AGRICULTURAL JOURNAL

MEDWELL PUBLICATIONS

# Characterization and Quality Assessment of Four Common Commercial Biofertilizers in Iran

Saeideh Ansari, Bahman Khoshru, Mohammad Reza Sarikhani and Amir Nobahar
*Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

**Key words:** Biofertilizer, quality control, PGP properties, enumeration, molecular identification

**Corresponding Author:**
Saeideh Ansari
*Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

Page No.: 1-8
Volume: 16, Issue 1, 2021
ISSN: 1816-9155
Agricultural Journal
Copy Right: Medwell Publications

**Abstract:** The beneficial effects of biofertilizers in agriculture rely on the quality of these biological products, hence, qualitative control of biofertilizer is a necessary process. Therefore, in this study, four common biofertilizers (including Barvar2, biosuperphosphate, Supernitroplus and Nitroxin) that are produced in Iran were used to assess the qualitative criteria such as bacterial populations, genus and strains identity confirmation and also the determination of some Plant Growth-Promoting (PGP) characteristics in each biofertilizer. Finally, the effect of these biofertilizers inoculation on maize (variety Single Cross 704) carried out under the greenhouse experiment. Bacterial enumeration showed that bacterial viable cell in the Supernitroplus and Barvar2 biofertilizers was ($10^8$ cfu $mL^{-1}$) and in the Nitroxin and Biosuper phosphate was $10^7$ and $10^6$ cfu $mL^{-1}$, respectively. Besides, the most solubilized P from tricalcium phosphate source related to biosuperphosphate and Barvar2 with close values to 400 and 350 mg $L^{-1}$. Nitroxin and Barvar2 had great potential in auxin production. N3, N5, Bio3, SN2 and SN1 isolates produce siderophore in qualitative (orange halo zone) experiments. The potassium releasing from mica minerals by these four type didn't significant effect and results of the production, showed Barvar 2 and Nitroxin had the highest amount. Overall, 13 isolates were obtained from biofertilizers and molecular identification results showed that these isolates belonged to *Pseudomonas*, *Bacillus*, *Pantoea*, *Acinetobacter* and *Citrobacter genera*. Results from the identifications revealed that two strains of the N3 and Bio2 are related to the *Citrobacter* and *Acinetobacter* genus, reflecting the lack of proper identification by producers. The result of the greenhouse experiment showed the effect of biofertilizers on chlorophyll index, N and K shoot uptake was significant ($\alpha \leq 0.05$). Both Barvar2 and

biosuperphosphate have increased N uptake in plant shoot where as super nitroplus and biosuperphosphate had maximum attribute in K uptake. None of the biofertilizers had no significant effect on the chlorophyll index.

## INTRODUCTION

Biofertilizers are mainly known as microbial inoculants which are artificially multiplied of certain soil microorganisms that can improve soil fertility and crop productivity. In different biofertilizer compounds well-known plant growth-promoting rhizobacteria (PGPR) from different genera such as *Bacillus*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Lactobacillus* or *Pseudomonas* and so on are used individually or mixed with other strains[1].

Using biofertilizers instead of chemical fertilizers can significantly decrease the need for chemical fertilizers and its high production costs. Due to the hopeful results of biofertilizers, amount of public and private units in the country is now producing them. Although, various findings in last years showed ample beneficial effects of soil microbes in enhancing plant growth and the possibility of a microbial strain to have more than one of functional traits[2-10], the overall effectiveness of mixed inoculants in farm fields and farmers acceptance on the products had not yet been evaluated. The most important quality feature of biofertilizers could be mentioned in the presence of recommended strain in requisite number and active form and if any of the above characters are missing in the product, the biofertilizer could be termed as substandard. The most well-studied PGPR biofertilizers correspond to nitrogen fixation and the utilization of insoluble forms of phosphorus.

According to the claim of companies, Barvar2 has been made of two Phosphates Solubilizing Bacterial (PSB) strains including *Pantoea agglomeranse* and *Pseudomonas putida* while Nitroxin has been formulated by Nitrogen-Fixing Bacteria (NFB) such as *Azotobacter* and *Azospirillum*. On the other hand, biosuperphosphate is being used as PS biofertilizer and contains *Pseudomonas* and *Bacillus* genera and supernitroplus biofertilizer contains *Pseudomonas*, *Bacillus* and *Azospirillum* (Table 1). Fortunately, in recent years as a result of Iranian researcher's attempts, some PGPR strains have been used as biofertilizers in agriculture with high satisfaction but still, there is not enough evaluation of their qualitative properties in the land.

