Plant growth promoting rhizobacteria (PGPR) as biofertilizer: Effect on growth of *Lycopersicum esculentus*

Ibiene AA[1], Agogbua JU[2], Okonko IO[1] and Nwachi GN[1]

[1]Department of Microbiology, University of Port Harcourt, East-West Road, PMB 5323 Choba, Port Harcourt, Rivers State, Nigeria
ibieneaa@yahoo.com; iheanyi.okonko@uniport.edu.ng
[2]Department of Plant Science and Biotechnology, University of Port Harcourt, East-West Road, PMB 5323 Choba, Port Harcourt, Rivers State, Nigeria; josyokoro@yahoo.com

Abstract: Plant growth promoting rhizobacteria (PGPR) are bacteria that colonize plant roots and encourage plant growth by a wide variety of mechanisms such as phosphate solubilization, phytohormone production, antifungal activity, etc. In this present study, effect of plant growth promoting rhizobacteria (PGPR) on *Lycoperscium esculentus* was examined. *Azotobacter* species, *Nitrobacter* species, and *Nitrosomonas* species were isolated and identified using standard methods. In-vitro screening of these PGPR was carried out to test their ability to produce phytohormones (siderophore, phosphate solubilization and indole acetic acid). Seed germination and seedling growth test were also conducted to evaluate the effect of PGPR on the germination of tomato seeds. The growth parameters (plant height, stem width, root length and the internode length of the plant) were monitored at 5 DAP (days after planting) interval from the day of sprouting. The findings of the study showed that the ability to solubilize phosphate was exhibited by *Nitrobacter* species and *Nitrosomonas* species while *Azotobacter* species produce indole acetic acid (IAA) and siderphore. It also showed that the consortium of the three isolates gave the best performance in terms of growth parameters (plant height = 15.8 cm, stem width = 1.0 cm, root length = 10.0 cm and the internode length = 3.8 cm) than the control (plant height = 11.0 cm, stem width = 0.5 cm, root length = 6.1 cm and the internode length = 2.5 cm) . Thus, the use of combined biofertilizers is advocated for excellent growth performance of plants.
[Ibiene AA, Agogbua JU, Okonko IO and Nwachi GN. **Plant growth promoting rhizobacteria (PGPR) as biofertilizer: Effect on growth of Lycopersicum esculentus.** Journal of American Science 2012;8(2):318-324]. (ISSN: 1545-1003). http://www.americanscience.org. 45

Keywords: *Azotobacter* species, *Nitrobacter* species, *Nitrosomonas* species, siderophore, phosphate solubilization, indole acetic acid, DAP, PGPR

1. INTRODUCTION

Tomato (*Lycoperscum esculentus*), according to the FAO, is the second most cultivated vegetable in the world, after the potato, with an annual production of nearly 108 t of fresh tomato in 3.7×10^6 ha worldwide, China, the USA and Turkey being the leading producers (FAO, 2004; Ordookhani et al., 2010). In addition to its economic importance, tomato consumption has recently been demonstrated to be beneficial to human health, because of its content of phytochemicals such as lycopene, β-carotene, flavonoids, vitamin C and many essential nutrients (Beutner et al., 2001; Ordookhani et al., 2010). This composition explains the high antioxidant capacity in both fresh and processed tomatoes (Gahler et al., 2003), associating the fruit with lower rates of certain types of cancer and cardiovascular disease (Rao and Agarwall, 2000; Ordookhani et al., 2010).

In the last century, chemical fertilizers were introduced and this made farmers to be happy of getting increased yield in agriculture in the beginning. But slowly chemical fertilizer started displaying their ill-effects such as leaching, polluting water basins, destroying microorganisms and friendly insects, making the crop more susceptible to the attack of diseases, reducing the soil fertility and thus causing irreparable damage to the overall system. One of the other most important effective factors in increasing plant yield is seed inoculation or priming with plant growth promoting rhizobacteria (PGPR) (Ashrafi and Seiedi, 2011). Also, plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Subba RaO 1999; Wu et al., 2005; Heidari et al., 2011).

