# Potential Use of Plant Growth Promoting Rhizobacteria in Organic Broccoli (*Brassica oleracea L., var. italica* ) Production

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## Abstract

*The continued use of chemical fertilizers and manures for enhanced soil fertility and crop productivity often results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface runoff of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems. Integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Microbial inoculants are promising components of such management systems. Field experiments was carried out to determine effects of plant growth promoting rhizobacteria (PGPR) [Bacillus cereus, ($N_2$-fixing), Brevibacillus reuszeri (phosphate solubilizing), and Rhizobium rubi ($N_2$-fixing and phosphate solubilizing)] on growth, chlorophyll content, yield and ionic composition of leaves of broccoli. The results showed that PGPR strain treatments stimulated plant growth and resulted in significant yield increase. Inoculation of broccoli plant roots and rhizosphere with PGPR strains also improved significantly uptake of mineral elements. The results of this study suggest that Bacillus cereus, Brevibacillus reuszeri and Rhizobium rubi have the potential to increase the yield, growth and nutrition of broccoli plant under organic growing conditions.*

**Keywords:** Bacterial inoculation, plant growth promoting rhizobacteria, fertilizer use efficiency

## Introduction

Fertilizers are essential components of modern agriculture because they provide essential plant nutrients. The use of fertilizers, including chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex system of the biogeochemical cycles. Fertilizer use has caused leaching and run-off of nutrients, especially nitrogen (N) and phosphorus (P), leading to environmental degradation. Important reasons for these problems are low use efficiency of fertilizers and the continuous long-term use (Adesemoye and Kloepper, 2009). One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth promoting rhizobacteria (PGPR). These bacteria exert beneficial effects on plant growth and development, and therefore may be used as biofertilizers for agriculture. The natural role of the PGPR in maintaining soil fertility is more important than in conventional agriculture where higher use of agrochemicals minimizes their significance (Canbolat et al., 2006; Adesemoye et al, 2009).

Bacteria are able to exert positive effects on plants through various mechanisms. Nitrogen fixation contributes organic nitrogen for plant growth, while the bacterial enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase hydrolyzes ACC (the immediate precursor of ethylene) and lowers the levels of ethylene produced in developing or stressed plants, promoting root elongation. Some bacteria solubilize insoluble minerals through the production of acids, increasing the availability of phosphorus and other nutrients to plants

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in deficient soils. Several bacteria improve plant growth through suppression of pathogens by competing for nutrients, by antibiosis, or by synthesizing siderophores, which can solubilize and chelate iron from the soil and inhibit the growth of phytopathogenic microorganisms (Caballero-Mellado et al., 2007).

Organic fertilization is very important in organic crop production due to use of inorganic fertilizers is not possible. Therefore $N_2$-fixing and phosphate solubilizing bacteria, including *Bacillus* sp., *Azotobacter* sp., *Azospirillum* sp., *Beijerinckia* sp., *Pseudomonas* sp. are widely used in organic plant growing (Lugtenberg and Kamilova, 2009). However, so far there has been limited attempt to study the effects of PGPR including $N_2$-fixing and phosphate-solubilizing bacteria on plant growth of broccoli. Therefore, in the present work, we report the effect of $N_2$- fixing and a phosphate-solubilizing bacterial strains treatments on broccoli plants growth.

## Materials and Methods

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. Broccoli 'Monet F1' (*Brassica oleracea* L., var. italica) plants were used as plant materials.

Seeds of broccoli were sown into plastic trays filled with peat (pH:5.5, EC:250 dS/m, N:300 mg/l, P: 131 mg/l, K: 333.33 mg/l, organic matter: 2%). The seedlings were initially grown in a greenhouse. Seedlings (about 1-month old) were transplanted in rows (0. 50 m apart with an intra-row spacing of 0.45 m). Each plot consisted of 25 plants. 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 (organic basal 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 Inc., 2004) statistical program. Mean values were separated according to Duncan test at $P$=0.05. Marketable broccoli heads were harvested in September. The inner rows were used for sampling and harvest. Entire plants were harvested at ground level from each plot when the terminal buds were swollen but not opened, and were weighed. The plants were then cut 20 cm below the top of head, which was trimmed to obtain a marketable product. Head diameter was measured across the widest part of head. Data on plant growth variables, such as stem diameter, were measured.

Because heading 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 heading time (five youngest leaves), 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). A portable chlorophyll meter (SPAD–502, Konica Minolta Sensing, Inc., Japan) was used to measure leaf greenness of the broccoli plants at 2 days before harvest. For each plant measurements were taken at four locations on each leaf, two on each side of the midrib on all fully expanded leaves and then averaged.

