Journal of Medicinal Plants and By-products (2020) 1: 19-31

Original Article

Investigating the Effect of Magnesium and Iron Oxide Nanoparticles on the Levels of Enzymatic and Non-enzymatic Antioxidants in Roselle

Hasti Kiapour, Payam Moaveni*, Behzad Sani, Faezeh Rajabzadeh and Hamid Mozafari

Department of Agronomy, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

Article History: Received: 04 October 2019/Accepted in revised form: 30 May 2020

Abstract

This experiment was conducted in order to investigate the effect of foliar application of magnesium oxide (MgO) and iron oxide (Fe_2O_3) nanoparticles on the amount of enzymatic and non-enzymatic antioxidants of Roselle (*Hibiscus sabdariffa*) as a medicinal plant by a factorial design in a randomized complete block design with four replications within the year of 2017 in Hashtgerd and Dehak areas. The first factor of the experiment consisted of different concentrations of MgO nanoparticles (zero (control), 0.01 and 0.03%), and the second factor included different concentrations of Fe_2O_3 nanoparticles (zero (control), 0.01 and 0.03%). The results of combined variance analysis of the two tested location indicated that the simple effect of each location treatment, different concentrations of MgO and Fe_2O_3 nanoparticles and the interactions of different concentrations of MgO and Fe_2O_3 nanoparticles on the values of hydrogen peroxide, flavonoids, anthocyanin and activity levels of the enzymes of catalase and peroxidase were significant. The final results of this study showed that MgO and Fe_2O_3 nanoparticles had an additive and significant effect on flavonoids, anthocyanin and the activity of catalase and peroxidase enzymes in comparison with the control treatment, and had a decreasing effect on hydrogen peroxide of this plant. Also, the results showed that the interactions of 0.03% MgO nanop for governing and/or coordinating plant growth under stress conditions articles and 0.03% Fe_2O_3 nanoparticles had an effect on the control treatment. It was also found that the Roselle bushes planted in Dehak region had the higher content of anthocyanin and flavonoids, and activity levels of catalase and peroxidase enzymes, and had the lower levels of hydrogen peroxide, compared with those planted in Hashtgerd area.

Keywords: Roselle, MgO, Fe_2O_3, ZnO, Nanoparticles, Antioxidant.

Introduction

Currently, one third of the medicine consumed by humans has plant origin. The growing demands of pharmaceutical manufacturers for raw materials and the necessity of preserving natural plant sources has duplicate the importance of studying the cultivation and processing of herbal medicine and aromatic plants [1]. In this regard, one of the most important medicinal plants that has a special position in the pharmaceutical industry, is Roselle (*Hibiscus sabdariffa*), widely grows in tropical areas of Iran. The anthocyanin found in the calyx of Roselle has a variety of biological effects including improving blood pressure and lipid profile. Furthermore, anthocyanin has a protective effect on LDL-C against oxidation and atherosclerosis [2]. The importance of this crop cannot be over emphasized. It is used for many different purposes, the most common of which are as a fiber crop, the young leaves are eaten as cooked vegetables especially with soup. The seeds are pounded into meal which is used as oily soup. Oil extracted from the seed is a substitute for castor oil. The fresh calyx is also

*Corresponding author: Department of Agronomy, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
Email Address: payam.moaveni@yahoo.com

rich in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron that are nutritionally important [3].

The shortage of nutrients such as iron and magnesium decreases the yield and quality of plants, and, by causing the hidden starvation and growing of diseases, human health is getting vulnerable [4]. Magnesium is the only metallic element in the chlorophyll of the plant, located in the center of it, therefore the lack of magnesium, reduces the amount of plant chlorophyll [5]. It is highly usable element that activates more than 300 enzymes (e.g., RNA polymerases, ATPase, protein kinase, phosphatase, glutathione synthase and carboxylase) and is also involved in the regulation, transport of ions, and cation balance in plants [6]. Magnesium is also essential for the metabolism of calcium, vitamin C, phosphorus and potassium, and it is very important in converting the blood sugar into energy, however, it is known as an antidote, thus the proper use of magnesium fertilizers in farms where deficiency of this element is observed, can be effective in improving the nutrition, health of human and livestock as well as raising crops [7].

Another element for the growth of plants is micronutrient. These elements are essential in small quantities in plants, but they have important effects on plants from the lack of these elements can sometimes act as a limiting factor for the absorption of other nutrients and growth, so it is necessary to pay more attention to their application. Although the use of micronutrients by foliar application can improve plant growth [8]. One of the most important low-consuming nutrients for plant growth is iron, which is a cofactor of more than 100 enzymes and proteins involved in cell division, nucleic acid metabolism and protein synthesis [9]. Iron is a vital element for plant metabolic processes and is necessary for DNA synthesis, photosynthesis and respiration also plays a key role in metabolism reactions [10]. Iron is also a key element in cellular metabolism and plays a role in the structure of the cytochrome molecule, which in conditions of iron deficiency, photosynthesis decreases sharply. In addition, iron is involved in the process of oxidation and enzymatic reduction, respiration and chlorophyll synthesis in plants [11]. Although the role of the iron is evident in nitrogen fixation and the activity of many enzymes, such as catalase, peroxidase and cytochrome oxidase, but there is still little information about its role in the production of secondary metabolites [12]. One of the ways to move towards advancement and development is to use new science and technology.

Given the many applications of nanotechnology, this technology revolutionizes various sciences, including agriculture, and in the near future will have significant impact on the world's economic plans, too. Considering the novelty of this science, Iran is not so far from the pioneering countries in this science comparing to other sciences, therefore, regarding the high potential of agriculture in Iran, by investing and planning for the application of this technology, it can lead to the development and progress of the country [13]. Nanotechnology is one of the novel technologies that have recently entered into all fields, including agriculture. Nanoparticle is an atomic or molecular particle with at least one dimension less than 100 nm in size, which has different physical and chemical properties in relation to their mass [14].

Nanoparticles can also absorb nutrients on their surface and act as an important food source for organisms, especially in cases where nanoparticles have a high surface area. In addition, due to their antimicrobial properties, nanoparticles can increase resistance and strength of plants against tension [15]. Yousefzadeh and Sabaghnia [16] in a study investigated the effects of various concentrations of iron nanoparticles (0, 1, 2 and 3 g/ L) on the traits of the dragonhead plant in the laboratory. Their results showed that the application of this nanoparticle had a positive and significant effect on the traits of flowering branches, the height of the first flowering branch, the number of lateral branches, stem diameter, percentage and yield of essential oil, content of chlorophylls a, b and total, total anthocyanin, flavonoids and yield of this plant. So that, the highest amount of dry matter and the plant essential oil derived from the use of 1 g/ L of iron nanoparticles.

