

## Enzyme activities and effect of plant growth-promoting rhizobacteria on growth in mountain tea

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### Abstract

This study aimed to evaluate possible effects and enzyme activities of seven N<sub>2</sub>-fixing and P-solubilizing microorganism based bio-fertilizers (*Paenibacillus polymyxa* RC05, *Pseudomonas putida* RC106, *Bacillus subtilis* RCK17, *Pseudomonas fluorescens* RCK1136, *Pantoea agglomerans* RK79, *P. fluorescens* RC77+B. *subtilis* RC63 and *P. fluorescens* RC77+Bacillus *megaterium* RC07) growth of mountain tea. Results indicate that the highest fresh shoot weight (84%) and fresh root weight (52%) were obtained from the application of RC77+RC63 combination. This combination also brings about the highest total chlorophyll content (51.2%). Other applications, especially RC77+RC07 and RC05, revealed statistically significant improvements in above mentioned parameters, when compared to control. Application of RC77+RC63, RC77+RC07, and RC05 produced statistically significant results in terms of G6PD, 6PGD, GR, and GST enzyme activity. In conclusion, application of RC77+RC63 and RC77+RC07 combinations and RC05 strain have great potential to be used in sustainable and organic mountain tea production.

**Keywords:** PGPR, Enzyme activity, bio-fertilizers, mountain tea

### 1. Introduction

*Sideritis* species are a group of plants known as “mountain tea” in Anatolia. The genus of *Sideritis*, a member of the family Labiatae is widely distributed in subtropical and moderate regions. These species are annual or perennial shrub plants (1). Some species are used as tonics for skin, for stomach disorders (2), and have anti-inflammatory (3), anti-anxiety (4), antibacterial, (5) antioxidant (6) and anticancer (7) effects. There aren't any reports about effect of PGPR (Plant Growth Promoting Rhizobacteria) in *Sideritis*. Bacterial species called plant growth promoting rhizobacteria (PGPR) are able to exert a beneficial effect upon plant growth, N<sub>2</sub> fixing and P solubilizing and playing a significant role as PGPR in the bio-fertilization of crops (8). These microorganisms are found in several genera including *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Rhizobium* and *Serratia* (9, 10, 11). Although the mechanisms of PGPR are not fully understood, they are thought to include the ability to produce plant hormones such as auxins (12), cytokinins (13) and gibberellins (14), asymbiotically fixate N<sub>2</sub> (15), solubilize inorganic phosphate and mineralize organic phosphate and/or other nutrients (12) and antagonism

against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics enzymes and/or fungicidal compounds, and competition with detrimental microorganisms (11, 16, 17). There are some reports with PGPR about promoting plant growth and yield in barley, sugar beet, tomato, pepper, apricot, rice, wheat, canola, maize and conifer species (8, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28). In the OPPP glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) reduce  $\text{NADP}^+$  to NADPH. During the process, the critical precursor used in the synthesis of phenolic secondary metabolites for plant growth and lignifications is produced (29). G6PD and 6-phosphogluconate dehydrogenase (6PGD) is important in cell division and salt responses in plants (30). In the oxidative pentose phosphate pathway (OPPP) is increased during nitrate assimilation in plant tissues. Reducing power for nitrate and nitrite reduction are provided by this process (31). Glutathione reductase (GR) catalyses the transformation of glutathione to reduced glutathione (32). During the regeneration of ascorbate and GSH GR plays important role GR has a key role in anti-oxidant defense and the plant's response to environmental stress. (33). Glutathione *S*-transferases (GST) comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification (34). GR and GST play an important role in glutathione metabolism and the adaptation of plant to stress conditions (35). Furthermore, plant GST has function in the cellular response to auxins and during the metabolism of plant secondary products (36). The aim of this study was to determine effects of mineral fertilizer (NPK) and seven  $\text{N}_2$ -fixing and P-solubilizing microorganism based bio-fertilizers growth of mountain tea. On the other hand, we examine the effects of anti-oxidant (GR and GST) and OPPP (G6PD and 6PGD) enzymes in the leaves of mountain tea.

## 2. Materials and Methods

### 2.1. Isolation and cultivation of the bacterial strains

A total of 5 bacterial strains and their of different combinations were tested for growth of mountain tea. They were isolated from rhizosphers of different plants. The bacterial cultures were grown on nutrient agar (NA) for routine use and maintained in Luria Broth (LB) with 15% glycerol at  $-80^\circ\text{C}$  for long-term storage (37), at the Department of Plant Protection, Faculty of Agriculture, Atatürk University.