The mechanisms by which PGPRs promote plant growth are not fully understood. But, several mechanisms have been suggested by which PGPR can promote plant growth and this include auxins (Egamberdiyeva, 2005), enhancing stress resistance, asymbiotic N2 fixation (Canbolat et al., 2006; Salantur et al., 2006), solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients (Cattelan et al., 1999; Jeon et al., 2003); increasing the supply or availability of primary nutrients to the host plant and antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of

antibiotics, enzymes or fungicidal compounds and competition with detrimental microorganisms (Lucy et al., 2004; Wu et al ,2005; Ahmad et al., 2006; Egamberdiyeva, 2007; Ashrafi and Seiedi, 2011). Kloepper and Beauchamp (1992) have been shown that cereal yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Strains of *Pseudomonas putida* and *Pseudomonas* fluorescens could increase root and shoot elongation in canola (Glick et al., 1997). Bashan et al. (2004) and Cakmake et al. (2006) reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in total plant biomass, nutrient uptake, plant height, leaf size, leaf area index and root length of cereals (Bashan et al, 2004; Ashrafi and Seiedi, 2011).

The soil microorganisms used in biofertilizers are phosphate solubilizing microbes, mycorrhizae, *Azospirilum* sp, *Azotobacter* sp, *Rhizobium* sp, sesbania, Blue green algae, *Nitrosomonas* sp, *Nitrobacter*, sp and *Azotolla* sp. Thus, the aim of this study was to determine the effect of biofertilizer bacteria - *Azotobacter* species; *Nitrosomonas* sp. and *Nitrobacter* sp. – isolated from Niger Delta soil on the growth of tomato plant *(Lycoperscum esculentus).*

2. MATERIALS AND METHOD

2.1. Collection of Soil Samples

Rhizosphere soil was collected from a tomato plant in a garden at Alakahia village Port-Harcourt. The soil sample was collected with a sterile trowel and transferred into a sterile bottle and then taken to the laboratory.

2.2. Isolation, Purification and Identification of Isolates

Nitrogen-free sucrose, ammonium and nitrite supplemented mineral salt agar media were employed for the isolation of *Azotobacter* sp., *Nitrosomonas* sp. and *Nitrobacter* sp., respectively (Okpokwasili and Odokuma, 1996 and Atlas, 1993). The test PGPRs were isolated from the soil sample using the spread plate method. Five grams of soil sample was mixed thoroughly in 45ml of sterile physiological saline. Following a 10-fold serial dilution of the soil suspension, 0.1ml of each dilution (10^{-1}-10^{-6}) was inoculated onto duplicate set of the various enrichment media. Plates were then incubated at 28 $\pm 2^0$C and examined after 7days for growth. Discrete colonies that developed were selected based on morpho-phenotypic characteristics (John et al.,1994 and Cheesebrough, 2006).

2.3. In Vitro Screening of Soil Bacteria for Plant Growth Promotion Activities

The isolated soil bacteria were screened for the production of indole acetic acid, phosphate solubilization, siderophore production. Siderophore production was tested qualitatively using chrome azural (CAS) agar as described by Alexander and Zuberer (1991). The bacterial isolate was streaked on the CAS agar plates and incubated at 28 $\pm 2^0$C for 24hrs. Orange halos around the colonies indicated siderophore-production.

Phosphate solubilization test was carried out by plating the bacteria on tricalcium phosphate agar medium (Chen et al., 2006). The presence of clearing zones around the bacterial colonies following incubation at 28 $\pm 2^0$C for 24hrs indicated positive for phosphate solubilization.

Bacterial indole acetic acid production was examined by growing isolates in nutrient broth supplemented with tryptophan (Ahmad et al., 2005). The growth cultures were centrifuged at 3,000rpm for 30mins and the resultant supernatant-filtrate mixed with salvakowski reagent in a ratio of 1:2. The mixture was then incubated for 30mins for the development of pink colour which indicated IAA production.

