IRON CHELATE AND RHIZOBACTRIA CHANGED GROWTH, GRAIN YIELD, AND PHYSIOLOGICAL CHARACTERISTICS IN MAIZE

M. Heidari[1], I. Salmanpour[1], H. Ghorbani[2], H.R. Asghari[1]

[1]Shahrood University of Technology, Agronomy and Plant Breeding Department, Shahrood, Iran
[2]Shahrood University of Technology, Soil Science Department, Shahrood, Iran

In order to investigate the effect of iron chelate and plant growth promoting rhizobacteria (PGPR) on maize, an experiment was conducted as completely randomized block factorial design with three replicates. The first factor included of bacterial strain: S_1= Control (without use of bacteria), S_2=Azotobacter chroococcum and S_3= Azospirillum brasilens and the second factor included of iron chelate: F_1= Control, F_2= soil application of Fe chelate, F_3= foliar application of Fe chelate, F_4= soil application of nano Fe chelate and F_5= foliar application of nano Fe chelate. The results showed that the highest grain yield and guaiacol peroxidase (GPX) enzymes activity were obtained at the S_3F_5 treatment and ascorbate peroxidase (APX) at S_1F_5 treatment. Except the content of phosphorus in leaves and carotenoid, PGPR had significant effect on biological yield, the content of chlorophyll 'a' and 'b', yield components (number of seed per row of the ear, number of rows per ear and thousand seed weight) and nutrient elements in both the seeds and leaves. However, iron chelate, increased the yield components, but among the iron chelate treatments, the highest amount of chlorophyll 'a' and 'b' in leaves and phosphorus in seeds were obtained at F_5. These results suggested that foliar application of nano Fe chelate and Azospirillum brasilens could be improvement of maize plant productivity.

Antioxidant enzyme activity, Iron chelate, Maize, Photosynthetic pigments, Rhizobacteria

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INTRODUCTION

Iron (Fe) is an element that used by crops in small quantities, yet is essential to normal plant improvement and play important roles in enzyme reactions, photosynthesis, improve the performance of photosystems, DNA transcription, RNA synthesis and auxin active. Iron deficiency is a widespread agricultural problem in many crops, especially in calcareous soils. In these soils, total Fe content is high, but occurs in chemical forms not available to plant root. Fe occurs mainly in the form of insoluble Fe^{3+}, especially in high pH and aerobic soils; therefore, these soils are usually deficient in the plant available form, Fe^{2+}. Because plants usually absorb Fe^{2+} from soil, Fe deficient soils lead to Fe deficient plants. Also, considering the soil-plant- animal- human food chain, Fe deficiency not only affects the growth and development of plants, but can also lead to anemia in animals and humans (L i et al., 2014). Plants respond to Fe limitation by inducing a series of physiological and morphological changes in the roots to facilitate the mobilization of sparingly soluble Fe compounds in the root environment (C a k m a k , 2002). The narrow range between phytotoxicity and deficiency of iron brings the need for defining appropriate rates to be used. Even on the world scale, it is estimated that Fe deficiency is widespread occurring in about 30 to 50% of cultivated soils (H a v l i n et al., 1999).

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of

nano-devices and nano-materials could open up novel applications in plant biotechnology and agriculture (Nair et al., 2010). Metal based nanoparticles (NPs) like ZnO and Fe_3O_4 can be used as foliar application for increasing intake of minerals by plant cells (Nair et al., 2010). In this regard, the use of nano fertilizers for accurate control of releasing nutrients can be an effective step towards achieving sustainable agriculture compatible with the environment (Cui et al., 2010). Among the nano fertilizers, nano iron chelated fertilizer, with hydrocarbon base, without ethylene bond and without hormones, releasing of Fe in a wide range of soil pH (3-11); it can be a certain source of bivalent iron supply for plants (Baghari, Farahani, 2013).

Upon uptake by plants, NPs can be transported and localize in various tissues. In fact, nanotechnology is a rapidly developing discipline substantially influencing every field of science and biology. Nanotechnology certainly holds the potential to rejuvenate agriculture. For instance, a significant amount of iron oxide NPs suspended in liquid media were shown to be taken up by pumpkin roots and translocated throughout the plant tissues (Hong et al., 2005). However, the NPs were primarily accumulated near the root and only a small percentage was detected in the leaves, due to the large size range (30 nm–1 μm) of the commercial iron oxide NPs (Hong et al., 2005).