### 2.2. Identification of the bacterial strains by Microbial Identification System [MIS]

Identification of the tested bacterial strains was confirmed by using MIS systems. Preparation and analysis of FAMES from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMES were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm, with cross-linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package (38).

### 2.3. Hypersensitivity tests [HR]

All of the bacterial strains were tested for hypersensitivity on tobacco plants (*Nicotiana tabacum* L. var. Samsun). The bacterial suspension ( $10^8\text{cfu/ml}$ ) was prepared in sterile distilled water and infiltrated into the inter-costal area of the leaves of tobacco plants by using a 3-cc syringe (Becton Dickinson, Franklin Lakes, NJ, U.S.A). The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24-48 h at  $20\text{-}28^\circ\text{C}$ . The presence of rapid tissue necrosis at the inoculation site was recorded within 24-48 h after infiltration. This test was repeated, at least three times, for each strain. For HR tests, sterilized distilled water ( $\text{sdH}_2\text{O}$ ) was used as a negative control.

#### 2.4. Phosphate solubilisation and Nitrogen fixation

Phosphate solubilization activity of the bacterial isolates was detected on Pikovskaya (PVK) and National Botanical Research Institute's phosphate growth medium (NBRIP-BPB). NBRIP-BPB contained (per-liter): glucose, 20 g;  $\text{Ca}_3(\text{PO}_4)_2$ , 10 g;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 5 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25 g; KCl, 0.2 g;  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 g, and bromophenolblue (BPB), 0.025 g. The pH of the media was adjusted to 7.0 before autoclaving, as described earlier (39). 5 ml of NBRIP-BPB medium was transferred to a sterile test tube and autoclaved. Autoclaved, uninoculated broth medium was served as control. The sterile liquid medium was inoculated with 500 µl suspension of the tested bacterial strains. The test tubes were incubated for 14 days at room temperature. At the end of the incubation period, change in pH of the culture broth was recorded. All the pure isolates were also tested in triplicate for their phosphate solubilizing capacity in sucrose-tricalcium phosphate agar media (40). Pikovskaya's medium contained per litre: glucose, 10 g;  $\text{Ca}_3(\text{PO}_4)_2$ , 5 g;  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 g; NaCl, 0.2 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g; KCl, 0.2 g; NaCl, 0.2 g;  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.002 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.002 g; yeast extract, 0.5 g. After incubation for 6 days, water soluble P was determined colorimetrically by the vanadomolybdophosphoric acid colorimetric method (41).

Each isolated strain was inoculated in plates containing NFb medium with or without addition of  $\text{NH}_4\text{Cl}$  as a unique nitrogen source (42). Plates were incubated at 28°C for 7 days and bacterial growth was observed as qualitative evidence of the atmospheric nitrogen fixation.

#### 2.5. Greenhouse studies

We used seven different potential PGPR rhizobacterial isolates, obtained from different plant rhizosphere on the basis of their SIM value,  $\text{N}_2$ -fixing and P-solubilizing ability. Bacterial cultures were grown on nutrient agar (NA) for routine use and for this experiment, a single colony was transferred to 500 ml flasks containing nutrient broth (NB) and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27°C (Merck, Germany). The bacterial suspension was diluted in sterile distilled water to a final concentration of  $10^8$  cfu/ml. Bacterial strains and fertilizers were tested for on growth and yield increasing potential under natural soil conditions by conducting pot experiments in Department of Field Crops greenhouse of Erzurum. The experiments were conducted in a completely randomized design with five replicates and repeated twice. The treatments included: 1: Control (without bacteria inoculation or mineral fertilizers), 2: NPK fertilizer (450 mg N + 300 mg P + 210 mg K/pot); 3: *P. polymyxa* RC05, 4: *P. putida* RC106, 5: *B. subtilis* RCK17, 6: *P. fluorescens* RCK1136, 7: *P. agglomerans* RK79, 8: *P. fluorescens* RC77+*B. subtilis* RC63, 9: *P. fluorescens* RC77+*B. megaterium* RC07. The bio-fertilizers application involved dipping the root system of the saplings into a suspension of each formulations for 60 min, prior to planting. Fresh and dry shoot weight, fresh and dry root weight, total chlorophyll content, number of leaves on 90th day and leaf area index were collected for all mountain tea rooted cuttings. An analysis of variance (ANOVA) and Duncan's multiple range test were performed to analyse statistical difference and to discriminate between means.

