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Research Paper

Evaluation Uptake and Translocation of Iron Oxide Nanoparticles and Its Effect on Photosynthetic Pigmentation of Chrysanthemum (*Chrysanthemum morifolium*) 'Salvador'

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Abstract

Recently, the use of superparamagnetic iron oxide nanoparticles (SPIONS) as a new and promising source of iron in agriculture has been suggested that further investigation is needed before extensive field use. In a greenhouse experiment, the effect of coated magnetite nanoparticles with humic acid (Fe_3O_4/HA NPs) was investigated on iron deficiency chlorosis and photosynthesis efficiency compared toiron chelates of Fe-EDTA (Fe-Ethylenediaminetetraaceticacid) and Fe-EDDHA [Fe-Ethylenediamine-di (o-hydroxyphenylaceticacid)] as control treatments in chrysanthemum cut flower (*Chrysanthemum morifolium*) in the open hydroponic cultivation system. The feasibility of absorption and translocation of nanoparticles in the plant was evaluated by vibrating sample magnetometry (VSM). The results of tracing by magnetization measurement was demonstrated that NPs penetrated in root and transferred to the aerial parts of chrysanthemum. The greenhouse experiment demonstrated that the application 20 mg/L Fe3O4/HA NPsin nutrient solution significantly ($P<0.001$) increased the content of chlorophylls a, b, total and carotenoids in the leaf 14.80, 12.15, 13.90 and 13.98 percent as compared with Fe-EDTA, respectively, but did not with Fe-EDDHA.Theequivalent ratio of chlorophyll a/b in all concentrations of nanoparticles with Fe-EDTA and Fe-EDDHA treatments, as traditional sources of iron in growth medium, demonstrated no significant difference in photosynthesis efficiency. Generally, Fe_3O_4/HA NPs transferred to plant aerial parts, increased the variety of photosynthetic pigments and obviated iron chlorosis.

Keywords: Chelate, Chlorophyll, Chlorosis, Humic acid, Nanofertilizer.

INTRODUCTION

One of the important tools in the field of modern agriculture is nanotechnology. Engineered Nanomaterials (ENM_S) have recently been widely used in various industrial, medical, and especially agricultural fields (Servin *et al.,* 2015; Verma *et al.,* 2018). Interest in the use of nanoparticles (NP_S) in various parts of agriculture is expanding. Promising advances have been made in the use of nanoparticles as nanofertilizers (NFS) in sustainable agriculture (Aslani *et al.,* 2014; Ditta and Arshad, 2016). Many studies have been conducted on nanoparticles and the effect on the plant and different results have been reported on the effect of nanoparticles on the plant germination, growth, and development) Lin and Xing 2007; El-Temsah and Joner, 2012; Yang *et al.,* 2015; Verma *et al.,* 2018). The mechanisms of toxicity of nanoparticles in plants are still not fully understood and sometimes are attributed to nanoparticles (Kim *et al.,* 2014(and the effects of oxidative stress, and sometimes ions released by nanoparticles in cells (Qian *et al.,* 2013 (or both (Li *et al.,* 2015; Navarro *et al.,* 2008(.

The oxidative stress caused by the production of reactive oxygen species (ROS) by nanoparticles can lead to the activation of the plant defense mechanisms against nano toxicity (Rico *et al.,* 2013; Yang *et al.,* 2017).

Iron is one of the essential elements of plants and plays a crucial role in the production of chlorophyll in photosynthesis. Chlorosis in the young leaves due to iron deficiency is the result of iron-induced chlorosis and it is present in most calcareous soils in arid and semi-arid regions. The high concentration of bicarbonate in the soil solution causes the conversion of Fe^{2+} to Fe^{3+}, and the inaccessibility of iron ions in rhizosphere, as well as in the form of precipitate in the plant vessels (Marshner, 2012(. Thus, in spite of the presence of sufficient and even a lot of iron in the plant, it does not provide the necessary physiological access to the plant by increasing the pH of the cell apoplast (Kosegarten *et al.,* 1999; Kosegarten and Koyro, 2001).

In order to solve this problem, iron chelate fertilizers are commonly used. Synthesis iron chelates, in addition to being expensive, cause direct and indirect damage (Nowack, 2002), including preventing precipitation and increasing the mobility of heavy and radioactive metals in the environment (Means *et al.,* 1978).

There is evidence of absorption and transfer of iron nanoparticles in the plant, and various reports that chlorophyll is increased or reduced due to the use of iron nanoparticles (Ghafariyan *et al.,* 2013; Shahrekizad *et al.,* 2015; Li *et al.,* 20160).

Different reactions of the plant growth against the use of these nanoparticles can be influenced by physio-chemical properties of the nanoparticle, type of coating, a method of application, and plant type (Zhu *et al.,* 2008). Unbeatable physicochemical properties of nanoparticles, such as the surface to volume ratio, high reactivity, the ability to adjust the size and shape of nanoparticles, give a special ability to elements that are not seen in the bulk solid material of the same element (Ditta and Arshad, 2016).

Today, magnetite nanoparticles have been considered for their unique properties, such as easy transfer, low cellular toxicity, good biocompatibility, relative facility of use and environment-friendly (Kong *et al.,* 2010; Zhu *et al.,* 2008).

The coating of nanoparticles has a definite effect on its effectiveness in a plant that should not be toxic in living organisms and must be biocompatible. Typically, negatively charged coatings with longer chain have less toxicity in living cells (Taran *et al.,* 2016).

Hence, the use of iron nanoparticles with natural coatings such as humic acid due to biocompatibility and the stability of nanoparticles with the lowest toxicity to living organisms cells (Bucak *et al.,* 2012(can be considered as a new method to supply iron in plant (Ghafarian *et al.,* 2013; Jalali *et al.,* 2016; Shafiee-Masouleh *et al.,* 2014).

Absorption and translocation of engineered nanoparticles into the plant cells is a complex

process. So far, its mechanism has not been well characterized, which can be attributed to different aspects of the plant anatomy and laboratory challenges for quantitative analysis of engineered nanoparticles in plants (Schwab *et al.,* 2016). The ability to penetrate of nanoparticles into the cell directly due to its very small size without the need for ionization reduces the energy consumption in the mechanisms of absorption and transfer elements into the cell (Brackhage *et al.,* 2013; DeRosa *et al.,* 2010).