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 version 4.5, 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. In the present study, P solubilising activities of the two *Brevibacillus reuszeri* and *Rhizobium rubi* were measured according to the qualitative methods (Mehta & Nautiyal., 2001).

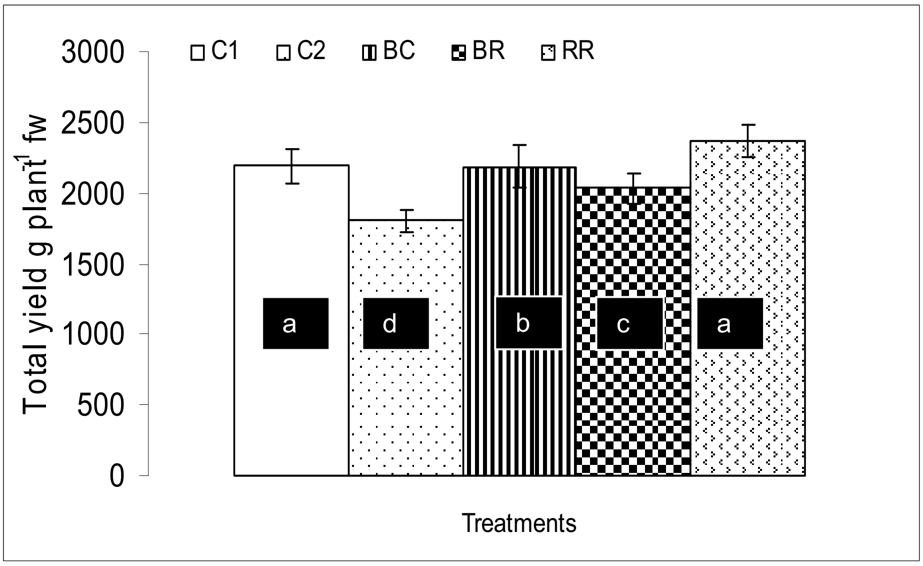
## Results and Discussion

*Yield and yield parameters:*

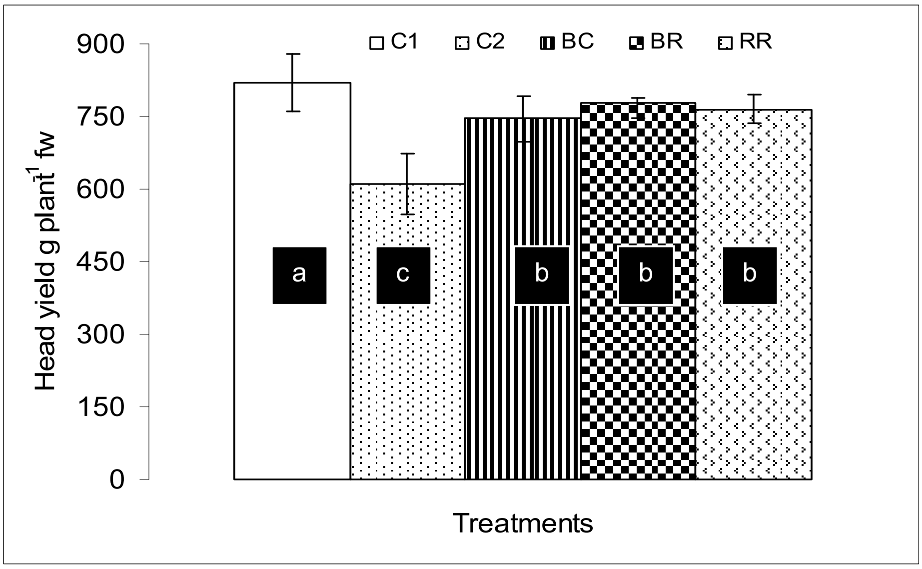
Bacterial inoculations improved the broccoli growth and growth parameters. The performance of the plants was better in inoculated treatments in comparison to the control 2. The results showed that total yield (TY), head yield (HY), head diameter (HD), and stem diameter (SD) of broccoli cultivars significantly increased by $N_2$-fixing and P-solubilising PGPR strains application compared with the control 2. The highest TY, HY, HD and SD were recorded in mineral basal fertilization application (C1), and the lowest TY, HY, HD and SD were determined 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 TY by 18.0%,12% and 31%, HY by 22%, 27%, and 25%, HD by 6%, 4%, and 16%, and SD by 1%, 8%, and 7%, respectively, due to increasing fertilizer efficiency (Figure 1). In other words, organic basal fertilization has significantly increased in TY, HY, HD and SD on condition that PGPR inoculation. When PGPR application with C2 is compared to each other, RR application with C2 was more effective to increase in the TY, HD and SD, while BR was most effective in the HY. 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;*