Soliman *et al.* [17] besides in a similar experiment, studied the effect of different concentrations of iron oxide nanoparticles (30, 60 and 90 mg/ L) on Moringa traits and the results showed that by increasing of the iron nanoparticles concentration to a concentration of 60 mg/ L, the activity of peroxidase enzymes, number of branches per plant and dry and fresh weight of the plants increased, but in the high concentrations of this nanoparticle, the values of these traits decreased significantly. Trankner *et al*. [18] in one investigation about the effects of different concentrations of magnesium (0.01, 0.1 and 0.4 mM) on the barley plant traits in hydroponic culture, were also found that the use of this element increased the values of the total dry weight, chlorophyll index, assimilation and dry weight of young and old leaves, and reduced the amount of hydrogen peroxide of the bushes. Jalali *et al*. [19] in a study investigated the effect of concentrations of 100 ppm of iron nanoparticles and 1 ppm of conventional iron (Bulk) on the characteristics of corn. Their results showed that corn bush treatments with this nanoparticle reduced the amount of hydrogen peroxide and peroxidation of the membranes of the plant. Therefore, due to the lack of information on the effect of nanoparticles on medicinal plants, the present study was conducted in order to investigate the effect of magnesium oxide and iron oxide nanoparticles on the amount of antioxidants in Roselle.

Material and Methods

In order to investigate the effect of foliar application of magnesium oxide and iron oxide nanoparticles on the amount of enzymatic and non-enzymatic antioxidants of Roselle (*Hibiscus sabdarifa*), an experiment was conducted in the year of 2017 on two farms (location) one in Dehak industrial town and another farm in Hashtgerd area were factorial in a randomized complete block design with four replications. The results were analyzed by combined variance analysis.

Hashtgerd area is located in west of Alborz province at an altitude of 1310 m and between longitude

35 °45'N and Latitude of 51°25'E.

Dehak area is located in Shahriyar city and Alborz province at an altitude of 2985.6 m and between latitude 40 °35'N and longitude of 51 °01'E. The average annual temperature and rainfall in Dehak and Hashtgerd were shown in Table 1 and 2.

The first factor consisted of different concentrations of magnesium oxide nanoparticles (zero (control), 0.01 and 0.03%), and the second factor included different concentrations of iron oxide nanoparticles (zero (control), 0.01 and 0.03%). Actions of land preparation were carried out in accordance with the custom of the area by implementing a plough and two perpendicular discs to each other before the planting.

Each experimental unit consisted of 6 planting lines 8 meters long, spaced 70 cm from each other. Prior to planting, a sample of one kilogram from 0 to 30 cm of soil layer from two locations was transferred to the soil and water laboratory to determine of physical and chemical characteristics of soil. Basic fertilizer recommendation such as nitrogen, potassium and phosphorus was used on the basis of the results of the soil test (Table 3). Seeds of Roselle made from Seed Company, were cultivated at intervals of 30 cm on row and 2-5 cm in depth, and also were cultivated on the 24th to 27th of May 2017. Based on the results of the soil test (Table 3), 30 tons. ha^{-1} of cow manure, 100 kg. ha^{-1} triple superphosphate, 150 kg. ha^{-1} potassium sulfate, 35 kg. ha^{-1} zinc sulfate and 200 kg. ha^{-1} ammonium sulfate were used. The first irrigation was leaked after planting, until the emergence stage, irrigation was carried out with a 5-day period and after that, the irrigation period increased from 5 to 7 days. At appropriate times, and mechanical weed control operation was conducted during plant growth by hand. The treatments as well as a foliar application with magnesium oxide and iron oxide nanoparticles were applied at the time of reproductive growth of the Roselle plants based on the recommended amount. 72 hours after foliar application, 10 leaves were randomly trimmed in each treatment and placed in the foil and liquid nitrogen in order to be transferred to the laboratory to determine the biochemical properties of the Roselle, in the freezer -80 °C.

Then, the amount of the activity changes of the antioxidant enzymes of catalase of Roselle was assayed through Aebi method [20], the amount of antioxidant peroxidase activity was assayed according to Chance and Maehley method [21], the amount of anthocyanin was assayed by Wanger method [22], the amount of plant flavonoids, was assayed by the method of Krizek *et al*. [23] and the amount of hydrogen peroxide plant tissue, was assayed according to Hung *et al*. method [24]. Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System and followed by Duncan's multiple range tests. Terms were considered significant at $P < 0.05$.

Results

Hydrogen Peroxide

The results of combined analysis of data showed that the simple effect of test site treatment on the amount of hydrogen peroxide was significant at the 5% level, while the simple effects of foliar application with each of the magnesium and iron nanoparticles, as well as the interactions of foliar application with magnesium nanoparticle on this trait indicated a significant difference statically at the 1% level (Table 4). Also, the results of the mean comparison of data demonstrated that the highest amount of hydrogen peroxide of the roselle (0.38 µmol. g^{-1} fw) was related to the control treatment or non-application of nanoparticles, and treatment of the interactions of 0.03% magnesium nanoparticle and 0.03% iron nanoparticle concentration (0.37 µmol. g^{-1} fw), while the least amount of this trait was observed in the interaction treatment of 0.01% concentration of magnesium nanoparticle and 0.03% concentration of iron nanoparticle treatment (0.15 µmol. g^{-1} fw) with regard to the amount of this trait there was not significant difference with the interaction of the 0.03% concentration of magnesium nanoparticle and concentration of 0.01 percent of iron nanoparticle (0.16 µmol. g^{-1} fw) and these treatments were considered together in one group of statistic (Fig. 1).

As the results of Table 5 indicate, the amount of hydrogen peroxide in the plant of the Roselle in Hashtgerd region with a value of 0.26 µmol. g^{-1}fw, was higher than the amount of hydrogen peroxide of this bushes in Dehak region (0.24 µmol. g^{-1} fw).

