Effect of Plant Growth-Promoting Bacteria on Mineral - Organic Fertilizer Use Efficiency, Plant Growth and Mineral Contents of Strawberry (*Fragaria x ananassa L.* Duch.)

H. Karlidag[1], E. Yildirim[1], M.Turan[2], M. F. Donmez[3]

Abstract

Biofertilizers are an alternative to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. The objective of this study was to evaluate possible effects of three plant growth promoting rhizobacteria (PGPR) strains as biofertilizer on growth, yield and ionic composition of leaves of strawberry plants. The application treatments included the control (without bacteria inoculation and mineral fertilizers), mineral fertilizers, and plant growth promoting rhizobacteria species [Bacillus cereus, (N_2-fixing), Brevibacillus reuszeri (phosphate solubilizing), and Rhizobium rubi (N_2-fixing and phosphate solubilizing)]. Data suggest that root inoculation of strawberry plants with PGPR strains tested increased root weight, shoot weight, ionic composition of leaves of strawberry and yield. The results of the study show that PGPR application may increase organic manure use efficiency and have capacity to stimulate strawberry growth and yield.

Keywords: Bacterial inoculation, plant growth promoting rhizobacteria, strawberry

Introduction

Recently, there has been a resurgence of interest in environmental friendly sustainable agricultural practices. In the development and implementation of sustainable agriculture techniques, bio-fertilization is of great importance in order to alleviate deterioration of natural and environmental pollution (O'Connell, 1992). A considerable number of bacterial species are able to exert a beneficial effect on plant growth. Such bacteria are generally designated as PGPR (plantgrowth-promoting rhizobacteria). The beneficial effects of these rhizobacteria on plant growth can be direct or indirect. Several mechanisms by which PGPR can act beneficially on plant growth are described. Examples of direct plant growth promotion that are discussed include (a) biofertilization, (b) stimulation of root growth, (c) rhizoremediation, and (d) plant stress control. Mechanisms of biological control by which rhizobacteria can promote plant growth indirectly, i.e., by reducing the level of disease, include antibiosis, induction of systemic resistance, and competition for nutrients and niches (Lugtenberg & Kamilova, 2009). The use of those bacteria as biofertilisers or biocontrol agents in agriculture has been a focus of research for a number of years.

Organic fertilization is very important in organic fruit production due to use of inorganic fertilizers is not possible. Therefore N_2-fixing and phosphate solubilising bacteria, including *Bacillus* sp., *Azotobacter* sp., *Azospirillum* sp., *Beijerinckia* sp., *Pseudomonas* sp. are widely used in organic plant growing (Lugtenberg & Kamilova, 2009). Strawberries have been grown in many countries with different ecological conditions and strawberry growing has spread quickly to areas where other agricultural crops have been grown previously for a long time. Production has increased significantly during the past decade (Dolgun, 2007). Strawberry is one of the most important fruit crops grown in the world and Turkey, with

[1] Atatürk University, Ispir Hamza Polat Vocational School, Dept. Organic Agriculture, Ispir, Erzurum, Turkey
[2] Department of Soil Science, Faculty of Agriculture, Atatürk University, Erzurum, Turkey
[3] Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

approximately 3,722,220 and 200,000 tons per year respectively is ranked as the fifth strawberry producing country in the world (FAO, 2007).

The aim of the study was to evaluate the efficiency of novel P-solubilizing and N_2-fixing bacterial strains so as to assess their possible use as inoculants for increasing productivity of strawberry in organically growing strawberry.

Materials and Methods

Location and Experimental Design Field trials were conducted at Atatürk University in Turkey in 2009. The soil at the experimental area had 28.3% sand, 32.2% silt and 39.5% clay. Relevant soil chemical characteristics were as follows: soil pH 7.3; organic matter 3.5%; Phosphorus (P); 22.5 kg P_2O_5 /ha and exchangeable Potassium (K) 2.6 meq /100 g soil. Approximately 30 ton/ha manure, which contained 2.2 % N, 1.7 % K_2O, 2.3 % P_2O_5, and EC of 6.2 dS/m, was applied to the beds before planting. Strawberry 'Fern' (Fragaria x ananassa L. Duch.) plants were used as plant materials. A randomized complete block design was employed as the experimental design with three replications. Treatments consisted of Control 1 (mineral basal fertilizer; nitrogen 120 k/ha as urea and phosphourus treatment- 180 kg/ha as triple super phosphate), Control 2 (orgnicbasal fertilizer; manure+ 100 kg/ha rock phosphate (including 18% P_2O_5), BC (manure+ rock phosphate+ *Bacillus cereus*), BR (manure+ rock phosphate+ *Brevibacillus reuszeri*), RR (manure+ rock phosphate+ *Rhizobium rubi*). The data were subjected to analysis of variance (ANOVA) using SPSS 13.0 (SPSS, 2004) statistical program. Mean values were separated according to Duncan test at P=0.05.

