate fertilizers, such as Fe-EDTA and Fe-EDDHA, are common practices to make Fe available to plants. However, not only are synthetic Fe chelates expensive, but they also incur direct and indirect damages (Nowack, 2002), such as preventing precipitation and increasing the mobility of heavy (Friedly *et al.,* 2002; Peng *et al.,* 2012; Ylivainio, 2010) and radioactive metals in the environment (Means *et al.,* 1978).

Recently, significant attention has been drawn to the use of nanofertilizers for more intelligent and efficient applications (Monreal *et al.,* 2015). The distinctive characteristics of these particles are that they have an extremely fine size, a high surface to volume ratio, and high surface energy (Ball, 2002; Nel *et al.,* 2006). Studies show that plants respond to the use of these nanoparticles differently, which can be due to the physicochemical characteristics of NPs, coating type, consumption method and plant species (Zhu *et al.,* 2008).

Today, magnetite NPs (Fe_3O_4) have been brought into attention because of their unique characteristics, including easy handling, low cytotoxicity, good biocompatibility, relatively inexpensiveness, and eco-friendliness (Kong *et al.,* 2010; Zhu *et al*., 2008). The coating of NPs has a definite effect on plants (Ghafariyan *et al.,* 2013; Shafiee-Masouleh *et al.,* 2014). It should not be toxic for living organisms and should also be biocompatible. Typically, coatings with a negative charge and longer chains are less toxic for living cells (Taran *et al.,* 2016).

Therefore, the use of natural coatings such as humic acid is a new method that can be considered a source of Fe to control Fe deficiency (Ghafariyan *et al*., 2013; Jalali *et al*., 2016; Shafiee-Masouleh *et al*., 2014) due to the biocompatibility and stability of magnetite NPs with the lowest toxicity for living organisms (Bucak *et al.,* 2012).

To the best of our knowledge, no similar study has been conducted on the effectiveness of Fe_3O_4/HA in plant nutrition. This study explores the effects of Fe_3O_4/HA NP_S in comparison with prevalent Fe chelates on the vegetative characteristics of chrysanthemum in hydroponic conditions.

MATERIALS AND METHODS

Laboratory experiment

Synthesis and characterization of Fe_3O_4/HA NPs

Magnetite NPs with humic acid coating were synthesized based on the modified method of Maity and Agrawal (2007) with a chemical coprecipitation method. Briefly, 0.022 M $FeCl_3.6H_2O$ and 0.015 M $FeSO_4.7H_2O$ were dissolved in 100 ml of deionized water and heated to 90°C, and then combined with 10 ml of ammonium hydroxide and 0.5 g of humic acid sodium

salt (Sigma-Aldrich, Steinheim, Germany). The black Fe_3O_4/HA precipitates were recovered by using an external magnet. Size, distribution, and magnetic properties of the NPs were determined by transmissive electron microscope (TEM) and vibrating sample magnetometer (VSM), respectively (Asadifard *et al.,* 2005).

Greenhouse experiment

Plant materials and growth conditions

The experiment was carried out in a hydroponic greenhouse at the Ornamental Plants Research Center in Mahallat City, Iran (33°54′30″N, 50°27′30″ E and 1747 m alt.), starting from spring. The cuttings of chrysanthemum (*Chrysanthemum morifolium*) variety 'Salvador' were rooted in the sand at the beginning of the spring. Then in June, they were planted in 3 L plastic pots with an inside diameter of 17 cm and a height of 15 cm filled with a mixture of perlite of medium size (2-5 mm) and fineness (0.5-1.5 mm) at the ratio of 50:50. The experiment was conducted in a completely randomized design with four replications.

Treatments consisted of different concentrations of pure Fe including 10, 20 and 40 mg/L from the source of Fe_3O_4/HA NP_S (62.06% Fe) (equivalent to 16.11, 32.16 and 64.22 mg/L NPs, respectively) and 1.4 mg/L pure Fe from two sources of Fe chelates including Fe-EDTA (Fe-Ethylenediaminetetraaceticacid) (13% iron, Van Iperen Co., Netherlands) and Fe-EDDHA [Fe-Ethylenediaminedi(O-hydroxyphenylaceticacid)] (6% iron, Agro Nutrition Co., France) (equivalent to 10.77 and 23.33 mg/L from Fe-EDTA and Fe-EDDHA, respectively) as control treatments in an open hydroponic cultivation system (Taweesak *et al.,* 2014). The treatments were used continuously in nutrient solution after three weeks from planting. Some physical and chemical properties of the medium used in the experiment are shown in table 1 (Fonteno and Bilderback, 1993).