Table 1 Average rainfall in the Dehak and Hashtgerd locations during the growing season of the roselle

Location	Average rainfall (mm)						
	May	June	July	August	September	October	November
Hashtgerd	35.2	0	0.6	0	0	12.2	3.2
Dehak	20.6	0	32	0	0	3.1	0.5

Table 2 Average air temperature in the Dehak and Hashtgerd locations during the growing season of the roselle

Location	Average air temperature (Ċ)						
	May	June	July	August	September	October	November
Hashtgerd	20.6	24.9	27.4	26.4	23	16.2	10.1
Dehak	23.7	27.8	30.1	29	25.6	19.3	13.6

Table 3 Analysis of the soil of the experiment site (Dehak and Hashtgerd)

Depth of soil 0-30 cm / Site of experiment	Electrical conductivity (dS/m)	Acidity	Clay (%)	Organic carbon (%)	Sand (%)	Silt (%)	Total nitrogen (%)	Magnesium (mg/kg)	Absorbable phosphorous (mg/kg)	Absorbable potassium (mg/kg)	Iron (mg/kg)
Hashtgerd	4.86	7.67	0.45	34.3	33.4	32.3	0.06	12.18	250.3	3.01	37
Dehak	4.58	7.55	0.57	30	40	30	0.08	22.10	165	3.98	51

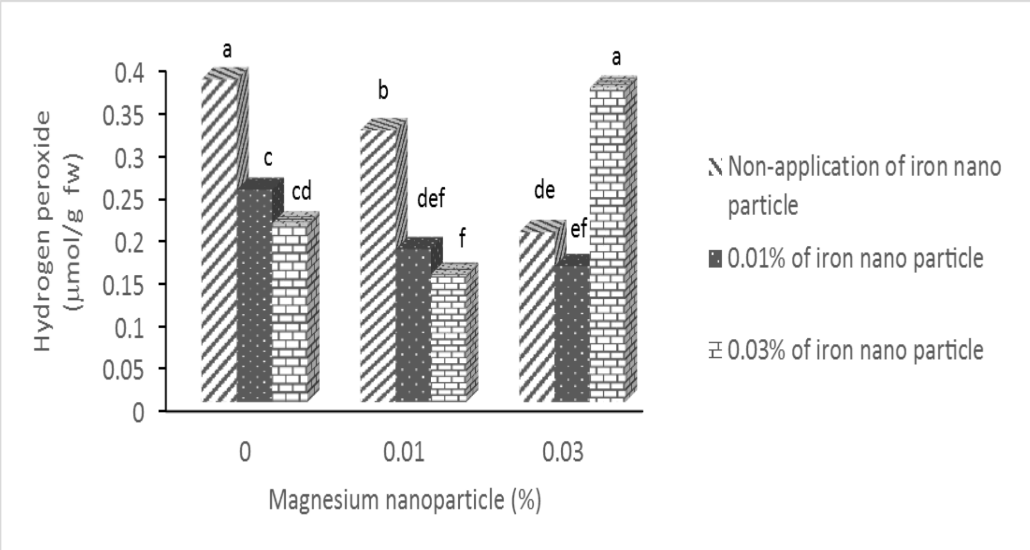

Fig. 1 Effect of interactions of magnesium and iron nanoparticles on hydrogen peroxide

Table 4 Combined variance analysis of biochemical and physiological traits of Roselle in two experimental sites

		Means square				
Sources of variation	df	Hydrogen peroxide	Flavonoids	Anthocyanin	Catalase enzyme activity rate	Peroxidase enzyme activity rate
Location(L)	1	0.0101 *	0.087 *	260.34 *	15.01 *	982.36 *
Error for location	6	0.0027	0.013	17.07	26.22	138.91
Magnesium oxide nano-particles (M)	2	0.0235 **	0.571 **	178.84 *	257.52 **	33355.87 **
Iron oxide nano-particles (I)	2	0.0618 **	0.060 *	274.68 **	150.56 **	7482.93 **
M×I	4	0.0801 **	0.164 **	258.67 **	149.49 **	27253.03 **
L×M	2	0.0042 ns	0.001 ns	3.10 ns	11.81 ns	19.84 ns
L×I	2	0.0019 ns	0.011 ns	9.11 ns	9.97 ns	4.45 ns
L×M×I	4	0.0005 ns	0.028 ns	6.28 ns	10.43 ns	16.21 ns
Error	48	0.0014	0.012	50.77	8.74	168.89
Cv (%)		15.11	7.92	12.60	10.85	8.58

Ns: Non-significant, *: Significant at 5% level and **: Significant at 1%

Table 5 Comparison of the main effects of magnesium and iron oxide nanoparticles on biochemical and physiological traits of Roselle in two experimental sites

Traits / Treatments	Hydrogen peroxide (µmol. g-^{1} fw)	Flavonoids (%)	Anthocyanins (µmol. g^{-1} fw)	Peroxidase enzyme activity (µmol.g^{-1}protein.min^{-1})	Catalase enzyme activity (µmol.g^{-1}protein.min^{-1})
Location					
Hashtgerd	0.26 a	1.36 b	54.61 b	147.75 b	26.40 b
Dehak	0.24 b	1.43 a	58.42 a	155.32 a	28.09 a

Note: Means in the same columns and rows, followed by the same letter, are not significantly difference (P<0.05)

Flavonoids

According to the results of the analysis of variance observed in Table 4, simple effects of location of experiment treatment and concentration of Nano particle of iron was significant at the 5% level on the amount of flavonoids content, while the simple effects of foliar application with magnesium nanoparticle, as well as the interactions of foliar application with magnesium and iron nanoparticle on this trait indicated a significant difference statically at the 1% level. As the results of the mean comparison (Fig. 2) showed, the highest flavonoid content of this plant (1.61%) belongs to the interaction treatment of 0.01% concentration of magnesium nanoparticle and concentration of 0.01% of iron nanoparticles and also interactions of 0.01% magnesium nanoparticle concentration and 0.03% iron nanoparticle concentration, which by considering amount of this trait,there was no significant difference with interaction of 0.03% magnesium nanoparticle and 0.01% iron nanoparticle treatment (1.54%).