Experimental Units and Data Analysis Planting beds (experimental plots) were 20 cm in height, 0.9 m wide, and 10 m long. Each bed consisted of 2 rows of strawberry and was replicated three times. So, in total there were 15 beds. Strawberry crowns were planted giving space of 30x30 cm in late April in 2009. Drip irrigation was applied to replace water lost through evapotranspiration. Marketable strawberry fruits were harvested with 5 day intervals from June 03 until September 05. Ten plants from each replicate were randomly harvested, and data on plant growth variables, such as shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were collected. Dry weights (after drying at 70°C) of root and leaf samples were measured.

Mineral Analysis Because flowering time is a good time to asses the relationship between plant nutrient content and soil nutrient pools (Jones *et al.*, 1991; Mengel & Kirkby, 2001), tissue subsamples were taken during flowering (five youngest leaves) while root samples were taken after harvest. In order to determine the mineral contents of leaves and root of strawberry, plant samples were collected from fully expanded leaves at the end of the growing season then oven dried at 68 ^{o}C for 48 h and ground and passed 1 mm sieve size. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996). Macro- (P, K, S, Ca Mg and Na) and micro-elements (Fe, Mn, Zn, and Cu) were determined after wet digestion of dried and ground sub-samples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three step (first step; 145ºC, 75%RF, 5 min; second step; 180ºC, 90%RF, 10 min and third step; 100ºC, 40%RF, 10 min) in microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005a). Tissue P, K, S, Ca, Mg, Na, Fe, Mn, Zn, and Cu were determined using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005b).

Bacterial Isolation, Culture and Inoculation Bacterial strains were originally isolated from the rhizosphere of tea plants naturally grown in Rize in Turkey, Turkey, and identified as

Bacillus cereus, *Brevibacillus reuszeri*, and *Rhizobium rubi* based on fatty acid methyl ester analysis using the MIDI system (Sherlock Microbial Identification System, MIDI, Inc., Newark, DE). For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 250 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (95 rpm) for 24 h at 27 ^{0}C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU ml^{-1}. Bacterial applications of *Bacillus cereus*, *Brevibacillus reuszeri*, and *Rhizobium rubi* were performed using dipping method in which plant roots were inoculated with the bacterial suspensions at the concentration of 10^8 CFU ml^{-1} in sterile water about 60 min prior to plantation. Control plants were dipped into sterile water. The bacterial strains *Bacillus cereus* and *Rhizobium rubi* were able to grow in N free basal medium indicating its N fixing potential. P solubilising activities of the two *Brevibacillus reuszeri* and *Rhizobium rubi* were measured according to the qualitative methods (Mehta & Nautiyal., 2001).

Results and Discussion

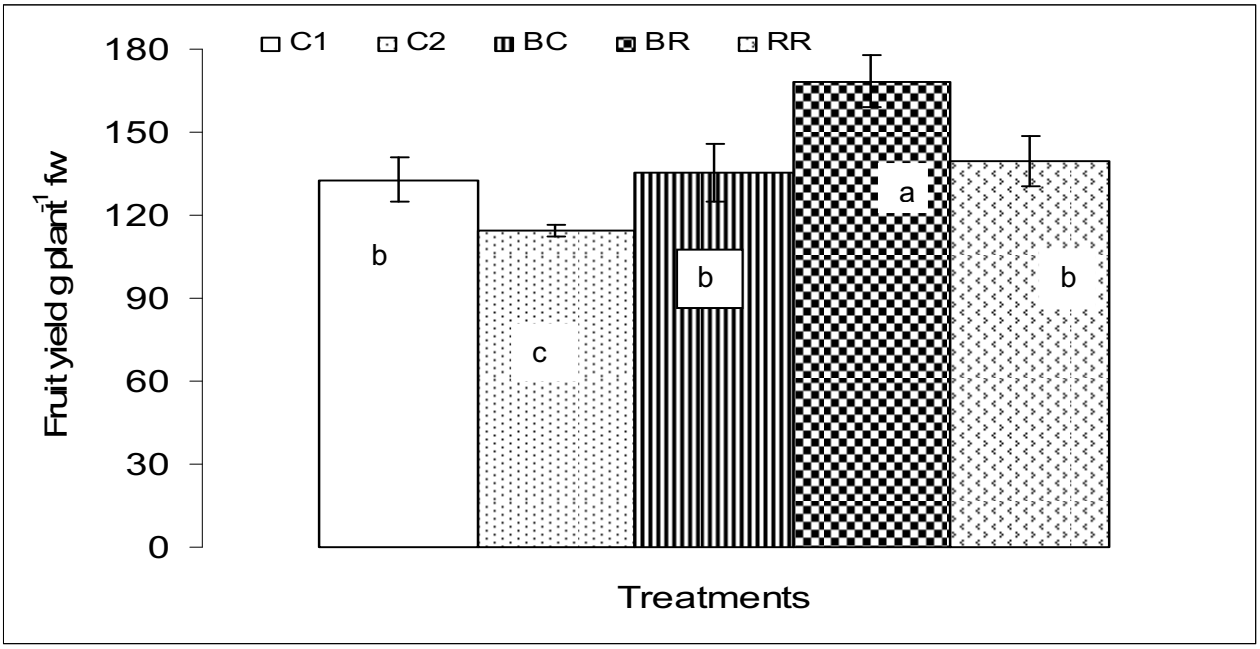
Yield and yield parameters Bacterial inoculations improved the strawberry growth and growth parameters. The performance of the plants was better in inoculated treatments in comparison to the control 2. The results showed that fruit yield (FY), shoot yield (SY), root yield (RY) of strawberry cultivars significantly increased by N_2-fixing and P-solubilising PGPR strains application compared with the control 2 (organic basal fertilization; manure+ 100 kg/ha rock phosphate). The highest FY, SY, and RY were recorded in mineral basal fertilization application (C1), and the lowest FY, SY, and RY were recorded in the organic basal fertilization (C2) treatment. On the other hand the bacterial inoculations BC, BR, and BR inoculation to organic basal fertilization (C2) increased FY by 18.0%, SY by 46.7%, and RY 22.0 %, respectively, due to increasing fertilizer efficiency (Figure 1). In other words, organic basal fertilization has significantly increased FY, SY and RY on condition that PGPR inoculation is treated. When BR application with C2 is compared to BC and RR with C2 treatment, BR application with C2 was more effective to increase the FY, SY and RY yield. In the current system, the results support increased organic fertilizer use efficiency if PGPR was added. This is different from the observations of Canbolat *et al*., (2006) and Elkoca et al., (2008), who reported no significant difference in root and shoot biomass of barley or seed yield and biomass of roots and shoots of chickpea, respectively, when inoculant alone or fertilizer alone was used.

