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Effect of iranian common biofertilizers inoculation on growth and uptake of elements by corn (single cross 704) and Bean *(Phaseolus vulgaris L.)* at green house condition

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Abstract
The use of native biofertilizers in Iran requires quality control of this type of fertilizer. Quality control of biofertilizers is a multi-step process and greenhouse testing is the most important step in using fertilizer. Accordingly, in order to investigate the effects of inoculation of conventional Iranian biofertilizers on corn and beans, a greenhouse experiment was conducted in a completely randomized design with 5 treatments (control, Barvar 2, Supernitroplus, Nitroxin and Biosuperphosphate) and 4 replications. The results showed that the use of biofertilizers had a significant effect on beans and corn. In Bean plant, the total fresh weight for Barvar2 treatment with an average of 67.10 g was the highest. Comparison of mean nitrogen concentration showed that Nitroxin biofertilizer treatment increased by 40.5% compared to control. In maize, nitrogen concentration was highest for Biosuperphosphate and Barvar 2 treatments with 0.91% and 0.81%, respectively. The chlorophyll index for Barvar 2 and Biosuperphosphate is the highest with an average of 4.945. Also, the comparison of the mean showed that the concentration of iron in the root of maize in the biosuperphosphate treatment (832.7 mg/kg^{-1}) was the highest and no statistical difference was observed between Barvar 2 and Nitroxin and control treatments.
Keywords: “biofertilizer, Barvar 2, Nitroxin, chlorophyll index, quality control ”.

Introduction

Biofertilizers contain a formulation of living microorganisms (algae, cyanobacteria, beneficial bacteria and fungi) or their production metabolites that can contribute to better soil quality and plant health beside increase active microbial population In the rhizosphere, nutrient availability may reduce plant soil diseases [1].

In recent years, unsuitable use of chemical fertilizers with nitrogen and phosphorus sources in agriculture has led to pollution of groundwater resources, farm soils and the environment. Although the use of chemical fertilizers has a significant effect on increasing the yield of agricultural products, but excessive use of these inputs has led to reduced soil fertility, imbalances in the natural ecosystem and environmental degradation. The use of nitrogen fertilizers such as urea in large quantities by farmers has led to irrecoverable damage to the agricultural sector, including water and soil pollution. Calcareous and gypsum soils, which also cover a large part of the area of agricultural and garden lands in our country, have led to the use of phosphorus fertilizers to be less efficient, and therefore farmers to achieve the desired result. Each year, they add large amounts of these chemical inputs to their farmland without seeing a significant increase in crop yield.The use of soil microorganisms that have the ability to dissolve insoluble phosphates and convert it into soluble phosphorus is one of the effective ways to increase the ability of the plant to absorb phosphorus. Biofertilizers consist of beneficial microorganisms that are derived from the rhizosphere of plants and help to improve plant nutrition through nitrogen fixation, phosphate dissolution, release of potassium ions, supply of iron and other elements, as well as reducing diseases. , Improving soil structure and other beneficial effects will further stimulate plant growth and increase crop quality [1]. A group of biofertilizers that are popular in the country include two groups of biofertilizers containing nitrogen fixers and insoluble phosphate solvents. Utilization of this type of biofertilizer in which the microorganisms in it are isolated and screened from the soil and are compatible with the climatic conditions and in accordance with the climatic conditions of the country, in addition to reducing the use of chemical fertilizers has been able to supply the nutritional needs of plants as well as increase crop yield in field conditions. The debate over the quality of these fertilizers is an important and challenging issue, and the extent to which these fertilizers have been able to supply these needs, requires laboratory and greenhouse studies, which is important and inevitable. In this regard, the aim of this study, was to investigate the effect of inoculation of Nitroxin, Supernitroplas, Barvar 2 and Biosuperphosphate biofertilizers on corn cultivar single cross 704 and bean in greenhouse conditions in the presence of native soil strains (non-sterile soil application).