To maintain a high-quality product, there must be an effective quality assurance or quality control program. Quality control can be defined as the process of measuring defined quality parameters of a product and its assurance is an overall check that quality control procedures and techniques are achieving what they intend to achieve. The enumeration and identification of selected microorganisms in biofertilizers are crucial to predict their effectiveness. Validating the identity and quantity of specific PGP microorganisms in the inoculants is a very important factor to make farmers certain about the product and its sufficient quality; thus, they can use the product with confidence under their local environmental conditions.

However, the preparation of the biofertilizer's inoculums with high quality is very important to achieve their benefits in agriculture[11-13]. We should consider that utilizing biofertilizers are not always effective and in some cases, it does not meet our expectations. Therefore, in the present study, we investigate to confirm the presence of claimed bacterial species in the biofertilizers and also their PGPR characteristics besides counting the microbial viable cells. Finally, the effect of these biofertilizers on physiological parameters and nutrient levels evaluated in maize.

## MATERIALS AND METHODS

**Biofertilizers:** The biofertilizers in this study, consisting of four common biofertilizers in Iran including Nitroxin, Supernitroplus, Biosuper phosphate (produced by industrial biotechnology company of Mehr Asia, all three in liquid forms) and Barvar2 (produced by Green Biotech, in solid form) were selected for investigations (Table 1). This study was conducted between 2012 and 2013 at an agricultural laboratory at the University of Tabriz.

**Determination of viable cell number in biofertilizers:** Estimation of total viable cell numbers was conducted by preparing the dilution series and transferring 100 µL of the final dilutions ($10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$ and $10^{-9}$ dilutions) to the general or selective solid media. General Luria and Bertani (LB) medium (for the overall bacterial growth), Sperber (for phosphate solubilizing bacteria), N-free Bromothymol Blue (NFB) and N-Free (NF) media (both for N2-fixing bacteria)[14] were used to count viable cell in biofertilizers. To prepare first dilution ($10^{-2}$), 1 mL of each biofertilizer (1 g of solid biofertilizer assuming the bulk density of 1 g $cm^{-3}$) was added to 99 mL of sterilized distilled water and other dilutions were prepared in the 9 mL containing sterilized distilled water tubes from bacterial suspensions up to $10^{-9}$ dilution.

**Molecular identification and biochemical tests:** Molecular identification of the isolates was carried out by PCR amplification of 16S rDNA gene using the universal primers 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' AAGGAGGTGATCCAGCCGCA 3'). Isolates

Table 1: Information listed on the examined biofertilizers packages

| Biofertilizer | Claimed bacterial genera and species | Characteristics | Validity date |
|---|---|---|---|
| Biosuperphosphate | *Bacillus* sp.<br>*Pseudomonas* sp. | $10^7$ bacterial colony for each bacterial genus in each mL of biofertilizer | Feb. 28, 2013-Sep. 1, 2013 |
| Barvar2 | *Pantoea agglomerans, P. putida* | Improvement soil structure, reduce soil pathogens and increase yield | Sep. 30, 2013 (c540 product series) |
| Nitroxin | *Azospirillum* sp., *Azotobacter* sp. | $10^8$ bacterial colony for each bacterial genus in each mL of biofertilizer | Feb. 26, 2013-Oct. 30, 2013 |
| Supernitroplus | *B. subtilis, Pseudomonas* sp.,<br>*Azospirillum* sp. | $10^7$ $N_2$ fixing bacteria and growth promoters in each mL of biofertilizer<br>$10^8$ spore and viable cell of Bacillus | Jan 8, 2013- July 1, 2013 |

were identified by the analysis of their 16S rDNA gene sequences and the online program BLAST-n was used in identifying the related sequences with known taxonomic information available at the data bank of NCBI[8]. Then, some of the biochemical tests were carried out to complementary studies for each isolate.

**PGPR properties of the biofertilizers**

**Phosphate solubilizing assay (Tricalcium phosphate):** The procedure to determine the amount of solubilized phosphorus with low solubility $Ca_3$ $(PO_4)_2$ by biofertilizers was conducted by adding 500 µL of $10^{-2}$ dilution from each biofertilizer in tree replications to Erlenmeyer containing 30 mL of liquid Sperber[15] culture. Erlenmeyer's were shaked at 150 rpm at 26°C for 7 days. After that, 2 mL of the supernatants were added to 10 $cm^3$ volumetric flasks which is containing 8 $cm^3$ of distilled water, after that they were individually centrifuged at 5000 rpm for 10 min then remove bacterial biomass. Available P in the supernatant was determined spectrophotometrically by the vanadate-molybdate method[8].