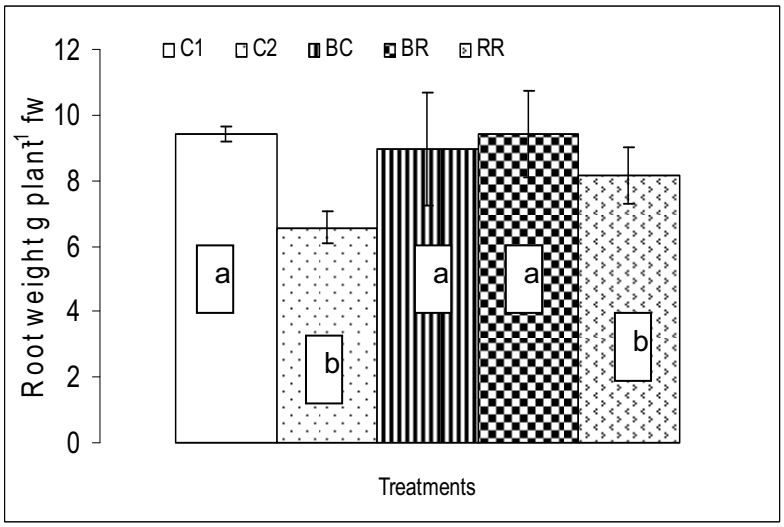
Effects of bio-fertilizer on plant nutrient element (PNE) contents of different parts of the *plant* N_2-fixing and P-solubilising PGPR strains application promoted PNE contents of different parts of the plant. Although the highest leaf, and root N, K, and P contents were obtained from C1, which increased by 15.5%, 14.3%, and 78.8 0% for leaf, 25.5%, 49.5%, and 85.8 0% were found for root respectively, compared with the control 2 treatment. On the other hand the bacterial inoculations BC, BR, and RR inoculation to organic basal fertilization (C2) increased also leaf, and root N, K, and P contents. While BR application is more effective P leaf and root content (78.8%, 85.3%), RR was most effective for N (18.0%, 21.0%) and K (21.1%, 19.1%) content of leaf and root respectively. The highest S, Ca, Fe, and Zn contents of plants leaves and root were obtained from RR treatment, and increase rate for leaf, and root of plant were 60.0%, 14.0% for S, 34.2%, 46.2% for Ca, 36.9%, 52.8% for Fe, and 34.5%, 56.7% for Zn, (Figure 1 and 2). The concentrations of plant nutrients measured were generally within the accepted critical levels (Jones *et al*., 1991; Mills & Jones,1996).The results presented here support the hypothesis that organic basal fertilizer with PGPR alone or in combinations and mineral fertilizer can improve plant growth and the nutrient contents part of the plants. Study result under field condition showed that organic basal fertilizer with PGPR alone and in combinations on plant yield