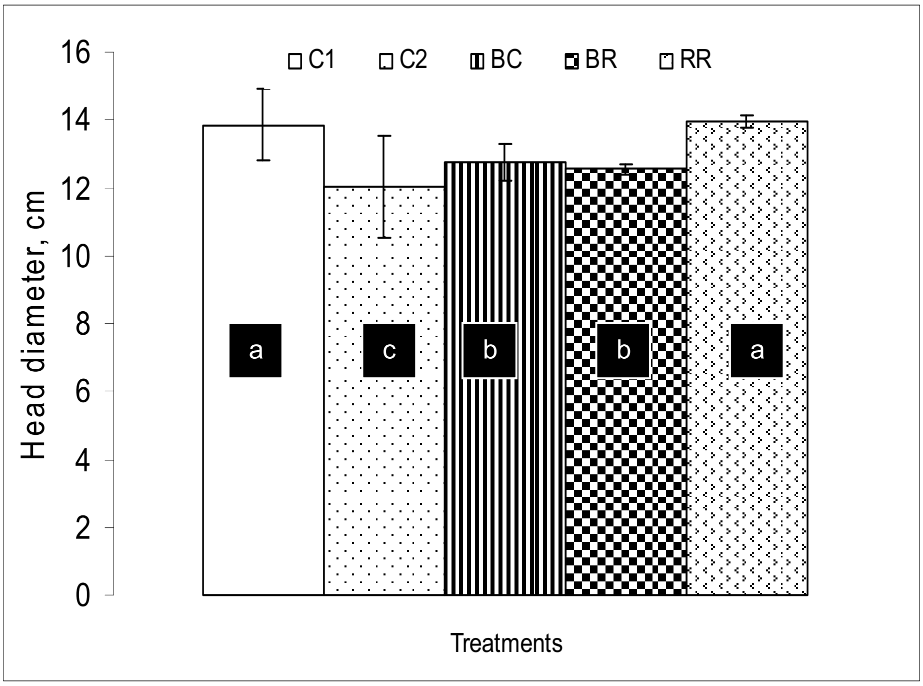
$N_2$-fixing and P-solubilising PGPR strains application promoted PNE contents of different parts of the plant. Although the highest N contents of leaf were obtained from C1, which increased by



C1
C2
BC
BR
RR
3000
2500
2000
1500
1000
500
0
Total yield g plant-1 fw
a
d
b
c
a
Treatments

C1
C2
BC
BR
RR
900
750
600
450
300
150
0
Head yield g plant-1 fw
a
c
b
b
b
Treatments