**Auxin assay:** Indole-3-Acetic Acid (IAA) production by biofertilizers was determined using Nutrient Broth culture in three replications. For this, 500 µL of $10^{-2}$ dilution series of the biofertilizers were added to 30 mL of NB medium (containing 50 mg $L^{-1}$ tryptophan or without it) and after 72 h incubation at 37°C, the suspension was centrifuged (5000 rpm, 10 min) and 2 mL of the supernatant was mixed with 4 mL of Salkowski's reagent ($H_2SO_4$ (12M)+$FeCl_3$ (0.5 M)). The mixture was kept in the dark for 30 min at room temperature and then IAA concentration was read using a spectrophotometer at 530 nm and calculated by standard curve by 0, 0.5, 1.5, 5, 7.5 and 10 µg $mL^{-1}$ of IAA[16].

**Released K assay:** Potassium release by biofertilizers was determined using Aleksandrov liquid medium containing Muscovite or Biotite in three replications. At the first, 500 µL of $10^{-2}$ dilution of each biofertilizer was inoculated to 30 mL of liquid Aleksandrov's medium containing white or black Mica were incubated for one week at 26°C and 150 rpm. After incubation, 2 mL of the supernatants were transferred to centrifuge tubes and were set to the final volume of 10 mL (by distilled water). After that they were centrifuged at 5000 rpm for 10 min and the supernatant liquid was used to determine the concentration of released K[5]. Asimilar method carried out for isolated strains.

**Qualitative assessment of siderophore production:** This test was carried out using Chrome Azurol S (CAS) media. CAS-agar medium was prepared and each petri plate was divided into three equal parts and 5 µL of fresh suspension of each isolate inoculated to the center of the plates by dot culture in three replications. Inoculated plates were incubated for 1,3,5 days at 26°C. The development of the yellow-orange halo zone around the bacterial colony is considered as positive for siderophore synthesis. Colony diameter and halo zone diameter and also their ratio was calculated.

**Greenhouse experiment**

**Soil analysis:** The soil used in this experiment was taken from the Agriculture Research Station of the University of Tabriz, Iran, at a depth of 0-30 cm. The soil texture was determined using the hydrometer method[17], $pH_e$ and $EC_e$[18], percentage of organic carbon[19], available phosphorus[20] and available potassium[21]. For the pot culture experiment, the soil was passed through 4 mm sieve and sterilized at 1 m and 121°C for 1 h. Finally, 2 kg of the soil was poured in each pot.

**Plant culture experiment:** To investigate the effect of biofertilizers on growth, yield and other traits such as nutrient status such as N, P, K and Fe in maize, a pot culture experiment with five treatments (control without microbial inoculation), N biofertilizers (Nitroxin and supernitroplus) and P biofertilizers (including Barvar2 and biosuperphosphate) were performed in 4 replications in a completely randomized design. It is noteworthy that the Nitroxin, supernitroplus and biosuperphosphate biofertilizers were liquid and were prepared by the Mehr Asia Biotechnology Company and can be used for 1 ha of land based on the recommendation of the company. Based on the soil weight used in each pot and the number of sown seeds, 10 mL of biofertilizer was used to inoculate the seeds per pot. The solid biofertilizer Barvar2 was obtained from Green Biotech Company and each 100 g package is recommended for 1 ha for uniformity testing assuming a density of 1 g $cm^{-3}$, $10^{-1}$ dilution of this

Table 2: Number of viable cells (cfu $mL^{-1}$ or cfu $g^{-1}$) in four biofertilizers in general or semi-selective media

| Biofertilizers | LB[a] | Sperber | NF[b] | NFB[c] |
|---|---|---|---|---|
| Barvar2 | $2.9\times10^{8}$ | $4.2\times10^{8}$ | - | - |
| Biosuperphosphate | $7.2\times10^{6}$ | - | - | - |
| Nitroxin | $3.2\times10^{7}$ | - | $3.7\times10^{7}$ | $4.2\times10^{7}$ |
| Supernitroplus | $1.3\times10^{8}$ | $2.8\times10^{8}$ | $7.1\times10^{7}$ | $8.5\times10^{8}$ |

[a]Luria and Bertani; [b]N-Free (NF) media; [c]N-free Bromothymol blue

fertilizer was prepared first and 10 mL per pot was used. To generalize the results to real conditions, the experiment was performed on non-sterile soil conditions using uninfected seeds.