#### 2.6. Preparation of enzyme extracts

Mountain tea (*Sideritis perfoliata*) leaves were harvested and stored at -80°C prior to use. 0.5 g leaves were ground in liquid nitrogen and 2 ml of the extract buffer was added containing: 50 mM Tris-HCl, 1 mM EDTA, pH: 7.5. The homogenate was centrifuged at 15,000 g for 20 min. The supernatants were used as a crude extract for determination of Glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), Glutathione reductase (GR), Glutathione S-transferase (GST) activities.

#### 2.7. Determination of protein concentrations and the enzyme activities

The protein concentrations of crude extract were determined by measurements of absorbance at 595 nm according to Bradford's method (43), with bovine serum albumin using as

a standard. G6PD (EC 1.1.1.49) and 6PGD (EC 1.1.1.44) activities were measured according to the Beutler's method (Beutler, 1984). Reaction medium contained 0.1 mM Tris-HCl buffer (pH:8), including 1 mM EDTA and 20 mM MgCl<sub>2</sub>, 0.2 mM NADP<sup>+</sup>, and 0.6 mM G6P for G6PD and 0.6 mM 6PGA for 6PGD in a total volume of 1 mL. The increase in A<sub>340</sub> nm was recorded. One enzyme unit was defined as the reduction of 1 µmol NADP<sup>+</sup> per min under the assay condition. GR (EC 1.8.1.7) was assayed by the fall in absorbance at 340 nm according to the method of Carlberg and Mannervik (1985). The assay system contained 0.5 mM Tris-HCl buffer pH 7.0, including 1 mM EDTA, 2 mM GSSG, and 0.2 mM NADPH in a total volume of 1 mL, one enzyme unit was defined as the oxidation of 1 µmol NADPH per min under the assay conditions. GST activity was measured using CDNB described by Habig et al. (1974). The assay system contained 0.1 M potassium phosphate buffer pH 6.5, 1.0 mM GSH, 1.0 mM CDNB, and 1% absolute ethanol in a total volume of 1.0 mL. The product of CDNB conjugation with GSH absorbs at 340 nm, one unit of activity was defined as the formation of 1.0 µmol product min<sup>-1</sup>. All assays were performed spectrophotometrically (Shimadzu Spectrophotometer UV-1800).

### 3. Results and Discussion

The MIS identification results of the bacterial strains, their similarity index (SIM), their nitrogen fixation, phosphate solubilization and hypersensitivity tests results are shown in Table 1. According to the MIS results, bacterial strains were identified as *P. polymyxa*, *P. putida*, *B. subtilis*, *P. fluorescens*, *P. agglomerans*, *P. fluorescens*, *B. subtilis* and *B. megaterium*. All isolates showed capacity to grow in nitrogen-free conditions and to solubilize phosphate. They also showed negative hypersensitivity tests results.

**Table1.** MIS identification results of bacterial strains, their similarity index (SIM), phosphate solubilization, nitrogen fixation and hypersensitivity reaction

Strain No	MIS results	SIM	PS	NF	HR
RCK-17	<i>Bacillus subtilis</i>	0.677	K+	Z+	-
RCK-1136	<i>Pseudomonas fluorescens</i>	0.530	K+	+	-
RK-79	<i>Pantoea agglomerans</i>	0.762	+	+	-
RC05	<i>Paenibacillus polymyxa</i>	0.809	+	K+	-
RC07	<i>Bacillus megaterium</i>	0.786	K+	Z+	-
RC63	<i>Bacillus subtilis</i>	0.600	Z+	K+	-
RC77	<i>Pseudomonas fluorescens</i>	0.833	+	K+	-
RC106	<i>Pseudomonas putida</i>	0.776	+	+	-

**SIM:** Similarity index, **PS:** Phosphate solubilization. **NF:** Nitrogen Fixation; **HR:** Hypersensitivity reaction:- negative reaction, +: positive reaction, **K+:** Powerful positive reaction, **Z+:** Powerful negative reaction