2.4. Preparation of Inoculum for Field Inoculation

The test organisms from the stock culture were resuscitated by sub culturing into enrichment media and incubated for 24hrs at 28 $\pm 2^0$C. After incubation, mineral salt medium was prepared by aseptically decanting the components into 1L of deionized water in four different 500ml conical flask. It was sterilized by autoclaving and allowed to cool. The test organisms (*Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp) were inoculated into each of the conical flask respectively and the fourth flask a mixture of the three organisms. It was incubated for 24hrs at 28 $\pm 2^0$C; the seeds for planting were soaked for 6hrs before taken to the field for sowing.

2.5. Collection and Preparation of Soil Sample

The soil sample (loamy soil) was obtained from a garden at Alakahia village, Port Harcourt, Rivers State, Nigeria. The soil was collected from the top 15cm depth with a trowel and evenly distributed into sterile planting pots. The planting pots were labeled appropriately and watered carefully awaiting the application of seeds.

2.6. Seed Viability Test

Seeds of tomato plant *(Lycopersicum esculentus)* were extracted and air dried for 5 days. The viability of the seeds were tested by planting about 50 seeds of tomato on a tray which had up to five small openings to drain out excess water. The seeds were spread on the tray and slightly covered

with the soil. The tray was covered with a plastic bag for two days to create humidity. After 5-6days, it was observed that about 90% of seeds germinated and this showed that the seeds were highly viable.

2.7. Seed Inoculation with the Bacterial Isolates

Seeds before sowing were treated with different bacterial suspension (*Azotobacter* sp, *Nitrobacter* sp, and *Nitrosomonas* sp) by aseptically soaking into the broth of each organism respectively and a mixture of the three organisms for about 6 hours when it has uniformly coated on the seeds. The seeds were removed and air dried in a shade and then sowed immediately into the appropriate planting pots.

2.8. Field Experimental Design

The planting pots were 1m apart from each other. The treatment consisted of A (control) – Garden soil (without biofertilizer), B– Garden soil + Biofertilizer (*Azotobacter* sp), C– Garden soil + biofertilizer (*Nitrobacter* sp), D – Garden soil + Biofertilizer (*Nitrosomonas* sp), and E – Garden soil + biofertilizer (*Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp).

2.9. Seedling Growth Test

Seedling growth test was carried out and the growth parameters were taken after five days interval for plant height (cm), stem width (cm), internode length and root length (cm) of the plant was taken 30days at the end of the experiment.

3. RESULTS ANALYSIS

3.1. Production of IAA, Siderophore and Solubilization of Phosphorus

The plant growth promoting properties of the test bacterial isolates were presented in Table 1. As shown in Table 1, isolates *Azotobacter* sp, induced the IAA production. *Nitrobacter* sp and *Nitrosomonas* sp had ability to solubilize the phosphorus. On the other hand only *Azotobacter* sp induced the siderophore production. It has been reported that IAA production by PGPR can vary among different species and it is also influenced by culture condition, growth stage and substrate ability (Mirza et al., 2001; Mishra et al., 2010). PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to *Lycopersicum esculentus* that represent a possible mechanism of plant growth promotion under field condition (Verma et al., 2001; Mishra et al., 2010). In comparison to non-rhizospheric soil, higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere (Mishra et al., 2010). Suresh et al. (2010) indicated that most of the isolates tested in their study possessed plant growth promoting traits and that these isolates can be used as potential biofertilizers and also as biocontrol agents.