The physical and chemical properties of the soil used in this experiment are given (Table 1). After preparing the plant bed media, the soil was used for the experiment and after soil analysis, the pot experiment was performed using pots with a capacity of 2 kg soil. Seed implantation and inoculation of microbial suspension for each biofertilizer were performed on the recommendation of the manufacturers. It is noteworthy that in the control treatment, the sterilized medium was used equally. After germination of maize seeds (variety Single Cross 704), four suitable plants were maintained in the pot and the rest were removed, pots were irrigated with distilled water up to 0.8 FC and at the end of growth period parameters such as fresh and dry weight of root and shoot, leaf chlorophyll index, phosphorus and potassium concentration in root and shoot, the nitrogen concentration of shoot and uptake of each of these elements by the maize plant was measured.

For determination of leaf chlorophyll index, mature and juicy leaves were selected from each plant and its chlorophyll content was measured with a Hansatech CL-01 model made in England at two wavelengths of 620 and 640 nm. Finally, by averaging the chlorophyll meterdata, the chlorophyll index for each pot was determined. To measure the percentage of phosphorus, potassium and iron in plant tissues by weighting 1 g of dry matter, 65% nitric acid digestion was used[22, 23]. To determine the phosphorus concentration after dilution of the main extract, Olsen and Sommers[24] method was used. Finally, the percentage of phosphorus of plant tissues at 882 nm was determined by Apel Japan PD-303 spectrophotometer. To determine the potassium of plant tissues after dilution for digested specimens, the concentration of this element was read using a Cornflakes 410 flame photometer[25]. Percentage of total nitrogen was determined by the Kjeldahl system by digestion of 0.5 g dry matter[23] and finally, the effect of applied treatments was statistically analyzed. Data were analyzed by MSTATC statistical software and plotted using Excel software. Comparisons were made by Duncan's test at 1% and 5% probability levels.

The results of the analysis of variance of the effects of biofertilizer treatments on maize plant parameters are presented (Table 2).

## RESULTS AND DISCUSSION

**Bacterial populations and PGPR properties:** The results of bacterial counts related to biofertilizers in four different media are given in this experiment (Table 2). Also, results of PGPR properties and presence confirmation of isolates in each biofertilizer are given (Table 3-5).

**Qualitative estimation of siderophore production:** Bacterial strains isolated from biofertilizers were further tested for their siderophore production. Qualitative detection of siderophore synthesis was carried out using the CAS agar medium and it was based on measurement of orange halo zone around the colonies and calculation of HD/CD ratio in the first, third and fifth days of inoculation (Fig. 1). Variance analysis results revealed that bacterial effect, time and also their interaction is significantly affecting the siderophore production ($\alpha \leq 0.01$). Over time, the siderophore synthesis rate significantly increased and the highest HD/CD ratio was observed in Bio2 and N3 isolates while Ba2 and N4 were not able to produce siderophore (Table 5).

There was a good correlation between quantitative measurements of siderophore synthesis[12] and qualitative method, as N3 and Bio2 were the best siderophore producers, Ba2 and N4 which were not able to make orange halo zone in the qualitative method, produced about 72 µM siderophore in the quantitative method[12].

Bric *et al*.[16] in a study on 53 isolates from different genera concluded that all isolates were able to release 1.3-7 mg $L^{-1}$ auxin. In a study in the Indonesian Soil Research Institute in 2003, plant growth-promoting bacteria leaning to PGPR features were isolated from 40 rhizospheric soils in the Philippines in *in-vitro* conditions and results revealed that out of 12 isolated efficient strains, 7 isolates were able to grow and make yellow halo zone and it has been reported that it is resulted by the complex chelating agent (siderophore) and ion $Fe^{+3}$ with CAS in CASagar.

**Potassium release from Mica minerals:** According to the analysis of variances, there was no significant difference in released potassium concentration from Muscovite and Biotite minerals by biofertilizers[3]. But isolated strains from biofertilizer had a means effect ($\alpha \leq 0.01$) on k releasing from Muscovite and Biotite minerals (Table 5). In a study on the solubilization levels of Microcline, Muscovite and Orthoclase minerals by *B. mucilaginosus* MCRCp1 reported that highest potassium release from Muscovite was 4.29 mg $L^{-1}$. It is many years that *B. mucilaginosus* is being used as potassium biofertilizer in some countries like China[25].