Table 1. Physical and chemical properties of the applied media.

pH	EC(dS/m)	Air capacity (%)	Water capacity(%)	Total porosity(%)	Particle density (g/cm^3)	Bulk density (g/cm^3)	Mass wetness (g/g)
8.10	0.12	23.60	47.09	70.69	0.59	0.17	2.71

The composition of the other elements in the nutrient solutions from the first to seventh weeks was: N 250, P 30, K 200, Ca 150, Mg 50, Fe 1.05, Mn 0.58, Zn 0.35, B 1.0, Cu 0.05, and Mo 0.05 mg/L. They changed to N 200, P 30, K 200, Ca 150, Mg 50, Fe 1.05, Mn 0.58, Zn 0.35, B 1.0, Cu 0.05, and Mo 0.05 mg/L until the end of the growth period. The pH of the nutrient solution was maintained at 5.5 ± 0.2 and the electric conductivity (EC) was kept within the range of 1.4-1.5 dS/m (Taweesak *et al.,* 2014). In order to adjust pH, 1 molar sulfuric acid solution was used.

The glasshouse light was set at 27000 lux with shading (it was 43000 lux without shading in the summer) and 28000 lux in the autumn without shading. The pots of chrysanthemum were placed on stages (25 plants/square meter) in the greenhouse. The hydroponic system was in open form with dripper irrigation system. The greenhouse cooling system was a fan and pad. The average day/night temperature was 25/16°C, and the optimum relative humidity was 60%. Aphids were controlled during the growth period.

Vegetative growth parameters

One month before the flowering stage, the number of branches and leaves per plant were counted. Stem height was measured with a ruler, stem diameter with a caliper, and shoot and root fresh weight (FW) and dry weight (DW) with a digital balance.

Physiological parameters

The chlorophyll concentration was measured by a Minolta SPAD-502 on the first fully expanded leaves of each plant. Five points were measured at different positions across the same leaf, and this was performed on three plants per plot (Rui *et al.,* 2016). Leaf proline content was measured by a spectrophotometer (Jenway, spec gene, UK) at 520 nm in the extraction of sulfa salicylic acid 3% (w/v) based on proline standard curve and blank of toluene (Bates *et al.,* 1973). Relative water content (RWC) (Volaire *et al.,* 1998) and electrolyte leakage (EL) (Lu *et al.,* 2009) were also recorded.

Nutrient elements

Oven-dried (72°C for 48 h) samples of the plants were ground separately and passed through a 40-mesh sieve. The concentration of nutrients including total nitrogen was measured by the Kjeldahl method (Bremmer and Malvancy, 1982) and phosphorus by vanadate/molybdate (the Yellow method) with a spectrophotometer (Chapman and Pratt, 1961). The ground plant samples were dry-ashed at 500°C for 4 h, the ashes were dissolved in 10 mL HCl (2N) and the volume was adjusted to 100 mL by adding distilled water. The concentrations of Ca, Mg, Fe, Cu, Mn, and Zn were measured by an atomic absorption device (Agilent technologies 200, series AA). Potassium was read by flame photometry (Jenway, PFP7, UK) after calibration with certain standards (Chapman and Pratt, 1961). The amount of B was measured by a spectrophotometer (Spectronic 20 Geneses, 4001/4, USA) via the colorimetric method (Wolf, 1971).

Statistical analysis

Analysis of variance was performed using the SAS version 9.1.3 software (SAS Inc., Carey NC), the treatment means were compared by Duncan's test, and the charts were drawn in the MS-Excel software package.

RESULTS

Characterization of Fe_3O_4/HANPs

NPs imaging and size distribution by The TEM

The magnetite NPs with humic acid coating were prepared successfully with a chemical coprecipitation method. The TEM imagery and the histogram of NP (Image processing software, Day Petronic Company) are shown in Fig. 1. The NPs were almost spherical in shape (Fig. 1a). The size distribution was relatively narrow with a diameter between 2 and 17 nm with an average of 8.38 nm (N = 200, Sd = ± 6.841) (Fig. 1b).