Table 1: Plant Growth Promoting Properties of the Test Bacterial Isolates

PHYTOHORMONES	*Azotobacter* sp	*Nitrosomonas* sp	*Nitrobacter* sp
Indole acetic acid	Present	Absent	Absent
Phosphate solubilization	Absent	Present	Present
Siderophore production	Present	Absent	Absent

3.2 MEASUREMENT OF GROWTH PARAMETERS

3.2.1. Plant height

The PGPR isolates significantly affected the height of *Lycopersicum esculentus* plants. Results reveal that the height increased in PGPR treated plants over uninoculated control. From Table 2, it showed that the control (A) measured 4.0cm on day 5 and increased to 11.0cm on day 30DAP after planting has the lowest measurement whereas E which was the combination of the three isolates (*Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp) measured 6.8cm on day 5 and increased to 15.8cm on day 30DAP and it has the highest measurement.

Table 2 shows plant height (cm) of tomato (*Lycopersicum esculentus*) recorded 5-30 days after planting (DAP). Similar results have been reported by Bashan et al (2004) and Ashrafi and Seiedi (2011). Bashan et al (2004) reported that inoculation of plants with Azospirillum could result in significant changes in various growth parameters, such as plant height. Ashrafi and Seiedi (2011) reported that that in order to increase of grain yield should be applied 9 plants m-2 in seed priming with *Azosprilium lipoferum* strain OF in conditions of Ardabil Plain.

Zaidi and Khan (2005) have suggested that seed priming with PGPR increased dry matter accumulation. The increase in dry matter accumulation with seed priming with PGPR indicates the favorable response of corn hybrids to seed priming with PGPR. Similar observations were also made by Golami er al. (2009) in corn. Perveen et al. (2002); Wani et al. (2007) have reported increase in dry matter accumulation due to inoculation with PGPR. Nezarat and Gholami (2009) in their third experiment showed that inoculation of maize seeds with all bacterial strains significantly increased plant height, 100 seed weight, and number of seed per ear and leaf area. Their results also showed significant increase in ear and shoot dry weight of maize.

3.2.2. Stem width

The PGPR isolates significantly affected the stem width of *Lycopersicum esculentus* plants. Results reveal that the stem width increased in PGPR treated plants over uninoculated control. The measurement of the stem width was taken 5 days

interval. From Table 3, it has been observed that the control had the lowest measurement ranging from 0.1-0.5cm on 5-30 days while the combination of the three isolates had the highest measurement ranging from 0.3-1.3cm on 5-30 days as in plant height. Table 3 shows stem width (cm) of tomato (*Lycopersicum esculentus)* recorded 5-30 days after planting (DAP). Nezarat and Gholami (2009) in their second experiment showed that leaf and shoot dry weight and also leaf surface area significantly increased by bacterial inoculation in both sterile and non-sterile soil. Their results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in nonsterile than sterile soil.

Table 2: Plant Height (cm) Of Tomato (*Lycopersicum esculentus) Recorded 5-30 Days after Planting (DAP)*

TREATMENT	5	10	15	20	25	30
A (Control)-Garden soil with biofertilizer	4.0	5.2	6.5	8.0	9.5	11.0
B-Garden soil + Biofertili (*Azotobacter*)	5.5	7.0	9.2	10.7	12.3	14.2
C-Garden soil + Biofertili (*Nitrobacter*)	5.0	6.8	8.6	10.5	12.0	14.0
D-Garden soil + Biofertili (*Nitrosomonas*)	5.3	7.1	9.0	10.6	12.2	14.3
E-Garden soil + Biofertili (*Azotobacter*, *Nitrobact Nitrosomonas*)	6.8	8.0	9.8	11.0	13.2	15.8

Table 3: Stem Width (cm) of Tomato (*Lycopersicum esculentus) Recorded 5-30 Days after Planting (Dap)*

TREATMENT	5	10	15	20	25	30
A (Control)-Garden soil with biofertilizer	0.1	0.2	0.2	0.3	0.4	0.5
B-Garden soil + Biofertili (*Azotobacter* sp)	0.2	0.3	0.4	0.5	0.6	0.8
C-Garden soil + Biofertili (*Nitrobacter* sp)	0.1	0.3	0.4	0.5	0.5	0.6
D-Garden soil + Biofertili (*Nitrosomonas* sp)	0.2	0.3	0.4	0.5	0.6	0.7
E-Garden soil + Biofertili (*Azotobacter* sp, *Nitrobacter Nitrosomonas* sp)	0.3	0.4	0.6	0.8	0.9	1.0