Table 3: Results of molecular identification of isolated strains from biofertilizers

| Biofertilizer | Genus and species of the bacteria claimed | Isolates | Result of the identification |
|---|---|---|---|
| Nitroxin | *Azospirillum* sp. | N1 | *Pseudomonas aeruginosa* |
| | *Azotobacter* sp. | N2 | *Pseudomonas fluorescens* |
| | | N3 | *Citrobacter freundii* |
| | | N4 | *Pseudomonas* sp. |
| | | N5 | *Pseudomonas aeruginosa* |
| Supernitroplus | *B. subtilis*, *Pseudomonas* sp., *Azospirillum* sp. | Sn1 | *Bacillus firmus* |
| | | Sn2 | *Pseudomonas aeruginosa* |
| Biosuperphosphate | *Bacillus* sp. | Bio1 | *Pseudomonas* sp. |
| | *Pseudomonas* sp. | Bio2 | *Acinetobacter johnsonii* |
| | | Bio3 | *Pseudomonas aeruginosa* |
| | | Bio4 | *Bacillus firmus* |
| Barvar2 | *Pantoea agglomerans* | Ba1 | *Pantoea agglomerans* |
| | *P. putida* | Ba2 | *Pseudomonas* sp. |

Table 4: Supplementary biochemical test results of isolated strains

| Biofertilizer | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nitroxin | N1 | + | + | + | + | - | + | - | + | + | - | - | - | + |
| | N2 | + | + | + | - | - | + | - | + | + | - | - | - | + |
| | N3 | - | - | + | + | - | + | + | - | + | - | - | + | - |
| | N4 | - | + | - | - | - | - | - | - | + | - | - | - | + |
| | N5 | + | + | + | + | - | + | - | + | + | - | - | - | + |
| Supernitroplus | Sn1 | - | + | + | + | + | - | - | - | - | - | - | - | + |
| | Sn2 | + | + | + | + | - | + | - | + | + | - | - | - | + |
| Biosuperphosphate | Bio1 | + | + | - | - | - | + | - | - | + | - | - | - | - |
| | Bio2 | - | + | - | - | - | - | - | - | - | - | - | - | - |
| | Bio3 | + | + | + | + | - | + | - | + | + | - | - | - | + |
| | Bio4 | - | + | + | + | + | - | - | - | - | - | - | - | + |
| Barvar2 | Ba1 | - | + | + | - | - | + | + | - | + | - | - | + | - |
| | Ba2 | + | + | - | - | - | - | - | - | + | - | - | - | + |

1 = Isolate; 2 = Oxidase; 3 = Catalase; 4 = Nitrate; 5 = Urease; 6 = Starch; 7 = Glucose; 8 = Glycerol; 9 = Fluorescent; 10 = Tryptophan; 11 = Indole; 12 = Proline; 13 = Sulfide; 14 = Gelatinase

Table 5: Solubilized phosphorous, auxin production by biofertilizers, quantitative comparisons of released K+, siderophore by isolated strains from biofertilizers

| Biofertilizer | Solubilized P (mg $L^{-1}$) | Auxin (mg $L^{-1}$) | Isolate | Qualitative siderophore (HD/CD) | K+ | |
|---|---|---|---|---|---|---|
| | | | | | Muscovite | Biotite |
| Nitroxin | $46.1^{d}$ | $19.7^{ab}$ | N1 | $1.26^{de}$ | $1.55^{cd}$ | $7.21^{b}$ |
| | | | N2 | $1.13^{e}$ | $2.22^{b}$ | $4.98^{d}$ |
| | | | N3 | $1.86^{b}$ | $1.73^{c}$ | $6.40^{c}$ |
| | | | N4 | $0.00^{g}$ | $1.36d^{e}$ | $0.90^{g}$ |
| | | | N5 | $1.39^{c}$ | $2.35^{b}$ | $7.08^{b}$ |
| Supernitroplus | $245.6^{c}$ | $10.8^{cd}$ | Sn1 | $1.45^{e}$ | $1.36d^{e}$ | $4.36^{e}$ |
| | | | Sn2 | $1.39^{cd}$ | $2.22^{b}$ | $6.71^{c}$ |
| Biosuperphosphate | $408.3^{a}$ | $13.7^{bc}$ | Bio1 | $1.18^{de}$ | $2.22^{b}$ | $7.76^{a}$ |
| | | | Bio2 | $2.37^{a}$ | $1.17^{e}$ | $1.39^{f}$ |
| | | | Bio3 | $1.38^{d}$ | $2.97^{a}$ | $6.71^{c}$ |
| | | | Bio4 | $1.33^{f}$ | $1.39^{de}$ | $4.30^{e}$ |
| Barvar2 | $367.1^{b}$ | $21.9^{a}$ | Ba1 | $1.23^{de}$ | $3.16^{a}$ | $7.64^{a}$ |
| | | | Ba2 | $0.00^{g}$ | $1.36^{de}$ | $1.21f^{g}$ |