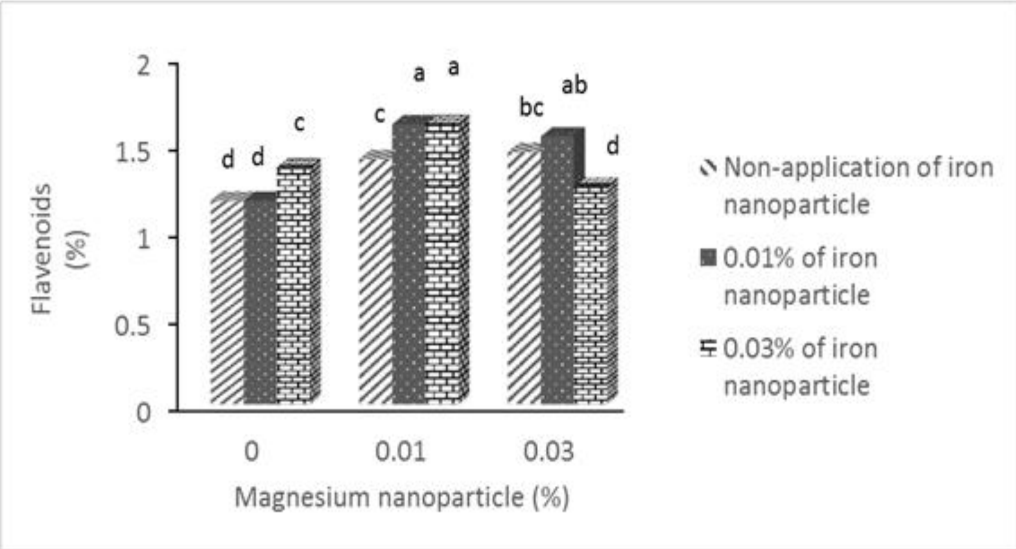

Fig. 2 Effect of interactions of magnesium and iron nanoparticles on flavonoids

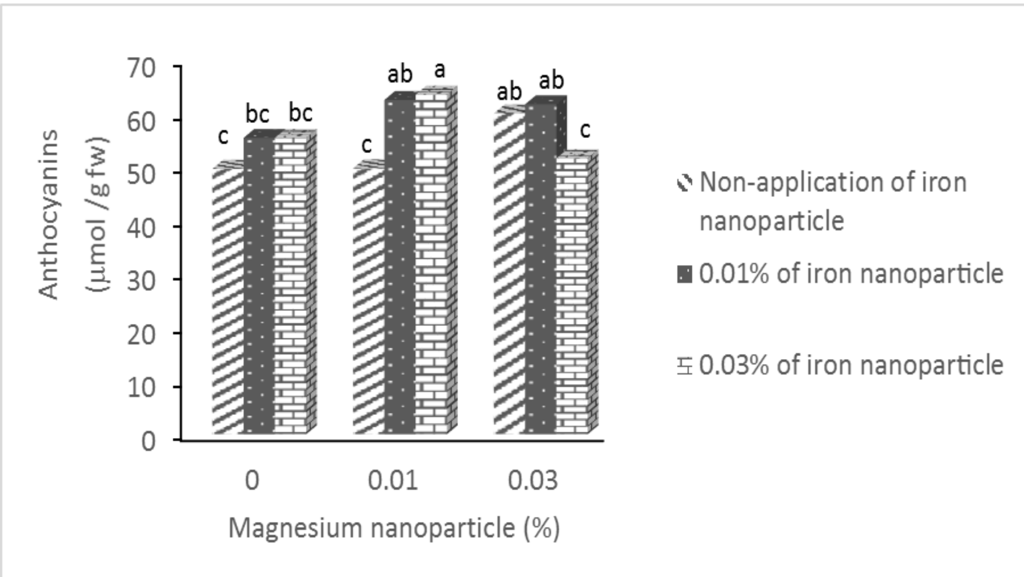

Fig. 3 Effect of interactions of magnesium and iron nanoparticles on anthocyanin

The lowest value of this trait was observed in the control treatment with the amount of 1.17%, and also interaction of non-application of magnesium nanoparticle and 0.01% concentration of iron nanoparticles treatment with the amount of 1.17% and also the interaction of the 0.03% concentration of magnesium nanoparticle and the concentration of 0.03% iron nanoparticles treatment (1.25%). In accordance with these results, the increase in the content of flavonoids in the medicinal plant in the treatment of iron nanoparticles was reported by Yousefzadehand Sabaghnia [16]. According to the results of Table 5, the amount of flavonoids of Roselle planted in Dehak region with the amount of 1.43% had higher amount of flavonoids than the plants that cultivated in Hashtgerd region (1.36%).

Anthocyanin

The results of the analysis of variance showed that the simple effect of iron nanoparticles and the interaction of magnesium nanoparticle × nanoparticle at a probability level of 1% and the simple effects of experimental location and magnesium nanoparticle, at the 5% level, had a significant effect on the content of the anthocyanin of the Roselle (Table 4).

According to the results of the mean comparison (Fig. 3), the highest amount of anthocyanin was related to the interaction treatment of 0.01% concentration of magnesium nanoparticle and 0.03% concentration of iron nanoparticles (63.40 μmol.g^{-1}fw) that with treatments of the interactions of 0.01% concentration of magnesium nanoparticles and 0.01% iron nanoparticle concentration (62.40 μmol.g^{-1}fw), and also interactions treatment of 0.03% magnesium nanoparticle and 0.01% concentration of iron nanoparticles (60.61 μmol.g^{-1}fw) and also interaction treatment of 0.03% magnesium nanoparticle and no application of iron nanoparticles (59.87 μmol. g^{-1}fw), had not significant difference and these treatments were in the same statistical group.

Furthermore, in this study, the lowest anthocyanin content of Roselle bushes was related to the control treatment (49.55 μmol. g^{-1}fw) and treatment of the interaction of 0.03% magnesium nanoparticle concentration and 0.03% iron nanoparticle concentration (52.51 μmol. g^{-1}fw), which did not have significant difference with the interaction treatment of non-application of magnesium nanoparticle and 0.01% concentration of iron nanoparticle (55.33 μmol. g^{-1}fw) and the interaction treatment of non-application of magnesium nanoparticle and the concentration of 0.03% of iron nanoparticle (55.32 μmol.g^{-1}fw). According to the results of Table 5, Dehak region with a value of 58.42 μmol. g^{-1}fw and Hashtgerd region with the amount of 54.61 μmol. g^{-1}fw had the highest and lowest levels of Roselleanthocyanin, respectively. In compliance with these results, in experiments of Mir and Dahmardeh [25] and Mir *et al*. [26], the highest amount of anthocyanin in the Roselle plant was obtained from the use of iron micronutrients.

Peroxidase Enzyme Activity Rate

Based on the results of the analysis of variance (Table 4), in this study, the simple effect of experimental location was significant at probability level of 5% and the simple effects of iron and magnesium nanoparticles concentrations and the interaction of magnesium nanoparticle × iron nanoparticle were significant at probability level of 1% on the content on the peroxidase enzyme activity rateof this plant.

According to the results of the mean comparison, the highest amount of this trait was related to the interaction treatment of magnesium and iron nanoparticles (Fig. 4), the treatment of 0.01% concentration of magnesium nanoparticle and the concentration of 0.03% iron nanoparticle (238.24 μmol/mg protein/min) had the highest level of enzyme peroxidase activity, and the least amount of this trait was associated withthe control treatment or non-application of nanoparticles (81.11 μmol/mg protein/min), treatment of interactions of 0.03% magnesium nanoparticle and 0.03% concentration of iron nanoparticle (89.16 μmol/mg protein/min) and also related to the interaction treatment of non-application of magnesium nanoparticle and 0.01% concentration of iron nanoparticle (93.45 μmol/g protein/min).