Before using any nanoparticle as a nanofertilizer on a large scale, it is necessary first to examine absorption, translocation, and its physiological effects on the plant. In this study, with the purpose of application superparamagnetic iron oxide nanoparticles ofFe_3O_4/HA as a nanofertilizer, we were investigating absorption and distribution of nanoparticles in different organs and the effect on photosynthetic pigments in chrysanthemum as compared with conventional iron chelates in hydroponic conditions.

MATERIALS AND METHODS

Synthesis and characterization of Fe_3O_4/HA NPs

Magnetite nanoparticles with humic acid coating were synthesized based on the modified method by 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 deionized water and heated to 90℃, then combined with 10 ml ammonium hydroxide and 0.5 g 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 nanoparticles were determined by transmissive electron microscope (TEM) (TEM, EM10C-100 KV, Carl Zeiss, Germany)and Vibrating sample magnetometer (VSM)(Danesh Pajoh Kashan, Meghnatice Co., Kashan University), respectively (Asadifard *et al.,* 2005).

Greenhouse experiment

This 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 2017. Cuttings of chrysanthemum (*Chrysanthemum morifolium*) cv. 'Salvador' were rooted in the sand at the beginning of the spring. Then in June, planted in 3 L plastic pots, with an inside diameter of 17 and the height of 15 cm, mixed from perlite of medium size (2-5mm) and fineness (0.5-1.5 mm) in the ratio of 50:50 were used. 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 source of Fe_3O_4/HA NP_S (62.06% Fe) (Equivalent to16.11, 32.16 and 64.22 mg/L nanoparticles,respectively) and 1.4 mg/L pure Fe from two sources of iron chelates which were Fe-EDTA (Fe-Ethylenediaminetetra acetic acid) (13% Iron, Van Iperen Co., Netherlands) and Fe-EDDHA[Fe-Ethylenediaminedi (O-hydroxyphenylacetate)] (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 the open hydroponic cultivation system (Taweesak*et al.,* 2014). 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). The composition of other elements in the nutrient solutions from the first week to the seventh week was: N 250, P 30, K 200, Ca 150, Mg 50, Mn 0.58, Zn 0.35, B 1.0, Cu 0.05, and Mo 0.05mg/L and then until the end of the growth period, contained N 200, P 30, K 200, Ca 150, Mg 50, Mn 0.58, Zn 0.35, B 1.0, Cu 0.05, and Mo 0.05 mg/L (Taweesak *et al.,* 2014). The pH of the nutrient solution was maintained between 5.5 ±0.2 and the electric conductivity (EC) was kept between 1.4 – 1.5 dS/m (Taweesak *et al.,* 2014). In order to adjust pH, 1 molar sulfuric acid solution was used.

Table 1. Physical and chemical properties of used 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 glasshouse light was 27000 Lux with shading (without shading43000 Lux in the summer) and 28000 Lux in the autumn without shading. Pots of chrysanthemum in the greenhouse were placed on the stages (25 plants/square meter). A hydroponic system was open form, with dripper irrigation system. The greenhouse cooling system was a fan and pad. The average daily and night temperature was 25 °C and 16 °C, respectively and optimum relative humidity was 60%. Aphids control was done during the growth period by deltamethrin 0.5 ml/L.

Absorption and translocation of NPs in plant

Evaluation of absorption and translocation of superparamagnetic iron oxide nanoparticles in the plant was performed in the experimental treatment of 40 mg/L pure Fe (NP_{40}) fromFe_3O_4/HA NPs compared with Fe-EDDHA (1.4 mg/L pure iron) as control by vibrating sample magnetometer(VSM) (Danesh Pajoh Kashan, Meghnatice Co., Kashan University) on samples of root, stem and leaf. After 90 days application of experimental treatmentsat vegetative stage, plant samples were dried in an oven (72 °C for 48 hours) (Imamy, 1996), then separately milled and passed through mesh 40 and used in the experiment of VSM (Asadifard *et al.,* 2005).

Chlorophyll content assay

After 90 days application of experimental treatments at vegetative stage, fresh mature leaves from the top of each branch of chrysanthemum (0.05g) were ground in 15ml of 80% acetone and then the obtained homogenates were centrifuged at 1000 rpm for 10 min. The supernatant was collected to determine the absorbance spectrum (A) at 470, 643, 647, and 663 nm with 80% acetone as a reference by Spectrophotometer (Spectronic 20 Geneses, 4001/4, USA). Evaluation for chlorophyll a, b, and total and total carotenoidscontent were determined by the following formula (Lichtenthaler, 1987):

$$\text{Chl a} = 12.25\ A_{663} - 2.79\ A_{647} \quad (1)$$

$$\text{Chl b} = 21.50\ A_{647} - 5.10\ A_{643} \quad (2)$$

$$\text{Total Chls} = \text{Chl a} + \text{Chl b} \quad (3)$$

$$\text{Total carotenoids} = (1000\ A_{470} - 1.82\ \text{Chl a} - 85.02\ \text{Chl b})/198 \quad (4)$$

Statistical analysis

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

RESULTS

Characterization of Fe_3O_4/HANPs

The magnetite nanoparticles with humic acid coating were prepared successfully by a chemical coprecipitation method. TEM images were showed the shape of nanoparticles was almost spherical and size distribution was relatively narrow with a diameter between 2 and 17 nm, with an average of 8.38 nm (N = 200, Sd = ± 3.84).

VSM curve of nanoparticles showed that magnetic saturation of nanoparticles became 55

emu/g. The lack of magnetic residual loop of nanoparticles was suggested the superparamagnetic properties (Fang *et al.,* 2012).

Magnetization in plants after treatment with Fe_3O_4/HA NPs

In order to determine the presence, relative rate and transfer of Fe_3O_4/HA from the root to aerial parts, after 90 days application of experimental treatments, plant samples were takenfrom different parts of treated plants with 40 mg/L of Fe_3O_4 NPs in comparison with control treatment of Fe-EDDHA iron chelate. Magnetic signals were not observed in the plant shoot and root samples of control treatment, due to lack of magnetic properties of iron ions (Fig.1a, b) (Ghafarian *et al.,* 2013). While theywere observed and measured in samples of different organs of the plant treated with nanoparticles (Fig.1c, d, e, f). In this study, the magnetism of each gram of nanoparticles, as mentioned previously, was 55.00 emu/g, which is consistent with the results of Zhu *et al.,* (2008) that reported 53.19 emu/g. Each memu/g is equal to 8.48×10^{11} magnetite nanoparticles per gram (assuming Fe_3O_4 particles have a density of 5.17 g/cm^3).