Application of single and mixed N<sub>2</sub>-fixing and/or P-solubilizing microorganism based liquid carrier-based bio-fertilizers promoted growth and yield of mountain tea under greenhouse conditions by conducting pot experiments at the Department of Field Crops, Faculty of Agriculture, Atatürk University of Erzurum. The best effects were obtained in plants grown in soils with mixed combination of the RC77+RC63, RC07+RCK77, NPK, RC05 (Table 2). P-solubilizing and N<sub>2</sub>-fixing bacteria improved and total chlorophyll content of *S. perfoliata*, but growth responses were strain-specific. The results showed that by using bio-fertilizers, the amount of chemical nitrogen and phosphorus can be reduced in *S. perfoliata* without negative effects on growth and yield. Tested microorganism based bio-fertilizers applications were found to be more effective in particular mixed combination suggesting that they can use as alternative for chemical fertilizers on the use of a single bacterium (15, 44).

**Table 2.** Influence of PGPR and fertilizer application on fresh and dry shoot weight, fresh and dry root weight, total chlorophyll content, number of leaves (on 90<sup>th</sup> day) and leaf area index of *Sideritis perfoliata*

Treatments	Shoot weight [g/plant]		Root weight [g/plant]		Total chlorophyll content (SPAD)	Number of leaves (90 <sup>th</sup> day)	Leaf area index
	Fresh weight	Dry weight	Fresh weight	Dry weight			
Control	46,9±4,0 f	5,08±0,4 e	21,8±1,9 e	2,96±0,3 e	37,8±0,6 f	33,2±5,5 e	4,5±0,5 e
NPK	71,4±12,4 bc	10,60±1,5 b	45,2±7,5 b	6,30±2,2 ab	47,5±1,6 bc	67,2±4,8 a	7,4±0,4 a
RC05	77,9±7,2 ab	9,77±0,9 b	39,9±3,7 b	5,28±0,7 c	47,6±3,4 bc	55,8±6,0 bc	6,7±0,4 b
RC106	47,1±7,9 f	5,03±0,9 e	21,6±3,8 e	2,93±0,5 e	37,9±0,7 f	31,8±3,8 e	3,7±0,5 f
RCK17	47,6±7,9 ef	5,15±0,9 e	22,2±3,9 e	3,0±0,5 e	42,6±2,9 de	52±3,4 b-d	5,8±0,4 c
RCK 1136	64,3±5,7 cd	7,95±0,8 c	32,1±4,0 c	4,16±0,6 d	43,6±3,0 de	54,6±6,3 bc	5,9±0,5 c
RK79	62,8±3,6 cd	7,90±0,5 c	33,2±2,5 c	4,20±0,3 d	45,2±4,3 cd	53,8±5,4 bc	5,8±0,7 c
RC77+63	84,0±5,5 a	10,64±0,7 b	52±3,4 a	6,9±0,5 a	51,2±1,3 a	57,8±4,5 b	7,4±0,3 a
RC77+07	82,9±7,4 a	12,36±1,5 a	42,4±3,8 b	5,95±0,6 bc	50,7±0,9 ab	57,8±8,5 b	7,2±0,6 ab

\*Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ( $P<0.05$ ).

\*control: without bacteria inoculation or mineral fertilizers; NPK fertilizer (450 mg N + 300 mg P + 210 mg K/pot); RC05, RC106, RCK17, RCK1136, RK79, RC63+RC77 and RC77+RC07 (*P. polymyxa* RC05, *P. putida* RC106, *B. subtilis* RCK17, *P. fluorescens* RCK1136, *P. agglomerans* RK79, *P. fluorescens* RC77+B. *subtilis* RC63 and *P. Fluorescens* RCK77+B. *megatherium* RC07).

Co-inoculation, frequently, increased growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients (45). Also, the few studies in the literature comparing the efficiency of combinations of beneficial rhizobacteria to single strain inoculation showed that mixed inoculation could lead to a competitive process by which plant growth promotion rhizobacteria. Results indicate that the highest fresh shoot weight (84%) and fresh root weight (52%) were obtained from the application of RC77+RC63 combination. This combination also brings about the highest total chlorophyll content (51.2%). Other applications, especially RC77+RC07 and RC05, demonstrated statistically significant improvements in above-mentioned parameters when compared to control. Application of RC77+RC63, RC77+RC07, and RC05 produced statistically significant results in terms of G6PD, 6PGD, GR, and GST enzyme activity.