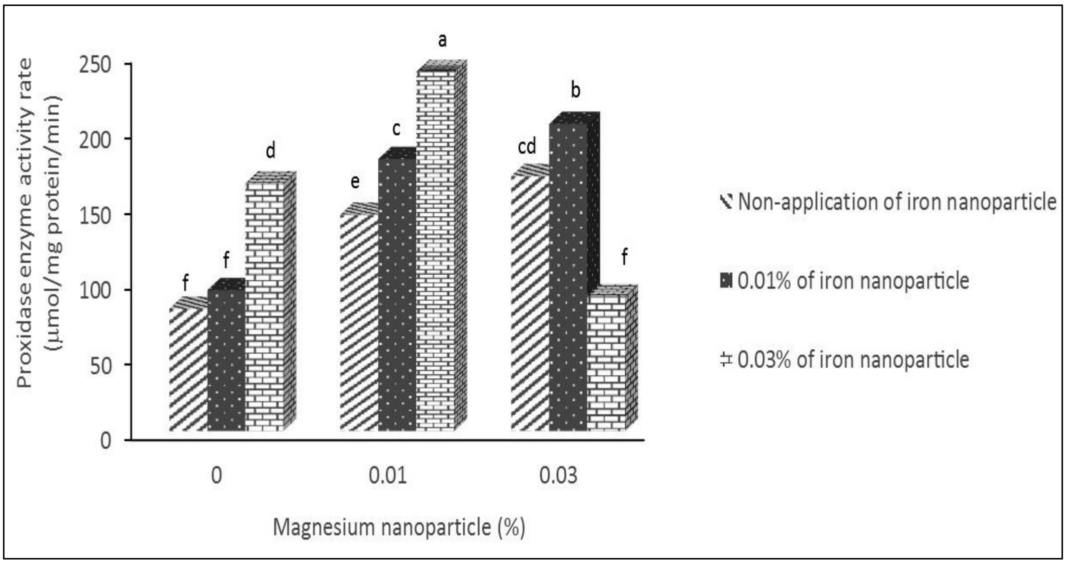

Fig. 4 Effect of interactions of magnesium and iron nanoparticles on peroxidase enzyme activity rate

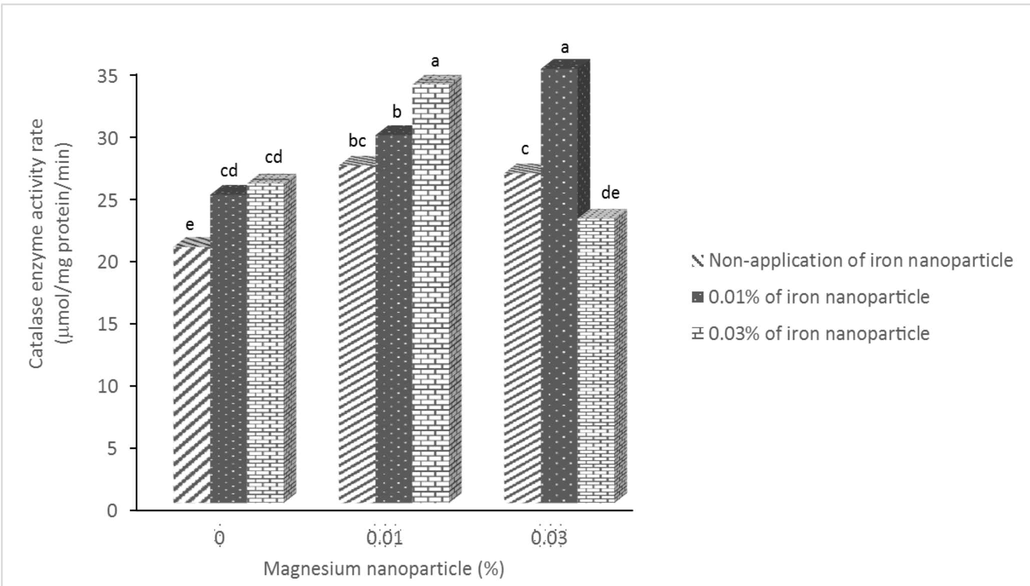

Fig. 5 Effect of interactions of magnesium and iron nanoparticles on catalase enzyme activity rate

Following to the Table 5 results, medicinal plant of Roselle that planted in Dehak area with 155.32 µmol/mg protein/min, had higher activity than the enzyme activity of plants that were planted in in Hashtgerd region with 147.75 µmol/mg protein/min. In parallel with these results, Salarpour *et al*. [27] also reported that the foliar application of *Moringa peregrina* plant with different concentrations of iron nanoparticles had a significant and increasing effect on the concentration of the enzyme peroxidase activity in this plant. Soliman *et al*. [17] also studied the effect of different concentrations of iron nanoparticles on the traits of medicinal plant Moringa, and their results showed that by increasing the concentration of iron nanoparticles specifically, the activity of the enzyme peroxidase increased in this plant, but in the higher concentrations of this nanoparticle, the amount of this trait decreased significantly. According to these results, Rui *et al*. [28] reported an increase in the activity of the enzymes of peroxidase in *Archis hypegea* plants by foliar application with iron nanoparticles.

Catalase Enzyme Activity Rate

The consequence of the combined analysis of data indicated that the simple effect of experimental location, treatment on the activity rate of catalase enzyme was significant at the 5% level statistical, while the simple effects of foliar application with each of the magnesium and iron nanoparticles, as well as the effects of the interactions treatments of magnesium nanoparticles × iron nanoparticles on this trait,had a significant difference at the 1% level (Table 4).

The results of the mean comparison of the data (Fig. 5) showed that the highest activity rate of the catalase enzyme of this plant in this study was pertinent to the interaction treatment of the of 0.03% magnesium nanoparticle concentration and the 0.01% concentration of iron nanoparticle (34.84 μmol/mg protein/min) and also interaction treatment of 0.01% concentration of magnesium nanoparticle and 0.03% concentration of iron nanoparticle (33.58 μmol/mg protein/min).

The lowest amount activity of this enzyme was observed in control or non-application of nanoparticles (20.56 μmol/mg protein/min), which had no significant difference with the interaction treatment of 0.03% magnesium nanoparticles and concentration of 0.03% iron nanoparticle (22.82 μmol/mg protein/min) (Fig. 5).