As shown in Fig. 1f, the strongest magnetic signal measured by VSM was 7.20 and 755 memu/g respectively for crown and root (Fig. 1e, f). The weakest magnetic signal was determined by the stem samples (Fig.1d). In comparison with the shoot, the strongest signals were observed from the root of the plant, as reported in previous studies, which can be due to the adsorption of more nanoparticles at the root surface (Miralles *et al.,* 2012) or accumulation behind the Casparian strip in the apoplast (Sun *et al.,* 2014). Hence, a small part of the nanoparticles entered the plant root central cylinder and shoot, which is consistent with the results of previous studies (Ghafarian *et al*., 2013; Li *et al.,* 2016; Zhu *et al.*, 2008).

Hence, measured magnetic signals in the crown of the plant were considered as an index of the number of nanoparticles introduced to the central cylinder. So, to calculate the ratio of iron transfer to the leaf from the root, it was used the ratio of the magnetic signals of leaf to crown (Ghafarian *et al.,* 2013). Therefore, iron transfer factor was determined 0.857.

The factors affecting the absorption and transfer of nanoparticles in the plant are the type of coating and its surface charge. So that, superparamagnetic nanoparticles of dextran-coated with a diameter of 9 nm at a concentration of 60 mg per liter of a nutrient solution were transferred to soybean shoot that absorption and transfer of nanoparticles with positive and negative charge exceeded uncharged nanoparticles. The maximum transfer factor was reported at 0.649 for nanoparticles of iron with a negative charge. Most of the magnetic signals were received from the root, indicating the accumulation of nanoparticles in the root (Ghafarian *et al.,* 2013) that was consistent with the results of this experiment, while in this study, transfer factor and the number of nanoparticles transferred to the leaf increased 32.05 % and 3.83 times, respectively.

Zhu *et al.* (2008) with investigating absorption and transfer of 20 nm magnetic nanoparticles by a VSM test in pumpkin (*Cucurbita mixta*) after 20 days under hydroponic conditions reported that while transferring nanoparticles from root to leaf, the highest magnetic signal was obtained from the root. On the other hand, Wang *et al*. (2011), stated that nanoparticles with a diameter of 25 nm could not be transmitted to pumpkin shoot (*Cucurbita mixta*), which could be due to the larger size of the nanoparticles, which had larger size NPs to penetrate problem through the cell wall and across transport in the cell membrane (Zhu *et al.,* 2009). It has been reported that carbon coated magnetic nanoparticles (d=10 nm) were transferred from roots to leaves in sunflower, wheat, chickpea and tomato seedlings in less than 24 hours (Cifuentes *et al.,* 2010). According to the diameter of cell wall cavities (5 to 20 nm) (Fleischer *et al*., 1999) and studies conducted, nanoparticles with a diameter of less than 20 nm can reach and cross the plasma membranes (Zhang *et al.,* 2008). It is also possible to increase the size of the cavities and enter through the endositose by the plasma membrane (Nair *et al.,* 2010).