Intensive agriculture relies on the use of chemical fertilizers to provide high quality and yield of crop plants. On the other hand, excessive use of chemical fertilizers causes problems not only in terms of financial cost but also in terms of the cost to the environment. The interest in sustainable agriculture recently has increased. The development and application of sustainable agricultural techniques and biofertilization are vital to alleviating environmental pollution (Vessey, 2003**)**. Many bacterial species, mostly associated with plant rhizosphere, have been tested and found to be beneficial for plant growth, yield, and crop quality. They have been called plant growth promoting rhizobacteria (PGPR) (Esitken et al., 2003).

PGPR may be important for plant nutrition by increasing N and P uptake by plants, and playing a significant role in the biofertilization of crops (Cakmakci et al., 2006).

Maize stimulates different N_2-fixers in its rhizosphere and the most abundant diazotrophs belonging to Enterobacteraceae and Azotobacteraceae families. Numerous studies have shown that inoculation of maize plants with PGPR strains caused significant increase in plant height, plant dry weight, root length and weight, yield, leaf area, and plant nutrient uptake of N, P, K, Fe, Zn, Mn and Cu (Sachin, 2009). Among the different bacterial genera that have been reported as PGPR (*Azospirillum*, *Agrobacterium*, *Rhizobium*, *Enterobacte*r, *Beijerinckia*, *Klebsiella*, *Xanthomonas*, *Phyllobacterium*) (Lucy et al., 2004), *Pseudomonas*, *Bacillus* and *Azotobacter* are the most widely reported (Đurić et al., 2011).

These bacteria reduce or prohibit some of deleterious effects of fungi or other phytopathogenic organisms by several different mechanisms. Also the direct mechanisms of PGPR for the plant growth enhancement include (1) facilitating the uptake of nutrients from the environment via the solubilization of phosphorus, nitrogen fixation, sequestering of iron from the soil by siderophores, (2) production of phytohormones such as gibberellin, auxin, cytokinin and (3) enzymatic lowering of plant ethylene concentrations (the most important of all) (Mayak et al., 2004).

PGPR are believed to increase the supply/availability of primary nutrients to the host plant, promoting the synthesis of antibiotics, enzymes and fungicidal compounds (Ahmad et al., 2006). Maize (*Zea mays* L.) is the most important crop among all cereal grain crops. It is important plant that is used as human food, livestock and poultry feed and as raw material in industry (Lin, Xing, 2008).

Effectiveness of PGPR inoculations on plant growth enhancement and crop yields depends upon their ability to survive and multiply in soils and is influenced by many abiotic and biotic factors including texture, soil pH, temperature, moisture content, soil type, soil amendment, nutritional status of the plant, plant species, plant age, microbial competition and predation (Marschner et al., 2004). The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements.

Another important effect of PGPR on plants is the improvement of leaf water status, especially under salinity and drought stress. Sarma, Saikia (2014) reported that *Pseudomonas aeruginosa* strain has improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. The ability of plants in utilizing water for growth depends on their stomatal apertures. The stomata on the plant leaf functions to balance the water content in leaf and water uptake by the roots. Ahmad et al (2013) reported that the stomata conductance (water vapor exiting through the leaf stomata) of plant leaf was higher in PGPR inoculated plants than non-PGPR inoculated ones under drought conditions. The finding from both studies proves that PGPR-inoculated plants tend to improve the water-use efficiency of plants. This finding could be beneficial to the environment in terms of reducing excessive usage of water. Some PGPR have been produced commercially as inoculants for agriculture to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity.

Due to the role of iron element in the growth of plants and importance of maize as an industrial crop, therefore, the aim of this study was to investigate the effect of PGPR and iron nano chelate on growth, grain yield and physiological responses of maize.

MATERIAL AND METHODS

Field experiment was conducted at the farm of Agricultural college Shahrood University of Technology, Iran (latitude of 36° 29′ N and longitude of 55° 57′ E, 1366 m above sea level) in the 2015. The field soil was sandy loam in texture, having pH, 7.9; electrical conductivity 1.6 $dS.m^{-1}$; 0.79% of organic carbon; 0.057% N, 3.4 and 144 mg/kg, of available P and K, respectively.

The experiment was laid out in a factorial design based on randomized complete block design with three replications. The first factor of study included three levels of bacterial strain consisting of: S_1=Control (without use of bacteria), S_2=*Azotobacter chroococcum* and S_3=*Azospirillum brasilens* and The second factor included iron chelate at five levels consist of: F_1=Control, F_2=soil application of Fe chelate, F_3=foliar application of Fe chelate, F_4=soil application of nano Fe chelate and F_5=foliar application of nano Fe chelate. Fe chelate explain EDDHA contains 6% of Fe.