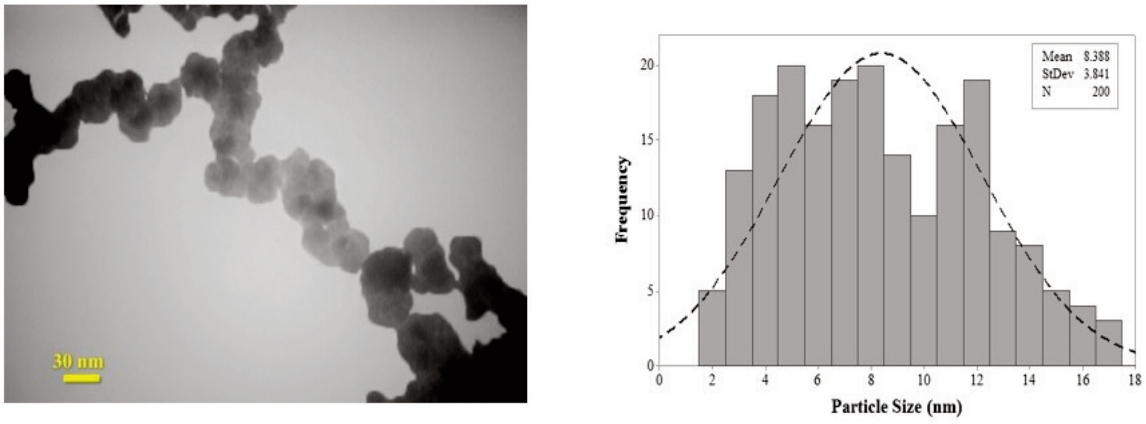

Fig. 1. (a) Transmissive electron microscope (TEM) imagery of Fe_3O_4/HA NP_S and (b) Histogram of NP_S size distribution.

Magnetic properties of nanoparticles

The magnetic properties of the magnetite NPs with humic acid coating and the magnetic residual loop were measured by a vibrating sample magnetometer (VSM) device at room temperature (Fig. 2). The VSM curve of NPs showed that when the magnetic field was applied, the magnet of the sample was increased sharply, but as the magnetic field was increased, the rate of the increase in the magnetization was decelerated until the level of about 2000 Oested (Oe) at which the sample reached its magnetic saturation (MS). Magnetic saturation of NPs became 55 emu/g. The lack of magnetic residual loop of NPs suggested the superparamagnetic properties (Fig. 2) (Fang *et al.,* 2012).

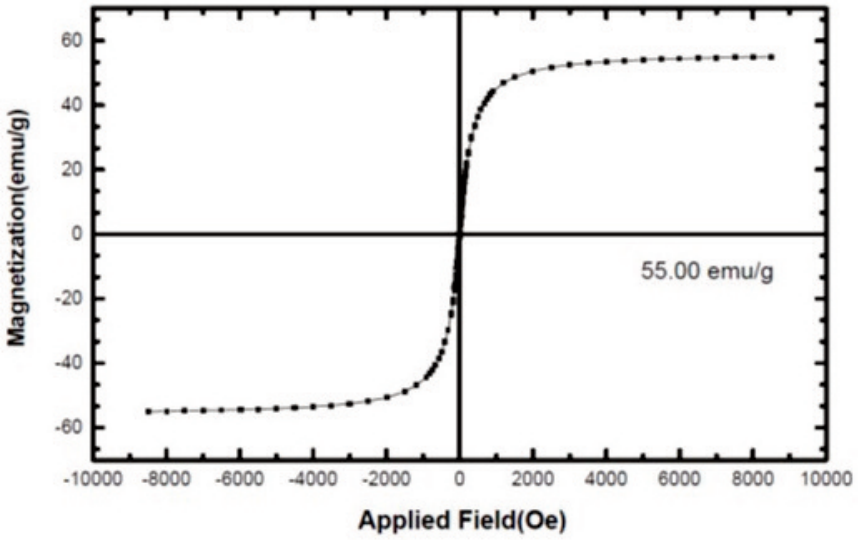

Fig. 2. Vibrating sample magnetometer (VSM) curve of Fe_3O_4/HA NP_S.

Effect of different Fe_3O_4/HA NP_S levels on vegetative parameters

The analysis of variance for the vegetative parameters of chrysanthemum indicated that the effect of different Fe_3O_4/HA NP_S levels on branch number per plant, stem height, leaf number, shoot fresh weight, root fresh and dry weight, and plant (root and shoot) fresh and dry weight was statistically significant (Table 2).

Table 2. Analysis of variance for the effect of different Fe_3O_4/HA NP_S levels versus Fe chelates on some vegetative characteristics of chrysanthemum.