*In each column, a similar letter between treatments is not significantly different at $p<0.01$ and $p<0.05$ of significance according to Duncan's multiple range test

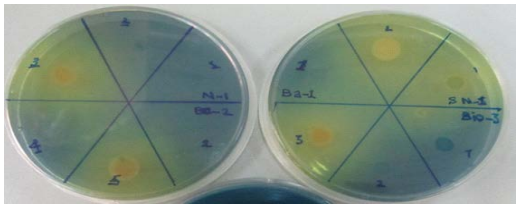

Fig. 1: Siderophore synthesis capability by isolated strains from biofertilizers in CAS-agar media

The present results indicate that inoculation with biofertilizers may increase the available nutrients for plants and help their growth, although, to be more confident about their effects in real conditions, field experiments are needed.

**Results of the pot experiment:** The physical and chemical properties of the soil used in this experiment are given (Table 6).

Table 6: Physical and chemical characteristics of soil used in pot experiment

| Soil texture | pH (1:1) | OC (%) | $CaCO_3$(%) | EC (ds $m^{-1}$) | P-available ( mg $kg^{-1}$) | K-available (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| Sandy loam | 7.8 | 1.28 | 33.71 | 1.2 | 25.4 | 372.3 |

Table 7: The effects of biofertilizers on chlorophyll index, N, K and P uptake by maize

| Biofertilizer | Chlorophyll index | Shoot N uptake (mg $pot^{-1}$) | Shoot K uptake (mg $pot^{-1}$) |
|---|---|---|---|
| Control | $3.9^{a}$ | $27.80^{c}$ | $128.9^{c}$ |
| Biosuperphosphate | $4.9^{a}$ | $69.59^{a}$ | $265.5^{ab}$ |
| Barvar2 | $4.9^{a}$ | $61.35^{ab}$ | $175.6^{bc}$ |
| Nitroxin | $1.7^{b}$ | $31.80^{b}$ | $109.8^{c}$ |
| Supernitrplus | $4.2^{a}$ | $45.51^{ab}$ | $275.8^{a}$ |

*In each column, a similar letter between treatments is not significantly different at $p<0.01$ and $p<0.05$ of significance according to Duncan's multiple range test

**Chlorophyll index:** The chlorophyll index of maize was significantly affected by inoculation ($\alpha \leq 0.05$). The chlorophyll index for Barvar2 and biosuperphosphate treatment had the highest mean of 4.9 which was statistically their percentage of effect on chlorophyll index at one level (Table 1). Besides, the supernitroplus was in the mean of 4.2 Chlorophyll index after these two biofertilizers but there was no statistically significant difference with chlorophyll index in the control treatment. Although, it was expected that Nitroxin and Supernitroplus biofertilizer had a significant effect on leaf chlorophyll index, it was due to the prevailing conditions for the experiment to use non-sterile soil (the presence of symbiotic fungi and native strains likely to have been increased interactions between soil microorganisms and bacterial genus in biofertilizers), reducing the range of biofertilizers affected. It is noteworthy that according to the manufacturer's claim that nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum* genus have been used in nitroxin and supernitroplus and are expected to be effective in providing nitrogen for the host plant and consequently Chlorophyll content improvement.

**N uptake:** Nitrogen uptake in the shoot was significantly affected by the inoculation of biofertilizers ($\alpha \leq 0.01$) (Table 7). Nitrogen uptake was the highest (69.59 mg $pot^{-1}$) for biosuperphosphate followed by Barvar2 and supernitroplus with 61.35 and 45.51 mg $pot^{-1}$, respectively (Table 7). These three treatments increased nitrogen uptake by 166.3, 134.7 and 74.1%, respectively.

A similar trend was observed for the percentage and nitrogen uptake using biofertilizers compared to the control but this increase was greater in the biosuperphosphate and Barvar2 biofertilizers. Biari *et al*.[26] reported that the inoculation of maize with growth-promoting bacteria (*Azotobacter* and *Azospirillum* genus) significantly increased nitrogen and phosphoruscontent levels compared to control. They stated that the results could be due to the application of nitrogen-fixing bacteria which by producing appropriate amounts of plant growth regulators such as auxin, gibberellin and cytokininthat improved plant rooting capacity and nutrient uptake. As a result, it increased the contentof nitrogen and phosphorus.