Azotobacter belongs to the Azotobacteriaceae family. These are Gram-negative, nonsymbiotic, aerobic diazotrophs. The size of a young rod-shaped cell vary from 2.0-7.0 to 1.0-2.5 µm and occasionally an adult cell may increase up to 10-12 µm, and be oval, spherical or rod-shaped cells. Azotobacter can grow well on simple N-free nutrient medium containing phosphate, magnesium, calcium, molybdenum, iron and carbon sources. Azotobacter contributes by adding of significant amounts of fixed N_2 in, on, or near plant (R e v i l a s et al., 2000). Azospirilla are Gram-negative free-living nitrogen-fixing rhizosphere bacteria. They display a versatile C and N metabolism, which makes them well adapted to establish in the competitive environment of the rhizosphere. Ammonium, nitrate, nitrite, amino acids and molecular nitrogen can serve as N sources. In unfavorable conditions such as desiccation and nutrient limitation, *azospirilla* can convert into enlarged cyst like forms (H a r t m a n n , Z i m m e r , 1994).

Seeds of maize were washed with distilled water then inoculation was performed by a suspension of any bacteria. Inoculated seeds and non-inoculated seeds were sown in 9 June 2015 at experimental plots of 3×4 m in dimensions. The cultivation rows were 75 cm apart in each plot (at 7 plants m^2 density). The Single Cross 704 cultivar, was used in this experiment. Weeds were removed by hand and plots were irrigated as required through the growing season.

Foliar application of nano iron chelate and iron chelate fertilizer (1.5 *1000 Littre water) were applied in two stages (4-6 leaf and before of tassel emergence). Iron nano chelated fertilizer included different microelements such as iron (8.9%), zinc (0.92%), manganese (0.96%) and sulfur (9.5%). About 15 kg/ha nano iron chelate and iron chelate fertilizer ware used for soil application before plant planting.Two weeks after foliar application of nano iron chelate and iron chelate fertilizer, to measure the photosynthetic pigments and enzymes activities in leaves, samples were taken from the young leaves.

Yield and yield components

The maize (*Zea mays* L.) was harvested at November 2015. At maturity, to determine grain yield and biomass yield, we removed and cleaned all the seeds produced within middle of the two central rows in each plot. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter ($g\ m^{-2}$) and quintals kg per hectare. Then yield components were calculated by using 5 plants per plot. The yield components included the number of seed per row of the ear, number of rows per ear and thousand seed weight.

Photosynthetic pigments and nutrient content

Chlorophyll ‘a’ and ‘b’ and carotenoid in the leaves were extracted with 80% acetone and determined according A r n o n ’s method (1967), wherein the chlorophyll spectrum absorptions were measured at 645 and 663 nm, respectively, and the carotenoid calculated at 440 nm.The contents of nitrogen in leaves and seeds was determined by kjeldahl method, Phosphorus by spectrophotometer and potassium content in leaves and seeds was determined by Jemway PFP7 Flam photometer.

Enzyme Assays

Antioxidant enzyme activities in the leaves were assayed from leaf samples collected in an ice bucket and brought to the laboratory. These samples were washed with distilled water and their surfaces wiped clean of moisture. The samples (0.5 g) were then homogenized in near zero degree 0.1 M phosphate buffers (pH 7.5) containing 0.5 mM EDTA using a pre-chilled pestle and mortar. Transferred to centrifuge tubes, the homogenate was centrifuged at 4 ºC in a refrigerated Beckman unit for 15 min. at 15000 rpm min^{-1}. The supernatant was transferred to 30 ml tubes and used sequentially as the enzyme extract.