On the basis of Table 5 results, it was determined that the activity rate of catalase enzymes in roselle planted in Dehak region with the amount of 28.09 μmol/g protein/min was higher than the activity of this enzyme in plants of Hashtgerd region (26.40 μmol/mg protein/min). According to these results, increasing of antioxidant enzyme activity was reported by Amiri-Nezhad *et al*. [29] and Rui *et al*. [28]. In the experiment of Azarbara [30], catalase activity of *Medicago sativa* (alfalfa) was not affected by the treatment of foliar application with magnesium nitrogen, while a decrease of catalase activity of wheat plant in case of deficiency of magnesium element was reported by Abbasi and Enayati [31]. Along with these results, Joshan *et al*. [32] also reported that application of iron nanoparticles in fodder corn increased the catalase enzyme activity of fodder maize.

Discussion

According to the result of soil test in two locations of this experiment (Hashtgerd and Dehak), the deficiency of iron and magnesium in both experimental farms is quite evident because the critical level of iron in the soil for different plants is 10-16mg.kg^{-1}of soil [33] and the critical level of magnesium in soil for different plants, is 74 mg/kg of soil [34], while the amounts of iron and magnesium in both of these farms (Table 3) is lower than the critical amount of these two elements. Therefore, both farms (Hashtgerd and Dehak) were deficient in two iron and magnesium elements, and in order to compensate for the deficiency, iron and magnesium in the form of nanoparticles were used in our experiment because the superiority of iron and magnesium nanoparticles to their bulk forms is due to small particles of iron and magnesium Nano lead to the formation of multiple complexes, therefore the solubility of these elements is increased, therefore, their absorption by plants is easier due to high specific surface as well as being light and small size of this nanoparticle [35]. Magnesium plays an important role in metabolic processes such as sucrose loading, activation of rubisco enzymes and chlorophyll biosynthesis.The lack of this element results in an increase in the production of various active oxygen species [36], besides magnesium deficiency, by reducing the stomatal conduction and production of photomasmilates, lead to reduction of the Rubisco enzyme activity in the Calvin cycle, therefore resulting in a reduction of the NADP +/ NADPH, H + ratio (Ort, [37]).

This is due to a reduction in the ratio of + NADPH, H in the Calvin cycle. For this reason, the carriers of the electron transport pathwayalso come in the form of resuscitation, and the electron leakage leads to incomplete oxygen regeneration and an increase in toxic oxygen species [38]. Whereas different types of active oxygen can attack vital cellular compositions such as unsaturated fatty acids, proteins, and nucleic acids. These reactions naturally reduce the features such as, membrane fluidity, ion transfer, enzymatic activity, and protein synthesis, leading to degradation of nuclear and mitochondrial DNA and ultimately cell death [39].

Therefore, higher levels of hydrogen peroxide were observed in control treatment plants and treatments that received higher amounts (in interaction mode of 0.03% concentration of both elements) or lower amounts of both iron and magnesium elements. According to these results, Trankner *et al*. [18] and Jalali *et al*. [19] investigated the effect of different concentrations of magnesium and iron nanoparticles that the treatment of barley and corn plants with these elements reduced the hydrogen peroxide of the plants.

To overcome the oxidative stress, there is a high efficiency antioxidant defense system in plants that can destroy, neutralize or remove free radicals. This system contains antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, phenol peroxidase and guaiacol peroxidase, as well as non-enzymatic antioxidant system including ascorbate, alpha-tocopherol, carotenoids, flavonoids, anthocyanin, proline and glutathione [40].The higher amounts of enzymatic antioxidants in treatments that received the desired amounts of iron and magnesium nanoparticle elements led to higher conversion of hydrogen peroxide to water by catalase and peroxidase enzymes, whereas reduction of the activity of these enzymes in the control treatment and treatments that received less or much more (in interaction mode of 0.03% concentration of both elements) magnesium and iron elements resulted in more accumulation of hydrogen peroxide in these treatments.

Consistent with the results, Soliman *et al*. [17] reported an increase in the enzyme peroxidation of the Moringa plant that affected by application of iron nanoparticles. It should also be emphasized that the deficiency of magnesium and iron highly affects chloroplasts among the organs [41]. This organ is a major source of active oxygen production, including free radical hydrogen peroxide (Edreva, [42]). Therefore, in our experiment, in the conditions of magnesium and iron deficiency in control treatment and interaction treatment of 0.03% concentration of magnesium and iron, the activity of catalase and peroxidase enzymes decreased and as result detoxification has done incompletely that led to an increase in hydrogen peroxide.

In this research, Roselle foliar application with iron and magnesium nanoparticles increased the flavonoids of this plant, but by increasing the concentration of each of the tested nanoparticles to the 0.03% level under the conditions of treatment of the interaction of these two nanoparticles, again the amount of flavonoids decreased significantly.The phenolic compounds of flavonoids, anthocyanin, and the tannins have antioxidant properties that, prevent the oxidation of vital biochemical molecules by collecting and regenerating reactive oxygen species, prevent oxidative stress or it prevents oxidative stress and reduces its effects on plant cells (Myung-Min *et al*., [43]).Consistent with these results, the researchers also reported that foliar application of Dragonhead with iron nanoparticles had a positive effect on the flavonoids rising of this medicinal plant [16].

Besides in our study, the application of certain concentrations of iron and magnesium nanoparticles treatments in comparison with the control treatment has a positive and additive effect on the amount of anthocyanin in the Roselle, which increasing of anthocyanin amount probably lead to photosynthesis growth by preventing damage to chlorophyll. However, interaction treatment of 0.03% concentration of iron and magnesium nanoparticles had a decreasing effect on the level of anthocyanin of this plant and probably the negative effect of 0.03% concentration of iron oxide and magnesium oxide nanoparticles was due to the fact that the concentration of 0.03% in interaction condition have the toxicity, deterioration or neutralization effects on the growth of this plant [44].In this study, the toxicity effect of the interaction treatment of 0.03% concentration of magnesium nanoparticles and0.03% concentration of iron nanoparticles, increased the concentration of hydrogen peroxide as a harmful substances and reduced the levels of enzymatic antioxidants of catalase,... and non-enzymatic antioxidants, while the interaction of 0.03% concentration of each nanoparticle (magnesium or iron) with