It seems that Barvar2 and biosuperphosphate biofertilizers possess plant growth-promoting bacteria from *Pseudomonas* and *Bacillus* genera through various mechanisms including production of plant hormones, phosphate solubilization and other positive effects on plant growth and development and nitrogen uptake[27]. Whereas Ansari and Sarikhani reported in vitro PGPR properties of these biofertilizers, the solubility of phosphate from tricalcium phosphate source for the two biosuperphosphate and Barvar2 was 408.3 and 367.3 mg $L^{-1}$, respectively. Barvar2 had the highest amount of auxin production in the presence and absence of tryptophan (230.26 and 9.9 mg $L^{-1}$, respectively). These properties make the biofertilizer have a greater role in the development of the aerial parts by increasing root branching and subsequently increasing nutrient uptake and transfer.

**K uptake:** Potassium uptake in the Maizeshoot wassignificantly affected by inoculation ($\alpha \leq 0.01$). Potassium uptake (Table 5) was the highest in the supernitroplus biofertilizer and then in the biosuperphosphate treatment which increased the potassium uptake by 105.87 and 95%, respectively compares to control. Change trend the uptake and potassium content in the aerial part of maize were similar and the amount of potassium uptake in Barvar2, although, not statistically different from the control treatment but was increased compared to the control.

Ansari and Sarikhani in the study of potassium release from Muscovite and Biotite minerals *in vitro* level, stated that all four biofertilizers used in this study had no significant effect on potassium release, however, when P13 strain alone was evaluated in Barvar2 biofertilizer was able to release potassium at 6.5 mg $g^{-1}$ and increased 27.24% compared to control but P5 strain was not different from control sample[9].

The type of response obtained from microbial inoculation seems to be influenced by the conditions of the experiment and the use of Barvar2 and nitroxin have not been effective in providing potassium for the plant.

Sundara *et al*.[28] reported that biofertilizers, especially phosphatesolubilizing bacteria, reduce soil pH by producing a variety of organic acids such as citric acids, glutamic, lactic and so on. The decrease in soil pH due to the use of biofertilizers reflects the fact that the acidification of soil by organic acids may be the main cause of greater access to stabilized elements such as phosphorus and potassium. As a result, increased phosphorus and potassium availability for the plant can be attributed to lower soil pH.

For biological and microbial products, quality control is an essential process including various stages from production to consumption. Quality control consists of operations that can be performed in the laboratory and the field. In the present study, we investigated the four biofertilizers belonging to two companies. Biofertilizer's quality control in this work included bacterial viable cell numbers, bacterial genus and strains and some plant growth promoting abilities such as strain's ability in siderophore production and also potassium releasing ability. The bacterial population wasunder standard level in biosuperphosphate biofertilizer and absence of claimed bacterial genus in Biosuperphosphate (Bio3) and Nitroxin (N3) biofertilizers are some cases that must be considered to make them appropriate for the market.

## CONCLUSION

Inoculation of biofertilizers containing plant growth-promoting bacteria resulted in the colonization of these bacteria in the rhizosphere of the Maize plant and increased some of the measured parameters meanly. Biosuperphosphate and Barvar2 have been effective in providing N or K. It should be noted, however, that in the leaf chlorophyll index, the biofertilizers and the control were in a statistical group. the conditions for testing whether soil, plant and other factors lead to different responses. Sometimes it is difficult to recommend a particular treatment in a variety of condition

## ACKNOWLEDGMENTS

The researchers would like to express their especial thanks to the University of Tabriz for the facilities and financial support of this research.