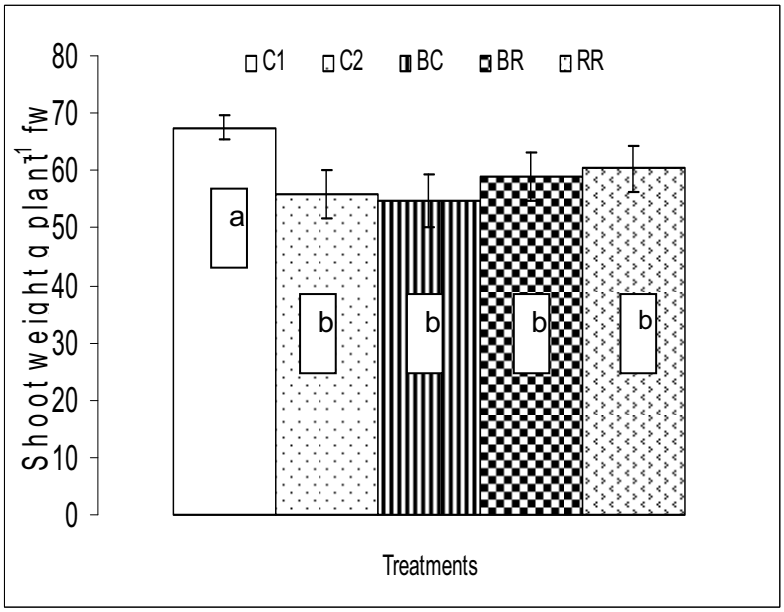
and nutrient content of strawberry in comparison to the without PGPR (C2) and BR most effective treatment for fruit yield and S, Ca, Fe, and Zn content of plant, following order was RR and BC treatment. When the percentage of recommended fertilizer was reduced and inoculants were used, plant FY, SY, RY and nutrient uptake were comparable to those with the basal mineral fertilizer without inoculants. After testing different PGPR inoculant alone or in combination to organic basal fertilizer increased organic fertilizer use efficiency rates, under these experimental conditions, organic fertilizer efficiency was the equal or over the mineral fertilizer application. Some of the previous studies with some of the same PGPR strains tested on chickpea, sugar beet, barley, corn, raspberry and tomatoes have been reported similar findings confirming our data in the present work. The use of the OSU-142 and M-3 in chickpea (Elkoca *et al.*, 2008), sugar beet and barley (Cakmakci *et al*., 2001; Sahin *et al*., 2004), corn (Ataoglu *et al*., 2004), raspberry (Orhan *et al*., 2006) and tomatoes (Turan *et al*., 2004) stimulated yield and quality parameters evaluated.

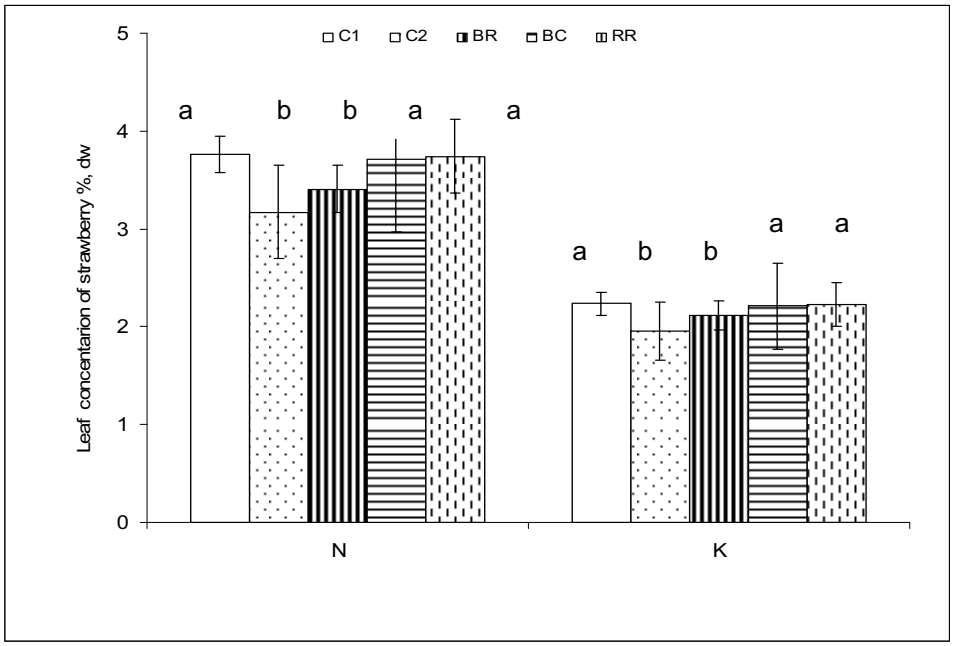
The yield and plant growth enhancement effects of bacteria used in this study on strawberry could be explained with N_2-fixing and P-solubilising capacity of bacteria. The positive effects of the PGPR on the yield and growth of crops such as chickpea, apricot, spinach, tomatoes, sugar beet, barley and wheat were explained by N_2-fixation ability, phosphate solubilising capacity, indole acetic acid (IAA) and antimicrobial substance production (Cakmakci *et al.*, 2001; 2007b; Esitken *et al.*, 2002; 2003; Turan *et al.*, 2005; Sahin *et al.*, 2004; Elkoca *et al.*, 2008). In the present study, it was also found that the inoculation of PGPR strains increased N, P, K, Ca, S, Fe, and Zn content of leaves and root of strawberry, which provide the additional evidence supporting the finding of previous study.