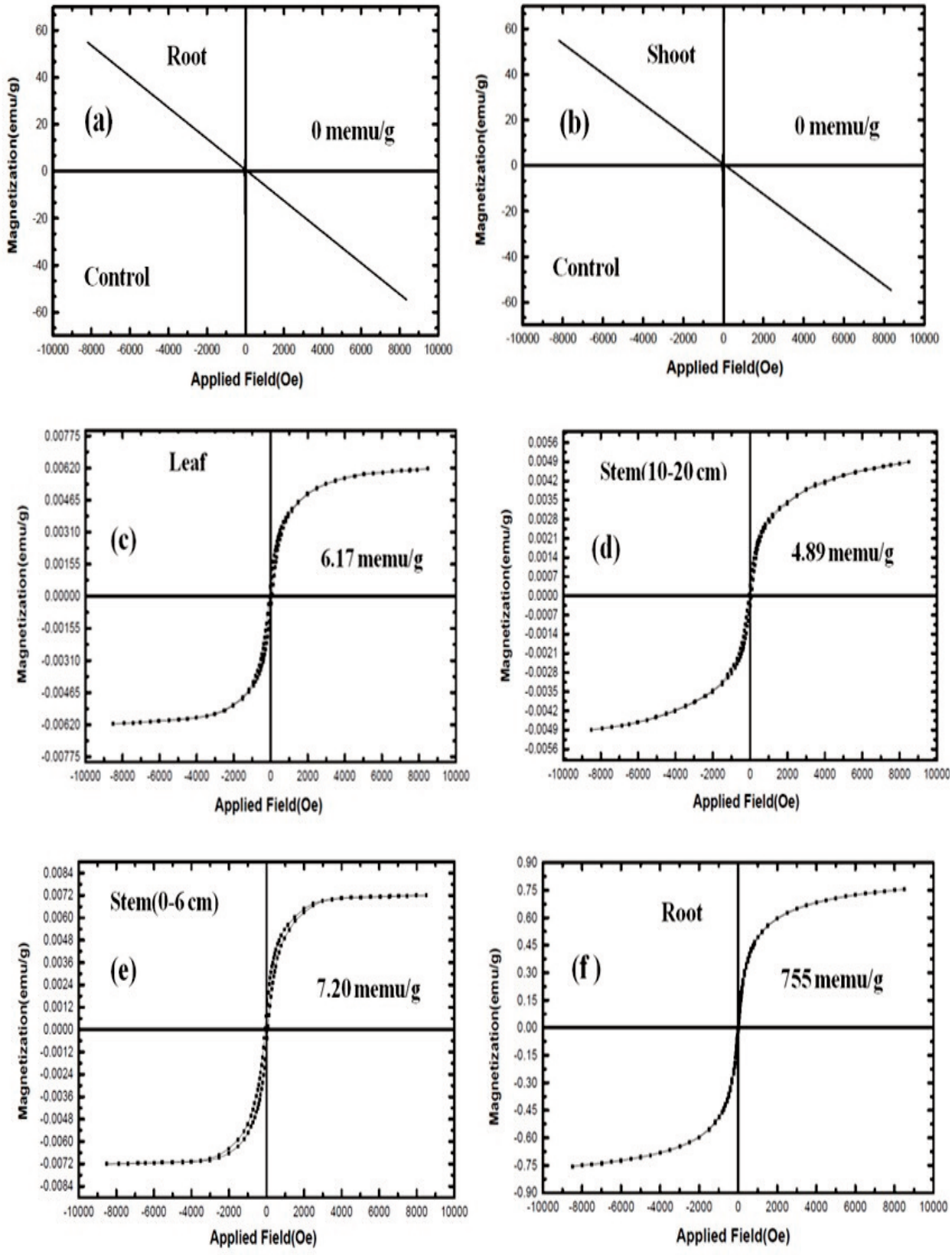

Fig.1. Selected VSM curves of chrysanthemum plant tissues for Fe_3O_4/HA NP_S: (a) control Fe-EDDHA (root), (b) control Fe-EDDHA (shoot), (c) treated plant leaf, (d) treated plant stem (10-20 cm), e) treated plant crown (0-6 cm) and (f) treated plant root. Symbols c, d, e, and f show the samples treated with 40 mg/L superparamagnetic Fe_3O_4/HA NPS.

Effect of Fe_3O_4/HA NPs on photosynthetic pigments and carotenoids

The analysis of variance for deferent kind of chlorophyll in chrysanthemum indicated that the effect of various Fe_3O_4/HA NP_S levels on Chl a, Chl b, carotenoids, total chlorophyll,and chlorophyll a+b to carotenoids ratio, was statistically significant, but it was not for Chl a / Chl b ratio (Table 2).

Table 2. Analysis of variance for the effect of different Fe_3O_4/HA NPs levels in relative to iron chelates on chlorophyll content in chrysanthemum leaf.

S.o.V	df	MS					
		Chla	Chl b	Carotenoids	Total chlorophylls	Chl a/Chl b	Chl (a+b) / carotenoids
Treatment	4	2.701***	0.642***	0.1393***	5.842***	0.0147 ns	0.106*
Error	15	0.208	0.062	0.0069	0.345	0.0152	0.035
CV (%)		4.37	5.28	3.09	3.86	5.59	3.31

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

Figs.2a to d illustrate that photosynthetic pigment content of chlorophyll and carotenoids in chrysanthemum depends on the concentration of Fe_3O_4/HA NPs in the growth medium. Increasing the concentration of iron nanoparticles motivated increasing the chlorophyll a, b, total and carotenoids at levels 20 and 40 in comparison with 10 mg/L and control treatments. 20 mg/LNPs compared to Fe-EDTA caused increasing chlorophyll a, b, total and carotenoids by 14.80, 12.15, 13.90, and 13.98% respectively, significantly but compared to Fe-EDDHA had no significant difference (Fig. 2a, b, c, d). Ghafariyan *et al.,* (2013) reported that magnetite nanoparticles with a diameter of 9 nm and dextran coating increased chlorophyll a, b and total of soybean by increasing the concentration of nanoparticles compared to Fe-EDTA. Also, chlorophyll of oak seedlings (*Quercus macdougallii*) treated with magnetite nanoparticles with citrate coating, with 6 to 10 nm diameter increased in the early stages of growth (Pariona *et al.,* 2017).

Jalali *et al.* (2016) reported that foliar application of magnetite nanoparticles a diameter of 15 to 20 nm and polyvinyl pyrrolidone coating increased total chlorophyll content in maize compared to Fe-EDDHA.

Also, magnetite nanoparticles with EDTA coating increased concentrations of chlorophyll a, b, and total and carotenoids in sunflower by soil application method (Shahrekizad *et al*., 2015).

Fig.2g and h demonstrated the linear regression relationship between chlorophyll a and b and carotenoids with a coefficient of determination of more than 90% ($R^2 \geq 0.90$ **), which indicates that Fe_3O_4/HA NPs and iron chelates treatments similarly affected on biosynthesis the main pigments of photosynthesis in leaf, which is consistent with previous results (Ursache-Oprisan *et al.,* 2011).

Light harvesting complex (LHC) II in photosystem II is an important part of the photosynthetic system in chloroplasts, which is considered as an index of the efficiency of the photosynthesis process, it is determined by the ratio of chlorophyll a/b(Hopkins, 1999). This important physiological parameter is shown in Fig.2e. In all treatments of nanoparticles in relative to Fe-EDTA increased LHC II index 7.98, 3.76 and 3.76 %, in 10, 20 and 40 mg/L treatments of iron nanoparticles, respectively, although the difference was not statistically significant.

The ratio of chlorophyll a + b to carotenoids did not also differ significantly at concentrations of 20 and 40 mg/L of nanoparticles with iron chelates as the control (Fig. 2f), although this