C1
C2
BC
BR
RR
16
14
12
10
8
6
4
2
0
Head diameter, cm
a
c
b
b
a
Treatments

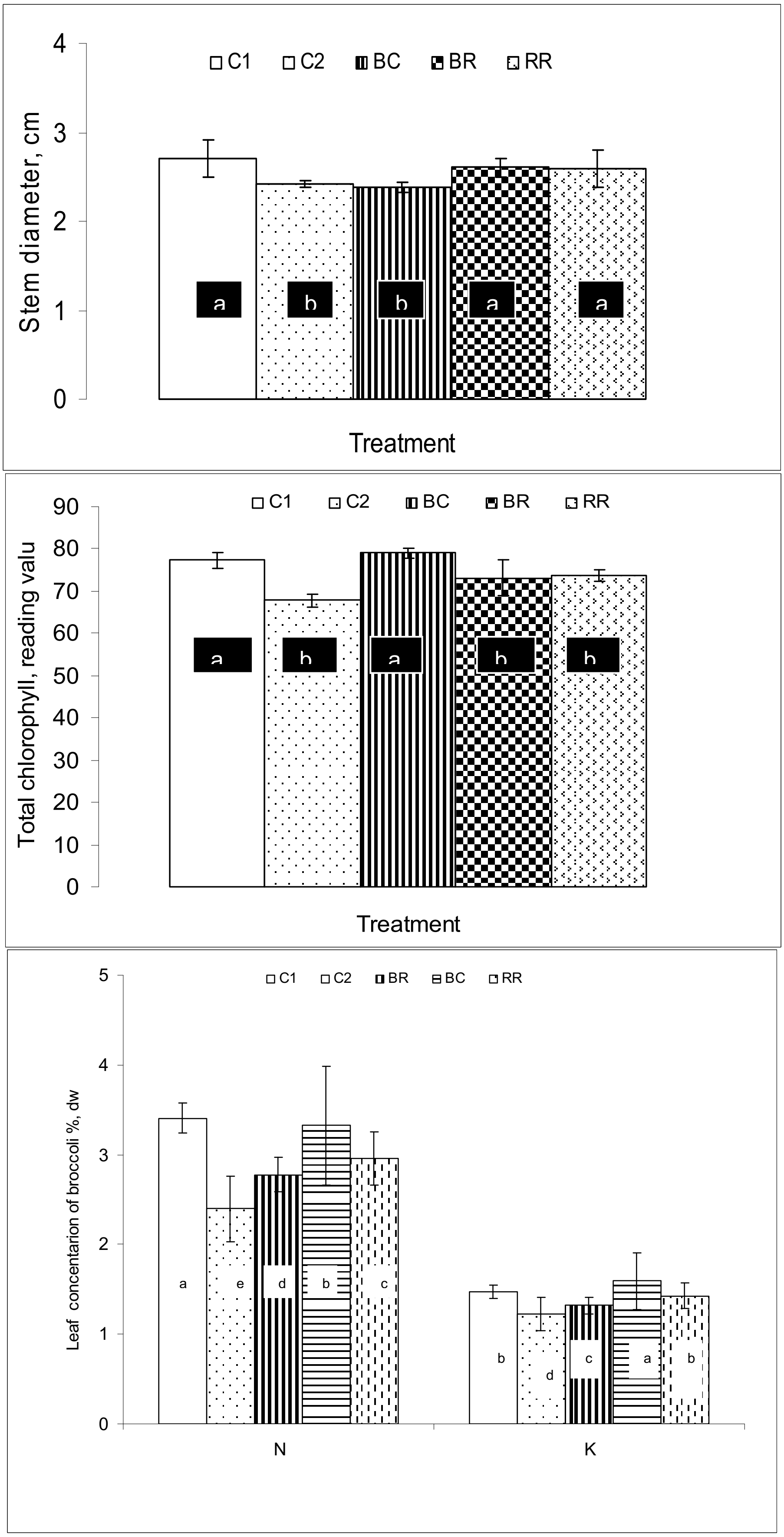



Figure 1: Effects of PGPR application on plant yield, yield parameters and total chlorophyll content of broccoli plant