In plants treated by PGPR strains, the PNE concentration of leaf and root may provide important information about the effect of bacterial inoculation in PNE uptake. In this study, it was found that bacterial treatments increased PNE contents part of strawberry plant. Generally, the enhancements in Ca, S, Fe, and Zn nutrient contents were more pronounced in organic basal fertilizer with PGPR treatment whereas mineral basal fertilizer (C1) were also resulted in significant N, P, K, nutrient increases in plant leaf, and root. Enhancement of mineral uptake by plants should result in an increased accumulation of both dry matter and minerals in the root and leaves of the plant. During the reproductive period, the accumulated minerals would be transferred to the reproductive parts of the plants (Bashan *et al.*, 1990). Some of the previous studies with the same PGPR strains tested on chickpea, barley, raspberry, and apricot have been reported similar findings confirming our data in the present work. The use of the OSU-142 and M-3 in chickpea (Elkoca *et al.*, 2008), barley (Cakmakci *et al.*, 2007a), raspberry (Orhan *et al.*, 2006), apricot (Esitken *et al.*, 2003), and strawberry (Güneş *et al*., 2009) stimulated macro- and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn, Cu.


C1
C2
BC
BR
RR
180
150
120
90
60
30
0
Fruit yield g plant-1 fw
b
c
b
a
b
Treatments


C1
C2
BC
BR
RR
12
10
8
6
4
2
0
Root weight g plant-1 fw
a
b
a
a
b
Treatments


C1
C2
BC
BR
RR
80
70
60
50
40
30
20
10
0
Shoot weight g plant-1 fw
a
b
b
b
b
Treatments