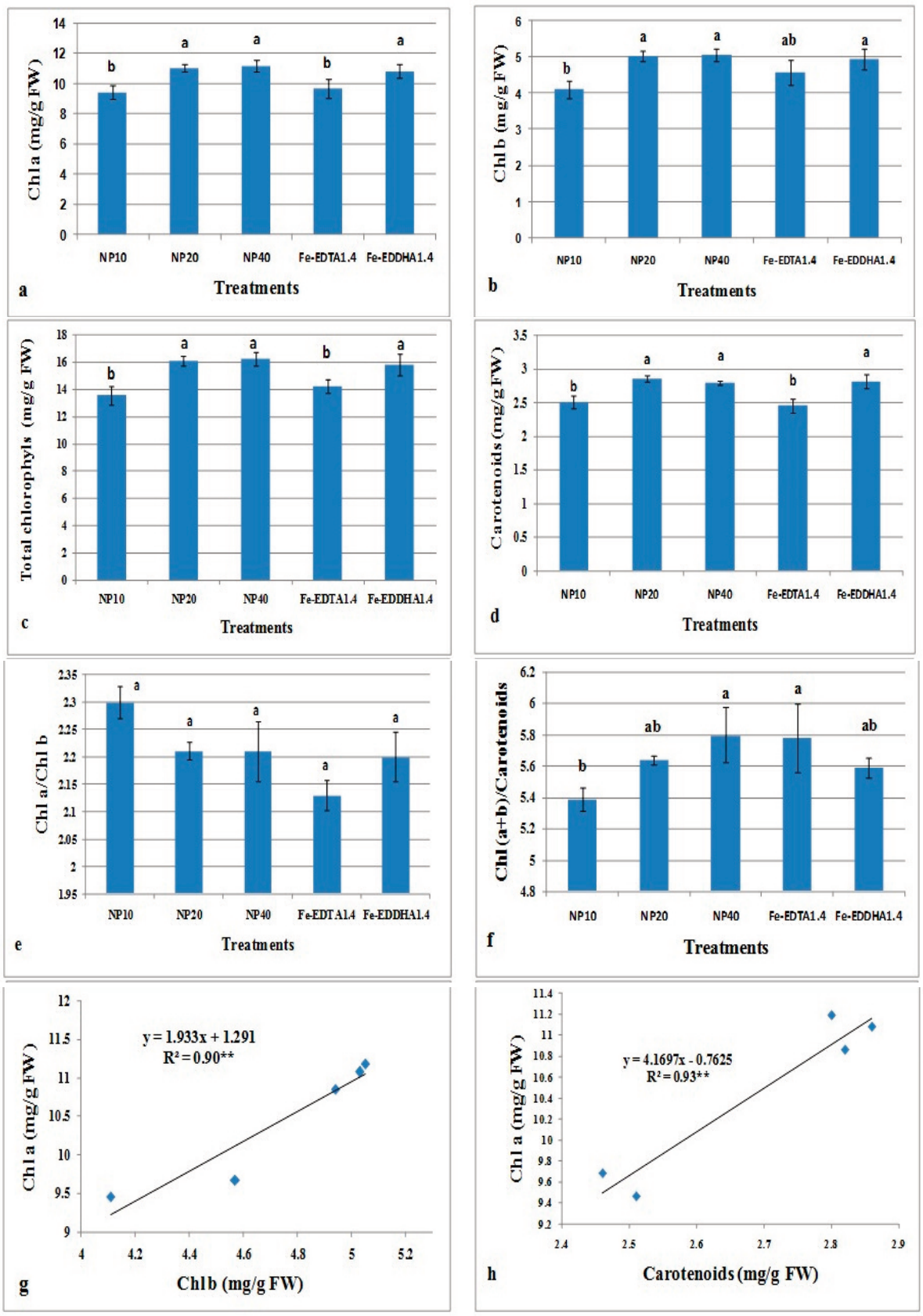

Fig. 2. Effects of different Fe3O4/HA NPS levels in relative to iron chelates on chlorophyll a (a) and chlorophyll b (b), total chlorophyll (c), total carotenoids (d) chlorophyll a to b ratio (e), chlorophyll (a+b) to carotenoids ratio (f), (Significant at 5% level of probability by Duncan test) and linear regression between chlorophyll a with chlorophyll b (g) and total carotenoids (h) in chrysanthemum leaves.

effect was related to the concentration of nanoparticles and in 10 mg/L of nanoparticles, a reduction was observed compared to Fe-EDTA control.

Despite the increase in chlorophyll a, b and carotenoids, a relative increase in chlorophyll a**,** as the most important component of chlorophyll was higher than the total carotenoids at concentrations of 10 to 20 and 40 mg/L of nanoparticles**.**

Previous studies showed that magnetite nanoparticles with EDTA coating, by foliar application, increased carotenoids and chlorophyll a + b ratios to carotenoids, but reduced LHC II index in sunflower compared to Fe-EDTA as control by soil application method (Shahrekizad *et al*., 2015). Ursache-Oprisan *et al*. (2011) reported that magnetite nanoparticles with sodium oleate coating reduced kind of chlorophylls, carotenoids and LHC II index in sunflower seedling compared to the control(without NPs). The difference in the results observed in this experiment can be related to the effect of the type of nanoparticle coating and the method of application(Liu *et al.,* 2005).

DISCUSSION

The use of nanoparticles with a new coating and knowledge of toxicity, absorption, and transfer to the plant as the most important component of the ecosystem are important for estimating the effect it on the environment and safe use.

In this experiment the reason for higher transfer factor of superparamagnetic Fe_3O_4/HA NPs(0.857, Fig. 1e, c) in relative to previous study (0.649, Ghafarian *et al.,* 2013) can be related with a negative surface charge of humic acid on iron nanoparticles that facilitates transfer in the plant (Ghafarian *et al.,* 2013), biocompatibility with the cell (Anastasia *et al.,* 2015), increase solubility and absorption of nutrients of the plant (Denre *et al.,* 2014), especially iron (Nyerges, 2005; Hajdua *et al.,* 2009), which can be a potential factor in improving absorption and transfer of iron, and physiological properties compared to Fe-EDTA and Fe-EDDHA iron chelates.

Previous studies indicated that low molecular weight humic acid fractions are taken up both actively and passively, whereas humic acid of molecular weight> 50000 daltons is taken up only passively (Vaughan and Ord, 1981). Humic materials increase the permeability of the cell membrane, resulting in an increase in nutrient uptake, especially iron (Shenker and Chen, 2005). Increasing the permeability of the cell membrane by humic acid is probably related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites (Chen and Schnitzer, 1978).

On the other hand, the widespread distribution of nanoparticles (diameter from 2 to 17 nm with a mean of 8.38 nm) can increase the probability of penetration and transfer of nanoparticles in this study in relative to a narrow range of 9 nm (single size).

The reasons of accumulation of nanoparticles in the root included penetration and transfer of iron oxide nanoparticles into the plant through apoplastic space from the epidermis to endoderm and accumulation in the plant cell vacuole (Li *et al.,* 2016; Pariona *et al.,* 2017). One of the obstacles to the movement of apoplastic nanoparticles can be Casparianstrip in the root cells. In studies conducted, it was found that Casparianstrip in zones called passage cells,penetration of nanoparticles is possible (Fahn, 1982; Luttge, 1971; Yamaji and Ma, 2014), and in addition, in young roots that still do not form Casparianstrip it is also feasible to transfer nanoparticles to the vascular system of the root (Sattelmacher and Horst, 2007; Zhang *et al.,* 2011; Schwab *et al.,* 2016). Nevertheless, TEM images showed that most magnetic iron oxide nanoparticles of -Fe_2O_3 accumulated in maize with a diameter of 17.7 nm in the root epidermis (Li *et al.,* 2016). The strong magnetic signals observed in the root clearly indicate the frequency of nanoparticles in surface absorbed / trappedin the root tissue, which can be due to absorption of macromolecules secreted from the root (such as proteins and polysaccharides) (Ghafarian *et al.,* 2013; Rauchand Creang,

2013; Westmeier *et al.,* 2016). The pore size also plays a decisive role in the transfer of nanoparticles to the shoot.