## REFERENCES

01. Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.
02. Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.
03. Liu, W., X. Xu, X. Wu, Q. Yang, Y. Luo and P. Christie, 2006. Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. Environ. Geochem. Health, 28: 133-140.
04. Husen, E.H., R.D.M. Simanungkalit, R. Saraswati and I. Irawan, 2016. Characterization and quality assessment of Indonesian commercial biofertilizers. Indonesian J. Agric. Sci., 8: 31-38.
05. Sarikhani, M.R., B. Khoshru and S. Oustan, 2016. Efficiency of some bacterial strains in potassium release from mica and phosphate solubilization under *in vitro* conditions. Geomicrobiol. J., 33: 832-838.
06. Nabti, E., B. Jha and A. Hartmann, 2017. Impact of seaweeds on agricultural crop production as biofertilizer. Int. J. Environ. Sci. Technol., 14: 1119-1134.
07. Nobahar, A., M.R. Sarikhani and N. Chalabianlou, 2017. Buffering capacity affects phosphorous solubilization assays in rhizobacteria. Rhizosphere, 4: 119-125.
08. Sarikhani, M.R., S. Oustan, M. Ebrahimi and N. Aliasgharzad, 2018. Isolation and identification of potassium releasing bacteria in soil and assessment of their ability to release potassium for plants. Eur. J. Soil Sci., 69: 1078-1086.
09. Sarikhani, M.R., B. Khoshru and R. Greiner, 2019. Isolation and identification of temperature tolerant phosphate solubilizing bacteria as a potential microbial fertilizer. World J. Microbiol. Biotechnol., Vol. 35, 10.1007/s11274-019-2702-1
10. Sarikhani, M.R., N. Aliasgharzad and B. Khoshru, 2020. P solubilizing potential of some plant growth promoting bacteria used as ingredient in phosphatic biofertilizers with emphasis on growth promotion of *Zea mays* L. Geomicrobiol. J., 37: 327-335.
11. Deaker, R., M.L. Kecskes, M.T. Rose, K. Amprayn and K. Ganisan *et al*., 2011. Practical methods for the quality control of inoculant biofertilisers. Australian Centre International Agricultural Research, Australia.
12. Khoshru, B., M.R. Sarikhani, N. Aliasgharzad and P. Zare, 2015b. [Assessment the important PGPR features of isolates used in biofertilizers barvar 2, biosuperphosphate, supernitroplus and nitroxin (In Persian)]. Applied Soil Res., 3: 39-52.
13. Khoshru, B., M.R. Sarikhani and N. Aliasgharzad, 2015a. [Molecular and biochemical identification of the bacterial isolates used in common biofertilizers in Iran (In Persian)]. J. Water Soil Sci., 25: 13-26.
14. Motsara, M.R. and R.N. Roy, 2008. Guide to Laboratory Establishment for Plant Nutrient Analysis. Food and Agriculture Organization of the United Nations, Rome, Italy, ISBN: 9789251059814, Pages: 204.
15. Sperber, J.I., 1958. Solution of apatite by soil microorganisms producing organic acids. Aust. J. Agric. Res., 9: 782-787.

16. Bric, J.M., R.M. Bostock and S.E. Silverstone, 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied Environ. Microbiol., 57: 535-538.
17. Gee, W.G. and D. Or, 2002. Particle-Size Analysis. In: Methods of Soil Analysis, Part 4: Physical Methods, Dane, J. and G.C. Topp (Eds.). Soil Science Society of America, USA., pp: 255-293.
18. Page, A.L., R.H. Miller and D.R. Keeney, 1982. Methods of Soil Analysis, Part 2. 2nd Edn., American Society of Agronomy, Madison, Wisconsin, USA.
19. Nelson, D.W. and L.E. Sommers, 1982. Total Carbon Organic Matter. In: Methods of Soil Analysis Part 2, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). 2nd Edn., American Society of America, Madison, Wisconsin, pp: 539-579.
20. Perez-Miranda, S., N. Cabirol, R. George-Tellez, L.S. Zamudio-Rivera and F.J. Fernandez, 2007. O-CAS, a fast and universal method for siderophore detection. J. Microbiol. Methods, 70: 127-131.
21. Thomas, G.W., 1982. Exchangeable Cations: Methods of Soil Analysis Part 2. In: Chemical and Microbiological Properties, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). 2nd Edn., Agronomy Monograph 9, ASA and SSSA, Madison, WI., pp: 159-165.
22. Rowell, D.L., 1994. Soil Science: Method and Applications. Addison Wesley Longman Limited, England.
23. Waling, I., W.V. Vark, V.J. Houba and V.D.J.J. Lee, 1989. Soil and Plant Analysis, a Series of Syllabi. Wageningen Agriculture University, Wageningen, Netherlands,.
24. Olsen, S.R. and E.L. Sommers, 1982. Phosphorus Avaibility Indices, Phosphorus Soluble in Sodium Bicarbonate, Methods of Soil Analysis, Part 2. In: Chemical and Microbiological Properties, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). American Soc. of Agronomy, Madison, Wisconsin, USA., pp: 404-430.
25. Jeon, J.S., S.S. Lee, H.Y. Kim, T.S. Ahn and H.G. Song, 2003. Plant growth promotion in soil by some inoculated microorganisms. J. Microbiol., 41: 271-276.
26. Biari, A., A. Golamii and H. Rahmani, 2007. Sustainable production and improvement uptake of corn nutrients in response to seed inoculation by growth promoting bacteria. Proceedings of the 2nd Iranian National Conference on Ecological Agriculture, October 25-26. 2007, University of Gorgan, Gorgan, Iran, pp: 25-26.
27. Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. Colloids Surf. B. Biointerfaces, 60: 7-11.
28. Sundara, B., V. Natarajan and K. Hari, 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. Field Crops Res., 77: 43-49.