3.2.3. Root and Internode length

A significant variation in root and internode length was observed in response to different PGPR isolates. In this study, the effectiveness of PGPR isolates on root length and internode length were investigated. The root length was taken at the 30th day of the experiment when a measurable value was gotten. A which was the control measured 6.1cm, B (treated with *Azotobacter* sp) measured 8.0cm, C (treated with *Nitrobacter* sp) measured 8.5cm, D (treated with *Nitrosomonas* sp) measured 8.3cm, and E (combination of *Azotobater* sp), *Nitrobacter* sp and *Nitrosomonas* sp) measured 10.0cm. From these values, the control had the lowest measurement 6.1cm while E which was treated with the three isolates had the highest measurement. Table 4 shows the Internode Length (cm) of tomato (*Lycopersicum esculentus)* recorded 10-30 days after planting (DAP).

The effects of plant growth promoting rhizobacteria (PGPR) on tomato plant (*Lycopersicon esculentum)* were clearly demonstrated. Bacterial inoculants (*Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp) were able to increase plant growth and germination rate, improve seedling emergence, responses to extended stress factors and protect plants from diseases.

Table 4: Internode Length (cm) Of Tomato (*Lycopersicum esculentus) Recorded 10-30 Days after Planting (Dap)*

TREATMENT	10	15	20	25	30
A (Control)-Garden soil without biofertilizer	1.0	1.3	1.7	2.2	2.5
B-Garden soil + Biofertilizer (*Azotobacter* sp)	1.5	2.0	2.6	3.0	3.2
C-Garden soil + Biofertilizer (*Nitrobacter* sp)	1.0	1.5	2.0	2.5	2.9
D-Garden soil + Biofertilizer (*Nitrosomonas* sp)	1.6	2.1	2.8	3.0	3.3
E-Garden soil + Biofertilizer (*Azotobacter Nitrobacter* sp, *Nitrosomonas* sp)	2.0	2.5	3.0	3.5	3.8

4. DISCUSSION

This study revealed that the tomato plants that were grown with combination of the three microbial inoculants (Treatment E) had greater value in all the growth parameters monitored such as plant height, stem width, root length, and the internode of the plant, than the plants that was treated with one microbial inoculant (Treatments, B, C, D) and also the control (Treatment A) which was not treated with any biofertilizer had the lowest value (Table 2-4).

These results were similar with the findings of Dobbelaere et al. (2003) who assessed the inoculation effect of PGPR *Azospirillum brasilense* on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering. Dobbelaere et al (2003) and Cakmakı (2005a) have been reported that PGPR can increase yield and leaf area index, shoot and root weight and delay leaf senescence. A similar result was reported by Vivas *et al*. (2003) who showed that inoculation of bacterial strain increased stomatal conductance and chlorophyll content of lettuce compared to a non- drought control. Ordookhani et al. (2010) reported that in all their treatments, shoot and fruit potassium increased when PGPR and Arbuscular Mycorrhiza Fungi (AMF) were used together. Ordookhani et al. (2010) also found that the application of Pseudomonas + *Azotobacter* + *Azosprillum* + AMF treatment had the most effect on lycopene, antioxidant activity and potassium contents on tomato. Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported by Biswas et al. (2000) and Asghar et al (2002).