For the mechanism of iron nanoparticles on the increase in the plant chlorophyll, it can be stated that the efficiency of the photosynthetic system is widely dependent on the iron ion. Iron deficiency leads to a reduction in the concentration of chlorophyll, which affects the metabolism of photosynthesis and plant development. Banijamali *et al.,* (unpublished data) reported that Fe_3O_4/HA NPs at a concentration of 20 mg/L of a nutrient solution in the hydroponic system increased the total iron absorption in chrysanthemum (2.30 mg per plant), and total biomass of the plant (43.75 g / plant), which increased by 42.86%, 22.99% and 35.91%, 38.54%, compared to Fe-EDTA and Fe-EDDHA, respectively. Iron ion supply to the photosynthetic system restricted can be due to proton secretion by rhizosphere of chrysanthemum root and iron ion release by iron nanoparticle for chlorophyll (Liu *et al.,* 2008). On the other hand, superparamagnetic magnetite nanoparticle in redox reactions of chloroplasts can provide iron ion for and biochemical reactions in chloroplast stroma, siderophores in thylakoid membranes(Racuciu and Creang, 2009) and photocatalytic reactions (Ghafariyan *et al*., 2013).

The mechanism of the effect of nanoparticles in addition to biochemical effects can also be due to the effect of the magnetic field of nanoparticles on enzymatic structures at different stages of enzymatic reactions (Atak *et al.*, 2007). Previous studies showed that low concentrations of iron magnetic nanoparticles in ferrofluids led to an increase in chlorophyll content in bean (Sala*,* 1999) and soybean seedlings (Atak *et al.*, 2007).

In conducted studies, iron oxide NPs in oak (Pariona *et al.,* 2017) and watermelon (Li *et al.,* 2013) motivated increasing growth and chlorophyll. The reason could be attributed to the appropriate amount of reactive oxygen species (ROS) production by Fe_3O_4 NPs magnetic nanoparticles is influenced by the application of optimal nanoparticle concentrations (Fig. 2a, b, c, d)(Sharma *et al.,* 2012; Yang *et al.,* 2017).

The positive effect of nanoparticles coating with humic acid in this experiment can be its role as an electron source that increases the reduction of iron nanoparticles. Liu *et al.,* (2005) reported that natural organic matter such as humic acid and organic fertilizers affected the mobility of iron oxide nanoparticles (Fe_2O_3) and transfer to the leaves of peanut and improved chlorophyll compared with iron nanoparticles with the citric acid coating. The usage of humic acid in nutrient solutions or foliar application in tomato and foliar application in begonia increased the concentration of chlorophyll and photosynthesis (Sladky, 1959; Sladky and Tichy, 1959). The application of mixed of peat and iron increased chlorophyll content in wheat, which did not have a significant difference with Fe-EDDHA treatment (Barak and Chen, 1982).

CONCLUSION

The study results demonstrated that the absorption of iron nanoparticles through the root and transmitted to the aerial parts of the plant was confirmed by magneto meter method. The presence of nanoparticles in the leaf increased the different kinds of photosynthetic pigments including chlorophylls a, b, total and carotenoids in relative to iron chelate of Fe-EDTA. No difference was observed in photosynthesis efficiency between iron nanoparticles and iron chelates treatments as iron sources. Generally, it seems that the coating of humic acid has improved biocompatibility and effectiveness of nanoparticles in chrysanthemum. Further studies are needed to understand the fate of nanoparticles in the ecosystem and their effects on plants.

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Literature Cited

Antisari, L.V., Carbone, S., Gatti, A., Vianello, G. and Nannipieri, P. 2015. Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide (CeO_2, Fe_3O_4, SnO_2, TiO_2) or metallic (Ag, Co, Ni) engineered nanoparticles. Environmental Science and Pollution Research, 22: 1841-1853.

Asadifard, R., Tilki, R., Rangbar, M., Dini, M., Arab, A., Gajavand, M. and Moradi, A. 2005. Introduction to nanotechnology laboratory equipment: Measurement and characterization. Nanotechnology Laboratory of Iran. Secretariat of the Headquarters for Nanotechnology Development. Science and Technology, Iran. 158 p. (In Persian)

Aslani, F., Bagheri, S., Muhd Julkapli, N., Juraimi, A.S., Hashemi, F.S.G. and Baghdadi, A. 2014. Effects of engineered nanomaterials on plants growth: An overview. The Scientific World Journal.Volume 2014, Article ID 641759, 28 pages.

Atak, C., Celik, O., Olgun, A., Alikamanoglu, S. and Rzakoulieva, A. 2007. Effect of magnetic field on peroxidase activities of soybean tissue culture. Biotechnology and Biotechnological Equipment, 21 (2): 166-171.

Barak, P. and Chen, Y. 1982. The evaluation of iron deficiency using a bioassay-type test 1. Soil Science Society of America Journal, 46 (5): 1019-1022.

Brackhage, C., Schaller, J., Bäucker, E. and Dudel, E.G. 2013. Silicon availability affects the stoichiometry and content of calcium and micronutrients in the leaves of common reed. Silicon, 5 (3): 199-204.

Bucak, S., Yavuzturk, B. and Sezer, A.D. 2012. Magnetic nanoparticles: Synthesis, surface modifications and application in drug delivery. *In*: Recent advances in novel drug carrier systems. In Tech. Chapter 7, 36 page.

Chen, Y. and Schnitzer, M. 1978. The surface tension of aqueous solutions of soil humic substances. Soil Science, 125 (1): 7-15.

Cifuentes, Z., Custardoy, L., de la Fuente, J.M., Marquina, C., Ibarra, M.R., Rubiales, D. and Pérez-de-Luque, A. 2010. Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the root of different crop plants. Journal of Nanobiotechnology, 8: 26.

Denre, M., Ghanti, S. and Sarkar, K. 2014. Effect of humic acid application on accumulation of mineral nutrition and pungency in garlic (*Allium sativum* L.). International Journal of Biotechnology and Molecular Biology Research, 5 (2): 7-12.