other concentrations of 0 and 0.01% of each nanoparticle (magnesium or iron), reduced the concentration of hydrogen peroxide and increased the amount of enzymatic antioxidants.
In our experiment, possibly increasing the concentration of iron and magnesium in the plant, disturbs the balance of the other elements in the plant, for example, the excessive use of magnesium fertilizers, not only disturbs the balance between magnesium and potassium, but also disturbs the balance between magnesium and calcium which causes a reduction in the absorption of potassium and calcium elements and affects the quality of the fruit [45]. In fact, if the content of magnesium in the plant is much increased, the ratio of magnesium to calcium changes, consequently the fertility of the plant as well as its yield will decrease [46], and may even high concentrations of both iron and magnesium through the foliar application of Roselle plants, by increasing the production of toxic free radicals and inducing oxidative stress, act as an inhibiting factor forplant growth and causing toxicity [47].
Consequently, according to the Kiani *et al*. [48], although, commonly antioxidant enzymes simply decompose hydrogen peroxide under common conditions. However, under high toxicity conditions orinteraction treatment of 0.03% concentration of magnesium nanoparticles and 0.03% concentration of iron nanoparticles, the inability to neutralize oxygen radicals and the presence of hydrogen peroxide in the plant, lead to the reaction of Fenton and Haber Weiss reaction, in which a dangerous radical hydroxyl is produced, which can be, consequently, unstable types of biological macromolecules, including lipids and proteins. The result of oxidative stress caused by the toxicity of iron and magnesium in plants, is the reduction of the amount of proteins, soluble sugars, chlorophylls and makes irreversible injuries to biological membranes and nucleic acids. However, the highest concentrations of iron oxide and magnesium oxide nanoparticles used in our experiment under the interaction conditions have a much lower effect compared to other concentrations of these nanoparticles and may have a better effect than the control treatment. According to these results, Soliman *et al*. [16] also reported that an excessive increase of iron and magnesium concentrations caused a reduction the amount of Moringa traits. In our experiment, also the significant effect of the experimental location on the characteristics of the Roselleplant may be related to the higher levels of iron and magnesium in Dehak farm compared to the Hashtgerd in before spraying time (Table 3).

Conclusion

The results of Present study indicated that showed that when the medicinal plant of the Roselle grows in the soil that is poor in iron and magnesium elements, this plant will respond to the addition of iron and magnesium fertilizers. Now, to add fertilizer to the plant, it is better to use a fertilizer that has a particle size equal to the nanoscale. So that the very small particles of iron and magnesium nanoparticles quickly and easily pass through the cell membranes of the plant and, by increasing the efficiency of the photosynthetic and enzymatic systems of the plant, increase the plant's growth. In our experiment we found that the simultaneous application of 0.01% concentration of magnesium and 0.03% concentration of iron nanoparticles treatment, due to the positive effect of increasing of the activity of enzymatic antioxidants (catalase and peroxidase) and non-enzymatic (anthocyanin and flavonoids) of this plant was the best treatment compared to the other treatments and is recommended for areas that Roselle plant cultivated in these two regions with similar conditions. However, the application of 0.03% concentration of iron and 0.03% concentration of magnesium nanoparticles by disturbing the balance of the Roselle plant elements, increased the free radical species in the plant, while the enzymatic and non-enzymatic system of this plant also did not remove the harmful effects of the hydrogen peroxide production and, therefore, in terms of efficiency, this treatment was equal to the control treatment. Further research studies are needed to determine of changes in the physical and chemical characteristics of Roselle under the influence of the interaction of each of the iron and magnesium nanoparticles with other high-consumption and low-consumption elements in the form of nanoparticles and different stress conditions and also this experiment with these treatments can be carried out at each of the test site in two consecutive years.

Acknowledgements

The authors wish to thank professors of agronomy department at the Shahre-Qods Azad University without whom this research couldn't be conducted.

References

1. Hajifaraji R, Sefidkon F, Kazemi F. Roman chamomile oil: comparison between hydrodistillation and supercritical fluid extraction. J Essent Oil-Bear. Plants. 2003;6:191-194.
2. Hajifaraji M, Matlabi M, Ahmadzadeh-Sani F, Mehrabi Y, Rezaee MS, Hajimehdipour H, Hasanzadeh A, Roghani K. Effects of aqueous extracts of dried calyx of sour tea (*Hibiscus sabdariffa* L.) on polygenic dyslipidemia: A randomized clinical trial. Avicenna J Phytomed. 2018;8:24-32.
3. Daudu OAY, Falusi OA, Dangana MC, Abubakar A, Yahaya SA, Abejide DR. Collection and evaluation of Roselle (*Hibiscus sabdariffa* L.) germplasm in Nigeria. Afr J Food Sci. 2015;9:92-96.
4. Mataei S, Amirnia R, Tajbakhsh M, Abdol-lahi Mandulakani B. Effect of iron, zinc and manganese and their method of application on phenology, yield and quality of sweet corn grain. J Crop Prod Process. 2014;4:231-239.
5. Beaicknejad S. The evaluation effects of potassium and magnesium on yield and some physiological traits of Soybean (*Glycine max* L.) based on some statistical analysis. J Biodivers Environ Sci. 2017;10:207-216.
6. Bose J, Babourina O, Rengel Z. Role of magnesium in alleviation of aluminium toxicity in plants. Journal of Experimental Botany. 2011;62:2251-2264.
7. Yaghubi M, Azizi Kh, Heydari S, Roham R, Norouziyan A. 2014. Study the effect of different methods of magnesium sulfate application and cultivar on percentage of seed magnesium and crude protein and growth index in lentil (*Lens culinaris* Med.) at Khorramabad climatic condition. Agron J (Pajouhesh & Sazandegi). 2014;27:12-22.
8. Movahhedy-dehnavy M., Modarres-Sanavy SAM, Mokhtassi-Bidgoli A. Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. Ind Crops Prod. 2009;30:82-92.
9. Yeritsyan N, Economakis C. Effect of nutrient solution's iron concentration on growth and essential oil content of oregano plants growth in solution culture. International Conference on Medicinal and Aromatic Plants. Possibilities and Limitations of Medicinal and Aromatic Plant Production in the 21st Century. Acta Hortic. 2002;576:277-283.
10. Barberon M, Zelazny E, Robert S, Conejero G, Curie C, Friml J, Vert G. Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) transporter controls iron uptake in plants. Proceedings of the National Academy of Sciences of the United States of America. 2011;108:450-458.
11. Zargar SM, Agrawal GK, Rakwal R, Fukao Y. Quantitative proteomics revealsrole of sugar in decreasing photosynthetic activity due to Fe deficiency. Front Plant Sci. 2015;6:1-4
12. Sun B, Jing Y, Chen K, Song L, Chen F, Zhang L. Protective effect of nitric oxide on iron deficiency induced oxidative stress in maize (*Zea mays*). J Plant Physiol. 2007;164:536-543.
13. Mohammadi A. The role of nanotechnology in the agricultural industry. Third International Seminar on Oilseed and Edible Oil, Tehran, Center for Knowledge-Coordination and Oil Seed Industry, December 1-2, 2010.
14. Monica, RC, Cremonini R. Nanoparticles and higher plants. Caryologia. 2009;62:161-165.
15. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH, Sigg L. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicol. 2008;17:372-386.
16. Yousefzadeh S, Sabaghnia N. Nano-iron fertilizer effects on some plant traits of dragonhead (*Dracocephalum moldavica* L.) under different sowing densities. Acta Agric Slov. 2016;107:429-437.
17. Soliman A, El-feky SA, Darwish E. Alleviation of salt stress on *Moringa peregrine* using foliar application of nanofertilizer. J Hortic For. 2015;7:36-47.
18. Trankner M, Jakli B, Tavakol E, Geilfus C-M, Cakmak I, Dittert K, Senbayram M. Magnesium deficiency decreases biomass water-use efficiency and increases leaf water-use efficiency and oxidative stress in barley plants. Plant Soil. 2016;406:409-423.
19. Jalali M, Ghanati F, Modarres-Sanavi AM, Khoshgoftarmanesh AH. Physiological effects of repeated foliar application of magnetite nanoparticles on maize plants. J Agron Crop Sci. 2017;203:593-602.
20. Aebi HE. (1983): Catalase. In*:* Bergmeyer, HU (eds.) Methods of Enzymatic Analysis, 3nd Ed, Verlag Chemie, Weinheim, Florida, 1983.
21. Chance B, Maehley AC. Assay of catalases and peroxidases. Method Enzymol. 1955;2:764-775.