The levels of GR, GST, G6PD, and 6PGD were determined in leaf extracts of young mountain tea plants, and the results are shown in Table 3. According to these results obtained; it was established that increasing at varying rates in GR, GST, 6PGD and G6PD enzyme activities occur in the application of bacteria. In the applications, made maximum GR 2.44 and RCK77+ 63 were obtained with the combination applications. This was followed by 1.80 with RC05, 1.76 with RCK77+07 applications. While the results of these three treatments in GR activity were statistically significant according to the negative control, they were found to be statistically insignificant in comparison to the positive control. 0.24, 0.21 and 0.19 results in 6PGD; 0,45, 0,33 and 0,30 results in G6PD were obtained respectively in the RCK77+63, RCK77+07 and RC05 applications. Similarly, the results of these three treatments in GR activity were found to be statistically significant according to the negative control, but they were found to be statistically insignificant. In GST activity, 1.02 was obtained from RCK77+ 63 application, 1.04 rate was obtained from the positive control (NPK) application. When the results obtained compared with the negative control and other applications, they were found to be statistically significant. However, the results obtained from the NPK application with the bacteria application were determined to have the same importance statistically. Consequently, when the results obtained from RCK77+63, RCK77+07 and RC05 bacteria applications used in the experiment compared with the control applications made, according to the negative control, these results were found to be statistically significant in all GR, GST, 6PGD and G6PD activities. This situation proves that the strain and/or strains used in this experiment in three applications have a high potential to be used as a bio-fertilizer. Among the bio-fertilizers, the

N<sub>2</sub>-fixing and P-solubilizing microorganism based mixed bio-fertilizers RC77+RC63, RC77+RC07 and RC05 have great potential to be used in sustainable and organic mountain tea production.

In recent studies have demonstrated that GR plays an important role in foliar growth in ryegrass and can be related to fresh mass and dry matter in spring wheat (46). Our study presented in this paper similar to other reports that GR activity increases with the nitrogen supply (32,35) and supports the hypothesis (47), suggesting that an increase in growth rate causes a deprivation of GSH and therefore increase in GR activity. Our research was screening that there is an effect of PGPR inoculation on growth and GR, GST, 6PGD, and G6PD activities in mountain tea leaves. Similarly, PGPR application of wheat and spinach on GR, GST, 6PGD, and G6PD enzyme activity have been found to be effective (32, 48, 49) demonstrated, in three plant species, that GR activity increased at high levels of N supply compared with low N. Ammonium-fed plants showed higher GR activities in maize and sunflower leaves, with the highest GST activity in maize (35). Also, G6PD and 6PGD activities are higher in roots supplied with NO<sub>3</sub><sup>-</sup> (31,50) and these activities associated with changes of plant growth and development (51). Changes in enzyme activities appeared to be triggered by the PGPR strain selected. PGPR inoculation gives rise to a differential increase in leaf 6PGD activity, as well as activation of other plant enzymes; this was a show that activation of these enzymes in mountain tea leaves would be differentially affected by different PGPR strain. Therefore, the growth and yield parameters of mountain tea could be improved by PGPR treatment due to increases in the activities of enzymes which play an important role in nitrate assimilation as well as in water and nutrient use efficiency. Finally, further studies are necessary to approve the effects of PGPR strains on the different enzyme activities responsible for plant nutrient uptake in other plant species under various conditions.

**Table 3:** The effect of PGPR on the activities of the antioxidant (GR and GST) and pentose phosphate oxidative cycle enzymes (G6PD and 6PGD) in the leaves of mountain tea.

Treatments	GR	6PGD	G6PD	GST
Control	0,67 a	0,06 a	0,09 a	0,20 a
NPK	1,75bc	0,23 b	0,48 c	1,04 d
RCK77+63	2,44 c	0,24 b	0,45c	1,02 d
RCK77+07	1,76 bc	0,21 b	0,33bc	0,58 c
RC05	1,80 bc	0,19 b	0,30bc	0,52 bc
RK79	1,54 a-c	0,17 ab	0,13 ab	0,35 a-c
RCK1136	0,96 ab	0,18 b	0,17 ab	0,48 bc
RCK17	0,92 ab	0,19 b	0,24 ab	0,44 a-c
RC106	1,00 ab	0,21 b	0,15 ab	0,34 a-c

G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; GR, glutathione reductase; GST, glutathione S-transferase; values are means  $\pm$  SE; values followed by different letters in a column were significantly different ( $p < 0.01$ ), using Duncan's multiple range test.