SoV	df	MS									
		Shoot FW	Shoot DW	Stemheight	Branch No.	LeafNo.	Root FW	Root DW	Total FW	Total DW	DW of shoot to root
Treatment	4	621.63**	34.96^{ns}	137.69***	0.807*	950.67*	782.2**	32.02**	2143.5**	111.38**	0.343*
Error	15	129.43	13.49	8.17	0.276	68.83	150.8	4.7904	516.4	23.34	0.086
CV (%)		11.44	15.34	7.22	17.28	11.18	12.98	15.57	11.71	13.27	14.76

*, **, *** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and insignificant, respectively.

The concentration of 20 mg/L Fe_3O_4/HA NP_S increased branch number by 25 and 50% compared to Fe-EDTA and Fe-EDDHA, respectively. This difference was significant as compared

with Fe-EDDHA. The highest stem height was observed at NP_S rate of 20 mg/L, which was significantly higher than that of plants treated with Fe-EDTA and Fe-EDDHA by 38 and 26%, respectively (Table 3). Leaf number per plant increased by increasing the concentration of Fe_3O_4/HA NP_S up to 20 mg/L of nutritional solution. Although there was no significant difference between different levels of Fe_3O_4/HA NP_S and Fe-EDTA in leaf number, it significantly increased (78%) versus Fe-EDDHA. In the same conditions, maximum shoot fresh and dry weight per plant was obtained from 20 mg/L Fe_3O_4/HA NP_S. This treatment brought about significant differences in shoot fresh weight as compared to control, but the difference it made in shoot dry weight was not statistically significant (Table 3).

Table 3. Effects of different Fe_3O_4/HA NP_S levels versus Fe chelates on some vegetative characteristics of chrysanthemum.

Treatments	Shoot FW(g)		Shoot DW(g)		Stem height(cm)		Branch No.		Leaf No.	
NP_{10}	95.19^{b1}	±6.17	23.49^{a}	±2.78	43.36^{a}	±3.02	3.00^{ab}	±0.16	77.50^{ab}	±16.52
NP_{20}	121.37^{a}	±3.64	28.93^{a}	±5.96	45.72^{a}	±3.64	3.75^{a}	± 0.29	97.00^{a}	±20.57
NP_{40}	94.37^{b}	±16.86	23.82^{a}	±2.82	42.16^{a}	±2.19	2.95^{ab}	± 0.52	74.00^{ab}	±23.02
Fe-$EDTA_{1.4}$	95.50^{b}	±10.14	21.43^{a}	±2.89	33.14^{b}	±1.98	3.00^{ab}	± 0.82	67.75^{ab}	±14.93
Fe-$EDDHA_{1.4}$	90.42^{b}	±11.96	22.05^{a}	±2.78	33.45^{b}	±3.10	2.50^{b}	± 0.58	54.75^{b}	±8.01
Treatments	**Root FW(g)**		**Root DW(g)**		**Total FW (g)**		**Total DW (g)**		**DW of shoot to root**	
NP_{10}	111.37^{a}	±16.5	15.82^{a}	±3.37	206.5^{ab}	±24.8	39.32^{ab}	±5.0	1.53^{b}	±0.32
NP_{20}	105.75^{a}	±15.3	14.82^{a}	±3.02	227.1^{a}	±30.1	43.75^{a}	±7.2	1.99^{a}	± 0.46
NP_{40}	93.12^{ab}	±11.9	12.16^{ab}	±1.64	187.5^{ab}	±24.5	35.99^{ab}	±4.4	1.96^{ab}	± 0.09
Fe-$EDTA_{1.4}$	84.87^{ab}	±5.3	9.92^{b}	±0.34	180.3^{b}	±7.5	31.36^{b}	±3.0	2.16^{a}	± 0.28
Fe-$EDDHA_{1.4}$	77.87^{b}	±8.8	9.53^{b}	±0.77	168.3^{b}	±19.9	31.58^{b}	±3.5	2.30^{a}	± 0.14

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using Duncan's test. NP_{10}, NP_{20} and NP_{40}: 10, 20 and 40 mg/L of pure Fe from the source of Fe_3O/HA NPS, respectively. Fe-$EDTA_{1.4}$ and Fe-$EDDHA_{1.4}$: 1.4 mg/L of pure Fe from two sources of Fe chelates which contain Fe-EDTA (Fe-Ethylene diamine tetra acetic acid) and Fe-EDDHA [Fe-ethylene diamine di (O- hydroxy phenyl acetate)] respectively.