Trials with Plant growth-promoting rhizobacteria indicated that yield and dry matter accumulation increase in wheat (De Freitas., 2000; Cakmakı et al., 2007), maize (Pal, 1998; Ashrafi and Seiedi, 2011; Sharifi et al., 2011); sugarcane (Sundara et al, 2002), rice (Sudha et al, 1999), and barley (Cakmakı et al., 2001; Fiahin et al., 2004). Numerous other studies have shown a substantial increase in dry matter accumulation and seed yield following inoculation with PGPR (Perveen et al., 2002; Wani et al., 2007; Mishra et al., 2010; Sharifi et al., 2011). Dilfuza (2007) suggested that inoculation of corn seeds with *Azospirillum brazilance* increased dry matter accumulation. Mishra et al. (2010) reported that most of isolates used in their study resulted in a significant increasing of shoot length, root length and dry matter production of shoot and root of *Cicer arietinum* seedlings. Application of PGPR isolates significantly improves the percentage of seed germination under saline conditions (Mishra et al., 2010).

The results of the study by Sharifi et al. (2011) showed that seed priming with Plant Growth Promoting Rhizobacteria affected grain yield, plant height, number of kernel per ear, number of grains per ear row significantly. Maximum of these characteristics were obtained by the plots which seeds were inoculated with *Azotobacter* bacteria.

5. CONCLUSION

The present study, therefore suggest that the use of PGPR isolates *Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp as inoculants biofertilizers might be beneficial for *Lycopersicum esculentus* cultivation. Biofetilizers are ecofriendly and pose no pollution threat to our environment unlike chemical fertilizer which causes environmental hazards such as water pollution, soil humus reduction, increased susceptibility to pests and diseases etc. Microbial inoculants play a significant role in regulating the dynamics of organic matter decomposition and availability of plant nutrients such as nitrogen, phosphorus, potassium.

From this study, it has been shown that the combined use of the three bacterial inoculants (*Azotobacter* sp, *Nitrobacter* sp and *Nitrosomonas* sp) had the highest value of the growth parameters monitored while the control (treatment A) had the lowest value measured. Biofertilizer has been widely used with excellent result for the growth of different kinds of plant and in several countries. Most of the isolates significantly increased plant length, root length and internode length root of *Lycopersicum esculentus*. Our results suggested that PGPR are able to enhance the production of IAA, solubilization of phosphorus, and siderophore production, thereby improving growth of *Lycopersicum esculentus* plant. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable *Lycopersicum esculentus* cultivation in Nigeria and other developing countries.

Correpodence to:
Dr. Abiye A. Ibiene
Department of Microbiology,
University of Port Harcourt,
East-West Road, PMB 5323 Choba,
Port Harcourt, Rivers State, Nigeria
Email: ibieneaa@yahoo.com; Tel.: +2348066720531

REFERENCES

1. Ahmad, F., I. Ahamad and M.S. Khan. (2006). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res*., 36:1-9.
2. Ahmad F, Ahmad I, Khan MS. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudmonas* in the presence and absence of tryptophan. Turk. J. Biol. 29 (2005) 29-34
3. Alexander, D.B. and D.A. Zuberer. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils 2: 39-45.
4. Asghar, H.N., Z.A. Zahir., M. Arshad and A. Khaliq (2002). Relationship between invitro production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea Bio. Fertile. Soil,* 35: 231-237.
5. Ashrafi V and Seiedi MN. Influence of different plant densities and plant growth promoting rhizobacteria (PGPR) on yield and yield attributes of Corn (*Zea maize* L.). *Recent Research in Science and Technology 2011, 3(1): 63-66.*
6. Atlas, R.M. 1993. Handbook of Microbiology Media. CRC Press, Boca Raton.
7. Bashan, Y., K. Holguin. and L.E. de-Bashan. 2004. Azospirillum-plant relationships: physiological, molecular, agricultural and environmental dvances. Can. J. Microbiol. 50:521–577.
8. Beutner S, Bloedorn B, Frixel S, Blanco IH, Hoffman T, Martin H (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. J. Sci. Food Agric., 81:559-568.
9. Biswas JC, Ladha LK, Dazzo FB (2000). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. J. Soil. Sci. 64:1644-1650.