Our results clearly indicate the beneficial effect of co-culturing the N<sub>2</sub>-fixer and P-solubilizer in inoculants production. Combined inoculations with N<sub>2</sub>-fixer and P-solubilizer have been reported to be more effective than single inoculation on promoting plant growth and providing a more balanced nutrition for various crops (15,52,53). In agricultural and forestry inoculation practices, mixing of two or more microbial species often has a more positive effect on plant growth may be reduced (54, 55).

## References

1. P.H. DAVIS. Flora of Turkey and East Eage an Island. Vol. 7. Edinburgh: Edinburgh University Press. (1982).
2. M.OZTURK, M.E. DURU, F. AYDOGMUS-OZTURK, M. HARMANDAR, M. MAHLICLI, U. KOLAK, A. ULUBELEN. GC-MS analysis and antimicrobial activity of essential oil of *Stachys cretica* subsp. *smyrnaea*. Nat. Prod. Commun, 4, 1, 109-114 (2009).
3. M. KHANANI, M. SHARIFZADEHB, A. HADJIAKHOONDIA, A. SHAFIEEC. Phytochemical investigation and anti-inflammatory activity of aerial parts of *Stachys byzanthina* C. Koch. J. Ethnopharmacol, 97, 463-468 (2005).

4. M. RABBANI, S.E. SAJJADI, H.R. ZAREI. Anxiolytic effects of *Stachys lavandulifolia* Vahl on the elevated plus-maze model of anxiety in mice. *J. Ethnopharmacol.* 89,271-276 (2003).
5. H. GRUJIC-JOVANOVIC, D. SKALTSIA, P. MARIN, M. SOKOVIC. Composition and antimicrobial activity of the essential oil of six *Stachys* species from Serbia. *Flavour Fragr J.* 19, 139-144 (2004).
6. A. AYDIN, B. SENER, I. ÇAKICI, N. N. TURAN, N. ERDEMOĞLU. Antioxidant activities of some Lamiaceae plant extracts. *Phytother Res.* 20: 91-93, (2006).
7. Z. AMIRGHOFRAN, M. BAHMANI, A. AZADMEHR, K. JAVIDNIA. Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. *Neoplasma*, 53,428-433 (2006).
8. R. ÇAKMAKCI, F. DÖNMEZ, A. AYDIN, F. SAHİN. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry*, 38, 1482-1487, (2006).
9. H. RODRIQUEZ, R. FRAGA. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv.* 17, 319-339 (1999).
10. A. DURSUN, M. EKINCI, M. F. DONMEZ. Effects of inoculation bacteria on chemical content, yield and growth in rocket [*Eruca vesicaria* subsp. *sativa*]. *Asian J. Chem.* 20, 3197-3202 (2008).
11. H. KARAKURT, R. KOTAN, F. DADASOĞLU, R. ASLANTAS, F. SAHİN. Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values and vegetative growth of sour cherry [*Prunus cerasus* cv. Kütahya]. *Türk J Biol.* 35, 283-291 (2011).
12. J.S. JEON, S.S. LEE, H.Y. KIM. Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol.* 41, 271-276 (2003).
13. I.E. GARCIA DE SALAMONE, R.K. HYNES, L.M. NELSON. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can J. Microbiol.* 47, 404-411 (2001).
14. F.J. GUTIERREZ-MANERO, B. RAMOS-SOLANO, A. PROBANZA. The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol Plantarum*, 111, 206-211 (2001).