Root fresh and dry weights of the plants were increased up to NP rate of 20 mg/L, but they were decreased at 40 mg/L although it was not statistically significant. The application Fe_3O_4/HA NP_S at all levels increased root fresh and dry weight versus the control treatments although it was increased significantly up to 20 mg/L in comparison with the control treatments (Table 3). The highest plant fresh and dry weight was obtained at 20 mg/L NP_S and increased significantly by 25.95, 34.93% and 39.50, 48.03% versus Fe-EDTA and Fe-EDDHA treatments, respectively.

Effect of different Fe_3O_4/HA NP_S levels on physiological parameters

The analysis of variance for physiological parameters indicated that the effects of different Fe_3O_4/HA NP_S levels on the electrolyte leakage, proline, and chlorophyll index of the leaves were statistically significant (Table 4).

Table 4. Analysis of variance for the effects of different Fe_3O_4/HA NP_S rates versus Fe chelates on electrolyte leakage, proline and chlorophyll index of leaf in chrysanthemum.

SoV	df	MS		
		Electrolyte leakage	Proline	Chlorophyll index
Treatment	4	3.092**	4.822***	53.11***
Error	15	0.454	0.423	2.659
CV (%)		6.010	11.57	2.77

*, ** and***: Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

The lowest leaf electrolyte leakage was observed at 20 mg/L Fe_3O_4/HA NP_S in the nutritional solution, which was reduced significantly when compared to the treatments of Fe-EDTA and Fe-EDDHA (Fig. 3a). Leaf proline content was the lowest at 20 mg/L Fe_3O_4/HA NP_S which showed a significant decrease as compared to 40 mg/L NP_S and Fe-EDDHA treatment (Fig. 3a). Chlorophyll index of the leaf was the highest at 20 mg/L Fe_3O_4/HA NP_S, which showed a significant increase versus Fe-EDTA treatment, but it did not show a significant difference with 40 mg/L Fe_3O_4/HA NP_S and Fe-EDDHA as control (Fig. 3b).

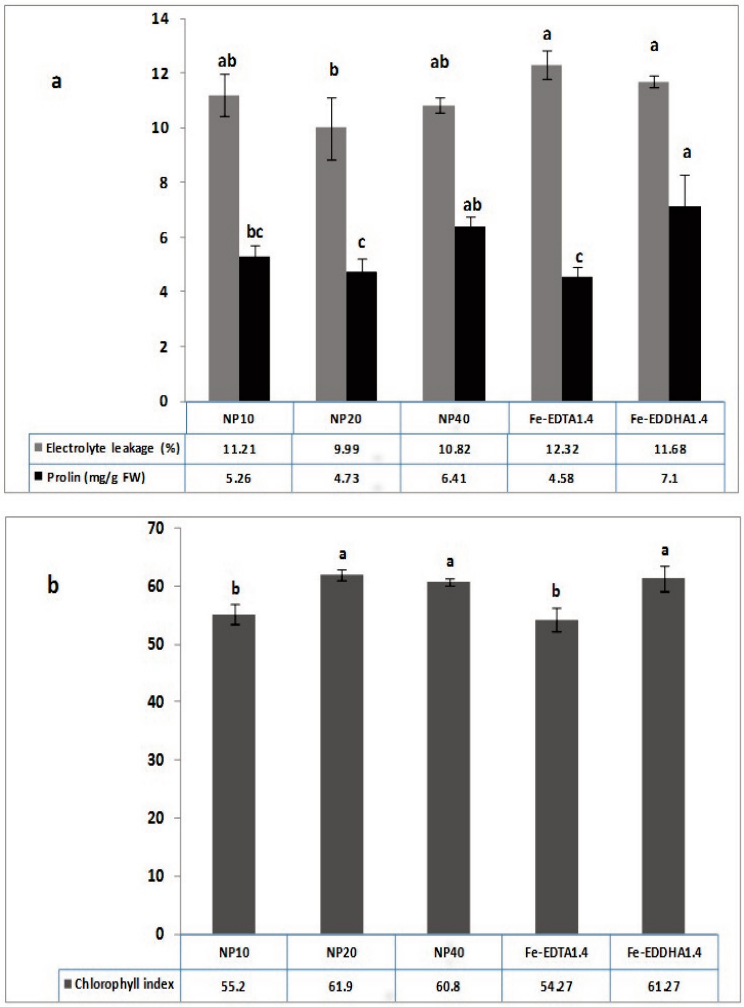

Fig. 3. Effects of different Fe_3O_4/HA NP_S levels versus Fe chelates on electrolyte leakage, proline content (a) and chlorophyll index (b) of chrysanthemum leaves (Significant at 5% level of probability by Duncan's test).