Materials and Methods

Four types of biofertilizers including Barvar 2, super nitroplus, nitroxin and biosuperphosphate were produced by Green Biotechnology Company and produced by Mehr Asia Biotechnology Company, were prepared. The soil used for potting was prepared from a depth of 0-20 cm Khalatpushan area of Tabriz university. To prepare the potting bed, 2 kg of soil was poured in each pot and the soil moisture was adjusted to 0.8 fc. Seed sowing and inoculation of microbial

suspension related to each biofertilizer was performed according to the recommendations of the manufacturers. In order to apply the treatments, 1 ml of liquid biofertilizers of super nitroplus, Nitroxin and biosuperphosphate were inoculated with 99 ml of sterile distilled water and 10^{-1} dilutions were prepared. For solid biofertilizer (Barvar2), 1 g of fertilizer was inoculated into 99 ml of sterile distilled water. The inoculation rate for each pot was 10 cc of 10^{-1} dilution. The treatments consisted of five treatments including (Barvar2, Super Nitroplus, Nitroxin, Biosuperophosphate and Control (Nutrient Broth) with four replications in a completely randomized design. At the end of the growth period, parameters such as fresh and dry weight of roots and shoots, fresh and dry weight of whole plant, chlorophyll index, percentage of phosphorus and potassium of roots and shoots, percentage of nitrogen in shoots, iron concentration and uptake of each of these elements for Both beans and corn as well as the number of tubers and fresh weight of tubers were measured for bean plant.

Results and Discussion

The physicochemical properties of the soil used for pot cultivation are given in Table 1.
According to the results obtained in this study, the application of biofertilizers had a significant effect on parameters such as total fresh weight, root dry weight, percentage of nitrogen and potassium uptake in the aerial part, on the fresh weight of the aerial part, root phosphorus uptake, root iron concentration and Root iron uptake in bean plants was significant.In the case of single cross 704 maize, the application of biofertilizers had a significant effect on parameters such as chlorophyll index, percentage of nitrogen and potassium in the shoot, nitrogen and potassium uptake in the shoot and also iron.

Table 1- physicochemical properties of used soil for pot culture

Soil texture	%OC	%$CaCO_3$	K available (mg/kg)	P available (mg/kg)	EC (ds/m)	pH
Sandy loam	۱.28	۳۳.71	۳۷۲.3	۲۵.٤	۱.۲	۷.8

Total fresh weight

Analysis of variance of data showed that the biofertilizer inoculation had a significant effect ($P < 0.05$) on the total fresh weight of bean plant. The total fresh weight for Barvar2 treatment with an average of 67.10 g was the highest, which led to an increase in the total fresh weight of this plant by 18.3% compared to the control. However, no significant differences were observed between plant treatments of Nitroxin and Biosupprophosphate compared to the control (Table 2).
Development of aerial parts of the plant due to the synthesis of some growth stimulant properties of microrganisms by increasing a) dissolution of insoluble phosphate b) production and synthesis of auxin and subsequent increase of root

uptake and transfer to the aerial part by increasing root branches c) increase nitrogen fixation. In the case of Barvar 2, the power of auxin synthesis and the solubility of insoluble phosphate (after Biosuperphosphat) in Sperber media had higher values than other biofertilizers (Ansari and sarikhani, 2016).In addition, Barvar 2 had the highest rate of root colonization due to high microbial population compared to other biofertilizers. It enhances their PGPR properties [2].

Also, analysis of variance of data showed that biofertilizer inoculation had significant effect ($P<0.01$) on root dry weight of bean plant. According to Table 2, root dry weight for control and Nitroxin treatment were the highest with mean values of 0.74 and 0.75 g, respectively. Nitroxin biofertilizer increased the dry weight of the roots of this plant by 1.35% compared to the control. Biosuperphosphate treatment with an average of 0.26 g had the lowest root dry weight. However, the use of these biofertilizers in maize had no significant effect on this parameter.

Table 2- Comparisons of the average parameters affected by the application of biofertilizer in Beans

Traits / treatment	Root iron concentration (mg/kg)	Root phosphorus take (mg/pot)	Shoot k uptake (mg/pot)	N Shoot (%)	Total fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Root iron uptake (mg/pot)
Control	۵۴۳ a	1.60 a	289.5 a	0.78 b	56.70 ab	0.76 a	48.35 ab	0.41 a
Barvar2	458.9 b	0.42 b	187.2 b	1.183 ab	67.70 a	0.29 ab	60.21 a	0.16 b
Biosuperphosphat	502.7 ab	0.41 b	186.9 b	0.98 ab	62.51 ab	0.26 b	45.56 ab	0.12 ab
Nitroxin	475.1 ab	1.29 ab	234.5 ab	1.405 a	55.53 ab	0.74 a	47.72 ab	0.34 ab
Supernitroplus	506.3 ab	2.18 a	178.4 b	0.89 ab	47.76 a	0.45 ab	42.96 b	0.22 ab

Perhaps one of the reasons for the failure of phosphate biofertilizers on root dry weight in this plant is the high amount of phosphorus in the soil used and the inefficiency of biofertilizers used to increase the available phosphorus for the plant. In a greenhouse experiment (cucumber and pepper cultivation) using sterile soil and poor in potassium and phosphorus available for the plant, simultaneous inoculation of phosphorus and potassium releasing strains increased the dry weight of roots and shoots in these treatments compared to Witnessed. While in the treatment of phosphate and potassium rocks, no significant effect was achieved in increasing the dry weight of roots and shoots compared to the control [3].