10. Cakmake, R.I., D.F. Aydm and A.F. Sahin. (2006). Growth promotion of plants by plant growth promoting rhizobacteria under green house and two different field soil conditions. *Soil Biol. Biochem*., 38: 1482-1487.
11. Cakmakı R (2005a). Bitki geliflimin de fosfat bakterilerin .nemi. Seluk Univ. Ziraat Fakutesi Dergisi 35: 93-108.
12. Cakmakı R, Kantar F, Fiahin F (2001). Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. J. Plant Nutr. Soil Sci. 164: 527-531.
13. Cakmakı, R., M. Erat, U.G. Erdo and M.F. Dnmez. 2007. The influence of PGPR on growth parameters, antioxidant and pentose phosphate oxidative cycle enzymes in wheat and spinach plants. J. Plant Nutr. Soil Sci. 170: 288-295.
14. Cattelan A. J., P. G. Hartel. And J. J. Fuhrmann. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J. 63: 1670-1680.
15. Cheesbrough M.(2006). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, U.K. p434
16. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology 34 : 33–41 (2006)
17. De Freitas JR (2000). Yield and N assimilation of winter wheat (*Triticum aestivum* L., var Norstar) inoculated with rhizobacteria. Pedobiologia 44: 97-104.
18. Dilfuza Egamberdiyeva (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Applied Soil Ecology. 36: 184-189
19. Dobbelaere S, Vanderleyden J, Yaacov Y, Okon L (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical Rev. Plant Sci. 22: 107-149
20. Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, Y. Okon and J. Vanderleyden, 2002. Effect of inoculation with wild type *Azospirillum brasilense* and A. irakense strains on development and nitrogen uptake of spring wheat and grain maize. Biol. Fertil. Soils, 36: 284-297.
21. Egamberdiyeva D. 2005. Plant-growth promoting rhizobacteria isolated from a Calcisol in a semi-arid region of Uzbekistan: biochemical characterization and effectiveness. J. Plant Nutrit. Soil Sci. 168: 94-99.
22. Egamberdiyeva, D., (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Appl. Soil. Eco.* 36: 184-189.
23. FAO, Crop Description and Climate (2004). Available: http://www.fao.org/ag/agl/aglw/cropwater/tomato.stm#- descrip [24 January 2005].
24. Fiahin F, Cakmak R, Kantar F (2004). Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. Plant and Soil.265: 123-129.
25. Gahler S, Otto K, Bohm V (2003). Alterations of vitamin C, total phenolics and antioxidant capacity as affected by processing tomatoes to different products. J. Agric. Food Chem., 51: 7962-7968.
26. Gholami A, Shahsavani S, Nezarat S (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Proceedings of Word Academy of Science. Engineering and Technology. 37: 2070-3740.
27. Glick B.R., L. Changping., G. Sibdas. and E.B. Dumbroff. 1997. Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium Pseudomonas putida GR12-2. Soil Biol. Biochem. 29: 1233-1239.
28. Heidari M, Mousavinik SM and Golpayegani A. Plant Growth Promoting Rhizobacteria (PGPR) Effect on Physiological Parameters and Mineral Uptake in Basil (*Ociumum basilicm* L.) Under Water Stress. ARPN Journal of Agricultural and Biological Science, 2011; 6(5): 6-11
29. Jeon J.S., S. S. Lee., H. Y. Kim, T. S. Ahn. H. G. Song. 2003. Plant growth promotion in soil by some inoculated microorganisms. J. Microbiol. 41: 271-276
30. John, G.H.; Neol, R.K.; Peter, H.A.; James, T.S.; Stanley, T.W. (1994). Bergey's Manual of Determinative Bacteriology (9th edition), Williams and Wickins, Maryland.
31. Jolt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. Williams, 1994. Bergey's manual of systematic bacteriology, 9 ed. Williams & Wilkins Co. Baltimore, Maryland, pp: 786.
32. Kloepper J.W. and C.J. Beauchamp. 1992. A review of issues related to measuring of plant roots by bacteria. Can. J. Microbiol. 38: 1219-1232.
33. Lucy M., E. Reed. B. R. Glick. 2004. Application of free living plant growth promoting rhizobacteria. Antonie Van Leeuwenhoek. 86(1): 1-25.
34. Mirza, M.S., W. Ahmad, F. Latif, J. Haurat, R. Bally, P. Normand and K.A. Malik. 2001. Isolation, partial characterization and effect of plant growth promoting bacteria on