Total catalase (CAT) was assayed by measuring the residual hydrogen peroxide (H_2O_2) by titanium reagent (T a r a n i s h i et al., 1974). Reaction mixture (3 ml) consisted of 1 ml of 6 mM H_2O_2 and 1.9 ml of 0.1 M phosphate buffer (pH 7.0) in test tubes, and the reaction was initiated by adding 0.1 ml of diluted enzyme extract. The reaction was stopped after 5 min by addition of 4 ml of titanium reagent, which also forms colored complex with residual H_2O_2. Reaction mixture without enzyme served as control and developed maximal color with titanium reagent. Aliquots were centrifuged at 10000 rpm for 10 min

Table 1: Results of two-way analysis of variance (ANOVA) for bacterial strain (S) and nano iron chelated fertilizer (F) effects and their interaction (S×F) for the parameters considered

Dependent variable	Independent variable		
	S	F	**S×F**
Grain yield	23581.31*	98541.31**	16485.67*
Biological yield	136467.59*	189697.24**	15805.43^{ns}
Seed per row of the ear	11.442^{ns}	105.62*	38.63^{ns}
Number of rows per the ear	0.308^{ns}	2.86*	0.91^{ns}
The weight of thousand seed	1324.15^{ns}	8888.13**	2193.20^{ns}
Chlorophyll a	18.839**	7.989*	4.041^{ns}
Chlorophyll b	2.057**	0.719*	0.506^{ns}
Carotenoid	2.118*	0.926^{ns}	0.828^{ns}
Ascorbate peroxidase (APX)	0.0026^{ns}	0.0007^{ns}	0.006**
Catalase (CAT)	0.003^{ns}	0.003^{ns}	0.006^{ns}
Gayacol peroxidase (GPX)	0.0022*	0.0018*	0.0014*
Nitrogen in seed	0.016^{ns}	0.127*	0.092*
Phosphorus in seed	0.00009^{ns}	0.001*	0.0002^{ns}
Phosphorus in leaves	0.00004^{ns}	0.0031^{ns}	0.0011^{ns}
Potassium in seed	0.302^{ns}	4.457*	2.087^{ns}
Potassium in leaves	4.474^{ns}	16.635*	9.114^{ns}

* *P<0.05*. ** *P<0.01* and ns.

Numbers represent *F* values at 5% level, ns: not significant.

and absorbance of supernatant was recorded at 415 nm in spectrophotometer.

Total Guaiacol peroxidase (GPX) activity was determined as described by Urbanek et al. (1991) in a reaction mixture (2.0 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 5.0 mM guaiacol, 15.0 mM H_2O_2 and 50 µL of the enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}). The results were expressed as µmol H_2O_2 $min.^{-1}$ g^{-1} DM, taking into consideration that 4 mol H_2O_2 are reduced to produce 1 mol of tetraguaiacol (Plewa et al. 1991).

Total APX activity was assayed according to Nakano, Asada (1981). The reaction mixture (1.5 mL) contained 50mM phosphate buffer (pH 6.0), 0.1 µM EDTA, 0.5mM ascorbate, 1.0mM H_2O_2 and 50 µL enzyme extract. The reaction was started by the addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 1 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate ($2.8mM^{-1}$ cm^{-1}) and the results expressed in µmol H_2O_2 min^{-1} g^{-1} DM, taking into consideration that 2 mol ascorbate are required for reduction of 1 mol H_2O_2 (McKersie, Leshem, 1994).

Statistical analyses

All data were analyzed with SAS Institute Inc. 9.2 software. All data were first analyzed by ANOVA to determine significant (P=0.05) treatment effects. Significant differences between individual means were determined using LSD test. Data points in the figures represent the means ± SE of three independent experiments at least three replications per treatment combination each.

RESULTS

Grain yield and yield components

Data analyses results given in Table 1 indicate that both plant growth promoting rhizobactria (PGPR) and iron chelate treatment had a significant effect on biological yield in maize. In the present investigation, PGPR treatment significantly increased the biological yield. The highest biological yield was recorded at the S_3(*Azospirillum brasilens*) and the lowest at the S_1 treatment. In comparison with control, S_3 treatment caused 15.5% increase of biological yield (Table 2). Biological yield in maize also increased significantly with the application of iron chelate fertilizer. Among

Table - 2. Grain yield, yield components, chlorophyll content, antioxidant enzyme activity and ion content in maize as affected by PGPR and iron chelate