Nitrogen

Analysis of variance of data showed that biofertilizer inoculation had a significant effect ($P<0.05$) on the percentage of aerial nitrogen in bean plant. Comparing the mean of this parameter showed that the treatment of Nitroxin biofertilizer (1.405%) compared to the control (0.78%) had a significant difference and led to an increase of 40.5% of this parameter compared to the control. The reason high amount for this parameter may be attributed to the nature of the strains used in this biofertilizer. This biofertilizer has Nitrogen fixing bacterial groups such as *Azotobacter* and *Azospirillum* jenus, and Nitrogen fixation by free and associated methods with their help may have a positive effect on the percentage of nitrogen in plant tissues.

In maize, analysis of variance of data showed that biofertilizer inoculation had a significant effect ($P<0.01$) on the nitrogen content and concentration of the aerial part. The percentage of nitrogen for biosuperphosphate and Barvar 2 treatment was the highest with 0.91% and 0.81%, respectively, which had a %121.2 and %97.56 increase in nitrogen compared to the control. The amount of nitrogen uptake for biosuperphosphate treatment was the highest (69.59 $mg.pot^{-1}$), followed by Barvar 2 and supernitroplus treatments with values of 61.35 and 45.51 $mg.pot^{-1}$, respectively. The three treatments increased the amount of nitrogen uptake by 166.32%, 134.78% and 74.16% compared to the control, respectively.

The beneficial effects of these biofertilizers are attributed to the synthesis of biologically active compounds, enhancing the presence of active rhizosphere microorganisms, producing inhibitors against plant pathogens, as well as increasing the availability of nutrients such as nitrogen, phosphorus, carbon and sulfur through BNF and mineralized organic residues [4,5].

Table 3- Comparisons of the average parameters affected by the application of biofertilizer in Maize.

treatment	Root iron concentration (mg/kg)	Shoot N uptake (mg/pot)	Shoot k uptake (mg/pot)	N -Shoot (%)	K -Shoot (%)	Chlorophyll index
Control	819.18 a	26.13 c	136.20 c	0.41 c	2.14 bc	3.91 a
Barvar2	769.3 ab	61.35 ab	162.5 bc	0.81 a	2.16 bc	4.94 a
Biosuperphosphat	832.7 a	69.59 a	265.6 ab	0.91 a	3.46 b	4.94 a
Nitroxin	804.6 ab	33.25 c	۱۰۹/۲c	0.53 bc	1.73 ab	1.73 b
Supernitroplus	722.6 b	45.51 c	280.4 a	0.71 ab	4.26 b	4.26 a

potassium

Analysis of variance of data showed that biofertilizer inoculation had a significant effect on the amount of potassium uptake in the aerial part of bean plant ($P < 0.05$). Comparison of the average amount of potassium uptake in the aerial part of bean plant showed that the control had a higher mean than the other applied treatments with 289.5 $mg.pot^{-1}$ and with the phosphate biofertilizer treatments (Barvar 2 and Biosuperphosphate) had a statistical difference. In maize, the amount of potassium uptake of Barvar 2 treatment, although had not statistically difference with the control, but showed a 0.9% increase compared to the control.

In a study conducted to investigate the effect of *Azotobacter chroococcum* and *Azospirillium lipoferum* on the amount of potassium uptake in the aerial part in two soils with deficiency and adequacy concentration of potassium, it was found that soils with adequacy range have higher dry weight and potassium concentration than soils with a range of potassium deficiency. Dry weight and potassium concentration in bacterial treatments of *Azotobacter chroococcum* had higher values than *Azospirillium* treatment, while *Azospirillium* itself was not significantly different from the control (soil with potassium deficiency. also, Ritika and Aptal (2014) reported that the use of different strains of *Azotobacter* jenus as biofertilizer led to increased growth and yield of crops such as sunflower and corn by 40% and 15-20%, respectively, compared to the use of chemical fertilizers.