DeRosa, M.C., Monreal, C., Schnitzer, M., Walsh, R. and Sultan, Y. 2010. Nanotechnology in fertilizers. Nature Nanotechnology, 5 (2): 91–94.

Ditta, A. and Arshad, M. 2016. Applications and perspectives of using nanomaterials for sustainable plant nutrition. Nanotechnology Reviews, 5 (2): 209-229.

El-Temsah, Y. S. and Joner, E.J. 2012. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environmental Toxicology, 27 (1): 42-49.

Fahn, A. 1982. Plant anatomy. Pergamon Press, Oxford UK.

Fang, M., Strom, V., Olsson, R.T., Belova, L. and Rao, K.V. 2012. Particle size and magnetic properties dependence on growth temperature for rapid mixed co-precipitated magnetite nanoparticles. Nanotechnology, 23 (14): 1-9.

Fleischer, A., Oneill, M.A. and Ehwald, R. 1999. The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. Plant Physiology, 121 (3): 829-838.

Fonteno, W.C. and Bilderback, T.E. 1993. Impact of hydrogel on physical properties of coarse-structured horticultural substrates. Journal of the American Society, 118 (2): 217-222.

Ghafariyan, M.H., Malakouti, M.J., Dadpour, M.R., Stroeve, P. and Mahmoudi, M. 2013. Effects of magnetite nanoparticles on soybean chlorophyll. Journal of Science and Technology, 47:

10645−10652.

Hajdua, A., Illes, E., Tombacz, E. and Borbath, I. 2009. Surface charging polyanionic coating and colloid stability of magnetite nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 347: 104–108.

Hopkins, W. G. 1999. Introduction to plant physiology. John Wiley and Sons. 528 page.

Imamy, A. 1996. Plant decomposition methods. Soil and Water Research Institute, Tehran Technical Bulletin, No. 982. (In Persian)

Jalali, M., Ghanati, F. and Modarres-Sanavi, A.M. 2016. Effect of Fe_3O_4 nanoparticles and iron chelate on the antioxidant capacity and nutritional value of soil-cultivated maize (*Zea mays*) plants. Crop and Pasture Science, 67: 621–628.

Kim, J., Lee, Y., Kim, E., Gu, S., Sohn, E.J., Seo, Y.S. and Chang, Y.S. 2014. Exposure of iron nanoparticles to *Arabidopsis thaliana* enhances root elongation by triggering cell wall loosening. Environmental Science and Technology, 48 (6): 3477-3485.

Kong, H., Song, J. and Jang, J. 2010. One-step fabrication of C-Fe_2O_3/polyrhodanine magnetic nanoparticles using *in situ* chemical oxidation polymerization and their antibacterial properties. Chemical Communications, 46 (36): 6735–6737.

Kosegarten, H. and Koyro, H.W. 2001. Apo plastic accumulation of iron in the epidermis of maize (*Zea mays*) roots grown in calcareous soil. Physiologia Plantarum, 113 (4): 515–522.

Kosegarten, H.U., Hoffmann, H. and Menge, K.1999. Apo plastic pH and Fe^{3+} reduction in intact sunflower leaves. Harald Plant Physiology, 121 (4): 1069–1079.

Li, J., Chang, P., Huang, J., Wang, Y., Yuan, H. and Ren, H. 2013. Physiological effects of magnetic iron oxide nanoparticles towards watermelon. Journal of Nanoscience and Nanotechnology, 13 (8): 5561-5567.

Li, J., Hu, J., Ma, C., Wang, Y., Wu, C., Huang, J. and Xing, B. 2016. Uptake, translocation and physiological effects of magnetic iron oxide (γ-Fe_2O_3) nanoparticles in corn (*Zea mays* L.). Chemosphere, 159: 326-334.

Li, L., Wu, H., Peijnenburg, W. and Gestel, C. 2015. Both released silver ions and particulate Ag contribute to the toxicity of Ag NPs to earthworm *Eiseniafetida*. Nanotoxicology, 9 (6): 792-801.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. Methods in Enzymology, 148: 350-382.

Lin, D. and Xing, B. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution, 150 (2): 243-250.

Liu, J.F., Zhao, Z.S. and Jiang, G.B. 2008. Coating Fe_3O_4 magnetic nanoparticles with humic acid for high efficient removal of heavy metals in water. Environmental Science and Technology, 42: 6949–6954.

Liu, X.M., Zhang, F.D., Feg, Z.B., Zhang, S.Q., He, X.S., Wang, R.F. and Wang, Y.J. 2005. Effects of nano-ferric oxide on the growth and nutrients absorption of peanut. Journal of Plant Nutrition Fertilizer, 11: 551-555.

Luttge, U. 1971. Structure and function of plant glands. Annual Review of Plant Physiology, 22: 23–44.

Maity, D. and Agrawal, D.C. 2007. Synthesis of iron oxide nanoparticles under oxidizing environment and their stabilization in aqueous and non-aqueous media. Journal of Magnetism and Magnetic Materials, 308: 46–55.

Marshner, H. 2012. Mineral nutrition of higher Plants. Third Edition. Publication of Elsevier, Oxford, 649 p.

Means, J.L., Crerar, D.A. and Duguid, J.O. 1978. Migration of radioactive wastes: Radionuclide mobilization by complexing agents. Science, 200: 1477-1481.

Miralles, P., Church, T.L. and Harris, A.T. 2012. Toxicity, uptake, and translocation of engineered

nanomaterials in vascular plants. Journal of Environmental Science and Technology, 46 (17): 9224−9239.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y. and Sakthi Kumar, D. 2010. Nanoparticulate material delivery to plants. Plant Science, 179: 54-163.

Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L. and Behra, R. 2008. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Journal of Environmental Science and Technology, 42: 8959-8964.

Nowack, B. 2002. Environmental chemistry of aminopolycarboxylate chelating agents. Journal of Environmental Science and Technology, 36 (19): 4009−4016.

Nyerges, E.I. 2005. Interaction of humic acid with magnetite nanoparticles: From soils to magnetic fluids. Ph.D. Thesis, Department of Colloid Chemistry, University of Szeged, Szeged, Hungary.

Pariona, N., Martínez, A.I., Hernandez-Flores, H. and Clark-Tapia, R. 2017. Effect of magnetite nanoparticles on the germination and early growth of *Quercus macdougallii*. Science of the Total Environment, 575: 869-875.