Treatments	Grain yield	Biological yield	Seed per rowof the ear	Number of rows per the ear	The weight of thousand seeds	Chlorophyll 'a'	Chlorophyll 'b'	carotenoid	Antioxidant enzyme activities			Nitrogen Seed	Nutrient content			
									APX	GPX	CAT		Phosphorus		Potassium	
													Seed	Leaf	Seed	Leaf
	g/m^2					(mg/g FW)	(mg/g FW)	(mg/g FW)	(μmol H_2O_2 min^{-1} mg^{-1} prot)			%	(mg/g Dry Weight)			
			Bio-fertilizer													
S_1	372.89 b	1031.88 b	24.21a	13.19a	181.24 a	7.87b	2.83b	5.39b	0.229a	0.134b	0.415a	1.51 a	1.096a	0.85 a	6.12a	a23.96
S_2	380.6 5 ab	1106.80 ab	24.90a	12.91a	a198.59	8.71b	3.20ab	5.75ab	0.211ab	0.152ab	0.392a	1.57a	1.092a	0.85 a	6.40a	a23.59
S_3	445.11 a	1221.28 a	25.98a	12.99a	196.18 a	10.09a	3.58a	6.14a	0.203b	0.158a	0.389a	1.54a	1.091a	0.86 a	6.30a	a22.90
			Nano iron chelated fertilizer													
F_1	340.27 bc	991.11 b	24.25b	12.58b	149.07 c	9.11 ab	3.10 abc	5.70a	0.215a	0.138b	0.424a	ab1.57	1.08b	0.86 a	6.76a	22.66ab
F_2	338.94 bc	1066.34 b	21.90b	12.33b	194.13 b	9.47ab	3.44ab	6.03a	0.200a	0.145b	0.413a	1.61ab	1.09ab	0.86 a	6.00ab	24.90a
F_3	415.83 b	1161.13 b	24.91b	13.18ab	b199.46	8.40 bc	3.00bc	5.73a	0.215a	0.138b	0.375a	1.37.c	1.09 ab	0.88 a	6.06ab	22.36b
F_4	327.54c	1027.67 b	23.35b	13.37ab	181.38 bc	7.53c	2.92c	5.27a	0.216a	0.144b	0.387a	1.67a	1.08b	0.84 a	7.16a	25.00a
F_5	576.16 a	1353.67a	30.83a	13.69a	235.98 a	9.93a	3.57a	6.07a	0.235a	0.173a	0.394a	1.48cb	1.10a	0.86 a	5.36b	22.40b

Means followed by the same letter are not significantly different within rows and column according to *Least Significant Difference* (LSD) ($P \leq 0.5$)

APX = ascorbate peroxidase, GPX = guaiacol peroxidase, CAT = total catalase, FW = Fresh Weight

to the iron chelate treatments, foliar application of nano-Fe chelate had the highest impact. The highest biological yield was obtained at the F_5 treatment (foliar application of nano Fe chelate) (Table 2). As to the grain yield, it was significantly affected by interaction between the two treatments (Table 1). In the treatments in this experiment, the highest grain yield was obtained at the S_3F_5 (*Azospirillum brasilens)* and foliar application of nano Fe chelate treatment (Fig 1).

The yield components studied in this experiment were the number of seed per row of the ear, number of rows per ear and thousand seed weight. Only the iron chelate treatment had a significant effect on these three yield components. The PGPR treatments had non-significant effect (Table 1). Among the iron chelate treatments, F_5 (foliar application of nano Fe chelate) had the greatest impact on these three yield components and the highest amount of the number of seed per row of the ear (30.83), number of rows per ear (13.69) and thousand seed weight (235.98 g) were obtained at the F_5 treatment. Foliar application of nano Fe chelate compared to the control treatment increased the number of seed per row of the ear, number of rows per ear and thousand seed weight about 21.4, 8.1 and 36.8% respectively (Table 2).

Enzyme Assays

Among the enzymes studied in this experiment, the interaction between plant growth promoting rhizobactria and iron chelate treatment had significant effect on APX and GPX enzymes activity in leaves tissues. As concerns CAT enzyme activity, the results showed that none of the treatments had any significant effect on it (Table 1). The highest activity of GPX and APX were obtained at S_3F_5 and S_1F_5 respectively (Figs 2 and 3). However, without the use of PGPR and iron chelate, the GPX activity was low. But by application of iron chelate, the GPX activity was increased. This increase was more prominent in foliar treatment than in soil application. The use of PGPR also increased the GPX activity and the highest GPX activity was obtained at the foliar application of nano Fe chelate and *Azospirillum brasilens* treatment (Fig 2).