Effect of different Fe_3O_4/HA NP_S levels on the uptake (total absorption) of nutrient elements

Analysis of variance for the uptake of nutrient elements indicated that the effect of different Fe_3O_4/HA NP_S levels on the uptake of N, K, Ca, Mg, Fe, Mn, Zn, and Cu was significant, but it was not significant for P and B (Table 5).

The highest uptake rates of N, K, Ca, Mg, Fe and Zn in plants was observed at 20 mg/L Fe_3O_4/HA NP_S so that N, P and Ca uptake rates in the shrub was significantly higher than those of the control treatments of Fe-EDTA and Fe-EDDHA, and so was Fe as compared with Fe-EDTA and Mg as compared to Fe-EDDHA.

Table 5. Analysis of variance for the effects of different Fe_3O_4/HA NP_S levels versus Fe chelates on the uptake of nutritional elements in chrysanthemum.

SoV	df	MS									
		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
Treatment	4	0.057^{**}	0.0002^{ns}	0.033^{*}	0.008^{*}	0.0016^{**}	0.028^{*}	0.035^{***}	0.0302^{***}	0.001^{***}	0.139^{ns}
Error	15	0.012	0.00008	0.010	0.0021	0.0003	0.009	0.003	0.003	0.0001	0.057
CV (%)		13.97	2.64	16.46	4.72	2.28	6.29	9.95	5.10	17.14	14.85

*, **, *** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and insignificant respectively.

The uptake of P and B in plants was higher than that of the control treatments although it was not statistically significant (Table 6).

Table 6. Effects of different Fe_3O_4/HA NP_S levels versus Fe chelates on the uptake of nutritional elements in chrysanthemum.

Treatments	N		P		K		Ca		Mg	
	g/plant									
NP_{10}	0.818^{ab1}	± 0.08	0.057^{a}	± 0.01	0.596^{b}	± 0.08	0.547^{b}	± 0.13	0.169^{ab}	± 0.04
NP_{20}	0.989^{a}	± 0.19	0.076^{a}	± 0.03	0.775^{a}	± 0.17	0.557^{a}	± 0.07	0.187^{a}	± 0.04
NP_{40}	0.797^{ab}	± 0.10	0.057^{a}	± 0.01	0.629^{ab}	± 0.05	0.448^{ab}	± 0.08	0.122^{ab}	± 0.01
Fe-$EDTA_{1.4}$	0.691^{b}	± 0.07	0.057^{a}	± 0.01	0.574^{b}	± 0.13	0.393^{b}	± 0.09	0.115^{b}	± 0.02
Fe-$EDDHA_{1.4}$	0.702^{b}	± 0.08	0.047^{a}	± 0.01	0.539^{b}	± 0.07	0.356^{b}	± 0.07	0.122^{ab}	± 0.02

	Fe		Mn		Zn		Cu		B	
	mg/plant									
NP_{10}	1.98^{ab}	± 0.17	0.649^{a}	± 0.04	0.781^{ab}	± 0.09	0.062^{b}	± 0.009	1.64^{a}	± 0.24
NP_{20}	2.30^{a}	± 0.54	0.593^{ab}	± 0.05	0.786^{ab}	± 0.22	0.058^{b}	± 0.012	1.78^{a}	± 0.30
NP_{40}	1.69^{b}	± 0.26	0.503^{bc}	± 0.07	0.559^{bc}	± 0.10	0.042^{b}	± 0.007	1.78^{a}	± 0.11
Fe-$EDTA_{1.4}$	1.61^{b}	± 0.18	0.538^{ab}	± 0.05	0.847^{a}	± 0.09	0.084^{a}	± 0.007	1.52^{a}	± 0.33
Fe-$EDDHA_{1.4}$	$1.87a^{b}$	± 0.28	0.399^{c}	± 0.05	0.411^{c}	± 0.05	0.059^{b}	± 0.014	1.34^{a}	± 0.12

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using Duncan's test. NP10, NP20 and NP40: 10, 20 and 40 mg/L of pure Fe from the source of Fe_3O_4/HA NPS, respectively. Fe-EDTA1.4 and Fe-EDDHA1.4: 1.4 mg/L of pure Fe from two sources of iron chelates which contain Fe-EDTA and Fe-EDDHA respectively.