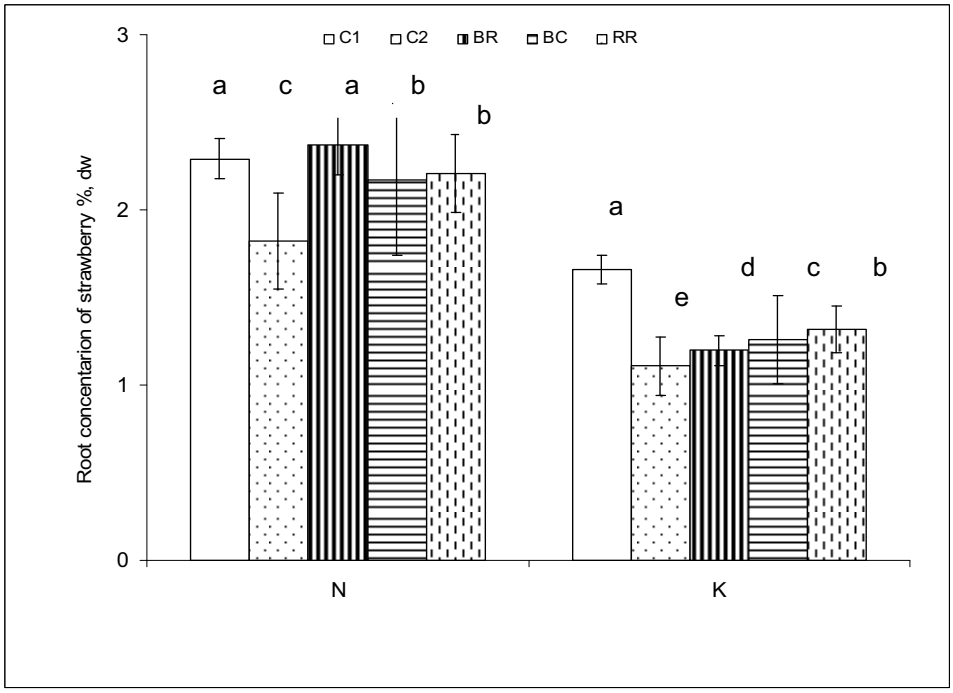
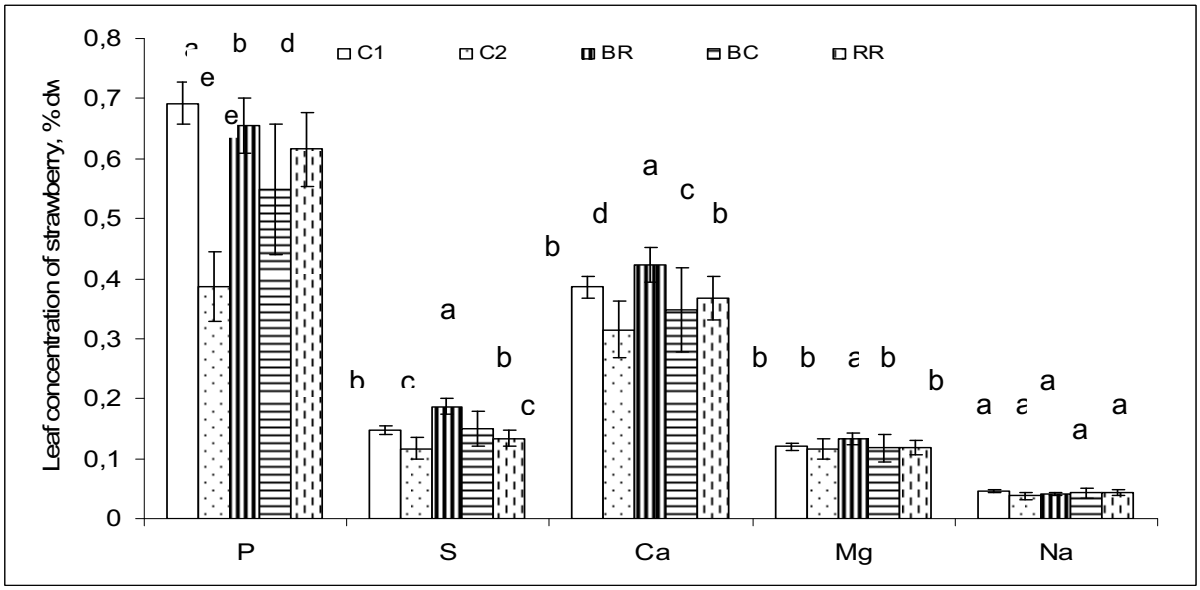
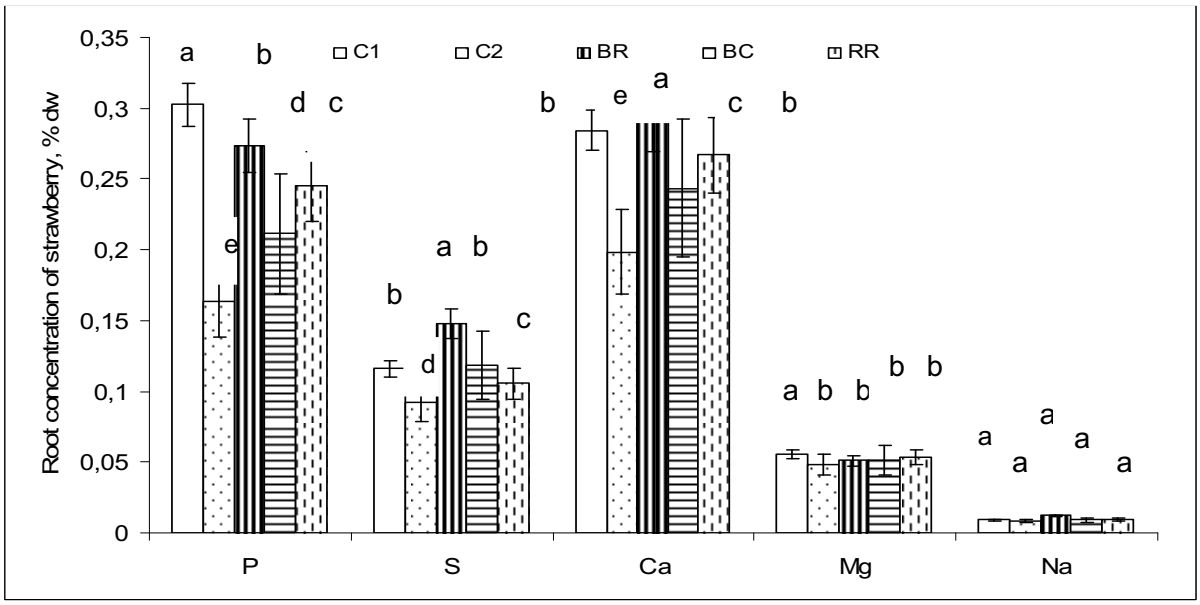
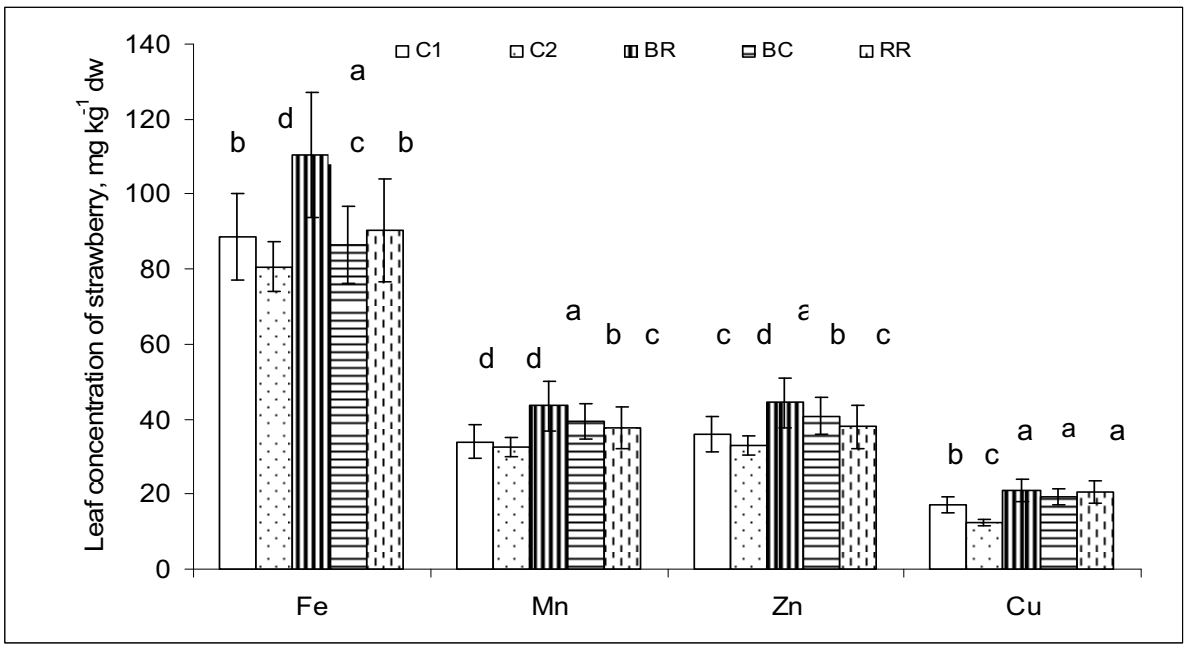