15. F. SAHİN, R. ÇAKMAKCI, F. KANTAR. Sugar beet and barley yields in relation to inoculation with N<sub>2</sub> fixing and phosphate solubilizing bacteria. *Plant Soil*, 265, 123-129 (2004).
16. R. DEY, K.K. PAL, D.M. BHATT. Growth promotion and yield enhancement of peanut [*Arachis hypogaea* L.] by application of plant growth-promoting rhizobacteria. *Microbiol Res.* 159, 371-394 (2004).
17. R. KOTAN, F. SAHİN, E. DEMIRCI. Evaluation of antagonistic bacteria for biological control of *Fusarium* dry rot of potato. *Phytopathology*, 89-41 (1999).
18. J.R. DE FREITAS. Yield and N assimilation of winter wheat [*Triticum aestivum* L., var. Norstar] inoculated with rhizobacteria. *Pedobiologia*, 44, 97-104 (2000).
19. S.S. PAL. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant and Soil*, 198, 169-177 (1998).
20. R. ÇAKMAKCI, F. KANTAR, O.F. ALGUR. Sugar beet and barley yield in relation to *Bacillus polymyxa* and *Bacillus megaterium* var. *phosphaticum* inoculation. *Journal of Plant Nutrition and Soil Science*, 162, 437-442 (1999).
21. D. CUPPELS, F. SAHİN, S.A. MILLER. Management of bacterial spot of tomato and pepper using a plant resistance activator in combination with microbial biocontrol agents. *Phytopathology*, 89-19, (1999).
22. R. KOTAN, F. SAHİN, E. DEMIRCI. Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biol. Control*, 50, 194-198 (2009).
23. S.N. SUDHA, R. JAYAKUMAR, V. SEKAR. Introduction and expression of the *cryIac* gene of *Bacillus thuringiensis* in a cereal-associated bacterium, *Bacillus polymyxa*. *Current Microbiology*, 38,163-167, (1999).
24. J.R. DE FREITAS, J.J. GERMIDA. Growth promotion of winter wheat by fluorescent *Pseudomonas* under field conditions. *Soil Biology and Biochemistry*, 24, 1137-1146 (1997).
25. E. BENT, C. BREUIL, S. ENEBAK, C.P. CHANWAY. Surface colonization of lodgepole pine [*Pinus contorta* var. *latifolia* Dougl. Engelm.] roots by *Pseudomonas fluorescens* and *Paenibacillus polymyxa* under gnotobiotic conditions. *Plant and Soil*, 241,187-196 (2002).
26. A. ESITKEN, H. KARLIDAG, S. ERCISLI. The effects of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot [*Prunus armeniaca* L. cv. Hacıhaliloglu]. *Aust. J. Agric. Res.* 54, 377-380 (2003).
27. E. ELKOCA, F. KANTAR, F. SAHİN. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth and yield of chickpea. *J. Plant. Nutr.*, 31, 157-171 (2008).
28. O.A. ALMAGHRABI, S.I. MASSOUD, T.S. ABDELMONEIM. Influence of inoculation with plant growth promoting rhizobacteria [PGPR] on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi Journal of Biological Sciences*, 20, 1, 57-61 (2013).
29. P. MCCUE, Z. ZHENG, J.L. PINKHAM, K. SHETTY. A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. *Proc. Biochem.* 35, 603-613 (2000).
30. J. HUANG, H. S. ZHANG, J. F. WANG, J.S. YANG. Molecular cloning and characterization of rice 6-phosphogluconate dehydrogenase gene that is up-regulated by salt stress. *Molecular Biology Reports*, 30, 223-227 (2003).