Ion content and photosynthesis pigment

The concentration of nutrient elements in seeds and leaves of maize plants was studied in this experiment. The statistical analysis showed that, among the treatments, PGPR treatment had significant effect on the concentration of nutrient in both the seeds and leaves. But iron chelate treatments had significant effect on concentration of nutrient elements neither in seeds, nor in leaves. Contrarily, the effect of the iron chelate treatment on the concentration of nutrient elements both in seeds and leaves was significant. The iron chelate treatment had significant effect on the nitrogen content in seed, potassium in seed and leaves, and phosphorus in seed (Table 1). In all the cases, the application of nano chelate had the greatest impact on the nutrient concentration in seeds and leaves. But comparing the two methods (nano Fe chelate application into soil or foliar application), except phosphorus in seed, the highest concentrations of nitrogen in seed and potassium in seed and leaves were obtained with F_4 or the soil application of nano Fe chelate treatment (Table 2).

Concerning the photosynthetic pigment, except carotenoid, PGPR and iron chelate treatments had a significant effect on chlorophyll 'a' and chlorophyll 'b' content in leaves (Table 1). PGPR and iron chelate application increased the photosynthetic pigments. Among the PGPR treatments, *Azospirillum brasilens*

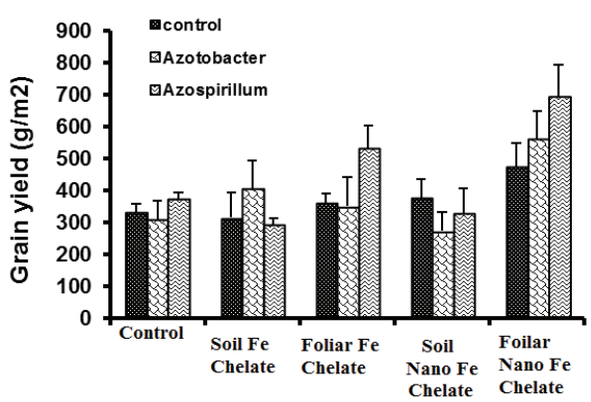

Fig. 1- Effect of iron chelate and plant growth promoting rhizobactria on grain yield

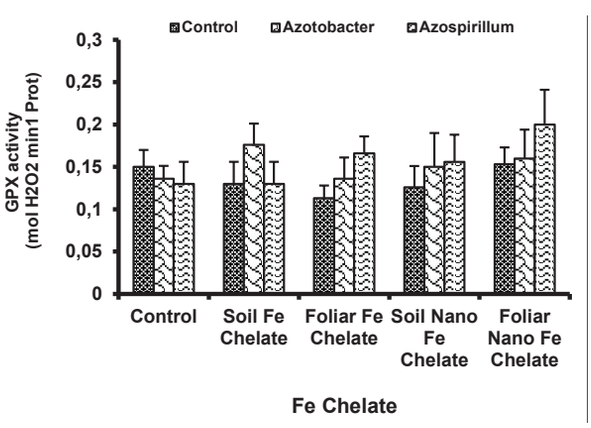

Fig. 2- Effect of iron chelate and plant growth promoting rhizobactria on guaiacol peroxidase (GPX) activity in leaves

and among the iron chelate treatments, foliar application of nano Fe chelate had the highest effects on the chlorophyll 'a' and chlorophyll 'b' content in leaves. The highest content of these pigments were obtained at the S_3 and F_5 treatments (Table 2).

DISCUSSION

Micronutrients consist of six essential elements: iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo). These elements are present in very small amounts in both soils and plants, but their role is regularly as important as that of the primary or secondary nutrient. They are playing an important role function in growth and development of plant (Steven, 2000). Iron is the fourth most abundant element in the Earth's crust (forming about 5.6%). Iron is an element relatively abundant in many cultivated soils with, on average, a total concentration of 20 to 40g kg^{-1}. Iron (Fe) is very insoluble in aerobic conditions at neutral and alkaline pH. It exists either in divalent (Fe^{++}) or trivalent status, while in the divalent status and to a lesser extent in the form of Fe chelates (Barker, Pilbeam, 2007).

Delgado, Sanchez-Raya (2007) reported that application of Fe fertilizer in sunflower resulted in the reduction of adverse effects of stress, and enhancement of NPK absorbed and consequently plant growth and yield. The results in this study showed that application of Fe chelate, increased grain yield in maize. But among the individual treatments of Fe chelates, foliar application of nano-chelate iron had the greatest impact on grain yield (Fig 1).

Ladan Moghadam et al. (2012) tested the effect of iron nanofertilizer on spinach and reported that application of 4 kg/ha iron nanofertilizer increased leaf weight by 58% and leaf area index by 47% compared with the control. In this case, not only grain yield, but also yield components (number of seed per row of the ear, number of rows per ear and thousand seed weight) were influenced by the foliar application of nano Fe chelate and and this application had the greatest impact on these yield components (Table 2).