Qian, H., Peng, X., Han, X., Ren, J., Sun, L. and Fu, Z. 2013. Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. Journal of Environmental Sciences, 25 (9): 1947-1956.

Racuciu, M. and Creang, D.E. 2009. Biocompatible magnetic fluid nanoparticles internalized in vegetal tissue. Romanian Journal of Physics, 54: 115-124.

Rauch, J., Kolch, W., Laurent, S. and Mahmoudi, M. 2013. Big signals from small particles: Regulation of cell signaling pathways by nanoparticles. Chemical Reviews, 113 (5): 3391-3406.

Rico, C.M., Hong, J., Morales, M.I., Zhao, L., Barrios, A.C., Zhang, J.Y., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. 2013. Effect of cerium oxide nanoparticles on rice: A study involving the antioxidant defense system and *in vivo* fluorescence imaging. Environmental Science and Technology, 47 (11): 5635-5642.

Sala, F. 1999. Magnetic fluids effect upon growth processes in plants. Journal of Magnetism and Magnetic Materials, 201 (1): 440−442.

Sattelmacher, B. and Horst, W.J. 2007. The apoplast of higher plants: Compartment of storage, transport and reactions–the significance of the apoplast for the mineral nutrition of higher plants. Dordrecht, Netherlands: Springer.

Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J. L. and Wiesner, M.R. 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants: Critical review. Nanotoxicology, 10 (3): 257-278.

Servin, A., Elmer, W., Mukherjee, A., Dela Torre-Roche, R., Hamdi, H., White, J.C., Bindraban, P. and Dimkpa, C. 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. Journal of Nanoparticle Research, 17 (2): 1-21.

Shafiee-Masouleh, S.S., Hatamzadeh, A., Samizadeh, H. and Rad-Moghadam, K. 2014. Enlarging bulblet by magnetic and chelating structures of nano-chitosan as supplementary fertilizer in *Lilium*. Horticulture, Environment, and Biotechnology, 55 (6): 437-444.

Shahrekizad, M., Gholamalizadeh Ahangar A. and Mir, N. 2015. EDTA-Coated Fe_3O_4nanoparticles: A novel biocompatible fertilizer for improving agronomic traits of sunflower (*Helianthus annuus*). Journal of Nanostructures, 5 (2): 117-127.

Sharma, P., Bhushan Jha, A., Shanker Dubey, R. and Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany, Volume 2012, Article ID 217037, 26 pages.

Shenker, M. and Chen, Y. 2005. Increasing iron availability to crops: Fertilizers, organo fertilizers, and biological approaches. Soil Science and Plant Nutrition, 51 (1): 1-17.

Sladky, Z. 1959. The effect of extracted humus substances on growth of tomato plants. Biologia Plan-

tarum, 1 (2): 142-150.
Sladky, Z. and Tichy, V. 1959. Application of humus substances to over ground organs of plants. Biologia Plantarum, 1 (1): 9-15.
Sun, D., Hussain, H.I., Yi, Z., Siegele, R., Cresswell, T., Kong, L. and Cahill, D.M. 2014. Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. Plant Cell Reports, 33 (8): 1389-1402.
Taran, M., Amoabediny, G. and Kashanian, F. 2016. The effect of coating on the toxicity of magnetic nanoparticles. Nanotechnology Monthly, 10: 21-27. (In Persian)
Taweesak, V., Abdullah, T.L., Hassan, S.A., Kamarulzaman, N.H. and Yusoff, W.A.W. 2014. Growth and flowering responses of cut chrysanthemum grown under restricted root volume to irrigation frequency. The Scientific World Journal, Volume 2014, Article ID 254867, 6 pages.
Ursache-Oprisan, M., Focanici, E., Creanga, D. and Caltun, O. 2011. Sunflower chlorophyll levels after magnetic nanoparticle supply. African Journal of Biotechnology, 10 (36): 7092-7098.
Vaughan, D. and Ord, B.G., 1981. Uptake and incorporation of ^{14}C-labelled soil organic matter by roots of *Pisum sativum* L. Journal of Experimental Botany, 32(4): 679-687.
Verma, S.K., Das, A.K., Patel, M.K., Shah, A., Kumar V. and Gantait, S. 2018. Engineered nanomaterials for plant growth and development: A perspective analysis. Science of the Total Environment, 630: 1413–1435.
Wang, H., Kou, X., Pei, Z., Xiao, J. Q., Shan, X. and Xing, B. 2011. Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. Nanotoxicology, 5 (1): 30-42.
Westmeier, D., Stauber, R.H. and Docter, D. 2016. The concept of bio-corona in modulating the toxicity of engineered nanomaterials (ENM). Toxicology and Applied Pharmacology, 299: 53-57.
Yamaji, N. and Ma, J.F. 2014. The node, a hub for mineral nutrient distribution in graminaceous plants.Trends in Plant Science, 19 (9): 556-563.
Yang, J., Cao, W. and Rui, Y. 2017. Interactions between nanoparticles and plants: Phytotoxicity and defense mechanisms. Journal of Plant Interactions, 12 (1): 158-169.
Yang, Z., Chen, J., Dou, R., Gao, X., Mao, C. and Wang, L. 2015. Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.). International Journal of Environmental Research and Public Health, 12 (12): 15100-15109.
Zhang, L., Feng, C. and Chen, Z. 2008. Super aligned carbon nanotube grid for high resolution transmission electron microscopy of nanomaterials. Nano Letters, 8 (8): 2564–2569.
Zhang, Z., He, X., Zhang, H., Ma, Y., Zhang, P., Ding, Y. and Zhao, Y. 2011. Uptake and distribution of ceria nanoparticles in cucumber plants. Metallomics, 3 (8): 816-822.
Zhu, H., Han, J., Xiao, J.Q. and Jin, Y. 2008. Uptake, translocation, and accumulation of manufactured iron oxide nano-particles by pumpkin plants. Journal of Environmental Monitoring, 10 (6): 713-717.
Zhu, R.R., Wang, S.L., Chao, J., Shi, D.L., Zhang, R., Sun, X.Y. and Yao, S.D. 2009. Bio-effects of nano-TiO_2 on DNA and cellular ultrastructure with different polymorph and size. Materials Science and Engineering, 29 (3): 691-696.

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