Figure 1: Effects of PGPR application on plant fruit yield, yield parameters and N, K content of leaf and root content of strawberry plant


Leaf concentration of strawberry, % dw
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
C1
C2
BR
BC
RR
P
S
Ca
Mg
Na


Root concentration of strawberry, % dw
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
C1
C2
BR
BC
RR
P
S
Ca
Mg
Na


Leaf concentration of strawberry, mg kg-1 dw
140
120
100
80
60
40
20
0
C1
C2
BR
BC
RR
Fe
Mn
Zn
Cu

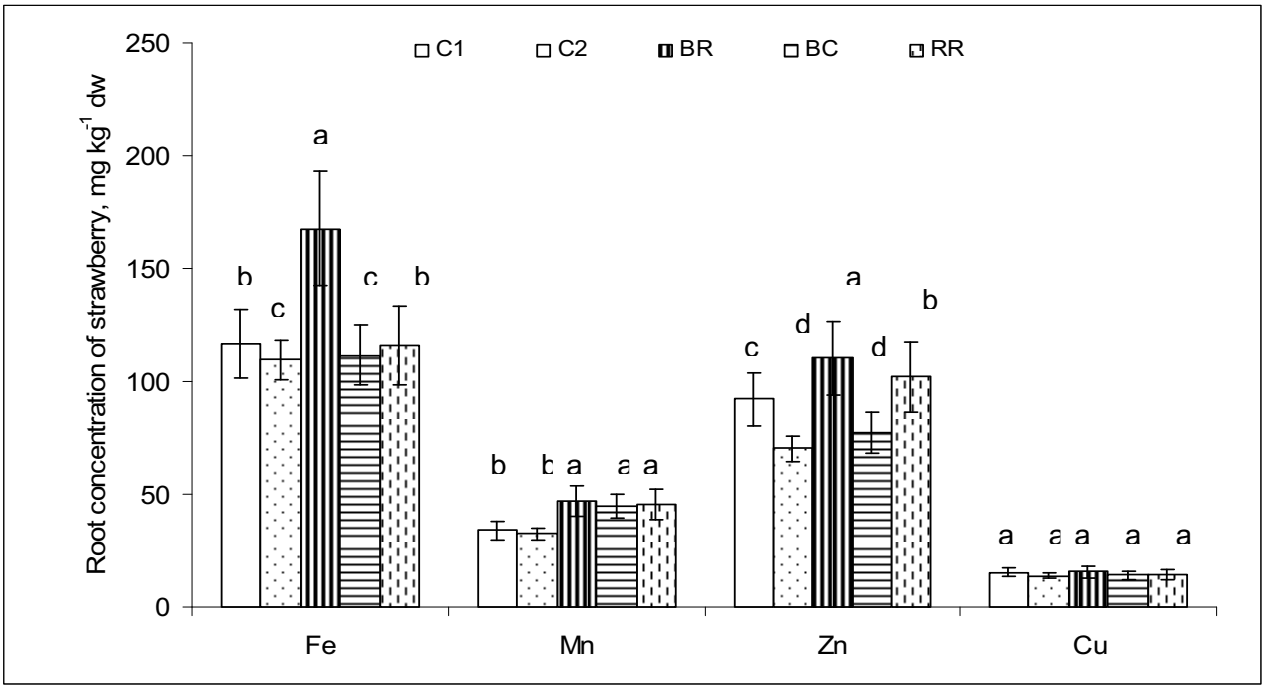


Figure 2: Effects of PGPR application on P, S, Ca, Mg, Na, Fe, Mn, Zn, and Cu content of leaf and root of strawberry plant