Amuamuha et al. (2012) also studied the effect of different concentrations of iron nanoparticles (1, 2, and 3 g l^{-1}) on marigold at three growth stages (stem elongation, flowering, and post-harvest). They reported that the highest flower yield and essential oil percentage were achieved when 1 g l^{-1} iron nanoparticles was applied at stem elongation stage.

Nazaran et al. (2010) also reported that yield components of wheat were affected by the foliar application of iron, and the grain yield increased, too.

Iron chelates based on EDDHA are stable in the soil and prevents iron from deposition for a reasonable period of time. Chelation agent EDDHA, stores ferric iron with high power and prevents from the deposition in soil. Thus the iron concentration in the soil increases, but using these fertilizers is very costly (Reazaei et al., 2014). With production of nano fertilizers, these nano compounds are rapidly and completely absorbed by plants and fix their nutrient shortages and needs. The use of a nano fertilizers leads to an increased efficiency of elements, reduces the toxicity of soil and reduces the frequency of fertilizers application (Nazaran et al., 2010). The comparison of the effect of nano Fe chelate with Fe chelate on growth parameters of *Ocimum basilicum* showed that the replacement of a common Fe fertilizer by the nano iron fertilizer, if applied in appropriate concentrations, can improve quantitative and qualitative plant characteristics (Peyvendi et al., 2011).

In this case, the use of PGPR with a positive impact on yield components increased the grain yield in maize (Table 2). The highest grain yield was achieved in the presence of *Azospirillum brasilens* (Fig. 1). The mechanisms by which PGPRs promote plant growth are not fully understood, but some of them included the ability to produce gibberellic acid, cytokinins, and ethylene, N fixation, solubilization of mineral phosphate and other nutrients (De Freitas et al., 1997).

In this experiment, the use of PGPR increased the concentration of inorganic elements such as nitrogen, phosphorus, and potassium in the leaves and seeds of maize. But this increase was not statistically significant (Table 1). In this case, among the Fe chelate treatment, foliar application of nano Fe chelate had the greatest effect on the concentration of these elements in two parts (Table 2).

However, PGPR are known to increase root system uptake properties of rhizobacteria colonized crops by facilitating ion nitrate adsorption, phosphate solubilization, and iron chelation (Islam et al., 2009). PGPR may be important for plant nutrition by increasing N

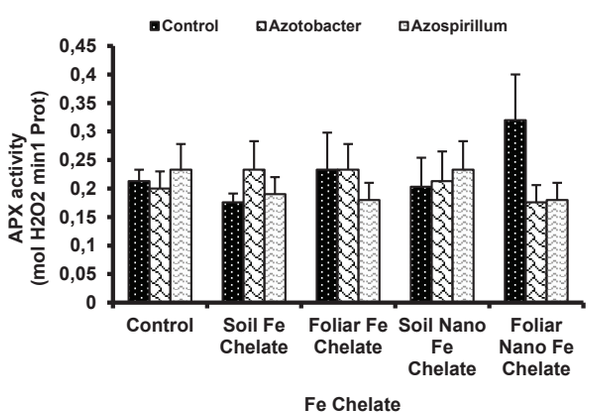

Fig. 3- Effect of iron chelate and plant growth promoting rhizobactria on ascorbate peroxidase (APX) activity in leaves

and P uptake by plants, and playing a significant role in the biofertilization of crops (Cakmakci et al., 2006). Farahani et al. (2015) reported that nano-iron chelate increased the content of macro elements in saffron (*Crocus sativus* L.). An excessive increase of this element in nano form had a reverse effect on dry and wet yield of saffron. Baghai, Farahani (2013) reported application of 5 kg nano iron chelate lead to an increase of 56% in wet yield of flower in saffron compared to the control.

Total iron uptake in shoot in nano fertilizer treatments significantly increased (by 11%) compared to microfertilizer. In addition to the change in absorbance, photosynthetic pigments have a huge impact on photosynthesis and production of dry matter and finally grain yield in plants. Identifying and measuring these pigments could provide insight into the photosynthesis process.

PGPR may be important for plant nutrition by increasing N and P uptake by plants, and playing a significant role in the biofertilization of crops (Cakmakci et al., 2006). These elements help improve photosynthesis. Inoculation of wheat with mycorrhiza fungi and PGPR in the medium containing heavy metals significantly increased the chlorophyll content. Thus, by increasing the amount of chlorophyll, photosynthesis increased (Gamal, 2005). The results of this experiment also showed that PGPR increased the amount of chlorophyll content in leaves of maize (Table 2).