micropropagated sugarcane *in vitro*. Plant Soil., 237: 47-54.
35. Mishra M, Kumar U, Mishra PK, Prakash V. Efficiency of Plant Growth Promoting Rhizobacteria for the Enhancement of *Cicer arietinum* L. Growth and Germination under Salinity. Advances in Biological Research 4 (2): 92-96, 2010
36. Nezarat S, Gholami A. Screening Plant Growth Promoting Rhizobacteria for Improving Seed Germination, Seedling Growth and Yield of Maize. *Pakistan Journal of Biological Sciences,* 2009; *12: 26-32*.
37. Okpokwasili GC, Odokuma LO. 1996. Response of *Nitrobacter sp.* to toxicity of drilling chemicals. *J. Pet. Sci.Engr. 16:*81-87.
38. Ordookhani K, Khavazi K, Moezzi A, Rejali F. 2010. Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. African Journal of Agricultural Research, 2010; 5(10): 1108-1116
39. Pal, S.S. 1998. Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 198: 169-177.
40. Perveen S, Khan MS, Zaidi A (2002). Effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiatus* L.). Ind. J. Agric. Sci. 72: 421-423.
41. Rao AV, Agarwall S (2000). Role of antioxidant lycopene in cancer and heart disease. J. Am. CollNutr., 19: 563-569.
42. Salantur A., A. Ozturk and S. Akten. 2006. Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria. Plant. Soil. Environ. 52(3): 111-118.
43. Sharifi RS, Khavazi K , Gholipouri A. Effect of seed priming with plant growth promoting Rhizobacteria (PGPR) on dry matter accumulation and yield of maize *(Zea mays* L.) hybrids. International Research Journal of Biochemistry and Bioinformatics, 2011; 1(3):076-083
44. Subba Rao, N.S. 1999. Soil Microbiology (Fourth Edition of Soil Microorganisms and Plant Growth). Science Publishers, Inc. USA.
45. Sudha SN, Jayakumar R, Sekar V (1999). Introduction and expression of the cry1Ac gene of Bacillus thuringiensis in a cereal associated bacterium, Bacillus polymyxa. Curr. Microbiol. 38:163-167.
46. Sundara, B., V. Natarajan and K. Hari. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yield. Field Crop Res. 77:43-49.
47. Suresh A, Pallavi P, Srinivas P, Kumar VP, Chandra SJ, Reddy SR. Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. African Journal of Microbiology Research, 2010; 4(14): 1491-1494.
48. Verma, S.C., J.K. Ladha and A.K. Tripathi, 2001. Evaluation of plant growth promoting and coloization, ability of endophytic diazotrophs from deep water rice. J. Biotechnol., 91: 127-141.
49. Vivas A., A. Marulanda., J. M. Ruiz-Lozano., J. M. Barea. And R. Azcon. 2003. Influence of a Bacillus sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG induced drought stress. Mycorrhiza. 13: 249-256.
50. Wani PA, Khan MS and Zaidi A (2007). Synergistic effects of the inoculation with nitrogen fixing and phosphate-solubilizing rhizobacteria on the performance of field grown chickpea. J. Plant Nutr. Soil Sci. 170: 283-287.
51. Wu, S.C., Z.H. Cao., Z.G. Li., K.C. Cheung and M.H. Wong. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma. 125:155–166.
52. Zaidi A, Khan MS (2005). Interactive effect of rhizospheric microorganisms on growth, yield and nutrient uptake of wheat. J. Plant Nutr. 28: 2079-2092.

1/2/2012