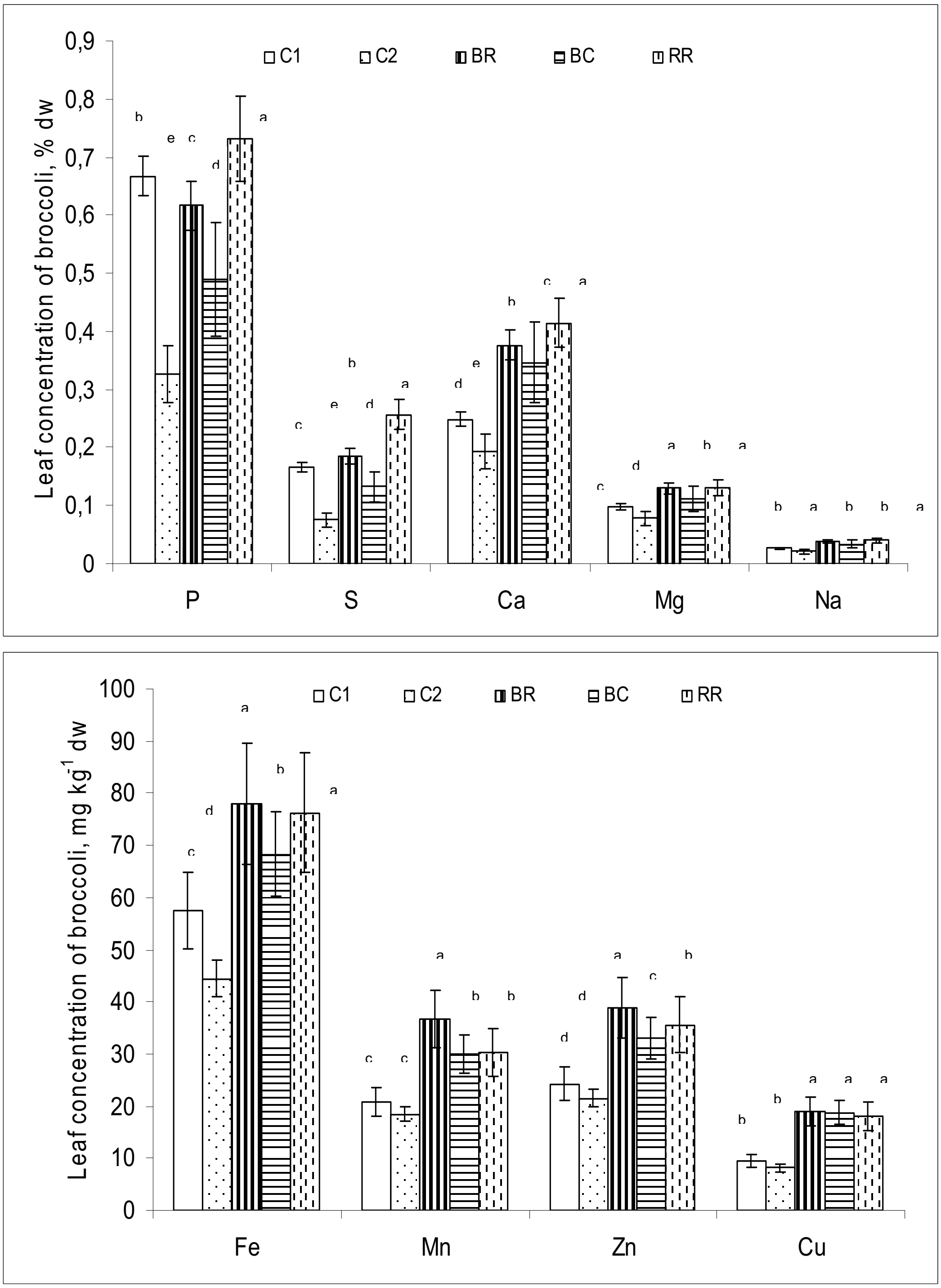



Figure 2. Effects of PGPR application macro-micro content of broccoli leaf

42.3%, compared with the control 2 treatment. On the other hand the bacterial inoculations BC, BR, and RR inoculation to organic basal fertilization (C2) increased leaf macro-micro element contents. While BR application is more effective for Fe (75%),Mn (98%), Zn (80), and Cu (134%) content of leaf, RR was most effective for P (125%), S (242%), Ca (114%), and Mg (68%) content of leaf. On the other hand the highest increase ration of total chlorophyll (16%) and K (30%) content was determined BC when compared with the control 2 treatment (Figure 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 broccoli in comparison to the without PGPR (C2) and RR most effective treatment for TY, HY, HD and SD and P, S, Ca, and Mg content of plant, following order was BR and BC treatment. 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), corn (Ataoglu *et al*., 2004), 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 broccoli 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, sweet cherry, 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.*, 2007b; Turan *et al.*, 2005; Elkoca *et al.*, 2008). In the present study, it was also found that the inoculation of PGPR strains increased macro-micro nutrient content leaves of broccoli, 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 of broccoli plant. Generally, the enhancements in macro-micro nutrient contents except for N were more pronounced in organic basal fertilizer with PGPR treatment whereas mineral basal fertilizer (C1) were also resulted in significant N nutrient increases in plant leaf. Enhancement of mineral uptake by plants should result in an increased accumulation of both dry matter and minerals in 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, apricot and sweet cherry 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), rocket (Dursun *et al.* 2008) and strawberry (Güneş *et al*., 2009) stimulated macro- and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn, Cu.

## 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

Adesemoye, A. O. & Kloepper, J.W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol. 85:1–12

Adesemoye, A. O. Torbert, H. A. . & Kloepper, J.W. (2009). Plant Growth-Promoting Rhizobacteria Allow Reduced Application Rates of Chemical Fertilizers. Microb Ecol (2009) 58:921–929.

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.

Caballero-Mellado, J., Onofre-Lemus, J., Estrada-de los Santos, P., & Martı´nez-Aguilar. L. (2007). The Tomato Rhizosphere, an Environment Rich in Nitrogen-Fixing Burkholderia Species with Capabilities of Interest for Agriculture and Bioremediation. Applıed and Envıronmental Microbıology, 73, 16. 5308–5319.

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., Donmez, M.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 Science* **170**: 288-295.

Dursun, A., M. Ekinci, M. & M. F. Dönmez, (2008). Effects of Inoculation Bacteria on Chemical Content, Yield and Growth in Rocket (Eruca vesicaria subsp. sativa). *Asian Journal of Chemistry*, **20(4):**3197-3202.

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.

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.

Mehta, S. & Nautiyal, C.S. (2001). An efficient method for qu. litative 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.

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.

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