In addition to PGPR, iron chelate also causes changes in the amount of chlorophyll in the leaves of maize plants. Among the iron chelate treatments, foliar application of nano Fe chelate had the highest effects on chlorophyll 'a' and chlorophyll 'b' content in leaves. The highest content of these pigments were obtained at the F_5 treatment (Table 2).

Among the different bacterial genera listed as PGPR, *Azospirillum, Agrobacterium*, *Rhizobium, Enterobacter, Beijerinckia, Klebsiella, Xanthomonas, Phyllobacterium, Pseudomonas, Bacillus*, and *Azotobacter* are the most widely reported (Duric et al., 2011). Growth promotion and disease control by these bacteria are complex interrelated processes involving mechanisms that include synthesis of some phytohormones (auxins, cytokinins, and gibberellins), production of siderophores, antibiotics, hydrogen cyanide, and volatile compounds. Indirect mechanisms include phosphosolubilization, competition and induced systemic resistance (Lugtenberg et al., 2002).

Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants. The study of Nazran et al. (2010) has shown that foliar application of the iron chelate fertilizer in the beginning of the trunk-related elongation in wheat enhanced the growth of leave and the efficacy of plant pigments such as chlorophyll and carotene, which improved plant growth.

Catalase and peroxidase enzymes have an important role in response to abiotic stresses in plants. The increase of iron concentration in plants and iron toxicity evoked greater production of reactive oxygen species. Plants respond to oxidative stress by increasing the production of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) or peroxidase. In this case, SOD is one of the early induced enzymes, and is responsible for the detoxification of the active superoxide radicals. In plants, the dismutation of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) is catalyzed by several isomeric forms of SOD. The conversion of H_2O_2 into water in peroxisomes is carried out by catalase (CAT), while that in cytosol and chloroplasts by ascorbate-glutathione cycle, which involves APX, ascorbate, and glutathione reductase (Foyer and Noctor, 2003).

The results of this experiment also showed that iron chelate and PGPR treatments influenced the activity of antioxidant enzymes in maize. The interaction between PGPR and iron chelate treatment had a significant effect on APX and GPX enzymes activity in leaf tissues (Table 1).

According to Priyadarshini et al. (2012) nano silver particles decreased H_2O_2 production and increased the efficiency of redox reactions. And a higher concentration of nano-silver reportedly enhanced the activity of H_2O_2 metabolizing enzymes.

It is well known that SOD is an enzyme that catalyzes the conversion of O_2^- to O_2 and H_2O_2. Enhanced SOD activity of leaves under the employed treatments may be interpreted as a direct response to augmented O_2^- formation. As previously suggested, the overexpression of SOD, if accompanied by the increment of H_2O_2 scavenging mechanisms like CAT, has been considered as a strategy to cope with oxidative damage (Hafis et al., 2011).

CONCLUSION

To conclude, plant growth promoting rhizobactria and iron chelate had a positive and significant impact on growth, yield, and physiological characteristics of maize plants. Among the kinds and application methods of iron chelate, the foliar application of nano-chelate iron had the greatest impact on grain yield, biological yield, photosynthetic pigments, and activities of antioxidant enzymes such as APX and GPX.

This study confirmed the plant growth enhancing ability of *Azospirillum brasilens.* The *Azotobacter* strain significantly affected growth, yield, yield components, and physiological parameters in maize suggesting that it can be applied as a biofertilizer improving maize production. In this case, the foliar application of nano-chelate iron in the presence of *Azospirillum brasilens* had a greater impact on corn growth and grain yield of maize plants. The highest grain yield and GPX activity

was obtained with the S_3F_5 treatment (*Azospirillum brasilens* and foliar application of nano Fe chelate). Therefore, it can be expressed *Azospirillum brasilens* can play benefit role in improving the growth and yield of maize under conditions of use of nano fertilizer. In this case, foliar application of nano-chelate iron in the presence of *Azospirillum brasilens* had more impact on growth and grain yield of maize plants.

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Corresponding author:

Mostafa H e i d a r i , PhD, Shahrood University of Technology, Faculty of Agronomy, Agronomy and Plant Breeding Department, Shahrood, P.O. Box 3619995161, Iran, e-mail: m_haydari@shahroodut.ac.ir