DISCUSSION

Recently, the tendency to application nanomaterials as nanofertilizer has been increased for smart and more efficient usage based on plant need with controlled release of the elements (Derosa *et al.,* 2010; Mukherjee *et al.,* 2016; Wang *et al.,* 2016). According to the present study, the consumption of 20 mg/L Fe_3O_4/HA NP_S in nutritional solution was effective in improving vegetative traits and led to an increase in the dry and fresh weight of root and shoot, stem height, and branch and leaf number per plant (Table 3).

At the same time, it caused a significant reduction in proline and electrolyte leakage of leaves and an increase in leaf chlorophyll index and absorption of nutrients in the plant as compared to prevalent Fe chelates as control (Tables 3 and 6; Fig. 3 a, b). Different effects of magnetite NPs on plant growth with different sizes and coatings have been reported. In this regard, several reports have been made on the positive effects of magnetite NPs on vegetative properties (Jalali *et al.,* 2016; Roosta *et al.,* 2015; Shahrakizade *et al.,* 2015). Li *et al.* (2016) reported that the effect of Fe_2O_3 iron oxide NPs on plants depends on their concentration. At a concentration of 20 mg/L, it

increases the growth of corn root significantly, but at 50-100 mg/L, it reduces the growth rate in the hydroponic system.

By foliar application of magnetite NPs with a diameter of 15-20 nm (concentration of 100 mg/L) with polyvinylpyrrolidone coating (PVP) to corns in calcareous soils, Jalali *et al.,* (2016) reported that magnetite NPs increased the vegetative growth of the plants and advanced reproductive phase in comparison with Fe-EDDHA. They also increased chlorophyll content and nutrient elements such as Fe and Ca to a greater extent than Fe-EDDHA treatment (Jalali *et al.,* 2017). In a study of the positive effect of soil application of magnetite NPs with EDTA coating at the concentration of 1000 mg/kg and alkaline pH in the root environment, it was found to increase total chlorophyll content, leaf number and Fe, Zn, P and K concentrations of sunflower versus the Fe-EDTA chelate, but it did not affect plant biomass and height (Shahrekizad *et al.,* 2015). But, in the present study the magnetite NPs with HA coating improved plant growth in the hydroponic system with acidic pH. It can be attributed to the effect of alkaline pH of rhizosphere on the NPs with EDTA coating, its effects on apoplastic acidity of the leaf and root cells ($pH \geq 6.3$) and decreased mobility and the reduction of the iron Fe^{3+} despite the increase in Fe content in the plant (Kosegarten *et al.,* 1999; Kosegarten and Koyro, 2001). It can also be caused by the positive effect of HA coating as a chelating agent on increasing the reduction of magnetite NPs as an electron source (Niu et al., 2011; Illes and Tombacz, 2006).

Liu *et al.* (2005) reported that natural organic matters such as HA and organic fertilizers influenced the mobility of Fe_2O_3 NPs and its translocation to peanut leaves and made an increase in the vegetative traits, chlorophyll content, and absorption rate of N, P and K as compared with Fe_2O_3 NPs with citric acid coatings. These results are consistent with reports from Hajdua *et al.* (2009) and Nyerges (2005), who reported that HA increased the solubility of Fe ions by complexing the resulting ions and it caused better Fe nutrition. Shafiee-Masouleh *et al.* (2014) reported that magnetite NPs ($d \leq 20$ nm) with chitosan coatings up to 15 mg/L improved growth, root and shoot fresh and dry weight, and bulblet fresh weight and diameter of *Lilium* in comparison with the mineral Fe salts in a hydroponic system. They explained that the reason was the better supply of Fe to the plants due to the chelating properties of chitosan coating.

The natural organic material coatings, including HA, have a protective effect on the stability and effectiveness of NPs on plants (Chekli *et al.,* 2014; Dickson *et al.,* 2012; Hu *et al.,* 2010; Pariona *et al.,* 2016). Ghafariyan *et al.* (2013) reported that the type of magnetite NP coatings (d=9 nm) had a significant effect on soybean chlorophyll content as compared with chelated Fe of Fe-EDTA so that the dextran coating with a negative surface charge was more effective than its neutral and positive counterparts.