Chlorophyll content

In maize, according to analyize variance Table 3, biofertilizer inoculation had significant effect on chorophyll index ($P<0.01$). the chlorophyll index for Barvar 2 and Biosuperphosphate with an average of values equal to 4.945 is the highest value. Although Nitroxin and Supernitroplus biofertilizers were expected to have a significant effect on chlorophyll index, due to the conditions of the experiment for which non sterile soil was used (presence of symbiotic fungi and native strains that may interact between Increases soil microorganisms and bacterial species in biofertilizers), reduces the effectiveness of these biofertilizers.

In a study conducted by Judges Hagh et al. 2015, it was found that inoculation of *Azospirillium* genus bacteria in maize, increased 7.99% of chlorophyll content and 10.25% of protein content in this plant.

phosphorus and Iron

Analysis of variance of data showed that root phosphorus uptake was affected by inoculation of biofertilizers in bean plant ($P<0.01$). Among the treatments, Supernitroplus treatment with 2.18 $mg.pot^{-1}$ had the highest amount of phosphorus uptake by roots. This treatment has a significant difference with other biofertilizers (Table 2).

Supernitroplus and Nitroxin biofertilizers contain *Azotobacter* and *Azospirillum* jenus, which in addition to Nitrogen fixation, also play a main role in phosphate dissolution and may increase phosphorus uptake by root plant. Among soil microorganisms, the *Bacillus* and *Pseudomonas* genera , as well as some species of *Azotobacter*, are better known for their phosphate dissolution and have a good ability to dissolve insoluble phosphate. A study conducted in Egypt, found that

the strains of *Aztobacter vinelandii* was able to dissolve more than 43% of the phosphorus in phosphate rock [6]

In the bean and corn plant, analysis of variance showed that inoculation of biofertilizers significantly affected the concentration and amount of root iron ($P<0.01$). Mean comparisons showed that the concentration of iron in bean root was the highest in the control treatment (543 mg kg^{-1}) and there was a statical difference between this treatment and Barvar 2 treatment (458.9 $mg.kg^{-1}$). The amount of root iron uptake was the highest for the control treatment with an average of 0.41 $mg.pot^{-1}$. Mean comparisons showed that the concentration of iron in the roots of corn was the highest in biosuperphosphate treatment (832.7 $mg.kg^{-1}$) and was not observed statistical difference between Barvar 2 and Nitroxin treatments and control. Siderophore producing bacteria can increase the concentration of iron in plants. Studies show that inoculation of plants with siderophore producing bacteria can improve the bio availibility of iron in plants and, consequently, improve the nutritional value of crops. Siderophore production is a means of increasing the competition of bacteria against other pathogenic microorganisms and thereby restricting their growth. Numerous studies have confirmed the role of these compounds. in controlling plant pathogens .In most studies, bacterial species that increase plant growth under abiotic stress conditions produce siderophores. In fact, *Azotobacter* species are able to significantly increase plant iron content by secreting siderophores and increase the dry biomass of maize under drought stress conditions compared to control plants.

Discusion

Application of biofertilizers in organic and sustainable agriculculture was the most importan in now years. Biofertilizers are including microorganisms that take from plant rhizosphere . these microorganisms are including different genus from bacteria, fungi and other microorganisms that can stimulate plant growth by multi mechanisms: a- free and associated Nitrogen fixation by *Azotobacter* and *Azospirillum* genus, b- insoluble phosphate solubilization by organic acid production and ligand and phosphatase enzyme (Bacillus and Psedumunase genus) , c- auxin production and increasing of nutrient up take by increasing root branch, d- sidrephore production and increasing iron uptake by plants, e- HCN production and removing phatogenic agent in plant rhizospher. Siderophore production is a means of increasing the competition of bacteria against other pathogenic microorganisms and thereby restricting their growth. Numerous studies have confirmed the role of these compounds in controlling plant pathogens . In most studies, bacterial species that increase plant growth under abiotic stress conditions produce siderophores. In fact, *Azotobacter* species are able to significantly increase plant iron content by secreting siderophores and increase the dry biomass of maize under drought stress conditions compared to control plants. Application of these biofertilizers led to increase physiological properties of maize and bean plants.in addition to these biofertilizers increased content and concentrations of nutrients such as nitrogen, phosphorus, potassium, and iron. Improving of this nutrients will increase yields of crops in form.

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