31. C. G. BOWSHER, D. P. HUCKLESBY, M. J. EMES. Nitrite reduction and carbohydrate metabolism in plastids purified from roots of *Pisum sativum* L. *Planta* 177, 359-366 (1989).
32. L.O. MEDICI, R.A. AZEVEDO, R.J. SMITH, P.J. LEA. The influence of nitrogen supply on antioxidant enzymes in plant roots. *Funct. Plant Biol.* 31, 1-9 (2004).
33. K. TANAKA, T. SANO, K. ISHIZUKA, K. KITTA, Y. KAWAMURA. Comparison of properties of leaf and root glutathione reductases from spinach. *Physiol. Plant.* 91, 353-358 (1994).
34. R. UDOMSINPRASERT, S. PONGJAROENKIT, J. WONGSANTICHON, A.J. OAKLEY, L.A. PRAPANTHADARA, M.C. WILCE, A.J. KETTERMAN. Identification, characterization and structure of a new Delta class glutathione transferase isoenzyme. *Biochem. J.* 388 (Pt 3): 763-71 (2005).
35. K. RIOS-GONZALEZ, L. ERDEI, S.H. LIPS. The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. *Plant Sci.* 162, 923-930 (2002).
36. K.A. MARRS. The functions and regulation of glutathione S-transferases in plants. *Annu. Rev. Plant Physiol. Plant. Mol. Biol.* 47, 127-158 (1996).
37. F. DADASOGLU. Isolation and identification of bacterial strains with insecticidal activities against green house and field pest. Ataturk University, Graduate School of Natural and Applied Science Institute, Department of Plant Protection, Master Thesis, 93 p. Turkey (2007).
38. L.T. MILLER. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *Journal of Clinical Microbiology*, 16, 584-586, (1982).
39. S. MEHTA, C.S. NAUTIYAL. An efficient method for qualitative nscreening of phosphate-solubilizing bacteria. *Curr. Microbiol.* 43:51-56 (2001).
40. R.E. PIKOVSKAYA. Mobilization of phosphates in soil in connection with vital activities of some microbial species. *Microbiol.* 17: 62-370 (1948).
41. M.L. JACKSON. Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd, New Delhi, 1973, (1989).
42. J. DOBEREINER. Isolation and identification of root associated diazotrophs, in: F.A. Skinner [Ed.], *Nitrogen Fixation with Non-Legumes*, Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 103-108 (1989).
43. M.M. Bradford. A rapid and sensitive method for quantitation or microgram quanlilies of protein ntili?ing the principle 01 protein-dychinding. *Anal. Biochem.* 72:248-254 (1976).
44. A.A. BELIMOV, A.M. KUNAKOVA, N.D. VASILYEVA, E.V. GRUZ-DEVA. Relationship between survival rates of associative nitrogen-fixers on roots and yield response of plants to inoculation. *FEMS Microbiol. Ecol.*, 17: 187-196 (1995).
45. Y. BASHAN. Inoculants for plant growth-promoting bacteria in agriculture. *Biotechnol Adv* 16: 729-770 (1998).
46. M. ŠPUNDOVÁ, K. SLOUKOVÁ, M. HUNKOVÁ, J. NAUŠ. Plant shading increases lipid peroxidation and intensifies senescence induced changes in photosynthesis and activities of ascorbate peroxidase and glutathione reductase in wheat. *Photosynthetica* 43, 403-409 (2005).
47. P.M. MULLINEAUX, G.P. CREISSEN. Glutathione reductase: regulation and role in oxidative stress, in Scandalio, J. G. T.: *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 667-713 (1997).
48. R. CAKMAKCI, M. ERAT, Ü. ERDOGAN, M.F. DONMEZ. The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. *J. Plant Nutr. Soil Sci.* 170, 288-295 (2007).
49. R. CAKMAKCI, M. ERAT, B. ORAL, Ü. ERDOGAN, F. SAHİN. Enzyme activities and growth promotion of spinach by indole-3-acetic acid-producing rhizobacteria. *Journal of Horticultural Science & Biotechnology*, 84 (4) 375-380 (2009).
50. D.P. WRIGHT, H.C. HUPPE, D.H. TURPIN. In vivo and in vitro studies of glucose-6-phosphate dehydrogenase from barley root plastids in relation to reductant supply for NO<sub>2</sub> assimilation. *Plant Physiol.* 114, 1413-1419 (1997).
51. S. ESPOSITO, G. MASSARO, V. VONA, V. DI MARTINO RIGANO, S. CARFAGNA, C. RIGANO. Ammonium induction of a novel isoform of glucose-6P dehydrogenase in barley roots. *Physiol. Plant.* 113, 469-476 (2001).
52. M. MADHAIYAN, S. POONGUZHALI, J.S., LEE, M. SENTHILKUMAR, K.C. LEE, S. ANDSUNDARAM. *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypicola* sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils. *Int J Syst. Evol Microbiol.* 60: 2451-2457 (2010).
53. J.Y. YU, Y. ZOU, S.T. KİM, T. LEE. The changing impact of ElNiño on US winter temperatures *Geophys. Res. Lett.* 39 (2012).
54. L. CHIARINI, A. BEVIVIONA, C. DALMASTRI, C. NACAMULLI, S. TABACCHIONI. Influence of plant development, cultivar and soil type on microbial colonization of maize roots. *Applied Soil. Ecol.*, 8:11-28 (1998).
55. A. PROBANZA, J.A. LUCAS GARCÍA, M. RUÍZ PALOMÍNO, B. RAMOS, F. J. G. MAÑERO. *Pinus pinea* L seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus (B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). *Applied Soil Ecology*, 20 (Suppl2): 75-84 (2002).