The benefits of natural and cheap humic acid coatings (Chekli *et al.,* 2014) include in creating a negative surface charge on magnetite NPs, which prevents the increase in the diameter of the magnetite NPs during synthesis (Illes and Tombacz, 2006), biocompatibility with cells (Chekanova, *et al.,* 2009), increasing solubility and absorption of nutrients by plant (Denre *et al.,* 2014), especially Fe (Nyerges, 2005; Hajdua *et al.,* 2009) which can be possible reasons for the improved physiological and vegetative traits of chrysanthemum versus chelated Fe of Fe-EDTA and Fe-EDDHA in this research.

A completely positive and significant correlation was observed between shoot dry weight and Fe uptake ($r = 0.914^{***}$) and other nutrients except for Cu. The reason behind the increasing absorption of macro- and microelements under the influence of Fe_3O_4/HA NP_S can be the direct role of Fe in the plants through its effect on the absorption of compounds such as nitrate and the effect on the activity of enzymes such as nitrate reductase as a cofactor. This enzyme plays a crucial role in the production of plant metabolites, such as chlorophyll, nucleic acids, proteins, and other plant materials, which consequently affects photosynthesis and plant growth and development.

So, improved Fe nutrition can indirectly affect the vital activities of plants, increasing cell membrane stability by reducing electrolyte leakage, decreasing plant stress along with the reduction of proline, and promoting plant growth. So, it increases the uptake of nutrients (Borlotti *et al.,* 2012). Magnetite NPs at optimum rates not only increased the chlorophyll content and absorption of nutrients, but they also increased water content of shoots in vegetative growth of chrysanthemum so that, at the optimal rate of magnetite NPs, shoot fresh weight increased significantly in comparison with Fe chelates, while shoot dry weight was not significantly different in similar treatments (Table 3). Zahra *et al.* (2015) reported that the increase in the moisture content of lettuce tissues by magnetite NPs as a nutrient was a result of the increased photocatalytic activity along with increasing chlorophyll content in the shoot of the plant. They also reported that the increased metabolic activity of the plants increased root secretions and reduced the pH of the rhizosphere, which increased nutrient absorption rates, including P, by the plant. Kole *et al.,* (2013) also investigated the effect of Fullerol [$C_{60}(OH)_{20}$] NPs on bitter melon (*Momordica charantia*) and found that this compound not only increased the number of fruits, biomass, fruit length and weight, but it also increased the water content of plant tissues by 128% as compared to the control.

In addition to intracellular biochemical effects, increasing chlorophyll content by using iron oxide superparamagnetic NPs ($SPION_S$) in chrysanthemum can be related to the formation of a magnetic field by NPs, which is effective in ion flow through ion channels of cell membranes and enzymatic structures in different stages of photosynthesis. Atak *et al.* (2007) exposed soybean seedlings to magnetic fluxes and magnetic field strength of 2.9 to 4.6 mT for 2.2 seconds and found that, as compared to the control, it increases fresh weight, chlorophyll content, and the total amount of RNA in the treated plants. The treatment with magnetite NPs reduced proline content and electrolyte leakage of leaf cells versus Fe-EDDHA. It has been documented that magnetite NPs have the potential for catalase- (Wu *et al.,* 2014; Chen *et al.,* 2012) and peroxidase-like activity (Pariona *et al.,* 2016) in cells, thereby reducing free radicals and oxidative stress.

In general, the possible reasons for the superiority of magnetite NPs with humic acid coatings over synthetic Fe chelates can be enumerated as rapid absorption and translocation of NPs from root to shoot without the need for ionization (Cifuentes *et al.,* 2010; Rico *et al.,* 2011), increasing the ratio of Fe^{2+} to Fe^{3+} during gradual ionization magnetite NPs as applicable Fe for physiological processes of plants (Tang and Lo, 2013), non-existence of ethylene compounds in the structure of magnetite NPs structure that was considered the inhibitory effect of ethylene on cell division and chlorosis of plants by synthetic Fe chelates (Albano and Miller, 2001; Dufkova, 1984), and the presence of humic acid along with NPs as a natural, non-toxic and biocompatible substance (Bucak *et al.,* 2012) which can chelate Fe and as electron sources for Fe redox reactions in cells (Vione *et al.,* 2004).

CONCLUSION

The results of this study indicated that Fe absorption in the shoot of the plant was increased significantly and it enhanced leaf chlorophyll content. This shows the successful absorption of magnetite NPs by the plant. At the optimal rate under hydroponic conditions, superparamagnetic NPs of magnetite with humic acid coating improved vegetative properties, decreased cell stress, and increased absorption of nutrients, especially Fe.

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