Conclusions

Our results indicated that PGPR application in organic farming can be use to increasing the fertilizer use efficiency similar to mineral fertilizer usage. In view of environmental pollution in case of excessive use of mineral fertilizers and due to high costs in the production of N and P fertilizers, bacteria tested in our study may well be suited alone or in combination to achieve sustainable and ecological agricultural production in the region. An important nutritional problem of developing countries is micro-nutrient malnutrition, also called hidden hunger. This paper supports the view that inoculations with PGPR have some potential to increase use efficiency of organic fertilizer in both organic and conventional farming.

References

Ataoglu, N., Turan, M.& Sezen, Y. (2004). Effects of phosphorus solubilizing bacteria (*Bacillus megatherium*) and growing media on growing performance and mineral contents of corn plant (*Zea mays* L.). In: Proceedings of the International Soil Congress on Natural Resource Management for Sustainable Development, 7-10 June, Erzurum, Turkey, pp. 10-18.

Bashan, Y., Harrison, S.K. & Whitmoyer, R.E. (1990). Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. *Applied and Environmental Microbiology* **56**: 769-775.

Canbolat, M.Y., Bilen, S., Cakmakci, R., Sahin, F. & Aydin, A. (2006). Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil *Soils*, **42**:350–357.

Cakmakci, R., Kantar, F. & Sahin, F. (2001). Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. *Journal of Plant Nutrition and Soil Science* **164**: 527-531.

Cakmakci, R., Donmez, F. & Erdogan, U. (2007a). The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish J. of Agr and For* **31:**189-199.

Cakmakci, R., Erat, M., Erdogan, U. & Donmez, M.F. (2007b). The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. *Journal of Plant Nutrition and Soil Sci.* **170**: 288-295.

Dolgun, O. (2007) Field performance of organically propagated and grown strawberry plugs and fresh plants. Journal of the Science of Food and Agriculture **87** ,1364-1367.

Elkoca, E., Kantar, F. & Sahin, F. (2008). Influence of Nitrogen Fixing and Phosphorus Solubilizing Bacteria on the Nodulation, Plant Growth, and Yield of Chickpea. *Journal of Plant Nutrition* **31**: 157–171.

Esitken, A., Karlidag, H., Ercisli, S., Turan, M. & Sahin, F. (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Australian Journal of Agricultural Research* **54**: 377-380.

FAO (2007) Statistical Database. — Available at: http://www.fao.org/corp/statistics/en/

Guneş, A., Ataoğlu, N., Turan, M., Eşitken, A., Ketterings, Q.M. (2009). Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. *Journal of Plant Nutrition and Soil Science* **172**: 385–392

Jones, T.A., Nielson, D.C. & Carlson, J.R. (1991). Development of a grazing-tolerant native grass for revegetating bluebunch wheatgrass sites. *Rangelands* **13**:147–150.

Lugtenberg, B., & Kamilova, F., (2009). Plant-Growth-Promoting Rhizobacteria. Annu. Rev. Microbiol. 63:541–556.

Mehta, S. & Nautiyal, C.S. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current microbiology*. **43**(1):51-6

Mertens, D. (2005a). AOAC Official Method 922.02. Plants Preparation of Laboratuary Sample. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter 3, pp1-2, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA.

Mertens, D. (2005b). AOAC Official Method 975.03. Metal in Plants and Pet Foods. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter 3, pp 3-4, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA.

Mills, H. A., & Jones, J. B. (1996). Plant Analysis Handbook II. Micromacro Publishing. 183 Paradise Blvd Ste 104, Athens, Georgia.

O'Connel, P: F. (1992). Sustainable Agriculture-a valid alternative. *Outlook on Agriculture* **21** (1), 5-12.

Orhan, E., Esitken, A., Ercisli, S., Turan, M. & Sahin, F. (2006). Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae* **111**: 38-43.

Turan, M., Ataoglu, N. & Sezen, Y. (2004). Effects of phosphorus solubilizing bacteria (*Bacillus megaterium*) on yield and phosphorus contents of tomato plant (*Lycopersicon esculentum* L.) III. In: Proceedings of the National Fertilizer Congress. Farming–Industry–Environment, 11–13 October, Tokat, Turkey.

Turan, M., Ataoglu, N. & Sahin, F. (2005). Evaluation the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *Journal of Sustainable Agriculture* **28**: 99-108.

Sahin, F., Cakmakci, R. & Kantar, F. (2004). Sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria. *Plant and Soil* **265**: 123-129.

SPSS Inc., (2004). SPSS Inc. SPSS® 13.0 Base User's Guide, Prentice Hall.