22. Wanger GJ. Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanis in protoplasts. Plant Physiol. 1979;64:88-93.
23. Krizek DT, Britz SJ, Mirecki RM. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of c.v. new red fire lettuce. Physiol Plant. 1998;103:1-7.
24. Hung SH, Yu CW, Lin CH. Hydrogen peroxide functions as a stress signal in plants. Botanical Bulletin of Academia Sinica. 2005;46:1-10.
25. Mir Z, Dahmardeh M. Determination of optimum levels of organic fertilizers and foliar application of iron on the quality indices of Roselle. Second National Congress on the Development a Promotion of Agricultural Engineering and Soil Science of Iran, Tehran, Foundation for the Development and Promotion of Basic Sciences and Techniques, June 27, 2016.
26. Mir Z, Dahmardeh M, Khomri A, Piri J. Determination of optimum levels of bio fertilizers and iron spraying on yield and quality indicators of roselle. Agroecol J. 2017;9:1194-1207.
27. Salarpour O, Parsa S, Sayyari MH, Alahmadi MJ. Effect of nano-iron chelates on growth, peroxidase enzyme activity and oil essence of cress (*Lepidium sativum* L.). Int J Agron Plant Prod. 2013;4(Special):3583-3589.
28. Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, Zhao Q, Fan X, Zhang Z, Hou T, Zhu S. Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). Front Plant Sci. 2016;7:1-10.
29. Amiri-Nezhad M, Akbari Gh, Baghi-Zadeh A, Alahdadi A, Shahbazi M, Naimi M. Effect of drought stress and foliar application of iron and zinc on some biochemical traits of Cumin seed. Agric Crop Manage. 2015;17:866-855.
30. Azarbara, A. The effect of magnesium nanoparticles on some morphophysiological and yield of alfalfa (*Medicago sativa* L.). Master's Degree Thesis in Agriculture, Islamic Azad University, Shahr-e-Qods Branch, 2017.
31. Abbasi A, Enayati V. Decrease of cell defense mechanisms efficiency and oxidative stress accruing in lake of Mg condition. J. Dryland Agric. 2013;1:41-51.
32. Joshan S, Sani B, Moaveni P. Investigation of the effect of foliar application of iron oxide Nano (Fe2O3) particle on some biochemical traits of fodder corn (*Zea mays* L.). The 3rd International Conference on Applied Research in Agricultural Sciences, Tehran, Applied Scientific University, January 30, 2016.
33. Sims JT, Johnson OV. Micronutrient soil test. In: Mortvedt J, Cox FR, Shuman LM, Walch RM. (eds.) Micronutrient in agriculture, 2nd Ed, Soil Science Society of America, 1991.
34. Kasinath BL, Ganeshmurthy AN, Nagegowda NS. Critical limit of soil and plant magnesium in tomato-growing soils of South Karnataka. J Hortic Sci. 2014;9:209-212.
35. Mazaherinia S, Astaraii A, Fotovat A, Monshi A. A comparison of uptake and concentration of iron (Fe) in wheat (*Triticum aestivum* L.) plant using ordinary and Nano iron oxides. Agron J (Pajouhesh & Sazandegi). 2010;92:103-111.
36. Cakmak I, Kirkby EA. Role of magnesium in carbon partitioning and alleviating photo oxidative damage. Physiol. Plant. 2008;133:692-704.
37. Ort D. When there is too much light?. Plant Physiol. 2001;125:29-32.
38. Beak KH, Skinner DZ. Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. Plant Sci. 2003;165:1221-1227.
39. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 2012;2012:1-26.
40. Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E. Heavy-metal induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Rev Environ Contam Toxicol. 2014;232:1-44.
41. Terry N, Ulrich A. Effects of magnesium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant physiol. 1974;54:379-381.
42. Edreva A. Generation and scavenging of reactive oxygen species in chloroplasts: A submolecular approach. Agric Ecosyst Environ. 2005;106:119-133.
43. Myung-Min H, Trick HN, Rajasheka EB. Secondary metabolism and antioxidant are involved in environmental adaptation and stress tolerance in lettuce. J Plant Physiol, 2009;166:180-191.
44. Feizi H, Rezvani Moqamat P, Fototo A, Tahmasebi N. Wheat seed reaction to different concentrations of titanium dioxide nanoparticles (TiO2) compared to non-nano particles. Second National Conference on Science of Seed Technology, Islamic Azad University, Mashhad Branch, November 4-5, 2011.
45. Ganjehi B, Golchin A. The effect of different levels of N, K and Mg on yield and growth indices of strawberry in hydroponic culture. J Sci Technol Greenhouse Cult. 2012;2:81-92.
46. Nourmandipour F, Farpoor MH, Sarcheshmehpour M. Effect of salinity and different Mg/Ca ratios on mycorrhiza colonization and vegetative traits of *Sorghum bicolor*. J Sci Technol Greenhouse Culture. 2014;5:81-92.
47. Alvareza A, Sierra MA, Lucena JJ. Reactivity of synthetic Fe chelates with soils and soil components. Plant Soil. 2002;241:129-137.
48. Kiani MH, Mokhtari A, Zeinali H, Abbasnejad A, Afghani Khoraskani L. Rosmarinic acid and anthocyanin content improvement by foliar application of Fe and Zn fertilizer in Lemon balm (*Melissa officinalis* L.). Int J Adv Biol Biomed Res. 2014;2:1525-30.